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Editor

Emerging Environmental Technologies

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Volume II

Edited by

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Preface

Within the span of last couple of years, the increasing human interference with various natural ecosystems and higher discharge of pollutants has presented numerous challenges to the society related to preserving the nature for a better tomorrow. The challenges also mount pressure on the scientific community to invent technologies that would provide solutions to the problems that are man made and also decrease the negative consequences that we are facing because of our own actions.

This edited book attempts to present eight technological innovations that have shown potential to provide answers to a few challenges. Like the previous collection, the described innovations in the current volume also cover a range of areas including water and soil pollution, bio-sensors and energy.

However, it is to be realized that no combination of technology can be enough to make a sizeable difference. As I said in my last collection, technological advances have to be integrated with a change in social behavior. The philosophy of sustainable development has to be the principle of future planning and growth. In this collection, I am pleased to include an article on noise pollution. Noise is a pollutant of our own behavior and can only be solved by a behavioral change. The change that is either voluntary or enforced by laws. As an environmental scientist noise is not normally a pollutant that would come in mind as a leading pollutant. After reading some articles on the increasing effect of noise pollution on human health, I invited Dr. Bronzaft and Dr. Hagler to contribute an article on the subject. The intention was to make the readers aware of the consequences of the noise pollution, some we may already be facing without realizing it. I hope that the article would make a good read for all the readers irrespective of their area of specialty.

All the articles that are presented through this book are peer-reviewed articles, and I wish to thank all the contributing authors, including the ones whose work did not make it through the peer-review process. I would also like to thank the reviewers of the articles for taking the time to provide their valuable comments.

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Chapter 1
Immobilization of Uranium in Groundwater Using Biofilms

Bin Cao, Bulbul Ahmed, and Haluk Beyenal

Abstract Uranium is one of the most common radionuclides in soils, sediments, and groundwater at radionuclides-contaminated sites. At these contaminated sites, uranium leaches into the groundwater, which has become a widespread problem at mining and milling sites across North America, South America, and Eastern Europe. The movement of groundwater usually transports soluble uranium contaminants beyond their original boundaries, causing a global problem in aquifers, water supplies, and related ecosystems and posing a serious threat to human health and the natural environment. In order to meet the EPA standards, extensive efforts have been made to assess and remediate uranium-contaminated sites. As a cost-effective technology with minimal disruption to the environment, bioremediation harnessing indigenous microbial processes for cleanup has been utilized for uranium remediation. In the first part of this chapter, various uranium remediation technologies are discussed. Emphasis is placed on the principles and mechanisms of uranium bioremediation and the key factors affecting it. The second part of this chapter focuses on the use of biofilms for uranium immobilization in groundwater from subsurface environments. Most of the literature studies on uranium bioremediation have been conducted with suspended microorganisms or enriched sediments, which were eventually spiked with micro- or nano-particles of other minerals. However, biofilms are the commonly found microbial growth pattern in natural soils and water-sediment interfaces. With heterogeneous and complex biotic, abiotic and redox conditions significantly different from those in bulk conditions, biofilms pose challenges in predicting the mobility of uranium. Although previous studies have improved our understanding of uranium immobilization processes in biofilms, in order to efficiently and sustainably immobilize uranium at contaminated sites using indigenous biofilms, more knowledge is needed on the complex interactions among uranium, biofilms, and various redox-sensitive minerals during *in situ* uranium bioremediation.

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Abbreviations

DU	Depleted Uranium
EPA	Environmental Protection Agency
MCL	Maximum Contaminant Level
PRBs	Permeable Reactive Barriers
DMRB	Dissimilatory Metal-Reducing Bacteria
LPS	Lipopolysaccharide
EPS	Extracellular Polymeric Substances
UMTRA	Uranium Mill Tailing Remedial Action
E_h	Electrochemical Potential
V_{SHE}	Potential Against Standard Hydrogen Electrode Potential
DIRB	Dissimilatory Iron-Reducing Bacteria
SRB	Sulfate-Reducing Bacteria
FBCR	Fixed Bed Column Reactor
TEM	Transmission Electron Microscopy
SAED	Selected Area Electron Diffraction
EDS	Energy-Dispersive Spectrometry
HRTEM	High-Resolution Transmission Electron Microscopy

1.1 Introduction

Uranium occurs naturally as a radioactive metallic element in soils and rocks, in concentrations generally varying between 0.5 and 5 ppm. It has three different isotopes – ^{238}U, ^{235}U, and ^{234}U, with natural abundances of 99.27, 0.72 and 0.005%, respectively [1, 2]. Among them, ^{235}U has the distinction of being a naturally occurring fissile isotope. It is used in nuclear weapons and nuclear power reactors. These applications require the enrichment of ^{235}U to a concentration of 3–5%. During the enrichment process, significant quantities of uranium that is depleted of ^{235}U but has a correspondingly increased fraction of ^{238}U, so-called depleted uranium (DU), are generated. The uranium enrichment process, combined with nuclear weapons testing and nuclear energy generation, have by now created large amounts of radionuclide wastes, which in some cases have been released into the environment accidentally and become one of the main sources of uranium contamination in the environment. Radioactive wastes produced during mining (ore extraction), milling (physical and chemical extraction of uranium from the ore), manufacturing and other human activities, such as the use of phosphate fertilizers and combustion from coal and other fuels [3, 4], are other sources of uranium contamination.

At contaminated sites, uranium leaches into the subsurface, which has become a widespread problem at mining and milling sites across North America, South America, and Eastern Europe [5, 6]. There are four different oxidation states of uranium in aqueous systems: U(III) (highly unstable), U(IV), U(V) (unstable), and U(VI). U(VI) is known to form complexes with carbonate, chloride, sulfate and various organic chelating agents such as acetate. Soluble U(VI) species are the predominant forms of uranium in contaminated groundwater and soils. The movement of groundwater usually transports the soluble U(VI) contaminant beyond its original boundaries, causing a global problem in aquifers, water supplies, and related ecosystems and posing a serious threat to human health and the natural environment [7, 8].

Uranium can enter the human body via inhalation (aerosols), ingestion (drinking and eating), and wounds (embedding) [9], and it poses a threat to human populations due to its radioactivity and chemotoxicity [10, 11, 12]. Although there are no conclusive epidemiological data correlating uranium wastes exposure to specific health effects, studies using cells and animals suggest the possibility of genetic, reproductive, and neurological effects from chronic exposure [13].

All uranium isotopes present in uranium contaminants are radioactive and chemically toxic [14]. It is generally accepted that when uranium enters the human body, radiation and chemical toxicity can increase the risk of cancers such as bone cancer and lung cancer and that uranium can accumulate in kidneys for a long period and cause renal dysfunction and structural damage [15, 9]. Milacic's report published in 2008 on health investigations of uranium waste clean-up workers in a DU-contaminated site in Serbia showed that although disease or tumors did not develop during the investigation period of four years, the total number of DNA alterations and damaged cells was higher after uranium decontamination [16].

Because of the threat of uranium radioactivity and chemotoxicity to human populations, it is very important to ensure that uranium contamination is under control. The U.S. Environmental Protection Agency (EPA) has established a Maximum Contaminant Level (MCL) of 30 μg/L for uranium in drinking water [17]. Guidance on implementation of the standard is provided by the EPA Office of Solid Waste and Emergency Response Directive no. 9283.1-14, "Use of Uranium Drinking Water Standards Under 40 CFR 141 and 40 CFR 192 as Remediation Goals for Groundwater at CERCLA Sites."

In order to meet the EPA standards, extensive efforts have been made to assess and remediate the uranium-contaminated sites. In the U.S., the total volume of all radionuclide wastes is 5.5 million m^3 and the volumes of contaminated soil and water have been reported to be 30–80 million m^3 and 1,800–4,700 million m^3, respectively. Uranium is one of the most common radionuclides in soils, sediments, and groundwater at these contaminated sites [18, 19, 4]. In the first part of this chapter, various uranium remediation technologies are discussed. Emphasis is placed on the principles and mechanisms of uranium bioremediation and the key factors affecting it. The second part of this chapter focuses on the use of biofilms for uranium immobilization in groundwater from subsurface environments.

1.2 Remediation Technologies

Generally, environmental remediation deals with the removal of pollution or contaminants from the environment, including soils, groundwater, sediment and surface water, for the protection of human health and the environment or for the redevelopment of brownfield sites. Various remediation technologies are available, including the more traditional physical and chemical approaches, such as "excavation and disposal" and "pump and treat," and the biological approaches, such as bioremediation and phytoremediation. The remediation technologies that have been used for uranium remediation are discussed in the following sections.

1.2.1 Physical and Chemical Remediation of Uranium

Among the physical and chemical remediation technologies, the pump and treat and permeable reactive barriers (PRBs) methods have been used in the field for uranium remediation [4]. In the pump and treat method, polluted groundwater is pumped to the surface, where it can be treated more easily. This traditional *ex situ* remediation approach has been used by the EPA at over 500 Superfund sites (hazardous waste sites placed on the EPA National Priority List based on a scoring process that rates current and potential health impacts) [20]. However, its application in uranium remediation is limited by poor extraction efficiency, the generation of large volumes of toxic uranium waste, and the increased public health and safety risks of bringing uranium contaminants up to the surface [18].

PRBs provide a relatively quick, economical *in situ* groundwater cleanup method. Reactive barriers such as zero-valent iron and phosphate minerals are placed in the subsurface to intercept a plume of contaminated groundwater that moves through it as it flows, typically on its natural gradient. As the contaminants move through the material, they are transformed into less harmful or immobile species. PRBs have been installed at more than 40 sites in the U.S. and Canada. This *in situ* remediation method has been applied to uranium-contaminated sites [21, 22, 23, 24]. However, when uranium is present at a high concentration, it precipitates and/or is absorbed in such large quantities that it causes the diversion of subsurface groundwater flow paths, exacerbating the subsurface containment issues by increasing the region influenced by the contaminant plume [25].

Although pump and treat methods and PRBs have been used in the field, their application on a large scale is limited by their high cost and low sustainability [4]. Thus, to circumvent the issues associated with these methods, bioremediation – the strategy of harnessing indigenous microbial processes for cleanup – has been extensively studied in the past decade. It is a cost-effective technology with minimal environmental disruption and has been utilized to achieve the goal of uranium remediation [18, 26, 27].

1.2.2 Bioremediation of Uranium

Bioremediation is defined as the use of microbes to detoxify contaminants present in the environment [28]. There are a number of *ex situ* and *in situ* bioremediation methods currently available [29]. Bioremediation usually works by either transforming or degrading contaminants into nonhazardous or less hazardous chemicals, so-called biotransformation and biodegradation, respectively [30]. Unlike organic compounds, metals and radionuclides such as uranium cannot be biodegraded [27]. Uranium bioremediation immobilizes uranium by converting soluble U(VI) species into insoluble U(IV) species. In general, laboratory tests and *ex situ* bioremediation applications have shown that microorganisms can change the oxidation states of various heavy metals and radionuclides, either increasing contaminant mobility to provide a route for removal from solid matrices such as soils, sediments, dumps and industrial waste, or immobilizing the metals and radionuclides, which enables transformation into insoluble, chemically inert forms which are thus removed from the aqueous phase [31, 27].

1.2.2.1 Uranium Bioimmobilization Mechanisms

Within the subsurface, microbial activity can be harnessed for uranium immobilization through various processes [32, 3, 12, 27]: (i) direct or indirect reduction of U(VI) to U(IV); (ii) biosorption of uranium onto the cells; (iii) precipitation of uranium by organic complexing ligands produced by the cells; (iv) bioaccumulation of uranium in the cytoplasm through chelating to polyphosphate bodies or forming needle-like fibrils. A schematic illustration of the various mechanisms of U(VI) bioremediation using bacterial cells is shown in Fig. 1.1.

Uranium Reduction

The microbial reduction of soluble U(VI) to insoluble U(IV), preventing its migration with groundwater, has been proposed for the immobilization of uranium [33, 34]. The reduction can be carried out directly by microbes or indirectly through electron transfer by metal-reducing bacteria [35]. Much research is going into efforts to develop *in situ* subsurface bioremediation technology for the U.S. Department of Energy Contaminated Sites. The first report on the microbial reduction of U(VI) appeared around 50 years ago: the reduction was demonstrated in crude extracts from *Micrococcus lactilyticus* (reclassified as *Veillonellar alcalescens*) [36]. Lovley and coworkers did pivotal work in establishing the reduction of U(VI) by dissimilatory metal-reducing bacteria (DMRB) such as *Desulfovibrio desulfuricans* and *Shewanella oneidensis* [33, 37, 34]. Currently, more than 25 phylogenetically diverse species of prokaryotes are known to be capable of U(VI) reduction [12]. Table 1.1 shows selected bacteria that are reported to be involved in U(VI) reduction.

The mechanism of electron transport during U(VI) reduction by DMRB has not been conclusively elucidated [27]. Recently, Renshaw et al. [38] demonstrated that, in *G. sulfurreducens*, single-electron reduction of U(VI) to U(V) followed

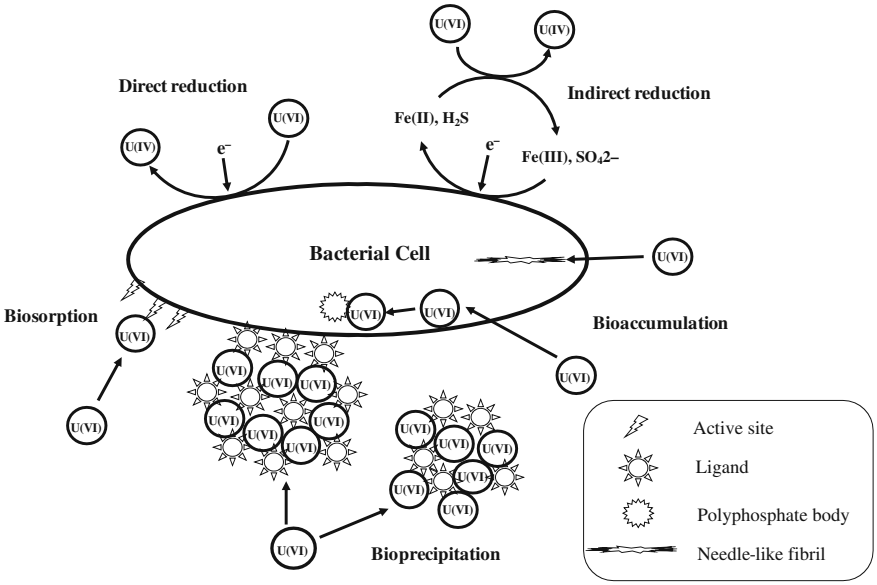


Fig. 1.1 Reducing and non-reducing mechanisms during the bioremediation of uranium using bacterial cells: direct and indirect reduction, biosorption, bioprecipitation, and bioaccumulation [32, 35, 3, 44, 27]

by disproportionation of the unstable U(V) complexes to U(IV) and U(VI) was the likely mechanism of uranium reduction. Due to the insoluble nature of U(IV) dioxide (UO_2), examination of the U(IV) deposition site provides an indication of the location of the enzymes responsible for U(VI) reduction. Although uraninite deposits within the cytoplasm in *Pseudomonas* sp., *D. desulfuricans*, and *D. aspoensis* have been reported [39, 3, 40], most research has found that insoluble U(IV) accumulates in the periplasm and on the outside of both Gram-negative and Gram-positive bacterial cells [41, 42, 37, 43], suggesting that U(VI) does not generally have access to intracellular enzymes. The enzymes responsible for U(VI) reduction would be electron-carrier proteins or enzymes exposed to the outside of the cytoplasmic membrane, within the periplasm, and/or in the outer membrane [27]. Recently, *c*-type cytochromes have been shown, in vitro and in vivo, to play an important role in the U(VI) reduction process, as summarized in Table 1.2.

Biosorption

Biosorption is defined as the metabolism-independent immobilization of heavy metals and radionuclides by physiochemical mechanisms (Fig. 1.1) [3, 44]. Although the biosorption of metal species is a metabolism-independent process and thus can be carried out by both living and dead microbial biomass, metabolic activity may

Table 1.1 Bacteria reported to reduce U(VI) to U(IV)

Bacterial species	Descriptions/comments	References
Anaeromyxobacter dehalogenans	Fumarate-grown cells reduced U(VI)–U(IV). Hydrogen was required as an electron donor and could not be replaced by acetate; the addition of nitrate caused reoxidation, but reduction resumed following consumption of the N-oxyanions. Reduction was inhibited by Fe(III) citrate or citrate; U(VI) reduction proceeded to completion at a much lower rate with amorphous Fe(III) oxide. No inhibition by fumarate or 2-chlorophenol was observed, and both were consumed concomitantly with U(VI). The coupling of U(VI) reduction to cell growth was established by monitoring the increase in 16S rRNA gene copy numbers using qPCR.	[112, 113]
Cellulomonas sp.	The oxidation of lactate or other unknown electron donors in the absence of lactate was coupled to U(VI) reduction. The reduction rate was slow: at least 150 h was required to reduce 0.2 mM U(VI) at a concentration of 500 mg/L total cell protein.	[114]
Clostridium acetobutylicum *Clostridium pasteurianum* *Clostridium* sp. *Clostridium sphenoides*	Uranyl-nitrate, -acetate, and -citrate complexes were reduced. The mechanisms remain unclear; optimizing the conditions for fermentation resulted in better U(VI) reduction.	[115, 116, 117]
Deinococcus radiodurans	Reduction of U(VI) occurred in the presence of humic acid analog anthraquinone-2,6-disulfonate; a recombinant strain harboring phoN, a gene encoding a nonspecific acid phosphatase, exhibited a high uranium precipitation ability even after exposure to 6 kGy of ^{60}Co gamma irradiation.	[118, 119]
Desulfotomaculum reducens	This was the first sulfate-reducing bacterium that could grow with U(VI) as the sole electron acceptor; it shared physiological properties with both the sulfate-reducing and metal-reducing groups of bacteria.	[120]
Desulfosporosinus sp. Desulfosporosinus orientis	No U(VI) reduction occurred in cell suspensions with 0.25% Na-bicarbonate or 0.85% NaCl; there was enzymatic reduction in cell suspensions depleted in bicarbonate and NaCl. U(VI) reduction was inhibited when 1 mM $CuCl_2$ was added to the cell suspensions.	[43]
Desulfosporosinus GBSRB4.2	This acid-tolerant sulfate-reducing bacterium enzymatically reduced U(VI) in acidic solutions (pH 4.2); it reduced U(VI) in groundwater more rapidly at pH 4.4 than at pH 7.1.	[121]

Table 1.1 (continued)

Bacterial species	Descriptions/comments	References
Desulfovibrio baarsii *Desulfovibrio sulfodismutans* *Desulfovibrio vulgaris*	The reduction of U(VI) resulted in the precipitation of uraninite. No reduction appeared to conserve enough energy to support growth.	[122]
Desulfovibrio sp.	This bacterium was capable of growing by reducing U(VI): it detoxified U(VI) and obtained energy *via* nitrate reduction.	[123, 124, 125]
Desulfovibrio desulfuricans	U(VI) was reduced with lactate or pyruvate as the electron donor.	[126]
Geobacter metallireducens	Dissimilatory Fe(III)-reducing bacteria obtained energy for growth by electron transport to U(VI).	[41, 34]
Geobacter sulfurreducens	The reduction of U(VI) associated with surfaces of synthetic hydrous ferric oxide, goethite, and hematite was comparable to the reduction of aqueous U(VI). The reduction of U(VI) adsorbed to natural Fe(III) oxide-bearing solids was slower and less extensive compared to that of synthetic Fe(III) oxide systems. The addition of anthraquinone-2,6-disulfonate enhanced U(VI) reduction.	[111]
Pseudomonas putida	Resting cells anaerobically reduced U(VI) with lactate as the electron donor. A slightly higher reduction was observed under aerobic conditions with glucose as the electron donor. Reduced uranium accumulated both on the surface and internally.	[39]
Pyrobaculum islandicum	U(VI) was reduced with hydrogen as the electron donor. The reduction was dependent upon the presence of cells and hydrogen.	[127]
Salmonella subterranea sp. Nov.	This bacterium was capable of using O_2, NO_3^-, $S_2O_3^{2-}$, fumarate, and malate as terminal electron acceptors and of reducing U(VI).	[128]
Shewanella alga	Lactate oxidation coupled to reduction of U(VI) under anaerobic conditions.	[129]
Shewanella oneidensis	This dissimilatory Fe(III)-reducing bacterium obtained energy for growth by electron transport to U(VI). It was formerly known as *Alteromonas putrefaciens*, then as *Shewanella putrefaciens.*	[34]

Table 1.1 (continued)

Bacterial species	Descriptions/comments	References
Thermoanaerobacter sp.	This bacterium was capable of using acetate, glucose, hydrogen, lactate, pyruvate, succinate, and xylose as electron donors while reducing U(VI).	[130]
Thermus scotoductus	This thermophilic bacterium used O_2, NO_3^-, Fe(III), and S^0 as terminal electron acceptors for growth and also reduced U(VI).	[131]
Thermoterrabacterium ferrireducens	Organotrophic growth was coupled to the reduction of sparingly soluble U(VI) phosphate. This was the first report of the microbial reduction of a largely insoluble U(VI) compound.	[132]

Table 1.2 *c*-type cytochromes and their role in U(VI) reduction

Bacterial species	Role/description/comments	References
Desulfovibrio vulgaris	Removal of cytochrome c_3 from crude extracts eliminated U(VI) reduction activity (in vitro). Reduced cytochrome c_3 was oxidized during U(VI) reduction.	[133, 134, 135]
Desulfovibrio desulfuricans	U(VI) reduction by a cytochrome c_3 mutant was inhibited by at least 90% with H_2 as the electron donor.	[136, 126]
Geobacter sulfurreducens	Elimination of two outer membrane cytochromes and two putative outer membrane cytochromes significantly decreased (50–60%) the ability of *G. sulfurreducens* to reduce U(VI).	[137]
Shewanella oneidensis	Deletions of outer membrane cytochromes MtrC and/or OmcA significantly affected the in vivo U(VI) reduction rate. There was a close association of the extracellular UO_2 nanoparticles with MtrC and OmcA.	[138, 71]
Thiobacillus denitrificans	Membrane-associated cytochromes c_4 and c_5 played a major role in nitrate-dependent U(IV) oxidation. Insertion mutations resulted in a decrease (<50%) in U(IV) oxidation activity. Complementation restored activity to the wild-type level.	[139]

affect the immobilization process by causing local changes in electrochemical potential (E_h) and pH, or by producing metal-complexing ligands [44]. A wide variety of microorganisms, including bacteria, fungi and algae, have been shown to possess capabilities of U(VI) biosorption [45–49]. Uranium can be biosorbed either to the cell wall or to extracellular components associated with the cell wall, such as polysaccharides, glycoprotein or lipopolysaccharides. Various functional groups, including carboxylate, phosphate, and hydroxyl groups, are usually found (Table 1.3) on the extracellular components.

Bioprecipitation

Microorganisms are also able to precipitate metals and radionuclides as carbonates and hydroxides through localized alkalization at the cell surface or to precipitate them with enzymatically generated ligands, such as carbonate, sulfide, and phosphate [50–53], providing an appreciable way to immobilize U(VI).

Bioaccumulation

Bacterial cells have been shown to have the ability to accumulate U(VI) intracellularly and then immobilize it through several mechanisms. The chelation of uranium by polyphosphate bodies is a well-studied mechanism [54]. McLean and Beveridge [39] speculated that the polyphosphate bodies in a bacterial cell might sequester uranium in the cytoplasm and form strong complexes with it to protect the cell. The accumulation of uranium in the form of needle-like fibrils in the cytoplasm is another type of bioaccumulation [55]. It has been suggested [44, 56] that this

Table 1.3 Biosorption of U(VI) by bacterial cells

Cellular structures		Descriptions	Sites for U(IV) immobilization	References
Cell Wall	S-layer	Part of cell envelope commonly found in bacteria. It consists of identical protein or glycoprotein subunits. It is 5–15 nm thick with pores 2-6 nm in diameter	Carboxylate and phosphate groups	[140]
	Peptidoglycan	Thick layer of 25 nm in Gram-positive bacteria and thin layer of 4 nm in Gram-negative bacteria	Carboxylate and phosphate groups	[141, 142, 143]
	Outer membrane	Outer membrane bilayer consisting of lipopolysaccharide (LPS) and protein outside the peptidoglycan layer in Gram-negative bacteria	Carboxylate and phosphate groups	[144, 145]
Extracellular polymeric substances (EPS)		Molecules from metabolism, organic effluents, and microbial lysis, including polysaccharides, proteins, humic substances, uronic acids, nucleic acids and lipids	Carboxylate, phosphate, amine, and hydroxyl groups	[146, 147]

intracellular uptake results from increased cell membrane permeability caused, for example, by the toxic effects of uranium.

1.2.2.2 Bioremediation Principles: From the Laboratory to the Field

Although the underlying principles of uranium bioremediation have been extensively studied using model systems (pure cultures) and proof-of-concept systems (laboratory studies of natural soils and sediments containing mixed microbial communities) [57, 58, 59], practical field-scale studies have only been carried out more recently [44]. Theoretically, both the immobilization of uranium in place (preventing further downgradient spreading of groundwater contamination) and its mobilization (allowing it to be more easily flushed) present opportunities for uranium bioremediation. Hazen and Tabak [32] suggested that mobilization can be a better long-term strategy because it provides a way to remove the contaminant from solid matrices, such as soils, sediments, dumps and other solid industrial wastes. However, it is difficult to justify to regulators and stakeholders at remediation sites because of its inherently greater risk if the mobile and usually more toxic contaminant fails to be adequately immobilized in the capture zone. Therefore, in uranium bioremediation, almost all of the reported field studies and deployments to date have used immobilization rather than remobilization.

The basic concept of uranium bioremediation through U(VI) immobilization is to harness indigenous microorganisms to reduce U(VI) and form sparingly soluble U(IV) minerals. This U(VI) bioremediation model has been shown to be feasible by multidisciplinary researchers. The reduction of U(VI) by microorganisms in groundwater and aquifer sediments has been demonstrated in the laboratory; however, electron donors are usually limited in natural aquifer systems [57–61]. Electron donors such as acetate, lactate, or ethanol are typically provided to stimulate the U(VI) bioreduction during *in situ* U(VI) bioremediation in field studies. Contaminated sites managed by the U.S. Department of Energy Uranium Mill Tailing Remedial Action (UMTRA) program at Oak Ridge and Rifle have been extensively studied. Representative field studies on the *in situ* bioremediation of U(VI) are summarized in Table 1.4.

1.2.2.3 Redox, Abiotic and Biotic Reactions

In order to enhance bioremediation efficiency in the field, a thorough knowledge of the local geochemistry and microbial metabolic activities is required because the mobility and fate of uranium in the environment are mainly controlled by redox reactions and biotic and abiotic processes. Redox reactions and abiotic processes are governed by geochemical parameters such as reduction potential E_h, pH, temperature and ligand concentrations [32, 27].

Redox Reactions of U(VI) and U(IV)

The redox reactions of U(VI) and U(IV) typically result in substantial changes in uranium solubility and bioavailability through: (i) immobilization of uranium when U(VI) is reduced to U(IV), forming insoluble U(IV)-bearing minerals such as uraninite UO_{2+x} and decreasing uranium bioavailability [62], and (ii) mobilization of uranium when U(IV) is oxidized to U(VI), increasing uranium solubility and bioavailability. In natural environments, the E_h of the U(IV)/U(VI) couple should fall in the range of –0.042 to 0.086 against the standard hydrogen electrode potential (V_{SHE}), depending on the Ca^{2+} and CO_3^{2-} concentrations [63]. For the reduction of U(VI), the development of a low redox potential is essential but not sufficient because other geochemical factors also play important roles in uranium solubility [64]. For example, under slightly acidic conditions, uranium immobilization is expected to be more sustainable. However, the pH should not be too low, because U(IV) may become soluble in an environment with a pH lower than 4 [32].

Abiotic Processes that Control Uranium Mobility

Abiotic U(IV) oxidation occurs when O_2, NO_3^-, Fe(III), and Mn(IV) are reduced [65–67]. H_2S, H_2, and Fe(II) reduce U(VI) at a slower rate compared to oxidation by O_2, Fe(III) and Mn(IV), and the reduction can be inhibited by uranium complexes. In fact, complexation with other chemical species such as carbonate is another important factor that determines uranium mobility and fate in environmental

Table 1.4 Selected field studies on the *in situ* bioremediation of U(VI)

Testing site	Method	Results/comments	References
An aquifer in Norman, Oklahoma	Push-pull test by injecting test solution containing U(VI) and bromide	Injected U(VI) (1.5 μM) was reduced in three days in the absence of an additional electron donor. The ability of indigenous aquifer bacteria to reduce U(VI) was demonstrated. This was not a typical U(VI)-contaminated aquifer because it had an established low redox potential but no preexisting contamination.	[59]
U(VI)-contaminated aquifer in a site at Rifle, Colorado	Biostimulation by injecting acetate	An initial growth of *Geobacter* species and decrease in U(VI) with the addition of acetate was followed by sulfate reduction associated with an increase in U(VI). Heterogeneity was a dominant control: local differences in hydrology led to an uneven distribution of the electron donor, microbial activity and U(VI) reduction. The microorganisms actively incorporating acetate into biomass and the bacteria involved in U(VI) reduction were identified.	[148, 149, 4, 8, 150]
U(VI)- and nitrate-contaminated aquifer in a site at Oak Ridge, Tennessee	Preconditioning using bicarbonate followed by biostimulation	In contaminated zones with a high level of nitrate (>100 mM) and low pH (~3.5), with the addition of adequate electron donor, denitrification followed by U(VI) depletion was observed. *Anaeromyxobacter* dominated before stimulation, and both *Anaeromyxobacter* and *Geobacter* were stimulated by the addition of the electron donor.	[85, 151, 152, 153, 59]
	Three-step approach: (i) nitrate removal from aquifer (acidic flush); (ii) nitrate removal from flush water; and (iii) reinjection of treated groundwater into aquifer in conjunction with the addition of an electron donor to stimulate *in situ* U(VI) reduction	Column studies demonstrated the feasibility of this three-step strategy and concluded that nitrate leaching out of the immobile pore space must be removed continuously by *in situ* denitrification to maintain favorable conditions for microbial U(VI) reduction.	[60, 154, 155]

settings [12, 27]. Mobile aqueous complexed species and insoluble uranium-bearing minerals are often formed by complexation [68]. As an example of the importance of complexation, it has been clearly demonstrated in the laboratory [18, 69, 34] and in a H_2S-rich stratified water column in the Black Sea [70, 12] that U(VI)-CO_3 complexes $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$, the dominant aqueous uranium species in most surface and subsurface settings, are not reduced homogeneously by chemical reductants such as sulfide and molecular hydrogen.

Biotic Processes that Control Uranium Mobility

U(VI)-reducing microorganisms typically reduce U(VI) or U(VI)-CO_3 complexes to form U(IV) oxides such as uraninite in the periplasmic space or nanoparticles in the extracellular material [71–73]. In natural systems, the mobility of uranium is determined by the interplay between biotic and abiotic processes [74, 27]. The electrons from the microbial oxidation of lactate can reduce U(VI):

$$2UO^{2+} + \text{lactate}^- + 2H_2O \rightarrow 2UO_2 + \text{acetate}^- + HCO_3^- + 5H^+ \qquad (1.1)$$

In principle, the oxidation of an electron donor coupled to the reduction of an electron acceptor with a higher redox potential is more favorable, because electrons always flow from the low redox potential to the high redox potential and this is favorable for microbial energy conservation. Generally oxygen is the ultimate electron acceptor because of its high redox potential. However, if oxygen is not present, in the presence of nitrate, nitrate is expected to be the next electron acceptor for microbial respiration:

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \qquad (1.2)$$

Under sulfate-reducing conditions, the microbes involved in the process of metal removal gain energy by coupling the oxidation of organic compounds with the reduction of sulfate ions, generating hydrogen sulfide as a by-product [27]:

$$2\ \text{lactate}^- + SO_4^{2-} + H^+ \rightarrow 2\ \text{acetate}^- + 2\ CO_2 + 2\ H_2O + HS^- \qquad (1.3)$$

$$HS^- + H^+ \Leftrightarrow H_2S \qquad (1.4)$$

$$HS^- \Leftrightarrow S^{2-} + H^+ \qquad (1.5)$$

Microbially generated H_2S dissolves in water and, being a diprotic acid, dissociates to bisulfide (HS^-) and sulfides (S^{2-}) that can reduce uranium [18]. In the presence of iron, the formation of iron sulfide minerals may decrease the amount of S^{2-} available for uranium reduction by sulfate-reducing bacteria.

Recent studies have demonstrated that redox transformations of uranium are governed by kinetic factors that are strongly controlled by microbial activity [12, 27]. Although abiotic uranium oxidation proceeds efficiently under aerobic

conditions, abiotic uranium reduction is inhibited by the formation of the U(VI)-CO_3 complexes that exist in groundwater. There are several bacteria capable of reducing U(VI)-CO_3 complexes enzymatically to uraninite. The abiotic reoxidation of uranium by Fe(III) and Mn(IV) oxides has been well demonstrated in the laboratory and in the field [12].

Biotic, Abiotic and Redox Conditions in Biofilms

In U(VI) bioremediation research, most of the studies have been conducted in the presence of suspended microorganisms or enriched sediments [75–78], eventually spiked with micro- or nano-particles of other minerals [69, 67]. However, in natural soils and water-sediment interfaces, microorganisms are commonly found in the form of surface-associated cells, or biofilms [79–81].

The metabolic activity of cells in biofilms, which is expected to be different from that of suspended cells, can affect the abiotic and redox reactions controlling the mobility of U(VI) in the environment. For example, when oxygen is introduced into a suspended cells system, one can expect to observe the reoxidation and remobilization of immobilized uranium in the system; however, when a biofilm is exposed to oxygen, some of the uranium near the interface of the biofilm and the bulk solution may be mobilized due to reoxidation while uranium in the deeper portion of the biofilm remains immobilized because oxygen does not reach there and the reducing conditions are still present inside the biofilm [82, 83]. In addition, the biotic reduction of nitrate to nitrogen occurring after the depletion of oxygen produces nitrite, nitrous oxide and nitric oxide and these intermediately oxidize U(IV) [84, 85, 66]. These situations can be important, especially if the contaminated site is mostly aerobic. The development of biofilm on surfaces may create conditions for nitrate reduction. All these combined biotic, abiotic and redox conditions pose challenges in predicting the mobility of uranium in biofilms. U mobility has been frequently described for bulk solutions and for planktonic cultures. However, uranium immobilization in subsurface-attached biofilms with heterogeneous local conditions can be significantly different from that in bulk conditions.

1.3 Biofilms Immobilizing Uranium

1.3.1 Definition of Biofilms

Figure 1.2 illustrates our definition of biofilms related to bioremediation. In Fig. 1.2a, single cells are shown attached to a surface. The presence of even a few cells will affect abiotic and redox reactions on the surface. When the cell number increases (Fig. 1.2b) the surface-associated bacteria will cover a significant amount of surface (Fig. 1.2c) and begin to control the chemistry on the mineral surface. Throughout this chapter we will use the term “biofilm” to refer to surface-associated cells as shown in Fig. 1.2. This definition is not new and has been described by

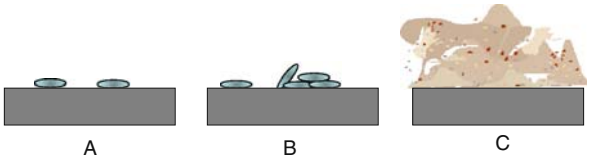


Fig. 1.2 A mineral with attached cells on the surface: (**a**) a few cells are attached to the surface; (**b**) the cells increase in number and start to cover the surface; (**c**) the surface is covered by layers of cells

Lewandowski and Beyenal [83] and by others [86]. The biofilm on the surface does not have to be very thick: the thickness can vary from a few micrometers to several hundred micrometers. We expect that when cells start growing on a surface, the metabolic activity of the cells will affect the abiotic and redox reactions. For example, the surface-associated cells can consume the available oxygen, which lowers the redox potential, influences the abiotic reactions and generates concentration gradients. These gradients will control the activity of the cells (biotic reactions) and the abiotic and redox reactions and will influence the bulk chemistry. The biofilms can also exchange electrons with the surface they are growing on, and these electron exchanges can influence uranium mobility. Therefore uranium immobilization must be investigated for redox-sensitive and redox-insensitive surfaces. Redox-insensitive surfaces do not exchange electrons with biofilms.

In the past several years, using sulfate-reducing biofilms as a model, researchers including our group have systematically studied the feasibility of U(VI) immobilization in biofilms growing on redox-sensitive and -insensitive surfaces with and without carbonate in the medium [18, 87, 73]. Recently, we have been studying uranium immobilization using dissimilatory iron-reducing bacteria (DIRB) *Shewanella oneidensis* MR-1 and other naturally growing species in uranium-contaminated subsurface sediments.

1.3.2 Uranium Immobilization Mechanisms Using Sulfate-Reducing Biofilms

Sulfate-reducing bacteria (SRB) are anaerobic microorganisms that utilize a variety of organic substrates as electron donors and sulfate as the terminal electron acceptor, resulting in the production of sulfide [88]. Due to the differences in solubility of metal sulfates and metal sulfides, SRB have been used to immobilize metals in groundwater and wastewater [88, 89]. Specifically, heavy metal immobilization using SRB biofilms has been shown to be an effective method in bioremediation. It has been reported that SRB also have the capabilities to reduce and immobilize metals and radionuclides, such as Cr(VI), U(VI) and Tc(VII) [35, 90].

Among the mechanisms we have discussed (Fig. 1.1), at least three are involved in U(VI) immobilization by SRB [18]: (i) biosorption of U(VI) to cell surfaces and

extracellular biopolymers; (ii) chemical reduction of U(VI) by microbially generated H_2S (indirect reduction); and (iii) enzymatic reduction in which U(VI) acts as the terminal electron acceptor (direct reduction). Mohagheghi et al. [91] hypothesized that the combination of biosorption and chemical reduction processes was responsible for U(VI) reduction. Lovley and coworkers [77], though, showed that the enzymatic reduction of U(VI) by sulfate-reducing bacteria was much faster than chemical reduction by sulfide, indicating that enzymatic reduction may be the dominant form of U(IV) immobilization in sulfidogenic environments. However, these conclusions were reached with respect to bicarbonate-buffered systems, in which it has been demonstrated that U(VI) is almost entirely complexed in the forms of $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$ [69], increasing the difficulty of U(VI) reduction by microbially generated H_2S and resulting in bioreduction as the prevailing U(VI) reduction mechanism [37, 34]. Interestingly, Spear et al. [92] showed that in a bicarbonate-buffered system growing cells of *D. desulfuricans* reduced U(VI) faster than U(VI) reduction under nongrowth conditions, suggesting that microbially generated sulfide may have been responsible for the increased rate of U(VI) reduction.

1.3.3 Uranium Immobilization Mechanisms Using DIRB Biofilms

In most DIRB biofilm studies, *S. oneidensis* MR-1 [93] has been used as the model biofilm-forming facultative anaerobic microorganism. There have been some studies of MR-1 biofilms, primarily focused on the structure and metabolism within the biofilms [94–96]. However, hitherto very few studies on U(VI) immobilization using *Shewanella* biofilms have been available. Recently, Sani et al. [7] reported their results on U(VI) removal by biofilms of *S. oneidensis* MR-1 in fracture-flow reactors. *S. oneidensis* biofilms were shown to have limited U(VI) immobilization capacity in both flow and batch modes. In a recent paper, McLean et al. [95] studied the kinetics and stratification of anaerobic metabolism within live biofilms of *S. oneidensis* MR-1 through a combination of noninvasive NMR microscopic imaging/spectroscopy and confocal imaging tools. It was suggested that, even under bulk aerobic conditions, MR-1 biofilms have the ability to perform anaerobic reduction as oxygen becomes scarce with depth in the biofilm: thus, U(VI) may be immobilized within the biofilm matrix and remain immobilized as long as the active biofilms are maintained and oxygen is depleted within the matrix.

In uranium bioremediation using DIRB biofilms, biosorption and bioreduction are the two main processes contributing to U(VI) immobilization. Both of these processes have been discussed earlier (Fig. 1.1). Here we want to emphasize the bioreduction process of MR-1. At least two distinct pathways have been proposed for the transfer of electrons to the mineral substrate by MR-1: (i) the direct transfer of electrons from the cell surface at the mineral-microbe interface through a network of *c*-type cytochromes [97, 98] localized in the periplasm [99], outer membrane [100, 71], and nanowires (pilus-like assemblages) [101] and (ii) the indirect transfer

of electrons from the cells to the mineral surface without direct contact through low-molecular-weight soluble redox mediators or electron shuttles [102, 103]. Studies have shown that redox shuttles such as quinone-containing humic acids can promote the reduction of Fe(III) oxides [104]. In U(VI) immobilization, humics have been demonstrated to play two functional roles: enhancing U(VI) bioreduction and increasing U(IV) solubilization and reoxidation by forming U(IV)-humics complexes [105]. In addition to these exogenous electron shuttles, endogenous electron shuttles, such as flavins secreted by *S. oneidensis* MR-1 in both planktonic cultures and biofilms, have been identified recently, and their potential to mediate extracellular electron transfer for mineral reduction has been confirmed [106, 107].

1.3.4 Biofilm Reactors for Studying Uranium Immobilization

Our research group generally uses two types of reactors in uranium immobilization studies: (i) flat plate reactors and (ii) fixed bed column reactors (FBCRs). The selection of the reactor depends on the research questions that need to be addressed. For example, if we are interested in the depth profiles of sulfite in SRB biofilms, a flat plate reactor will be used because this reactor allows us to monitor the location of the microelectrode and image the biofilm structure simultaneously. However, if we are only interested in quantifying uranium immobilization in biofilms, FBCRs are preferred because we can select a wide range of minerals to study how mineral type affects uranium immobilization.

1.3.4.1 Flat Plate Reactor

A flat plate reactor is typically a rectangular channel used to grow biofilms for quantifying relations between biofilm structure and activity [83]. This type of reactor allows us to (i) grow biofilms under well-defined hydrodynamic conditions, (ii) use a variety of microscopic techniques to quantify biofilm structure, and (iii) use microsensors to quantify chemical gradients in the biofilms.

A flat plate reactor typical of those we use in our biofilm studies is shown in Fig. 1.3. The polycarbonate channel is 2.5 cm wide, 4.0 cm deep and 34.5 cm long. The working volume of the reactor is around 150 mL including the tubing volume. All fittings except the output line have a 3/8” opening width with a 1/8” plastic pipe thread centered and placed near the edge. The output line is placed above the recycle line, and the latter is used to control the total flow rate by recycling part of the effluent to the reactor. The residence time in the reactor is determined by the flow rate in the nutrient line only because the flow in the recycle line does not leave the system and hence has no effect on the residence time.

The following protocol is used to operate the flat plate reactor to grow biofilms:

1. Autoclave the tubings, reactor, flow breakers and connectors.
2. Sterilize the entire system by fully filling the reactor with bleach (20% v/v) and recycling for at least 2 h.

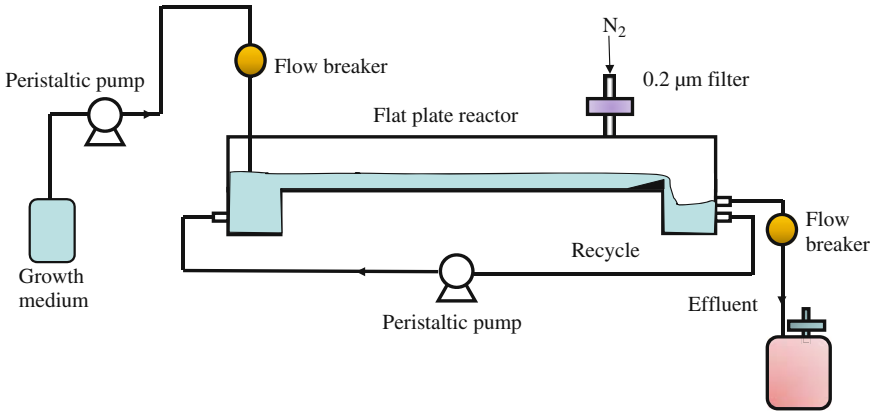


Fig. 1.3 Schematic illustration (not to scale) of a flat plate reactor used to image biofilms and study uranium immobilization and local chemistry in biofilms

3. Drain the reactor, and then fill it with sterile deionized (DI) water. Reverse the recycle line and drain it again. Repeat the fill and draw procedure at least 5 times.
4. Drain the reactor again and fill it with growth medium. Repeat the fill and draw procedure for growth medium at least three times.
5. Inoculate the reactor aseptically with 30 mL of inoculum through slow manual injection using a syringe.
6. Stop the recycle line and wait for 30 min to allow the initial attachment of the bacteria. Then start the recycle at a very slow rate and increase gradually up to the operating recycle flow rate within a few minutes.
7. Operate the reactor overnight in batch mode to allow the bacteria to grow.
8. Start the nutrient pumps and deliver the nutrient solution at the operating conditions to grow the biofilm.
9. Uranium can be added the medium after biofilm establishment. The growth medium should not react with uranium. Otherwise it is difficult to identify the microbiological factors involved in uranium immobilization.

1.3.4.2 Fixed Bed Column Reactor

FBCRs are usually used to simulate subsurface biofilms growing on various surfaces to investigate biofilm-mineral interactions. These reactors are usually operated for several months and then disassembled for various measurements of the biofilms grown on the mineral surfaces.

A FBCR typical of those used in our studies is shown in Fig. 1.4A. It is made of a clear polycarbonate tube with an internal diameter of 2.5 cm and a length of 40 cm. A typical column is filled with selected minerals and then sealed. To ensure the uniform delivery of growth medium, flow distributors made of glass beads (0.5 cm in diameter) are entrapped between two plastic sieves and these are placed in the inlet

and in the outlet of the column reactor. In order to prevent backflow and contamination, flow breakers are used between the feed line and the reactor inlet as well as between the reactor outlet and the waste collection line. The feed is provided from the bottom of the reactor, and effluent is collected from the top. A mixing chamber (250 mL of volume) is located in the recycle line when needed. A mixing chamber is used only when we need to achieve a high flow rate in the columns. If the operational flow rate is small, a single pass is used. A single-pass FBCR is called a flow-through column reactor (Fig. 1.4B).

The following protocol is used to operate FBCRs to grow biofilms:

1. Autoclave the tubings, reactor and mixing chambers.
2. Sterilize the entire system by filling it with ethanol (70% v/v) and recycling for three hours. This is done if the filling materials do not react with alcohol.
3. Drain the reactor, and then pump 10 L of sterile deionized (DI) water through the system to wash out the traces of alcohol.
4. Drain the reactor again and fill it with the appropriate growth medium.
5. Inoculate the reactor aseptically with 30 mL of inoculum. In the case of natural sediments, we sterilize everything except the natural sediments and we do not inoculate, since the naturally growing bacteria will be stimulated with the additional growth medium.
6. Operate the reactor in batch mode for at least 8 h or overnight, and then start the flow of nutrient solution. When a single-pass reactor is used we do not run it in batch mode. The reactor is fed with the appropriate medium all the time.

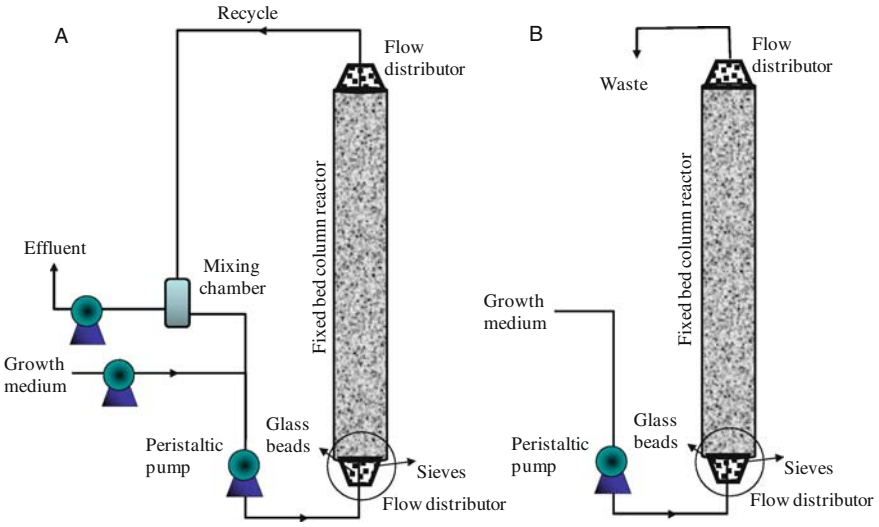


Fig. 1.4 Experimental setup (*a single column*). (**a**) A polycarbonate column filled with minerals is operated continuously with a recycle. The recycle provides higher flow rates in the column. (**b**) A flow through a column reactor

7. After a few days of operation, biofilm should be visible on the packing material; its presence can be confirmed by sulfide production when SRB are used. The time needed for a visible biofilm growth depends on the amount of the substrate and the microorganisms added to the reactors.

1.3.5 Uranium Immobilization Using Biofilms Grown on Various Surfaces

Biofilms grown on both redox-insensitive and redox-sensitive surfaces have been used in uranium immobilization studies. As redox-insensitive surfaces, quartz and glass have been used to evaluate U(VI) immobilization by SRB biofilms [18, 73] and *S. oneidensis* MR-1 biofilms in flat plate reactors, and to evaluate U(VI) removal by biofilms of *S. oneidensis* MR-1 in fracture-flow reactors [7]; as redox-sensitive surfaces, synthetic minerals such as hematite, calcite, and dolomite have been used to test whether U(VI) can be reduced in a subsoil formation by SRB biofilms in the presence of carbonates in an efficient and sustainable way [87]. In addition to well-controlled laboratory conditions, natural sediments with biofilms of stimulated indigenous bacteria have also been evaluated for U(VI) immobilization [108, 66, 109].

1.3.5.1 Biofilms Grown on Redox-Insensitive Surfaces

A redox-insensitive surface is a surface that does not exchange electrons with biofilms. We generally use quartz as a redox-insensitive surface. When a redox-insensitive surface is used, electrons are only exchanged among electron donors, electron acceptors and redox mediators.

SRB Biofilms on Quartz Surface

Beyenal et al. [18] measured U(VI) immobilization and microbial activity using SRB biofilms composed of *D. desulfuricans* G20 grown on quartz surfaces in flat plate reactors (Fig. 1.5). Lactate and sulfate were used as the electron donor and the electron acceptor, respectively. A medium that minimizes metal complexation was used. Microbial activity was determined by measuring the input and outlet lactate concentrations or by measuring the H_2S production in the biofilm. Since it was also expected that H_2S could abiotically reduce U(VI), H_2S profiles measured using microelectrodes in the biofilms were selected as an indicator of microbial activity and H_2S production.

The H_2S concentrations and flux were found to be higher when the reactor was not fed with U(VI) (Fig. 1.5a). After inactivating bacterial metabolism with NaN_3 (after which no H_2S was produced), the U immobilization was quantified. The total amount of U immobilized in biofilms exposed to U(VI) was higher than

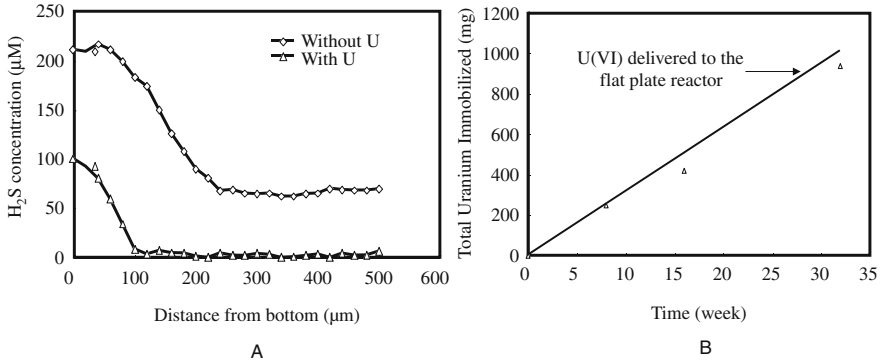


Fig. 1.5 (**a**) H_2S concentration profiles in three-week-old biofilms grown without U(VI) and with U(VI). (**b**) Uranium accumulated in SRB biofilms. Reprinted "in part" with permission from Beyenal et al. [18]. Copyright 2007 American Chemical Society

that measured in biofilms exposed to both U(VI) and NaN_3 (8.5 ± 1.1 vs. 6.2 ± 0.6 μmol/cm^2), suggesting that some of the microbially generated sulfide may have reacted with U(VI).

To separately determine the effect of sulfide on U(VI) reduction, Beyenal et al. performed abiotic experiments in batch reactors containing U(VI) and sulfide in PIPES buffer (30 mM, pH 7) [18]. Under anoxic conditions, both the sulfide and U(VI) concentrations decreased with time, which was not observed in the U(VI)- and sulfide-free control experiments [18]. This demonstrates that U(VI) is abiotically reduced by sulfide in the absence of carbonate buffer. It was also found that when the medium was buffered with 30 mM carbonate, sulfide did not reduce U(VI) [18]. Recently, other research groups have shown that microbially generated sulfide can reduce uranium in a medium with 15 mM carbonate buffer, and that the lower the carbonate buffer strength, the higher the uranium reduction rate [110].

The mass of the U immobilized in the sulfate-reducing biofilm was also estimated to quantify the immobilization dynamics. The total amount of U immobilized in the biofilm was found to increase linearly with time during the operation of the reactor (Fig. 1.5b). U removal of at least 88% was obtained consistently using a sulfate-reducing biofilm in the reactor.

It has been shown that SRB biofilms have the ability to immobilize U(VI) for significant amounts of time as the result of both enzymatic and chemical reduction of U(VI) to uraninite. However, natural groundwater is usually buffered with carbonates, where the chemical reduction of U(VI) was considered difficult because of the formation of complexes with carbonates. In addition to SRB it has been reported that DIRB can reduce U(VI) to U(IV) enzymatically in the presence of a carbonate buffer [111, 41].

Marsili et al. [73] tested the ability of SRB biofilms to remove U(VI) from contaminated carbonate-buffered groundwater, in flat plate reactors (Fig. 1.3) with biofilms of *D. desulfuricans* G20 grown on glass slides operated for 5 months at

concentrations of carbonate that reflected those most commonly encountered in natural waters (10 mM) [73]. Biofilm activity and uranium removal were evaluated during the operation of reactors supplied with 12.6 μM (3-mg/L) and 126 μM (30-mg/L) U(VI). The reactor with a feed of 126 μM U(VI) immobilized 72.3% of the uranium. It was expected that in the reactor fed with less U (12.6 μM) all of the U would be immobilized. However, their results showed that 30.4% of the uranium was removed from that reactor. The difference in U(VI) removal between the two reactors could be attributed to biofilm detachment, which was observed but not quantified. In general, the uranium removal extent and rate were surprisingly satisfactory in the presence of bicarbonate buffer.

Transmission electron microscopy (TEM) and selected area electron diffraction (SAED) analysis showed that in both reactors uranium accumulated mostly on microbial cell membranes and in the periplasmic space [73]. Energy-dispersive spectrometry (EDS) analysis revealed that the deposits contained uranium and phosphates. High-resolution transmission electron microscopy (HRTEM) showed that the deposits had amorphous or poor nanocrystalline structures [73].

S. oneidensis MR-1 Biofilms on Glass Surfaces

To compare U immobilization by biofilms of SRB and DIRB, *S. oneidensis* MR-1 biofilms were grown in identical flat plate reactors and the reactors were operated under comparable conditions. The mass of U immobilized in *S. oneidensis* MR-1 biofilms is shown in Fig. 1.6. The total amount of U immobilized in the biofilm was found to increase linearly with time during the operation of the reactor. At least 96% of the U was immobilized consistently using *S. oneidensis* MR-1 biofilms. The experimental data from the first five days showed that U immobilization using sulfate- and iron-reducing biofilms growing on redox-insensitive surfaces in the presence of carbonate was comparable.

In *in situ* bioremediation, fractures are usually present in contaminated subsurface sites, which can change the local substrate flux and redox conditions [7].

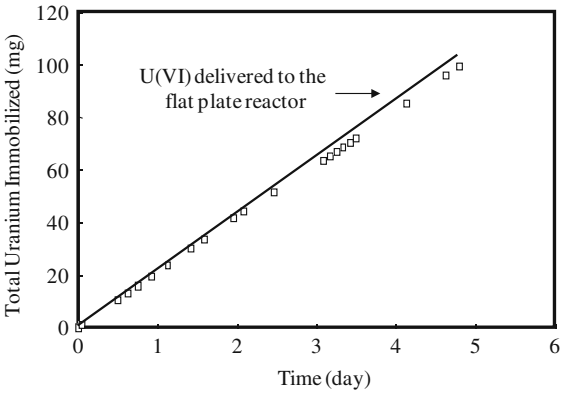


Fig. 1.6 Total amount of uranium accumulated in *S. oneidensis* MR-1 biofilms. The flat plate reactor was fed with 126 μM U(VI) at a flow rate of 30 mL/h. The continuous line shows the cumulative amount of uranium delivered to the reactor (or maximum possible uranium accumulation)

To better understand U(VI) immobilization in subsurface fracture-flow systems, Sani et al. [7] recently reported their results on U(VI) removal by biofilms of *S. oneidensis* MR-1 in fracture-flow reactors. *S. oneidensis* biofilms were shown to have limited U(VI) immobilization capacity in both flow and batch modes due to plugging of the fracture-flow reactors.

Biofilms Grown on Redox-Sensitive Surfaces

A surface that can exchange electrons with the cells in a biofilm is called a redox-sensitive surface. A redox-sensitive surface can be a synthetic mineral such as hematite or a mineral from contaminated sediment. The use of synthetic minerals allows us to have reproducible surfaces for well-controlled experiments with high reproducibility. However, it is difficult to have homogeneous and well-defined surfaces when we use natural contaminated minerals.

SRB Biofilms on Synthetic Minerals

In a previous study by Marsili et al. [87], the immobilization of U(VI) using biofilms of *D. desulfuricans* G20 growing on synthetic carbonate-bearing minerals was studied to test whether U(VI) can be reduced in a subsoil formation by SRB biofilms in the presence of carbonates in an efficient and sustainable way. To this end, three FBCRs (Fig. 1.4), each filled with calcite, dolomite, or hematite, were operated using lactate as the electron donor [87].

In the systems filled with carbonate-bearing minerals (calcite and dolomite), the growth medium was not buffered, while 10 mM of carbonate was used to buffer the system filled with hematite, a non-carbonate-bearing mineral. The total amount of uranium accumulated in the biofilms increased linearly with time in all three reactors (Fig. 1.7). In these experiments, 87.2% (calcite-filled column), 82.4% (dolomite-filled column), and 72.5% (hematite-filled column) of uranium was removed from the bulk solution. The results demonstrate that the SRB biofilms grown in all the reactors were able to immobilize uranium efficiently, despite the presence of U-complexing carbonates when biofilms were grown on redox-sensitive surfaces. Figure 1.8 shows HRTEM images of SRB biofilms grown on one redox-insensitive surface (quartz) and several redox-sensitive surfaces (calcite, dolomite, and hematite). The darker areas in the images show uraninite which was confirmed using EDS [87]. In all cases, uranium was mostly reduced in the periplasmic space. However, when the biofilm was grown on calcite, it was noticed that some of the U was reduced in the extracellular material. This could be because the chemistries of the extracellular matrices in biofilms grown on different minerals are different. The immobilization of U in the extracellular matrix could be responsible for the higher overall uranium removal efficiency in the column reactor filled with calcite.

Figure 1.9 shows a SEM image of biofilms growing on a dolomite surface. Although the cells produced a significant amount of extracellular material, the TEM image (Fig. 1.8c) shows that most of the uranium was immobilized within the

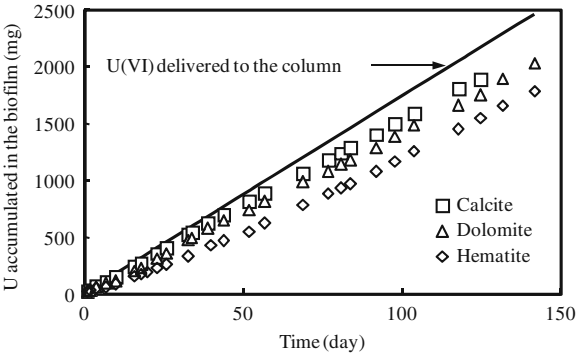


Fig. 1.7 Uranium accumulation in SRB biofilms in reactors filled with various minerals. The *continuous line* shows the cumulative amount of uranium delivered to each reactor (or maximum possible uranium accumulation). Modified from reference [87]. Reprinted with permission from Marsili et al. [87]. Copyright 2007 American Chemical Society

periplasmic space. However, we do not currently know the quantitative contribution of the EPS and the cells to uranium immobilization in biofilms.

Based on the results obtained, possible mechanisms contributing to U removal by SRB biofilms grown on minerals have been proposed and are schematically shown in Fig. 1.10. Although the figure shows biofilms grown on hematite only, similar mechanisms should be active in biofilms grown on other redox-sensitive surfaces, including calcite, dolomite and natural sediments. In U immobilization using biofilms grown on Mn-bearing minerals, Mn(II) and Mn(IV) may have effects similar to those of Fe(II) and Fe(III) (Fig. 1.11).

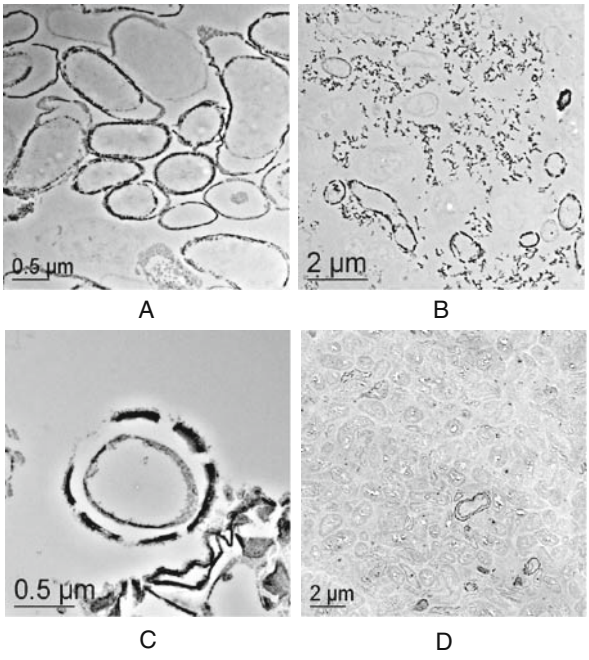


Fig. 1.8 TEM images of thin cross sections of biofilms deposited in the reactors filled with (**a**) a redox-insensitive surface, quartz, and redox-sensitive surfaces: (**b**) calcite, (**c**) dolomite and (**d**) hematite. Reprinted with permission from Marsili et al. [87]. Copyright 2007 American Chemical Society

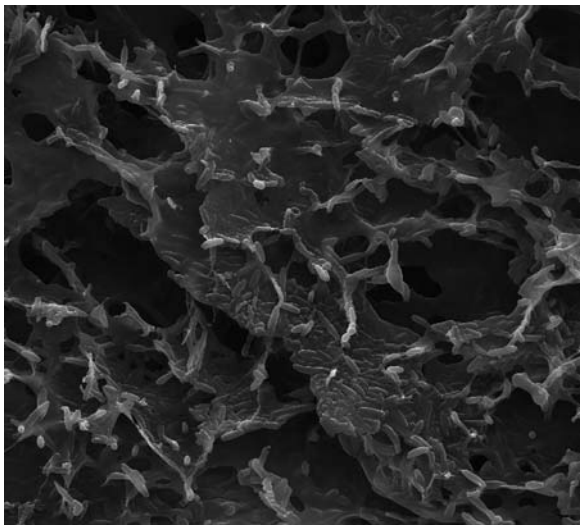
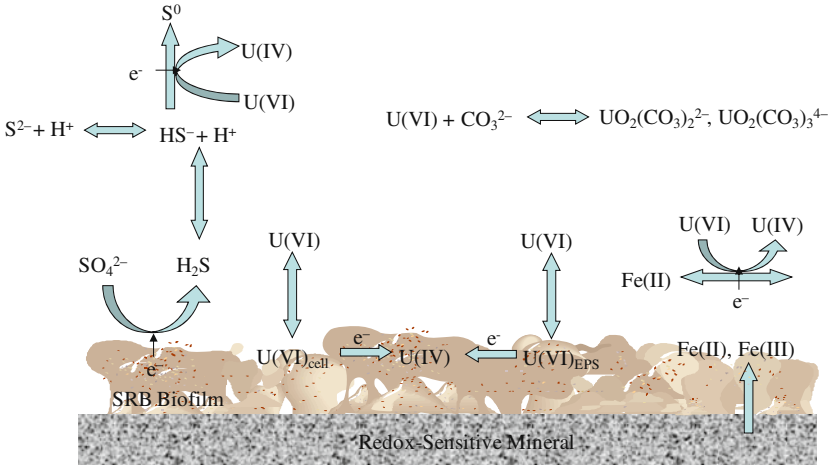


Fig. 1.9 SEM image of biofilms growing on a dolomite surface

The feasibility of U(VI) immobilization using SRB biofilms for bioreduction and the stability of bioreduced U in carbonate-buffered groundwater are still under discussion, especially for contaminated sites containing carbonate-bearing minerals. Although the mineral type on which biofilms are grown and the total carbonate concentration affect U removal efficiency, literature results demonstrate that SRB biofilms precipitate U and that the precipitated U is stable over a long period of time



$U(VI)_{cell}$: U(VI) adsorbed or intaken by cells; $U(VI)_{EPS}$: U(VI) accumulated in EPS.

Fig. 1.10 Possible mechanisms of U immobilization and remobilization using SRB biofilms grown on iron-bearing surface. Reprinted ("adapted") with permission from Marsili et al. [87]. Copyright 2007 American Chemical Society

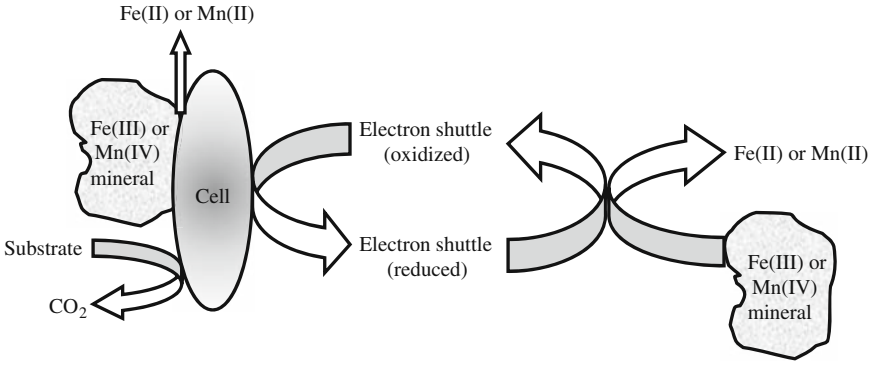


Fig. 1.11 Fe(III) and Mn(IV) as competing electron acceptors in U(VI) reduction [156, 65, 157]

(4–5 months) as long as sulfate-reducing conditions are satisfied in the reactor. The dominant mechanism of U removal in biofilm reactors is thought to be enzymatic reduction.

Stimulated Indigenous Biofilms on Natural Sediments

Recently, our research has focused on using biofilms of naturally growing facultative bacteria grown on sediments from uranium-contaminated sites in the Hanford 300 Area to evaluate U(VI) immobilization in the presence of naturally occurring redox-sensitive minerals.

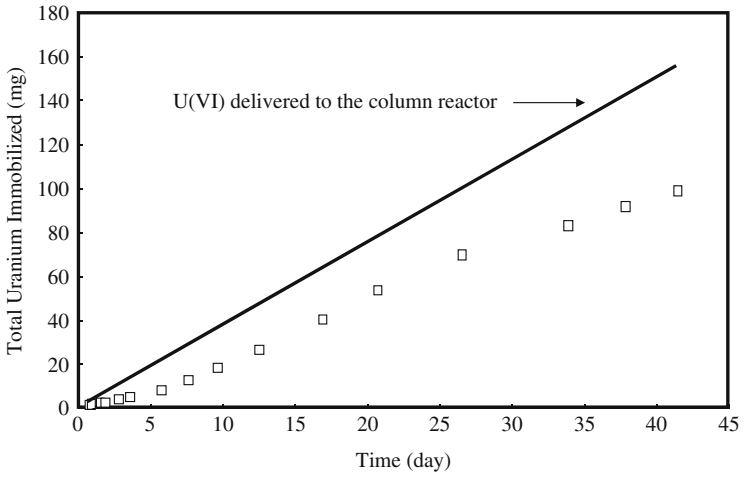


Fig. 1.12 Total amount of uranium accumulated in lactate-stimulated indigenous biofilms on sediments from the Hanford 300 Area. The reactor was fed with 126 μM U(VI) at a flow rate of 125 mL/day. The *continuous line* shows the cumulative amount of uranium delivered to the reactor (the maximum possible uranium accumulation)

A column filled with sediments from the contaminated sites was used in a FBCR (Fig. 1.4b). The FBCR was stimulated with lactate, succinate, malate, and fumarate in artificial groundwater. Samples were taken periodically from the column outlet and analyzed. The total uranium accumulated in the biofilm reactor over time is shown in Fig. 1.12. We found that 63.5% of the uranium was removed in around 40 days, which is somewhat lower than what we expected based on previous studies using pure culture and synthetic minerals in the laboratory. During the U(VI) bioremediation in this biofilm reactor, we found that Fe(III) and Mn(IV) were released from the sediments. Both Fe(III) and Mn(IV) have been shown to have the capability of competing with U(VI) as electron acceptors, which may have contributed to the discrepancies between these results and those of previous studies using minerals containing no Fe or Mn oxides. In addition to the mechanisms of U immobilization and remobilization illustrated in Fig. 1.11, the release of Mn(IV) and Mn(II) may have similar effects to those of Fe(II) and Fe(III) during U immobilization using indigenous biofilms grown on natural sediments. Extensive research work will be needed to elucidate the complex interactions between biofilms and redox-sensitive minerals.

1.4 Conclusion

Uranium is one of the most common radionuclides in soils, sediments, and groundwater at the DOE contaminated sites and must be remediated. Because indigenous microorganisms are readily available at the contaminated sites, bioremediation through natural attenuation by microbial processes has become a preferred strategy for *in situ* uranium remediation. The basic concept of uranium bioremediation is to immobilize U(VI) by harnessing indigenous microorganisms in groundwater and aquifer sediments to reduce U(VI) and form sparingly soluble U(IV) minerals, which has been shown to be feasible by multidisciplinary researchers.

Most uranium immobilization studies have been conducted in the presence of suspended microorganisms or enriched sediments, eventually spiked with micro- or nano- particles of other minerals. However, in natural soils and water-sediment interfaces, microorganisms are commonly found in the form of surface-associated cells, or biofilms. In the past several years, using SRB biofilms as a model, the feasibility of U(VI) immobilization in biofilms growing on redox-insensitive surfaces (quartz or glass slides) and redox-sensitive surfaces (carbonate-bearing minerals) has been studied. In the absence of bicarbonate, SRB biofilms have been shown to immobilize U(VI) for significant amounts of time as the result of both enzymatic and chemical reduction of U(VI) to insoluble uraninite. In the presence of carbonate buffer, the uranium removal extent and rate were satisfactory in the SRB biofilm reactors after 5 months of operation. In addition, SRB biofilms grown on carbonate-bearing minerals have been shown to be able to immobilize uranium and the precipitated uranium has been shown to be stable as long as sulfate-reducing conditions are satisfied in the biofilm reactor over a long period of time (4–5 months).

Recently, we have been extending these laboratory and well-controlled pure culture studies to the field by studying uranium immobilization using biofilms of indigenous microorganisms grown on sediments from the Hanford 300 Area. Uranium accumulated in biofilms of the lactate-stimulated naturally occurring bacteria in this system with less efficiency than what we expected on the basis of previous pure-culture studies using SRB biofilms grown on glass slides or carbonate-bearing minerals.

As the ubiquitous growth pattern of indigenous microorganisms in soils and water-sediment interfaces at contaminated sites, biofilms play an important role in the *in situ* bioremediation of uranium. The metabolic activity of cells in biofilms, differing from that of suspended cells, affects the abiotic and redox reactions controlling the mobility of U(VI) in the environment. The complex biotic, abiotic and redox conditions in biofilms pose challenges in predicting the mobility of uranium because uranium immobilization in biofilms with heterogeneous local conditions can be significantly different from that in bulk conditions. In order to efficiently and sustainably harness indigenous heterogeneous biofilms to immobilize uranium at contaminated sites, much more research work will be needed to elucidate the complex interactions among uranium, biofilms, and various redox-sensitive minerals during *in situ* uranium bioremediation.

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Chapter 2
Encapsulation of Potassium Permanganate Oxidant in Biodegradable Polymers to Develop a Novel Form of Controlled-Release Remediation

Stephanie Luster-Teasley, Patrick Onochie, and Vestel Shirley

Abstract This research seeks to develop controlled release biodegradable polymers that release chemical oxidants at controlled or sustained rates to extend the longevity of treatments for water and soil. The present work explores the ability to encapsulate chemical oxidants in biodegradable polymers for environmental remediation. Several polymer/oxidant formulations were tested by combining hydrophobic and hydrophilic polymers to create pellets with various release rates for potassium permanganate. Potassium permanganate is a crystalline chemical oxidant that is currently dissolved in water for use in drinking water treatment and in the remediation of chlorinated solvents. Prototype pellet structures ranging in 0.3–0.5 mm size were produced. The pellets were capable of slowly releasing potassium permanganate over a 44–47 day period of testing. This technology provides the potential for new methods for chemical remediation using biodegradable polymers including creation of subsurface permeable reactive barriers, wells packed with oxidation pellets, or reactors filled with pellets for pump and treat systems. This paper will summarize the development of this technology, release rate studies, and preliminary remediation studies for trichloroethylene using the controlled release prototypes.

Keywords Potassium permangante · Encapsulation · Biodegradable polymers

2.1 Introduction

Controlled release (CR) methods to deliver drugs and chemicals are increasingly being employed for various fields such as surgery, pharmacology, and agriculture [1–8]. Examples of CR designs in the pharmaceutical industry include small nontoxic devices implanted under the skin and biodegradable CR polymers implanted

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in humans subjects [1, 9–14]. In agriculture, CR biodegradable polymers and waxes are used for insecticide, fungicide and pesticide applications [3, 4, 6, 7]. Despite extensive medical and agricultural research for controlled release systems using environmentally benign and biodegradable polymers, few studies have investigated implementing these methods for environmental engineering applications. Several researchers have developed controlled release systems for soil and water remediation using clay, waxes, gels, and waxy polymers. Examples include slow release oxygen polymers for bioremediation, phosphate buffer encapsulation for polymers for pH control during denitrification of groundwater and sediment, alginate gel for Fenton photochemical oxidation, and encapsulation of bacterial cells [15–19].

Exploring the ability to use biodegradable polymers featuring controlled release capabilities for environmental remediation and treatment is an emerging concept. Like medical and agricultural systems, many environmental engineering treatments rely on mass transfer and delivery of chemicals. Fundamental research of CR applications for environmental protection, decontamination, and remediation therefore merits investigation. CR technologies can extend treatment methods for soil and water remediation. The existing body of literature for biodegradable polymers focuses on seeking affordable replacements for non-degradable, synthetic plastics and methods to increase physical properties such as tensile strength for the packaging industry [2,20,21]. For environmental engineering applications, however, the benefit of using polymers which naturally degrade to deliver treatment chemicals provides a method for in-situ remediation that would not need to be removed after treatment is completed.

Using the advantageous characteristics of biodegradable polymers and controlled release capabilities to improve mass transfer, delivery, and longevity of chemical and biological treatments in environmental remediation is an intriguing approach. A variety of biodegradable polymers serve as potential candidates for developing controlled release structures. The broad classifications for non-toxic, biodegradable polymer groups include poly(esters), poly(orthoesters), poly(anhydrides), poly(amides), and poly(saccharides) [2, 12, 14, 22]. Biodegradable polymers can be classified based on the mechanism for polymer breakdown. These mechanisms include: water-soluble polymers made from hydrolytically unstable cross links (type 1); linear polymers which are solubilized by hydrolysis ionization or protonation but without backbone cleavage (type 2); or water insoluble polymers which breakdown into smaller soluble products by backbone cleavage (type 3) [23].

Based on the environmental media, the type of contaminants present in the target treatment system, and physical conditions, biodegradable polymers can be selected or designed with specific physicochemical properties. Criteria for polymer selection include compatibility with the selected delivery chemical and the ability for the polymer to erode or dissolve at a slower rate than the target delivery chemical. Therefore, multiple combinations of biodegradable polymers and types of chemicals are possible for controlled release design. Table 2.1 provides examples of five groups of non-toxic, biodegradable polymers that represent candidates for controlled release development for environmental remediation. These general polymer groups offer different physical and chemical attributes that can be used to construct CR structures with polymer erosion, diffusion controlled chemical

Table 2.1 Summary of biodegradable polymers to consider for CRBP

Polymer groups	Group examples	Beneficial controlled release characteristics
Poly(esters)	Poly(lactic acid) (PLA) Poly(glycolic acid) (PGA) Copolymer Poly(lactic acid-co-glycolic acid) (PLGA) Homopolymers Poly(d-Lactic acid) (PDLA) Poly(l-lactic acid) (PLLA) Poly(ethylene glycol) (PEG) also referred to as poly (ethylene oxide) (PEO)	Bulk degradation with random hydrolytic scission of polymer backbone. Degradation rates vary based on structure
Poly(ortho esters)	(DETOSU)-based poly(ortho esters) Cross-linked poly(ortho esters)	Allows for diffusion mechanisms after the polymer chains at the surface are degraded
Poly(anhydrides)	Sebacic acid (SA) P-(carboxyphenoxy)propoane (CPP) P-(carboxyohenoxy)hexane (CPH) Poly(anhydride-imides) Poly(anhydride-esters)	Hydrophobic with water sensitive linkages – heterogeneous surface erosion
Poly(amides)	Poly(amino acids) Polymers with combinations of amino acids Examples: poly(glutamic acid) or poly(aspartic acid)	Hydrophilic with degradation rates dependent upon amide bond
Poly(saccharides)	Natural polymers made with starch, cellulose, and chitosan	Natural polymers

release, and combined control mechanisms. Figure 2.1 depicts the mechanistic designs to enable the controlled release of treatment chemicals from biodegradable polymers.

2.2 Controlled Release Chemical Oxidation and Literature Review

This research seeks to investigate controlled release methods using biodegradable polymers to deliver chemical oxidants for environmental remediation. Chemical oxidants are highly reactive, non-selective chemicals that generate hydroxyl radicals

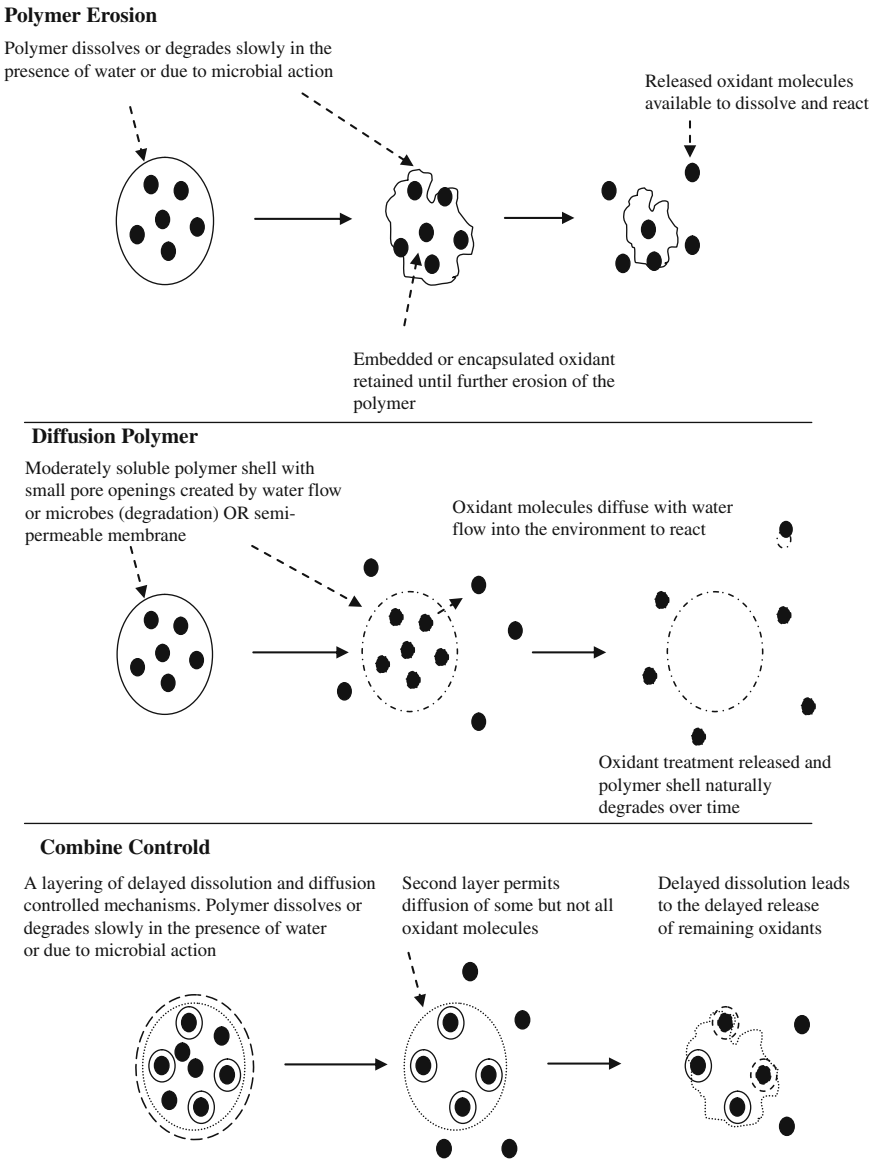


Fig. 2.1 Schematic for mechanisms influencing CRBP structure design (modified from Uhrich et al. 1999)

able to degrade environmental contaminants. In current chemical oxidation methods, highly reactive liquids or gases are injected into a contaminated zone. Chemical oxidants rapidly react to oxidize and breakdown subsurface contaminants to less toxic byproducts. Examples of oxidants used in chemox include ozone gas, Fenton's

Reagent and crystalline permanganate or persulfate dissolved in water. Despite the effectiveness of chemical oxidation, there are drawbacks to current chemox technologies. These drawbacks include the need (1) to inject very strong gases or copious volumes of chemox liquids into the ground, (2) to reapply the oxidants into the subsurface to fully clean sites due to side reactions occurring with natural organic matter or plugging of the subsurface system with chemox precipitates, (3) to prevent explosion hazards caused by the rapid reactions, and (4) to protect workers from the health and safety hazards associated with use of these chemicals.

Oxidants encapsulated and stabilized in polymers for remediation provides a novel technique for chemical oxidation treatments for water and soil due to the ability to slow down the delivery of the oxidant to the contaminated area. The development of a controlled release delivery system for oxidants from a degradable shell has several advantages: (1) it stabilizes the solid oxidants for emplacement in soil, sediment or subsurface applications. The placement may vary from ones that form permeable reactive barriers, packing in a series of wells where contaminated water will flow past the pellets for remediation, packing in a reactor for a pump and treat system, emplacement in sediment or surface water, or augured into soil. (2) It reduces the need for maintenance associated with the gaseous and liquid oxidants. (3) It reduces the dangers associated with handling the oxidant by workers. (4) It extends the release of an oxidant without the need to re-inject the gas or liquid solutions. Therefore this new method has the potential to effectively extend the ability to provide chemox treatment in a fashion similar to using biodegradable medical implants to deliver drug formulations in the body to provide controlled and extended release of their active components.

This research focuses on the development of controlled release with the chemical oxidant potassium permanganate. Potassium permanganate is a purple crystalline oxidant that can be used to remediate trichloroethylene and was selected as the initial target oxidant for prototype development [16–18]. Several researchers have investigated the ability to encapsulate the chemical oxidant potassium permanganate for slow release remediation using waxes and chlorine based polymers [16–18]. Kang et al. [16] encapsulated $KMnO_4$ (EPGs) in paraffin wax pellets 0.5–5 mm in size. Using a melt-dispersion method, paraffin wax was melted and uncoated, milled $KMnO_4$ (UMPP) was gradually mixed into the wax. The paraffin and milled $KMnO_4$ were then cooled to produce the EPGs. The granules were milled for 20 minutes to form the final product called encapsulated $KMnO_4$ (EPP). The EPP demonstrated a biphasic release of $KMnO_4$ where an initial rapid release of $KMnO_4$ (<10 min) was observed due to dissolution of $KMnO_4$ on the surface or partially embedded in the wax. Following the rapid release, a sustained release for the remaining four days was observed in reagent grade water. EPP encapsulation resulted in 10–45% release of $KMnO_4$ over a 4-day period. Kang observed the release of wax encapsulated $KMnO_4$ in the presence of perchloroethylene (PCE). The solubility of paraffin wax in PCE resulted in complete dissolution of the wax within 3 min resulting in the rapid release of oxidant from the encapsulated matrix.

Schwartz et al. [17] investigated use of wax encapsulated $KMnO_4$ as the active component for a permeable reactive barrier. Controlled release $KMnO_4$ was manufactured by dispersing $KMnO_4$ in paraffin wax. The wax was cooled in cylindrical molds at room temperature to produce candle shaped rods 2.5 cm in diameter and 5 cm long. Each rod contained 70 g of $KMnO_4$. The rods were inserted into 2.6 (i.d.) $\times$ 10 cm long delivery wells used in the flow-tank experiments. Release studies were performed in column tests where $KMnO_4$ concentrations were measured for flowing water (19–21 mL/min) through a 4.8 $\times$ 15 cm Chromaflex glass column. Biphasic release of $KMnO_4$ was observed where a high concentration of $KMnO_4$ was released followed by an extended release over 28 days of testing. In the second part of the study by Schwartz, two delivery wells containing six $KMnO_4$ wax rods (2.5 $\times$ 10 cm) were inserted in the into a glass tank filled with silica sand. Water was uniformly pumped into the tank at an inflow rate of 19.2 L/day. Small multi-level wells were emplaced along the center of the tank to permanganate samples from the tank. In the study, $KMnO_4$ delivery through the tank and remediation of water contaminated with trichloroethylene (TCE) were measured. The study demonstrated that the controlled release method is capable of destroying dissolved trichloroethylene (TCE) in a long-term, controlled manner. Incomplete destruction of TCE was observed suggesting that placement of delivery wells to facilitate lateral spreading and mixing of permanganate with the dissolved contaminated plume is necessary.

Ross et al. [18] produced microcapsules ranging in size from 0.06 to 2 mm using chlorine-based waxy polymers which released oxidants for up to 20 days in an aqueous batch system. Potassium permanganate was encapsulated in blend of Boler way, Piccolyte resin S115, Epolene C-16, and Clorez 700. Microcapsules were fabricated by producing a slurry of $KMnO_4$ and wax by heating the material above the melting point of the waxes. The wax and $KMnO_4$ were sonicated and the slurry was formed into droplets using a spinning disk. The process produced two types of microcapusles. The first prototype contained a single grain core (SGC) of $KMnO_4$ encased in a polymer shell. The second prototype had 5–10 multiple grains of $KMnO_4$ in the core (MGC). The grain size for SGC ranged from 0.06 to 1 mm and the MGC ranged from 0.06 to 2 mm. The mass ratio of $KMnO_4$ to shell material ranged from 0.25 to 0.50. Ross et al. investigated the use of MGC microcapsules for TCE remediation in batch aqueous studies. TCE degradation using the MGC demonstrated effective degradation of TCE in aqueous media over several weeks.

The studies by Kang, Schwartz, and Ross et al. suggest the development of encapsulated oxidants to create controlled release systems for remediation warrants further investigation. In particular, $KMnO_4$ solutions have been used in various field studies and remediation projects to remediate TCE contamination. The reaction between $KMnO_4$ and TCE involves cleavage of TCE to yield CO_2, manganese dioxide (MnO_2), potassium chloride (KCl), and hydrochloric acid (HCl). The reaction is as follows

$$C_2Cl_3H + 2KMnO_4 \rightarrow 2CO_{2(aq)} + 2MnO_{2(s)} + 2KCl + HCl$$

2.3 Experimental Discussion

In the present work, several biodegradable polymers and polymer blends were investigated for their potential to produce slow release $KMnO_4$ for extended periods of time. These prototypes ranged in diameter size from 0.3 to 0.5 cm solid pellets capable of releasing $KMnO_4$ for over one month duration. This article presents the preliminary laboratory research conducted to produce encapsulated $KMnO_4$ using biodegradable polymers and examining the feasibility of this technology. Prototype development was based on three criteria: (1) polymer shell compatibility with the oxidant, (2) the ability for diffusion of the oxidant through the shell boundary, and (3) the ability to form pellet structures feasible for use as fill material for a permeable reactive barrier or in a reactor system.

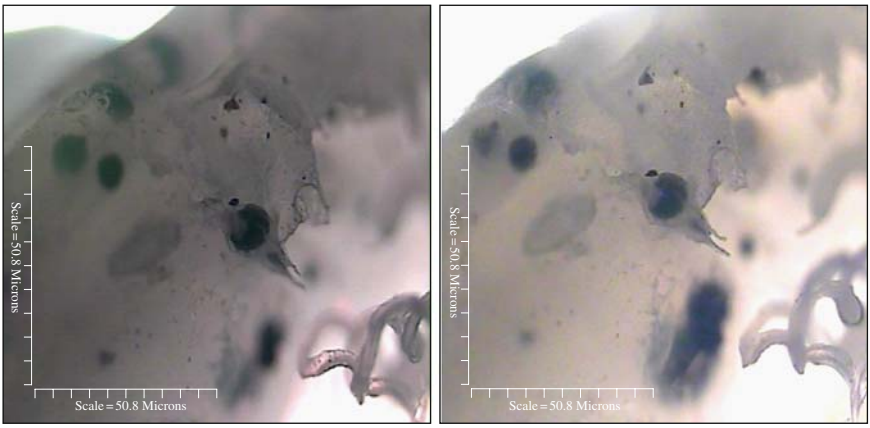
Prototypes with various diffusion and erosion rates were investigated using batch studies with aqueous media. Studies were conducted to evaluate the prototype reaction with the encapsulated oxidant, release of the oxidant from the polymer, and remediation of 500 ppm trichloroethylene in batch reactors. Criteria for biodegradable polymer selection included the compatibility with the selected oxidant, the ability to form solid pellet structures ranging in size less than 0.3–0.5 cm, and the ability of the polymer to degrade or dissolve to release the oxidant.

2.3.1 Materials

Certified ACS $KMnO_4$ (Fisher Chemical, Fair Lawn, NJ) was encapsulated in a blend of inert biodegradable polymers. Due to the proprietary nature of the work, the specific biodegradable polymers and blends selected for the prototype design will not be disclosed in this article; however, the prototype development primarily investigated the use of polymers from the polyester polymer group and polyvinyl alcohol. The structures, henceforth referred to as controlled-release biodegradable polymers (CRBP), Polymer A (a hydrophobic biodegradable polymer), and Polymer B (a hydrophilic biodegradable polymer) were formed into 0.3–0.5 cm in size solid pellets capable of controlled-release of the oxidant. The polymer blends for Polymer A and Polymer B were designed based on three mechanisms presented in Fig. 2.1. These mechanisms include: CRBP type 1 – Polymer erosion, CRBP type 2 – diffusion controlled, and CRBP type 3 – combined control. In a type 1 polymer erosion based system, the polymer had a higher capability of dissolving in water to release $KMnO_4$. The type 2 diffusion controlled system was designed for a higher capability for the $KMnO_4$ to diffuse through the shell into water. The type 3 combined control is a combination of type 1 and type 2 systems. Potassium permanganate concentrations in solution were measured using a Spectronic Spectrophotometer at 525 nm wavelength. Trichloroethylene concentrations were analyzed using a HP 5890 Series II gas chromatograph with a flame ionization detector (GC/FID) and a capillary column (J&W Scientific DB-624, 0.53 mm id $\times$ 30 m length).

2.3.2 Stability of $KMnO_4$

In this study, $KMnO_4$ was tested for reactivity with the biodegradable polymers and polymer blends. One concern for shell material is to ensure the oxidant was not reactive with the polymer. If the $KMnO_4$ is reactive with the shell, visible evidence would be a brown discoloration caused by manganese oxide formation or visible degradation of the shell material. To determine the stability, $KMnO_4$ was encapsulated in biodegradable polymer shells and mounted on a glass slide. Photos at 40x and 100x were taken of the pellets using a digital microscope to observe any reactions or discoloration of the shell. Little to no reactivity was seen in the biodegradable polymers used to produce the CRBP prototypes. Figure 2.2 shows a photo spanning the initial encapsulation and 7 months after of encapsulation. The $KMnO_4$ in the polymer appears to be stable and non-reactive for the polymer prototypes. Little to no reaction appears between the polymer material and the $KMnO_4$ demonstrating the shell is inert and able to stabilize the oxidant.



(a) Polymer with oxidant immediately following encapsulation

(b) Polymer with oxidant after 7 months

Fig. 2.2 Photo of polymer with $KMnO_4$ 7 months after encapsulation

2.3.3 Release Studies for Encapsulated $KMnO_4$

Biphasic $KMnO_4$ release was observed from the encapsulated oxidant pellets. This is consistent with the observations seen in previous studies using wax and chlorinated polymers [16, 18]. Figure 2.3 shows the polymer pellets and the oxidant diffusing from the shell. The speed for diffusion of the oxidant across the pellet surface was controlled by the polymer blend. A rapid release of $KMnO_4$ occurred during the initial 1–3 days followed by a controlled and slower release of $KMnO_4$. Oxidant release was delayed by increasing the ratio of hydrophobic Polymer A

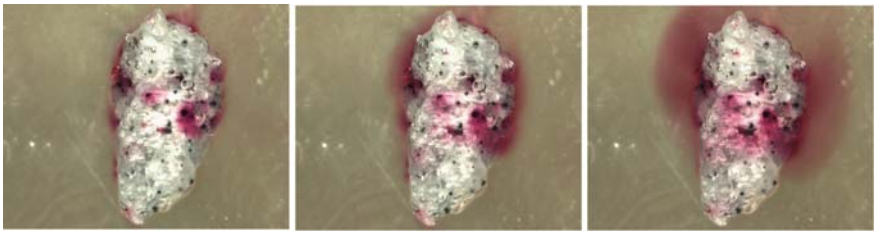


Fig. 2.3 Time series photos of pellet with oxidant diffusing from shell

to hydrophilic polymer B in the polymer blend. Prototype blends were identified as PAB-50, PAB-60, PAB-90, and PAB-100 contained 50, 60, 90, and 100% of Polymer A to Polymer B, respectively. Therefore PAB-50 represented a 1:1 ratio of Polymer A to Polymer B. Figure 2.4 shows a graph comparing the release rate for the polymer blends. PAB-50 was observed to release $KMnO_4$ at a faster rate than PAB-60, PAB-90 and PAB-100. PAB-60 and PAB-90 appeared to exhibit similar release rates for $KMnO_4$ with PAB-90 releasing the oxidant slower during the first 20-min. PAB-100 demonstrated the slowest release of the oxidant. Uniform mixing of the polymers and oxidant proved to be important to ensure diffusion of the oxidant from the shell at a controlled rate because the hydrophilic polymer will readily dissolve in water leaving pores in the hydrophobic shell. For example, an excess amount of hydrophilic polymer on the pellet surface led to rapid diffusion of the oxidant from the polymer. Pellets designed with only the hydrophobic polymer demonstrated the slowest rate of oxidant diffusion.

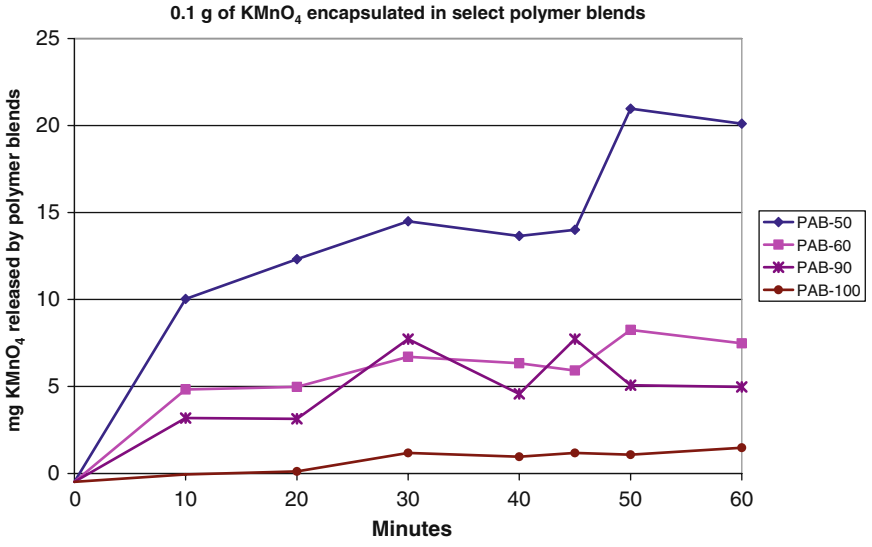


Fig. 2.4 Prototype KMnO4 release for the hydrophilic and hydrophobic polymer blends

The diffusion based prototypes containing Polymer A proved to be the most effective $KMnO_4$ delivery without the formation of MnO_2 precipitates. The erosion based prototypes with Polymer B formed manganese oxide precipitates. The diffusion based and the combined prototypes were determined to be more feasible for long-term release of the oxidant subsurface. The prototypes with a higher ratio of Polymer B produced MnO_2 precipitate which decreased in formation as the ratio of Polymer A polymer increased in the pellet design. Because manganese oxide is a precipitate known to inhibit the ability to use $KMnO_4$ for trichloroethylene remediation, the PAB-100 prototype was evaluated for the media replacement, continuous release, and TCE remediation studies.

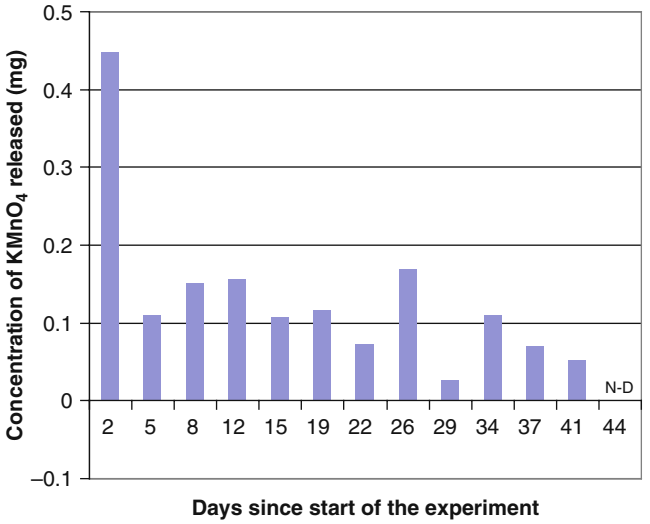
2.3.3.1 Replacement Media Study

In the replacement media study, PAB-100 prototypes containing 2 and 20% $KMnO_4$ by mass to polymer ratio were encapsulated in the polymer shell. In batch reactor bottles, 50 ml of DI water and 0.5 g of prototype pellets were sealed and stored for 3–4 days at 28°C and in the dark. After 3–4 days, the $KMnO_4$ concentration was measured using a Spectronic Spectrometer at 525 nm wavelength. Following the $KMnO_4$ concentration measurement, the pellets and bottles were rinsed with DI water three times and 50 ml of fresh DI water was added to the bottles with the same pellets. This process was then repeated by storing the reaction bottles for another 3–4 days and a $KMnO_4$ measurement was performed. This replacement media study was designed to measure the steady-state release rate of $KMnO_4$ prototypes.

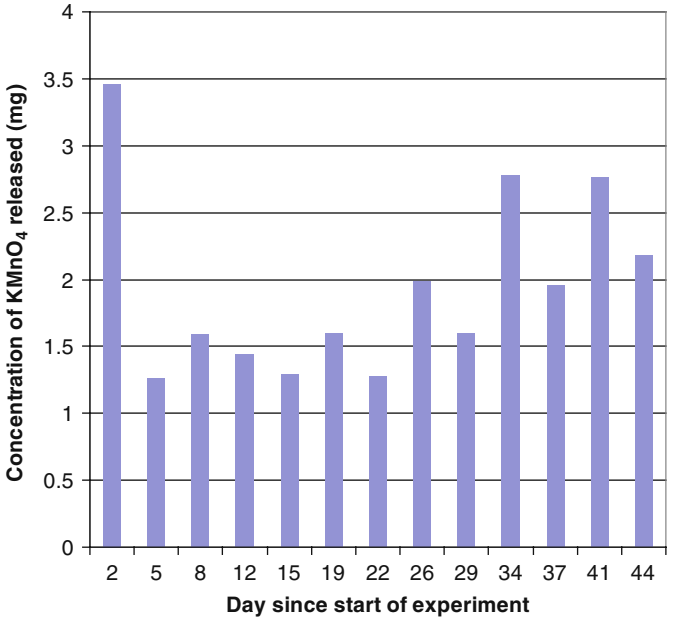
The prototypes demonstrated an initial rapid release of the oxidant followed by a slower, steady state release (Fig. 2.5a and b). The 2% prototype exhausted the encapsulated $KMnO_4$ diffusing from the shell within 44 days. The 20% $KMnO_4$ structures however had not exhausted the oxidant within the 44 day period of the experiment. It is hypothesized this prototype would be able to continue to release $KMnO_4$ release for a significantly longer period due to the higher concentration of KMnO4 encapsulated in the polymer prototype.

2.3.3.2 Continuous Release Study

In the continuous release studies, PAB-100 prototypes containing 2 and 20% $KMnO_4$ by mass to polymer ratio were encapsulated in the polymer shell. A mass of 0.5 g of pellets were added to 50 ml of DI water in reaction bottles. Measurements for $KMnO_4$ were conducted every 3–4 days using a Spectronic Spectrometer at 525 nm wavelength. Following the measurements, the prototypes and the liquid sample were returned the bottles, capped, and stored for another 3–4 days at 28°C in the dark before the next measurement. In the continuous batch study, $KMnO_4$ release was observed to extend over a 46–48 day period where 2 and 20% $KMnO_4$ by mass in polymer prototypes were measured for $KMnO_4$ concentration in water (Fig. 2.6a and b).

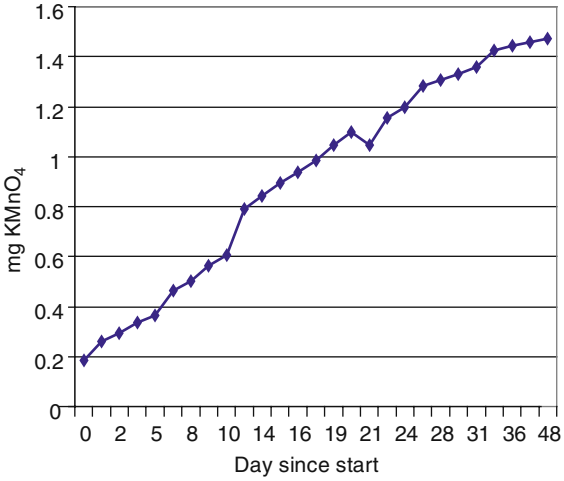


(a)

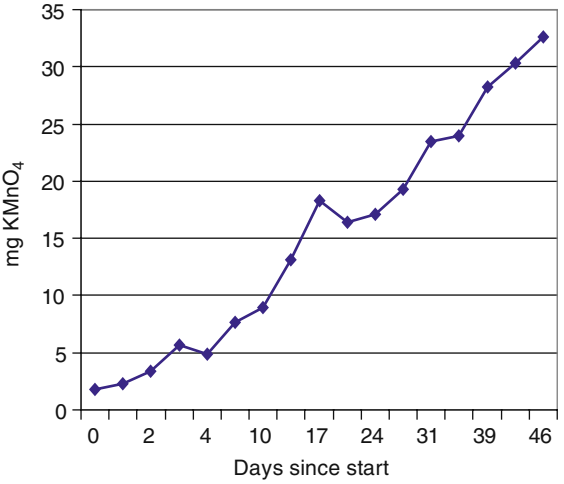


(b)

Fig. 2.5 (**a**) Release of $KMnO_4$ from the 2% $KMnO_4$ by mass prototype for water media replaced every 3–4 days to determine a steady state release from the pellets. Day 44 measured a non-detectable (N-D) level of $KMnO_4$. (**b**) Release of $KMnO_4$ from the 20% $KMnO_4$ by mass prototype for water media replaced every 3–4 days to determine a steady state release from the pellets



(a)



(b)

Fig. 2.6 (**a**) KMnO4 concentration released from the 2% $KMnO_4$ by mass prototype over a 47 day period. (**b**) $KMnO_4$ concentration released from the 20% $KMnO_4$ by mass prototype over a 47 day period

Future studies to evaluate $KMnO_4$ release will entail optimizing the concentration of $KMnO_4$ (greater than 20% by mass) that can be encapsulated in the polymer structures and reducing the biphasic release observed in the prototypes. Additionally, alternative hydrophobic and hydrophilic polymer blends will be investigated. The surface area of the polymer structures can be enhanced using various shapes and geometries such as cylinder columns, saddles, or hollow tubes.

2.3.4 Reaction with Trichloroethylene

The prototypes demonstrated the ability to degrade trichloroethylene (TCE) in a batch system. The TCE contaminated water (500 ppm) was added to 25 ml serum bottles with 0.5 g of either the 2, 20, or 60% $KMnO_4$ prototypes. The bottles were overfilled and capped to prevent headspace air from being sealed in the bottle. This ensured all TCE would be dissolved in the liquid phase without the need to analyze headspace gas. TCE concentrations were analyzed using a HP 5890 Series II gas chromatograph with a flame ionization detector (GC/FID) and a capillary column (J&W Scientific DB-624, 0.53 i.d. × 30 mm length). Measurements were conducted every seven minutes for a total of 77 minutes. Trichloroethylene degradation for the 2% pellets reached 50% removal after 70 minutes while the 20% pellets reached 50% removal of TCE within 47 min. The TCE concentration reached 50% reduction in the 60% KMnO4 prototypes within 13 min however there was a large formation of MnO_2 (Fig. 2.7). The TCE and $KMnO_4$ reaction demonstrated first order kinetics which is consistent with literature [24].

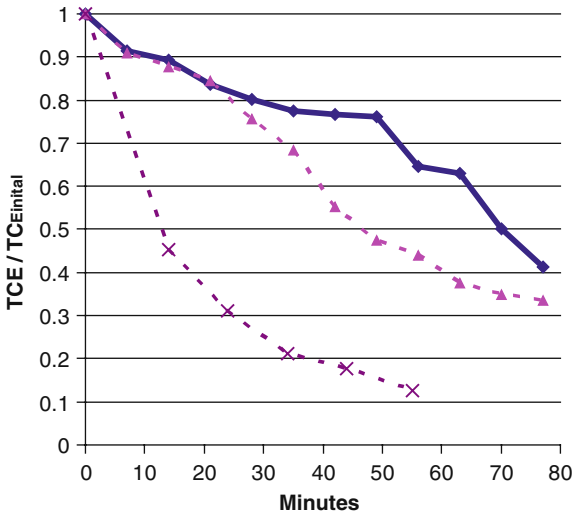


Fig. 2.7 TCE degradation by $KMnO_4$ released from the 2% (*solid line*) and 20% (*dotted line with triangles*) $KMnO_4$ by mass prototype; 60% KMnO4 by mass (*dotted line with x*)

2.3.5 Potential Challenges for CRBP $KMnO_4$ Remediation

There may be several factors that affect or impair the system performance. These factors include the quantity of potassium permanganate that will diffuse from the system, the production of MnO_2, and reaction efficiency may be limited. Prototype design and determination of an adequate quantity of pellets to remediate contaminants should help overcome these challenges. CBRP can overcome the need to inject copious amounts of liquid $KMnO_4$ into the subsurface which is the common

practice currently used for $KMnO_4$ and TCE remediation. The formation of MnO_2 precipitates which, is also a significant problem for traditional $KMnO_4$ remediation, still remains an important consideration and potential challenge to overcome for CRBP remediation.

Literature reports one of the limiting factors for chemical oxidation methods is the formation of precipitates that will "clog" the soil particles and reduce the effectiveness of transporting chemox liquid to the subsurface [25–27]. In previous liquid $KMnO_4$ studies, the system formed solid manganese oxide (MnO_2) precipitates which act as a barrier in the destruction of TCE. Preliminary data suggest the erosion based pellets produced MnO_2 precipitates within 24 h which supports the need to design CRBP which minimizes MnO_2 production. The precipitate production, however, subsided for the diffusion based prototypes using the hydrophobic polymers.

Literature also suggests that permanganate can react with soil constituents such as natural organic matter; this reaction with natural organic matter must be accounted in the determination of the optimal concentration of $KMnO_4$ to deliver from the pellets [25]. These side reactions are characteristics of all chemox treatments (i.e. ozone, Fenton's Reagent, $KMnO_4$) because of their ability to non-discriminately react with other chemicals. This characteristic is why chemical oxidants are applied in higher than stoichometrically required ratios to account for oxidant loss. This aspect of chemox is inherent to the use of highly reactive chemox compounds. Therefore it may be a characteristic that will not be easily altered but should not decrease the interest in using chemox methods. To overcome this difficulty, current chemox methods increase the molar ratio of oxidant injected into the system based on the premise that side reactions will occur decreasing oxidant efficiency. For permanganate reactions with TCE, 2-mole of $KMnO_4$ will stoichiometrically react with 1-mole of TCE. The system for $KMnO_4$ delivery from the pellets can be adjusted by providing a higher dose of $KMnO_4$ in the treatment system than the 2-mole $KMnO_4$: 1 mole TCE thus making this method comparable to liquid and gaseous oxidant reactions occurring in the field. Literature reports the observed stoichiometric ratio for permanganate to TCE as 3.5–5 moles $KMnO_4$ for every mole of TCE using waxy pellets in aqueous systems [24, 28, 29]. Preliminary studies show the polymers are moderately soluble in TCE. This finding was also supported by research conducted with the chlorine-based polymers used in Ross et al. [18]. This observation may facilitate the release of the potassium permanganate from the pellets in the presence of TCE and additionally some TCE may diffuse into the pellets and become entrapped within the polymer thus aiding with remediation.

2.4 Future Considerations and Conclusion

Biodegradable polymers offer an attractive alternative for improving remediation methods for environmental engineering applications. Exploring the ability to use biodegradable polymers featuring controlled release capabilities for environmental

treatments offer a new realm of research similar to medical research being conducted for use of polymers for drug delivery and human implants. One of the major considerations however is the cost for biodegradable polymers. Many major biodegradable polymer producers have stopped or limited production of their biodegradable polymers brands due to the higher cost for the use of a biodegradable polymer compared to traditional polymeric materials.

Controlled release methods for environmental engineering can have a widespread impact as alternative chemicals are considered for use in extended remediation. The delivery of solid oxidants from a degradable shell has several advantages. (1) It stabilizes the solid oxidants for emplacement in the subsurface to form permeable reactive barriers, packed in a series of wells where contaminated water will flow past the pellets for remediation, or packed in a reactor for a pump and treat system. (2) It reduces the need for maintenance associated with the gaseous and liquid oxidants. (3) It reduces the dangers associated with handling the oxidant by workers. (4) It extends the release of an oxidant without the need to re-inject the gas or liquid solutions. Preliminary results support the potential for this technology in environmental engineering remediation. The possible applications of this technology include use of the prototypes to form permeable reactive barriers. Such barriers have been shown to be effective in recent remediation studies using zero-valent iron [30, 31]. Packing screened wells in a treatment wall helps diffuse the chemical oxidants in contaminated plumes. This technology will also work well in a reactor system to treat contaminated effluent or as a pump-and-treat technique for remediation. We are interested in exploring both subsurface, surface water, and land application uses for the technology.

Our future work for using chemical oxidants encapsulated in biodegradable polymers will include exploring alternative chemicals for encapsulation and investigating alternative hydrophobic and hydrophilic polymer blends to control degradation rates for the polymer/chemical pellets. We will investigate optimization of the chemical to polymer ratio, controlling and modeling chemical diffusion from polymer shells, encapsulation methods (i.e. micro-based and nano-based), and structure geometry. In particular, we are interested in exploring the use of alternative oxidants for treatment of environmental contaminants including various groundwater contaminants, pathogens, and pesticides.

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Chapter 3
Decontaminating Heavy Metals from Water Using Photosynthetic Microbes

Daniel D. Lefebvre and Chad Edwards

Abstract Elevated levels of heavy metals in our environment can pose serious problems to a wide variety of living organisms, including humans. This is because transporters in cell membranes can absorb toxic non-essential metal ions and essential metal ions to excess, both of which can deleteriously affect important metabolic processes. Organisms have responded to this threat by evolving coping mechanisms that biotransform the metals into forms possessing low toxicity. By their very nature these mechanisms also act to make metals less bioavailable in the environment, and it is this property that can be exploited for bioremediation purposes. Sulfur and its metabolism is often central to these coping mechanisms. It is absorbed by cells in the form of sulfate that in turn is converted to sulfite and subsequently into thiols via energy input and reduction. Metal ions can bind to these thiol groups in cysteine, glutathione and metallothioneins rendering them essentially detoxified. Furthermore, some organisms such as the sulfate reducing bacteria biotransform metal ions into metal sulfides that have very low solubilities and hence, very low bioavailabilities. However from the perspective of applying metal bioremediation, the sulfate reducing bacteria require anoxic environments that would be unlike the conditions associated with most anthropogenic sources of heavy metals. Recently, photosynthetic microbes have also been shown to form metal sulfides. Here, we present the potential of these microbes for the effective aerobic bioremediation of heavy metals.

Keywords Heavy metals · Metal biotransformation · Bioremediation · Cyanobacteria · Algae · Sulfur metabolism · Thiol · Metal sulfide

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3.1 Introduction

Many heavy metals occur naturally at elevated levels in the earth's crust and environmental pollution from heavy metals has become widespread as industrial activities have increased over the last two centuries. During this period these pollutants have arisen from a variety of anthropomorphic sources such as urban and agricultural runoff, industrial effluents, sewage treatment plants, mining operations and refining of fossil fuels. As a consequence, detrimental effects are now witnessed in a wide assortment of ecosystems [1, 2].

When heavy metals are introduced in their elemental forms or in organic-metalloid compounds, they can have dramatic health implications for human populations. Exposure has been linked to neurological impairment and cellular senescence [3] renal and hepatic failure [4] and carcinogenesis [5–7]. Consequently, it is not surprising that the United States Environmental Protection Agency (EPA) has ranked mercury, cadmium, copper, lead, nickel and zinc on the priority list for hazardous pollutants [8]. Bridges and Zalups [9] provide a worthwhile review of the medical implications of heavy metal toxicity.

Heavy metal decontamination has been studied in a wide variety of species across all phylogenic kingdoms [10–13]. Many species of various taxa contain genes encoding metallothionein proteins and peptides that actively bind to heavy metals [14–19]). Prokaryotic, algal and fungal capacities to cope with metal stress have been extensively investigated because of their relative biological simplicity, ease of culture, and the molecular similarity of their decontamination mechanisms to mammalian counterparts [20]. These mechanisms are known to deal with metals such as Zn(II), Cu(II), and Cd(II) [21, 22]. Furthermore, prokaryotic species possess *mer* operons capable of regulating the stress response associated with ameliorating high concentrations of mercury [15, 23]. Prokaryotes, algae and fungi also have the ability to biotransform heavy metals into metal sulfides that are relatively unavailable biologically because of their insolubility [24].

Vascular plants, including aquatic macrophytes, are known to possess the ability to bind and detoxify heavy metals, and much of this knowledge has been applied to understanding heavy metal detoxification in algae [25, 26]. For example, various algal species have been investigated for their abilities to accumulate heavy metals and for their increased biomass by comparison with metal tolerant aquatic macrophytes [25]. Nevertheless, both macrophytes and algae have been given attention because they can remove and retain these harmful contaminants from the environment. For example, cadmium resistant strains of *Chlamydamonas reinhardtii* have been identified and studied for their potential to bind large quantities of heavy metals [27–30].

This chapter focuses on the process of heavy metal tolerance and bioconversions in micro-organisms with particular emphasis being placed on the mechanisms of decontamination in the photosynthetic micro-organisms, cyanobacteria and algae. These organisms do not require fixed carbon as a source of energy, possess aerobic metabolisms and, under the appropriate conditions, can be very effective at the biotransformation of heavy metal ions. Because there is a strong possibility for similar

mechanisms of metal decontamination to occur between these micro-organisms and plants, and there is a wealth of information on the latter, examples of higher plant studies have been included in this article.

3.2 Membrane Transport of Heavy Metals

There are several known mechanisms for heavy metal absorption into cells. These vary depending on the species of metal as well as the compounds with which metals may be associated. Metals can be imported into cells through their natural transport processes or by molecular and ionic mimicry through competition for binding sites within carrier proteins for other ions or compounds, such as Ca(II) channels [9]. Negative correlations between intracellular concentrations of metals have shown that absorption of different metals can occur through the same process. For example, the red alga, *Cyanidium caldarium*, has been shown to be extremely tolerant when grown in media with Al(III) concentrations ranging from 50 to 100 mM [31]. Even though it can grow in such high concentrations of aluminum, internal concentrations remain relatively low, thus suggesting that there is selective exclusion of aluminum. However, at higher temperatures the ratio of absorption between Fe and Al shifts towards the latter, suggesting a loss of metal specificity in Fe transporters.

Table 3.1 presents a list of transport processes for heavy metals other than in mammalian species.

Heavy metals can also bind extracellularly with low molecular weight thiols such as glutathione and, as a consequence, can be transported across the membrane through the thiol transporters [44, 45]. Silver has been shown to be transported readily into the algae, *Chlamydomonas reinhardtii* and *Pseudokirchneriella subcapitata*, when it is bound to thiosulfate [46], apparently crossing the plasma membrane intact [38]. Metals bound with thiols are more stable and less likely to cause oxidative damage, although high levels can still be deleterious.

Heavy metals can also bind with metallothioneins, including phytochelatins, followed by entry into cells through endocytosis. In a reverse process, these chelators can become a means of metal excretion because they release metals into the exterior medium by dissociation. In addition, to prevent the build up of heavy metals within cells, active metal efflux occurs through metal specific ATPase pumps [47].

3.3 Uptake and Assimilation of Sulfate

Sulfur is an essential component of the amino acids, cysteine and methionine. It is taken up from the environment by organisms in the form of inorganic sulfate that is absorbed by active transport systems – bacteria [39], algae [48], yeast [49], and higher plants [50, 51]. After uptake by the cell, sulfate is transferred through the cytoplasm into organelles and, in plant cells, excesses become stored in vacuoles.

Table 3.1 Toxic metal mimicry in membrane transport

Metal	Toxic mimicry	Transporters	Species	
Fe(III)	Al(III)	Fe Transporters	*Cyanidium caldarium* R-11	[31]
Fe(II)	Cu(II),Zn(II), Mn(II),Co(II)	Fet4p	*Escherichia coli*	[32]
			Saccharomyces cerevisiae	[33]
Fe(II), Ca(II)	Pb(II)	DMT1 (Fe transporter)	*S. cerevisiae*	[20]
			S. cerevisiae	
PO_4^{3}	AsO_4^{3}	Pho84 and Pho87 Pi Transporter	*S. cerevisiae*	[34]
			Holcus lanatus	[35]
SO_4^{2-}	SeO_4^{2-}	Sulfate permease	*Selenastrum capricornutum*	[36]
	AgS_2O_3	ABC Transporter	*Thalassiosira pseudonana*	[37]
	Cr(IV)	SulP	*Chlamydomonas reinhardtii*	[38]
		SulT (ABC)	*E. coli*	[39]
Hg(II)	Hg(II)	MerT and MerP	*E. coli*	[40]
Cu(I)	Ag(I)	Type1- P-type ATPases (monovalent)	*Arabidopsis thaliana*	[41] [42]
Ca(II),Cu(II) Zn(II),	Co(II), Cd(II), Pb(II)	Type 1-P-type ATPases (Divalent)	*A. thaliana*	[41] [42]
Zn(II) Cu(II)	W(II)	ABC Transporters; TupA, TupB	*Eubacterium acidaminophilum*	[43]
X(GS)[a]	$Hg(GS)_2$ Cd(GS)	Ycf1p	*S. cerevisiae*	[44] [45]

[a]Glutathione conjugate.

The regulation of the sulfate assimilation pathway has been identified to be associated with three genes in the green alga *Chlamydomonas reinhardtii*: *sac1*, *sac2*, and *sac3* [52]. The *Sac1* gene encodes an integral protein that has conserved homology with a dicarboxylate transporter [53]. Sac1 may regulate the sulfur concentration within the cell, and be involved in activating the sulfate assimilation pathways.

For sulfate reduction to occur in algae and cyanobacteria, it is first converted to adenylylsulfate by ATP sulfurylase. APS is then reduced further by APS reductase to produce sulfite. Sulfite reductase acts on free sulfide to incorporate it into cysteine [54] (Fig. 3.1).

Cysteine serves as a cellular pool for reduced sulfur within cells [54] to be employed in the formation of thiol containing compounds such as glutathione and, along with methionine, in protein synthesis. A group of proteins and peptides that contain high thiol contents from cysteine are the metallothioneins involved in metal binding.

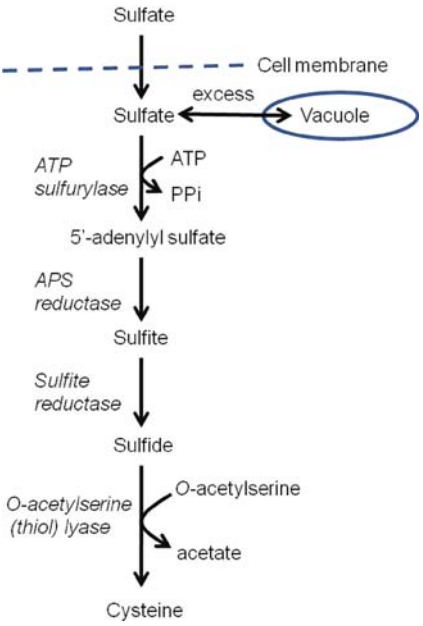

Fig. 3.1 Flow diagram of sulfate reduction pathway in phytoplankton. Adapted from [54]

3.4 Metallothioneins

Metallothioneins are peptides and relatively small proteins containing cysteine residues that bind to metals such as zinc, copper and cadmium. They may also be intimately involved in metal sulfide production [55, 56].

When eukaryotic algal species are exposed to heavy metals, an induced stress response is activated causing the synthesis of class II metallothioneins (MtII) (identified in cyanobacteria, algae and higher plants) and class III metallothioneins (MtIII) (found in most algal species, higher plants and fungi). The latter are also known as phytochelatins [57]. The classes of these metal binders differ in their positions and numbers of cysteines. Class I, the archetypal mammalian protein group, and class II are encoded by their respective genes whereas class III, the phytochelatins, are enzymatically synthesized.

3.4.1 Class II Metallothioneins

The processes governing how and when class II metallothioneins are employed by organisms are not entirely clear. Their regulation may be linked to the age of the organism, enzyme sensitivities to specific heavy metals, and the essential or non-essential nature of the heavy metals, themselves [58]. Interestingly, MtII tends to preferentially bind with zinc ions, and thus the types of metallothioneins appear to have metal specificity [59].

The cyanobacterium, *Synechococcus* PCC 7942, synthesizes a 56 amino acid cysteine-rich protein, SmtA, that is strongly activated during exposure to cadmium, copper, and zinc [59]. Similar proteins have also been identified in the algae *Chlorella* and *Euglena* [60]. The transcription of *smtA* tends to be maximally induced by the presence of zinc in *Synechococcus* with copper and cadmium exhibiting lower inductive capabilities [59]. Mutants that lack functional *smtA* have a several fold decrease in their zinc tolerance [61]. Other prokaryotic species [62] as well as algae [60] also possess similar metallothioneins.

3.4.2 Class III Metallothioneins

Some species and ecotypes of algae have adapted to live in the presence of toxic metal concentrations that are normally lethal. Perales-Vela and colleagues [57] listed ten divisions and 24 genera of algae that possess metal-MtIII complexes as their main contingency for heavy metal stabilization. MtIII production appears to play an major role in the adaptive ability of these species to cope with the heavy metals. Gekeler et al. [60] first determined that phytochelatin (MtIII) synthesis is ubiquitous to algae and preferentially induced by high concentrations of Cd(II) and Cu(II). These metallothioneins, also known as phytochelatins, are enzymatically synthesized and composed of short chain polypeptides rich in cysteine. The most potent activator of their production is Cd(II), followed by Pb(II), Zn(II), and Cu(II) [57]. The metalloids, As and Se, may act as weak activators. Steffens et al. [26] determined that the promotion of a class III metallothionein synthesis seems only to be linked with heavy metal and metalloid presence.

The gamma bond present between glutamate and cysteine in phytochelatins cannot be formed during protein translation. Instead, the bond is made by phytochelatin synthase, an amylcysteine dipeptidyl transpeptidase [63, 64]. This enzyme has the general mechanism of $[\gamma\text{Glu-Cys}]_n\text{-Gly} \rightarrow [\gamma\text{Glu-Cys}]_{n+1}\text{-Gly+Gly}$ 136 [60, 65]. Respective genes have been isolated from *Schizosaccharomyces pombe* [66], *Arabidopsis thaliana* [67], and *Triticum aestivum* [68]. Thus far, however, regulatory mechanisms governing the induction of phytochelatins remain unclear. Mutants of *Arabidopsis thaliana* that are unable to make phytochelatins show increased sensitivity to Cd(II) [69]. Therefore, MtIII peptides play a particularly important role in stabilizing intracellular heavy metals.

3.4.3 Labile and Non-labile Phases of Metals

Metals such as Cd(II), Pb(II), Zn(II), Cu(II) and Co(II) within cells form labile and non-labile phases [70]. Cadmium may be present in both phases within algal species. Labile Cd(II), bound to phytochelatins, is capable of being mobilized and exported. Non-labile phase metals that are bound to cytoplasmic proteins and membranes are relatively unavailable for export. Lee [70] observed in the marine diatom,

Thalassiosira weissflogii, that efflux of phytochelatins resulted in a physiological removal of Cd(II) from the cells.

Cytosolic fractions taken from species of cyanobacterial *Nostoc* after exposure to Cd and Zn can contain up to 30% of the metals bound to proteins [71]. Their findings correspond with those of Bierkens et al. [72] and Torres et al. [73] in algae of metallothioneins binding to Cd and converting it to a less harmful form. Furthermore, Class III metallothioneins can exist as low and high molecular weight variants. In low molecular weight forms the metal is bound to thiol groups, whereas in the high molecular weight forms, additional inorganic sulfur is incorporated into the MtIII complexes. This sulfur forms particles that are in the range of nanometres in diameter [56]. The presence of the inorganic sulfur appears to stabilize the MtIII complex and improve detoxifying capabilities, though their origin is not known. Vande Weigh and Ow [74] have proposed that in *Schizosaccharomyces pombe* the supply of inorganic sulfur is controlled by a sulfide oxidoreductase that maintains a sulfide to metal equilibrium. This is of particular interest with respect to the recent findings concerning mercury sulfide production in cyanobacteria and algae [75].

3.4.4 Sequestration and Compartmentalization of Phytochelatins

Metal-MtIII complexes can be sequestration into vacuoles has been observed in the microalga *Dunaliella bioculata* [76] and *Schizosaccharomyces pombe* [77]. In the green alga, *Tetraselmis suecica*, heavy metals have also been shown to accumulate in the cell wall and within organelles, with precipitation of Cd(II), Ca(II) and S(II) being detected in the vacuole [78]. This can also happen in other species exposed to Cd (II), Cu (II), Hg (II) and Cr (II) [61, 71, 79, 80]). Metals can accumulate in the mitochondria and chloroplasts of species devoid of vacuoles [81, 82].

To add to the complexity of metallothionein partitioning into organelles, there are three possibilities for the formation of MtIII compounds present in chloroplasts and mitochondria. Firstly, the MtIIIs may be synthesized in the cytosol where they bind to heavy metals. These are then transported into the mitochondria and chloroplasts. Secondly, MtIIIs may be synthesized inside the chloroplasts and mitochondria, where they then sequester metals, forming heavy molecular weight complexes with inorganic sulfur. Thirdly, both of these pathways may co-exist and MtIII may be synthesized in each of the three cellular compartments. Interestingly, cDNAs encoding MtIIIs have been identified in *Chlamydomonas reinhardtii* where 60% of Cd(II) was found within the chloroplast, however it is worth noting that the chloroplasts of this species are large structures that compose most of the cell [82].

3.4.5 Cellular Exportation of Phytochelatins

Phytochelatin-metal complexes can be exported from the cell via exocytosis. Once exported, however, these complexes do not appear to remain stable as studies have shown that Cd(II) and Pb(II) disassociate to their free ionic forms in the media [70, 83]. Although the process of stabilization of heavy metals by MtIIIs is adequate

when these complexes are stored intracellularly, MtIII metal complex formation may not be ideal for bioremediation purposes. This is mainly owing to the fact that MtIII metal complexes can be exported and dissociated when cytosolic heavy metal concentrations become elevated [70, 83]. Regrettably, under these circumstances the freed ionic metals return to bioavailable forms.

3.5 Toxicity of Heavy Metals

Metals in their elemental ionic forms elicit quite variable effects on cells. Each metal has been shown to have its means of entry, however how toxic stress is caused remains largely unclear. Heavy metals have been demonstrated to cause membrane depolarization and acidification of the cytoplasm, disrupting homeostasis [84]. Depolarization alters ion gradients required for the proper function of within cellular compartments including organelles. In addition, heavy metals promote oxidative stress by causing an increase in the concentration of reactive oxygen species [85] and suppressing cellular antioxidation mechanisms [86]. This is the case for Fe(II) and Cu(II) in sunflower where these metal ions decrease the activities of antioxidizing enzymes [87]. It is evident that a paradox exists with respect to cellular maintenance of metal concentrations because high levels of even essential metal ions can cause widespread oxidative damage. The paradox arises from these metals also playing crucial roles in enzymes that are responsible for the removal of reactive oxygen species, including Cu-, Zn-, and Fe-superoxide dismutases [47]. Elevated levels of these three metals actually induce oxidative stress. Although antioxidant production has been thoroughly studied in higher plants [88], the study of antioxidant synthesis at the molecular level has not been investigated in photosynthetic microbes to the same extent [89, 90].

Several pieces of evidence suggest that the presence of heavy metal ions of Cd, Hg, Pb, and Cu in high concentrations disrupts mitochondrial and chloroplast function [91]. The intense electron fluxes within these cellular compartments of algae elevate oxygen and metal ion concentrations, putting these organelles at particular risk of oxidative damage [47]. Cadmium has been extracted from chloroplasts and mitochondria in relatively high concentrations by comparison to that in other cellular organelles [29]. Oxidative damage can severely impede algal photosynthetic and metabolic activity as well as affect the overall electrical gradient of the cell. Furthermore, an abundance of light can cause an increase in the amount of reactive oxygen species within cells by causing a proportional increase in the number of excited molecules, such as triplet state chlorophyll and singlet state oxygen with the latter being able to strongly oxidize other molecules [47].

3.6 Genetic Transformation Studies

The first gene transfer study in plants used cauliflower mosaic virus as the vector for a mammalian MtI gene in turnip leaves that became resistant to elevated Cd(II) [92]. In another study using photoautotrophic organisms, *Anabaena* PCC 7120,

Synechocystis PCC 6803, and *Synechococcus* PCC 7942 were transformed with a MtI cDNA gene under the regulation of a metal inducible promoter [93]. These transgenic cyanobacteria were tolerant to up to 6 times the Cd(II) concentration of the wildtypes.

A phytochelatin synthase-like protein has been detected in the cyanobacterium *Nostoc* PCC 7120 and, when its gene was expressed in *E. coli,* it yielded a protein with MtIII homology [94]. Furthermore, NCBI-BLAST search enquiries revealed that multiple MtIII synthase genes exist in this species. Despite this, cyanobacteria do not actually appear to have their MtIII production enhanced through exposure to metals. The fact that cyanobacteria possess these phytochelatin synthase genes supports the notion that they may be constitutively produced at effective metal detoxifying levels. Tsuji and colleagues [94] suggested that other organisms subsequently evolved regulatory processes for activating these genes in the presence of excess metals.

3.7 Metal Sulfide Biotransformation

Certain photosynthetic microbes [24, 95] and anaerobic bacteria [2] share the ability to biotransform metal ions into sulfides, apparently through a common ability to reduce sulfate into sulfide.

3.7.1 Anaerobic Metal-Sulfide Production

Sulfate reducing bacteria possess the ability to form hydrogen sulfide in the anoxic zone of wetlands that, in turn, can act to precipitate metal ions into insoluble metal sulfides [2]. This heavy metal binding process has already been incorporated into bioremediation efforts using up-flow anaerobic packed bed reactors and other industrial decontamination procedures [96, 97]. These anaerobic bacteria form sulfides with Fe(III), U(VI), Cr(VI), Te(VII), Mo(VI) and Pd(II) [98]. Interestingly, conversion into insoluble metal sulfides can inhibit further sulfur metabolism by sterically preventing sulfate and organic compounds from coming into contact with relevant enzymes [99].

One drawback to the use of these bacteria in bioremediation lies in the conundrum that even low levels of free metals, such as Cd(II), Zn(II) or Ni(II) in concentrations as low as 20 μM, can be toxic [100]. Furthermore, these organisms require anoxic environments in order to function properly and maintaining these conditions in an open system can be problematic.

3.7.2 Aerobic Metal-Sulfide Biotransformation

Aerobic metal-sulfide biotransformation has been studied for mercury in algae, cyanobacteria, and fungi [24, 101]. The spread of mercury has resulted from industrial processes acting as point sources, the volatile mature of Hg(0), and the fact that rainfall precipitation is a driving force in mercury's mobility. Therefore,

mercury eventually accumulates in watershed ecosystems where organisms must cope with it. The widespread assumption that Hg(II), as determined in animals and higher plants, is bound to thiol chelates such as the metallothioneins discussed previously, does not appear to be universal. Kelly and colleagues [75] discovered that the main mercury compound in several algae, cyanobacteria and fungi was mercury sulfide. It was concluded that these organisms can convert mercury into meta-cinnabar and Hg(0) [101]. However, the pathway for the former conversion remains to be elucidated [75] and at lower exposure rates the production of Hg(0) became negligible. The cyanobacterial species *Limnothrix planctonica*, *Synechococcus leopoliensis* and *Phormidium limnetica* biotransformed Hg(II) under pH stable and aerated conditions to meta-cinnabar as well as a relatively small amount of the volatile Hg(0) [24]. Furthermore, these species did not produce methyl-mercury under these conditions.

Biotransformation studies with several fresh water eukaryotic algae revealed similar results demonstrating the synthesis of mercury sulfide. *Selenastrum minutum*, *Chlorella fusca* var. *fusca*, *Galdieria sulphuraria* and *Navicula pellicosa* were all tested for their ability to biotransform mercury provided as $HgCl_2$ [75]. All of the cultures were capable of biotransformation of the mercury into meta-cinnabar, however the rates at which the transformations occurred was dramatically different among the species. For *S. minutum*, *C. fusca* var. *fusca*, and the diatom *N. pelluclosa,* all of the mercury within the cultures was biotransformed in a period of hours, whereas *G. sulphuraria* completed the transformation within a matter of minutes [95].

When *G. sulphuraria* was exposed to 100 ppb Hg(II) it transformed 90% into β-HgS within 20 min [95]. This species is the only eukaryotic algae tested thus far that can convert Hg(II) at such a rapid rate and this may be a testament to the conditions to which the species is adapted, including volcanic and acidic areas throughout the world [102, 103]. Volcanic activity can be associated with the release of high amounts of mercury [102], and extremophiles such as *G. sulphuraria* would thus be required to exhibit high Hg(II) biotransformation rates in order to survive.

The aerobic production of metal sulfides occurs in two apparent phases after metal exposure [75]. When first exposed to the metal ions, there is a rapid phase of metal sulfide formation. This rapid phase has been proposed to be dependent on a readily available endogenous pool of sulfur present within the cell for direct sulfide production [95]. Following this rapid phase, the production of metal sulfides slows considerably and may be proportional to the rate by which such a pool is synthesized by the organism. It is interesting to speculate that the sulfur in the metal sulfide may be that which has been shown to be associated with and possibly derived from the high molecular weight form of phytochelatin [56].

3.8 Metal Bioremediation

The cost for conventional remediation of metal-contaminated environments is high, especially when dealing with removal of low concentrations in order to satisfy regulatory requirements. The advantage of using biological organisms to treat

metal contamination is that these organisms employ metabolic processes to create detoxifying chemical intermediates from simple nutrients. This can greatly reduce cost while maintaining and even enhancing the rate of remediation over that of physicochemical processing [104]. Summaries of the few metal bioremediation systems that exist are provided below. These provide a basis of comparison with the anaerobic metal-sulfide bioremediation technology proposed at the end of this section.

3.8.1 Packed-Bed Bioreactor

Wagner-Dobler [105] developed a packed-bed bioreactor in which elemental mercury produced by bacterial mercury reductase enzymes collects outside of the bacterial cells. Mercury is a liquid at 22°C and virtually insoluble in water. These researchers prevented its contact with air to remove any potential volatilization of Hg(0) so that droplets of mercury form [106]. Their packed-bed bioreactor accumulates mercury from waste water as it is converted into Hg(0) inside the reactor [105–107]. A biofilm of mercury reducing bacteria formed on the packed bed composed of inert carrier material (e.g., siran or pumice granules). Wastewater amended with nutrients to feed the bacteria was passed through the bed in an up-flow mode such that there was a hydraulic retention time of 15–60 min. This bioreactor was effective on both synthetic mercury chloride solutions and chlor-alkali cell waste water [106, 108, 109]. Recovery values were between 93 and 100% [105].

The major drawbacks of this system is that the treatment rate is severely limited by (1) the limited flow rates needed to prevent volatilization of Hg(0), (2) by effluent mercury concentration from the source, and (3) the mercury collected must be removed by distillation, a dangerous and expensive process.

3.8.2 Other Metal Bioremediation Systems

At present very few other bioreactor systems have been developed beyond the experimental stage for heavy metal bioremediation. These include (1) the Homestake Mine (Lead, South Dakota) in which zinc and copper are adsorbed to microbial biomass, (2) the sulfur reducing bacterial systems such as Thipaq at the Budelco zinc refinery in the Netherlands, (3) Metex anaerobic sludge reactor in Linde, Germany, and (4) the Bio-Substrat anaerobic micro-carrier reactor, also in Germany [105].

Absorption to microbial biomass has been implemented by the Homestake Mine (Lead, South Dakota) for the removal of zinc and copper from mining runoff. This process exploits the ability of biomass to absorb metals [110]; i.e. inactivated biomass acts as a matrix to which ionic metals adhere. After the matrix has captured its capacity of metals, these can then be washed off by changing the liquid conditions such as lowering of the pH to remove the metal ions. The process can

be repeated a number of times. Although relatively effective, this process is not as efficient at metal removal as a bio-precipitation process.

Sulfur reducing bacterial systems include the use of Thipaq at the Budelco zinc refinery in the Netherlands, the Metex anaerobic sludge reactor in Linde, Germany, and the Bio-Substrat anaerobic micro-carrier reactor, also in Germany. All of these processes share the requirement for anaerobic conditions needed by sulfate reducing bacteria to produce insoluble metal sulfides [97, 111, 112]. This biotransformation into precipitates detoxifies the metals by making them biologically unavailable while at the same instance removing them from solution onto bacterial support matrices. These systems all require anoxic environments thus complicating bioreactor design.

Two bio-techniques have been implemented in the field for soils and wetland sediment bioremediation. In one system, a combination of leaching sediments with acid followed by bacterial conversion has been used by Nakamura and colleagues [113]. This was achieved by employing an indigenous Minamata Bay bacterial strain, *Pseudoalteromonas haloplactis* [114]. In the other system, Daly and his group combined *mer* activity with thiol-containing metallothionein production in bacteria to form Hg(II) that binds to metallothionein proteins in subsurface treatments [115, 116]. These techniques have the disadvantages of either releasing substantial amounts of Hg(0) into the atmosphere or of not being developed for effluent treatment purposes.

3.8.3 Potential Aerobic Metal-Sulfide Bioremediation

Bioremediation processes using aerobic precipitation of metals as sulfides may not have the limitations of the systems discussed above. It should be pointed out that metal sulfide biotransformation is quite a distinct process from that of metal ion-exchange mechanisms documented in some algae [25, 117] and cyanobacteria [118–120]. Potential algal and cyanobacterial bioremediation systems using metal sulfide production do not require anoxic environments, thereby greatly facilitating bioreactor design. In addition, the phototrophic capabilities of these organisms provide the advantage of using light as their energy source and they possess enhanced aerobic metabolisms over anaerobic bacteria.

Mercury sulfide synthesis occurs in cyanobacteria such as *Limnothrix planctonica* [24], and is particularly efficient in the red alga *Galdieria sulphuraria* [75, 95]. It is not known if other photosynthetic organisms that effectively biosorb metal ions such as the cyanobacterium spirulina [121, 122] also form metal sulfides. *Galdieria sulphuraria* can rapidly biotransform over 90% of Hg(II) into β-HgS ($K_{1/2} \approx 20$ min). This species' extraordinary metabolic adaptability makes it a key prospective organism to develop for bioremediation purposes as it can adjust to extreme conditions and it is very tolerant to toxic metal exposure [123]. This may have particular importance because many contaminated sites contain more than one metal source and thus the ability for the bioremediating species to be tolerant to a

wide variety of metal stressors is vital. Studies in this species with metals other than mercury are needed.

The source of sulfides in organisms such as cyanobacteria and algae could be directly or indirectly from an organic pool. For example, MT IIIs are known to be associated with nanocrystalline sulfide particles in which metal ions are bound [55, 56, 124, 125]. Apparently, these nanoparticles stabilize the MT III complexes and, as a consequence, improve resistance to metals. It remains to be seen if aerobic metal sulfide production is mainly through this latter mechanism. The pathway has not been determined for the source of sulfur used in metal sulfide biotransformation even though thiol containing compounds have been implicated [24]. Kelly et al. [75] showed that the synthesis of mercury sulfide followed two distinct phases. The initial efficiency of the biotransformation process appears to be reliant on the size of the initial phase and the effectiveness of longer term metal bioremediation should be proportionate to the biotransformation rate associated with the slower second phase. These are suspected to be reflected in the initial size and rate of replenishment of a pool of readily available sulfur from compounds such as cysteine, glutathione or metallothioneins.

3.9 Future Considerations

Aerobic microbiological processes offer attractive alternatives for the clean-up of metal-containing waste water produced by mining, gas-scrubbing, soil-washing, and processing of fossil fuels as well as from municipal waste. However, aerobic photosynthetic biotransformation has yet to be utilized as a method for bioremediation of heavy metal contaminated sites. Further investigations are required to advance the work spearheaded by Kelly and colleagues [24, 75]. In order to effectively implement this biotechnology the efficiency of the process must be determined with respect to a range of contaminating metal ions of varying concentration. Careful consideration must also be given to choosing which organisms are best suited for bioremediation of the different heavy metals and their combinations. These studies would lay the groundwork for further research and development of bioreactors designed for large scale industrial applications.

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Chapter 4
Noise: The Invisible Pollutant that Cannot Be Ignored

Arline L. Bronzaft and Louis Hagler

Abstract Noise is arguably the most widespread and least controlled environmental pollutant. Noise has been recognized since the time of the Romans as unwanted and intrusive. It was the Industrial Revolution and the rise of cities that greatly accelerated noise pollution to current levels, which continue to increase. The main sources that underlie noise continue to be population growth and urbanization; technology has added to the din. Among its many adverse effects, noise damages hearing, disturbs communication, disrupts sleep, impairs cardiovascular function, interferes with teaching and learning, reduces productivity, harms relationships, provokes unwanted behaviors, and increases accidents. It is a significant source of recurring and often unrecognized stress, which, itself, degrades both health and the quality of life.

Controlling noise will require efforts at several levels. First, government must act responsibly to protect human health and well-being. This means enacting rational noise control laws, seeing they are implemented, and enforcing them as necessary. Education of lawmakers and the public will be an ongoing part of this effort. Business must recognize its role in generating noise and must be part of the solution in noise reduction. Technology will have to play a role in designing and manufacturing all sorts of machines and devices that produce noise levels that do not adversely affect health. The public must play a part by recognizing the hazards of noise pollution, by being unwilling to tolerate it, and by demanding legislative action and enforcement in their federal, state, and municipal governments. Each of us will have to cease being sources of unnecessary noise. As a society, at all levels, we must turn down the volume.

Keywords Noise and health · Noise and quality of life · Noise mitigation · Noise legislation · Noise and children

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4.1 Introduction

In identifying the many sources of noise, Annette Zaner [1] noted that although noise has intruded on people for thousands of years, it was the Industrial Revolution and the rise of cities that accelerated the growth of noise pollution. She went on to say that noise producing and noise-related technology were advancing so rapidly that it was difficult to catalogue noise sources. One might expect that continued advances in technology would make noise even more ubiquitous; this has proven to be the case. Apart from technology, the United States Environmental Protection Agency (EPA) documented that noise levels in communities were directly related to population density. Noise from road, rail, and air traffic, construction sites, household appliances, and from an increasing number of highly mobile, powerful, and varied noise polluting devices has contributed to this growth in noise [2–5]. Thus, population growth, progressive urbanization, and technology all contribute to current noise levels. Some people view noise as a nuisance, accepting this nuisance as the price we pay for living in the modern world. Others, and their numbers are increasing, are concerned about the growing evidence that noise can lead to adverse physiological and psychological effects that degrade both health and well-being. Dr. William H. Stewart, former Surgeon General, stated, "Calling noise a nuisance is like calling smog an inconvenience. Noise must be considered a hazard to the health of people everywhere" [5].

Despite Dr. Stewart's comments, made 30 years ago, health professionals, environmentalists, educators, and government agencies have not moved as assertively as they should have in curbing noise. In turn, legal and administrative actions, educational efforts, and technological advancements to control noise have lagged behind. To encourage the desire in readers of this chapter to engage in activities that will lessen environmental noises, it will be necessary for them to learn about the differences between sound and noise, why people react negatively to noise, and how noise can adversely influence physiological and psychological well-being. Just as technology has contributed to the increase in the noise around us, it is hoped that this chapter will promote technologies that will lower the noise level.

4.2 Defining Sound and Noise

Sound begins as a vibrating object causes the movement of air molecules, setting up alternate bands of compression and expansion in the air that then strike the ear drum. The mechanisms of the middle ear carry the vibrations to the hair cells of the inner ear (the Organ of Corti) where they are converted into electrical impulses that are transmitted to the brain. The brain decodes these transmissions into what we perceive as sound – its nature, pitch, volume, source, and duration. The brain also provides emotional evaluation of the sound. In other words, the listener can determine what the sound represents and whether the sound evokes pleasure or

discomfort. When the sound is judged by the brain to be unwanted, discordant, or disagreeable, then we commonly describe it as noise.

The two major properties of sound waves, independent of whether the sound is deemed wanted or unwanted, are the speed at which the sound vibrates and the intensity of the vibrations. The frequency of vibration defines the pitch of the sound and is measured in hertz (Hz). The human ear responds to sound between 20 and 20,000 Hz but the body may respond to sound below 20 Hz. It is essentially the intensity of each vibration that gives us the sense of loudness. Loudness of sound is measured on a decibel (dB) scale, which is based on the physical measurement of sound pressure. The dB scale is not linear but rather logarithmic (based on powers of ten) so that an increase in 10 dB represents a sound that is perceived as being twice as loud as the preceding sound. The ear is not equally sensitive to sounds at all frequencies. Thus, a special frequency-dependent scale (designated dBA) has been devised to compensate for this variable sensitivity. The dB scale ranges from zero, the lowest point, to over 170 dB. Whispers can be measured at 20 dBA, a quiet home between 30 and 40 dBA, normal conversation at 60 dBA, and some household appliances can range between 60 and 85dBA. New York City subway stations can reach over 90 dBA, rock concerts over 120 dBA, jet take-offs over 140 dBA, and military weapons may exceed 170 dBA.

Noise has been generally been understood to be unwanted, uncontrollable, and unpredictable sound that is interpreted by the listener at the cognitive level as intrusive. A sound need not be loud to be intrusive. The dripping faucet is noise while you are trying to fall asleep. Your upstairs neighbor's footsteps produce noise that awakens you. The backup beeps of the garbage truck below your window in the morning are also intrusive. Certain sounds judged to be intrusive by some may be pleasant to others. For example, the music emanating from a parked or passing vehicle that interrupts your sleep or study may be highly enjoyable to the occupants of the vehicle. Some have thought it difficult to assess the influence of sound on the physiological well-being of humans because individuals differ in their interpretation of – and response to – the sound that surrounds them. To the contrary, for those listeners who define the incoming sounds as noise, one can still measure the adverse effects just as we ascertain the effects of allergens on those who are susceptible.

4.3 Effects of Noise on Hearing

Sound, whether deemed to be wanted or unwanted, can indeed harm the listener. It is generally accepted that a loud sound can damage the inner ear, which, in turn, can lead to a loss of hearing. There is general agreement that exposure to sound less than 70 dBA does not lead to hearing loss. When levels of sound exceed 85 dBA, especially when exposure lasts for more than eight hours, there is the potential of hearing loss [6–11]. However, a single exposure to a very loud sound can impair hearing. It has been said that former President Ronald Reagan lost some of his hearing on a movie set when a loud gun rang out next to his ear.

At one time, exposures to sounds above 85 dBA were limited to occupational settings, but in modern society, sounds that we hear in our everyday environment, e.g., passing traffic, roaring construction drills, loud subway stations, and sirens, among others, are exposing our ears to levels exceeding 85 dBA. Such continuous exposure over many years does affect the aging ear. The League for the Hard of Hearing in New York City collected hearing data over a period of 19 years. The League took measurements of over 27,000 older citizens, ranging in age from 60 to 89 years and found that the percentage of individuals who failed the hearing screening test increased with each passing year. It was hypothesized that the city's increased noise over the years accounted for the accelerated hearing loss in the older citizens [12].

Actually the threat to hearing begins early in life. Over 20 years ago, health professionals began to recognize the hazards of loud sounds in neonatal intensive units. Infants who were exposed to the loud sounds of these units suffered some hearing loss and delayed growth and development [13]. Studies suggest that children are more vulnerable to loud sounds than adults. That is one reason Nancy Nadler of the League for the Hard of Hearing warned parents to listen to toys before purchasing them. She noted that rattles and squeaky toys for very young children and the drums, horns and electric guitars preferred by older children can emit very loud sounds [14]. Brookhouser reported that as many as 80% of elementary school children use personal music players, many for extended periods of time and at potentially dangerously high volumes [15]. This is a practice that parents should strictly discourage. In 2001, it was estimated that 12.5% of American children aged 6–19 years had impaired hearing in one or both ears [16]. In a more recent study, Agrawal, et al. found that hearing loss was growing in the United States, with the rise not just amongst older people, as might be expected, but amongst people between the ages of 20 and 29 years. The authors also found that the risk for hearing loss was greater in people who smoked; were exposed to occupational, leisure-time, or firearm noise; or had hypertension or diabetes [17]. Plakke, in his study of two video arcades, found that certain games reached levels of 111 dBA [18]. Like youngsters in the eighties, young people today still enjoy visiting video arcades and, undoubtedly, are subjecting their ears to high levels of sound. Thus, it shouldn't be surprising that Holgers and Petterson reported that leisure time noise exposure correlated with tinnitus in students aged 13–16 years [19]. Also, young adults responding to a web-based survey reported experiencing tinnitus or impaired hearing after exposure to loud music at concerts or in clubs [20]. The Royal National Institute for the Deaf found that the hearing of nearly 70% of the people who visited nightclubs were adversely affected, with loud music damaging the hearing of nearly 50% of pub goers and up to 90% of young people experiencing symptoms that suggest hearing damage, such as tinnitus or decreased auditory acuity after a night out [21].

People working in clubs, bars and other places of entertainment are also at risk for hearing loss [22]. Nearly a third of students who worked part time (bar staff or security staff) in a university entertainment venue were found to have permanent hearing loss of more than 30 dB [23]. Pete Townsend of the WHO band had often talked of his hearing loss and hearing impairment is often seen in amateur

pop/rock musicians who fail to protect their hearing [24]. We have long recognized that leisure use of firearms, which produce very loud impulsive sounds, can damage the hearing of shooters if the ears are not protected [3]. One can ask if we have we paid sufficient attention to the large number of soldiers and marines in Afghanistan and Iraq who are exposed to roadside bombings and firefights, which come suddenly and unexpectedly, leaving military personnel little or no time to protect their hearing. Hearing loss and tinnitus have become serious problems among U.S. troops. As reported by the National Institutes of Health, nearly 70,000 of our military who have served in two war zones are collecting disability for tinnitus and more than 58,000 are on disability for hearing loss [25].

Hearing loss also interferes with one's ability to communicate and interact socially. Difficulties with hearing and communication can impair job performance, interfere with new job opportunities, and may result in decreased earning power. Sometimes people with hearing deficits refrain from interacting readily with others and, in some cases, hearing loss can lead to a sense of isolation. Even if one's hearing is fine, a noisy environment does not allow one's hearing to function optimally and as a result interferes with conversations and social interaction. As noted by the EPA, "For millions of Americans residing in noisy urban areas, the use of outdoor areas for relaxed conversation is virtually impossible" [5]. A noisy environment may also make it difficult to hear sirens and other warning sounds essential to safety.

4.4 Noise and Annoyance

Annoyance is a common reaction to sound that the hearer judges to be undesirable. Annoyance can be defined as a feeling of displeasure associated with any agent or condition that adversely affects the individual. As early as 1974, Miller viewed annoyance as a common response to noise independent of the loudness of the sound [26]. Earlier, Borsky examined a list of factors that he believed affected the acceptability or rejection of certain sounds. Amongst the factors he listed was the fear of the sound, e.g., believing that the loud jet overhead may crash into your home. Borsky believed that the dislike of the individual responsible for the noise, e.g., your troublesome next-door neighbor, would contribute significantly to one's annoyance [27]. Also, living in a neighborhood that has other problems increases the tendency to be annoyed by community noises. One major factor, stressed by Berglund and Lindvall [3] and Bronzaft, et al. [28], is that the noise intrudes on ongoing activities such as television viewing, conversations, reading, and resting. Furthermore, Borsky [29] and Weinstein [30] reported that individuals did not adapt to the intrusive noise; in other words, over time, the noise continued to annoy them.

As to the sources of noise, Zaner in her review of survey data collected in the 1970's reported that motor vehicles and aircraft were found to be principle sources of noise complaints [1]. That transportation is a major source of noise complaints has been long recognized [3]; this has been recently underscored by the lawsuits

filed against the Federal Aviation Administration (FAA) for revision of its airspace rules that has brought increased aircraft noise to five states in the northeastern part of the United States [31]. Additionally, there are numerous media reports of residents around Heathrow airport who protested the expansion of this airport because of the potential of increased noise to their community. Bronzaft et al. [32] found that transportation generated noises headed the list of complaints by participants who completed a community noise survey. However, it should be pointed out that people in this survey also complained about noise from neighbors, pets, construction sites, power garden equipment, as further evidenced from the results of three on-line surveys of neighborhood noise conducted by Bronzaft and Van Ryzin [33]. Their surveys indicated that annoyance was the number one response to intrusive noises amongst the New Yorkers who responded to the survey as well as the respondents from across the United States.

There has been some doubt cast on the accuracy of data collected from surveys with respect to assessing how annoying noise is. Some say that people who are more sensitive to noise are more likely to respond to noise questionnaires. This criticism can be overcome by providing data on large samples. However, even if we were to accept that there are individuals who are more sensitive to the noises around them, as there are those who are less sensitive, most people would probably fall in the middle of the normal curve and would very likely be annoyed by certain noises. Kryter, well-recognized for his books on noise, at first questioned the reliability of attitude surveys of noise but finally concluded that the reliability was greater than he had originally thought. After examining a number of studies, Kryter stated, "Thus, although noise annoyance can perhaps be a burden that creates problems for highly sensitive people, it appears that it creates the most annoyance for persons engaging in normal behavior; that is, it occurs most often in normal people [6].

Annoyance to intrusive noise very often involves communities, e.g., noise from a nearby airport, from a new construction, from urban mass transit systems, or from passing boom cars with community members complaining that noise interferes with use of back yards and terraces, prevents them from keeping their windows open, interferes with concentration, and disturbs sleep. Berglund and Lindvall [3], after reviewing many surveys dealing with effects of noise on annoyance, have concluded that annoyance in response to noise is prevalent amongst a majority of residents and that annoyance is probably the most common response to a noisy environment. They go on to say that noise complaints are commonly referred to government agencies. In fact, in fiscal '05 and '06 over 350,000 noise complaints came into New York City's 311 complaint line (personal communication to Bronzaft from 311 staff).

Annoyance does not begin to cover the wide range of negative reactions associated with noise pollution which includes anger, disappointment, dissatisfaction, withdrawal, agitation, exhaustion, and helplessness [3, 33]. If the noise continues unabated, and the sufferer gives up hope of relief, then the individual may experience what psychologists call "learned helplessness." Learned helplessness is a psychological response to a lack of control over the noise. The individual gives up trying to remedy the situation and stops complaining but inwardly feels disgusted, unhappy, and angry. Thus, the reaction goes beyond annoyance, eliciting a

variety of emotional responses. Learned helplessness may also explain why too few of them complain despite the growing numbers of community residents who are disturbed by noise [6]. Without a fuller understanding of the psychological factors to predict annoyance across communities, Staples [34] believes that government agencies, e.g., FAA, are unable to set standards to protect community groups affected by surrounding noises.

Low frequency noise, though not necessarily loud, e.g., the music coming from a downstairs bar, a passing boom car, an entertainment center; nearby ventilation and air conditioning units; industrial machinery, or vehicles whose mufflers no longer muffle, are especially annoying to people living in urban environments [3, 35]. These lower frequency sounds, below 200 Hz, are often accompanied by vibrations. Individuals begin to hear their doors or windows rattle or feel some movement in their beds. Annoyance to low frequency noises are exacerbated by these accompanying vibrations [36]. Leventhal [35] noted that regulatory agencies generally measure noise levels on the A weighted dB scale, which is not sensitive to low frequency sounds; he concludes that the contribution of low frequency noise is ignored because of the way sound is measured.

4.5 Effects of Noise on Physical Health and Well-Being

The human ear responds directly to sound and sound that is too loud, as discussed earlier, can damage the ear resulting in a loss of hearing. However, unwanted sound can affect other organs and systems in the body in an indirect way. Sound, which is not necessarily loud, e.g., footsteps of upstairs neighbor or a dripping faucet, may still be deemed bothersome because it is unwanted sound. The body responds to unwanted, uncontrollable, and in some cases unpredictable noise through a complex set of physiological changes typically identified as stress or arousal. The various adverse health effects of noise, many of which are stress induced, has recently been reviewed [36]. Stress leads to an outpouring of so-called stress hormones (epinephrine, norepinephrine, and cortisol). These hormones produce an increase in blood pressure, an increase in heart rate, an increase in respiratory rate, and a slowing of digestive activity. A single stressful noise exposure is followed by a return of these hormones to baseline levels. However, with repeated stressful noise exposures, there may not be time for all the various affected systems to return to normal or baseline levels. Thus, repeated exposure to noise can bring about long-lasting physiological changes in the cardiovascular system and other systems. We can safely say that noise, like other forms of stress, can adversely affect physical health.

The Office of Noise Abatement and Control (ONAC) in the EPA recognized noise as a health issue when it published its 1978 document *Noise: A Health Problem* [5]. This document stated that noise may contribute to heart and circulatory disease even though more studies were called for to validate the link between noise and heart and circulatory ailments. The document concluded that there was enough information

to warn people about the health hazards of noise as more research is undertaken to identify the specific links. It should also be pointed out that the Noise Control Act of 1972 had assigned responsibility of curtailing noise to ONAC and this document was published in keeping with the mandate of the Noise Control Act, which was to protect Americans from the harmful effects of noise.

Subsequently, additional studies on the health effects of noise were conducted, largely in communities disturbed by noises from nearby airports, railroads and highways, and they did indeed find a relationship between noise exposure and subsequent cardiovascular disease [3, 8, 9]. Several years ago, Babisch [37] reviewed epidemiologic studies that examined the relationship between environmental noise and cardiovascular risks (mean blood pressure, hypertension, and ischemic heart disease) and concluded that the risk of cardiovascular ailments had increased since his last review. More recently, a panel of European noise researchers [38] met to review the burden of disease from environmental noise and estimated that 3% of all deaths due to ischemic disease across the European Union were attributable to community noise. Lars Jarup and his associates examined exposure to noise near airports and found statistically significant effects on blood pressure of night-time aircraft noise and average 24 h road traffic noise exposure, the latter especially for men. Jarup et al. conclude that: "Hypertension is an important independent risk factor for myocardial infarction and stroke and the increased risk of hypertension in relation to aircraft and road traffic noise near airports demonstrated in our study may therefore contribute to the burden of cardiovascular disease" [39].

Not only are infants in neonatal intensive care units (NICU) at risk for hearing loss, but studies also found that the noise to which infants in NICU were exposed, much of it from staff activities, also triggered undesirable physiological responses such as abrupt fluctuations in blood pressure, heart rate, respiratory rate, and oxygen saturation. Concern over such physiological changes in the infants in NICU resulted in recommendations to minimize the surrounding noises to which they are exposed not only in the NICU but following discharge from the hospital [13]. Noise exposure may also put older children at risk. Children who live in noisy environments have been shown to have elevated blood pressure and elevated levels of stress-induced hormones [8, 40]. Systolic blood pressure was found to be significantly higher in children living in noisy residences when compared to children living in quiet environments [41]. Yet, van Kempen and her associates [42] looking at the results of effects of aircraft and road traffic noise exposure on children's blood pressure and heart rate believed the data were not yet sufficient to prove that noise produced adverse effects on children's blood pressure; thus calling for further research in this area. It might be worthwhile to follow youngsters who were exposed to noisy environments into adulthood to learn whether or not they were indeed prone to higher blood pressure and cardiovascular disorders.

With respect to the effects of noise on the immune system, Passchier-Vermeer and Passchier [43] concluded that the small number of studies on this topic did not permit them to arrive at conclusions about a causal relationship. Similarly, it is difficult to draw conclusions about causal relationships from the occasional report of adverse effects of noise on other physiological systems.

Yet, good health is more than the absence of physical ailments. If we give it a broader definition that includes a decent quality of life, we will recognize that noise has a deleterious effect on health. The World Health Organization does recognize the adverse effects of noise on quality of life. Noise leads to stress in individuals that indeed affects their physical well-being. In a study of airport-related noise, four questions related to noise were embedded in a health-related questionnaire that was distributed to a community living within a flight path (subjects did not know the purpose of the study). A similar questionnaire was distributed to a community in a non-flight area. There were significant differences between the two groups with a higher percentage of those living in the flight path reporting that noise interfered with life activities, e.g., talking on the phone, conversations at home, radio and television, and sleep. Indeed their quality of life was diminished by the airport-related noise. Those bothered by the airport-related noise also reported themselves to be in poorer health, another indication that they were not experiencing a decent quality of life [28].

Although there is still a need to conduct additional studies examining the noise–health link, there appears to be a sufficient and increasing body of evidence to support the warning that noise may be damaging to your health. It should be reiterated that Dr. William H. Stewart, former U.S. Surgeon General had the foresight as early as 1969 to state: "Noise must be considered a hazard to the health of people everywhere" [5].

4.6 Effects of Noise on Children's Language, Cognition and Learning

A large body of research confirms the deleterious effects of noise on children's cognitive development, language development, and learning skills. Language development starts in the home and too many young children are exposed to noises within the home itself as well as intrusive noises from outside rail, road, and air traffic. Wachs and Gruen [44] found that noisy households can impede a child's cognitive and language development, whereas, Bronzaft, in her study, found that high academic achievers, all members of Phi Beta Kappa, when asked about their childhoods, noted quiet times in their homes to read, do homework, and think [45]. Evans and Lepore [40] in their review of effects of noise on children concluded that residential noise delays early cognitive development.

When children attend schools that are near vehicular, rail, or air traffic they are exposed to noises that disrupt the learning process. Bronzaft and McCarthy found that passing elevated trains disrupted teaching time in the classrooms of a nearby school by at least 11%. When the reading scores of 2nd, 4th and 6th graders attending classes near elevated train tracks were compared to children attending classes on the quiet side of the building, the children exposed to train noise did more poorly, with 6th graders being nearly one year behind in reading. In addition to missed teaching time, the children exposed to train noise, when interviewed, said they found

it more difficult to think and to do their work in their classrooms [46]. Evans and Lepore [40] believe that noise exposure interferes with auditory discrimination and attentional mechanisms, making it more difficult for children to learn to read in noisy environments. Two other factors, namely lack of motivation and feelings of helplessness, may also contribute to poorer reading scores in children exposed to noise in their classrooms. A few years later, Bronzaft had the opportunity to revisit the school in which her earlier study was done to examine the effect of two noise abatement procedures, namely, the installation of acoustical tiles in the ceilings of the classrooms adjacent to the tracks and the installation of noise-absorbing materials on the tracks, which reduced noise in the class 6–8 dB. She found that children on both sides of the building were now reading at the same level, indicating that when noise is lessened children's learning improves [47].

In their review of more than 20 studies, the Federal Interagency Committee on Aviation Noise [48] concluded that children's reading, language, and memory skills can be negatively affected by aircraft noise. Similarly in their London study, Haines et al. [49] found chronic aircraft noise exposure was associated with impaired reading comprehension. In their cross-national and cross-sectional study of over 2800 school children attending schools in different countries, Stansfeld et al. [50] concluded that aircraft noise could impair cognitive development in children, especially in reading comprehension. They did not find similar results for road traffic noises, as had been found in other studies, but believed the low noise levels in this study may have been a factor.

The studies linking noise to decrements in children's learning and achievement has resulted in the Acoustical Society of America partnering with the Noise Pollution Clearinghouse (www.nonoise.org) to create a web page to inform parents, teachers, and school administrators about the importance of quieter school learning environments. Information on the National Standard for Classroom Acoustics is included on this web page as well as strategies for achieving good classroom acoustics.

4.7 Noise and Sleep

Noise can disrupt sleep, leading to increased awakenings during the night. Significant numbers of residents living with the roar of overnight jets have reported that noise disrupts their sleep [28, 51]. Passchier-Vermeer and Passchier [43], after reviewing a number of epidemiological studies on night time noise disturbances, report that noise does indeed increase awakenings. With a good night's sleep being a prerequisite for normal physiological and psychological functioning, one can conclude that such awakenings may lead to health problems. In addition, noise-induced sleep loss may impair job performance the next day as well as make one less receptive to cues of danger [3, 43, 52].

However, noise can adversely affect health even if it does not awaken the individual. With references to a body of evidence that includes both field and laboratory

studies, Berglund and Lindvall [3] and Maschke and Hecht [53] note that noise exposure during sleep can increase blood pressure, increase heart rate, change respiratory rate, and bring about changes in circadian rhythms. Such changes may have long-term health implications. Several additional studies [54, 55] note that night time noise elicits stress hormones that may have a deleterious effect on health as well.

One recent investigation measured blood pressure and collected health data using a questionnaire during home visits for nearly 5,000 individuals who had lived at least 5 years near one of six major airports [39]. The authors found statistically significant effects on blood pressure with exposure to night time aircraft noise and average 24 h road traffic noise. Since hypertension is a risk factor for myocardial infarction and stroke, the authors of this study believe residents near airports may be at risk for cardiovascular disease. Ohrstrom et al. [56],in a field study, looked at the effects of road traffic noise on sleep on children and adults and found a significant relationship between noise levels from road traffic and sleep quality and awakenings for parents. Sleep quality and problems with sleepiness during the daytime were found for children exposed to noise exceeding 55 dBA.

The growing number of studies indicating that noise impairs a good night's sleep should serve as a warning; night time noise interferes with body's ability to restore itself mentally and physically.

4.8 Mental and Social Effects of Noise

That noise annoys, bothers, and disturbs people attests to its adverse effects on normal mental health and well-being. In the community noise survey cited earlier [32], Bronzaft et al. asked individuals to identify emotional responses to noise. Over 70% stated that noise annoyed them; 43% responded that noise made them angry, and 30% reported that noise upset them (subjects could list more than one emotional response). Hiramatsu et al. also reported that respondents to their survey indicated that noise made them emotionally unstable, depressed, and nervous [51]. The proposed expansion of the Heathrow airport, and the worry about increased noise exposure, has elicited angry responses from nearby citizens and residents. Similarly in the United States, the FAA confronts angry residents when they meet with communities to discuss airport expansions or the redesign of air routes that will bring more noise to nearby communities [57]. The methodology of studies linking noise to increased admissions to mental hospitals has been challenged and thus, we cannot at this time state that noise leads to mental illness. However, the annoyance, anger, and frustration expressed by people complaining about the myriad sources of noise in their lives, as well as the anger of groups fearful of being exposed to increasing levels of noise, does permit us to state that noise adversely affects our mental well-being.

Noise also elicits asocial or aggressive behavior in some people. Stories about fights erupting because of noisy disputes amongst neighbors can be accessed from the following websites: www.boomcars.org, www.nonoise.org, www.noiseoff.org.

Thirty years ago the EPA in its *Noise: A Health Problem* referred to early laboratory and field studies that found that noise heightened social conflicts both at home and at work and that people were less likely to help others in a noisy setting [5]. Well-known American psychologist Stanley Milgram noted city dwellers were so overwhelmed by stimuli as they traversed crowded and noisy streets that they often behaved in ways that appeared to be less helpful and rude. The reason for this asocial response; it wasn't rudeness but the desire to get away from the noise [58].

4.9 Lessening the Noise: Legislation, Technology, and Education

4.9.1 The Role of Legislation in Noise Mitigation

Zaner [1] acknowledged that noise had intruded upon the lives of people for thousands of years [1]. She recognized, as did Karin Bijsterveld that it was the Industrial Revolution and the rise of cities that accelerated the growth of noise pollution. One could say that noise pollution was the price society paid for the advances of modern civilization. Yet, were citizens willing to trade off some peace and quiet for modern technology? Professor Bijsterveld, in her book Mechanical Sound, states that citizens objected to intrusive noises long before industrialization. Bylaws existed that "targeted singing and shouting on Sundays, barking dogs, crying vendors, nightly whistling, street music and making noise in the vicinity of churches, hospitals and other institutions." She cites ordinances that protected workers in noisy occupations as well as bylaws against environmental noise that were passed in England, Antwerp, Bern and Amsterdam long before the twentieth century. People were not willing to accept the noises that accompanied the growth of cities and did not welcome the added noises that came with advanced technology [59].

Cities such as New York, Washington, DC, Chicago, and London published city noise surveys in the 1920s and the anti-noise organizations that arose in the 1930s in Europe and the United States used the data from these surveys to support their requests for anti-noise legislation [59]. Some of their efforts did indeed lead to legislation that attempted to lessen the surrounding din, e.g., banning of motor horns at night, and prescribing a muffler to reduce exhaust noise. However, legislation was no match for the "quantitative increase in traffic" that followed in the years to come. On the other hand, Bijsterveld notes that there were also campaigns in New York and London that coupled noise with excitement and joy [59]. So even seventy years ago, noise and pleasure were linked as they are today by certain groups, e.g., motorcyclists, who couple noise with excitement and freedom.

New York City was a leader in identifying noise sources and passing legislation to deal with these sources as early as the 1930s. However, as the noise increased, stronger legislation to curb noise was introduced when the New York Noise Control Code was passed in 1972. This Code, not only attempted to deal with noise from

motor vehicles, circulation devices, refuse compacting vehicles, commercial music, etc. but it also asked the New York City Department of Environmental Protection to study airport noise, essentially controlled by the federal government, and transit noise, under the purview of the state government. Federal or state control of a particular source of noise does not prevent a city from studying the effects of that noise source on its citizens. Data from such local studies can in turn influence federal or state noise control legislation. In July 2007, New York City updated its lengthy 1972 noise code to better cope with the multitude of noises to which its residents are now exposed daily. However, it is not just New York City that is responding to the demand by citizens for less noise. Across the United States, ordinances have been passed by states, counties, and cities in an attempt to control environmental noise. Some of these ordinances make it illegal for sounds to be audible at certain distances; some link dB restrictions to certain times of the day or night; and some require the use sound meters to ascertain whether disturbing sounds exceed specific decibel limits. Using a different approach, California and Illinois, among others, employ the clearly audible standard in determining illegality of amplified sounds emanating from motor vehicles. For additional information, see the following websites: www.noiseoff.org, www.nonoise.org.

The United States federal government passed the Noise Control Act in 1972 to protect its citizens from noise and ONAC was charged with enforcing this act. ONAC had undertaken efforts to educate citizens to the dangers of noise with its many publications and encouraged states to develop anti-noise programs. ONAC was about to launch efforts to urge manufacturers to quiet their products when former President Ronald Reagan defunded the office [60]. The act that established ONAC has not been rescinded but without funding, ONAC cannot function and efforts to refund ONAC have not been successful. Essentially, the federal government is "... legally responsible for developing and coordinating a national noise policy, reviewing the noise-related policies and regulations of federal agencies, and establishing noise-emission standards and labeling for products distributed in commerce" [61]. However, the federal government has opted to ignore its own policy regarding noise and it has been left to cities and states to limit environmental noise. However, passing legislation, as the American cities and towns have done, is not enough; laws must be enforced.

Thirty years ago, the United States federal government acknowledged that noise could be controlled at its source, which is the most desirable way to eliminate environmental noise. The EPA then claimed that it would "... soon be requiring noise labels on consumer products that will enable you to compare the loudness of appliances before you buy them" [62]. Russell Train, the then EPA administrator, stated: "The aircraft manufacturers can and should make aircraft substantially quieter than they are today" [63]. In the 1970s the United States government believed that technology could be developed to mitigate unwanted sounds at the source. Airplanes, motor vehicles, trains, and appliances could be made quieter but without the pressure and/or encouragement of ONAC, manufacturers proceeded at their own, slower pace. It seems clear that legislation and government involvement are essential elements in driving technology.

In 2002, the European Union adopted a Directive which set out for its member nations a community approach to manage and evaluate ambient noise in order to protect public health [64]. This directive asked the member countries to map noise especially in their large cities, implement action plans to curb noise based on these maps, and provide information to the public on the dangers of noise. The Directive focused on environmental noise and did not deal with noises in the workplace, between neighbors or from military aircraft. A working group from the EU developed an inventory of noise mitigation methods, laying out the broad requirements for a program of noise mitigation at the national level [65].

Legislation or directives will give rise to legal measures that will include standards to control and monitor noise. One example of this is the federal rule that allows the creation of quiet zones at railroad grade crossings. Such measures can be used to create noise maps, identify noise exposure levels in different situations, establish land use and zoning, develop noise codes for construction, and establish enforcement measures. Legislation will be largely abetted by existing technology that can lessen the ambient noise. With legislation providing the "will" to abate noise, technology will find the "way" to mitigate noise.

4.9.2 The Role of Technology in Noise Mitigation

Mitigation of noise can take place in one of three fundamental ways: at the source, along the path of transmission between the source and the person who hears the noise (the receiver), and at the receiver.

4.9.2.1 Noise Mitigation at the Source

Control of noise at the source, it is generally agreed, is the most desirable way to eliminate environmental noise. Controlling noise at the source involves the application of methods and technologies that insulate, absorb, dampen, or isolate the vibrations that produce sound. During the past thirty years, jet noise in the United States has decreased about 20 dB, and this has been due to the great emphasis placed on quieting aircraft engines. However, this past year the rise in fuel prices and the desire to emit less harmful gases in the environment has speeded up the design of quieter aircraft. Pratt and Whitney boasted that their new engine will feature a geared fan that will spin independently of the main turbine which, in turn, will lower fuel consumption and noise [66]. On August 2, 2008, Port Authority of New York and New Jersey officials welcomed the inaugural A380 from Emirates Airlines which boasted better fuel economy and less noise within and outside the cabin; this plane will generate half the noise of other aircraft at takeoff. Boeing has also announced that its new airplanes will emit less harmful gases and make less noise, in addition to consuming less fuel. Thus, the "will" to produce quieter planes was sparked by the high cost of fuel and the fear of global warming and the technology followed.

Sometimes consumers can push for quieter products and, undoubtedly, purchasers of air conditioners who were not pleased that cooler rooms meant adapting

to noisy units demanded quieter units. Anti-noise groups can also force government agencies to seek out quieter products. From the time the New York City transit trains began to operate in the 1880s, citizens complained about the noise of these trains. Citizens became especially vocal about the noise in the late 1970s and learning that new trains were coming into the system they asked the Authority to bring in trains with quieter traction motors. At first, the manufacturers claimed that they could not meet New York's specifications and offered the Authority a noisier traction motor instead. When New York City pointed out that quieter motors had been delivered to other transit properties and that these less noisy motors could be adapted to New York's trains, the manufacturers agreed to exert some effort to provide the quieter motors, provided New York City paid them to develop the "quieter motors." These quieter motors did not cover the first 350 of the 1,100 cars ordered but when they were installed in the remaining cars, they proved to be quieter and longer lasting [67]. It should be noted that sometimes quieting products results in products with longer shelf live to counteract the often heard statement that the problem of noise mitigation is that it carries a high cost.

Looking at train noise further, we note that one of the major sources of train noise results from the interaction of steel wheel and steel rails. This interaction sets up noise producing vibrations in the wheels, rails, track support and ground and within the train structure itself. Flat wheels and rails that are not continuously welded can produce vibrations and increased noise. Wheel truing lessens noise but maintaining the wheel flange contour leads to a longer life for the wheels and rails and places less stress on the car trucks. Welded rail similarly dampens the noise but this too leads to less stress on the rail cars. Adding rubber rail seats between the rail and the track results in a quieter train. It also maintains the integrity of the supporting structure. Investing dollars in quieting train noise actually saves dollars as we note the benefit to the integrity of the rail cars, the rails and the structure as a whole. Added to this is the not insignificant societal benefits that accrue from quieter rail operations.

The noise of train horns at crossings has become a growing environmental problem for the increasing numbers of people who now live near railroads. The minimum levels established by the Federal Railroad Administration still encroach upon nearby residents, leading to many complaints from these people to their public officials. The introduction of wayside horns, which aim sound directly at oncoming vehicular traffic in both directions perpendicular to the path of the train, would produce a smaller footprint and be far less intrusive on neighboring communities. The technology to correct at the problem exists but has not yet been sufficiently utilized in American communities.

The most important sources of noise arising from motor vehicles are the engines, transmissions, exhaust systems, and the interaction of tires with the roadway. Vehicular noise, or rolling noise, results from the interaction of tires with the roadway. Rolling noise can be reduced by altering the material from which the tire is made, its speed of rotation and the type of tread. Additionally, rolling noise can be lessened by improved roadway surfaces and maintaining surfaces which causes less wear and tear on the automobile itself. The desire to use cleaner, less expensive fuels has resulted in quieter sanitation trucks [68] and quieter hybrid automobiles

[69], again underscoring that the "way" to reduce noise can be found if the "will" is there.

Machines and other frequently used tools and products produce noise. Noise from these sources can be mitigated by improved design and proper maintenance. The New York City Department of Environmental Protection lists on its website those machines and types of building equipment that produce less noise (www.nyc.dep) and, as a result, intrude less on the quality of life of those residing near construction sites. Similarly, within the European Union, sound labeling provides consumers with information about the noise emissions of products considered for purchase. According to several different EU directives, most consumer products must display an EU Energy Label when offered for sale. This label contains, among other things, information about the noise emitted by the product [70].

4.9.2.2 Noise Mitigation Along the Path of Transmission

Protecting the individual from the source of noise is another approach to mitigating environmental noise. Architects have had a major role in designing buildings in a way that protect inhabitants from sources of noise from within and outside the structure; such protection is vital as our modern cities grow more compact and dense. Increased transportation (air, rail, road) demands technology to protect people from the noise that has accompanied this growth in travel. Barriers have been erected alongside highways to limit noise of passing cars; homes near airports have installed double-glazed windows and air conditioning to limit noise from external sources; and air traffic and flight operations can be designed to carry less noise to residential communities. According to Owens-Corning, homes should be acoustically attractive as well as visually pleasing. Owens-Corning recognizes that certain rooms in the house may be noisier, e.g., laundry rooms and rooms in which children practice on musical instruments. There is also recognition that certain rooms require quiet, e.g., offices and bedrooms. Owens-Corning has developed a set of products for the home that will control noise originating outside the home as well as noise arising within the home and moving from one room to another [71].

Concert halls, theaters, and movie theaters are venues that require a quiet environment for enjoyment of the performances and so they receive acoustical treatment to keep out external noises. Hospitals and schools are treated acoustically to minimize both external and internal noise; this may be costly, but the adverse effects of noise upon health and well-being may lead to greater health care and educational costs.

4.9.2.3 Noise Mitigation at the Receiver

Environmental noise is an inescapable part of our modern society but judging from the articles reporting on noise in our national parks and quiet areas, it has become increasingly difficult to escape. As people walk down major thoroughfares, ride in subways, travel in noisy jets, or attend rock concerts, they have learned to depend on ear plugs or other personal noise attenuating devices to protect themselves from

the surrounding noise. Adolescents should protect their ears when visiting arcades where certain games reach levels exceeding 110 dBA, sounds comparable to those of loud jack hammers [18].

It is also essential to point out that one way to protect ears is to limit the amount of sound to which they are exposed. Sound, rather than noise, is being used here because the individuals choose to listen to sounds at high levels which can be potentially damaging to the ears. Manufacturers of radios for use in the home or automobile, manufacturers of personal sound systems, and manufacturers of television sets, compact disc players, and other sound producing devices should build in protection that would guarantee that the device does not produce sound at levels that could be harmful to hearing. As an example of this, the Ford Motor Company recently announced a smart key solution. Starting next year, the company will offer smart keys that allow parents to impose limits on their teen drivers. With the smart key in place, amongst other features, is one in which the stereo won't go above half-volume.

There is, of course, an element of personal responsibility in the effort to control personal noise. People can cut down on noise by demanding that manufacturers make noise-producing equipment as quiet as possible and by purchasing the least noisy of all available options. There is little doubt that consumer demand could provide the impetus to design and build quieter hair dryers, vacuum cleaners, leaf blowers, and a host of other time and labor saving devices that are noise-producing.

4.10 Education

Education is a key element of noise abatement programs. Educational efforts alert people to the dangers of noise and to the ways they can protect themselves from these dangers. Publishing the latest research on the dangers of noise as well as the successes of mitigation programs also serves to inform professionals and the public at large about the hazards of noise and technologies that exist to abate it. Such publications in turn initiate new research and additional technology. Noise pollution, as it now stands, does not appear to have the visibility required if we are to move to a quieter, healthier environment. This conclusion is supported by the findings of Vincent and Lambert who cite studies conducted in Spain, under the guidelines of the European Union, in which they found citizens lacked knowledge about sound levels, noise abatement plans, sources of noise and the adverse effects of noise [72]. According to Vincent and Lambert, this lack of information about noise and effects stemmed from the fact that local authorities concentrated on transmitting information through a website rather than television, newspapers and periodic mailings; apparently the decision-makers did not give much thought to ways of educating and informing the public.

The European Union in its 2002 Directive discussed the value of education, information and public awareness in promoting acceptance of – and compliance with – noise regulations. Noise labeling on products is a prerequisite for noise control but

the public's awareness of the dangers of noise must be heightened to encourage the demand for and purchase of quieter products [64]. Before ONAC was essentially "defunded" in the United States in 1982, it was about initiate a program called "Buy Quiet." Although certain products, e.g., refrigerators, air conditioners, have become quieter in the United States, it was the result of public asking for quiet, not the U.S. federal government asking companies to quiet their products.

When ONAC was functioning, it had produced excellent booklets and pamphlets educating people to the dangers of noise. ONAC had produced a *Public Education and Information Manual for Noise* in 1980 that was distributed to neighborhood and community-based organization, local governments, schools and the media. Thirty years ago, the United States had recognized that education was an important tool in promoting a "less noisy" environment but, unfortunately, the noise education arm of the EPA no longer exists. Some of these excellent materials produced by ONAC can be viewed at the website of the Noise Pollution Clearinghouse (http://www.nonoise.org). Today, anti-noise citizen groups have taken on an educational role by building websites packed with information on noise including health effects, existing legislation, and ways to alleviate noise. Such groups include the Noise Pollution Clearinghouse (www.nonoise.org) and Citizens Coalition Against Noise Pollution (www.noiseoff.org) in the United States, the United Kingdom Noise Association (www.ukna.org), and the Right to Quiet Society (www.quiet.org) and Noise Watch (www.noisewatch.netfirm.org) in Canada. These citizen groups reach beyond their websites because they provide information that is frequently quoted in the media.

The European Inventory on Noise Mitigation Methods recognizes the importance of education, information and public awareness [64]. The United States Department of Transportation [4] reported on the general health effects of transportation noise in an attempt to educate the public on the dangers of noise. The EPA is now preparing a paper on Noise and the Adverse Health Effects on Children and possibly this paper will trigger off additional anti-noise activities at the federal level. The Toronto Public Health Department is distributing a brochure entitled *Noise and Children*, focusing on reducing the level of noise in the home and the New York City Department of Environmental Protection plans to distribute a book entitled *Listen to the Raindrops* (author: Arline L. Bronzaft and illustrator: Steven Parton) that teaches young children about the beauty of good sounds and the dangers of noise to all the city schools. This book will be accompanied by a lesson plan on noise and the effects on health. The Council on the Environment of New York City works on environmental issues with high school and college students and its educational curriculum includes lesson plans on noise pollution (www.cency.org).

4.11 Concluding Comments

Noise pollution produces direct and cumulative adverse effects on mental and physical health and degrades residential, social, working and learning environments. Noise robs people of an acceptable quality of life. By understanding the adverse

effects of noise, citizens will be more likely to ask for legislation and technology to limit and control noise. Thus, legislation, and technology are significant partners in noise abatement programs. This is not to say that certain sounds (emergency vehicles) do not serve a societal purpose, but even the noises that they make can be mitigated. It is important for business to recognize its contribution to noise and consider ways to lessen the noise. However, another element in the effort to reduce noise must be the recognition that individuals themselves can be a source of noise pollution as they fail to respect the rights of others to quiet. To create a quieter society, all of us can do one thing immediately – turn down the volume. We can all keep the volume on our radios, televisions, and personal music systems on lower settings; we can keep our pets quiet; we can forego honking horns except in an emergency; we can keep our automobiles and truck engines, air conditioners and appliances in good working order; and we can keep our voices lower as we speak on cell phones in public places. Acting respectfully and responsibly toward others will not only create a quieter society but a more civil one as well.

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Chapter 5
Energy Production from Food Industry Wastewaters Using Bioelectrochemical Cells

Abhijeet P. Borole and Choo Y. Hamilton

Abstract Conversion of waste and renewable resources to energy using microbial fuel cells (MFCs) is an upcoming technology for enabling a cleaner and sustainable environment. This chapter assesses the energy production potential from the US food industry wastewater resource. It also reports on an experimental study investigating conversion of wastewater from a local milk dairy plant to electricity. An MFC anode biocatalyst enriched on model sugar and organic acid substrates was used as the inoculum for the dairy wastewater MFC. The tests were conducted using a two-chamber MFC with a porous three dimensional anode and a Pt/C air-cathode. Power densities up to 690 mW/m^2 (54 W/m^3) were obtained.

Analysis of the food industry wastewater resource indicated that MFCs can potentially recover 2–260 kWh/ton of food processed from wastewaters generated during food processing, depending on the biological oxygen demand and volume of water used in the process. A total of 1960 MW of power can potentially be produced from US milk industry wastewaters alone. Hydrogen is an alternate form of energy that can be produced using bioelectrochemical cells. Approximately 2–270 m^3 of hydrogen can be generated per ton of the food processed. Application of MFCs for treatment of food processing wastewaters requires further investigations into electrode design, materials, liquid flow management, proton transfer, organic loading and scale-up to enable high power densities at the larger scale. Potential for water recycle also exists, but requires careful consideration of the microbiological safety and regulatory aspects and the economic feasibility of the process.

Keywords Microbial fuel and electrolysis cell · Electricity · Wastewater resource · Hydrogen · Power density

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5.1 Introduction

The food processing industry produces significant amounts of residual organic wastes as well as wastewater. The residual wastes include leftover food from residences and commercial establishments such as restaurants, cafeterias, etc. and discarded waste generated from food production operations. The amount of total leftover food waste in the US estimated for 2002 was about 43.6 million tons [1]. The total food waste in the state of California alone was estimated to be about 4 million dry metric tons [2]. About half of this was from the food processing industry and the other half from municipal solid waste streams going to landfill and composting facilities. The state of California has developed a bioenergy development program to increase generation of electricity from renewable resources [3]. Production of energy from food waste is included in the program and has become a part of California's renewable portfolio standard (RPS). Conversion of food waste into biogas makes about 4.7% of the state's renewable portfolio. Similar incentives are also expected from other states in coming years.

The wastewater volume produced by the food industry is quite large. The biological oxygen demand (BOD) of the wastewater depends on the industry and spans a wide range [4]. The dairy industry produces wastewater with BOD ranging from about 1000 mg/L (milk or cheese plant wastewater) to 35,000 mg/L (whey wastewater). The meat industry wastewater has a BOD ranging from 400 to 11,000 mg/L. The sea food, edible oil, confectionary food and brewery processing industries, similarly have a wide range of BOD from few hundred to several thousand mg/L. The fate of the wastewater depends on the location as well as the specific industry. The wastewater streams typically enter a common municipal wastewater stream, but in many cases, the food processors are charged a sewer surcharge, especially for streams with high BOD [2, 4].

The food processing wastewater streams contain carbohydrates, fats, oils and proteins, which are much easier to degrade compared to raw biomass, such as agricultural plant residues, forest product residues, etc. Release of the streams into the environment without proper treatment can result in adverse impact on the environment. The nutrients and carbon present in the wastewater can be a valuable resource for energy production. Use of biological methods such as anaerobic digestion has been investigated for treatment of wastes from the food industry [4, 5]. While anaerobic digestion is a suitable option for very high BOD liquid and solid wastes, it may not be the best option for low BOD wastewaters. Alternative emerging technologies such as microbial fuel cells (MFCs) offer a potential solution for such wastewater streams [6].

Microbial fuel cells are devices which oxidize organic matter and produce electricity [7]. Extracting useful energy from wastewaters laden with organic matter, while cleaning the water, is one of the exciting aspects of this technology. Several wastewaters have been investigated for their potential to generate electricity. Removal of volatile fatty acids [8, 9], lactate [10–12], glycerol [13], proteins [14], as well as treatment of wastewaters from vegetable processing [15], swine processing [16, 17], and other food industry wastewaters has been demonstrated [16, 18–20].

Several hurdles remain in commercialization of the MFC technology, such as cost of construction materials, scale-up issues, and ability to harness low voltage output. The key to being able to commercialize this technology depends on improving designs of the MFCs to achieve power densities in the range 400 to 1000 W/m^3. Recent improvements in MFC designs have shown power densities approaching these goals [12, 21, 22]. Investigations into the effect of the exoelectrogenic microorganisms and the microbial enrichment processes in influencing the MFC performance have also shown potential in improving power densities [12, 23, 24].

Potential for production of hydrogen instead of electricity from food industry wastewaters also exists [25–28]. A modification of the MFC process, termed as microbial electrolysis cell (MEC) process, involves application of additional voltage (0.3 V minimum) and use of a hydrogen-producing catalyst within an anaerobic cathode chamber resulting in hydrogen production instead of electricity [29]. Hydrogen production has been demonstrated from sugars and organic acids [30] and has been proposed as a method for energy production from renewable resources [31–33]. Hydrogen, which is a higher value product, offers a distinct economic advantage over electricity as the primary product of the bioelectrochemical process, since it helps compensate the high capital costs in implementing this technology [30, 34].

A second aspect of the cleanup of food industry wastewater streams is the possibility of water reuse and recycle. The quality of water has to approach drinking water levels in order to consider its reuse; however, recycle of the water to other operations within the industry may be possible. Several wastewater streams in the food industry contain quite low levels of organic carbon. The MFC technology may have an advantage for these streams over anaerobic digestion due to its potential to remove contaminants to very low levels and the ability to process the water at high flow rates using biofilm-based catalysts [12]. Currently, water reuse in the food industry is limited due to legislative constraints and hygiene concerns. Increase in energy costs and scarcity of the water resource in some locations has prompted rethinking of the water use practices [35]. The regulatory, technological, monitoring, verification and ethical aspects associated with microbiologically safe reuse of water need to be considered for any technology that is considered for the wastewater treatment. An alternative set of guidelines and regulations have been recently developed for use of water other than potable water for application in the food industry [36].

In this work, we assess the potential for electricity and hydrogen production from food industry wastewaters and discuss the potential application of this technology as the need and prospects for energy production from waste and renewable resources increase. The niche of this technology is evaluated for treatment of low BOD wastewaters. Energy production from high BOD wastewaters is also evaluated. As a case study, we investigate electricity production from wastewater obtained from a local milk dairy plant (Mayfield, Athens, TN). The objective of this experimental study was to test electricity production from the wastewater and to determine the maximum power density possible using an air-cathode MFC. The need for pretreatment/amendments into the raw wastewater was examined to identify potential limitations. The results from this study, along with reported literature values,

were used to get a realistic view of the current state of the technology and assess the potential of the technology for electricity and hydrogen production from food industry wastewaters.

5.2 Materials and Methods

5.2.1 Calculations to Determine Electricity Production Potential

Wastewater characteristics for various food industry wastewaters were obtained from the literature (See Table 5.1). The total wastewater volumes were available for dairy and the brewery industries, for which total energy production potential

Table 5.1 Estimated power and hydrogen production potential from food industry wastewaters

Category	Sub-category	COD, g/L[a]	Wastewater flow rate, gal/ton[b]	Product volume, million tons/year[c]	kWh/ton of product	Total MW potential	Hydrogen production potential, m^3/ton of product
Milk products							
	Milk/Cheese	3.2	3600	17	23	46	24
	Whey	35	3600	67	256	1960	267
Brewery							
	Beer	33	3120	18	42	44	44
Seafood							
	Catfish	0.7	*1000* [d]		1.4		1.5
	Shrimp	1.2	*1000*		2.4		2.5
Meat							
	Mixed	9.6	4200		82		85
Edible oil							
	Palm oil	50	*1000*		101		106
	olive	120	*1000*		243		254
Confectionary							
	Potato peel	10	*1000*		20		21
	Bakery/bread	1.5	720		2.2		2.3
Fruits and vegetables							
	Green beans	*1*	14,500		29		31
		1	4200		9		9
		1	4680		9		10

[a]Obtained from Digman and Kim [4]
[b]Obtained from McIlvane Company: http://www.mcilvainecompany.com/generic_examples/food.htm. An average flow was calculated based on the range given at the website.
[c]Obtained from Zhang et al. [5].
[d]The values shown in italic in this Table are assumed (for lack of data availability).

was determined. For other food wastewater sources, energy production per ton of the product was calculated.

Equation (5.1) gives the current production potential per ton of product, $I_{\mathrm{MFC},i}$ (Ampere-hours/ton or A h /ton).

$$I_{\mathrm{MFC},i} = \frac{1}{3600} * \frac{\mathrm{COD}_i}{M_{\mathrm{COD}}} * Q_i b_i * \frac{n_e}{n_{\mathrm{COD}}} * F\eta_c \tag{5.1}$$

This can then be used to determine the electricity production potential per ton of product, $E_{\mathrm{MFC},i}$ (Watt h /ton or W h/ton), as per Eq. (5.2).

$$E_{\mathrm{MFC},i} = I_{\mathrm{MFC},i} V \tag{5.2}$$

Where,

COD	Chemical oxygen demand for wastewater stream i, g/L
M_{COD}	Molecular weight of oxygen (COD) = 32 g/mole
Q_i	Wastewater flow rate per ton of product i, L/ton
b_i	biodegradability of organic carbon in wastewater type i
$n_{\mathrm{e}}/n_{\mathrm{COD}}$	Electrons released per mole of COD during anodic degradation of organic carbon in MFC = 4
F	Faraday constant = 96485.3 C/mole. One Ampere is equivalent to one C/s.
η_{c}	Coulombic efficiency of the MFC
V	Voltage generated in MFC, Volts

The anodic and cathodic half reactions for the degradation of carbohydrate, as an example of organic carbon present in wastewater, can be given as:

$$\text{Anode: } \mathrm{CH_2O + H_2O \rightarrow CO_2 + 4H^+ + 4e^-} \tag{5.3}$$

$$\text{Cathode: } \mathrm{4H^+ + 4e^- + O_2 \rightarrow 2H_2O} \tag{5.4}$$

The following assumptions were made based on the justification given below:

Biodegradability of organic carbon (COD) in wastewater = 0.8 [4],
Voltage generated in MFC = 0.4 volts,
Coulombic efficiency (CE) = 50%.

The voltage generated in an MFC is a function of the external electrical load. An MFC will typically be operated close to its maximum power density, which in turn decides the voltage output. The voltage at maximum power density in MFCs can range from 0.2 to 0.5 V depending on the design of the MFC and its performance. In this analysis, a voltage output of 0.4 V is used, which is reasonable considering that better MFC designs will emerge which can provide a steady voltage output ranging up to 0.5 V during a continuous MFC operation. The coulombic efficiencies (CE) reported in literature range from 5 to 59% for wastewaters containing

complex organic matter [25, 37–39] or model streams containing fermentable substrates such as glucose. Improvements in accessibility of insoluble substrates to the anodic biofilm microorganisms can lead to better CEs. The CE for acetate and other non-fermentable substrates has been shown to reach higher than 90% [37]. Thus, considering the limited studies conducted with complex organic wastewaters, a CE of 50% was assumed for the assessment of power production from food industry wastewaters.

5.2.2 Calculations to Determine Hydrogen Production Potential

The calculations for hydrogen production (L/ton product) were done using Eq. (5.4).

$$H_{\mathrm{MEC},i} = \frac{\mathrm{COD}_i}{M_{\mathrm{COD}}} * Q_i b_i * \frac{n_{H2}}{n_{\mathrm{COD}}} * M_{\mathrm{H2}} \eta_{\mathrm{H}} \tag{5.5}$$

Where,

$H_{\mathrm{MEC},i}$	Hydrogen production in L/ton of food product processed.
$N_{\mathrm{H2}}/n_{\mathrm{COD}}$	Ratio of number of moles of hydrogen obtained per mole of COD = 2.
M_{H2}	Molar volume of hydrogen, L/mol
η_{H}	Efficiency of hydrogen production or hydrogen yield

The molar volume of hydrogen was assumed to be 22.4 L/mole. The efficiency of hydrogen production was assumed to be 50%. Realistically, higher hydrogen yields (up to 95%) have been obtained with soluble model substrates [30], however, this may not be the case for hydrogen production from complex organic matter.

5.2.3 MFC Application in a Dairy Industry – an Experimental Study

Application of MFCs for energy production from dairy industry wastewater was investigated. The MFC was constructed using a 4 cm diameter × 1.27 cm thick anode chamber using a carbon felt electrode. The electrode completely filled the anode chamber, leaving no dead volume. The cathode was made up of a 4 cm diameter Pt-coated carbon gas diffusion electrode obtained from Fuel Cell Store, San Diego, CA. The cathode electrode was a typical air-cathode directly exposed to air. The cathode was separated from the anode by a Nafion 115 membrane. The details of the design are given elsewhere [12]. The anode nutrient medium flowed upwards through the anode in a recirculation mode.

The anode chamber was inoculated with a culture from an operating MFC using glucose and lactate as the carbon and energy source [12]. A sample of the biofilm

along with any planktonic organisms was collected by dislodging the cells from the electrode with a needle, followed by withdrawal of the liquid sample from the anode exit. The original inoculum for the MFC was from a Municipal wastewater treatment anaerobic digestor reactor. The anode biocatalyst for the MFC in this study was established using a synthetic wastewater containing minerals and a mixture of carbon source containing glucose and lactate. A preliminary test with the Mayfield wastewater as the carbon source indicated slow growth, as evidenced by negligible current production over a period of 8 days. A glucose and lactate mixture was used as the substrate for biocatalyst growth over a period of 5 days. After this period, the defined medium was replaced with wastewater. Acclimation of the biocatalyst to the wastewater was carried out by starting with 25% wastewater (balance being defined medium with no amended carbon source). This was followed by operation with 100% wastewater as the liquid phase. The MFC system used in the study is shown in Fig. 5.1.

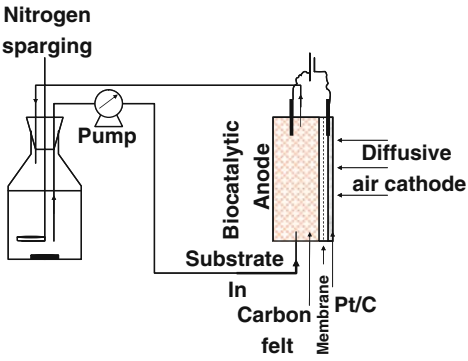

Fig. 5.1 Schematic of the MFC and the recirculation set up

5.2.4 Wastewater Collection and Use

The wastewater was collected from a local milk dairy plant operated by Mayfield Dairy Farms in Athens, TN. The plant generates about 190,000 gallons of wastewater per day with an average BOD of 2250 mg/L. The wastewater is released into the sewer to be treated by the municipal wastewater treatment plant. The wastewater was collected from the discharge pipe of the plant and stored at 4°C until use. The solids were allowed to settle to the bottom and the supernatant was used as the substrate for the MFC.

5.2.5 Electrical and Analytical Measurements

The voltage output from the MFCs was measured using a Hewlett Packard HP 3468B multimeter. The data were continuously collected using a 4-port DATAQ DI-158 USB data acquisition device. This was interfaced via USB cable into a computer

running the WinDaq data acquisition software. A variable-load resistor (0–5,000 Ω) was used to generate power density curves.

Analysis of glucose, lactate, and by-products of bioconversion by the anode biocatalyst was conducted using a Hitachi LaChrom Elite HPLC system, with a Bio-Rad AminexHPX-84H column and an RI detector. The column temperature was 65°C and the detector temperature was 50°C. The flow rate of the mobile phase was 0.5 ml/min.

5.3 Results and Discussion

5.3.1 Electricity Production Potential from Food Industry Wastewaters

The estimated electricity production potential was calculated per ton of the product for various food industry wastewaters. These results are shown in Table 5.1. The total wastewater volume for production within the US was available for the dairy and the brewery industry, for which the total MW production potential is given. As seen from the results in Table 5.1, power production, by itself, may not offer a sufficient incentive to warrant use of the MFC technology. Other advantages, such as potential for water reuse and production of alternative form of energy such as hydrogen may need to be considered.

5.3.2 Hydrogen Production Potential From Food Industry Wastewaters

The microbial electrolysis cell process has potential to produce hydrogen from wastewaters that contain easily degradable organic carbon. The potential amount of hydrogen that can be produced from the wastewater streams is shown in Table 5.1. Hydrogen is considered as the fuel of the future and is also an important reagent in the chemical industry. While it may not be economical to produce hydrogen from all of the wastewater streams in individual food processing plants, potential integration of food processing plants with future biorefineries may offer a niche for hydrogen production, especially if hydrogen is required on-site [40]. Of special interest for this application are the cereal, edible oil, and brewery industries. Utilization of wastes and wastewaters to produce this versatile chemical can help develop sustainable energy use alternatives to existing practices.

5.3.3 Electricity Production from Dairy Wastewater

A preliminary characterization of the wastewater from the Mayfield dairy plant by HPLC revealed presence of various sugars as well as organic acids. Based on known

standards, presence of fructose, citric acid, lactic acid, acetic acid, and butyric acid was detected. In addition, a few other peaks were observed, which were not characterized due to lack of appropriate standards. It was found that the anode biocatalyst growth was slow using the wastewater as the sole nutrient source. The biocatalyst was therefore established using a defined nutrient medium containing glucose and lactate at 0.2 g/L, each. After the biocatalyst was established, as indicated by a current of 1 mA at a load of 250 Ω (Fig. 5.2), the MFC was exposed to the wastewater. The biocatalyst was first acclimated to 25% wastewater, mixed with the nutrient medium (without amended carbon source), followed by operation with 100% wastewater. A current of 2.1 mA was obtained at a load of 100 Ω with the 25% wastewater stream. A similar current was obtained with the full strength wastewater as well. However, the current rapidly dropped below 1 mA. The decrease in current was due to a drop in pH. The wastewater was not ammended with any buffer salts which was the primary reason for the drop in pH to below 6.0, within the two day period. Adjustment of the pH to 7.0 reinstated the current output (Fig. 5.2). Thus, pH control would be necessary for the MFC application. The MFC produced electricity continuously from 200 mL of wastewater for a period of 7 days at above 400 mW/m^2 power density (37 W/m^3 of total anode volume). A power density analysis indicated a maximum of 470 mW/m^2 (Fig. 5.3). Operation of the MFC for three additional days increased the power density to 690 mW/m^2 (54 W/m^3). No further increase in the power density was observed over the next month. An analysis of the internal resistances of the MFC via electrochemical impedance spectroscopy (EIS) indicated that the ohmic resistance was less than 10 ohms.

Typical power densities obtained using food industry wastewater as the energy source have been in the range of 80–370 mW/m^2 [25, 39] (see further discussion below). The power density obtained in this study is relatively higher than those reported in literature. The primary reason for this is the MFC architecture which lowers the internal resistance of the MFC as indicated by the low ohmic resistance observed for this design. Further work on the assessment of the impedances via EIS is in progess and will be reported elsewhere.

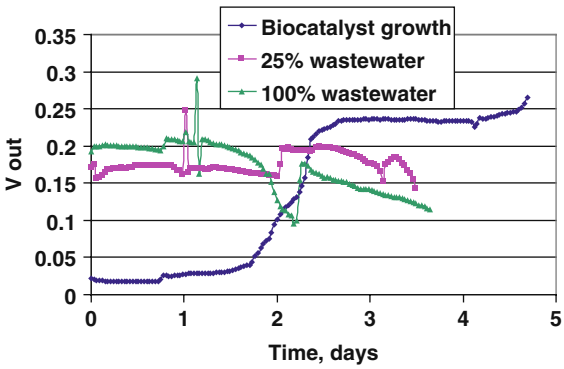

Fig. 5.2 Voltage output during biocatalyst growth and during use of wastewater as the fuel source. The load on the MFC was 250 and 100 Ω during the growth period and the wastewater testing period, respectively

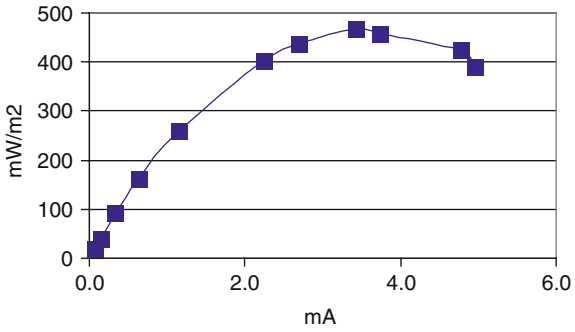

Fig. 5.3 Power density of the MFC using dairy wastewater as the energy source

5.3.4 Complex Organic Matter in Dairy Processing Wastewater

The Mayfield wastewater potentially contained complex organic carbon in the form of dissolved as well as suspended particulate matter. The HPLC analysis indicated a total of about 0.5 g/L dissolved solids in the wastewater. This amount of organic carbon would provide continuous power for a period of 8 days, assuming 100% coulombic efficiency and a current output of 2.1 mA. This is based on a total volume of 230 mL (maximum volume of MFC system, including the reservoir). The BOD of the wastewater was approximately 2.25 g/L (equivalent to 2.12 g/L carbohydrate). Thus, about 1.62 g/L of the carbohydrate-equivalent organic matter was present in a complex organic form.

5.3.5 Assessment of MFC/MEC Application for Food Industry Wastewater Treatment

In application of bioelectrochemical systems for treatment of food processing wastewaters, a number of factors need detailed assessment, especially if the goal is to maximize energy generation. In the following subsections, we review and discuss energy production from complex organic matter, potential changes to improve power densities and pursue practical applications and potential for water reuse in the industry.

5.3.5.1 Deriving Energy from Complex Organic Matter

Electricity production from starch, molasses, cellulose, protein and other complex carbon sources has been reported using MFCs [14, 39, 41–44]. Deriving energy from the complex, soluble as well as suspended organic matter in food processing waters therefore should be possible, but further work is required to demonstrate the degree of conversion of these wastewater constituents. The suspended particulates may require modified MFC designs to handle solids or pretreatment to solubilize

the insoluble constituents. One such modification of the anode was reported by Niessen et al. [41]. A platinized electrode was combined with a fermentative organism (*Clostridium beijerinckii or C. butyricum*) to demonstrate utilization of starch and molasses as the carbon source for electricity production at high current densities (between 1 and 1.3 mA/cm^2). Another study investigating utilization of cellulose in MFCs reported use of enzymatic hydrolysis to solubilize the cellulose, with effective electricity production with cellulase as the substrate [41]. The process of conversion of complex organic matter into electricity or hydrogen can be broken down into three steps. The first step is the depolymerization or breakdown of the complex organic matter into its monomeric constituents. The second step, (using carbohydrates as an example), is fermentative degradation of the sugars into volatile fatty acids and hydrogen. The third step is the conversion of the VFAs and hydrogen to electricity. Use of MECs can result in conversion of the VFAs to hydrogen. Direct conversion of sugars to electricity is also possible [45]. However, when using microbial consortia a recent study demonstrated that the primary route for electricity production from glucose was via VFA and hydrogen formation [46]. This study used an inoculum enriched on acetate. A similar study using an microbial consortia enriched on glucose or sugars is needed to determine the path of conversion of sugars to electricity. In MFCs using consortia enriched on acetate, potential for conversion of either the VFAs or hydrogen to other products such as methane (via methanogenesis), has been demonstrated [46]. Methane is unsuitable as a substrate for exoelectrogens, which leads to lower coulombic efficiencies. The role of fermentative and methanogenic bacteria is therefore quite important while considering electricity or hydrogen production from complex organic matter, as in the case of food processing wastewaters. Use of methods to minimize methanogens in MFC anodic communities, such as intermittent aeration, have shown some success [34] but long term studies have not been conducted.

The overall COD removal from most wastewaters in MFCs is usually very high (approaching 90% or higher), which indicates that degradation of the organic carbon is not a problem. The problem is related to the electron acceptor used for the bioconversion process. In addition to the diversion of the electrons towards methanogenesis, other paths to electron oxidation also exist via use of nitrate, sulfate and oxygen as electron acceptors. Nitrate may be present at high concentrations in wastewaters originating from fermentation operations such as brewery wastewater. Sulfate may be an issue in food operations using groundwater as the source of washwater. Oxygen leakage into the anode chamber also impacts coulombic efficiencies (CEs), and becomes significant in membrane-free MFCs. In a study investigating electricity production from starch processing wastewater, the COD removal efficiencies of 96–98% were reported, while the CE was only 7% [39]. The huge inefficiency was essentially attributed to oxygen diffusion, although it also included contribution of other electron acceptors in the wastewater. CEs for MFCs fed with glucose have ranged from 28 to 59% [37, 46, 47]. Studies using complex organic matter or wastewaters as the substrate report CEs in the range of 5–40 [25, 37–39]. The relatively lower CEs for the latter may also be due to the

presence of organic matter that may not be easily degraded by the microbial consortia used in the studies. Typically, the studies have used consortia enriched using model substrates such as glucose or acetate. These consortia may not contain organisms capable of degrading complex organic matter at sufficient levels. The study by Lu et al. [39] demonstrated that the use of microorganisms in the wastewater itself, which are potentially pre-enriched in degrading the complex organic matter leads to high COD removal (96–98%). Control of oxygen leakage into the system, and methanogenesis can lead to CEs of 50% or higher for food processing wastewaters. The relative importance of oxygen leakage vs. methanogenesis is not very well studied for food industry wastewaters, and further work is needed in understanding these effects.

5.3.5.2 Potential for Enabling Higher Power Densities and Practical Applications

The range of power densities obtained in MFCs processing industrial wastewaters relevant to the food industry are shown in Table 5.2. Assessment of the commercial feasibility of MFCs has revealed that the power densities need to be in the range of 400–1000 W/m^3 for application consideration [21, 22]. MFCs with power densities approaching this range have been reported, but mostly with model soluble substrates [12, 23, 48–50]. The power densities for MFCs fed with insoluble substrates are lower compared to those with soluble substrates. Thus, accessibility to the solid substrate is an issue and requires novel designs to improve their conversion. Since food industry wastewaters contain a significant amount of soluble sugars and organic acids, the potential for application of MFCs for deriving energy from

Table 5.2 Power densities reported in MFCs processing wastewaters

			Power density		
Type of wastewater	Primary substrate	MFC description	mW/m^2	W/m^3	Reference
Starch processing	Starch	Carbon paper electrodes with Nafion 117 membrane	239	14	[39]
Synthetic wastewater	Starch, peptone, and fish extract	Stackable cartridge-type MFC	899	129	[42]
Cereal-processing	Complex organic matter	Two-chamber MFC	81	8.8	[25]
Cereal-processing	Fermented wastewater	One-chambered MFC	371	13.4	[25]
Paper processing	Cellulose	Two-chamber MFC	100	0.6	[59]
Dairy processing	Soluble components from wastewater	1.27 cm thick carbon felt anode with air-cathode	690	54	This study

food industry wastewaters is high. However, realization of the complete potential requires further work with insoluble substrates. There are several technical issues that need to be considered for application of MFCs to wastewater treatment. These can be classified into two main categories: MFC design or engineering parameters and process or operational parameters. The MFC design parameters include electrode spacing, type of membrane, ratio of anode to cathode surface area, porosity of electrodes and chemical vs. biological cathode. The MFC design parameters have been reviewed in several recent reviews [7, 21, 22] and the issues related to cathode systems have been discussed in two recent reports [51, 52]. The operating parameters include ionic strength, buffering capacity, flow rate of liquid through the anode and/or cathode chamber, oxygen content of the wastewaters and organic loading.

The ionic strength of the wastewater has been shown to affect the power density of MFCs [53, 54]. The buffering capacity is a related parameter, which also affects stable power generation as observed in the Mayfield wastewater case study. Continuous power generation at high rates is possible by amending the wastewater with buffering salts to maintain the ionic strength and buffering capacity. However, this may not be a practical option due to the cost of the buffer salts, especially because the salts cannot be easily recovered from the treated wastewater. Use of membrane-free MFCs alleviates the problem of buffering capacity to some degree, since it minimizes pH polarization [55]. However, the buffering capacity will still be an issue in larger scale systems. In absence of membranes, diffusion of oxygen towards anode and carbon source towards cathode become significant resulting in reduced coulombic efficiency.

The anode flow rate controls the carbon and nutrient supply to the microbes typically present as biofilms on the electrodes. Presence of pH gradients, which occurs in biofilms and non-porous electrodes can also be minimized by designing systems with flow-through capability. Use of porous, three-dimensional anodes incorporating these principles has been reported to result in higher power densities [11, 12]. The flow rate is also know to affect biofilm formation and its subsequent impact on power densities [56]. In a pilot-scale study with brewery wastewater as the feed, several operating parameters were investigated [57]. One of the parameters was the dissolved oxygen present in the incoming stream. The study reported formation of thick biofilms which was a problem for stable, continuous operation of the MFC. The presence of dissolved oxygen can promote aerobic growth of biofilms which is undesirable for optimum MFC performance. The study also found a number of other process and design parameters including proton transfer, liquid flow management, electrode conductivity, electrical contacts, issues related to scale-up and handling of operational upsets, which need to be carefully considered and optimized in order to achieve power densities similar to those observed in the laboratory.

5.3.5.3 Potential for Water Reuse and Recycle

One of the selling points of the MFC technology has been its ability to treat wastewater while generating energy. While removal of organic carbon from wastewaters

has been demonstrated, and quite often to levels approaching 90% or more [58], the research to demonstrate the ability to clean the water sufficiently to enable reuse has been very limited. A recent publication proposed use of the MFC technology to enable recycle of water in a biorefinery via application of MFCs to remove byproducts and other contaminants accumulating in the process [58]. The potential for water reuse in the food industry has been considered, although not via implementation of the MFC technology [58]. Typical processes investigated for water cleanup to enable reuse have been a combination of filtration and disinfection. The water streams considered for reuse are mostly low organic carbon containing streams such as cooling water, condensate, chiller water and wash water. Removal of any organic carbon present in the water has been via use of diatomaceous earth filters (soluble as well as insoluble matter) and micro- or ultra-filtration (for particulate matter). The potential for water recycle (i.e., use of cleaned wastewater in a different operation but within the same industry), is greater than the potential for reuse. The consideration of MFCs for wastewater clean-up can potentially open the door for many other streams containing higher levels of organic carbon ($\sim$ 500–5000 mg/L). Water reuse in the food industry requires careful consideration of the microbiological safety aspects and related regulatory, technological and economic factors [35]. Any use of MFCs for this application has to be done using non-hazardous microorganisms. One of the characteristics of exoelectrogenic anodic microorganisms used in MFCs is their tendency to form biofilms, which can minimize but not eliminate the presence of microorganisms in effluents from the MFCs. Recent changes in the guidelines for water reuse in the food industry [36] may allow consideration of emerging technologies such as MFCs for wastewater cleanup.

5.4 Conclusions

The food industry wastewaters contain significant amount of easily degradable organic carbon which can be used for energy production. MFCs offer a potential solution for treatment of low BOD wastewaters. MFCs can produce electricity in the range of 2–260 kWh/ton of product from the wastewater used for processing the food products, depending on the BOD and volume of water used in the process. A total of 46 MW of power can potentially be produced from wastewaters from milk dairy farms (low BOD wastewater) in the US. In comparison, up to 1960 MW of electricity can be produced from high BOD wastewater from the dairy industry. Hydrogen is an alternate form of energy that can be produced using bioelectrochemical cells from the food industry wastewaters with potential for generation of 2–270 m^3/ton of the food product. Application of MFCs for treatment of food processing wastewaters requires further investigations into electrode design, materials, liquid flow management, organic loading and scale-up to enable high power densities at commercial scale.

Our experimental study investigating treatment of milk dairy wastewater demonstrated electricity production of 54 W/m^3 from wastewater using an MFC system.

Removal of soluble organic matter was demonstrated with simultaneous production of electricity. Important parameters for consideration of continuous generation of power using the MFC included the buffering capacity and ionic strength of the wastewater stream.

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Chapter 6
Needle-Type Multi-Analyte MEMS Sensor Arrays for *In Situ* Measurements in Biofilms

Jin-Hwan Lee, Youngwoo Seo, Woo Hyoung Lee, Paul Bishop, and Ian Papautsky

Abstract Biofilms are colonies of microbial cells in a polymeric matrix. Formation of biofilms has been associated with a broad range of industrial problems at the annual cost of billions of dollars. For example, biofilms are ubiquitous in water distribution systems and control of their growth have been a great challenge, with many water utilities in the US reporting biofilm survival in water distribution systems despite the continuing presence of disinfectants. In addition to being a nuisance, biofilms may also harbor various types of microorganisms including opportunistic pathogens and thus can threaten public health. The conventional methods for studying biofilms include microelectrode sensors fabricated from pulled glass micropipettes. However, fragility, difficulty to manufacture and operate, and susceptibility to electrical interference limit their use to specialized laboratories under highly controlled conditions. Thus, there is a critical need for robust microelectrode sensors that can be used *In Situ* to study biofilms.

This chapter describes the use of microelectromechanical systems (MEMS) technologies to develop needle-type sensors for *In Situ* measurements in biofilms. The individual needle-type sensors for measuring oxidation reduction potential (ORP), dissolved oxygen (DO), and phosphate were integrated into a single multi-analyte sensor array. All three sensors were extensively characterized, exhibiting higher sensitivity, faster response time, and higher stability with smaller tip size than the conventional sensors. The multi-analyte sensor was successfully applied to *In Situ* evaluation of microprofiles in multi-species biofilms. The major advantages of these new MEMS sensors include the ability to penetrate samples to perform measurements, the small tip size for *In Situ* measurements, array structure for higher robustness, and possibility of multi-analyte detection. The sensors demonstrated monitoring of local concentration changes in small structures with a high spatial resolution, and offer the versatility of the microelectrode technique as well as the capability for repetitive measurements. Ultimately, this research will enable *in situ*

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measurements in a wide variety of small sample applications in environmental engineering and life sciences.

Keywords Needle-type sensors · Multi-analyte sensors · MEMS · *In situ* measurements · Biofilms · Dissolved oxygen sensor · Oxidation reduction potential sensor · Phosphate sensor

List of Abbreviations

ASTM:	American society for testing and materials
COM:	Commercially available millielectrode
CCD:	Charge couple device
COD:	Chemical oxygen demand
DO:	Dissolved oxygen
EBPR:	Enhanced biological phosphorus removal
EDM:	Electrical discharge machining
EPS:	Extracellular polymeric substances
FISH:	Fluorescent *in situ* hybridization
HOC:	Hydrophobic organic compound
HRT:	Hydraulic retention time
IC:	Integrated circuit
ISFETs:	Ion-sensitive field-effect transistors
LOC:	Lab-on-a-chip
ME:	Conventional pulled-glass pipette microelectrode
MEA:	Microelectrode Array
MEMS:	Microelectromechanical systems
MLSS:	Mixed liquid suspended solids
ORP:	Oxidation reduction potential
PAOs:	Phosphate accumulating organisms
PCB:	Printed circuit board
SBR:	Sequencing batch reactor
SRT:	Sludge retention time
UEA:	Utah Electrode Array

6.1 Introduction

Microbial cells attach firmly to almost any surface in soil or in aquatic environments. The immobilized cells grow, reproduce, and produce extracellular polymeric substances which frequently extend from the cell forming a tangled matrix of fibers that provide structure to the assemblage. This structure is termed a *biofilm* [1], and its formation can be categorized into three steps, as shown in Fig. 6.1. First, free bacteria attach to substratum and form a thin layer called “micro-colonies.” In the second step, the attached bacteria aggregate together on the substratum with the help of extracellular polymeric substances (EPS). Finally, fully developed biofilms

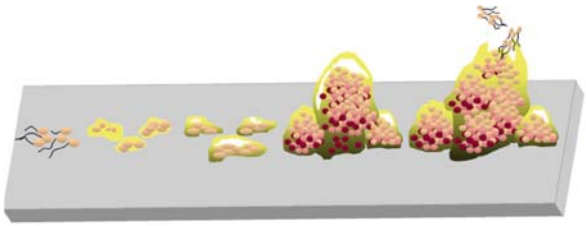

Fig. 6.1 Basic steps in biofilm formation. (Adapted from Francolini et al. [79])

are formed by bridging cells and EPS in conjunction with non-cellular substances, such as trapped inorganic particles, to form an environment.

Formation of biofilms has been associated with a broad range of problems and costs billions of dollars to industry each year [2]. In water distribution systems, biofilm formations are ubiquitous and controls of their growth have been great challenges. Reports from many water utilities in the US have shown that biofilms survive in water distribution systems, despite the continuing presence of disinfectants [3, 4]. They also harbor various types of microorganisms including pathogenic and opportunistic pathogens and threaten public health [5, 6]. In addition to water distribution systems, biofilm formations cause metal corrosion, material deterioration, and fouling of heat exchangers, membranes and ship hulls. Corrosion of metals caused by sulfate reducing biofilm is a universal problem. In the medical industry, biofilm formation on human lung, medical devices, and artificial organs also have been of great concern [7, 8]. The various problems associated with biofilms are summarized in Table 6.1.

Table 6.1 Problems associated with biofilm formation

Environment	Problem location	Consequences	References
Industrial	Membrane filtration units	Total flux loss, energy loss, reduction of membrane life	[77]
	Heat exchanger	Loss of heat exchange efficiency, energy loss	[75, 78]
	Ship hull	Energy loss	[79]
	Water distribution pipe	Decreased disinfection, corrosion, health threat	[3, 4, 6, 76]
	Petroleum reservoir	H_2S souring, increasing refinery cost, pipe clogging	[77]
Medical	Medical devices and implants	Infection in medical devices and implants, cystic fibrosis (CF) in human lung	[7, 8]
	Teeth	Dental plaque and cavity	[80]

6.1.1 Industrial Applications of Biofilms

While removing biofilms has been one of the great industrial challenges, biofilms have also found many applications. For instance, biofilms have been successfully used in waste and water treatment due to their advantages over the use of suspended bacteria [9]. Biofilms have the ability to support a variety of microbial populations at various locations within the biofilm. Thus, diversity of microorganisms in the biofilm can induce degradation of different organic substances [1, 9]. Biofilms also have high microorganism populations in a small unit volume. This enables high substrate removal rates, making it possible to build small, effective reactors [9].

In soil bioremediation of xenobiotic compounds, field scale permeable reactive barriers using biofilms have been tested for the treatment of contaminated groundwater [10]. In biobarrier systems for contaminated soil and groundwater, the biofilm EPS plays an important role in the sorption of organic pollutants [11]. Hydrophobic organic compound (HOC) sorption is the primary chemical process in subsurface aquifer systems [12, 13] and is important for stable operation of engineered bioremediation systems. Therefore, an increased biomass and the EPS content of the biofilm can create a strong affinity for HOCs.

Due to the increased sorption capacity, the biofilm EPS can trap organic compounds in the groundwater; the adsorbed organic compounds can then be desorbed and diffused out from the EPS, permitting subsequent degradation of the HOCs by the attached microorganisms in the biofilm matrix. The increased mass of HOCs sorbed to the biofilm matrix can also induce an increase in the number of degraders present. A stable removal of HOC may be achieved through entrapment and simultaneous degradation of HOC in biofilm. In addition to the sorption capacity of biofilm, recent studies observed direct biofilm growth on toxic chemical crystals (pyrene and phenanthrene) without any other available carbon source [14–16]. In this aspect, formation and structure of the biofilm is crucial for the soil and groundwater remediation [10]. However, biofilm formation in a subsurface aquifer has sometimes produced an adverse effect. Currently, most studies have focused on the removal of HOC through the biobarrier [17–19] and the mass transport mechanisms, and structural forms of the biofilm exposed to the HOC are not well understood.

6.1.2 Biofilms in Environmental Systems

Biofilm formation and development in natural and industrial systems solely depend on electron donors (organic substrates) and electron acceptors (oxygen, nitrate and sulfate). Thus, monitoring and controlling the electron donors and acceptors is one of the approaches used to control biofilms in both natural and industrial systems.

Recent developments in the field of molecular biology are beginning to enable scientists to study the spatial distribution, diversity, and activity of microorganisms in biofilms [20]. Along with molecular tools, microelectrode techniques have been applied for *in situ* measurement of chemical transport in biofilms [21, 22]. Initially,

microelectrodes were developed for intracellular analysis by animal physiologists, but recently they have been used in environmental research. Microelectrodes for nitrous oxide-oxygen [23], nitrate [24], and sulfide [25, 26] have been used for nitrification and denitrification studies. In addition, concentration gradients of dissolved oxygen (DO), ammonium, pH, and oxidation reduction potential (ORP) could be monitored by microelectrodes with no disturbance to the biofilm structure [23–26]. These microprofiles which indicate the target constituent concentration gradients from bulk solution to inside biofilm give many significant benefits for understanding the biofilm process or mechanism inside biofilm, providing the spatial distribution and change of microbial activities within biofilms. DO concentration microprofiles was used to study the external mass transfer resistance [21]. Microprofiles of nitrate, pH, ORP in the nitrification and de-nitrification biofilm process showed oxygen and alkalinity utilization, and supported the hypothesis that denitrifying biofilms are stratified into an anoxic layer and an anaerobic layer [27]. Phosphate micro-profiles, combined with other constituent profiles including pH, DO, ammonia, and ORP, in the flocs can be used to elucidate the dynamic activity of microbial processes in the EBPR process and can be valuable for designing operating systems or modeling efforts for biological nutrient treatment [28]. Phosphate microprofiles can also be used for investigation of phosphate effect as a corrosion inhibitor in the drinking water distribution system biofilms. Overall, with the current development of molecular methods and microelectrode techniques, *in situ* structural and functional analysis of biofilm communities can be achieved and this information will give the more understanding of the mechanism of microbial biofilm process for designing modeling and/or biofilm control strategies in the drinking water distribution system.

6.2 Needle-Type Microelectrode Array (MEA) Sensor

6.2.1 Overview and Rationale

Traditional environmental monitoring methods use electrode probes with tips approximately 1–3 cm in diameter [27–30]. Typically, samples are collected from the field site, transported to laboratory, and analyzed in a well-controlled setting. However, this traditional approach is clearly not applicable to biofilms where *in situ* measurements are necessary. Sample properties, such as redox potential and dissolved oxygen content, may change during transport from the collection site to the analysis laboratory. Further, due to their large size, traditional sensors can be used to monitor bulk liquid concentrations when there is sufficient volume to wet the electrode contacts, but are often inappropriate for measurements in small volumes of samples.

In recent years, the development of *in situ* environmental sensors has become an important research topic [31–34], and microelectrode sensors with tip diameters of ~10 μm have been developed for *in situ* studies of small samples such as biofilms [35].

Microelectrodes can be fabricated in a number of ways [31, 32], most commonly by pulling a glass micropipette, inserting a metal wire (such as Pt or Au), and then filling with a low-melting-point alloy. Alternatively, a metal wire can be inserted into a glass micropipette first and then the metal/glass assembly pulled under heat to simultaneously decrease the wire diameter and tightly seal the metal within the glass capillary. Fine Au or Pt wires can also be sealed into glass by inserting them into a glass capillary and melting it around the wire. Such "conventional" microelectrodes have been used to investigate microscale distribution of oxygen consumption [21, 36–38], photosynthesis [39], sulfate reduction [25, 26, 40, 41], and nitrification and de-nitrification [39, 42].

Although these microelectrode fabrication methods are well-established, a number of inherent disadvantages still exist, such as low success rate, poor reproducibility, fragility, and difficulty in making a multi-sensor device [43–45]. Further, these microelectrode sensors are susceptible to electrical interference and have to be operated in specialized laboratories inside a Faraday cage [42–44]. Therefore, a need for robust, sensitive, and easy-to-fabricate sensors for *in situ* measurements still remains.

Microelectromechanical systems (MEMS) miniaturization technologies offer many advantages for fabrication and integration of sensor components [46]. These include reduced costs due to batch fabrication, increased integration, and potentially reduced power consumption due to smaller size. The use of MEMS fabrication techniques can also reduce complexity and increase reproducibility of the fabrication process. The most important advantage of using MEMS fabrication, however, is the increase in sensor reliability due to redundancy and better process control.

Many researchers that apply MEMS technologies to solving sensor problems have focused on microfluidic lab-on-a-chip (LOC) systems [47–49]. Such systems typically contain microfluidic channels for sample collection, preparation, or transport with planar sensing areas for specific target analytes. Others have developed ion selective sensors based on field-effect transistors (or ISFETs) [50]. These sensors also are based on planar electrodes, often integrated with a microfluidic system. Nevertheless, they key drawback of such systems is of course that samples still must be extracted from the site of interest, which often is not acceptable. In order to perform *in situ* measurements, 3-D microelectrode sensors are needed to be capable of penetrating directly into samples such as soil pores or biofilms.

MEMS technologies have been used to develop penetrating 3-D microelectrode sensors for neuroscience applications. Fofonoff et al. [51] combined wire electrical discharge machining (EDM) with a chemical etching process to fabricate titanium microelectrode arrays for neural activity recording in mice. Several structures such as a 10×10 rectangular shape and a honeycomb pattern containing 1141 electrodes were demonstrated with a device that was 1 mm long and 80 μm wide. Motta and Judy [52] developed neural microprobes using a 3-D continuous electroplating process which yielded 22 mm long microprobes. A 3-D flexible microprobe array was designed by Takeuchi et al. [53] using polyimide deposited on a 250 μm thick silicon substrate. Deep reactive ion etching (DRIE) and XeF_2 etching processes removed the silicon substrate to achieve flexible polyimide probes.

The 2 × 3 probe array was fabricated with a 1.2 mm height and a 160 μm width. The external magnetic field was used to align probes toward the same direction. Glass/silicon composite electrode arrays, known as The Utah Electrode Array (UEA), were developed with 100 silicon needle-type electrodes in a 10 × 10 array [54]. The needles were 1.5 mm in length and 80 μm in diameter. Glass was melted to electrically isolate individual electrodes on the back side of a silicon substrate and then the sawing process produced tall silicon columns, which were sharpened and tapered using an acid etching procedure. However, all of these needle-type MEMS electrodes have been designed for neural recording and can only measure potential (e.g., neuronal activity). Consequently, development of MEMS needle-type sensors capable of *electrochemical* measurements is still a necessity.

The proposed needle-type sensor system concept is illustrated in Fig. 6.2. The sensor system is divided into two key components: (1) a multi-analyte microelectrode array (MEA) sensor for *in situ* electrochemical measurements; and (2) an IC chip and circuitry for signal acquisition, processing, and transmission to a data storage/display device. By intimately integrating the sensors and the electronics, signal-to-noise ratio can be improved drastically. Such an integrated sensor system will be ideally suited for on-site applications, capable of rapidly and accurately sensing multiple analytes *in situ* for environmental applications.

This chapter describes development of the needle-type multi-analyte MEA sensor. More specifically, research work to miniaturize and characterize individual sensors for measurements of the oxidation reduction potential (ORP) [55, 56], dissolved oxygen (DO) [57, 58], and phosphate [59, 60] is described. These sensors were integrated into a single sensor array to demonstrate a proof-of-concept multi-analyte MEA, which was then applied to *in situ* evaluation of biofilms. Microelectrode miniaturization and integration was performed using MEMS technologies, which offer the advantages of accurate fabrication methods, reduced complexity of the fabrication process, increased reliability and reproducibility, reduced cost, and possibility of batch fabrication for large scale production. It

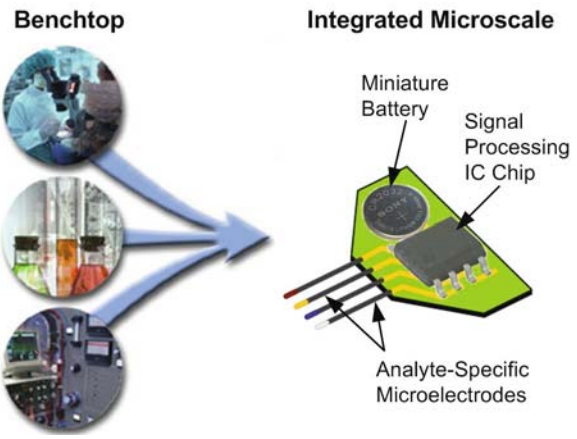

Fig. 6.2 Concept of an integrated multi-analyte MEMS sensor array for *in situ* monitoring in biofilms and the environment [57]

is expected that the demonstrated multi-analyte MEA sensor will enable *in situ* measurements and analyses in environmental applications. Ultimately, these sensors offer the ability to penetrate samples, due to the small tip size, for *in situ* measurements, as well as providing an array structure for higher robustness and multi-analyte measurements.

6.2.2 MEA Fabrication

The needle-type MEA was fabricated from 175 μm thick, 45 × 50 mm borosilicate glass wafers (Erie Scientific, Pittsburgh, PA). Batch fabrication was used to facilitate fabrication of a large number of sensors, reduce costs, and increase yield. Twelve MEAs could be fabricated from a single glass wafer. The process, illustrated in Fig. 6.3, has five major steps: dicing, etching, metallization, packaging, and sensor tip formation.

Dicing. Glass wafers were cleaned with sulfuric peroxide solution (H_2SO_4 and H_2O_2 in a 7:3 (v/v) ratio) and cut with a dicing saw to yield an array of glass probes (Fig. 6.3a). A 10 mil thick, 45 μm diamond grit resinoid blade (K&S Micro-Swiss, Fort Washington PA) was used to form 900 μm center-to-center spacing between each glass probe, 2 cm in length. Alternatively, dicing can be outsourced (e.g., American Dicing Co., Syracuse, NY). Longer 2.5 cm cuts were made between every four probes to define the edges of individual MEAs. In a later process step, an additional cut across the arrays would be made to separate individual MEAs. However, keeping MEAs together at this time permits batch processing, increasing yield and reducing fabrication costs. The cut wafer was then annealed at 550ºC for 10 min in a programmable box furnace (Lindberg/Blue M, Thermo Scientific, Norwood, MA) to relieve stress from the dicing process. Three cooling steps were used to reduce thermal shock: 480ºC was reached at ~12ºC/min, followed by 24ºC/min cooling

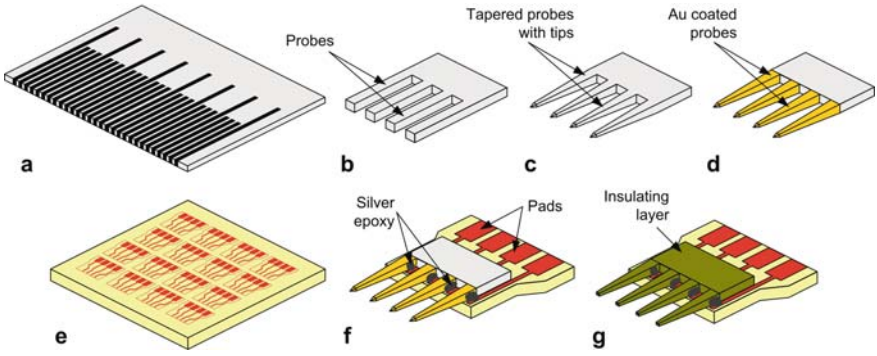

Fig. 6.3 Microelectrode array (MEA) sensor batch fabrication sequence: (**a**) dice glass wafer, (**b**) form glass probes by dicing, (**c**) use meniscus etching to sharpen probes, (**d**) deposit Au conductive layer, (**e**) pattern PCB, (**f**) use silver epoxy to establish electrical connections, and (**g**) coat microelectrodes with parylene insulating layer and fabricate recessed tips [55, 57]

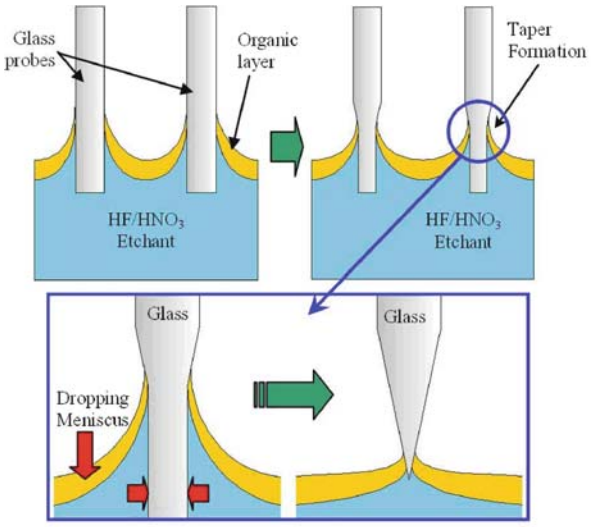

Fig. 6.4 Fabrication of sharp MEA tips using meniscus etching. Meniscus height falls dynamically to form a taper with geometry dependant on the initial meniscus height [55, 57]

to 400ºC, followed by cooling to ambient temperature. After annealing, the diced wafer was cleaned with acetone, methanol, and DI water for 5 min each.

Etching. The glass probes were sharpened into needle-type microelectrodes using the chemical sharpening process, termed *meniscus etching*, which uses surface tension force at the glass-etchant interface. The process is schematically illustrated in Fig. 6.4. Glass probes are immersed into HF-based etchant with an organic layer on top, typically paraffin oil or vegetable oil, to modify contact angle at the glass-etchant interface. The etchant wets the surface of the probes and gradually reduces their dimensions. The surface tension force at the glass-etchant interface reduces with the diminishing dimensions, forcing the height of the meniscus to decrease with time until the etching front reaches the center of the probe, and forming a sharp tip. The process is self-terminating. The balance of two opposing forces, the surface tension and the weight of the etchant, determines the final tip geometry. This process has been used previously in fabrication of optic probes [61] as well as redox potential sensors [55, 56] and dissolved oxygen sensors [57, 58].

The probes were first etched in the microelectrode etchant solution for 20 min with agitation to smooth the diced surface and reduce the probe dimensions to ~95 μm in width. The microelectrode etchant solution was prepared by mixing HF, HNO_3, and H_2O in a 10:7:33 (v/v/v) ratio. The lateral and transverse etching rates were ~2.5 and ~2.3 μm/min, respectively. The ~10% increase in lateral etch rate was due to the greater roughness of the unpolished vertical sidewalls of the glass beams formed by dicing, offering a greater surface area for the etching process [61]. At the end of this etch step, the cross-section of individual probes was 85 × 90 μm due to the starting probe dimensions of 175 μm in thickness and 190 μm in width [55].

Next, the glass probes were gradually pulled out using a computer-controlled (via LabView v.7) motorized linear translation stage (Newport Corp., Irvine, CA) to taper them down to 20 × 20 μm at the tip. In the final etch step, approximately 1 mm length of the tapered probe was immersed into the same etchant for further sharpening by meniscus etching, yielding ~200 nm tips [55, 62,]. This final etch step was self-terminating, which permitted consistent and reliable fabrication of microelectrode sensor tips. Following this final etching step, the sharpened glass probes were cleaned in sulfuric peroxide solution (H_2SO_4 and H_2O_2 in a 7:3 (v/v) ratio), followed by acetone, methanol, and DI water for 5 min each. The total etch time was approximately 40 min for one glass wafer.

Metallization. The tapered glass probes with sharpened tips were metalized on all sides by thermal evaporation (Fig. 6.3d). A 200 nm thick layer of gold (Au) was deposited as a conductive layer on top of a 20 nm thick layer of titanium (Ti) adhesion layer [57]. A glass cover slip was used to mask the microelectrode array base on both sides to prevent metal deposition and electrically isolate individual microelectrodes.

Packaging. Following the batch fabrication steps, the metalized glass wafer was cross-cut to separate individual MEAs. For easier handling and establishing electrical connections with individual sensors, MEAs were packaged with copper-clad laminate glass-epoxy (also known as printed circuit board or PCB) carriers. Using PCB is also beneficial for future system integration with IC circuitry. The laminate was 790 μm thick (D&L Products, Inc.) with a 35 μm thick layer of copper and a 33 μm thick layer of dry film negative photoresist. The copper layer was photolithographically patterned and etched in ferric chloride to define electrical traces on the carrier surface. Following photolithography, carriers were cut to the exact size from the patterned board by circuit milling (Quick Circuit 5000, T-Tech). Individual microelectrodes were fixed to carriers using UV-cured epoxy (3301, Loctite, Rocky Hill, CT). Conductive silver epoxy (Ablebond 8700E, Emerson & Cuming, Billerica, MA) was used to establish the electrical connections to individual microelectrodes with copper strip lines on PCB (Fig. 6.3f).

To insulate individual microelectrodes, a 1.5 μm thick layer of Parylene C was coated over the entire substrate (PDS 2010 Parylene Labcoter, Specialty Coating Systems) (Fig. 6.3g). Parylene C is a well known biocompatible polymer material [63, 64]. It is inert and is optically transparent. Using simple vapor deposition, Parylene C is deposited easily and uniformly on any substrate. The surface roughness is very low, similar to that of Teflon, and the surface is pinhole free.

Sensor tip formation. Microelectrode tips were first beveled (BV-10 Beveler, Sutter Instrument Co.) at 45° above horizontal (for better penetration) for 30 min on a rotating plate under visual control through a microscope to remove Parylene C and Ti/Au layers. The resulting structure with exposed Au, schematically shown in Fig. 6.4(b), formed the solid-state ORP sensor. Gold gives more reliable measurement of ORP than platinum for this application, as platinum may catalyze some additional reactions at its surface [45, 65].

The DO sensors are polarographically recessed cathode Au electrodes. Thus, for DO microelectrodes, a recess was created at the tip of each microelectrode (Fig. 6.4c). To create the recess, the glass core and Ti exposed by beveling were etched using HF-based etchant for 5 min. The beveling and etching steps permit precise control of the recess opening size and depth. The exposed Au was etched in a 1:4:40 (m/m/v) mixture of I_2, KI, and H_2O for 3 min to relocate the Au sensing area inside the formed recess. Microelectrodes were cleaned ultrasonically in DI water after each etching step. For both sensors, tip diameters were on the order of 1–2 μm.

Since Au exposed to the solution enables ORP measurements, both structures in Fig. 6.5b and c could be used as an ORP sensor. The recessed structure in Fig. 6.5c not only can measure ORP but also DO. However, the beveled ORP sensor in Fig. 6.5b has the glass core structure and thus is more robust than the recessed ORP microelectrode in Fig. 6.5c for penetrating samples.

For phosphate sensors, cobalt (Co) was electrodeposited on the exposed gold tips (Fig. 6.5d). Two cobalt plates, 3 × 5 cm, were used as anodes. Plates were cleaned in 20% HCl for 15 min to remove any cobalt oxide layer. An electrolyte solution was prepared by dissolving 33 g $CoSO_4$ and 3 g H_3BO_3 in 100 mL of water. The MEA was placed between the cobalt plates at 1 cm distance from each. Cobalt was electrodeposited to ~0.2 μm thickness on gold tips using a current density of 10 mA/cm^2 for about 2 min.

The needle-type multi-analyte MEAs integrating ORP & DO & phosphate were successfully fabricated, as illustrated in Fig. 6.6. This new MEA sensor consisted of four 2-cm long probes at 900 μm center-to-center spacing packaged on a PCB carrier. The single MEA sensor contained DO, ORP, and phosphate sensors within the array structure.

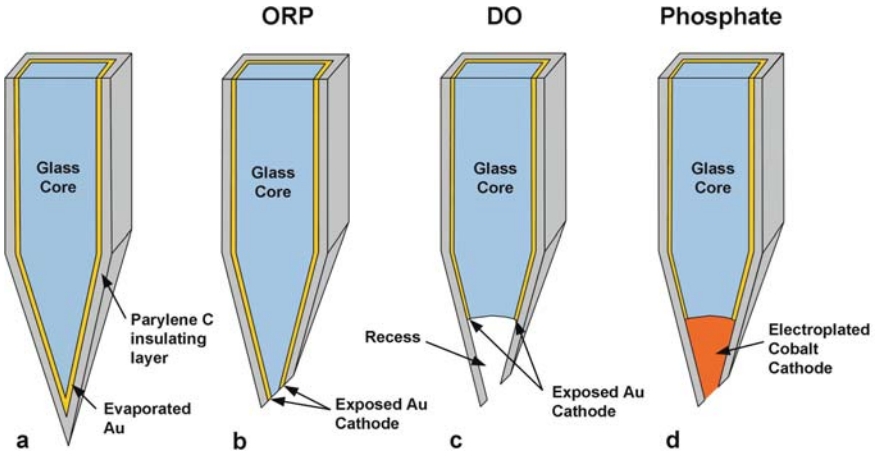

Fig. 6.5 Tip structures of the multi-analyte MEA sensor: (**a**) Parylene C insulating layer covering the entire microelectrode, (**b**) beveled and exposed glass and gold cathode, (**c**) glass and Au etching for the recessed oxygen sensor, and (**d**) cobalt electroplating for the phosphate sensor

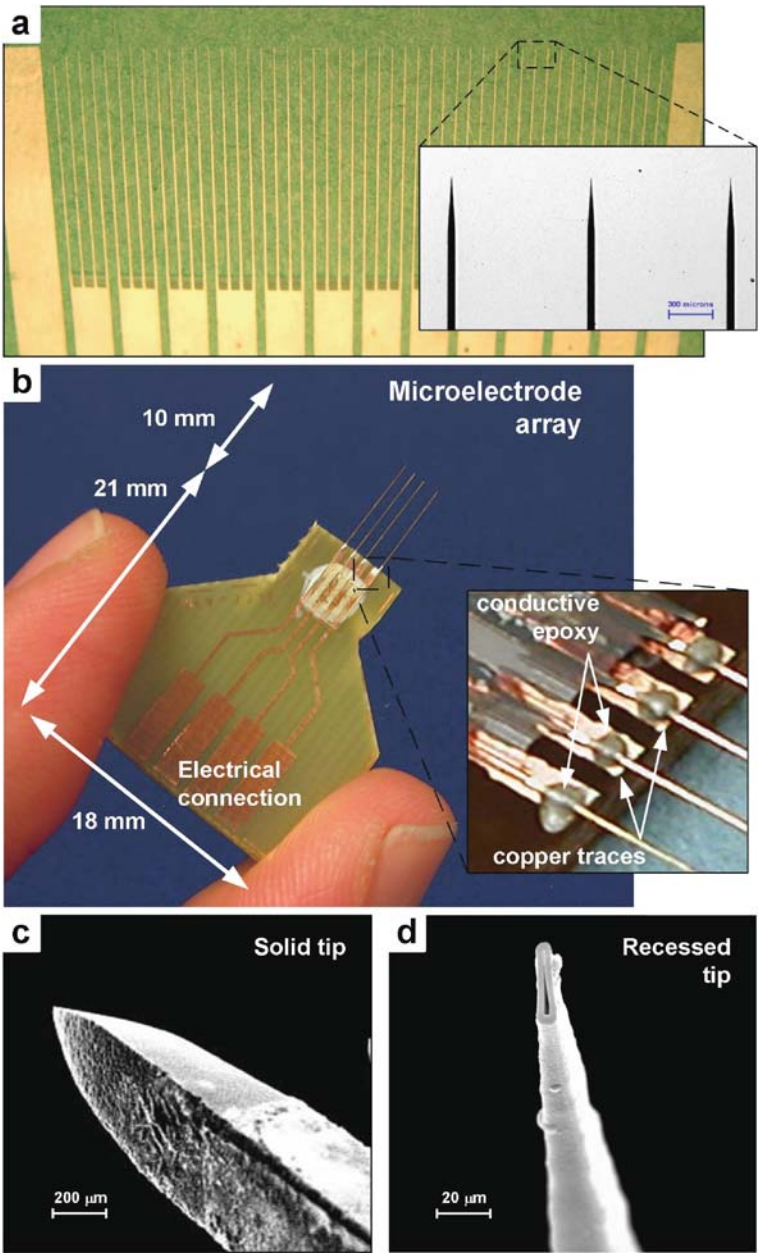

Fig. 6.6 Photographs of (**a**) an etched glass wafer with probe tips at the center and near the edges. Close-up shows parallel tip ends at 900 μm center-to-center spacing, and (**b**) the fabricated DO microelectrode array sensor packaged on a PCB carrier. Close-up shows the design of the MEA packaging using conductive silver epoxy. Scanning electron micrographs (SEMs) of (**c**) sharpened solid tip, and (**d**) recessed tip at 45° beveling angle. (Adapted from [57–59])

6.2.3 ORP MEA Sensor

ORP electrodes directly measure the potential of a solution. This is in contrast to the ion selective electrodes that measure a potential that is proportional to the concentrations of the chemical species in a solution. Thus, an ORP electrode is not specific, and measures the oxidized and reduced forms of all chemical species in the solution. Consequently, it is standard practice to verify the performance of an ORP electrode against standard and reference solutions. Three ORP reference solutions of 450, 228, and 90 mV at 25°C (Sensorex Corp., Garden Grove, CA) and Orion ORP standard solution of 221 mV (Thermo Fisher Scientific, Beverly, MA) were used to investigate performance of the OPR sensors. A commercial Ag/AgCl milli-electrode (MI-401, Microelectrodes Inc.) was used as reference. The American Society for Testing and Materials (ASTM) standard D1498 [66], recommends that measured redox potentials should be within 10 mV of the nominal redox potentials for a good redox electrode.

The standardization curve of the ORP sensor is shown in Fig. 6.7a. The MEA sensor is compared with a commercially available millielectrode (COM) and a conventional pulled-glass pipette microelectrode (ME) against the four redox standard or reference solutions. The slopes of the three curves are very close to the theoretical value of 1.00, which clearly indicates that the ORP MEA compares very well with the conventional two electrode types and is deemed acceptable for measurements of redox potentials.

The ORP values can be correlated to the logarithm of the hydrogen concentration (i.e., pH) with a linear relationship. This is illustrated in Fig. 6.7b. The ORP MEA sensor showed a log-linear ORP response down to a hydrogen concentration of 10^{-10} M (pH 10). The sensitivity of the ORP MEA sensor (change in redox potential per pH unit) is calculated to be ~61.5 mV/pH, which is very close to the ideal calculated slope of 59 mV/pH as reported by Pang and Zhang [45].

The response time of the MEA was substantially faster than that of the commercial milli-electrode (COM) due to smaller tip size and simple thin film structure. Figure 6.7c illustrates the representative results. Overall, the MEA reached 99% of the final stable reading in less than 1 s for the ferrous-ferric standard solution, in approximately 10 s for both the pH 4 quinhydrone reference and the Orion ORP standard solutions, and in less than 30 s for the pH 7 quinhydrone reference solutions. Under the same conditions, the response times for the commercial milli-electrode were 2 min for the ferrous-ferric standard solution, approximately 5 min for both the pH 4 quinhydrone reference and the Orion ORP standard solutions, and more than 10 min for the pH 7 quinhydrone reference solution [55, 56]. In *in situ* monitoring, fast response time means lower power consumption for the sensor system, especially if a power-down protocol between measurements is used.

The MEAs proved to be extraordinarily stable. The stability of the MEA was evaluated by continuously measuring redox potential of the Orion ORP standard solution (Fig. 6.7d). Both MEA and an Ag/AgCl reference electrode were in the standard solution for the duration of the experiment, while potential measurements

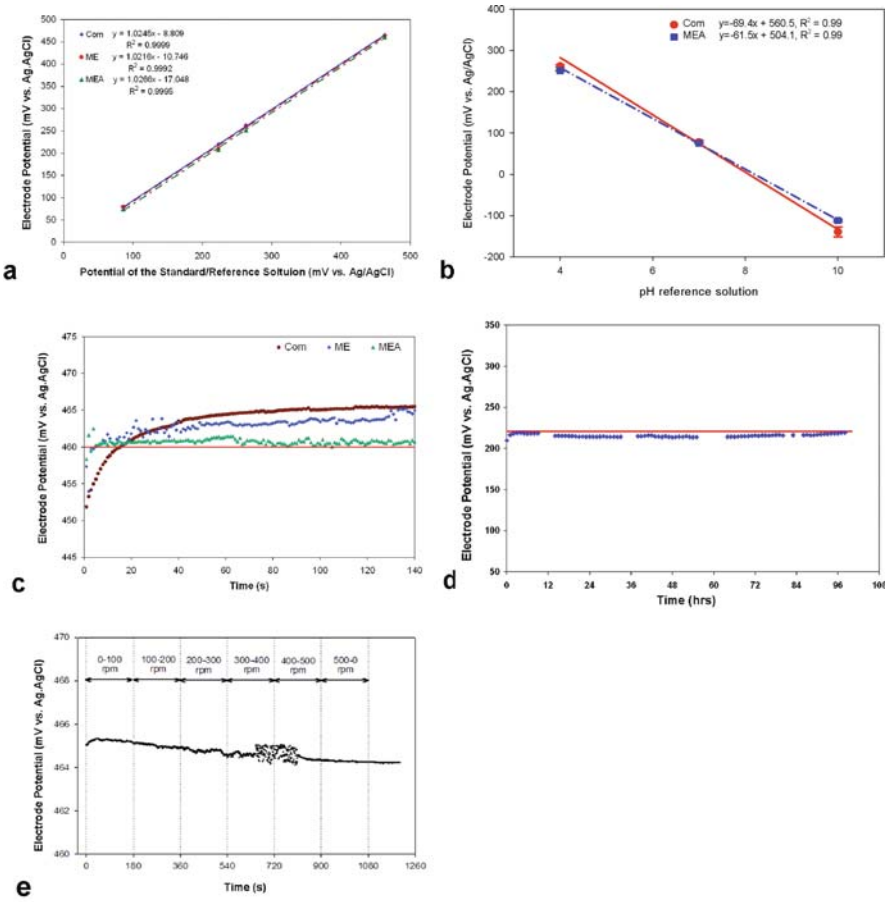

Fig. 6.7 Characterization of ORP MEA sensor: (**a**) standardization curves of the three ORP electrodes against four redox standard or reference solutions with respect to Ag/AgCl reference electrode, (**b**) *pH sensitivity of the ORP MEA and the commercial milli-electrode in ferrous-ferric standard solution*, (**c**) response times in ferrous-ferric standard solution at 23°C, (**d**) redox potential of Orion ORP standard solution measured at 23°C, and (**e**) performance in ferrous-ferric standard solution for changing mixing intensity. From [55, 56]

were recorded hourly. After four days, the average measured ORP was 215.5 mV with a standard deviation of 1.7 mV, which is well within the ±10 mV specification of the ASTM [66].

To evaluate the long term stability, the MEA was rinsed with water, dried with nitrogen, and stored on a shelf from several days to several months. Repeated measurements following storage showed accurate and reproducible measurements without special cleaning or reconditioning procedures. The most common problem reported for aqueous samples is that readings can differ by a significant margin, as much as 50–100 mV, even though the sensors are in the same solution. Here, the

ORP MEA sensor produced a very stable response over the course of several days without the need of a Faraday cage.

The stirring effect on ORP measurements was also investigated to verify the sensitivity, the reliability, and the stability of the ORP MEA [55, 56]. For *in situ* monitoring, convection of sample medium is a critical factor in measurement error. The experiment was carried out bysequentially inserting the integrated microelectrode into standard ORP solutions using five different stirring velocities. As shown in Fig. 6.7e, the redox potential profile exhibited a trend of a very gradual decrease, as stirring intensity increased. The slightly unstable potential profile between 300~500 rpm occurred when the stirring bar began bumping the beaker wall. The stirring effect (Re) can be calculated as [67]:

$$\mathrm{Re} = \left[\frac{(V - V_1)}{V}\right] \times 100\% \tag{6.1}$$

where V_1 is the potential measured from the unstirred sample, and V is the potential measured from the stirred sample. Even with artificial turbulence at 300–500 rpm, the measured ORP variability was less than 1 mV which is Re ~ 0.2%. Thus, it can be concluded that the signal was not substantially influenced by stirring.

6.2.4 DO MEA Sensor

The DO sensor calibration set up is schematically illustrated in Fig. 6.8. The DO MEA sensors were polarized and calibrated with a commercial Ag/AgCl reference electrode (MI-401, Microelectrodes Inc.). The polarization voltage and current were supplied by a Chemical Microsensors II potentiostat (Diamond General Development Corp., Product No 1231). The –750 mV polarization voltage was applied to the oxygen microelectrodes against the Ag/AgCl reference electrode for at least several hours prior to calibration. Negative applied voltage can reduce the amount of O_2 on the cathode of the microelectrode surface, and given sufficient potential and time, O_2 concentration can be reduced to zero. Thus, the residual oxygen which can cause measurement errors is removed by a polarization process to permit measurement of more accurate DO values. Following polarization, a test solution was prepared by aeration of 0.85% saline solution with pure nitrogen gas (0% O_2 or 0 mg/L DO), a gas mixture containing 10% O_2 and 90% N_2 (10% O_2 or 4.1 mg/L DO), and air (21% O_2 or 8.7 mg/L DO). The aeration was applied for at least 20 min to establish a stable concentration. A commercial oxygen milli-electrode (MI-730, Microelectrodes Inc.) was used to verify the concentration of oxygen in bulk solution and during calibration.

The electrolyte in saline solution plays an important role in an electrical connection between the working electrode and the reference electrode [64]. The maximum solubility of dissolved oxygen in natural water at 25°C is 8.7 mg/L (or 21% O_2).

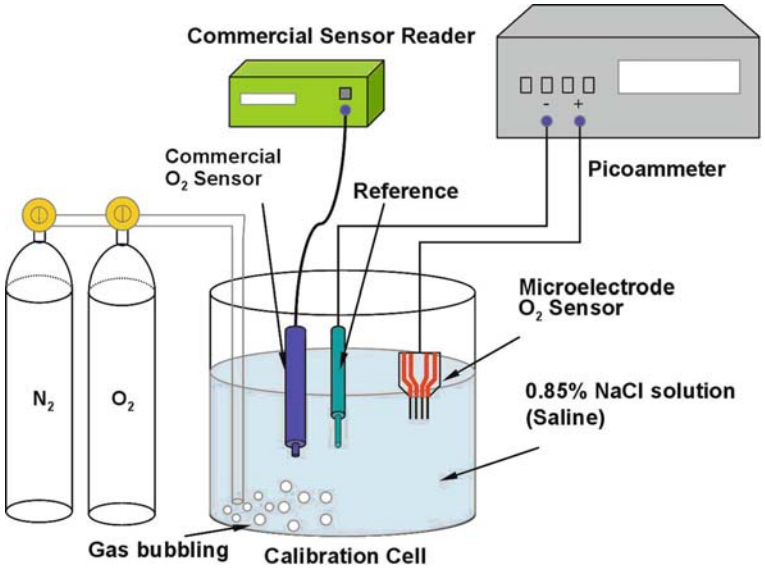

Fig. 6.8 Schematic diagram of the calibration cell for DO MEA characterization. A picoammeter was used to measure current directly with respect to a commercial Ag/AgCl reference electrode

Therefore, this characterization range represents all soluble water in the environment. For example, DO concentration in tap water measured using a commercial DO electrode under normal laboratory conditions (25°C) was ~17% O_2 or ~7 mg/L.

A cyclic voltammetry test was performed in two different DO concentrations of saline solution at a scan rate of 10 mV/s (Fig. 6.9a). Pure nitrogen gas was used to prepare the 0 mg/L (0% O_2) in the saline solution, and the 8.7 mg/L (21% O_2) in the saline solution at ambient temperature was used in comparison. Initial and switching potentials were 0.2 V and –1.2 V, which were applied to a recessed DO working microelectrode. The cyclic voltammogram shows good oxidation and reduction curves to verify the reliable working electrode and also confirms that the applied bias of –750 mV was appropriate. The 0 mg/L saline solution curve is lower due to the lack of oxygen. It is clear that the measured current is proportional to the DO concentration.

Response of a sensor as a function of DO concentration is shown in Fig. 6.9b. Calibration curve shows a linear relationship between the current response and the DO concentration, with high correlation coefficients. The sensor performed linearly, and exhibited a high sensitivity of ~200 pA/mg/L in saline. Standard deviations in these measurements were very low, indicating low variability and high stability. The time for 90% response (t_{90}) was typically less than 20 s, which is much shorter than that of macroscale commercial oxygen electrodes. Figure 6.9c illustrates response of a sensor to a challenge with three different concentrations of DO. These characteristics are a substantial improvement over the previously reported microelectrodes constructed from pulled glass pipettes which exhibited similar response times but

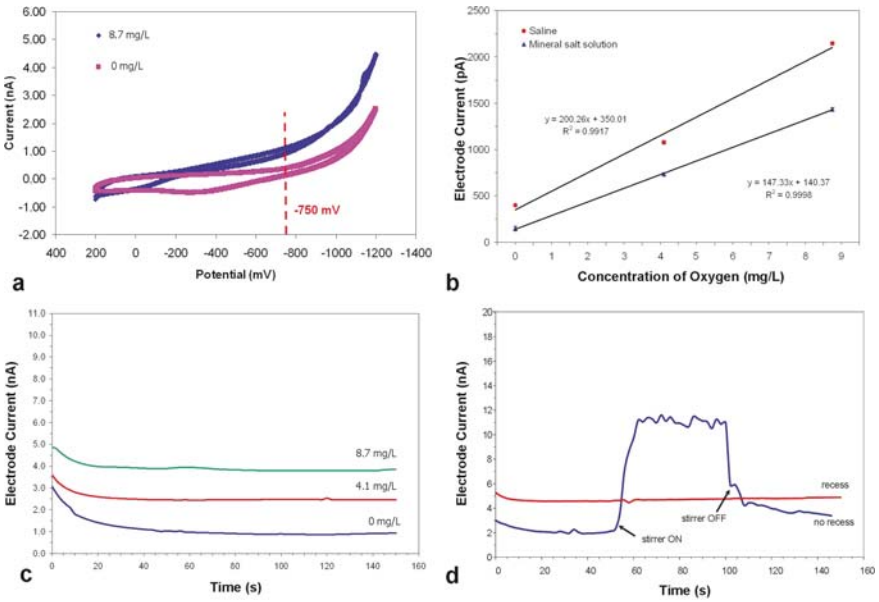

Fig. 6.9 Representative results of DO MEA sensor characterization. (**a**) Cyclic voltammogram (CV) curve for 0 mg/L and 8.7 mg/L DO in saline solution at a scan rate of 10 mV/s. (**b**) Calibration curves in 0.85% NaCl saline and mineral salt solutions. (**c**) Representative current response for an MEA sensor at three DO concentrations. (**d**) Representative stirring effect results that compare response of recessed and non-recessed sensor tips at 500 rpm agitation. Adapted from [57, 58]

lower sensitivities of ~13 pA/mg/L [26]. This increased sensitivity is attributed to the differences in recess dimensions and electrode surface areas.

The stirring effect is an important factor for the sensitivity, the reliability and the stability of this recess-type DO sensor. The stirring effect test compared DO measurements of open (Fig. 6.4b) and recessed (Fig. 6.4c) tips at 500 rpm agitation. For the exposed open tip the current signal changed drastically when the test solution was stirred, and rebounded back to the initial current level when the stirrer was turned off (Fig. 6.9d). The stirring effect (Re) can be calculated in this case as [64]:

$$\mathrm{Re} = \left[\frac{(I - I_1)}{I}\right] \times 100\% \tag{6.2}$$

where I_1 is the current measured from the unstirred sample, and I is the current measured from the stirred sample. The test result reported that the non-recessed MEA (i.e., prior to etching steps) exhibited a strong variation in signal due to stirring at 500 rpm, with Re ~ 23%. The recessed cathodes exhibited no change in signal, with Re ~ 0%. This stirring effect result agreed with the previous reports [68–70]. Thus, the recessed structure with the inner cathode improves sensitivity and stability of the DO microelectrode.

6.3 Phosphate MEA Sensor

Cobalt (Co) metal was used as the electrode material for the phosphate measurements [59]. This direct measurement approach is based on the formation of $Co_3(PO_4)_2$ precipitate on Co surface [71–74]. The sensing mechanism involves dissolution of cobalt on the electrode surface and formation of oxide film [72]:

$$2Co + 2H_2O \leftrightarrow 2CoO + 4H^+ + 4e^-$$

$$O_2 + 4H^+ + 4e^- \leftrightarrow 2H_2O$$

$$2Co + O_2 \leftrightarrow 2CoO$$

Phosphate present in solution leads to the formation of cobalt phosphate on the electrode surface, depending on the solution pH [72]:

$$3CoO + 2H_2PO_4^- + 2H^+ \leftrightarrow Co_3(PO_4)_2 + 3H_2O \qquad \text{(at acidic pH)}$$

$$3CoO + 2HPO_4^{2-} + H_2O \leftrightarrow Co_3(PO_4)_2 + 4OH^- \qquad \text{(at neutral pH)}$$

$$3CoO + 2PO_4^{3-} + 3H_2O \leftrightarrow Co_3(PO_4)_2 + 6OH^- \qquad \text{(at basic pH)}$$

These coupled reactions show the shift in the equilibrium potential that is dependent upon oxidation of cobalt, reduction of oxygen, and $Co_3(PO_4)_2$ precipitate forming on the electrode surface. This leads to a shift of the mixed potential to more negative, while keeping other factors constant. The shift is related to the phosphate concentration since equilibrium potentials are governed by the Nernst equation [75]. Thus, a linear potential response may be expected with exponential change in phosphate ion concentration (at constant levels of dissolved oxygen) [76]. Such Co-based phosphate sensors are highly selective, stable, and inexpensive, and can detect both the inorganic and organic phosphate [72–74].

The cobalt-coated phosphate MEA was characterized with six different concentrations of standard solution ranging from $10^{-5.1}$ to 10^{-3} M KH_2PO_4 at ambient temperature [59, 60]. Based on the pH value in which typical biological nutrient removal systems are operated, the pH of each standard solution was adjusted to pH 7.5 by adding potassium hydroxide. At pH 7.5 standard solutions have dihydrogen phosphate ion and hydrogen phosphate ion. The phosphate MEA was oxidized by immersing in DI water along with a Ag/AgCl reference electrode for 30 min, followed by 30 min in 10^{-4} M KH_2PO_4 solution. The data acquisition system included the Denver Instrument pH/mV meter (model 225) and BalanceTalk Software (Labtronics Inc.). The long-term stability, stirring effect, oxygen sensitivity, and interference tests were conducted to characterize sensor performance at room temperature.

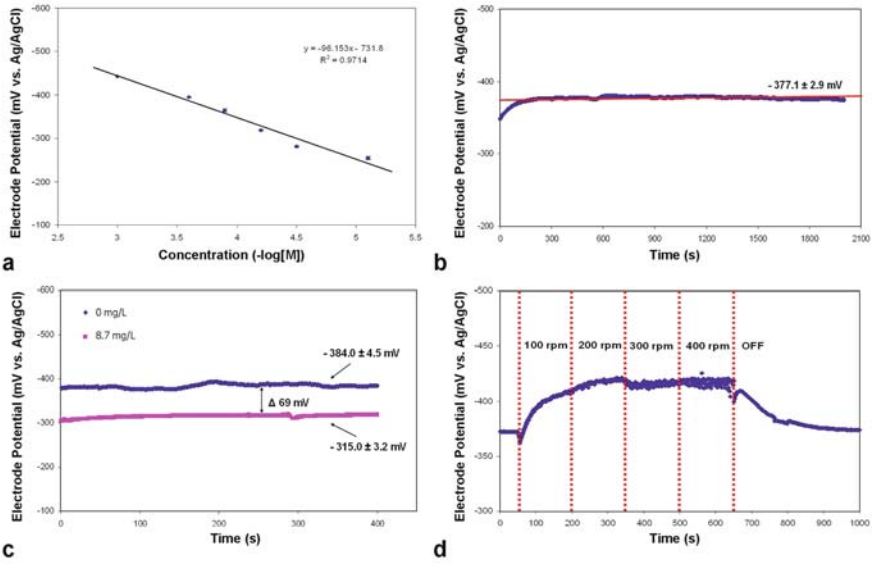

Fig. 6.10 Characterization of phosphate MEA sensor at pH 7.5: (**a**) calibration curve in various concentrations of KH_2PO_4, (**b**) long-term stability test in $10^{-3.9}$ M KH_2PO_4, (**c**) the oxygen effect in $10^{-3.9}$ M KH_2PO_4, and (**d**) the stirring effect in $10^{-3.9}$ M KH_2PO_4 [59]

Electrochemical characterization of the phosphate MEA showed a linear response over a wide range of concentrations. The data is plotted in Fig. 6.10a. The sensor exhibited a high sensitivity of about 96 mV per decade of KH_2PO_4 concentration in the $10^{-5.1}$ to 10^{-3} M range. Others have shown phosphate sensors based on cobalt rods and wires to have a linear response in the 32–55 mV per decade change of phosphate concentration [71–74]. A phosphate sensor recently demonstrated using Co thin film in a planar microfluidic chip exhibited a lower sensitivity of ~35 mV per decade change of concentration [77]. This significantly increased sensitivity of the phosphate MEA is most likely due to the 3-D thin film structure of the sensor and the simple electrical interface. The response time (t_{90}) of the MEA ranged from ~1 to 30 s as KH_2PO_4 concentration was decreased from $10^{-3.1}$ M to $10^{-5.1}$ M at pH 7.5.

The long-term stability of the phosphate MEA was monitored by continuously measuring potential of the $10^{-3.9}$ M KH_2PO_4 solution. The results are shown in Fig. 6.10b. After 30 min, the average measured potential was –377.1 mV with a standard deviation of 2.9 mV. The sampling frequency was 1 Hz. The phosphate MEA produced a very stable response without a Faraday cage.

Oxygen has been reported to affect activity of phosphate ions [69, 70]. The sensor response to $H_2PO_4^-$ ions should decrease with increasing oxygen concentration. The effect of dissolved oxygen was evaluated using 0 mg/L (0% DO) and 8.7 mg/L (21% DO) concentrations in a $10^{-3.9}$ M KH_2PO_4 solution. Nitrogen gas and air were bubbled for more than 20 min to produce 0 and 21% DO solutions

using the same experimental setup for DO MEA characterization as described previously. A commercial oxygen milli-electrode (MI-730, Microelectrodes Inc.) was used to verify concentration of oxygen in the test solution and during calibration. Figure 6.10c shows ~69 mV offset between 0 and 21% DO in the same $10^{-3.9}$ M KH_2PO_4 solution. This is a substantial change, significantly higher than the measurement variations (standard deviations are <4.5 mV). Thus, oxygen calibration should be performed before or with phosphate measurements. In this work, all phosphate measurements were performed in ambient conditions with ~17% DO (measured using the commercial DO sensor).

The effect of stirring in $10^{-3.9}$ M KH_2PO_4 at pH 7.5 was also investigated to verify the stability of the phosphate MEA. The experiment was performed by measuring sensor output at four sequentially increasing stirring velocities. As shown in Fig. 6.10d, the electrode potential profile exhibited an immediate change of about 30 mV when 100 rpm agitation was applied, yielding Re ~6%. However, higher velocities (200, 300, and 400 rpm) showed a stable potential with a slightly increased Re ~11%, independent of stirring intensity. The electrode potential returned to non-stirring potential immediately when stirring was turned off. Given the demonstrated sensitivity to the dissolved oxygen, this stirring effect sensor response is expected as agitation influences oxygen diffusion on the sensor surface.

Meruva and Meyerhoff suggested the mixed potential response mechanism of cobalt electrode sensors toward inorganic phosphate. According to this mechanism, a slow oxidation of cobalt occurs at the electrode surface coupled with simultaneous reduction of oxygen and the formation of $Co_3(PO_4)_2$. Thus, the sensor detects mixed potential due to both oxidation and reduction. This mechanism characteristic provides a reasonable explanation of the cobalt-based phosphate sensor, and especially for the effects of dissolved oxygen and stirring.

As discussed earlier, cobalt-based electrodes have been reported not to be susceptible to interference from Cl^-, NO_3^-, and SO_4^{2-} ions [68, 69]. In order to confirm it, the phosphate MEA was used to measure phosphate ions while subjected to interference from several ions typical in wastewater. The ion concentration in each test solution was 0.015 g/L of CH_3COO^-, 0.043 g/L of Cl^-, 0.196 g/L of NO_3^-, and 0.196 g/L of SO_4^{2-}. The concentrations were based on those typical of an activated sludge sample [70]. The phosphate MEA sensor was used for the above tests and stored in a dry desicator for one month prior to performing these interference tests. Results in Fig. 6.11 show a good linear relationship between the electrode potential and the phosphate concentrations for each interference ion tested. The "no-ion" condition was the control test without presence of interference ions. The exhibited sensitivities of the phosphate MEA per decade change of KH_2PO_4 concentration were 67.3 mV for CH_3COO^-, 72.1 mV for Cl^-, 75.8 mV for NO_3^-, 65.4 mV for SO_4^{2-}, and 70.1 mV for the "no-ion" control. Thus, the interference tests yielded a sensitivity of 70.2 ± 4.7 mV per decade of KH_2PO_4 concentration, which compares very well to the 70.1 mV per decade value measured for the control. Overall the interference effect was not significant, but for accurate measurements, pre-calibration should be performed.

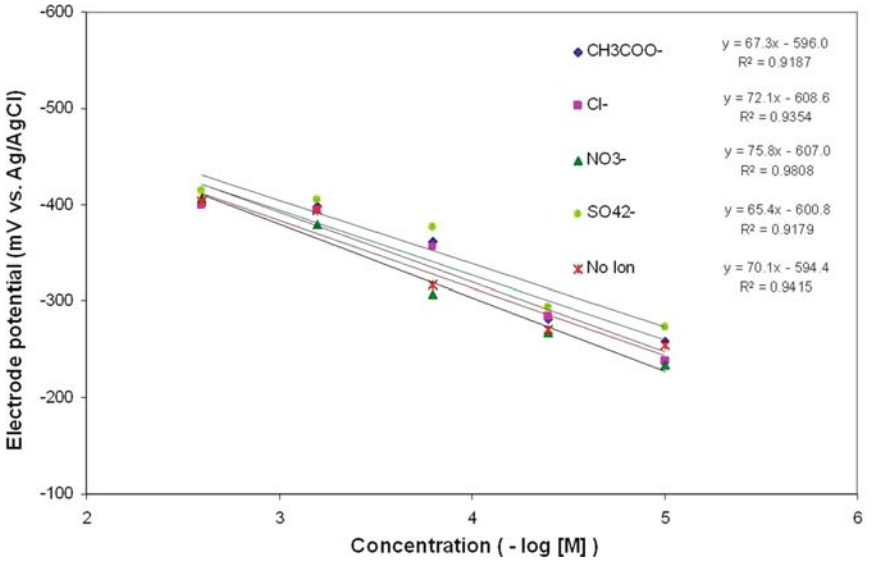

Fig. 6.11 Results of the interference tests by the phosphate MEA sensors in various concentrations of KH_2PO_4 at pH 7.5. No meaningful differences were found, indicating no interference susceptibility to the tested ions [59]

6.3.1 DO and ORP Microprofile Measurements in Biofilms

A multi-species biofilm was developed for the DO and ORP microprofile measurements using activated sludge from a municipal wastewater treatment plant aeration tank (Mill Creek WWTP, Cincinnati, OH) [58]. Activated sludge was decanted, washed with DI water several times, and transferred to Petri dishes. Frosted glass slides (12-544-5CY, Fisher Scientific) were placed inside of the Petri dishes for biofilm formation and growth. After 24 h, biofilm containing glass slides were suspended from the top of a closed reactor, schematically illustrated in Fig. 6.12a. A two ring polycyclic aromatic hydrocarbon, naphthalene, was used as the sole carbon source. In order to obtain a stable influent concentration of naphthalene (around 18 ~ 20 mg/L), a 20 L glass jar was used as a feed solution tank, containing an excess amount of crystal naphthalene (4 g/L) mixed with mineral salt solution. The mineral salt solution was prepared by mixing 32 mg/L of $NaNO_3$, 10 mg/L of NH_4Cl, 40 mg/L of Na_2HPO_4, 10 mg/L of KH_2PO_4, 1.4 mg/L of $CaCl_2$, 3.8 mg/L of $MgSO_4$, 0.65 mg/L of $FeCl_3$, 11.2 μg/L of $MnSO_4$, 0.7 μg/L of $CuSO_4$, 0.4 μg/L of $NaMoO_4$, and 12 μg/L of $ZnSO_4$. To remove naphthalene particles from the influent, a fabric filter was attached to the outlet line of the naphthalene tank and the flow was cycled through the closed biofilm reactor with a peristaltic pump (Cole-Parmer Instrument Co., Chicago, IL). Feed solution with naphthalene was prepared in advance; naphthalene was allowed to dissolve for three days before it was used. The feed solution was changed every three days. Hydrogen peroxide

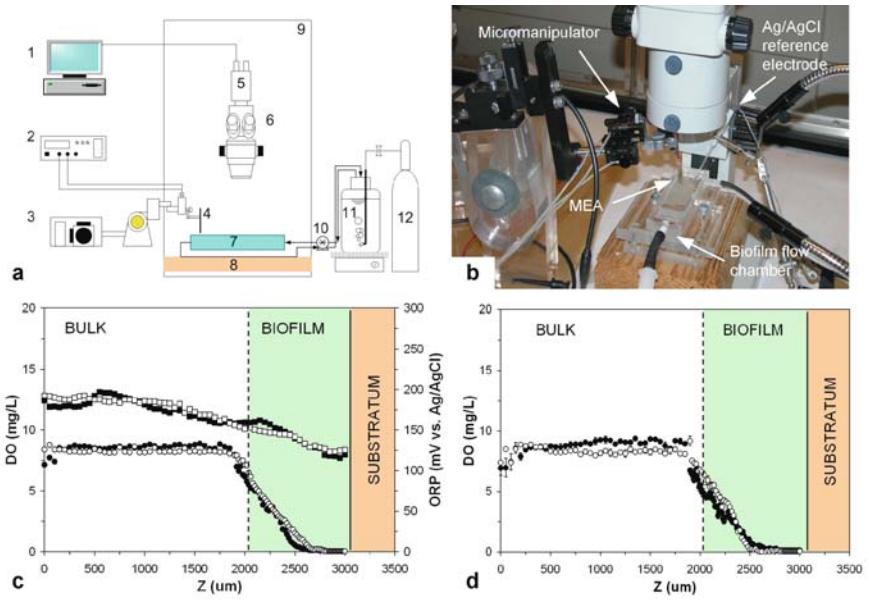

Fig. 6.12 DO and ORP measurements in Biofilm: (**a**) schematic diagram of experimental setup (*1*. video display; *2*. data acquisition system; *3*. 3-D micromanipulator; *4*. ME or MEA; *5*. charge-coupled device (CCD) camera; *6*. stereomicroscope; *7*. open-channel chamber; *8*. vibration isolation table; *9*. Faraday cage; *10*. peristaltic pump; *11*. feed tank; and *12*. oxygen cylinder), (**b**) photograph of the experimental setup, (**c**) microprofiles of dissolved oxygen (DO, ●○) and oxidation-reduction potential (ORP, ■□) in biofilm. Filled shapes represent microprofiles with the MEA (one sensor of each type) and empty shapes represent microprofiles with the ME. The data is the average of six measurements, and (**d**) DO microprofile recorded with the MEA during the biofilm insertion (●) and withdrawal (○). The data is the average of six measurements. From [58]

(0.5%) was added as a supplemental oxygen source to maintain 5.0 ± 0.5 mg/L DO in the reactor.

Biofilm microprofile measurements. Figure 6.12b shows the experiment setup for the microprofile measurements inside the biofilm [58]. Multispecies biofilm grown on slides were taken from the closed reactor and placed in the open channel test chamber to obtain oxygen and redox potential profiles. The open channel chamber (7.85 cm L, 2.9 cm W, 1.2 cm H) was mounted under a stereo microscope with a charge-coupled device (CCD) camera (Model JE-3662 HR, Javelin Elec., Torrance, CA) situated on a Micro-g series high-performance vibration isolation table (63-527-01, TMC, Peabody, MA) inside a Faraday cage (TMC, MA). Feed solution with the same composition as in the closed biofilm reactor was continually aerated and recycled (2.35 mL/min) through the open channel chamber with a peristaltic pump mounted outside of the Faraday cage. The biofilm sample was placed in the chamber for at least 30 min before performing measurements. Microelectrodes were mounted and positioned using a motor-driven 3-D micromanipulator (Model 11N, Narisige, Japan). Oxygen profiles were measured at 10–50 μm intervals into the biofilm; ORP

profiles were measured at 50 μm intervals. To monitor the reproducibility of the electrodes and any possible damage to the biofilm by the microelectrode penetration, the oxygen profile measurements in the biofilm were conducted during both the microelectrode penetration and withdrawal stages and compared.

To demonstrate the versatility of the new MEA electrode technique in biofilm studies, DO and ORP microprofiles were obtained and compared using both the MEA and the conventional ME under the same conditions. Multi-species biofilm grown on slides for one month was taken from the closed reactor and placed in the open channel test chamber in order to obtain oxygen and oxidation redox potential profiles. The same biofilm was used with both MEA and ME sensors. Identical electrode positioning was achieved by using a small marker on the biofilm and a stereo microscope with a CCD camera. In the case of the conventional MEs, DO and ORP electrodes were separately prepared and mounted on the 3-D micromanipulator before each measurement. The MEA was constructed with integrated DO and ORP sensors in one body, and thus could obtain DO and ORP profiles simultaneously. The entire measurement process was monitored using a color monitor connected to a stereomicroscope with a video camera; the image was lit from above with a high intensity lamp during video observation. The bottom substratum of the biofilm and biofilm thickness were defined as the point where the electrode hit the substratum and visually bent. The whole system had clean electrically-grounded lines.

DO Microprofile. Figure 6.12c shows the microprofile changes obtained using both MEA and ME sensors [58]. The DO in the bulk solution was determined to be around 8.5 mg/L and decreased through the biofilm's mass transfer boundary layer to 5.9 mg/L with the MEA or to 6.2 mg/L with the ME at the biofilm surface. The thickness of the DO mass transfer boundary layer was estimated to be around 200 μm, and dissolved oxygen decreased by about 2.6 mg/L in this region. Inside the biofilm, oxygen decreased continually and was totally depleted at 700 μm depth, according to both the MEAs and MEs. This result confirms that an oxic zone inside of the mixed species biofilm is several hundred micrometers thick. At the biofilm depth of 300 – 500 μm, small concentration differences of about 1 mg/L DO concentration were observed between the two electrodes. It is not clear whether this was caused by the heterogeneity of the biofilm, due to slight differences of positioning of the electrodes (microelectrode spacing in the MEA is 900 μm center to center), or to signal differences between the MEA and ME. Nevertheless, there is a strong correlation between the MEA and ME measurements ($r\,(82) = 0.98$, $p < 0.01$). The DO MEA electrode has a larger gold surface area that produces approximately 10 times larger current signals than the ME.

To monitor reproducibility of the microprofile measurements and any possible damage to the biofilm by the MEA penetration, the DO measurements in the biofilm were performed during both penetration and withdrawal (i.e., in-and-out technique). Figure 6.12d illustrates that the same microprofile is obtained using the MEA during both penetration and withdrawal. Correlation analysis on these data yields a coefficient of $r\,(82) = 0.97$, $p < 0.01$. No structural damage to the biofilm was observed during these measurements.

ORP Microprofile. The ORP in the bulk solution was approximately 180–190 mV (1000 μm from the surface of the biofilm) and decreased gradually to 160 mV at the biofilm surface (Fig. 6.12c) [58]. In the biofilm, the redox potential profiles provided by both the MEA and the ME also exhibited a gradual decrease from the surface to the substratum. The MEA measured 119.6 mV near the substratum, which compares well with the 125.3 mV measured by the ME. The ORP profiles revealed that both the MEA and ME performed similarly. The ASTM standard D1498 [63] states that the measured redox potentials for a good redox electrode should be within ±10 mV of the nominal redox potential. Both electrodes behaved within the error range. A correlation analysis on these data yields a coefficient of $r\,(64) = 0.96$, $p < 0.01$, indicating a nearly perfect correlation. Among the 66 measurement points, all but five differed by less than 10 mV. As with DO measurements, the difference between electrodes is possibly due to the biofilm heterogeneity or due to slight differences of positioning of the electrodes.

6.3.2 Phosphate Microprofile Measurements in Biofilms

For phosphate microprofile measurements, a Sequencing Batch Reactor (SBR) was operated for more than six months in an Enhanced Biological Phosphorus Removal (EBPR) process mode to grow phosphate accumulating organisms (PAOs); these microorganisms are responsible for the high removal efficiency in the phosphate removal system [60]. The sludge retention time (SRT) of the reactor was 10 days, and the hydraulic retention time (HRT) was 8 h, including 2 h in the anaerobic zone and 3 h in the aerobic zone. The concentration range of the Chemical Oxygen Demand (COD) was 200 ~ 250 mg/L in the influent and 5 ~ 10 mg/L in the effluent. The average influent phosphorus concentration and the effluent phosphorus concentration were about 2.6–3.3 mg/L as P and 0.2 mg/L as P, respectively. During the experiment periods, Mixed Liquid Suspended Solids (MLSS) was 1900–2000 mg/L.

Biofilm Microprofile Measurements. The microprofile measurements were carried out inside a Faraday cage (TMC, MA) to minimize signal noise; nitrogen gas was injected into the feed tank to establish anaerobic conditions [60]. An up-flow chamber with laminar flow conditions was used during microprofile measurements so that the floc was kept suspended but stationary while the microelectrode was inserted into the floc in the flowing liquid [78]. The up-flow chamber was mounted under a stereo microscope with a CCD camera (Model JE-3662 HR, Javelin Elec., Torrance, CA) situated on a Micro-g series high performance vibration isolation table (63-527-01, TMC, Peabody, MA) inside the Faraday cage. The flow rate was controlled with a needle valve and the water inside the up-flow chamber overflowed evenly though four outlets at the upper part of the chamber, as shown in Fig. 6.13a. By controlling the velocity into the up-flow chamber, the floc could be stabilized in suspension. A stereomicroscope, a CCD camera and color monitor were used to monitor the stabilization of the flocs and location of the microelectrode's tip. Positioning and movement of the MEMS MEA tip toward the floc was conducted

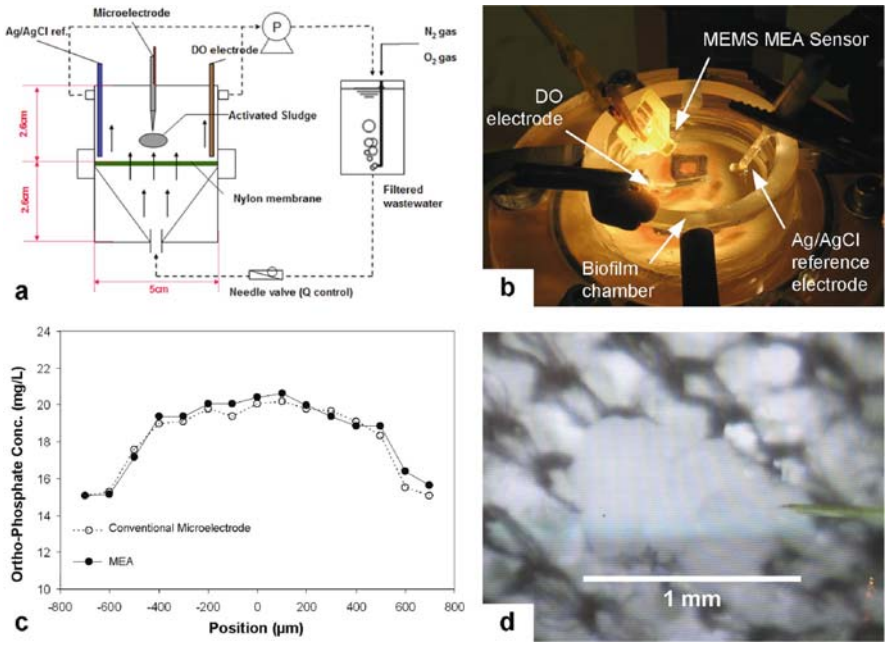

Fig. 6.13 Phosphate measurements in Biofilm: (**a**) schematic diagram of up-flow chamber (adapted from [73]), (**b**) photograph of up-flow chamber, (**c**) microprofiles of phosphorus in flocs from the EBPR process, and comparison with the phosphorus microprofiles from conventional microelectrode (ME) and phosphate MEA, and (**d**) penetration of microelectrode through the floc during microprofiling [60]

using a 3-D micromanipulator (Model 11 N, Narisige, Japan) controller which was located outside the Faraday cage. A pH meter (Model 215, Denver Instruments, Denver, CO) was used to obtain potentiometric signals (mV) and a Balance Talk SLTM (Labtronics Inc., Guelph, Ontario, Canada) spread logger was used to record these electrode response (mV) continuously for monitoring the phosphate response. An Ag/AgCl milli-electrode (MI-401, Microelectrodes Inc., Bedford, MA) was used as a reference electrode. A commercial oxygen mini-electrode (OM-4 Oxygen sensor, Microelectrodes Inc., Bedford, MA) was used to measure the oxygen concentration in the up-flow chamber while monitoring phosphate in the anaerobic phase in the EBPR process.

Phosphate Microprofile Measurements. During the EBPR process, PAOs in the reactor released P to increase the 3.0 mg/L of influent phosphate to 15.0 mg/L as P at the end of the anaerobic phase, and then performed luxury uptake in the aerobic phase. At the end of the aerobic phase, the phosphate concentration was down to 2.0 mg/L as P. The *in situ* measurement of the phosphate MEA sensor was conducted in the anaerobic phase where the dissolved oxygen was below 1 mg/L and thus caused no interference on the selectivity of the phosphate. Three to five microprofiles of each microbial floc were measured in the up-flow chamber in a Faraday cage,

and nitrogen gas was injected into the feed tank to establish anaerobic conditions. The flocs for phosphate measurement were taken at the end of the anaerobic stage of the EBPR process. Therefore, it was assumed that enough phosphorus release had occurred throughout the reactor and reached a pseudo steady-state condition. The measurement of profiles was taken within 30 min. The microelectrode readings were recorded at 100 μm intervals. Well-defined phosphate profiles across the flocs were observed under the anaerobic conditions, during which phosphorus was released from the flocs, as determined using the MEMS microelectrode (Fig. 6.13c).

Figure 6.13d shows the picture of penetration of MEMS MEA array microelectrode into the floc [60]. The diameter of the floc was about 1000 μm, and the center of the floc is depicted as a depth of 0 μm in Fig. 6.13c. Based on the profile measurements, the phosphate concentration of 15.1 mg/L as P in the bulk solution increased as the floc was penetrated toward the center of the floc.

Compared with the microprofiles obtained using the conventional cobalt-based microelectrode, the results show very similar patterns of phosphate concentration through the flocs, according to the depth (Fig. 6.13c). Phosphate concentration in the bulk solution was about 15 mg/L as P in the anaerobic bulk phase and increased to 20.6 mg/L as P with penetration toward the center of the floc. Wang and Bishop [71], using microelectrode and fluorescent *in situ* hybridization (FISH) techniques, indicated that the higher phosphate concentration in the floc center was due to a higher density of PAOs at the center of the floc than that at the edge of the floc. The diffusion boundary layer in which the phosphate concentration started to change near the floc was defined as 100 μm thick.

The developed MEMS MEA sensors were thus able to penetrate biological samples in order to perform phosphate measurements, and will enable *in situ* analysis in many biological applications for measurement of phosphate. These microelectrode sensors can be effective research tools for elucidating the transport phenomena occurring within biofilm reactors by measuring the concentration microprofiles of species of interest in the biological aggregates without destroying the biofilm structures.

6.4 Conclusions

This chapter described the development of the needle-type multi-analyte MEMS sensor arrays for *in situ* measurements in biofilms. A batch fabrication approach was used to increase yield and consistency of the sensor. The key fabrication technology was the HF-based meniscus etching process to sharpen and recess sensor tips. MEMS technologies offer the advantages of large-scale production, low cost, and increased reliability, and can be used to overcome limitations of conventional sensor fabrication.

Overall, novel needle-type multi-analyte sensors for *in situ* measurements of ORP, DO, and phosphate have been successfully developed and integrated into a single sensor array. The major advantages of these new sensors include the ability

to penetrate samples to perform measurements; the small tip size for *in situ* measurements; array structure for higher robustness; and possibility of multi-analyte detection. The MEMS sensors demonstrated monitoring of local concentration changes in small structures with a high spatial resolution, and offer the versatility of the microelectrode technique needed for biofilm studies as well as the capability for repetitive measurements. Ultimately, this research will enable environmental scientists to perform *in situ* measurements at Superfund sites or in drinking water and wastewater systems which need *in situ* monitoring. The developed sensors may also have potential uses in biomedical applications for measurements in small sample volumes or in cell engineering.

In the future, the sensors will be integrated with an Ag/AgCl reference electrode in the same array. A good reference electrode is an essential component of any electrochemical system and is a prerequisite for achieving truly reliable performance. Using separate reference and working microelectrodes works well for laboratory use and is relatively easy to fabricate, but their use requires good shielding and grounding to minimize electrical interference. In addition, the developed sensors will be further integrated with an IC chip for signal acquisition and processing, which is expected to yield improved signal to noise ratio and potentially enhanced sensitivities and detection limits. Overall, a fully-integrated sensor is expected to overcome many of the shortcomings of today's conventional microelectrodes and may be used to obtain direct information from measurements inside heterogeneous biological systems.

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Chapter 7
Fundamentals and Applications of Entrapped Cell Bioaugmentation for Contaminant Removal

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Abstract Entrapped cell bioaugmentation is an addition of gel or rubber matrices embedded with microorganisms to increase biological activities. The technology is an integration of cell entrapment and cell bioaugmentation techniques. In the last decade, this technology has been frequently studied for its applications in the environmental field for removing collective and specific contaminants. The technology not only provides sufficient contaminant-degrading cultures but also prevents them from environmental stresses and being transported out of the target systems. This paper provides a review on the uses of entrapped cell bioaugmentation for contaminant removal including background of the technology, principles of cell entrapment techniques, types and preparation procedures of selected cell entrapment matrices, and studies on the applications of the technology for wastewater treatment and site remediation. Future perspectives of the technology are also discussed.

Keywords Bioaugmentation · Biodegradation · Bioremediation · Cell entrapment · Wastewater treatment

7.1 Introduction

Engineered and natural biological processes sometimes do not perform well or take long time in removing contaminants such as nutrients, heavy metals, phenolic compounds, and chlorinated compounds because they have inappropriate types and/or insufficient numbers of contaminant-degrading cultures [1–7]. A technique called cell bioaugmentation, was developed to overcome these problems [1–9]. Cell bioaugmentation is the addition of adequate numbers of effective contaminant-degrading microbial strain(s) to remove contaminants. The cell bioaugmentation

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technique has been applied to remove several contaminants, such as 3-chloroaniline, 2,4-dichlorophenoxyacetic acid, and 3-chlorobenzoate, in wastewater and contaminated sites [2, 3].

The key attributes for the success of cell bioaugmentation are the viability and retention of the bioaugmented cells in the target systems [2, 4, 7, 10]. In field applications, the augmented cells might experience biotic and abiotic environmental stresses, such as predation and competition with indigenous species and presence of inhibiting compounds [11]. Moreover, the augmented cells sometimes leave the systems along with the effluent or groundwater flow for the cases of wastewater treatment or site remediation, respectively.

Cell entrapment, a cell immobilization method by embedding microorganisms in a porous polymeric matrix, can be used to alleviate the shortcomings associated with the traditional planktonic (suspended or free) cell bioaugmentation scheme. Some of the common natural and synthetic polymeric materials used as cell entrapment matrices include calcium alginate (CA), carrageenan (CN), polyvinyl alcohol (PVA), and cellulose triacetate (CTA). Cell entrapment has been studied and applied mainly as stand-alone wastewater treatment processes for the removal of collective pollutants, such as organic carbon [12–20] and nitrogen [21–27], as well as specific contaminants such as phenol [1], dyes [28–31], and cyanide [32]. Recently, the technique was combined with cell bioaugmentation resulting in a new process, known as entrapped cell bioaugmentation, for removing pollutants, such as nitrogen, herbicide, and other hazardous compounds in wastewater and contaminated sites [6, 7, 33, 34]. The entrapment matrix can protect the augmented cells against environmental stresses and prevent their loss from the target systems making entrapped cell bioaugmentation a more reliable technology compared to the traditional planktonic cell bioaugmentation.

This article reviews the basics and applications of entrapped cell bioaugmentation for contaminant removal. The principles of cell entrapment, types and preparation procedures of selected cell entrapment matrices including CA, CN, PVA, and CTA, and advantages and drawbacks of entrapped cells compared to suspended or free cells are described. Previous studies on the applications of entrapped cell bioaugmentation for wastewater treatment and site remediation including success, concerns, and future perspectives of the technology are also discussed.

7.2 Cell Entrapment

Entrapment is one of the cell immobilization techniques in which microorganisms are embedded within porous polymeric supporting materials (Fig. 7.1f) [35–37]. Some other common cell immobilization techniques include physical adsorption, ionic binding, covalent binding, cross-linking, and encapsulation (Fig. 7.1a–e). In entrapment and encapsulation, microorganisms are not directly bonded but enclosed in supporting porous matrices. The cell entrapment technique confines microbial cells within the pores and voids of immobilization matrix while the cell

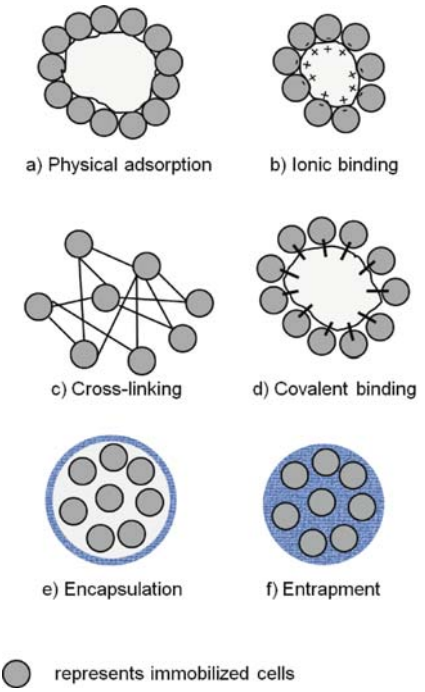

Fig. 7.1 Cell immobilization techniques [65], Reprinted by permission of the publisher

encapsulation technique wraps the cells inside a shell (matrix) as shown in Fig. 7.1e. As a result, the two techniques provide more protection to the cells and are sometimes grouped together as a single category of cell immobilization. There are a number of successful applications of entrapped and encapsulated cells in environmental, pharmaceutical, and food industries. Entrapment matrices are known to be more durable than encapsulation matrices and therefore are more suitable for field applications.

7.2.1 General Principles of Cell Entrapment

Cell entrapment procedures normally consist of two steps: (1) mixing of cells and viscous-liquored matrix and (2) gelation (Fig. 7.2) [38]. The mixing of cells and matrix is performed by dispersing the cells in the matrix, which can be accomplished by simple blending techniques such as magnetic stirring and propeller mixing. There are two common approaches for gelation: droplet and plated gelations. In droplet gelation, the mixture of cells and matrix is dropped into a gel formation solution to produce spherical beads using a syringe or a peristaltic pump (Fig. 7.2). In plated gelation, the mixture of cells and matrix is poured into a tray containing a gel formation solution and the formed gel is cut into small cubes (Fig. 7.2).

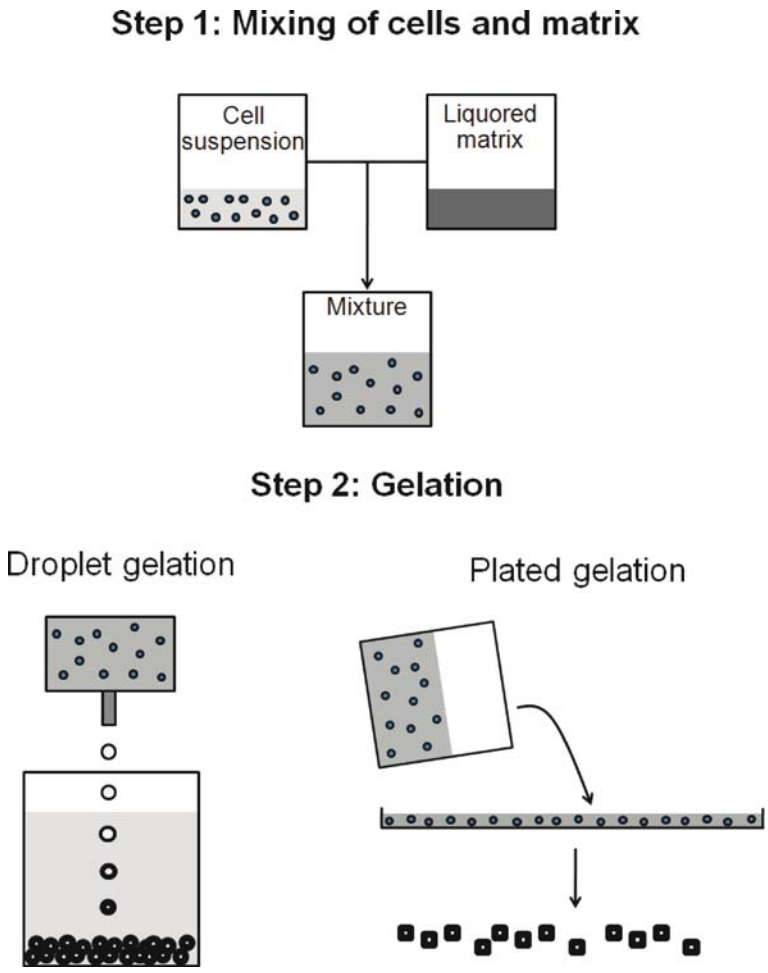

Fig. 7.2 General cell entrapment procedures

The gelation takes place via several processes including ionictropic gelation, temperature-induced gelation, organic polymerization, and phase separation depending on entrapment matrices [39]. Ionictropic gelation is a cross-linking between a matrix (polyionic polymer) and a cation in the gel formation solution. For example, calcium alginate is formed through ionictropic gelation. Temperature-induced gelation is a phase transition at different temperatures. The examples of the temperature-induced gelation are agarose and gelatin formations. Organic polymerization occurs through a reaction between monomers. Common cell entrapment matrices such as polyacrylamide, polymethacrylate, and PVA are the products of organic polymerization. During polymerization, a cross-linking agent may be added for a better gel network. The last process is a phase separation which the cells are

extracted by a gel formable solvent. The phase separation process is quite limited in use since the gel formable solvent could severely damage the viability of the cells.

7.2.2 Widely Used Cell Entrapment Matrices and Procedures

For environmental applications, cell entrapment matrices can be categorized into two types: natural and synthetic. Natural matrices, such as CA, CN, agarose, and gelatin, are polysaccharides produced from algae or seaweed while synthetic matrices are man-made polymers, such as PVA, CTA, polyethylene glycol, and polyacrylamide. The criteria for matrix selection are summarized in Table 7.1. The principles, descriptions, and cell entrapment and de-entrapment procedures for only selected matrices including CA, CN, PVA, and CTA are reviewed below. Note that the de-entrapment process is needed for evaluating the cell number and growth inside the matrix.

Table 7.1 Criteria for cell entrapment matrix selection

Property	Criterion	Reference
Surface area	Large	Kourkotus et al. [65]
Handling and regeneration	Easy	Kourkotus et al. [65]
Cell retention	High	Kourkotus et al. [65]
Cell viability	High	Kourkotus et al. [65]
Biological activity	High	Jen et al. [35] Kourkotus et al. [65]
Porosity/Diffusivity	High	Jen et al. [35] Kourkotus et al. [65] Leenen et al. [79]
Mechanical and chemical stability	High	Kourkotus et al. [65] Leenen et al. [79]
Preparation procedure	Easy	Kourkotus et al. [65] Leenen et al. [79]
Solubility	Low	Leenen et al. [79]
Biodegradability	Low	Leenen et al. [79]
Cell growth	Possible	Leenen et al. [79]
Cost	Low	Leenen et al. [79]

7.2.2.1 Calcium Alginate

Calcium Alginate Chemistry and Gelation

Alginate is one of the pioneer materials used for cell entrapment. It is a non-toxic natural polysaccharide extracted from brown algae, seaweed and bacteria such as *Laminaia hyperborean*, *Macrocystis pyrifera*, *Ascophyllum nodosum*, and *Azotobacter vinelandii* [40–42]. It is a chain of 1–4 linked β-D-mannuronate (M) and α-L-guluronate (G) in different compositions, sizes, and patterns depending

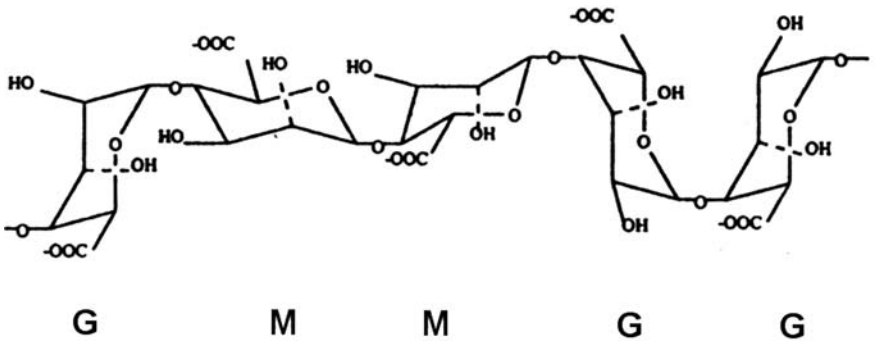

Fig. 7.3 Chemical structure of alginate [42]

on the sources (Fig. 7.3). Alginate is commercially available as a sodium salt of alginate.

Gelation of alginate is a cross-linking of alginate with divalent cations, such as Ca^{2+}, Ba^{2+}, and Sr^{2+}. Calcium is the most widely used cross-linker and the CA entrapment is simple, quick, and inexpensive. Normally, CA gel is prepared by the droplet gelation method. When the sodium alginate solution is in contact with the Ca^{2+} solution, a semi-solid structure is formed immediately in its outer layer. The Ca^{2+}solution then passes through the outer layer to form the gel structure for the entire alginate bead. The chemical structure of alginate affects its properties, stability, and biodegradability [42]. Alginate containing high G content, especially with a long GG structure, provides high gel strength and low shrinkage. This is because the GG block favors more cation bindings, which consequently lead to higher gel stability.

The CA gel is stable in broad ranges of pH (pH of 3–10) and temperature (up to 85°C) [41]. The drawbacks of the CA gel are gel abrasion and swelling under some conditions [4, 40, 41, 43]. The CA gel beads are demolished in the environment containing high concentrations of divalent cations (except Ca^{2+}), phosphate, and chelating agents and swell in the presence of monovalent cations.

Procedures of Calcium Alginate Cell Entrapment and De-Entrapment

The CA cell entrapment procedures are similar in most previous studies. The following procedure is one of the successful methods which was used in several environmental applications [20, 44, 45]. Sodium alginate powder is dissolved in deionized water (DI) at 2% (w/v). To prevent agglomeration, the powder is slowly added into stirred DI. The solution is stirred until all the powder is totally dissolved, which could take up to 12 h. A liquid medium containing microbial cells is centrifuged at 7000 rpm for 10 min to obtain concentrated cells, which are then uniformly mixed with the sodium alginate solution. The mixture is dropped into a calcium chloride solution of 3.5% (w/v) using a peristaltic pump at a flow rate of 3 mL/min (bead size of 2.0–5.0 mm depending on the pump head). The droplets

remain in the solution for 2.5–3.0 h for the formation and hardening of spherical beads.

As mentioned earlier about the variation of the CA entrapment procedure, the chemical concentrations, centrifugation speed, dropping rate of the cell-alginate mixture, and hardening time may be modified for enhancing the bead durability and/or convenience of bead preparation. For instance, the hardening time of the CA gel beads normally ranges from 0.5 to 3.0 h; however, it has been shown that more durable beads can be obtained by increasing the hardening times to overnight [20]. The CA entrapped cells can be de-entrapped by vigorous vertical shaking in a 0.3 M sodium citrate solution at pH 5 [20, 46] or 50 mM phosphate buffer at pH 7 [40].

7.2.2.2 Carrageenan

Carrageenan Chemistry and Gelation

Carrageenan is another common matrix for cell entrapment. It is produced from red seaweed [38, 43, 47]. Its structure contains 1,3-linked β-D-galactose and 1,4-linked 3,6-anhydro-α-D-galactose. There are three types of CN based on the number and position of sulfonation: kappa (κ), lambda (λ), and iota (ι) (Fig. 7.4). Lambda-CN is water soluble; therefore, it is not suitable for cell entrapment. Between κ and ι-CN, κ-CN is a better cell entrapment matrix since it has a stronger gel network.

The CN gelation process can be either ionictropic or temperature-induced (cooling) gelations. Similar to CA gel, κ-CN gel can be formed with different ions, such

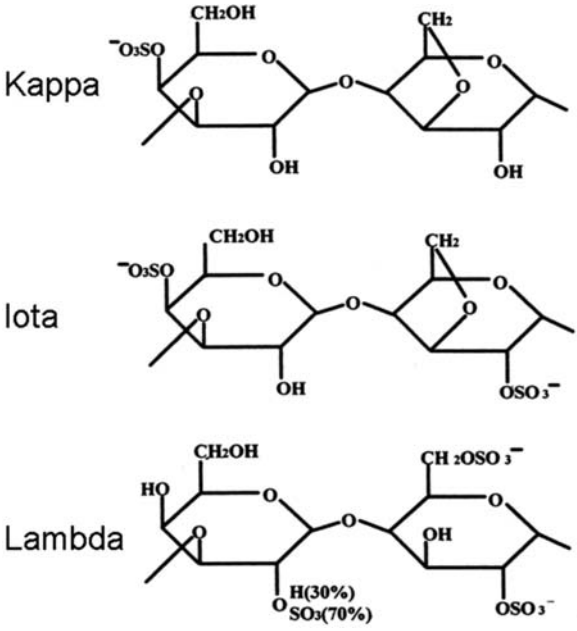

Fig. 7.4 Chemical structure of carrageenan [47]

as K^+, NH_4^+, Ca^{2+}, Cu^{2+}, Mg^{2+}, Fe^{3+}, and amines [38, 43, 47]. Additionally, the strength of κ-CN gel can be improved by adding polymers such as galactomannans, locust bean gum, and taragum [38, 43]. Normally, κ-CN entrapped cells have relatively high biological activities and in turn broad utilizations. However, the applications at high temperatures are not suitable because κ-CN gel dissolves [48].

Procedures of κ-Carrageenan Cell Entrapment and De-Entrapment

The κ-CN cell entrapment can be accomplished by both droplet and plated gelations. The droplet method is suitable for a laboratory scale since the setup is simple. However, the droplet method would be time-consuming for preparing a large volume of entrapped cells; the plated method is more appropriate for it. The following procedure is a general droplet method implemented in several studies [20, 47, 49]. Kappa-CN powder is dissolved in stirred DI at a temperature of 50°C and the solution is allowed to cool down to 35°C. Then, concentrated microorganisms are mixed with the κ-CN solution. The mixture is dropped into 0.3 M potassium chloride and 0.18 M calcium chloride solutions for gel formation and gel hardening, respectively. The final κ-CN concentration is about 2–5% (w/v). The de-entrapment procedure for the κ-CN entrapped cells involves continuous shaking in a 1% sodium citrate solution at 37°C [20, 27].

7.2.2.3 Polyvinyl Alcohol

Polyvinyl Alcohol Chemistry and Gelation

PVA is a polymer that can be prepared in the forms of film and hydrogel with high mechanical strength and durability [4, 21, 50]. Similar to CA and CN, PVA is non-toxic even though it is a synthetic polymer. Therefore, it does not negatively affect both microorganisms and environment. Raw PVA appears in a white and free-flowing granule. The chemical structure of PVA is shown in Fig. 7.5. The properties of PVA are based on the polymer chain length (molecular weight) and degree of hydrolysis. Polyvinyl alcohol with high molecular weights and degrees of hydrolysis has high mechanical stability and low water solubility [51].

Several gelation techniques are available for producing PVA gels for cell entrapment including boric acid-PVA (BPVA), freezing and thawing of PVA (FPVA), and phosphorylated-PVA (PPVA) methods. The BPVA technique is the simplest and most economical. The technique is a one-step droplet gelation method [52]. The BPVA gelation process is a cross-linking of boron and PVA as shown in Fig. 7.6. The BPVA hydrogel beads present high mechanical strength and durability. However,

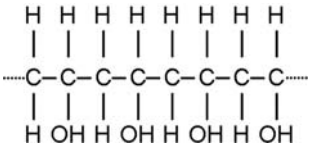

Fig. 7.5 Chemical structure of PVA

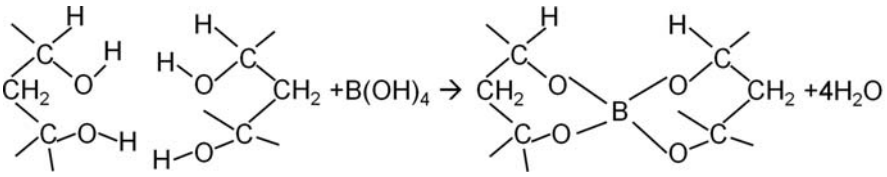

Fig. 7.6 Reaction of PVA-boric gelation

there are two potential problems associated with the technique: cell damage in the boric acid solution and PVA bead agglomeration [21, 53]. Several researchers modified the procedure to solve these problems, such as additions of calcium alginate and activated carbon [53, 54].

The FPVA technique is based on physical cross-linking during temperature-induced condition. Under cryotropic conditions, hydrogen bonds between OH groups of the PVA polymer chain(s) occur either within the chain (intramolecular) or between two chains (intermolecular) [55]. Although this technique provides a strong PVA cryogel, the freezing condition could affect cell viability.

Chen and Lin [21] developed a PPVA method that reduces the boric acid contact time and consequently cell damage associated with the boric acid-PVA method. This modified technique not only decreases the cell damage by boric acid but also increases the strength and durability of entrapped cell beads. The PPVA technique is a two-step droplet gelation method including spherical bead formation and hardening. In the first step, spherical bead formation, the PVA-boron cross-linking occurs according to the reaction shown in Fig. 7.6. In the second step, bead hardening, spherical beads are left in a sodium phosphate solution to increase the surface gel strength through PVA phosphorylation (Fig. 7.7) [56].

Procedures of Phosphorylated-Polyvinyl Alcohol Cell Entrapment and De-entrapment

As mentioned above that BPVA and FPVA entrapment protocols may affect cell viability, therefore, only PPVA cell entrapment is reviewed here. The following PPVA cell entrapment procedure is according to Siripattanakul et al. [57]. The procedure was modified from Chen and Lin [21] for preventing PVA bead agglomeration during the PVA-boron cross-linking step. The modified cell entrapment procedure begins with dissolving PVA in stirred DI at temperature of 60–80°C and letting the solution cool down to room temperature. Microbial cells are centrifuged at 4000×*g* for 10 min and then mixed with the PVA solution. The mixture is dropped into a saturated boric acid solution in a 1-l cylinder and remains in the solution for 30–45 min

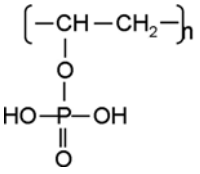

Fig. 7.7 Structure of PVA phosphorylation [56, p. 654], Copyright (2004 and John Wiley & Sons, Inc.); Reprinted with permission of John Wiley & Sons, Inc

to form spherical beads. Note that in the method by Chen and Lin [21], the cell-PVA mixture is dropped into a stirred boric acid solution for 10–120 min. Then, the formed hydrogel beads are then soaked in a 1.0 M sodium phosphate solution at pH 7 for 60 min for hardening. The final PVA concentration is 10% (w/v).

The de-entrapment procedure of the PPVA involves adding the PPVA entrapped cells into DI and heating to about 60°C. As mentioned above, the de-entrapment process is normally performed for measuring cell number and/or growth inside the matrix. This thermal de-entrapment may damage the cell viability making plate counting of the de-entrapment product an unsuitable method for quantifying the cells in the PPVA matrix. Measuring the cell mass (represented by volatile suspended solids) after the de-entrapment is an alternative to this limitation [20].

7.2.2.4 Cellulose Triacetate

Cellulose Triacetate Chemistry and Gelation

Cellulose is a natural polymer extracted from plants. Its structure is a chain of organic compounds containing glucose molecules of which the number and location in the chain vary based on the sources [43]. Natural cellulose itself is not appropriate for cell entrapment; however, modified cellulose compounds via chemical processes, such as esterification and etherification, can form fiber networks. Examples of the modified cellulose compounds are cellulose azide, diazo cellulose, and CTA. Cellulose triacetate has been applied as a cell entrapment matrix since 1980s. The CTA entrapped cells were first developed for food technology applications. The CTA cell entrapment matrix is rubber-like, which is different from the other entrapment media described earlier. The CTA entrapped cells have very high mechanical strength. It was reported that the CTA entrapped cells can be used continuously for more than 8 years [13, 14].

Procedure of Cellulose Triacetate Cell Entrapment

The CTA entrapped cells are prepared by the plated gelation. A procedure to prepare CTA entrapped cells was introduced by Kolarik et al. [58]. Later, Yang and See [59] modified it to ease the preparation. The modified procedure has been used in several studies [6, 12–14, 34]. Cellulose triacetate powder is dissolved in methylene chloride at a concentration of 10% (w/v). Concentrated microbial cells are uniformly mixed with the CTA solution. Then, the mixture is plated into toluene for hardening. The hardened CTA sheet is cut to small cubes and washed with water to rinse the residual chemicals. Currently, there is no procedure for CTA de-entrapment.

7.2.3 Advantages and Drawbacks of Entrapped Cells

There are several advantages of entrapped cells over suspended cells [60, 61]. Basically, cell entrapment leads to the enhancement of both biological and mechanical stabilities. The entrapment matrix can alleviate physicochemical challenges, such as temperature, pH, solvents, shear, and heavy metals. Other advantages of entrapped cells include high biomass concentration, no need for cell separation,

increased product yield and stability, increased reaction selectivity, and versatility in the selection of the reactor. Several studies reported that entrapped cells provided better waste treatment performances and/or are more durable than free cells [7, 20, 62–64]. For example, in a previous study, PPVA entrapped cells were used for removing total organic carbon compared to free cells. The results indicated that the PPVA entrapped cells had substantially higher specific growth and substrate utilization rates [20]. The main drawbacks of entrapped cells are metabolic changes, cell morphology changes, substrate and chemical growth factor diffusion limitations, and inconsistent growth pattern [61, 64].

7.3 Applications of Entrapped Cell Bioaugmentation

In the past two decades, bioaugmentation and cell entrapment processes have been separately applied in the environmental field. Examples of bioaugmentation applications include removal of 2,4-dichlorophenoxyacetic acid, 3-chlorobenzoate, 3-chloroaniline, diesel (oil spills) [2, 3, 64] whereas the cell entrapment has been applied for removing phenol, dyes, and cyanide [1, 28–32]. Although both processes alleviate several problems associated with traditional contaminant removal schemes, the roles of the two processes are different. Bioaugmentation provides a number of specific or acclimated contaminant-degrading cultures whereas cell entrapment maintains the cultures in the system and protects them from stresses.

Combining bioaugmentation and cell entrapment results in a novel process, called entrapped cell bioaugmentation, which inherits the benefits of both processes. Entrapped cell bioaugementation has been studied for environmental applications only in recent years. The applications involved the degradation of collective and specific pollutants in wastewater treatment plants and contaminated sites. Although entrapped cell bioaugmentation has not been applied at field scales since it is relatively new, the bench-scale results thus far are very promising. The technique can retain effective contaminant-degrading cultures within the target systems and the matrices can protect the cells from environmental stresses. Table 7.2 presents a summary of previous studies on entrapped cell bioaugmentation for environmental applications. Since the technology has been studied mainly for wastewater treatment and site remediation, only these two categories of applications are reviewed below for each cell entrapment matrix separately. For matrices that have not been used for entrapped cell bioaugmentation, their technological outlook is provided.

7.3.1 Wastewater Treatment

7.3.1.1 Calcium Alginate Entrapped Cell Bioaugmentation

Calcium alginate is the most common matrix studied in the applications of entrapped cell bioaugmentation for wastewater treatment. There were several successful applications of the CA entrapped cell bioaugmentation for removing toxic compounds in domestic and industrial wastewater such as oil, phenol, and cresol, as listed in Table 7.2. The bioaugmented cultures were pure or enriched mixed cultures.

Table 7.2 Summary of studies on entrapped cell bioaugmentation for contaminant removal

Entrapment matrix	Microorganism	Contaminant	Environmental medium	Reference
CN	*Pseudomonas* sp. UG30	Pentachlorophenol	Water	Cassady et al. [76]
CN	*Pseudomonas* sp. UG30	Pentachlorophenol	Soil	Cassady et al. [77]
CA	Enriched mixed cultures	Phenol and cresol	Wastewater	Guiot et al. [66]
CA	Enriched mixed cultures	Phenol and cresol	Wastewater	Hajji et al. [78]
FPVA	Enriched microorganisms	Diesel	Soil	Cunningham et al. [64]
BPVA modified by sodium alginate or activated carbon	*Zoogloea* sp.	Phenanthrene and pyrene	Soil	Li et al. [54]
CA and agar	*Rhodobacter shaeroide* S *Rhodobacter shaeroide* NR-3	Cooking oil	Synthetic wastewater	Takeno et al. [68]
CA	*Rhodococcus erythropolis* NI86/21	Atrazine	Water and soil	Vancov et al. [33]
PVA	Denitrifying sludge	Nitrate	Agricultural drainage	Hunt et al. [74]
CA	Mixed culture	Nitrate	Synthetic agricultural infiltrate	Siripattanakul et al. [45]
PPVA	Acclimated mixed culture and *Agrobacterium radiobacter* J14a	Atrazine	Synthetic wastewater	Siripattanakul et al. [57]
CA	*Sphingomonas chlorophenolica* PCP-1	Pentachlorophenol	Groundwater	Yang and Lee [24]
CA	Recombinant *Escherichia coli*	Coumaphos, chlorferon, and diethylthiophosphate	Waste cattle dip solution	Ha et al. [18]

Table 7.2 (continued)

Entrapment matrix	Microorganism	Contaminant	Environmental medium	Reference
PPVA	*Agrobacterium radiobacter* J14a	Atrazine	Synthetic agricultural infiltrate	Siripattanakul et al. [7]
PPVA	Mixed culture	Atrazine	Synthetic agricultural infiltrate	Siripattanakul et al. [75]

The calcium alginate matrix contains numerous pores while providing a strong network for cell restriction and proliferation as shown in scanning electron microscopic (SEM) images in Fig. 7.8. Large numbers of cells are present both inside and on the surface of the matrix. It has been reported that CA entrapped cell bioaugmentation greatly improves the wastewater treatment operation and efficiencies. For example, Guiot et al. [66] examined phenol and cresol removal by the bioaugmented CA entrapped acclimated mixed cultures in an upflow anaerobic sludge blanket reactor (UASB). The results showed that the UASB with the bioaugmented CA

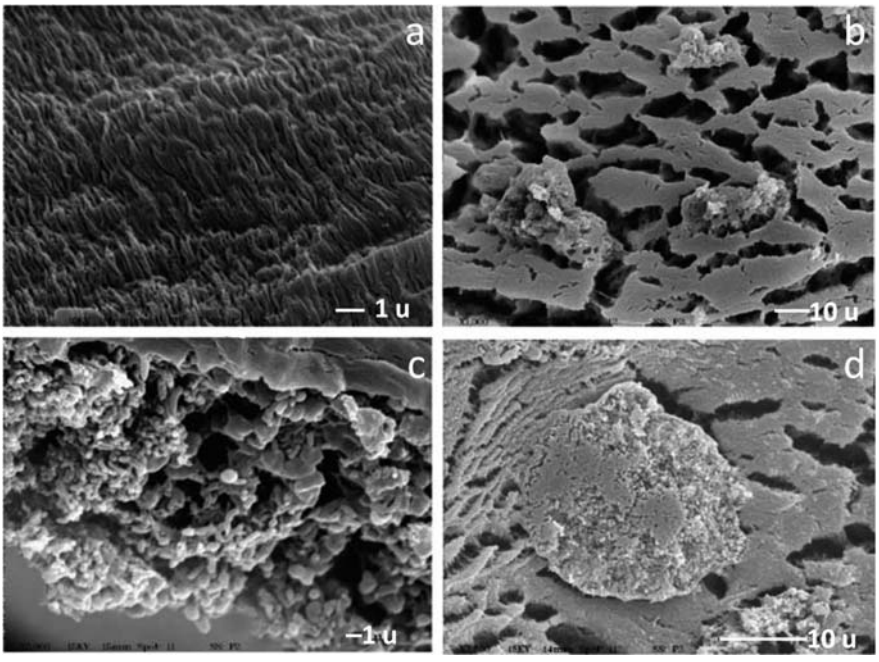

Fig. 7.8 SEM images of calcium alginate entrapped nitrifying bacteria: (**a**) Bead surface after entrapment, (**b**) Interior bacterial floc after entrapment, (**c**) Surface rupturing bacterial colony after experiments, and (**d**) Interior bacterial colony after experiments [44]

entrapped cells could remove the contaminants much better than the UASB alone. The maximum specific phenol, *p*-cresol, and *o*-cresol treatment activities of the system with the entrapped cells were approximately 13, 16, and 8 times higher than those without the entrapped cells. Additionally, the bioaugmented system reached a steady state much quicker and provided higher process stability.

Although CA is a popular entrapment material, there have been reports on its susceptibility to degradation [4, 67]. However, some studies found that the CA entrapped cells are durable and perform well after several rounds of reutilization. A good example is a study by Moutaouakkil et al. [30] which reported that the CA entrapped cells removed toxic azo dye at high concentrations efficiently even after reutilizing them 7 times. Lately, the modified or amended CA was developed for improving contaminant removal efficiencies and the matrix stability. Activated carbon, bentonite, and skim milk are among the amendments [17].

7.3.1.2 Carrageenan Entrapped Cell Bioaugmentation

The utilization of CN entrapped cells is relatively limited among the selected matrices. To date, there has been no CN entrapped cell bioaugmentation application for wastewater treatment. There are only a few basic studies on the uses of the CN entrapped cell inoculation as a treatment scheme by itself. For example, κ-CN entrapped cells were applied to remove total organic carbon [20] and glucose [49] in liquid systems.

The limitation on the CN entrapped cell applications may be attributed to the weakness of the material. The traditional κ-CN entrapped cell preparation requires warm temperatures (35–55°C) for dissolving the κ-CN powder, which could damage the viability of cells. Additionally, the κ-CN entrapped matrix is sensitive to cations, such as K^+ and NH_4^+ leading to easy gel abrasion. However, the κ-CN structure is appropriate for cell proliferation. Godia et al. [69] reported a large number of proliferated cells in κ-CN after 1-day fermentation (Fig. 7.9). The modified κ-CN matrices by clay or skim milk amendments were developed for better gel strength [70]. Through advancements in material science and technology, it is possible that

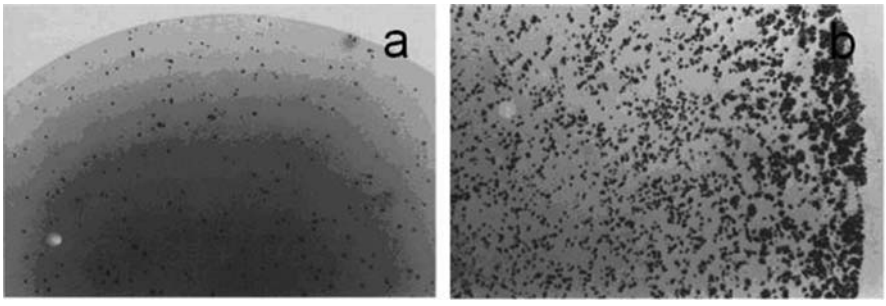

Fig. 7.9 Images of cell distribution in carrageenan matrix: (**a**) After entrapment and (**b**) After 1-day fermentation [69]

the strength of κ-CN will be improved in the near future and more applications of κ-CN entrapped cells including bioaugmentation will then take place.

7.3.1.3 Polyvinyl Alcohol Entrapped Cell Bioaugmentation

Up to date, there has been no work on PVA entrapped cell bioaugmentation for wastewater treatment. Based on several previous successful cases of PVA entrapped cell applications as a stand alone wastewater treatment process, PVA entrapped cell bioaugmentation will likely be attempted soon. The PVA entrapped cells successfully removed more than 90% of contaminants such as organic carbon, nitrogen, 2-methylnaphthalene, and phenol [21, 28, 71–73]. Sharanagouda and Karegoudar [72] reported that 2-methylnaphthalene removal efficiencies by PVA entrapped cells (60 to > 90%) are higher than those by corresponding free cells (20–60%).

The PVA matrices provide a proper microstructure for the contaminant-degrading cultures [57]. Figure 7.10 presents the SEM images of PPVA entrapped atrazine degraders, which reveals two porous bead layers. The outer layer has less porosity providing an effective structure for cell retention. Additionally, PVA matrices were proven to be a good entrapment material in terms of mechanical, chemical, and biological stabilities. The matrices were found unbroken after 6-month utilization [28], reusable more than 30 times without losing degradation ability for

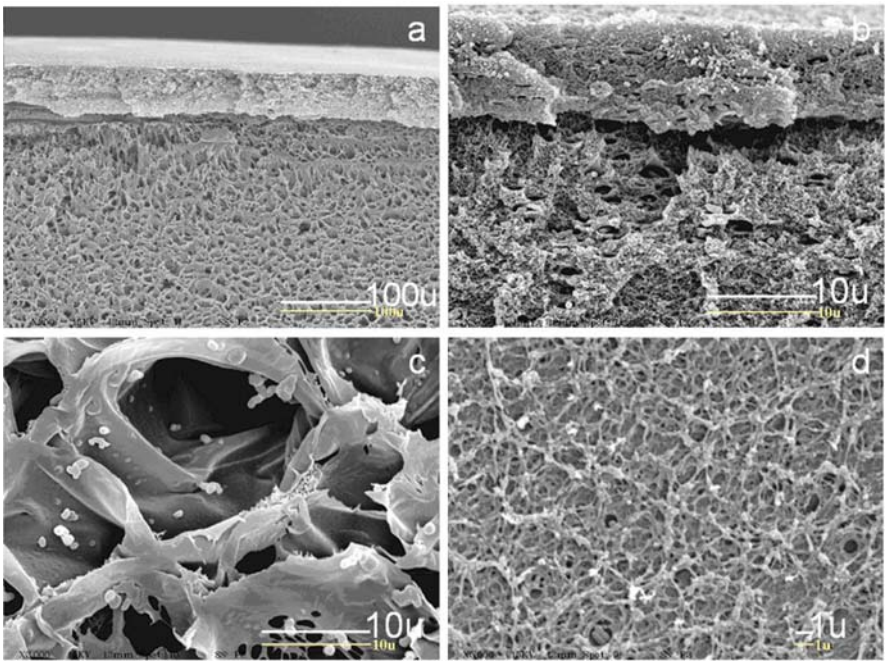

Fig. 7.10 SEM images of PPVA entrapped cells: (**a**) Cross-section at 250×, (**b**) Exterior layer at 3000×, (**c**) Interior layer at 3000×, and (**d**) External surface at 5000× [57]

2-methylnaphthalene [72], and reusable more than 50 times at various pH and temperatures [73]. The PVA entrapment process only slightly affects the cell viability based on the viable plate count and a fluorescence based assay [28].

7.3.1.4 Cellulose Triacetate Entrapped Cell Bioaugmentation

There have been less numbers of applications of CTA entrapped cells for wastewater treatment compared to CA and PVA. This could be because the CTA entrapped cell preparation procedure involves the use of toxic chemicals as mentioned above. The chemicals may severely damage the contaminant-degrading cultures and pose health risk to the personnel involved. In addition, hazardous wastes are generated from the procedure. Advantages of the CTA matrix include very high mechanical and chemical strengths. The entrapped cells can be used for longer than eight years without the breakage of the matrix. Even though the entrapment procedure could be very harmful to microorganisms, high contaminant removal efficiencies by CTA entrapped cells have been reported [6, 12–14, 34, 59].

Cellulose triacetate entrapped cell bioaugmentation is utilized in a novel wastewater process called immobilized cell augmented activated sludge (ICAAS), which was developed to improve the ability of activated sludge process to degrade contaminants (Fig. 7.11). The ICAAS system is an activated sludge system with an off-line enricher reactor growing CTA entrapped cells, which are induced to have specific activities such as toxic contaminant degradation, nitrification, and denitrification [6, 34]. The enriched entrapped cells are used for bioaugmentation in the aeration tank. Once they are less active due to unfavorable conditions in the aeration tank such as the absence of the target contaminants and/or competition with indigenous microorganisms, they are returned for reactivation in the enricher reactor and in the mean time replaced by the active cells from the enricher reactor.

Jittawattanarat et al. [6] investigated pentachlorophenol (PCP) removal by completely mixed activated sludge (CMAS) and ICAAS processes (Fig. 7.12). Synthetic wastewater containing PCP at 40 mg/L was used. The ICAAS systems with and without powder activated carbon (PAC) entrapped along with the cells removed PCP more than the CMAS system (no bioaugmentation) as shown in Fig. 7.12.

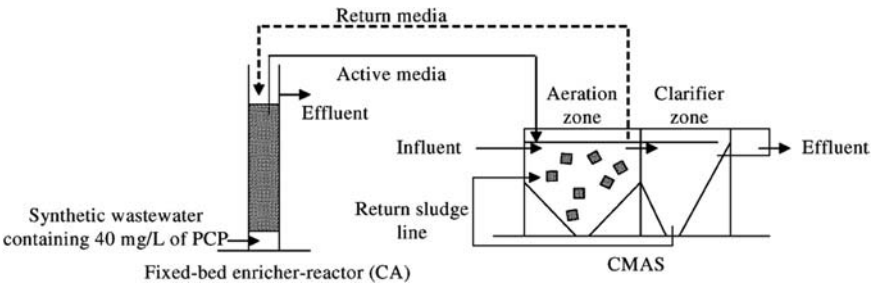

Fig. 7.11 A diagram of immobilized cell augmented activated sludge system [6], Reprinted by permission of the publisher

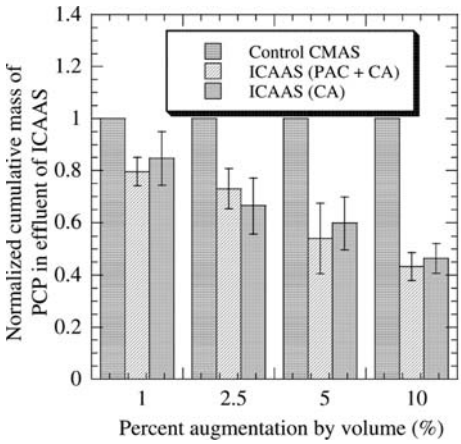

Fig. 7.12 Normalized pentachlorophenol concentrations in effluent from CMAS and ICAAS systems at different bulk volumetric bioaugmentation ratios [6], Reprinted by permission of the publisher

At 10% bioaugmentation by volume, the cumulative mass of PCP in the effluent of ICAAS was about 50% less than that of CMAS. The results further indicated that PCP biodegradation and adsorption took place in the ICAAS systems but biodegradation by the bioaugemented entrapped cells was the main removal mechanism.

7.3.2 Site Remediation

7.3.2.1 Calcium Alginate Entrapped Cell Bioaugmentation

There have been four studies on CA entrapped cell bioaugmentation for site remediation. The target contaminants were atrazine, nitrate, PCP, coumaphos, chlorferon, and diethylthiophosphate (Table 7.2). Calcium alginate entrapped cells were bioaugmented for removing contaminants in soil, groundwater and infiltrate. Siripattanakul et al. [45] studied the use of CA entrapped cell bioaugmentation for denitrifying synthetic agricultural infiltrate using a laboratory sand column setup at $20 \pm 2°C$. The CA entrapped cells achieved nitrate removal of more than 90% within 8 hr compared to about 50% by corresponding free denitrifiers.

7.3.2.2 Polyvinyl Alcohol Entrapped Cell Bioaugmentation

Only a few studies dealing with the uses of PVA entrapped cell bioaugmentation for site remediation have been reported. As presented in Table 7.2, PVA entrapped cell bioaugmentation was used for phenanthrene, pyrene, and atrazine treatment in soil and infiltrate [7, 54, 75]. Phosphorylated polyvinyl alcohol entrapped cell bioaugmentation is a potential method for site remediation since the matrix is durable and has no negative effects on microorganisms and environment [4, 21, 57].

Siripattanakul et al. [7] introduced a PPVA entrapped cell bioaugmentation scheme for removing atrazine in agricultural infiltrate and in turn protecting groundwater quality. In their laboratory-scale sand column study, *Agrobacterium*

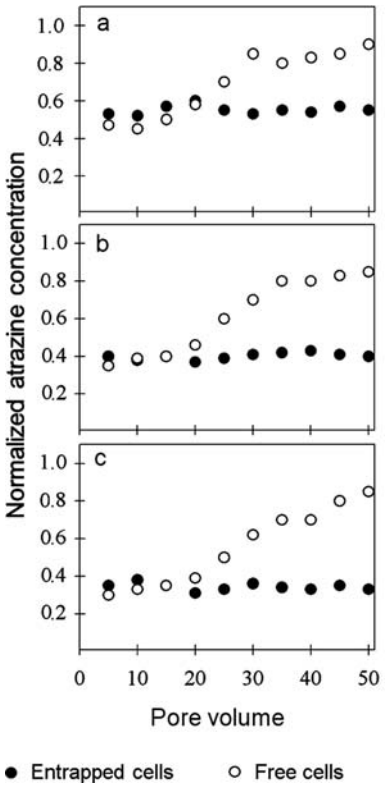

Fig. 7.13 Long-term column experimental results of cell bioaugmentation for treating atrazine in infiltrate using PPVA entrapped and free cells at cell loadings of (**a**) 300, (**b**) 600, and (**c**) 900 mg dry cells/L empty bed volume [7], Reprinted by permission of the publisher

radiobacter J14a (J14a), a known atrazine degrader, entrapped in PPVA was applied on a top sand layer and its ability to treat atrazine in a synthetic infiltrate was compared to bioaugmented free J14a cells. For a short term experiment (6 pore volumes), the atrazine removal efficiencies of up to 99% were achieved for both the free and entrapped cells. However, for a long-term experiment (50 pore volumes), the entrapped cell system provided consistent atrazine removal efficiency while the atrazine removal by the free cells declined gradually because of the cell loss (Fig. 7.13).

7.3.2.3 Carragenan and Cellulose Triacetate Entrapped Cell Bioaugmentation

Two investigations on the use of κ-CN entrapped cell bioaugmentation for site remediation have been conducted [76, 77]. Pentachlorophenol was the contaminant in both studies which focused on different environmental media, soil and water. Entrapped cells removed PCP from synthetic wastewater 3-times higher than free cells [76]. For the soil study, PCP removal using free cells, entrapped cells, CN matrices without cells, and sterile soil (no cells) was compared [77]. The results indicated that the bioaugmented entrapped cells performed more efficiently reducing PCP concentration about 64% while PCP removal in the systems with free cells,

CN matrices, and sterile soil systems ranged 16–18%. However, based on the CN chemistry, the κ-CN entrapped matrix is sensitive to cations, the application for site remediation may not be practical because environmental media (soil, infiltrate, and groundwater) normally contain several types of ions.

There has been no work on CTA entrapped cell applications for site remediation. The CTA matrix should be suitable for practical use in site remediation because it is very durable. However, the toxic chemicals used for the CTA entrapped cell preparation may cause additional contamination to the environmental media.

7.4 Conclusions and Future Perspectives

Entrapped cell bioaugmentation is a potential technology for contaminant removal. It provides better cell retention and tolerance compared to traditional planktonic cell bioaugmentation leading to higher contaminant removal efficiencies. In the last decade, studies on the applications of entrapped cell bioaugmentation for wastewater treatment have been mainly on xenobiotic treatment enhancement. The drawbacks associated with entrapped cell bioaugmentation, such as effect of entrapment procedure on cell viability, substrate diffusion limitation, and durability of entrapped cells were discovered and solved. For site remediation, the entrapped cell bioaugmentation investigations have been on remediating runoff, infiltrate, and soil contaminated with urban, industrial, or agricultural residues. Previous work examined the contaminant degradation performance, and augmented cell retention and tolerance under different environmental stresses.

Entrapped cell bioaugmentation is a technology that has been tested at bench scales. The technology has been verified under controlled laboratory conditions for its potential for both wastewater treatment and site remediation applications. Recent laboratory research efforts have been on testing the technology under different environments such as contaminated soils and agricultural infiltration. In the future, entrapped cell bioaugmentation will likely move on to pilot and field scales. Additionally, more environmental applications of the technique may be studied. For example, the technique can be used for in-situ treatment of landfill leachate and degradation of organic solid waste (bioaugmented landfill bioreactors).

The uses of entrapped cells for contaminant removal regardless of the scheme have been in a black-box manner. In-depth investigations on important aspects of entrapped cells including growth, metabolism, morphology, and genetics compared to those of free cells are needed. These understandings at the cellular and molecular levels of entrapped cells would enable more accurate prediction of their behaviors and effective bioaugmentation. Most of the work on entrapped cell bioaugmentation has been limited to laboratory scales. A lack of low-cost and industrial-scale production of entrapped cells is a major impediment for practical applications of the technology. If this issue could be resolved, entrapped cell bioaugmentation, which is a technically capable process, would turn into a sustainable practice and consequently one of the commonly used contaminant removal technologies.

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Chapter 8
Biofuels for Transport: Prospects and Challenges

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Abstract Environmental issues, the growing demand for energy, political concerns and the medium-term depletion of petroleum created the need for the development of sustainable technologies based on renewable raw materials. The so-called biofuels might help to meet the future energy supply demands as well as contributing to a reduction of green house gases emissions. In this work, we aim to provide the latest update of the production and potential of biofuels in the transport sector including type of biofuel, feedstocks and technologies as well as some realistic engine tests for the widespread use of biofuels in our society.

Keywords First generation biofuels · Biodiesel · Biogas · Bioethanol · Biobutanol · Second generation biofuels · Synthetic fuels · Engine performance · Emissions

8.1 Introduction

There is now a general scientific consensus that observed trends in global warming are been caused by fossil-fuel combustion and anthropogenic emissions of greenhouse gases (GHG) including nitrous oxide (N_2O) and specially carbon dioxide (CO_2) and methane (CH_4) [1]. Initial concerns about the impact of GHG in our society led to the development of the United Nations Framework Convention on Climate Change (1992) which in turn resulted in the 1997 Kyoto Protocol as a way to tackle the problem. In 2002 the European Union ratified the Kyoto Protocol and emphasised the potential for scientific innovation as a means of countering GHG emissions. Neither these targets nor the Kyoto targets have so far been met. However, judging from the figures of energy consumption over the last few years, the current scenario is believed to complicate in the future. Transport has shown the

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highest rates of growth in GHG emissions in any sector over the last ten years (20% global CO_2 emissions, 25% UK emissions), with a predictable 80% higher energy use and carbon emissions than now by 2030 [2].

Oil is the world's primary source of energy and chemicals with a current demand of about 12 million tonnes per day (84 million barrels a day) [3] and a projection to increase to 16 million tonnes per day (116 million barrels a day) by 2030. While a 30% of the global oil consumption accounts for transport, a striking 60% of the rising demand expected for 2030 corresponds to transport [4]. With the transport sector expanding in the US and Europe and specially developing in the newly industrialised and emerging economies of China and India, these values can easily be underestimated. The availability of conventional oil is again becoming geographically restricted and a general agreement now is that the era of cheap and secure oil (cheap energy) is over [5, 6]. Several alternatives are currently been explored, including a range of carbon free and renewable sources (photovoltaics, wind and nuclear power, hydrogen), in an attempt to replace natural gas, coal and oil in the electricity generation sector. However there is not such equivalent in transportation yet, since fuel cells, electric/hydrids and natural gas based cars are still a long way from becoming mainstream vehicles.

A short and medium term alternative is needed. Crop-based fuels denoted as biofuels including biodiesel and bioethanol, emerged as a real alternative to the use of gasoline and conventional diesel in transportation. There has been a relatively high acceptance from general public, governments, producers and part of the agricultural sectors in promoting the expansion of biofuels in our society in an attempt to switch from the petrol-based industry we have been relying on for the last 50+ years to a biobased industry and society that can guarantee a more secure energy supply. An exponential increase in the consumption of these biofuels has taken place in the last few years (Fig. 8.1).

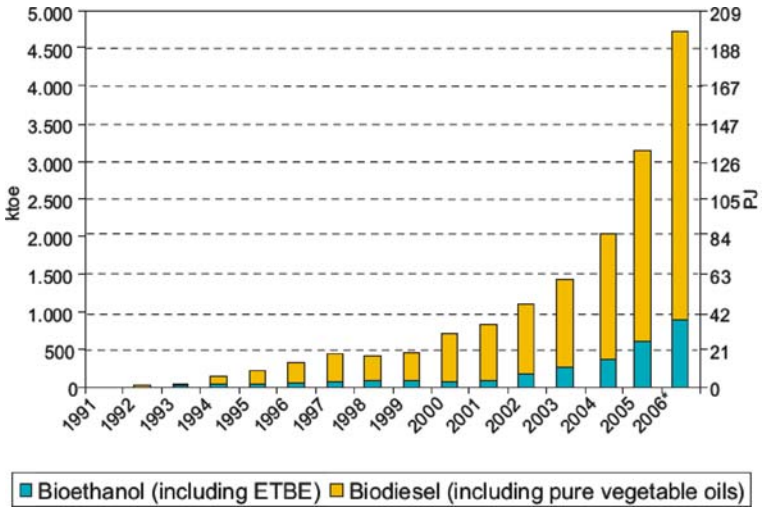

Fig. 8.1 Biofuels consumption (1991–2006) in the EU27
Source: REFUEL, IEA, Eurovserv' ER. Reproduced with permission of Marc Londo.

Ideally, such oil alternatives should reduce (or even remove) the dependence of oil as well as contributing as much as possible to meet the GHG emissions target. However, it is also widely accepted that joint efforts from politics, regulators, scientists and consumers will be needed to support an independent oil/GHG controlled scenario in the future.

On the view of the predictions, the need for a secure energy supply for transportation make essential to explore biofuels as alternative to mineral oil based fuels addressing and evaluating socio-economic and environmental consequences originated in their implementation.

From a wider context, there are three main drivers for the promotion, development and implementation of biofuels in our society. In principle, these are energy independence, climate change and rural development [3]. The political motivation to support biofuels arises from each individual driver or combinations of them. The main theoretical reasons for the promotion of biofuels can be summarised as follows:

1. *Biofuels can improve independence and energy security*. Local, national or global production of energy can avoid the reliance on politically and/or socially unstable energy suppliers [7]. In addition, the global oil demand is increasing exponentially and there is a need to find alternatives to fossil fuels derived from petroleum.
2. *Biofuels may contribute to a reduction in carbon emissions (aka climate change mitigation).* They have been often considered a solution to climate change. In fact, net emissions from biofuels have been reported to be remarkably lower than those generated from the combustion of fossil fuels [8, 9]. Nevertheless, the GHG emissions from the production of biofuels are a key issue that needs careful attention as they arise from every single stage in the supply chain from feedstock production and transport to conversion, biofuels distribution and end use (Fig. 8.2). Recently, some studies point out the CO_2 reduction may be far less than originally thought due to the inclusion of crop production costs including fertilisers, machinery, etc. as well as harvesting, transformations and distribution [3].
3. *Biofuels can help to increase farm income and contribute to rural development.* With a growing biofuels market, many countries will be able to grow more type of crops to cover national or foreign demands on energy crops. The increasing demand for agriculture due to the labour-intensive plant derived technologies [10] is expected to improve farm income, which in countries with oversupply can also help to reduce the need for subsidies. Traditionally deprived rural areas could experience a renaissance through the implementation of biofuels and biorefineries. In addition, there is also a lot of controversy over food vs fuel and the growth of specific crops to be transformed into biofuels. The resolution of this process will necessarily take place on the basis of very different variables over time [11].

With a wide array of potentially renewable energy resources, the concept and proposed benefits evolving from the use of biofuels are inspiring; therefore they

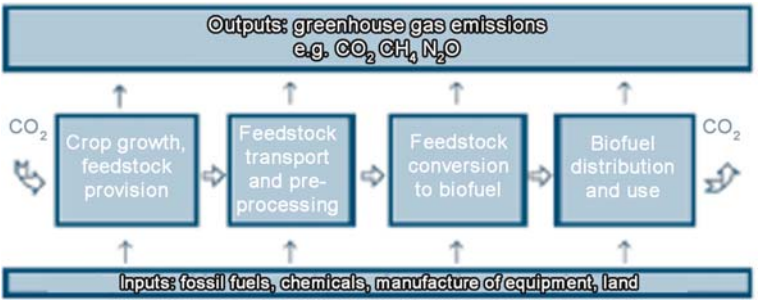

Fig. 8.2 Greenhouse gas emissions from production and utilisation of biofuels
Source: Sustainable biofuels: prospects and challenges, The Royal Society. Policy document 01/08, ISBN 978 0 85403 662 2. Reproduced with permission of the Royal Society.

need to be taken into account in order to contribute to a sustainable and energy secure future.

In this contribution, we aim to provide a general overview of the state of the art in the production and potential of biofuels for transport. Several technologies have been disclosed for the preparation on biofuels. Depending on the technology and the feedstock, we can classify the biofuels into different generations from simpler and conventional technologies and feedstocks (1st generation) to more advanced technologies (2nd generation and a potential 3rd generation in the horizon) in the so-called *biofuels ladder*.

8.2 Biofuels: Processes and Technologies

8.2.1 First Generation Biofuels

The first generation biofuels referred to biofuels manufactured from readily available energy crops including sugar, starch and oil crops (*edible feedstocks*) using conventional technologies. The most common first generation biofuels are biodiesel and bioethanol. Some other biofuels in this category including biofuels integrating glycerol, biofuels from catalytic cracking and biobutanol will also be briefly discussed. The various biofuels will be grouped according to the technology employed for their preparation. These are *chemical* and *biological* conversion.

8.2.1.1 Biofuels Produced by Chemical Conversion

Biodiesel

First generation biodiesel is currently the most common biofuel in Europe. It remains in the political and economic arena and is playing a part in the biofuels expanding process as the awareness alternative fuel spreads through the consciousness of the general public. Only in 2007, 19 biodiesel plants in the new EU member

states were starting operations, or were under construction/planning. Relatively large plants can be found in Lithuania, Poland and Romania, with capacities of 100,000 tonnes/year. The conventional methodology for the production of biodiesel involves the transesterification of triglycerides (TG) from vegetable oils (palm, corn, soybean, rapeseed, sunflower, etc.) with short chain alcohols including methanol and ethanol to yield fatty acid (m)ethyl esters (FAM/EE) and glycerol as by product (Figs. 8.3 and 8.4).

Several reviews on the preparation of biodiesel from different feedstocks can be found in the literature [12–15]. A very good overview of such technologies has been

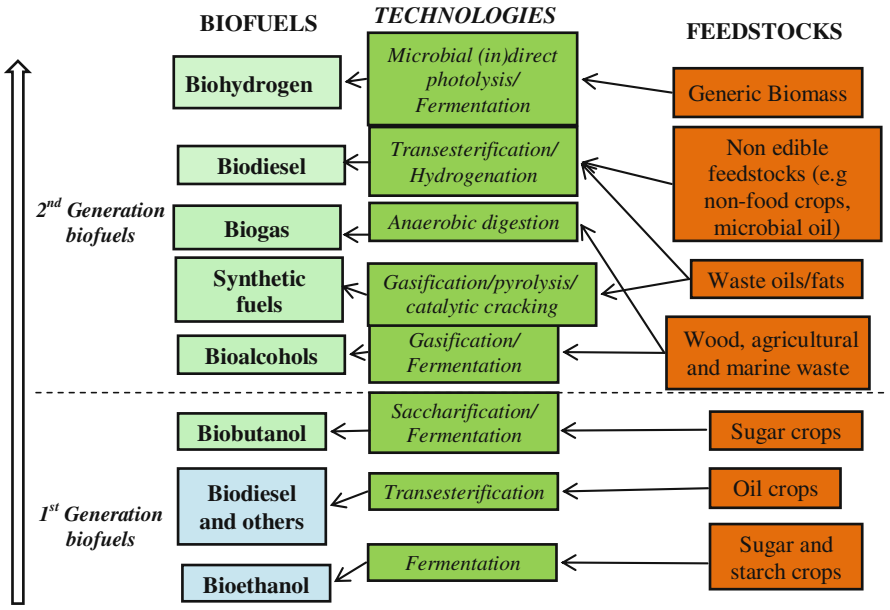

Fig. 8.3 The biofuels ladder. Road map of biofuels production from feedstocks and technologies

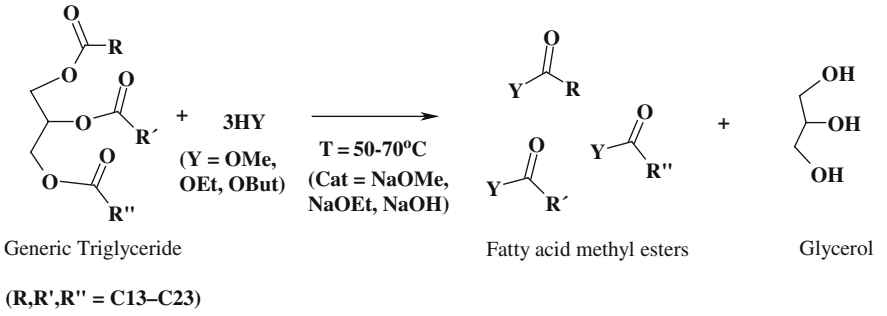

Fig. 8.4 Conventional transesterification of TG for the production of biodiesel

recently published by Al-Zuhair [16]. The methods of preparation of biodiesel can be classified in: chemical catalytic (base- or acid catalysis), biocatalytic (enzyme catalysis) and non-catalytic processes.

Biodiesel Produced by Chemical Catalytic Methods

a. Homogeneous catalysis. The conventional and traditional methodology for the production of biodiesel primarily involved the transesterification of the vegetable oils using NaOH and KOH [17–21] or mineral Brönsted acids (sulphuric, phosphoric or hydrochloric acids) [22–24] as homogeneous catalysts and vegetable oils or waste oils and fats as feedstock at relatively mild temperatures (50–80°C). Few reports on the production of biodiesel using a variety of homogeneous catalysts including guanidines [25] and different amines as catalysts (yielding conversions higher than 98% in a one-step reaction, minimizing the production of waste water) [26] can also be found.

Regardless of the limitations of the methodology, the process is also far from being environmentally friendly. The final mixture needs to be separated, neutralised and thoroughly washed, generating a great amount of salt, soaps, and waste water which need to be further purified or treated. The catalyst cannot also be recycled. These several additional steps certainly put the total overall biodiesel production costs up, reducing at the same time the quality of its main by-product (glycerol). This phase needs to be separated from the biodiesel for further washing/drying to remove the traces of glycerol aand from the fuel to comply with EU quality standard regulations (EN 14214). The standard prescribes 0.02% or lower glycerol content must be present in the biodiesel.

The acid catalysed homogeneous transesterification has not been widely investigated compared to the alkali-catalysed process due to its slower reaction rates, the need of harsher conditions (higher temperatures, methanol to oil molar ratios and quantities of catalysts) and the formation of unwanted secondary products such as dialkyl or glycerol ethers [15]. These drawbacks make impractical its successful implementation with hardly any examples of commercial processes available [27].

b. Heterogeneous catalysis. Several reports can be recently found on the production of biodiesel involving other chemically catalysed protocols as greener alternatives using vegetable oils using solid bases [28–32] and solid acids [24, 28, 33–37]. Di Serio et al. have recently reviewed the use of heterogeneous catalysts for biodiesel production [28]. The advantages of the heterogeneously catalysed protocols from the green chemistry standpoint are that the catalyst may be recycled and subsequently employed in the reaction. The biodiesel prepared has improved properties compared to the homogeneously catalysed process. The elimination of the pre-treatment steps and the minimisation of waste, avoiding the production of waste salts, also improves the green credentials of the reaction. Excellent yields of FAME/FAEE can be obtained under relatively mild conditions with many of these heterogeneous catalysts. However, the separation, disposal or use of the glycerol generated in the process as well as the washing of the crude biodiesel obtained

to remove the traces of glycerol to meet the EEC regulations are often a problem associated to the chemical production of biodiesel.

Biodiesel Produced by Biochemical Catalytic Methods

The increasing environmental concerns have led to a growing interest in the use of enzyme catalysis as it usually produces a cleaner biodiesel under milder conditions. It also generates less waste than the conventional chemical process. Many authors have reported a wide range of efficient and low energy intentive protocols obtaining very promising results with lipases (in both free and immobilised form) [38–43] and combining lipases with alkali catalysts [44].

The limitations of the industrial use of enzymatic methodology is mainly due to their high production costs, which may be overcome by molecular technologies to enable the production of the enzymes in higher quantities as well as in a virtually purified form [45, 46].

Biodiesel Produced by Non-catalysed Processes

The most common and simple non-catalysed biodiesel production process has been performed using supercritical methanol via simultaneous transesterification of triglycerides and esterification of fatty acids [47, 48]. The supercritical alcohol conditions are essential because a very low reaction rate is obtained under subcritical conditions.

The procedure has been claimed to be very effective yielding high FAME contents in a very short time of reaction (typically less than 30 min). The presence of water also facilitated the formation of the methyl esters. Nevertheless, the supercritical methodology is still very expensive and the implementation of such costly technology in industry is currently a challenge.

8.2.1.2 Biofuels Produced by Biological Conversion

Bioalcohols

Bioethanol

Bioethanol is the other common first generation biofuel that is generally used as a blend that can go up to 85% content (E85) [49]. It is the most employed biofuel on a world level with the US currently being the world's largest producer and Brazil the largest exporter, accounting together for 70% of the world's production and 90% of ethanol used for fuel [49]. In Sweden and the US, a high-proportion bioethanol blend E85 (85% ethanol and 15% petrol) is being used in Flexible Fuel Vehicles (FFVs) with modified engines that are able to run on either E85 or petrol, or any mixture of the two.The E85 can nowadays be also purchased in several petrol stations in the UK (Fig. 8.5). Neat ethanol (E100) has also been employed in large scale in Brazil in specially modified engines.

Fig. 8.5 E85 bioethanol blend can be found at cost-competitive prices in many petrol stations all over the UK (May 2008)

The common feedstocks employed for the production of first generation bioethanol are energy crops including sugar cane, corn, wheat, maize and sugar beet ("food" crops) although a great potential of grain or sweet sorghums in replacing maize and sugar cane, respectively, has been reported [50].

First generation bioethanol is generally obtained by biological conversion involving two key steps: hydrolysis and fermentation, followed by a distillation and dehydration of the bioethanol produced to obtain a higher concentration of alcohol to make it suitable for its use as automotive fuel.

Hydrolysis (saccharification). The digestion of the feedstock is normally performed via enzymatic hydrolysis using mixtures of amylases enzymes to convert the starch into sugars. Sugar cane and beet directly produce sugars that can be directly fed into the bioreactor.

Fermentation. The released sugars are subsequently fermentated to ethanol using yeast (e.g. *Saccharomyces cerevisiae*) using a similar process to that used in beer and wine-making [51, 52]. The invertase enzymes present in the yeast catalyse the conversion of sucrose into glucose and fructose that are subsequent transformed into ethanol and carbon dioxide by the zymases enzymes (Fig. 8.6).

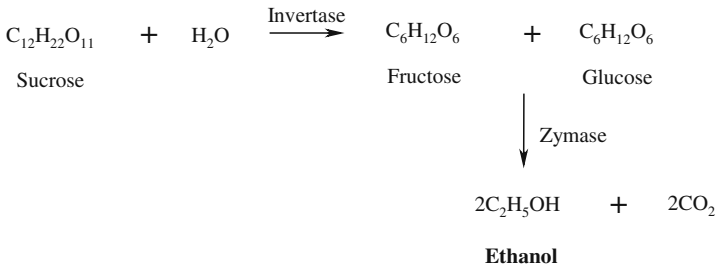

Fig. 8.6 Production of bioethanol via fermentation of hydrolysed sugars from energy crops

Bacteria strains such as *Zymmomonas mobilis* have been demonstrated as an alternative to yeats offering several advantages in the fermentation including higher specific productivity, ethanol yield and alcohol tolerance [3].

Biobutanol

Biobutanol (also denoted as biogasoline) is another interesting candidate that recently entered the battle of the alcohols and has the potential to become one of the key biofuels of the future due to its interesting properties [53–56].

The biobutanol is produced via fermentation in which the sugars from the source (so far from edible feedstocks) are firstly converted to butyrate and hydrogen, then turned into butanol via fermentation using various bacteria strains [53, 54]. The process has been reported to work with a wide range of bacteria and biomass [53–57]. Four main species have been in use, namely *C. acetobutylicum*, *C. beijerinckii*, *C. saccharoperbutylacetonicum* and *C. saccharobutylicum*. Most data are available from *C. acetobutylicum* that has been widely employed in the fermentation of starchy raw materials [53, 54].

Dupont and BP announced a partnership in 2006 to develop the next generation of biofuels, with biobutanol as first product [56, 58, 59]. A biobutanol demonstration plant has recently started to be built at an existing BP site in the UK that is expected to start test production of biobutanol by 2009 using sugar beet as feedstock [58, 59]. Similar biobutanol pilot plant projects are also ongoing in the US [60].

8.2.2 Second Generation Biofuels

Alternative feedstocks, generally *non-edible feedstocks* including waste vegetable oils and fats, non-food crops and biomass sources, and/or technologies were implemented/developed in an attempt to overcome the major shortcomings of the production of first generation biofuels. The biofuels obtained from such technologies have been denoted as second generation biofuels [61]. In theory, these can solve these problems and can supply a larger proportion of fuel supply in a more sustainable and reasonably priced way with greater environmental benefits (Fig. 8.7).

Several advances have been made in the last few years/months. The majority of the second generation biofuels processing technologies are not yet available on a fully commercial scale so the biofuels are expected to enter the market within a few years. Moreover, the development of many other approaches are currently ongoing and many more are to be reported, so the list included below, far from being exhaustive, provides the most interesting technologies reported until very recently. Second generation biofuels will be classified in various groups depending on the technologies employed for their preparation. In a similar way to those of the first generation biofuels, these are prepared by *chemical, thermo-chemical and biological* conversion.

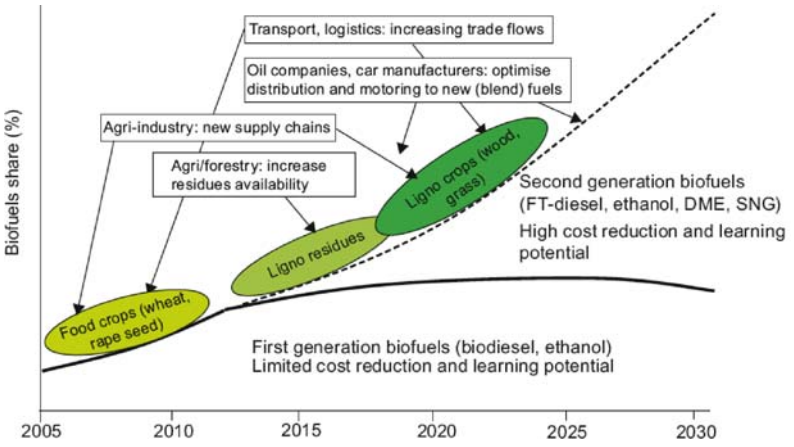

Fig. 8.7 Road map of potential development pathways for first and second generation biofuels, including implications for different markets
Source: REFUEL. http://www.refuel.eu/fileadmin/refuel/user/docs/REFUEL_final_road_map.pdf. Reproduced with permission of Marc Londo.

8.2.2.1 Biofuels Prepared by Chemical Conversion

Biodiesel from Non-edible Feedstocks (Via Transesterification)

Biodiesel from Non-food Crops

A second type of feedstock becoming relevant for the production of biodiesel is the so-called non-edible raw materials including non-food crops and waste oils and fats. Non-food crops, generally not suitable for human consumption or animal feed, have comparable or even higher oil yields (27–40% w/w) and lower resource consumption (i.e. cultivation inputs) than conventional 'food' crops [62], making then specially suitable for a more sustainable biodiesel production, in terms of a more efficient use of resources, minimal interaction with food crops and expected lower environmental impact [63].

Examples of these crops including *Brassica carinata* [64–67] and *Jatropha curcas* [63, 66–68] for the preparation of biodiesel have recently been reported. *Jatropha* is a particularly good example of a non-food crop for biofuel production since it thrives on poor soil and land unsuitable for food crops, actually creating topsoil, and gives a high oil yield.

The preparation of biodiesel from non-food crops is very similar to the chemical transformations (transesterifications) previously described for the use of traditional vegetable oils from food crops. Brazil opened in summer 2007 its first commercial Jatropha biodiesel facility (Compahnhia Productora de Biodiesel de Tocantins) with an estimated production of 40,000 tonnes biodiesel/year by the end of 2008 [69]. Some other *Brassica* and *Jatropha* projects including pilot plants in India, Africa and South America are also ongoing.

Biodiesel from Used Vegetable Oils (UVO) and Fats

UVO and animal fats are also considered as very attractive feedstocks for the production of biodiesel due to their lower market value compared to virgin oils and the fact of being recycled materials from other industrial sectors [70, 71]. The processing of the oil often requires a reduction of the high content FFA via acid catalysed esterification before the actual raw material can be transesterified to biodiesel [50].

Kulkarni et al. have recently reported the use of a heterogeneous solid acid catalyst that is able to carry out a simultaneous esterification of the free fatty acids and transesterification of the triglycerides, giving high FAME yields [72]. Efficient and low energy intentive protocols of the production of biodiesel from waste oils and animal fats combining lipases with alkali catalysts have also been reported [73].

Biodiesel from Microbial Oil (Via Transesterification)

Biodiesel from algae oil. Research is currently ongoing into the production of biodiesel from microalgae, which are believe to afford greater oil yields than any known feedstock as has been recently reported [74, 75].

Microalgae are sunlight-driven cell organisms that convert atmospheric CO_2 (via photosynthesis) into a plethora of chemicals including methane, hydrogen, polysaccharides and oil [74–76]. The production of microalgal oil is remarkably more efficient compared to conventional oil crops, providing higher oil yields (up to a 75% dry weight) and lower land area utilisation (Tables 8.1 and 8.2).

The process involves the extraction of the oil from microalgae and subsequent transesterification with alcohols using homogeneous or heterogeneous catalysts (in a similar way to that of biodiesel obtained from (non) edible feedstocks) to give biodiesel.

Significant advances in the field have been recently reported with biodiesel from microbial oil. Cellana, a joint venture of Shell and HR Biopetroleum recently started the construction of a pilot facility in the Kona coast of Hawaii Island to grow algae as biofuel feedstock [77].

Biodiesel from other microbial oils. Many reports can be found on the subject using different microbes including various yeast and bacteria [78–80]. A summary of the main reported microorganisms and their respective oil yields have been included in Table 8.3.

Table 8.1 Microbial oil content (% dry weight) of various algae species [74, 75]

Microalgae	Oil content (% dry wt)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Cylindrotheca sp.	16–37
Nannochloropsis	31–68
Nitzschia sp.	45–47
Schizochytrium sp.	50–77

Table 8.2 Comparison of oil yield vs required land for different biodiesel feedstocks in the US [74, 75]

Crop	Oil yield (L/ha)	Required land (M ha)[a]
Microalgae[b]	136,900	2
Microalgae[c]	58,700	4.5
Oil palm	5950	45
Jatropha	1,892	140
Canola	1190	223
Soybean	446	594
Corn	172	1540

[a]To meet 50% of all US current transport consumption;
[b]70% (w/w) oil yield in biomass;
[c]30% (w/w) oil yield in biomass.

Table 8.3 Oil production (oil content and yield) of different microorganisms grown on various carbon sources [79, 80]

Microorganism	Carbon source	Biomass (g/L)	Oil content (%)	Oil yield (g/L)	References
Trichosporon fermentans	Molasses	36.4	35.3	12.8	[78]
Lipomyces starkeyi	Sewage sludge	9.4	68.0	6.4	[79]
Mortierella isabellina	Starch	10.4	36.0	3.7	[80]
	Pectin	8.4	24.0	2.0	
Cunningamella echinilata	Starch	13.5	28.0	3.8	
	Pectin	4.1	10.0	0.4	

In general, the cultivation of such microorganisms is not dependent on seasons or climate. They can also be easily grown on a variety of inexpensive substrates including waste residues from agriculture and industry [79], providing they have the nutrients needed for the microorganisms.

8.2.2.2 Biofuels Produced by Thermo-(Chemical) Conversion

Biofuels included under this headline are also prepared from various non-edible biomass feedstocks. Thermo-chemical conversion pathways include processes such as gasification and pyrolysis (Fig. 8.8) [81–83].

Biofuels from Gasification

The process involves the partial combustion of the feedstock to produce syngas (a mixture of carbon monoxide (CO) and hydrogen (H_2) denoted as bio-Synthetic Natural Gas, bio-SNG) via conventional or alternative gasification processes. Then, bio-SNG is subsequently transformed into liquid hydrocarbons (mostly diesel and kerosene-type fuels) and/or gases via different processes, leading to a variety of biofuels that will be outlined. Such prospective liquid/gas biofuels for transport

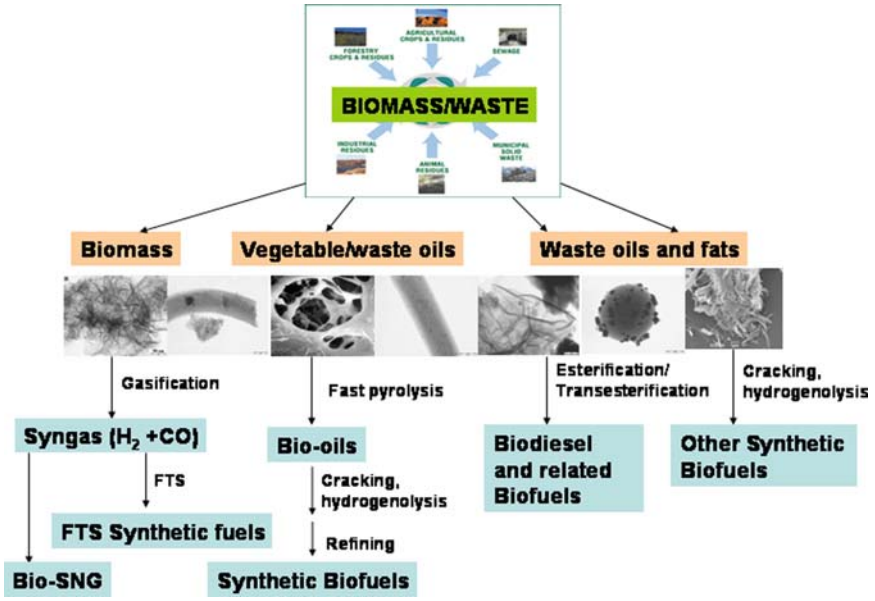

Fig. 8.8 Biomass gasification and pyrolysis routes to synthetic biofuels
Source: Sustainable biofuels: prospects and challenges, RS Policy document 01/08, ISBN 978 0 85403 662 2. Reproduced with permission of the Royal Society.

(Fig. 8.8) include bioalcohols (methanol, ethanol and linear higher chain alcohol mixtures) and synthetic biofuels.

At this point is worth mentioning that although bio-SNG could be classified as *synthetic biofuel,* it comes first since all the reported biofuels from gasification are obtained from it and thus the technologies (up to the preparation of the syngas) are very similar.

Bio-SNG

Bio-SNG can be produced by a conventional gasification process (methanation) at high temperatures (800–1000°C) aiming at producing large quantities of methane. The current technology employed allows the use of a wide range of biomass feedstocks including wood chips and waste wood [84–86]. The conventional gasification process involves various steps (Fig. 8.9). Firstly, the biomass undergoes endothermal steam gasification (reaction 1) to give a mixture of CO and H_2, which is subsequently converted into methane, CO_2 and hydrogen (reactions 2 and 3). The net overall reaction from biomass to methane and CO_2 (reaction 4) is slightly exothermic. However, the main drawback of the conventional gasification technology is the formation of tars and char [87].

Interestingly, the gasification of biomass can be performed at lower temperatures (250–400°C) in supercritical water. It has currently been reported at lab scale, employing different Ni and Co based catalysts [86]. In this process, the biomass

(1) $CH_{1.49}O_{0.67}(s) + 0.33\ H_2O(g) \longrightarrow CO + 1.08\ H_2$

(2) $0.52\ CO + 1.56\ H_2 \longrightarrow 0.52\ CH_4 + 0.52\ H_2O(g)$

(3) $0.48\ CO + 0.48\ H_2O(g) \longrightarrow 0.48\ CO_2 + 0.48\ H_2$

(4) $CH_{1.49}O_{0.67}(s) + 0.29\ H_2O(g) \longrightarrow 0.52\ CH_4 + 0.48\ CO_2$

Fig. 8.9 Reactions involved in the conventional gasification of biomass

disintegrates in supercritical water forming a mixture of carbon dioxide, carbon monoxide and methane (SNG). The technology is expected to be especially suitable for wet (polluted) biomass and has higher efficiency than the conventional gasification process at lower temperature [86]. Bio-SNG can also be produced from biogas.

Various projects in the Netherlands, including the largest existing bio-SNG plant located in Buggenum, currently produce bio-SNG from the co-gasification of biomass with coal at different proportions [88].

Bioalcohols

Biomethanol. Biomethanol can be produced from synthesis gas [89] via conventional gasification of biomass (partial oxidation) at high temperatures (800–1000°C) and subsequent catalytic synthesis of the $CO+H_2$-in a 1:2 ratio-under high pressures (4–10 MPa) [89–91].

The biofuel can be blended with petrol up to 10–20% without the need of any engine modifications [90, 91]. Several feedstocks including bark, woodchips, bamboo, waste wood and pulp [89–91] and even glycerol [92] have been reported to be used in the process.

There are a few biomethanol pilot plants under development, mainly in the US (e.g. North Shore Energy Technologies, 40 MMgy plant) and Japan (e.g. Norin Green no1, MAFF and Mitsubishi heavy industries) [90].

Bioethanol. Bioethanol can be also obtained via conventional thermal gasification of biomass to syngas combined with catalytic processes in similar way to those for the production of biomethanol (Fig. 8.11, left side) [3].

Alternatively, following biomass gasification, the syngas can be directly fermented to ethanol using anaerobic bacteria (Fig. 8.11) [93]. This eliminates the need of the hydrolysis step to break up the cellulose and hemicellulose fractions of the biomass. The lignin fraction can also be converted into ethanol. The process has been reported at lab scale and is still under development [3, 93]. However, the efficient delivery of the syngas to the microorganisms still remains a challenge [3].

There are some examples of ongoing industrial processes. An operating lignocellulosic bioethanol production plant is located in Ottawa (Canada), run by the IOGEN Corporation [94]. The demonstration plant produces up to 3 million litres of bioethanol per year. The feedstocks employed are wheat, oat and barley

straw. A bioethanol plant in Ulmea (Sweden) is running using waste stream of cellulose-based materials and another pilot plant production for the preparation of bioethanol from lignocellulosic materials (e.g. Norway Spruce) has recently started production [95].

Linear bioalcohol mixtures. Mixed linear alcohols (i.e., mixtures of mostly ethanol, propanol and butanol, with some pentanol, hexanol, heptanol and octanol) can also be produced from syngas in a similar way to that described for methanol and ethanol [96]. One of such linear alcohol mixtures denoted as Ecalene^{TM} is currently registered with the US Environmental Protection Agency per 40 CFR 79.23 as a fuel blending additive [96].

Synthetic Biofuels

Synthetic biofuels can be defined as fuels prepared from syngas via different processes. Bridgwater and Demirbas have recently reported comprehensive overviews of the development of these technologies for the preparation of biofuels [87, 89].

Under this headline we can include a selection of some interesting options such as biofuels obtained by steam reforming, HydroThermalUpgrading (HTU) and Fischer-Tropsch Synthesis (FTS).

Biofuels obtained by steam reforming. Steam reforming can be applied to various solid waste materials including organic waste, sewage sludge, waste oils, black liquor and agricultural waste to produce biofuels [89]. Steam reforming of natural gas (often referred as steam CH_4 reforming) is the most common method to produce commercial H_2 [89].

Biohydrogen can therefore be produced from a biomass feedstock via conventional gasification at high temperatures to syngas to obtain methane (reaction 4, Fig. 8.9) and subsequent steam CH_4 reforming at high temperatures (700–1100°C) using Ni supported catalysts (e.g. Ni/Al_2O_3, Ni/MgO) at 3–25 bar pressure (Fig. 8.10, reaction 1) [89, 97]. For the production of high purity H_2, the reforming of the biofuel that includes multiple catalytic steps is followed by two water gas-shift (WGS) reaction steps (Fig. 8.10, reaction 2), a final CO purification and removal of the remaining CO_2 by pressure swing adsorption or ceramic membrane separation [89, 97].

Alternatively, the gasification step of biohydrogen can also be performed in supercritical water (in a similar way to that of the bio-SNG) with the advantages of the direct use of wet biomass without drying and a high gasification efficiency at lower temperature [89, 98]. However, the cost of H_2 production using this technology is several times higher than the current price of H_2 obtained from steam reforming [89].

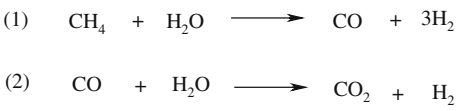

Fig. 8.10 Steam CH_4 reforming (1) and WGS (2) reactions for the preparation of biohydrogen

Biofuels obtained via HydroThermal Upgrading (HTU). HTU-diesel can be produced from various feedstocks including dry (wood and lignocellulose) [99] and wet (beet pulp, sludge or bagasse) biomass [100–102]. The methodology involves the hydrothermal treatment of biomass that is converted into a mixture of hydrocarbons at relatively low temperatures (250–350°C) and moderate (autogenous pressure) [99] to high (120–180 bar) pressures [100, 101].

The biocrude obtained is a heavy organic liquid immiscible with water that contains a wide range of hydrocarbons including acids (e.g. acetic acid), alcohols (e.g. isopropyl alcohol) and phenolic derivatives (in the particular case of lignocellulosic materials) [99–102]. Often, the hydrocarbon mixture obtained needs further processing [via catalytic hydro-de-oxygenation (HDO)] to yields a liquid biofuel similar to fossil diesel that can be blended with fossil diesel in any proportion without the necessity of engine or infrastructure modifications [102]. HTU research has been mainly performed in The Netherlands, with an HTU demonstration plan in Amsterdam that is able to generate over 12,000 tonnes of biocrude (including ash) per year [100, 101].

Biofuels obtained via Fischer-Tropsch Synthesis (FTS). The Fischer-Tropsch (FT) process is one of the advanced biofuels conversion technologies. It has been known since 1923 when German scientists Franz Fisher and Hans Tropsch aimed to synthesize long-chain hydrocarbons from a CO and H_2 gas mixture, but it was mainly used in the past for the production of liquid fuels from coal or natural gas [103, 104].

Prior to the FTS, the gasification of biomass feedstocks takes place in a similar way that described for the production of bio-SNG (Fig. 8.9). Then, a cleaning and conditioning step of the produced syngas is normally performed to remove all the impurities present prior to the catalytic reaction to minimise the poisoning of the catalyst [105, 106].

The FTS process is then carried out. It comprises of various steps described by the set of equations in Fig. 8.11, where x is the average length of the hydrocarbon chain and y is the number of H_2 atoms per carbon.

The first step involves the reaction of CO with H_2 in the presence of a Co or Fe catalyst (Fig. 8.10, top reaction) to afford a hydrocarbon chain extension ($–CH_2–$) that is a building block for the formation of longer hydrocarbons. Typical operation conditions are temperatures between 200 and 400°C and 15–40 bar pressures, depending on the process [89, 103, 104].

All reactions are exothermic and the product is a sulphur free mixture of different predominantly linear hydrocarbons (primarily alkanes and alkenes) that frequently undergoes upgrade and refining steps to be turned into automotive fuels, namely FT-diesel (main product) and gasoline-like biofuels (by-products) [89]. The FT

$$x\mathrm{CO} + 2x\mathrm{H_2} \longrightarrow (\text{-}\mathrm{CH_2}\text{-}) + x\mathrm{H_2O}$$

$$x\mathrm{CO} + 2(x{+}1)\mathrm{H_2} \longrightarrow \mathrm{C_xH_{2x+1}} + x\mathrm{H_2O}$$

$$x\mathrm{CO} + (x + y/2)\mathrm{H_2} \longrightarrow \mathrm{C_xH_y} + x\mathrm{H_2O}$$

Fig. 8.11 FTS reactions for the production of linear long-chain hydrocarbons

catalysts are mainly based on iron and cobalt [89, 103, 104, 107, 108]. Cobalt catalysts have the advantage of a higher conversion rate and they are also more reactive in hydrogenation, producing less unsaturated hydrocarbons and alcohols compared to iron catalysts that produce higher alkenes and oxygenates content [89, 103, 104].

The process using biomass as feedstock is currently under development. In theory, there are no restrictions in the type of biomass that can be used as feedstock. Woody and grassy materials and agricultural and forestry residues have been investigated in the process [107–109].

Pilot production facilities for Fischer-Tropsch liquids from biomass are currently in operation in Germany (e.g. Lurgi and Choren) and the Netherlands [88].

8.2.2.3 Biofuels Produced by Biological Conversion

Bioalcohols

Bioethanol. Second generation bioethanol is usually produced from a range of alternative readily abundant and inexpensive cellulosic biomass feedstocks including woody biomass, grasses, forestry and agricultural waste [110, 111]. Very interesting reviews about the progress in bioethanol and lignocellulosic processing have been recently reported [51, 112, 113].

An overview of the production routes of second generation bioethanol is included in Fig. 8.12.

The process (Fig. 8.12, right hand side) is identical to that described in the production of first generation bioethanol: decomposition of the material into fermentable sugars (hydrolysis) and transformation of the sugars into bioethanol (fermentation).

The main changes are the processing technologies and the feedstocks that usually account for the majority of the plant cost. Cellulosic biomass comprises of two main components. Cellulose and hemicellulose (complex carbohydrate polymers), accounting roughly for about a 70–75 wt% of the lignocellulose. A mixture of enzymes (cellulases and hemicellases) different from those of the first generation bioethanol production are employed in the hydrolysis step. In the particular case of lignocellulosic (woody) materials, lignin is obtained as by-product of the process. Lignin can be burned to produce heat and power for the processing plant and potentially for surrounding homes and businesses and it is to be hoped that

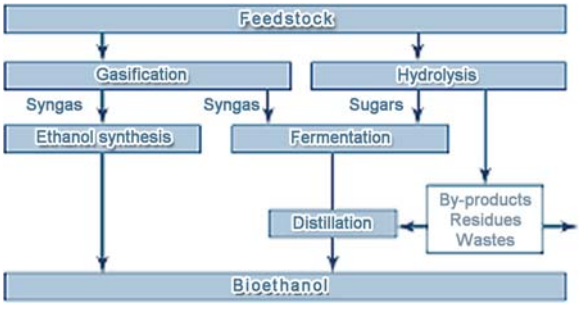

Fig. 8.12 Thermochemical and biological routes to second generation bioethanol Source: Sustainable biofuels: prospects and challenges, RS Policy document 01/08, ISBN 978 0 85403 662 2. Reproduced with permission of the Royal Society.

it can become a future source of aromatic chemicals and materials. Alternative organisms need also to be employed due to the impossibility of the traditional yeast and bacteria to process the pentose (C5) sugars derived from hemicellulose [114].

Branched alcohol mixtures. The preparation of a branched-longer chain alcohol mixture with a potential use as biofuel has been recently reported by Liao et al. [115]. Such alcohol mixture with high isobutanol content is produced via synthetic non-fermentative pathway employing metabolic engineered bacteria (e.g. *E. coli*) and glucose as carbon source. This strategy diverts the 2-ketoacid intermediates in the aminoacid biosynthetic pathway of *E. coli* for alcohol synthesis, converting them into aldehydes (by 2-ketoacid decarboxylases) and then to alcohols (by alcohol dehydrogenases) [115]. The process has been already licensed to Gevo (spin-off company from Pasadena, US) that hopes to begin commercial scale production within a few years [116, 117].

Biogas

Biogas is an environment friendly, clean, cheap and versatile fuel, composed of a mixture of CH_4 and CO_2 that is usually generated from bacterial digestion of biomass in absence of air between 10 and 72°C [83, 118]. Almost any type of organic matter (e.g. sewage sludge, animal wastes, industrial effluents) is suitable for the production of biogas, which can be directly utilised in cooking and heating systems [119]. The process is carried out in anaerobic digesters that can vary in size from 1 m^3 (small household unit) to as large as 2000 m^3 [120], involving a step-wise series of reactions that require the cooperative action of several microorganisms. Initially, a group of microorganisms (acidogens) break down the organic matter into a digestible form (usually simpler fatty acids) that can be utilised by methane-generating anaerobic bacteria (methanogens) that produce biogas as metabolic byproduct [121, 122].

The use of biogas as transport fuel has been explored in its application in explosion engines. Biogas has shown a great potential for its uses in Brazil [123] and in places such as Sweden, has been use in urban buses since 2004 [124]. Also in Sweden some studies have evaluated the economic and environmental feasibility of biogas as a renewable source of energy in large scale showing positive results in its applicability CHP (centralised heat and power) [125].

Biohydrogen

Various authors have recently reviewed the prospects and potential in the production of biohydrogen [126–130]. Biohydrogen can be produced by three different biological pathways: fermentation and direct or indirect (bio)photolysis.

Fermentation. Dark and photo fermentation are technologies under development (currently at lab scale) to produce biohydrogen from wet biomass (e.g. molasses, organic wastes, sewage sludge) using (an)aerobic hydrogen fermenting bacteria [130, 131]. The advantage of the dark fermentation is that the biohydrogen is produced directly without formation of methane [127, 130]. During dark fermentation,

various organic acids are also produced. These compounds can subsequently be converted to hydrogen by a process denoted as photo fermentation.

Direct photolysis. In this approach, the process takes advantage of the photosynthetic capability of algae and cyanobacteria to split water into O_2 and H_2 via direct absorption of light and transfer of electrons to two groups of enzymes that participate in biological hydrogen metabolism: hydrogenases and nitrogenases [129].

Indirect photolysis. Alternatively, biohydrogen can be prepared through the use of some microorganisms (algae) that can directly produce hydrogen under certain conditions [126, 127, 129]. Most specifically, sealed cultures of green algae become anaerobic in the light under deprivation of sulfur nutrients and spontaneously induce the "hydrogenase pathway" to photosynthetically produce hydrogen [127]. Substantial rates of hydrogen production were obtained over 60 h in the light although the hydrogen production leveled off reaching a point (after 100 h) in which the algae go back to the normal photosynthetic pathway in order to restore the consumption of internal starch and proteins that takes place in the course of the hydrogen production [132].

8.3 Engine Performance of Biofuels

8.3.1 Diesel Engines Performance Using Biodiesel

Short- and long-term performance tests in diesel engines using biodiesel (mainly, ethyl and methyl esters from fats or vegetable oils including soybean, rapeseed and sunflower oils) have revealed an increase in the volumetric brake specific fuel consumption, due to the lower volumetric calorific value. Engine power and torque differ slightly or remain unchanged, while smoke emissions of biodiesel are much lower compared to diesel fuel [133–137]. Some of these properties of biodiesel compared to diesel fuel are summarised in Table 8.4.

To improve the combustion properties and cold-weather behavior, several investigations have recommended the use of biodiesel blended with diesel fuel in different percentages [139–142]. The heating value of biodiesel mixtures becomes higher than that of biodiesel due to the lower heating values and stoichiometric air/fuel ratios of biodiesel compared to diesel fuel [143]. A wide range of diesel engines of different sizes and types has been tested. Direct injection, turbocharged, and

Table 8.4 Fuel Specifications of biodiesel and mineral diesel fuel [138]

Property	Diesel fuel (EN-590)	Biodiesel (EN 14214)
Density at 15°C (kg/m^3)	820−860	860−900
Kinematic viscosity at 40°C (mm^2/s)	2−4.5	3.5−5
Flash point (°C)	> 55	> 120
Cetane number (CN)	> 46	> 51
Gross heating value, GHV (MJ/kg)	45−46	30−42

4-cylinder diesel engines have been the most frequently employed [144]. Since engine characteristics might have some influence on the effects of biodiesel, this information has to be taken into consideration.

8.3.1.1 Effect of Biodiesel on Engine Performance Properties

Brake effective power and power output. The power output delivered with biodiesel is reduced with respect to that delivered with diesel fuel at full- and partial-load conditions even with the accelerator fully pressed down. Although reductions around 8% (corresponding to loss of heating value) would be expected in most cases, results show some variations according to literature reports. Kaplan et al. [133] compared sunflower-oil biodiesel with diesel fuel at different engine speed and load regimes, in a 2.5 L, 53 kW engine. The loss of torque and power varied from 5% (at low speed) to 10% (at high speed). Çetinkaya et al. [136] compared waste-oil biodiesel with diesel fuel in a 75 kW 4-cylinder common rail engine under full-load conditions. The loss of torque was in the 3–5% range when biodiesel was used to replace diesel fuel. The authors pointed to the reduced heating value in biodiesel as the most plausible explanation for this reduction. Similar results were achieved by Lin et al. [142] in a naturally aspirated 2.84 L diesel engine running with diesel fuel, biodiesel from palm-oil and a 20% biodiesel blend. The loss of power at full load was around 3.5% with pure biodiesel and 1% with the blend. Similar results in terms of power loss have also been reported elsewhere [145, 146].

Some authors have claimed that there is a relationship between power losses and the reduction in heating value. Yücesu and Ilkiliç[147] measured reductions in torque and power of 3–8% when pure biodiesel from cottonseed was utilised. Interestingly, they also reported for biodiesel a heating value 5% inferior to that of diesel fuel. Difficulties in the fuel atomization (rather than the loss of heating value) was claimed to be the cause of the power loss. Other tests using biodiesel from waste cooking oil in a marine outboard 3-cylinder naturally aspirated engine at full load resulted in a power loss of 7.14% as compared with diesel fuel [148]. The difference in the biodiesel/diesel heating values was interestingly very close to this value. Dorado et al. [138] found a slight increase (5.7%) in the maximum engine power using waste olive oil methyl esters instead of diesel fuel. Only after the engine run on biodiesel for 50 h, a minor 2% loss in maximum power was observed.

There are also some publications reporting unexpected increases in engine power and torque when using biodiesel. Altiparmak et al. measured a 6.1% increase in maximum torque compared to diesel fuel when 70% tall-oil biodiesel blended with diesel fuel was used [149]. Although the increased cetane number was used to explain these findings, the unusually high values of density and viscosity of the tested biodiesel (922 kg/m^3 and 7.1 cSt at 40°C, respectively) could also partially explain such results. Similarly, an increase in torque and power was observed in an indirect injection diesel engine running at 1500 and 3000 rpm on different blends of diesel fuel with biodiesel from tobacco seed oil (with a lower heating value of 39.8 MJ/kg) [150]. The 17.5% biodiesel blend showed the highest values of torque, power, density, viscosity and improved combustion, despite the reduced heating value of biodiesel.

Various reasons have been reported to explain the torque and power recovery at full load (corresponding to the loss of heating value) of biodiesel with respect to diesel fuel.

The higher viscosity of biodiesel may affect the engine brake effective power, especially under full-load operating conditions. The increased injected volume has also been attributed to the increase in viscosity [150].

The higher bulk modulus and sound velocity of biodiesel, together with its higher viscosity, lead to an advanced start of injection [151]. This fact, together with an increase in the cetane number, may slightly advance the start of combustion. To reduce pressure and temperature peaks in the combustion chamber, and thereby nitric oxide formation, current diesel engines need to have delayed combustion. This delay involves a loss of thermal efficiency and consequently of brake effective power. If the start of injection, and thus that of combustion, is advanced, the combustion process is then re-centered and the power output increases [149, 152, 153].

The higher lubricity of biodiesel could also reduce the loss of friction leading to an increased brake effective power. Several researchers have used this argument to explain the increased thermal efficiency or power recovery in spite of the unknown origin of this improvement (reduction of mechanical losses in the injection pump and cylinder walls) [153]. In any case, it seems very unlikely that the lubricity can contribute to the torque and power recovery.

The concept of thermal barrier coatings may be useful to limit the effect of the high viscosity of biodiesel. Engines with thermal barrier coating are called low heat rejection (LHR) engine. The LHR concept is based on suppressing heat rejection to the coolant and recovering the energy in the form of useful work. Insulating the combustion chamber components of LHR engines can reduce heat transfer between in-cylinder gas and cylinder liner, thus enhancing engine power and torque due to the increased exhaust gas temperatures before the turbine inlet [143, 154].

Brake-specific fuel consumption (BSFC). BSFC is the ratio between mass fuel consumption and *Brake effective power*, being inversely proportional to the thermal efficiency. According to literature reports, the biodiesel specific fuel consumption is expected to increase around 10–20% in relation to diesel fuel, corresponding to the increase in heating value in mass basis. In other words, the loss of heating value of biodiesel has to be compensated with a higher fuel consumption. An indicator of the loss of heating value is the oxygen content in the fuel [144]. A correlation between BSFC and oxygen content has been found and the conclusions are the increase in BSFC is due to the oxygen enrichment from the fuel, but not from the intake air [155, 156].

Fuel consumption seems to behave proportionally to the loss of heating value, whether heavy-duty or light-duty engines were tested. Turrio-Baldassarri et al. tested a 6-cylinder 7.8 L engine with a 20% rapeseed-oil biodiesel (with a glycerin content of 1.15%)/diesel fuel blend [157]. They measured a BSFC increase of 2.95% with 95% statistical confidence. A similarly sized engine (6 cylinders and 170 kW of rated power) was tested by Hansen and Jensen with pure rapeseed-oil biodiesel measuring a 14% increase in BSFC [135].

Similar results have been reported considering a wider range of vehicle engine sizes. An increase of 2.5 and 14% in BSFC were obtained in a diesel engine running on 20% biodiesel/diesel fuel blend and pure biodiesel, respectively [158]. The performances of biodiesel from soybean and waste-oils compared in a 57 kW engine showed the feedstock did not have any influence on the BSFC [158]. Senatore et al. tested a 1.9 L diesel engine with rapeseed-oil biodiesel, and found that the increase in BSFC with biodiesel was proportionally related to the decrease of the lower heating value [159]. Similar results were reported for such biodiesel in a single-cylinder diesel engine tested in three steady modes [160].

Many research efforts have carried out in order to ascertain the implications of the properties of biodiesel in the BSFC. The increase in BSFC was reported to be similar to the loss of heating value in biodiesel from waste oils tested on a 2.2 L diesel engine [161]. Monyem and Van Gerpen tested a 4.5 L diesel engine with differently oxidized soybean-oil biodiesel [162]. The increase in BSFC was 13.8 and 15.1% for non-oxidized and oxidized biodiesel (peroxide index of 340 meq/kg). This difference was attributed to the different heating value of both fuels. Most authors have explained these increments by the loss of heating value, although some others attributed them to the different densities of biodiesel and diesel fuel [163]. Nevertheless, some reports moved away from these correlations and claimed no proportionality between the increase in BSFC and the loss of heating value. A 3.3 and 16.7% increase in BSFC (compared to the use of diesel fuel) were observed when using a 20% blend and pure palm-oil biodiesel, respectively [142]. Similarly, Hess et al. [164] found a 18% increase using pure biodiesel from soybean oil. In contrast to these findings, Silva et al. reported no significant changes in BSFC in a 6-cylinder 9.6 L diesel engine fueled with 5 and 30% sunflower-oil biodiesel/diesel fuel blends [146]. Similarly, Dorado et al. [138] evaluated the use of waste olive oil methyl esters during a 50-h short-term performance test in a 3-cylinder 2.5 L diesel engine and found a very slight BSFC increase. Nevertheless, the statistical analysis showed no important differences between biodiesel and No. 2 diesel fuel tests. Kaplan et al. also claimed that the fuel consumption decreased with biodiesel, causing a reduction in the emitted smoke and soot (smoke opacity) [133].

8.3.1.2 Diesel Engine Exhaust Emissions Using Biodiesel

Several approaches have found that, in general, biodiesel lead to less emissions (e.g. CO_2) of the most regulated pollutants compared to standard diesel fuel. Biodiesel may then contribute to reduce greenhouse gas emissions [165–167]. Provided its oxygenated structure, biodiesel causes lesser particulate formation and exhaust emissions compared to diesel fuel, resulting in substantially lower unregulated emissions of carcinogenic compounds (i.e. ketones, benzene and aromatic compounds).

Smoke opacity is a direct measure of smoke and soot. Various studies show that smoke opacity for biodiesel is generally lower [133, 168, 169] with much lower emissions of hydrocarbons. This is also due to oxygenated nature of biodiesel where more oxygen is available for burning and reducing hydrocarbon emissions

in the exhaust [169]. Nitrogen oxides (NO_x) can be very important in polluted air. Regionally high NO_2 concentrations can cause severe air quality deterioration. Practically all anthropogenic NO_x emissions enter the atmosphere as NO generated from the combustion of fossil fuels in internal combustion engines. Like carbon monoxide, NO binds to hemoglobin and reduces the oxygen transport efficiency. However, the concentration of NO normally is much lower than that of carbon monoxide so that the effect on hemoglobin is less significant [170]. In general, reported results have revealed an increase in NO_x emissions for biodiesel [137, 157, 171–173]. Dorado et al. tested a diesel direct injection Perkins engine fueled with waste olive oil methyl esters at several steady-state operating conditions. Results revealed that the use of biodiesel resulted in lower emissions of CO, CO_2, NO, and SO_2, with an increase in NO_2 emissions [138]. The efficiency of the combustion remained constant using both biodiesel and diesel fuel [137]. Recent reports pointed out the formation of higher quantities of NO_x can be correlated to the higher temperatures and quantities of oxygen expected in the combustion chamber due to the improved biodiesel combustion [174, 175]. However, the lower sulfur content present in biodiesel may allow the use of designer control technologies (e.g. catalysts) to the abatement of NO_x emissions that cannot be otherwise employed with conventional diesel.

The fact of the improved combustion process is partially believed to be a result of the advanced injection derived from the optimum physical properties of biodiesel (viscosity, density, compressibility, sound velocity) [175], in good agreement with a variety of reports [152, 153, 159]. Monyem and Van Gerpen [162] found a good correlation between the start of injection and NO_x, independently of the fuel used, which suggests this is the only reason for NO_x increase. Another plausible explanation points to the electronic improvements in the injection pump when biodiesel is used instead of diesel fuel [176]. Thus, it seems that the main reasons for the increase of NO_x emissions using biodiesel as fuel are injection-related.

The acid rain, caused by the deposition on the earth's surface of acids (e.g. SO_2), can be mostly attributed to industrial operations emissions and fossil fuel combustion. As a result of its widespread distribution and effects, it is an air pollutant that may pose a threat to the global atmosphere [170]. Nevertheless, since biodiesel is sulfur-free, less sulfate emissions and particulate reduction are reported in the exhaust and thus it may contribute to reduce the problem of acid rain due to transportation fuels [174].

8.3.2 Spark Ignition Engines Performance Using Bioethanol

Bioethanol is an oxygenated fuel containing 35% oxygen that exhibits a higher octane number (108), lower cetane number (less than 10), broader flammability limits, higher flame speeds and higher heats of vaporization than gasoline [51, 177]. These properties lead to a higher compression ratio, shorter burn time and leaner burn engine, enhancing the theoretical efficiency over gasoline [178]. The autoignition temperature and flash point of ethanol are higher than those of gasoline, which

Table 8.5 Some properties of ethanol, gasoline and diesel fuel [51, 180]

Property	Gasoline (EN 590)	Diesel fuel (EN 590)	Ethanol
Specific gravity (at 15°C)	0.73	0.82	0.79
Boiling point (°C)	30–225	190–280	78.3
Specific heat (MJ/kg)	43.5	43.0	27.0
Heat of vaporization (kJ/kg)	400	600	900
Octane number	91–100	–	108
Cetane number	Below 15	40–60	Below 15
Flashpoint (°C)	–40	64	13
Auto ignition temperature (°C)	300	230	366

makes it safer for transportation and storage. Some properties of ethanol, compared to diesel fuel and gasoline are showed in Table 8.5. However, bioethanol has several drawbacks including a 66% lower energy density compared to gasoline, corrosiveness, low flame luminosity, lower vapor pressure (making cold starts difficult) and its miscibility with water and relative toxicity to ecosystems [51, 179].

Due to its lower volatility and photochemical reactivity in the atmosphere compared to gasoline, there is a reduced smog formation from evaporative emissions in pure ethanol [181]. Bioethanol can also be conveniently blended with gasoline to improve the octane number as well as to promote a more complete combustion. The power output of the engine fueled with low ethanol/gasoline blends is higher compared to gasoline-fueled engines. In general, 10% ethanol addition increases the engine power output by 5%. However, even for low percentage ethanol/gasoline blends (e.g. 10%), undesirable interactions between ethanol and gasoline may cause the vapor pressure to increase. To compensate this effect, the vapor pressure of the gasoline may be reduced. Bioethanol has a very low toxicity compared to other petroleum-based fuels and is readily biodegradable in water and soils (>70% biodegradable compared to diesel fuel), reducing the penetration of plumes of smoke from leaks and environmental concerns as a consequence of spills.

8.3.2.1 Effect of Bioethanol on Diesel Engines Performance Properties

Alcohols can enhance the combustion in compression ignition engines. There are a number of fuel properties that are essential for the optimum performance of a diesel engine. The addition of ethanol to diesel fuel may affect key properties of the blend with particular reference to blending stability, viscosity and lubricity, energy content and cetane number. The properties of ethanol–diesel fuel blends have a significant effect on safety, engine performance, durability, and emissions [177].

Homogeneous charge compression ignition (HCCI) engines tipically exhibit a rapid combustion. However, the components in fuel mixtures do not ignite in unison or burn equally. The combustion of fuel blends in HCCI engines may find a preferential combustion of some the components of the blend [182].

The aromatic content of diesel fuel can also affect the solubility of ethanol in its blend and therefore the effectiveness of emulsifiers and co-solvents [183]. The polar nature of ethanol induces a dipole in the aromatic molecule allowing it to interact

with the ethanol, while the aromatics remain compatible with other hydrocarbons in diesel fuel.

The addition of ethanol to diesel lowers the viscosity and lubricity of the final blend. Lower fuel viscosities lead to greater pump and injector leakage, reducing maximum fuel delivery and ultimately power output. Hot restart problems may occur also as a consequence of the insufficient fuel injected at cranking speed (when fuel leakage in the high-pressure pump is amplified) due to the reduced viscosity of the hot fuel. Fuel viscosity also affects the atomization and spray characteristics in the combustion chamber [184].

With the inverse relationship of octane number and cetane number, ethanol exhibits a low cetane rating (inferior to 10). Hence, increasing concentrations of ethanol in blends proportionately lower the cetane number.

An increase in fuel consumption approximately equivalent to the reduction in energy content of the fuel can also be expected when using ethanol/diesel fuel blends. The energy content in the blends decreases by approximately 2% for each 5% of ethanol added, by volume, assuming that any additive included in the blend has the same energy content as diesel fuel [185]. In any case, no noticeable differences in engine performance, compared to diesel fuel, have been reported with ethanol contents up to 10% [186].

8.3.2.2 Effect of Bioethanol on Spark Ignition Engines Performance Properties

Extensive research efforts have been devoted to investigate the effect of ethanol as a pure fuel and its blends with gasoline on engine performance [187]. Currently, the ethanol consumption in the Brazilian transportation sector represents 44% of the overall gasoline consumption used for transport [188]. Nevertheless, the use of ethanol and ethanol-gasoline blends on spark ignition engines may originate some problems. Upon increasing the ethanol content in the fuel (up to 10%), the heating value of the blends decreases and then the Reid vapor pressure (RVP, a common measure of the volatility of gasoline) increases (which indicates indirectly increased evaporative emissions, while CO tailpipe decreases) to a maximum and then decreases. The heating value of ethanol is lower than gasoline. Therefore, in order to achieve the same energy output, 1.5–1.8 times more ethanol is needed. This leads to higher volumetric fuel consumption compared to petrol, which causes an increase of the BSFC [174]. Other problem related to the use of ethanol-gasoline blends is the phase separation, in the presence of water. Alcohols with 3–8 carbon atoms have better water solubility in blends [189].

Bioethanol has a higher octane rating mixture than branched alcohols, but is far more volatile owing to the formation of minimum temperature azeotropes with the hydrocarbons of gasoline and thus presenting a higher vapor pressure than mixtures of ethers in gasoline [190].

The rapidly increasing use of ethanol as a biofuel in blends with gasoline provides an opportunity to expand its further use as petrol-fuel replacement, with the potential to expand markets for agricultural commodities used to produce ethanol.

However further work is required in specifying acceptable fuel characteristics, confirming the long-term effects on engine durability, and ensuring safety in handling and storing ethanol-diesel blends [177].

8.3.2.3 Engine Exhaust Emissions Using Bioethanol

Bioethanol used in combustion engines has a tremendous potential for a net reduction in the emissions of greenhouse gases. Life-cycle emissions predict the great environmental benefit that can be achieved from the use of bioethanol as transport fuel. Ethanol and others biofuels are considered as "climate friendly", even when considered on a life-cycle basis [191, 192].

CO_2 is released into the atmosphere when a fuel is burned in the engine. However, it is recycled into organic tissues during plant growth. Only about 40% or less of the organic matter is actually removed from farm fields for ethanol production [174]. Bioethanol is believed to give a 70% carbon dioxide reduction compared to petrol [51].

CO is formed by the incomplete combustion of fuels, most readily produced from petroleum fuels, which contain no oxygen in their molecular structure. Since ethanol and other oxygenated compounds contain oxygen, their combustion in automobile engines is more complete. The result is a substantial reduction in CO emissions (up to 30%), depending on the type and age of engine/vehicle, the emission control system used, and the atmospheric conditions in which the vehicle operates.

The addition of bioethanol to diesel fuel has also a beneficial effect in reducing particulate matter (PM) emissions [193]. The degree of improvement varies from engine to engine and also within the working range of the engine itself. While there is considerable value in being able to use the fuel directly in an unmodified engine, small adjustments to fuel injection characteristics may result in further gains in reducing emissions [177].

Because of its high octane number, the addition of bioethanol to gasoline leads to the reduction or removal of aromatic hydrocarbons (e.g. benzene), and other hazardous high-octane additives commonly used to replace tetraethyl lead in gasoline [194]. Clear trends of reduced hydrocarbons and CO emissions and increased NO_x emissions have been observed with increasing percentages of ethanol in the blend (from 0 to 20%). A standard vehicle operates at air/fuel ratios significantly richer than stoichiometric, with an average air/fuel ratio running on gasoline of approximately 12.2:1. For leaner base conditions, the trend could be the opposite, with increasing hydrocarbon emissions and reduced NO_x emissions with increasing ethanol contents [195]. Acetaldehyde emissions are also superior with increasing ethanol contents in the blend as this compound can be produced from ethanol via oxidation under certain operating conditions. Interestingly, such emissions have also a close relationship with the engine load and the ethanol content in the blend. With increasing loadings from idling, the acetaldehyde emissions gradually decrease to their minimum at medium loads, then increase again at high engine loads [192].

Toxic unregulated emissions (i.e. formaldehyde, propionaldehyde, 1,3-butadiene, acrolein, linear alkenes and aromatics) and fine particulate should be considered to

ascertain the impact of ethanol-blended fuels. Researchers report benzene emissions reduction up to 50% with the ethanol-blended fuels. Emissions of 1,3-butadienes were also substantially decreased in the range from 24 to 82% [196].

8.3.3 Effect of Ethers as Biofuels in Spark Ignition Engine Performance Properties

The industrial production of tert-alkyl ethers has a rising interest for refineries, due to reformulated gasoline obtained with respect to EURO standards applied in Europe, Australia and New Zealand [197]. The asymmetric ethers are synthesized through an addition reaction between the alcohols and the tertiary olefins of high reactivity which may be found in the hydrocarbons flow coming out of destructive processes such as catalytic cracking and pyrolysis [197]. In this way, ethers including ETBE (C_4H_9–O–C_2H_5), and TAEE (C_5H_{11}–O–C_2H_5) can be prepared as alternative fuels. In France, Spain and Germany, ETBE is usually mixed with gasoline in proportions up to 15 vol% [198]. It is produced by the etherification of isobutene, usually present in a mixture of C4 isomers, with ethanol [188, 190, 197, 198]. ETBE is an adequate substitute for methyl tert-butyl ether (MTBE), an oxygenating additive that is currently prohibited in many countries due to its toxicity and contamination of underground waters [199]. These ethers offer several advantages with respect to ethanol in terms of low latent heat of vaporization, low solubility in water and higher combustion enthalpy [200].

8.4 Future Prospects and Challenges

8.4.1 Future Prospects: 1st Vs 2nd Generation Biofuels

Various interesting conclusions can be drawn from the use of first and second generation biofuels. 1st generation biodiesel and bioalcohols have many advantages as petrol-fuel replacements but also important disadvantages. The main concern related to the production of first generation biofuels comes from the fact that the conventional biofuel production process generally involves the use of ‘food’ crops. This issue has generated much controversy in a world where the limited area of arable land and grain reserves may contribute to skyrocket the food prices if we carry on using such food crops extensively for biofuel production. That and other issues that arise related to deforestation, global warming and biodiversity threatening, in particular in developing countries (e.g. Malaysia as a consequence of the use of vegetable oils (e.g. palm oil) for the production of biodiesel) encouraged the search for alternative technologies and feedstocks for biofuels production and the development of second generation biofuels.

The production of biofuels from second generation biofuels from non-edible feedstocks has interesting features. Non food crops can be cultivated in alternative

lands included the so-called "wastelands", tropical zones or even arid regions as they are more likely to proliferate at relatively extreme conditions (e.g. plagues and dry environments) with a low fertiliser input. Many of the biomass feedstocks are also self-seeding crops (they do not need to be planted and re-seeded after harvested) and require little or virtually no fertilizer input. These approaches can therefore be applied to "marginal lands" where the soil cannot/should not support food crops [201]. In this way, they will not interfere with the land dedicated to food crops. They can also provide a solution for the production of quality biofuels in developing countries (e.g. India) where, for example, a blend of biodiesel obtained from jatropha and palm has been reported to have a right balance of physical properties conferring the product with an adequate cold low performance and oxidation stability [62], also falling within the acceptable by the American and European biodiesel standards.

However, the switch to these non-edible feedstocks poses various concerns. The cultivation patterns of the crops are still under investigation and early studies have shown relative differences depending on the approach taken to crop cultivation and oil production management [202]. The crops have only been employed by local communities for different uses (e.g. soap production and natural crop protection for the inedible nature of the oil and toxicity of the seeds, respectively) [203, 204]. Therefore, the evaluation of the sustainability index needs of more data to estimate the real global impact of these crops. Furthermore, the technologies available for the majority of the second generation biofuels are still in their infancy and need major developments to be able to sustain a scaled-up production of biofuels for transport. The economics of the processes may play a key role in the successful implementation of many of these technologies.

A full discussion on these important topics, with a thorough evaluation of socio-economic, environmental and related issues, has been recently reported [205]. Some of the most relevant prospects and challenges for the future of second generation biofuels will now be detailed.

8.4.1.1 Second Generation Biodiesel

The use of cheap feedstocks (e.g. waste oils and fats) and the potential commercialisation of glycerol (and glycerol derived products) can considerably reduce the biodiesel production costs, specially taking into account that 70–90% of the biodiesel cost arises from the cost of the oil [206]. However, the use of high temperatures in the transesterification, incomplete conversion and variability of the incoming feedstock (with marked differences in water content and FFA depending on the source, location and usage) are problems related to such feedstocks for biodiesel production.

The production of methyl esters from algal oil has also recently attracted a great deal of attention. The enormous diversity of species of algae with high oil content that require a tiny land utilisation compared to oil crops offers an interesting possibility of industrial exploitation of such organisms in the production of biodiesel.

However, there are major limitations in their successful implementation, being the economic feasibility of the technology the most important. Firstly, the recovery of such bio-oil from algae is very challenging task. The algal broth produced in the biomass production generally needs to be further processed to recover the biomass [207] and then the concentrated biomass paste is extracted with an organic solvent (e.g. hexane) to recover the algal oil that can be transesterified into biodiesel.

Secondly, microalgal oil is rich in long-chain polyunsaturated acids including eicosapentaenoic (20:5 n-3, EPA) and docosahexaenoic acids (22:6 ω-3, DHA) which are generally undesirable in conventional biodiesel due to the negative impact of the polyunsaturations on oxidation stability. The presence of EPA and DHA is not contemplated in the EU (EN 14214 and EN 14213, biodiesel for transport and heating) and US (ASTM D6751) quality biodiesel standards that specify a limit of 130 g (EN 14213) and 120 g (EN 14214) iodiene/100 g biodiesel (iodine value). The storage issues arising from the oxidation instability may either be overcome through chemical transformations (e.g hydrogenations) of the polyunsaturated compounds [208]. It is yet unclear how the presence much more saturated FAME will affect cold performance (CFPP) of the biodiesel.

These main drawbacks will undoubtedly put up the costs of an already costly process in which problems related to capital infrastructure costs, contamination through open pond systems and costs associated with harvesting, drying and valorisation of the rest of the algae may have also a major contribution. A full and precise estimation of the economics of the process, that have been argued to be far too good from what Chisti [74, 75] originally reported, is needed in order to demonstrate its feasibility [76, 207].

8.4.1.2 Second Generation Bioalcohols

There are two critical issues that need to be addressed for the succesful development of the second generation bioalcohols from biomass via biological conversion. Firstly, the development of an efficient pre-treatment process in order to break up the fibre structure of the biomass is needed because the methodologies investigated (mechanical, thermal, chemical, enzymatic-cellulase- and combinations of them) have been proven to be unsuitable due their high costs, low yields, produced waste or undesired by-products. Secondly, an efficient microorganism for the fermentation of pentoses, present in hemicellulose, needs to be developed. These strategies may also open up interesting possibilities to employ more user-friendly microorganisms (e.g. *Saccharomyces cerevisiae*) for biofuels production. Therefore, there is a need for a joint effort from chemists, microbiologists and chemical and biochemical engineers in order to demonstrate the potential of second generation bioethanol via biological conversion.

Bioalcohols obtained from the gasification of biomass does not have significant differences in properties compared to that obtained by biological conversion. However, the processes are remarkably dissimilar. The conventional gasification step is a costly process compared to the relatively inexpensive biological conversion. Another important issue that needs to be addressed is the lack of standards for

producers and users. Nevertheless, the biomass feedstock can effectively be turned into syngas (without the need of any microorganisms) and subsequently into bioalcohols [3]. Compared to bioalcohols produced by biological conversion, this protocol avoids issues such as the inefficient degradation of biomass to fermentable sugars as well as dealing with the processing of the pentoses (C5) generated in the hydrolysis of biomass.

8.4.1.3 Biogas

Biogas might be of relevance in renewable energy markets both for transport and for generation of electricity. It is also a realistic alternative to the accumulation of waste in landfill as new sites can be specially configured to optimise gas output (as high as 1000 m^3/h biogas. However, LCA studies have identified an impact to GHG in its production, associated to the generation and emission of CO_2 and N_2O in the process [126, 127].

8.4.1.4 Biohydrogen

Biohydrogen is believed to be one of the biofuels for the future, combining its ability to potentially reduce the dependence of foreign oil and contribute to lower the GHG emissions from the transportation sector. However, storage (biohydrogen has to be compressed, liquefied, or stored in metal hydrides), transportation and use (fuel cell vehicles are not commercially available yet and a distribution infrastructure for hydrogen cannot be realised in the short term) as well as the technological advances needed for its successful implementation limit bio-hydrogen only as a longer-term option for the transport sector.

8.4.1.5 Bio-SNG

Bio-SNG has various advantages but also a number of challenges for the future. Its octane number is very high, but the cetane number is very low, which means that bio-SNG has to be used in spark ignition engines, which need to be adapted for its use. Storage is also a challenge for the future as bio-SNG is also a gas at room temperature so it needs to be compressed or liquefied to be used as an automotive fuel. Furthermore, larger storage and fuel tanks are needed due to the lower volumetric energy content of the fuel.

The supercritical water low-temperature gasification technology may overcome some of the main technological barriers in the process. Nevertheless, gas cleaning (especially tar removal) and catalyst development are important technological issues, although if active and selective catalysts are used (e.g. Ru/C), no significant quantities of tars or char have been reported to form. However, the cost of the supercritical water production of bio-SNG is several times higher than that of the conventional gasification process [89].

8.4.1.6 Synthetic Biofuels

Synthetic biofuels have several advantages as they can be used in unmodified diesel engines [88, 89] and they are cleaner that traditional fuels due to the removal of all contaminants to avoid the poisoning of the catalysts used in the processing steps [3, 105, 106]. These biofuels can have excellent autoignition characteristics as they have similar energy content, density and viscosity that of fossil diesel as well as higher cetane number and lower aromatic content (which results in lower particle emissions). They are also S-, N-free and less corrosive than other biofuels (e.g. bioethanol and biodiesel) therefore being more environmentally friendly than fuels produced from crude oil. Some of them (e.g. FT-diesel) have been proved to reduce the CO, NOx and particulate matter compared to diesel fuel [88, 107, 108].

However, the production of synthetic biofuels faces a similar technological barrier to that of the gasification-derived biofuels (i.e. bioalcohols and other synthetic biofuels): the production of the synthesis gas has to be adapted to the higher reactivity and different properties of biomass with respect to coal. This includes two key steps in the process that need thorough improvements: biomass pre-treatment (via torrefaction and/or pyrolysis) to avoid the aggregation of the biomass particles that can plug the feeding lines and economically viable inferior temperatures of gasification (e.g. *via* supercritical water gasification) that have been reported to provide higher efficiencies.

For instance, the FTS biofuel production can be more cost effective reducing both the capital and the operating costs of the plant [209], being the purification of the syngas the most expensive section to take into account for costs in an FT plant. The development of active and selective catalysts and the utilisation of by-products including electricity, heat and steam are some other inputs that need to be addressed.

8.5 Conclusions

The potential for biofuels has been recognised throughout the twentieth century but the new century has brought with it a widespread realisation that the petroleum age is coming to an end. The use of petrol-fuel replacements has generated a lot of controversy; ideally they should contribute to global sustainability, ensuring the energy supply and meeting the GHG targets (as well as being profitable and cost competitive as much as possible) without compromising the economies, culture, societies and the environment of our future. More thoughtful analysis is now showing that many of these so-called first generation biofuels are little better than traditional fuels in terms of overall carbon footprint and environmental damage. Second generation biofuels and more widely, biomass exploitation, have a great potential to improve these values and the future aims should focus on redoubling our efforts to produce later generation biofuels based on low value and waste biomass, using the greenest and efficient technologies and with properly measured and reported environmental impacts. A joint effort from politics, economists, environmentalists and scientists is needed now, more than ever, to address the issues of the progressive incorporation

of biofuels in our society and to come up with alternatives, policies and choices to advance the key technologies for a more sustainable future.

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Chapter 9
Floating Vegetated Mats for Improving Surface Water Quality

Robert K. Hubbard

Abstract Contamination of surface and ground waters is an environmental concern. Pollution from both point and nonpoint sources can render water unsuitable for use. Surface waters of concern include streams, rivers, ponds, lakes, canals, and wastewater lagoons. Lagooned wastewater from confined animal feeding operations (CAFOs) represents an extreme in water quality problems. Wastewater lagoons are used for primary treatment which includes settling of solids and loss of gases by volatilization. Additional methods are often used to treat the wastewater from the lagoons. These methods include passing the wastewater through constructed wetlands, where both plant uptake and biological processes such as denitrification remove or retain nutrients, and application of the wastewater to agricultural or forestry land. A new concept for improving surface water quality including that of wastewater lagoons is to grow vegetation on floating platforms in the water body. Little research has been conducted in this area, although this technology basically is application of hydroponics using floating platforms for the vegetation which then utilizes nutrients contained in the contaminated waters. Research conducted by USDA-ARS and the University of Georgia at Tifton, GA has focused on determining the feasibility of growing vegetation to produce biomass and remove nutrients from contaminated surface water bodies. The research has shown that different plant species can be found to grow on floating platforms in a range of different water qualities. In the most contaminated water tested thus far, anaerobic swine lagoon wastewater, it was determined that plants remove nutrients to their maximum capacity such that total removal of nutrients from the water body is a function of biomass produced. This chapter explains the concepts and techniques involved in using floating vegetated mats on contaminated water bodies for nutrient removal, reports results from completed studies, discusses ongoing projects, and identifies research needs for this emerging technology.

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9.1 Introduction

Clean water is a crucial resource for drinking supply, irrigation, industry, transportation, recreation, fishing, hunting, and support of biodiversity. Pollutant enrichment of surface water bodies is often attributed to production areas where applied inorganic fertilizers or animal wastes have moved via surface runoff or leaching from the point of application. This enrichment may result in eutrophication of the water body, which then places environmental and/or economic burdens on society when remediation is required. Eutrophication caused by excessive inputs of phosphorus (P) and nitrogen (N) is the most common impairment of surface waters in the United States [1], with impairment measured as the area of surface water not suitable for designated uses. Eutrophication accounts for approximately 50% of the impaired lake area and 60% of the impaired river reaches in the United States [2], and is the most widespread pollution problem of U.S. estuaries [3]. Freshwater eutrophication has been a growing problem for decades [4, 5]. For most temperate estuaries and coastal ecosystems, N is the element most limiting to primary production and most responsible for eutrophication [3, 6–8]. For inland waters P is generally the most limiting nutrient for ecosystems with excess P resulting in eutrophication of the water body. Eutrophication has many negative effects on aquatic ecosystems [9]. The most obvious consequences are the increased growth of algae and aquatic weeds that interfere with use of water. Oxygen (O_2) shortages caused by senescence and decomposition of nuisance plants cause fish kills.

9.1.1 Nitrogen

Nitrogen (N) contamination of surface and groundwater is a health concern for both humans and animals. Elevated nitrate (NO_3-N) concentrations in drinking water have caused infant death from the disease methomoglobinemia, and toxic effects on livestock [10, 11]. Infants are most sensitive because bacteria that live in an infant's digestive tract can reduce NO_3-N to nitrite (NO_2-N), causing conversion of hemoglobin into methemoglobin, which interferes with the oxygen-carrying ability of blood [11]. Formation of potentially carcinogenic nitrosamines in the soil from nitrite (NO_2-N) and secondary amines is also a health concern. Both NO_3-N and NO_2-N have been shown to negatively affect the metabolism of domestic animals [10, 11].

Nitrate levels greater than 10 parts per million (public health standard) have been documented in groundwater associated with agricultural activities in New York [12], Wisconsin [13], Nebraska [14, 15], Arkansas [16], Ontario [17], England [18, 19], Georgia [20, 21], and Oklahoma [22, 23]. These high concentrations were associated

primarily with fertilizer use. Nitrogen contamination of groundwater from animal waste has been linked to both grazing animals and to land application of wastewater from Confined Animal Feeding Operation (CAFO) lagoons [20, 24–28].

9.1.2 Phosphorus

Phosphorus is of environmental concern because excess amounts in surface water bodies may cause eutrophication. Phosphate (PO_4-P) is a soluble agricultural chemical that may be moved from point of application by surface runoff or move out of the soil surface with percolation. In general, PO_4-P is considered to be of concern primarily for surface runoff since it binds to Fe, Al or Ca in the soil depending on pH and is not readily leachable. Soluble PO_4-P and PO_4-P associated with sediment in surface runoff have been found to vary linearly with P application rate [29]. Low concentrations of dissolved PO_4-P have been found in runoff from deep incorporation of fertilizers [30]. Movement of PO_4-P through the soil profile varies with soil texture. For nonsandy soils, the leaching of PO_4-P with percolating water is extremely low or undetectable. The PO_4-P content of percolate from non-sandy soils can be within an order of magnitude of 0.1 mg L^{-1} [31]. Numerous investigators [31–34], however, have shown that in very sandy soils, PO_4-P will move down the profile to a considerable depth (>1.0 m). On the basis of diffusion studies, Olsen and Watanabe [35] concluded that there was an eight-times greater risk of PO_4-P pollution of groundwater from sands than from clays. The contribution of P from animal wastes can represent a significant fraction of the P circulating in agricultural systems. Where fecal matter is deposited into farm ponds or streams the direct effect may be noticeable.

9.1.3 Wastewater Lagoons

A number of methods have been designed to handle animal wastes from CAFOs. Most systems involve primary treatment in wastewater lagoons for settling of solids and loss of gases by volatilization. Anaerobic treatment systems (lagoons) are used widely for practical treatment and storage of swine manure [36, 37]. These lagoons are typically earthen and rely on bacteria to stabilize organic material [38]. Lagoons are relatively simple to operate and maintain, and are relatively inexpensive compared with other treatment methods [39]. Dairy operations may use a two lagoon system with aerators commonly used in the second lagoon. Animal wastewater lagoons are extreme examples of contaminated surface water bodies.

Aquaculture operations for production of fish or shrimp may also produce wastewater. The water in the ponds must be periodically replaced to avoid disease, low O_2, algae, and/or pH problems associated with the accumulation of waste. Organic solids, mainly generated from feed residue and fish excreta, are the primary pollutants to be removed from an aquaculture effluent or in a recirculating aquaculture system [40]. These solids can become an additional source of O_2 demand,

NH_4-N, and P as they decompose, decreasing the quality of receiving waters or the water in a recirculating aquaculture system [41, 42]. Liao and Mayo [43] estimated that 70% of the NH_4-N in aquaculture wastewater is associated with organic solids. Bergheim et al. [44] estimated that 47–84% of the total phosphorus (TP) in aquaculture wastewater is particle-bound. Furthermore, high concentrations of suspended solids (SS) may directly cause gill damage in fish by fouling. Gill damage increases stress and susceptibility to diseases in fish [45].

Algal growth typically occurs in outdoor fishponds as a result of photosynthetic productions [41, 42]. Large amounts of algae can increase pH levels, which can harm the fish in a fishpond. Also, fishpond discharges contain algae as organic SS, thus increasing O_2 demand in the receiving waters by bacterial degradation [46]. Therefore, algae removal is also essential for aquaculture wastewater treatment [41, 42].

9.2 Methods of Addressing Water and Wastewater Concerns

Generally the quality of surface water bodies is addressed through limiting point or non-point pollution entering the water. Point source contamination of water bodies may be from sources such as improper discharge from CAFOs. Point source pollutant discharges tend to be continuous, with little variability over time, and often can be monitored by measuring concentrations and discharge periodically at a single point [9]. Nonpoint inputs can also be continuous, but are more often intermittent and linked to seasonal agricultural activity or irregular events, such as heavy precipitation or major construction. Nonpoint inputs are the major source of water pollution in the United States [2]. Control of nonpoint pollution centers on land management practices.

9.2.1 Land Application

Wastewater from lagoons generally is land applied. Land treatment systems may include application of wastewater to crops or pasture [47–49], forest, or vegetative buffer systems [50, 51]. A number of investigators have used dairy wastewater on forage systems [52–55]. At Tifton, GA, research investigated the utilization of dairy lagoon wastewater on a frequent, around-the-year basis in an attempt to reduce manure storage and its associated cost and potential for nutrient loss, odor and overflow; maximize recycling of nutrients in crops; and reduce labor demands associated with seasonal manure application [49]. Two systems were investigated: a mixture of Abruzzi rye and crimson clover overseeded in the autumn into a Tifton 44 bermudagrass (*Cynodon dactylon*) *sod* (for spring haylage), minimum tillage silage corn (*Zea mays*) seeded after rye/clover harvest, and bermudagrass hay harvest in summer; and conventional minimum tillage (no living cover crop) rye and clover established in the autumn (for haylage), a first crop of temperate corn in spring and a second crop

of tropical corn in summer (both for silage). These systems were investigated at field scale under a pivot irrigation system and in replicated small plots, and included comparisons between manure and commercial fertilizer that was applied at rates based on soil tests following each crop. Newton et al. [49] showed that dairy lagoon wastewater could be successfully used for triple cropping systems including both cropland and winter grazing of pasture.

Hubbard et al. [56] showed that vegetated buffer systems can effectively assimilate N from swine lagoon wastewater. In a study on replicated 30X4 m plots they tested three different vegetated buffer treatments at two different wastewater rates. The treatments were (1) 10 m grass buffer draining into 20 m existing riparian zone vegetation; (2) 20 m grass buffer draining into 10 m existing riparian zone vegetation; and (3) 10 m grass buffer draining into 20 m maidencane (*Panicum hematomon* Schult 'Halifax'). The wastewater, which contained an average N concentration of 160 mg L^{-1} N was applied to the plots either once per week (1285 L $plot^{-1}$) or twice per week (2570 L $plot^{-1}$). Nitrogen concentrations in surface runoff and shallow groundwater increased over time at the top ends of the plots but showed little increase at the bottom ends of the plots. Overall, all three vegetative treatments were successful in assimilating N from the wastewater. In a similar study, but at the farm scale with highly contaminated wastewater from the anaerobic lagoon of a commercial hog farm, Hubbard et al. [57] found that NO_3-N concentrations in shallow groundwater 20–30 m downslope from the overland flow application point were still near background levels after five years of wastewater application.

9.2.2 Constructed Wetlands

Another method of treating contaminated surface waters is by using constructed wetlands. Constructed wetlands, that is, the integrated physical, chemical, and biological processes that occur in the substratum soil (or gravel)-water-plant ecosystem, have grown in popularity for wastewater treatment since the early 1970s [58]. During the past three decades, constructed wetlands have been used to treat municipal wastewater, acid mine drainage, industrial wastewater, agricultural and storm runoff, and effluent from livestock operations [41, 42]. Their advantages include moderate capital costs, very low energy consumption and maintenance requirements, and benefits of increased wildlife habitat [59]. Many researchers have demonstrated that natural treatment systems can remove significant amounts of SS, organic matter (OM), N, P, trace elements, and microorganisms (including algae) from wastewater [60–63].

Lin et al. [41] used a pilot-scale wastewater treatment system consisting of free water surface (FWS) and subsurface flow (SSF) constructed wetlands arranged in series for treatment of aquaculture wastewater. Their study was conducted to examine system start-up phenomena and to evaluate system performance in removing inorganic N and P from aquaculture wastewater under various hydraulic loading rates (1.8–13.5 cm day^{-1}). They found excellent N removals. The efficiencies were 86–98% for NH_4-N and 95–95% for total inorganic nitrogen (TIN) and removal

efficiencies were affected little by the hydraulic loading trials. Phosphate removal of 32–71% occurred with the efficiencies being inversely related to hydraulic loading. The FWS wetland removed most inorganic N whereas the SSF wetland removed PO_4-P at a rate equal to or even greater than the FWS. Removal of NH_4-N and NO_3-N (effluent concentrations <0.3 mg NH_4-N L^{-1} and 0.01 mg NO_2-N L^{-1}) were sufficient for recycle in the aquaculture system without danger of harming the fish.

In the same study Lin et al. [42] found that macrophyte density was a critical factor affecting the reduction of SS and chlorophyll for the FWS wetland, but not for the SF wetland. Suspended solids removals in both of the wetlands and the combined system (47–86%) decreased significantly as the hydraulic loading rate increased, strongly following the first-order mass-decrease equation. Phytoplankton solids (biomass and detritus) were a primary source of SS in the aquaculture wastewater. Both chlorophyll reduction (76–95%) and chemical oxygen demand (COD) removal (25–55%) in the constructed wetland systems were apparently not affected by hydraulic loading.

Maine et al. [64] used a free water surface wetland to treat wastewater containing metals (Cr, Ni and Zn) and nutrients from a tool factory in Santo Tome, Santa Fe, Argentina. They found that water hyacinth (*Eichhornia crassipes*) was dominant with a free water surface during the first year but then decreased as the water depth was lowered. Cattail (*Typha domingensis*) then became dominant. While water hyacinth was dominant, the wetland retained 62% of the incoming Cr and 48% of the Ni. Nitrate and NO_2-N were also removed (65 and 78%, respectively), while dissolved inorganic PO_4-P and NH_4-N were not removed. When cattail became dominant retention was 58% Cr, 48% Ni and 64% dissolved P, while 79% NO_3-N, 84% NO_2-N, and 13% NH_4-N were removed. Maine et al. [64] also found that NH_4-N showed a different behaviour at different phases of vegetation development.

Several different studies have shown denitrification to be a major pathway in wetlands. Mass balance in the Maine et al. [64] study suggested that N retained by plants represented a minor fraction of the N removed from the incoming wastewater in the small-scale wetland. They concluded that denitrification may have been the major removal process. D'Angelo and Reddy [65] determined that most of the ^{15}N-NO_3 (roughly 90%) applied to sediment-water cores was lost by denitrification. Reddy et al. [62] measured large denitrification rates in the rhizosphere of emergent macrophytes of deltaic marshes. Matheson et al. [66] performed ^{15}N balances in wetland microcosms, and estimated that denitrification accounted for 61% of the NO_3-N load; 25% was retained in the soil, and 14% was stored in the vegetation biomass.

Emergent macrophytes are known to release O_2 from the roots producing a strong positive effect on nitrifying bacteria in the rhizosphere [67]. Sliekers et al., [68] showed that anaerobic NH_3 oxidation is a qualitatively important pathway in wastewater sediments. Maine et al. [64] concluded that in their system they had simultaneous occurrence of partial NH_4-N removal through the water hyacinth decline period and the cattail dominance phases. They also concluded that dissolved P might have been adsorbed onto Fe oxy hydroxides and later settled on the bottom sediment. This occurred because enhanced phytoplanktonic and periphytic growth

and O_2 transfer from the atmosphere as well as O_2 release by emergent macrophyte roots probably created niches of high redox potential where this adsorbtion occurred.

Surrency [63] reported on constructed wetland research from four locations in the southeast and concluded that giant cutgrass (*Scirpus californicus* and *S. validus*), maidencane, pickerelweed (*Pontedieria cordata*), arrowhead (*Sagittaria lancifolia*), and cattail (*Typha latifolia*) are the best aquatic plants to use in constructed wetlands for treating wastewater from dairy and swine operations and for municipal constructed wetland systems. In an in-situ containerized field study, Hubbard et al. [69] evaluated growth and nutrient uptake response of the species Dahoon holly (*Ilex cassine*), buttonbush (*Cephalanthus occidentalis*), Virginia sweetspire (*Itea virginica*), saltmeadow cordgrass (S*partina patens*), soft rush (*Juncus effuses*), and maidencane when swine lagoon wastewater was applied. They found that buttonbush and saltmeadow cordgrass were best at removing nutrients as compared to the other species.

9.2.3 Hydroponics

A number of researchers have investigated using hydroponics to purify wastewater from agriculture [70–74]. Snow and Ghaly [71] used barley (*Hordeum vulgare*) for the purification of aquaculture wastewater in a hydroponics system. They found that total solids (TS), COD, NH_4-N, NO_2-N, NO_3-N, and PO_4-P reductions ranged from 52.7 to 60.5%, from 72.9 to 83.1%, from 66.0 to 76.0%, from 97.6 to 99.2%, from 76.9 to 81.6% and from 87.1 to 95.1%, respectively. The effluent produced from the hydroponics system had slightly higher levels of TS (420–485 mg L^{-1}) than the 480 mg L^{-1} recommended for aquatic animals. Snow and Ghaly [72] also tested water hyacinth, water lettuce (*Pistia stratiotes*), and parrot's feather (*Myriophyllum aquaticum*) for the hydroponic purification of aquaculture wastewater. With these species they found that the TS, COD, NH_4-N, NO_2-N, NO_3-N, and PO_4-P reductions ranged from 21.4 to 48.0%, from 71.1 to 89.5%, from 55.9 to 76.0%, from 49.6 to 90.6%, from 34.5 to 54.4% and from 64.5 to 76.8%, respectively. In this study they found that in terms of COD, NO_3-N and PO_4-P, the effluent leaving the hydroponics system was suitable for reuse in aquaculture. However, the effluent had slightly higher levels of TS, NH_4-N, NO_2-N and pH after treatment. Snow and Ghaley [70] found that at hydraulic retention times of 6 and 12 days, the average biomass of water hyacinth, water lettuce and parrot's feather were 83, 51 and 51 g m^{-2} and 49, 29 and 22 g m^{-2}, respectively. Wen and Recknagel [74] examined the use of parrot's feather for treatment of agricultural drainage waters and reported an average growth rate for parrot's feather of 7.12 g m^{-2} day^{-1}.

Jo et al. [73] evaluated the growth of water hyacinth and water lettuce plants for 30 days on effluent from an intensive recirculating aquaculture system and reported biomass yields of 6402 and 10188 g m^{-2}, respectively. At water temperatures of 30–38.5°C the water lettuce and water hyacinth plants in this system reduced the concentrations of NH_4-N in the wastewater from 2.3 to 0.4 mg L^{-1} and 0.6 mg L^{-1}

over a 48 h period. Nitrite and NO_3-N concentrations were reduced from 0.197 to 0.024 and 0.029 mg L^{-1} and from 21.4 to 17.4 and 17.9 mg L^{-1} in aquaria containing water lettuce and water hyacinth, respectively.

A number of investigators have examined use of hydroponics for improving water quality of municipal or factory effluents [75–78]. DeBusk et al. [75] evaluated the use of a water hyacinth based treatment system for nutrient removal from secondarily treated municipal wastewater and reported an average plant productivity of 16 g m^{-2} day^{-1}. Over a three month period the water hyacinth reduced the NH_4-N, NO_2-N, and NO_3-N concentrations in the wastewater from 2.57 to 0.03 mg L^{-1}, 1.32 to 0.08 mg L^{-1}, and 4.12 to 0.26 mg L^{-1}, respectively.

Nuttall [76] examined the ability of parrot's feather for nutrient reduction from a secondarily treated municipal wastewater over a 13 month period and reported suspended solids removal efficiencies ranging from 12.8 to 65.0%. John [77] investigated the use of water hyacinth for TS removal from rubber factory and palm oil mill effluents. They reported TS reductions of 16.9, 39.4 and 57.0% at hydraulic retention times (HRTs) of 5, 10, and 15 days when the water hyacinths were grown on undiluted raw rubber factory effluent and 32.4, 42.9, and 44.7% at HRTs of 10, 20 and 25 days when they were grown on an anaerobically treated palm oil mill effluent.

From rubber factory effluent John [77] reported COD reductions of 69.0, 80.2, and 88.7% at hydraulic retention times of 5, 10, and 15 days. When water hyacinths were grown on an anaerobically treated palm mill effluent, COD reductions of 76.7, 83.1 and 87.3% were observed at HRTs of 10, 20 and 25 days, respectively.

Dedes and O'Shaughnessy [78] investigated the use of duckweed (*Lemna minor*) for treatment of domestic wastewater over 74 days under 5 different hydraulic retention times (2.0, 2.7, 5.5, 5.6 and 11.7 days) and reported that the fraction of NH_4-N removed remained relatively constant at approximately 54–58% despite changes in hydraulic retention time. The fraction of NO_3-N removed ranged from 17 to 36% and increased with longer retention times.

Awuah et al. [79] evaluated the potential use of water lettuce for pollutant removal from a low-strength, anaerobically treated domestic sewage and reported NO_3-N reductions of 70% after 6 months of operation. Cloris and Araujo [80] examined the use of a water hyacinth based system for tertiary treatment of domestic sewage and reported a PO_4-P reduction of 88% over a 4 month period. Xu et al. [81] evaluated the ability of a water hyacinth based treatment system for removal of nutrients from domestic wastewater and reported PO_4-P reduction of 75–95%. Jing et al. [82] investigated the use of water lettuce for nutrient removal from an artificially prepared wastewater over a 30 day period and reported average PO_4-P removal efficiencies in the controls and in the compartments containing water lettuce of 8.0, 33.3, 42.3, and 31.6%, and 14.3, 53.9, 73.2, and 55.6 % at hydraulic retention times of 1, 2, 3 and 4 days, respectively.

Tripathi and Shukla [83] used a three stage system in the laboratory to treat wastewater from Varanasi city, India (city sewage mixed with industrial effluents). Their system had water hyacinth in the first and third stages and algal culture in the second stage. Their three stage system resulted in very high reductions of BOD

(96.9%), SS (78.1%), total alkalinity (74.6%), PO_4-P (89.2%), NO_3-N (81.7%), acidity (73.3%), NH_4-N (95.1%), COD (77.9%), hardness (68.6%) and coliform bacteria (70%). An increase in the concentration of dissolved O_2 was also observed.

9.3 Floating Vegetated Mats

9.3.1 Concept

Additional methods for utilizing and removing the nutrients contained within animal wastewater lagoons and other contaminated water bodies are needed. One potential method for removing nutrients is to have floating vegetation growing in the lagoon, farm pond, canal, stream, or river which is periodically harvested. With the removal of biomass from the mats, nutrients taken up by the plants are then removed from the water body. Successful implementation of this concept may provide water managers with an additional tool for treating or utilizing the nutrients contained in contaminated waters.

A number of different species of plants commonly grow in water bodies. Examples include water lilies (*Nymphaeaceae*), water hyacinth, duckweed (*Lemnaceae*), and many different species of algae. Although these take up nutrients from the water, if not harvested they fall to the bottom of the water body after death. There is no removal of nutrients from the system, only cycling. Although it is possible to harvest free floating species, it may not be economically feasible on large water bodies due to the need for specialized harvesting equipment. Also, algae generally are considered undesireable in water bodies because they lower dissolved O_2 levels causing a negative effect on fish populations.

A different concept for growing vegetation in water bodies for the purpose of utilizing nutrients and improving water quality is to have floating islands from which the vegetation can be harvested. While most naturally floating vegetation is at or slightly above the water surface, use of a floating platform to support the plants allows for growth of relatively tall vegetation. Tall vegetation can produce considerable amounts of biomass which thus removes significant amounts of nutrients from the water body.

Floating islands of vegetation are known to occur naturally. Van Duzer [84] reported on lush floating vegetated islands found in the sinkholes on El Rancho Azufrosa near the small town of Aldama in the state of Tamaulipas in northeastern Mexico. The water in the sinkholes was highly mineralized, smelling strongly of sulfur, and was also quite warm, with average temperatures ranging from 28.3 to 33.8°C. The flora of the floating islands was dominated by a grass known as "zacate," and in fact it is the distinctive islands of zacate that give the sinkhole its name "Zacaton." The names "zacate" and "zacaton" are applied to several different species, including *Muhlenbergia robusta, Festuca amplissima,* and *Sporobolus wrightii,* as well as other species in these genera. A small number of shrubs and cacti also grow on these islands.

Historical reports also exist of floating vegetated islands formed on travertine rafts. A lake now called Lago della Regina, formerly known as Lacus Albuleus, La Solfatra, or Lago delle Isole Natanti, near Tivoli, Italy, once had vegetated floating islands formed on floating masses of travertine. These were famously described by Athanasium Kircher [85] and Francesco Lana [86] in the seventeenth century, and in more detail by Sir Humphry Davy [87] in the nineteenth century. Lana [86] described these floating islands as follows: "I myself saw several of these islands in a small lake of sulfurous water not far from the Tiber; they were mostly circular or oval, and rose four or six inches above the water. Their surface is flat and grassy, and at the edges of some of them a few larger plants grow, which act as sails, so that even the slightest breeze pushes the islands from one part of the lake to another. The largest of them are a few yards in diameter, yet nonetheless can sustain several men standing upon them."

9.3.2 Water Improvement Processes

Floating vegetated mats can improve water quality through several different processes. The primary process by which floating vegetated mats improve water quality is through nutrient removal by the plants. It is also possible that denitrification may occur in anaerobic zones of the mat. Another benefit of use of the mats is shading of the water. This is of benefit when the entire surface of the water body is covered because it may reduce populations of undesireable algae which are dependent on sunlight reaching the water. Although not yet researched, and not a water quality improvement process, it can be hypothesized that for wastewater lagoons a complete cover by floating vegetated mats might improve odor problems. This would be somewhat analogous to manufactured lagoon covers which are used to trap both undesirable odors and methane.

9.3.3 Requirements for Successful Use of Floating Vegetated Mats

There are several requirements that must be met for successful implementation of floating vegetated mat systems. The first requirement for a floating vegetated system is a platform for supporting the growing vegetation. The platform must allow plant roots to attach, grow through, and reach into the water. The platform also must be durable and long lasting in relation to the quality of the wastewater. Secondly, appropriate vegetation must be selected for the body of water. Vegetation that grows well in moderately impacted water may not do well at all in severely contaminated wastewater. The reverse is also true. Vegetation that grows prolifically on a floating mat in water with high concentrations of N and P may not survive in waters with low levels of these nutrients. In addition to selection of floating island platform and appropriate plant species, the platforms must be sized appropriately for harvesting of biomass. One major difference between floating mats and constructed wetlands

is the removal of nutrients from the system through constant cutting and removal of the biomass from the floating mat system. In contrast, most constructed wetland systems have permanent vegetation which means that plant tissue ultimately falls to the bottom of the wetland as it senesces.

9.3.4 Small Scale Study Using Secondary Stage Swine Lagoon Wastewater

A study was designed in late 2000 to test the hypothesis that plant species commonly found in ponds or wetlands could produce biomass and remove nutrients from swine lagoon wastewater. The idea for the study was a follow-up from a presentation made by Mr Chris Hoag of USDA-NRCS Plant Materials Laboratory, Aberdeen, ID, showing a study to improve farm pond water quality using vegetation growing on platforms constructed of PVC pipe and chicken wire. The scientific question that came from that presentation was whether or not there were any plant species which would grow on a floating platform in swine lagoon wastewater.

A replicated study was conducted by USDA-ARS and the University of Georgia from 2001 to 2002 using aquaculture tanks at a field site approximately 760 m from the University of Georgia Coastal Plain Experiment Station main swine research facility at Tifton, GA [88]. The floating platforms were built using 0.64 cm diameter PVC pipe, chicken wire, and fibrous matting material. Each frame had an outer square and an inner cross constructed of PVC pipe. Attached to the sides and supported by the middle T-cross were chicken wire and fibrous matting. Each individual platform was 1 m^2 and was built to float inside of a tank capable of holding 1285 L of wastewater.

There were three different nutrient treatments, three different plant species, and four replicates of each combination, for a total of 36 floating mats each contained within an individual tank. The three different nutrient treatments were full-strength wastewater, ½-strength wastewater (swine lagoon wastewater mixed with well water), and inorganic nutrients (1/4-strength Hoaglund solution) [89]. The full-strength wastewater contained on average total nutrient concentrations of 160 mg L^{-1} N, 30 mg L^{-1} P, and 45 mg L^{-1} K, while the ½ strength wastewater contained half this amount. Total nutrient concentrations for the ¼ - strength Hoaglund solution were 53 mg L^{-1} N, 8 mg L^{-1} P, and 59 mg L^{-1} K. The ¼-strength Hoaglund solution was designed to provide sufficient N and P for the plants so that they would not die, but insufficient for rapid growth. Every two weeks, half of the liquid in each tank was replaced with new liquid of the appropriate nutrient level (full-strength wastewater, ½-strength wastewater, or ¼-strength Hoaglund solution), except during the winter months when the nutrient replacement interval was spread out to every three weeks, since the plants were either dormant or very slow growing.

Three different wetland plant species were selected for the study: cattail (*Typha latifolia L.*), soft rush (*Juncus effuses*), and maidencane. The cattail and rush species were selected for the test because of published information concerning their use in constructed wetlands [90–99] while the maidencane was selected because it had

been successfully used in an overland flow vegetated buffer study where swine lagoon wastewater was applied [56].

The study started in June 2001 with sprigging of plant material on each floating mat. The plant biomass on each floating mat was then periodically harvested from August 2001 through September 2002. The plants were cut so as to leave about 5 cm of green material above the base of each floating platform for regrowth. Measurements of total biomass, and percent N, P, and K in the plant tissue were made at each plant harvest.

Mean biomass per cutting for the cattail ranged from a low of 106 g m^{-2} for the wastewater treatment on 30 Sept. 2002 to a high of 5794 g m^{-2} on 3 June 2002 (Table 9.1). Although there were some statistically significant differences in cattail biomasses between treatments for individual cutting dates, there were no significant differences in the overall totals. Mean biomass per cutting for the rush ranged from a low of 78 g m^{-2} for the wastewater treatment on 15 Oct. 2001 to a high of 2493 g m^{-2} for the inorganic treatment on 3 June 2002. As with cattail, there were no significant overall differences in biomass due to treatment, although one cutting of the rush (14 March 2002) did have significant treatment effects. No biomass data is shown for the rush on 29 July 2002 or 30 Sept. 2002 for the mixture or full-strength wastewater treatments. This is because the rush growing on these mats did not survive. Mean biomass per cutting for the maidencane varied from 453 g m^{-2} for the inorganic treatment on 15 Oct. 2001 to 5903 g m^{-2} for the mixture on 3 June 2002. Although not always significantly different from the other treatments, the mean biomass for the maidencane grown on the mixture was numerically greater than that of the other treatments for all sampling dates. Overall, the biomass results showed that both the cattail and maidencane were suitable species for growing on floating mats on lagoon wastewater at the nutrient level used for this study, but that the rush was unsuitable for growth in this wastewater.

Mean total N removal with cattail biomass by sampling date ranged from a low of 7.3 g m^{-2} with wastewater on 30 Sept. 2002 to a high of 176.4 g m^{-2} on 3 June 2002, also with wastewater (Table 9.2). The mean total N removal by sampling date for the cattail varied among the inorganic, mixture, and full-strength wastewater, with no consistent pattern as to which nutrient treatment had the greatest N removal. The mean total N uptake by the rush was lower than that of either the cattail or maidencane. As discussed earlier, the rush grown on the mixture and full-strength wastewater had died by the cutting of 20 July 2002. Mean total N removal per cutting by the maidencane ranged from a low of 16.9 g m^{-2} for the inorganic treatment on 30 Sept. 2002 to a high of 157.7 g m^{-2} for the plants grown on the mixture on 3 June 2002. Numerically, the mean total N removal by the maidencane grown on the mixture was generally greater than that of the other two treatments.

Mean total P removal by the cattail ranged from a low of 1.1 g m^{-2} for the wastewater treatment on 30 Sept. 2002 to a high of 28.3 g m^{-2} for the wastewater treatment on 20 Aug. 2001 (Table 9.3). Overall, there were no significant differences among treatments in mean total P removal by cattail for the entire study period. The rush species receiving the mixture or full-strength wastewater removed P up until sickness or death of the plants. Mean total P removal by the maidencane was

Table 9.1 Mean biomass (all values in g m^{-2})

	Cattail						Rush						Maidencane					
	Inorganic		Mixture		Waste		Inorganic		Mixture		Waste		Inorganic		Mixture		Waste	
Date	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
08/20/2001	1753^{cd*}	809	4443^{a}	1441	5223^{a}	1489	715^{de}	141	834^{de}	135	183^{e}	136	689^{e}	391	3301^{b}	431	2343^{bc}	640
10/15/2001	1599^{bcd}	1452	3911^{a}	1800	2875^{ab}	1387	275^{e}	90	811^{cde}	381	78^{e}	47	453^{de}	311	1760^{bc}	381	1607^{bcd}	294
03/14/2002	–	–	–	–	–	–	1523^{ab}	977	2264^{a}	2414	266^{bc}	204	–	–	–	–	–	–
06/03/2002	4544^{ab}	4576	2500^{abc}	2710	5794^{a}	2073	2493^{abc}	1677	1759^{bc}	1408	81^{c}	115	4061^{ab}	2854	5903^{a}	674	2830^{abc}	1459
07/29/2002	2543^{ab}	2699	931^{bc}	1096	2513^{ab}	1479	183^{c}	121	–	–	–	–	2559^{ab}	1721	3768^{a}	778	1421^{bc}	486
09/30/2002	599^{bcd}	793	186^{cd}	222	106^{d}	136	270^{cd}	351	–	–	–	–	1136^{bc}	632	3188^{a}	1707	1550^{b}	857
Totals	11038^{abc}		11971^{abc}		16511^{ab}		5459^{cd}		5668^{cd}		608^{d}		8898^{c}		17920^{a}		9751^{bc}	

*Letters denote T tests-Least Significant Difference (LSD) in the same row. Where letters are the same, means are not significantly different at the 0.05 level.

Table 9.2 Mean total N removed (all values in g m^{-2})

	Cattail						Rush						Maidencane					
	Inorganic		Mixture		Waste		Inorganic		Mixture		Waste		Inorganic		Mixture		Waste	
Date	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
08/20/2001	59.6^{c*}	26.9	155.8^{a}	51.1	168.7^{a}	44.7	15.8^{d}	3.4	19.6^{d}	4.0	4.8^{d}	3.0	25.3^{d}	14.7	100.6^{b}	13.9	73.2^{bc}	46.4
10/15/2001	46.4^{cd}	41.8	112.5^{a}	48.5	97.3^{ab}	43.2	7.3^{e}	2.4	24.2^{cde}	11.0	2.5^{e}	1.5	18.7^{de}	3.8	55.0^{cd}	12.3	59.2^{bc}	11.0
03/14/2002	–	–	–	–	–	–	46.2^{a}	29.1	60.9^{a}	59.4	11.4^{a}	3.9	–	–	–	–	–	–
06/03/2002	144.1^{abc}	92.3	65.0^{cde}	67.9	176.4^{a}	64.8	49.7^{de}	23.8	46.5^{de}	41.5	3.6^{e}	2.5	85.4^{bcde}	60.7	157.7^{ab}	23.7	97.4^{abcd}	46.1
07/29/2002	79.1^{ab}	57.4	36.0^{bc}	27.6	84.8^{ab}	48.5	4.1^{c}	2.7	–	–	–	–	53.3^{abc}	35.4	102.2^{a}	22.8	46.6^{abc}	13.1
09/30/2002	20.3bc	10.8	8.6^{c}	–	7.3^{c}	4.1	4.9^{c}	5.9	–	–	–	–	16.9^{bc}	7.7	79.2^{a}	25.6	46.3^{ab}	24.3
Totals	349.5^{cd}		377.9^{bc}		534.5^{a}		128.0^{ef}		151.2^{ef}		22.3^{f}		199.6^{de}		494.7^{ab}		322.7^{cd}	

*Letters denote T tests-Least Significant Difference (LSD) in the same row. Where letters are the same, means are not significantly different at the 0.05 level.

Table 9.3 Mean total P removed (all values in g m^{-2})

	Cattail						Rush						Maidencane					
	Inorganic		Mixture		Waste		Inorganic		Mixture		Waste		Inorganic		Mixture		Waste	
Date	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
08/20/2001	9.3^{c*}	4.4	24.1^{a}	7.8	28.3^{a}	8.8	2.5^{d}	0.7	3.3^{d}	0.5	0.7^{d}	0.5	2.6^{d}	1.7	18.2^{b}	2.0	12.3^{bc}	3.6
10/15/2001	7.7^{bcde}	6.9	19.8^{a}	9.6	14.3^{ab}	6.8	1.0^{ef}	0.4	3.7^{cdef}	1.4	0.3^{f}	0.2	2.5^{def}	0.8	10.1^{bc}	2.1	8.5^{bcd}	1.5
03/14/2002	–	–	–	–	–	–	5.3^{a}	3.8	8.3^{a}	8.3	1.3^{a}	0.5	–	–	–	–	–	–
06/03/2002	23.4^{a}	16.4	9.7^{abc}	10.2	22.5^{ab}	8.7	8.4^{bc}	6.1	6.3^{c}	4.5	0.9^{c}	0.6	13.0^{abc}	9.7	22.3^{ab}	2.8	13.3^{abc}	6.3
07/29/2002	16.5^{a}	11.5	6.9^{bc}	5.6	12.9^{ab}	7.1	0.6^{c}	0.4	–	–	–	–	6.3^{bc}	4.4	13.6^{ab}	2.9	5.7^{bc}	2.0
09/30/2002	4.9^{ab}	3.2	1.5^{b}	–	1.1^{b}	0.5	1.0^{b}	1.2	–	–	–	–	2.7^{b}	1.4	11.8^{a}	6.1	7.8^{ab}	3.6
Totals	61.8^{abc}		62.0^{abc}		79.1^{a}		18.8^{f}		21.6^{ef}		3.2^{f}		27.1^{def}		76.0^{ab}		47.6^{cde}	

*Letters denote T tests-Least Significant Difference (LSD) in the same row. Where letters are the same, means are not significantly different at the 0.05 level.

greatest with the mixture. The greatest removal of P by plants growing on full-strength wastewater was by the cattail.

Mean total K removed by the cattail ranged from a low of 10.4 g m^{-2} on 30 Sept. 2002 for the wastewater treatment to 213.5 g m^{-2} for the inorganic treatment on 3 June 2002 (Table 9.4). There were no statistically significant differences between treatments for the cattail cuttings on specific dates except for the cutting of 20 Aug. 2001, when the plants grown on the inorganic treatment had significantly less removal of K than the other two treatments, and the cutting of 15 Oct. 2001, when the inorganic treatment had significantly less K removal than the plants grown on the mixture. Mean total removal of K per cutting by the rush was much lower than that removed by the cattail and maidencane. Mean total K removal by the maidencane was generally greatest for the mixture. The total removal of K by the cattail was significantly greater than that removed by the rush and the maidencane except for the mixture treatment of the maidencane. Overall, the cattail removed the greatest K from full-strength wastewater.

Mass balance calculations were made of the total percent nutrient removal by the floating vegetated mats (Table 9.5). For these calculations, it was assumed that the root zone depth was 15 cm for both the rush and maidencane, and that it was 30 cm for the cattail. It was also assumed that the total available nutrients to the plants was the sum of the nutrients contained in the 26 applications of the solutions for the total wastewater volume corresponding to the thickness of this root zone. In reality, this is not true, since nutrients in solution below the assumed root zone thickness could freely mix with the solution in the root zone. Hence, where the mass balance calculations (Table 9.5) show percentages greater than 100, natural mixing of the solutions within the tanks clearly provided nutrients in excess of those calculated using our root zone thickness assumptions.

Mass balance calculations for N removal ranged from a low of 4% for the rush grown on wastewater to a high of 157% for the maidencane grown on the mixture. For both the cattail and rush, the percent N removal decreased in the order inorganic > mixture > wastewater. For P, the percent removal ranged from 3% for the rush grown on wastewater to 130% for the maidencane grown on the mixture. For K, mass balance calculations showed a different pattern than that observed for N and P. Both the cattail and maidencane removed as much or more K than that contained in the root zone thickness.

Overall, the mass balance calculations showed that we were meeting the nutrient needs for the cattail and maidencane with the inorganic treatment, had an excess of N and P with the full-strength wastewater, and that with greater growth on ½-strength wastewater, the maidencane utilized N in excess of that from the assumed root zone thickness. For both the cattail and rush, more K was removed than was available from the assumed root zone thickness. Clearly, a wastewater lagoon with continuous inputs of fresh animal waste will in most cases provide nutrient amounts such that the plants on the floating mats are in a luxury uptake situation, and removal of nutrients from the lagoon will be a function of biomass produced and the maximum plant nutrient uptake levels.

Table 9.4 Mean total K removed (all values in g m^{-2})

	Cattail						Rush						Maidencane					
	Inorganic		Mixture		Waste		Inorganic		Mixture		Waste		Inorganic		Mixture		Waste	
Date	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
08/20/2001	66.9^{bc*}	31.0	173.2^{a}	82.3	201.3^{a}	74.0	13.8^{cd}	2.8	11.2^{d}	3.4	2.9^{d}	2.8	18.0^{cd}	13.7	78.1^{b}	17.5	56.2^{bcd}	26.5
10/15/2001	60.0^{bc}	52.4	160.4^{a}	87.3	105.6^{ab}	53.2	6.3^{cd}	2.4	24.0^{cd}	12.1	1.8^{d}	1.0	17.2^{cd}	2.5	45.6^{cd}	5.1	37.6^{cd}	10.8
03/14/2002	–	–	–	–	–	–	39.4^{a}	26.5	62.9^{a}	73.8	7.8^{a}	2.7	–	–	–	–	–	–
06/03/2002	213.5^{a}	141.8	109.6^{abc}	111.0	158.3^{ab}	50.6	56.6^{bc}	43.9	35.4^{bc}	31.0	2.8^{c}	1.7	105.9^{abc}	78.3	98.5^{abc}	17.1	56.5^{bc}	30.6
07/29/2002	158.2^{a}	107.5	76.3^{ab}	72.1	86.9^{ab}	52.8	4.2^{b}	2.8	–	–	–	–	56.8^{b}	38.1	77.9^{ab}	20.3	36.1^{b}	13.1
09/30/2002	41.4^{ab}	26.2	18.8^{ab}	–	10.4^{b}	7.8	6.7^{b}	9.2	–	–	–	–	24.3^{ab}	13.6	62.9^{a}	33.1	36.4^{ab}	20.1
Totals	540.0^{a}		538.3^{a}		562.5^{a}		127.0^{cd}		133.5^{cd}		15.3^{d}		222.2^{bcd}		363.0^{abc}		222.8^{bcd}	

*Letters denote T tests-Least Significant Difference (LSD) in the same row. Where letters are the same, means are not significantly different at the 0.05 level.

Table 9.5 Calculated percent nutrient removal from the root zone of the replicated tank study with swine lagoon wastewater

	Nitrogen			Phosphorus			Potassium		
	Inorganic (%)	Mixture (%)	Wastewater (%)	Inorganic (%)	Mixture (%)	Wastewater (%)	Inorganic (%)	Mixture (%)	Wastewater (%)
Cattail	84*	61	43	99	53	34	117	307	160
Rush	62	48	4	60	37	3	55	154	9
Maidencane	97	157**	52	87	130	41	97	413	127

*Calculations of percent nutrient removal were made assuming rooting zone depths of 15 cm for the rush and maidencane, and 30 cm for the cattail. It was also assumed that the total available nutrients were the sum of 26 applications of the solution for the thickness of this root zone.

**Where calculations result in percentages greater than 100, natural mixing of the nutrients within the tank would have provided nutrients in addition to those calculated just for the root zone thickness.

The study showed that floating mats of vegetation can be grown in lagoon wastewater, and that the cattail was the best plant species for biomass production and nutrient removal on this wastewater. At the wastewater strength used for this study, both the maidencane and cattail survived and removed nutrients. The rush, although initially showing promise, ultimately died and also showed growth problems with the inorganic control treatment. Total biomass produced on full-strength wastewater during the study was 16,511 g m^{-2} and 9751 g $m^{-1}$for the cattail and maidencane, respectively. Total N, P, and K removed on full-strength wastewater were 534, 79, and 562 g m^{-2} for the cattail and 323, 48, and 223 g m^{-2} for the maidencane, respectively. Using an assumed root zone thickness of 30 cm for cattail and 15 cm for maidencane, mass balance calculations showed that, on full-strength wastewater, the cattail removed 43, 34, and 160% of the applied N, P, and K, respectively, while the maidencane removed 52, 41, and 127%. More K was needed than that calculated as being supplied by the wastewater in the root zone. This K clearly came from mixing of wastewater within the tanks. The mass balance calculations showed that N and P were in excess of plant needs for the full-strength wastewater for the assumed root zone thickness. Since root zone thicknesses used for the mass balance calculations were 15 or 30 cm, and lagoons are commonly in excess of 2 m deep, in most cases, lagoon nutrients will be greatly in excess of potential plant uptake and removal.

9.3.5 Floating Vegetated Mat Study on a Single Anaerobic Wastewater Lagoon at a Commercial Hog Farm

A floating vegetated mat study was conducted from 2005 to 2008 on a commercial swine farm (Southern Select) located near Omega, GA. The producer flushed all wastes from approximately 3000 hogs to a single anaerobic wastewater lagoon adjacent to the swine houses. All wastes were flushed from the houses using fresh water.

The floating platforms for this study were designed by Maryland Aquatic Nurseries and Charleston Aquatic Nursery (Fig. 9.1). In our previous study [88] we used PVC support frames covered with chicken wire and a coir mat. During pre-study plant tests at the Southern Select lagoon in 2004 we determined that PVC and chicken wire platforms (Fig. 9.2) were unsuitable for the highly concentrated wastewater. The chicken wire rusted out in 2–3 months and the vegetation would then fall through the platform into the lagoon. Maryland Aquatic Nurseries and Charleston Aquatic Nursery designed a platform consisting of material cut in a square with an inner hollow portion. The hollow portion was then covered with fibrous coir materials and attached to the outer frame with plastic fasteners and a stainless steel bar. These were tested in 2004 and found to work well on the lagoon wastewater without deterioration due to poor water quality.

During summer-fall of 2004 through mid-summer 2005 a pre-study test was conducted to determine which, if any, plant species could survive in this single stage

Fig. 9.1 Mat platform used in experiment at commercial hog farm

swine wastewater lagoon. We included wetland, grass, and horticultural species. The species tested included cattail, soft rush, maidencane (*Panicum hematomon*), willow trees (*Salix caroliniana*), common bermuda grass (*Cynodon dactylon* L. Pers.), Tifton 85 bermuda grass (*Cynodon dactylon* L. Pers.), St. Augustine grass (*Stenotaphrum secundatum*), giant reed (*Arundo donox*), napier grass (*Pennisetum purpureum*), and wild millet (*Panicum milliaceum*). The cattail, soft rush, maidencane, willow trees, and napier grass all immediately died. The common bermuda grass, Tifton 85 bermuda grass, St. Augustine grass, giant reed, and wild millet all survived and grew during summer 2004–mid-summer 2005. These five species were selected for the 2005–2008 study.

The experimental design for the 2005–2008 lagoon study was completely randomized, with four replicates of each of the five plant species. The study started in

Fig. 9.2 Platform of PVC pipe and chicken wire tested at commercial hog farm

Fig. 9.3 Floating platform with grass about one month after sprigging at commercial hog farm

September 2005 with sprigging of live plant materials onto the floating platforms designed in cooperation with Maryland Aquatic Nurseries and Charleston Aquatic Nursery (Fig. 9.1). Each replicate platform was tethered by rope to an iron stake pounded into the side of the lagoon dam (Figs. 9.3 and 9.4). The vegetation on the platforms was harvested eight times during the study. The harvesting process consisted of pulling each platform to shore and cutting the vegetation by hand with a motorized hedge trimmer. At each cutting the vegetation on each platform was cut so as to leave about 8 cm of plant stalk for regeneration. The harvested vegetation was dried, weighed, and then ground for subsampling for analyses for N, P, and K. The wastewater averaged about 300 mg L^{-1} N with most of it in the NH_4-N form.

The plants were harvested on an as-needed basis in the spring, mid-summer, and fall of 2006 and 2007. The harvesting dates in 2006 were 6/23/06, 8/17/06, and 11/14/06, while those in 2007 were 6/27/07, 8/14/07, and 10/8/07. The plants either died back or were dormant during the winters of 2005–2006 and 2006–2007 and

Fig. 9.4 Replicated study at commercial hog farm

then, for the species which performed well, grew rapidly during the spring months with the result that harvesting was needed by late June of each year. After the first cutting, sufficient growth occurred each summer for two additional cuttings. During 2005–2006 the platforms had pure stands of the planted species. By 2007 weediness was a problem. Weeds included the wild millet and common bermuda grass along with common agricultural weeds such as ragweed and thistle. This problem was addressed by spraying with roundup. By spring 2008 all platforms were extremely weedy. It was decided to continue the experiment during 2008 but use this data as a measure of biomass and element uptake that occurred with a heterogeneous mixture of the original planted species and many weeds. For many of the platforms the wild millet, which was on the edge or in the wastewater lagoon prior to the start of the study, became the dominant vegetation. The two cutting dates in 2008 were 5/28/08 and 8/11/08.

The 2005–2008 replicated study showed that St. Augustine grass was completely unsuitable for producing biomass on highly contaminated wastewater. Only a small patch on one platform survived past mid 2006. The giant reed initially appeared to be an excellent candidate for producing biomass. However, after the second cutting of 2006 it began dying and was completely dead by 2007. The common bermuda grass, Tifton 85 bermuda grass, and wild millet all proved to be good producers of biomass. Mean total biomass produced from 2005 to 2007 was 3213, 3559, and 3082 g m^{-2} for the common bermuda grass, Tifton 85 bermuda grass, and wild millet, respectively (Table 9.6). There were no significant differences among these plant species.

The mean N content of the biomass harvested from the floating platforms ranged from 2.78 to 4.72% (Fig. 9.5). There were few statistical differences among the plant species on any of the harvesting dates. The greatest values were found in the St. Augustine grass. Most of the mean N percentages for all species were in excess of 3%. This was comparable to what was observed with cattail, soft rush, and maidencane with full strength swine lagoon wastewater in the 2001–2002 floating mat study. It would appear that given unlimited N availability from wastewater, plant species which can survive the poor quality wastewater (including weeds) will contain 3–4% N.

Mean P in biomass grown in the swine lagoon wastewater ranged from 0.46 to 3.16% (Fig. 9.6). Significant differences in mean P contents only occurred on 11/14/06 and 6/27/07. On 11/14/06 the St. Augustine grass had significantly more P than the giant reed, while on 6/27/07 the wild millet had significantly more P than the giant reed. Most of the cuttings had P percentages in the range of 0.5–1.0%. The sprigged plants had greater P percentages on 11/14/06 than on any of the other cutting dates. The weeds cut on 8/11/08 also had relatively high P percentages. In general, the P concentrations found in the cuttings from this study were greater than those observed in the 2001–2002 study, where the range for cattail, rush, and maidencane was from 0.35 to 0.51%. Greater uptake of P by the 5 species tested for this study than the three species tested in the earlier study may be related to species differences.

Table 9.6 Mean total biomass and growth rate by plant species and harvesting date

	Plant species																			
	Common bermuda grass				Tifton 85 bermuda grass				Wild millet				St. Augustine grass				Giant reed			
	Total biomass ($g\ m^{-2}$)		Growth rate ($g\ m^{-2}\ d^{-1}$)		Total biomass ($g\ m^{-2}$)		Growth rate ($g\ m^{-2}\ d^{-1}$)		Total biomass ($g\ m^{-2}$)		Growth rate ($g\ m^{-2}\ d^{-1}$)		Total biomass ($g\ m^{-2}$)		Growth rate ($g\ m^{-2}\ d^{-1}$)		Total biomass ($g\ m^{-2}$)		Growth rate ($g\ m^{-2}\ d^{-1}$)	
Harvesting date	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
6/23/06	397a*	314	1.35a*	1.06	487a	262	1.65a	0.89	139ab	80	0.14b	0.08	16b	31	0.05b	0.10	463a	201	1.54a	0.70
8/17/06	732a	252	13.31a	7.68	689a	304	12.53a	5.53	380ab	123	6.90ab	2.23	208b	267	3.77b	4.84	610a	156	11.09a	2.83
11/14/06	579a	322	6.51a	3.61	849a	407	9.53a	4.57	555a	209	6.24a	2.34	32b	68	0.36b	0.73	22b	42	0.24b	0.48
6/27/07	548b	162	2.43b	0.72	797a	162	3.55a	0.72	690ab	126	3.07ab	0.56	181c	300	0.81c	1.33	177c	208	0.78c	0.93
8/14/07	518a	156	10.81a	3.25	342ab	278	7.12ab	5.79	614a	86	12.78a	1.78	22b	42	0.44b	0.89	123b	246	2.56b	5.12
10/8/07	439ab	318	7.98ab	5.77	395ab	288	7.18ab	5.24	704a	72	12.81a	1.32	206b	321	3.74b	5.84	0b	0	0b	0
Total	3213a	1322			3559a	675			3082a	277			665b	577			1376b	645		
5/28/08	408a	183	1.75a	0.79	344a	182	1.48a	0.78	275a	234	1.18	1.00	313a	190	1.35a	0.81	204a	57	0.87a	0.24
8/11/08	460b	152	6.13b	2.03	794a	129	10.58a	1.72	632ab	170	8.43ab		534b	113	7.12b	1.51	573b	163	7.65b	2.17
Total	868a	173			1138a	298			907a	386			847a	210			777a	119		

*Letters denote T-tests-Least Significant Difference (LSD) in the same row for Total Biomass or Growth Rate. Where letters are the same, means are not significantly different at the 0.05 level.

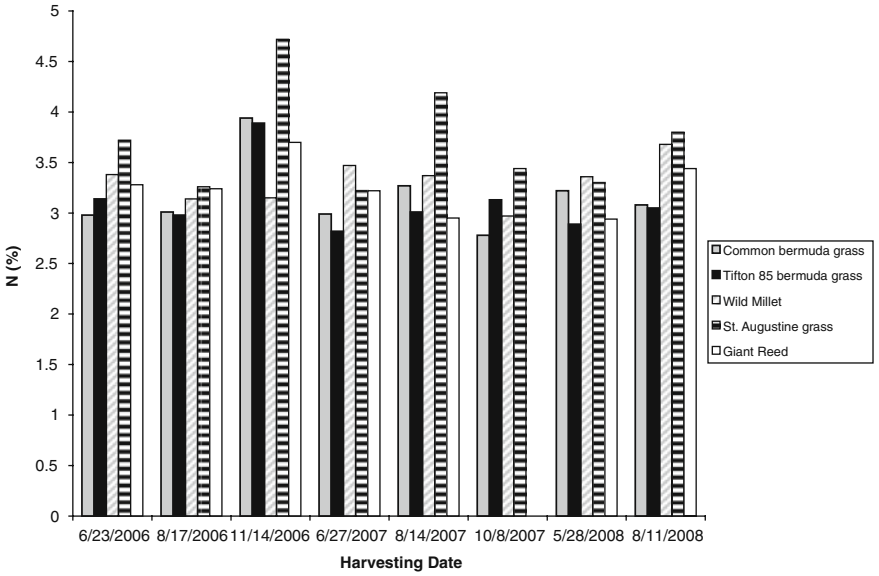

Fig. 9.5 Nitrogen content of species grown at the commercial hog farm

The mean K content of the biomass from the platforms ranged from 1.31 to 4.98% (Fig. 9.7). Some of the greatest values were found in the weeds cut in 2008. The 2001–2002 study indicated that most of the K values for cattail, rush or maidencane ranged from 3 to 4%. Some of our values for the 2005–2008 study were lower, particularly for the common bermuda grass. The uptake of K by the weeds in 2008 was more consistent with the uptake by the plant species grown for the 2001–2002 study.

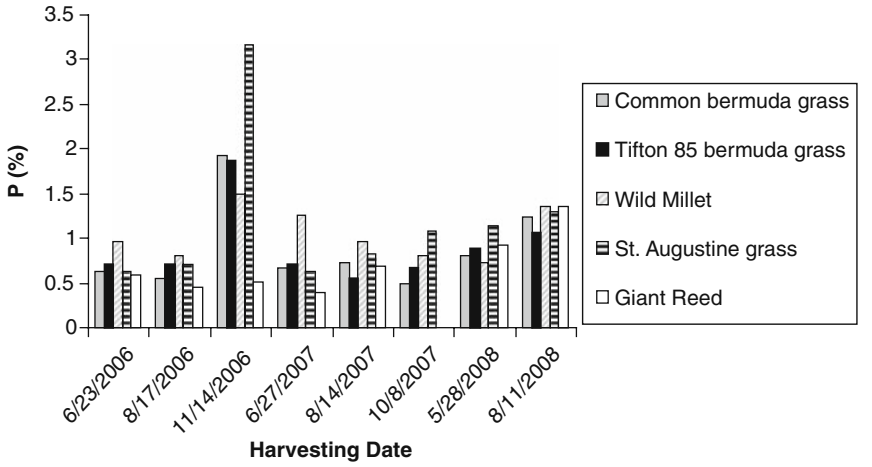

Fig. 9.6 Phosphorus content of species grown at the commercial hog farm

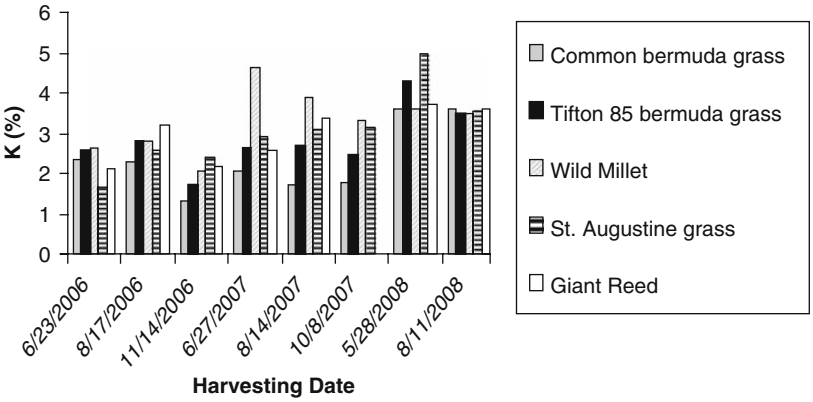

Fig. 9.7 Potassium content of species grown at the commercial hog farm

9.3.6 New Research

A research project is being implemented in 2009 at the University of Georgia Aquaculture research unit in Tifton, GA to evaluate the feasibility of using floating vegetated mats to improve the quality of aquaculture wastewater. In commercial aquaculture the water in the ponds must be periodically replaced with fresh water to avoid build up of wastes from the fish or other aquatic species and accompanying algal growth. The project is being conducted in 36 aquaculture tanks (the same ones used for the first floating mat study). During 2008 a test was made to determine plant species which potentially will grow well in aquaculture wastewater (Fig. 9.8). Species tested included cattail, soft rush, maidencane, iris, canna lilies, border grass, Tifton 85 bermuda grass, common bermuda grass, and bamboo.

Fig. 9.8 Iris growing in aquaculture wastewater

The aquaculture wastewater is quite different from the swine lagoon wastewater in that it is relatively low in N and P, although algae will grow profusely.

The test during 2008 showed that many of the species which grew well in moderately or severely contaminated swine lagoon wastewater could not survive in the aquaculture wastewater. Three species which did grow well in 2008 were iris, cattail, and soft rush. This study will measure both the nutrient uptake by the plants and the quality of the water in the tanks. The ultimate objective is to see if floating vegetated mat systems can be used to improve aquaculture wastewater quality sufficiently so that the treated water can be recycled back to the aquaculture ponds.

9.3.7 Research Needs

Considerable research is needed on floating mat technology. Research needs include studies to determine relationships between water quality and specific plant species to find ones which will thrive on a given water body, studies which will determine the potential for production of biomass, studies to develop engineering technologies for handling the platforms and vegetation, studies of how best to utilize the plant materials (either living or harvested), and economic feasibility studies. Our work at Tifton, GA has shown that plant species are very sensitive to water quality. Grasses that grew well in severely contaminated low O_2 swine lagoon wastewater at a commercial farm died when placed on wastewater from aquaculture ponds. We also found that there were local adaptations among the same species according to available nutrient levels. We successfully grew cattail in our first study with moderately contaminated swine lagoon wastewater. The cattail we used were found growing naturally along the sides of ponds on the University of Georgia Animal Science Farm. When we used cattail from the same source for our studies with aquaculture wastewater, they died. However, we found native cattail growing on the sides of the aquaculture ponds (low nutrient level water) which worked well on the floating platforms in tanks containing aquaculture wastewater. Also, different plant species produce different amounts of biomass and have different overwintering patterns, so determination of best species to produce biomass is needed.

Engineering research needs to be done to develop methods for handling vegetated mat systems. Methods need to be developed for completely mechanizing mat systems. At the research scale we have been moving mats and harvesting vegetation mostly by hand. This is labor intensive and not very economical. If a system of sprigging, moving the mats on and off of a water body, and then harvesting and transporting the vegetation can be developed then floating mat systems become much more economically viable.

Research is also needed to study alternate platforms for floating mats. Our research to date has been with platforms built from PVC, chicken wire, and a fiber mat, or from polypropylene with a hollowed out area covered with coir fiber blanket as supplied by Maryland Aquatic Nurseries and Charleston Aquatic Nursery. Another platform type for floating mats which has been developed and is being marketed comes from Floating Island International (Fig. 9.9). The platforms from

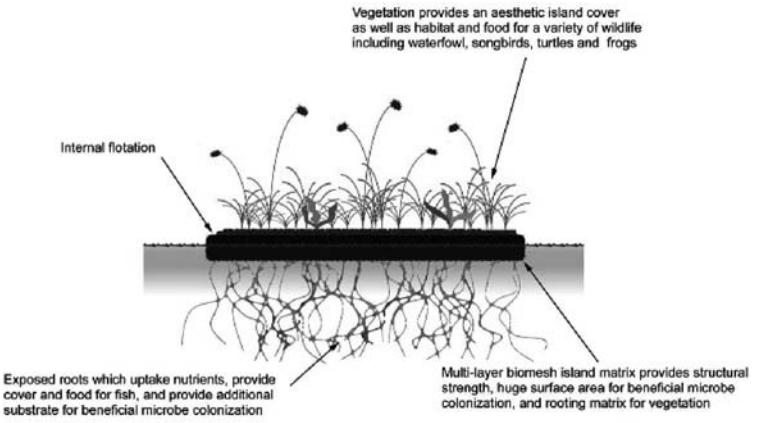

Fig. 9.9 Schematic of floating platforms from Floating Island International and Floating Island International Southeast

Floating Island International and Floating Island International Southeast use a different concept than either the PVC/chicken wire ones or the ones from Maryland Aquatic Nurseries or Charleston Aquatic Nursery. They are thicker and contain a vertical zone which has periphyton and bacteria which retain or utilize nutrients from the water. This zone may also contribute to a significant amount of denitrification. These platforms also allow for significant plant root growth in the water body (Fig. 9.10). Plans are in progress for tests with these platforms in both swine lagoon and aquaculture wastewater to quantify nutrient removal.

Research also needs to be done regarding utilization of the harvested material. There is potential for composting the biomass and using it as a soil amendment. This would add both nutrients and carbon to the soil. Potential also exists for transplanting material grown on the floating platforms. We have successfully removed bermudagrass sod intact from the floating platform by rolling up the coir mat with

Fig. 9.10 Plant and root growth on Floating Island International platforms

Fig. 9.11 Mat with bermuda grass being transported for transplanting

the sod growing on and through it and then moving the mat of sod to a land site (Fig. 9.11). The grass then grew quite well on the site to which it was transplanted.

Complete economic analyses of growing biomass for composting or bioenergy, or grass and/or horticultural species for transplant on floating mats to improve water quality is needed. The analyses should include both the production costs associated with sprigging, harvesting, and transporting the material and the value of the biomass after composting, using as a bioenergy feedstock, or transplanting. Economic analyses also should factor in the number of farm ponds, canals, lakes, streams, rivers, etc. which could use this technology, and the value associated with improving water quality by removing nutrients from the water body.

9.4 Conclusions

Methods are needed to protect and improve water quality. Technologies for wastewater commonly include using constructed wetlands or land application. A new concept is to use floating vegetated mats to cover part of the surface water body. The mats grow on floating platforms. Roots of the plants reach into the water and take up nutrients. Biomass can be harvested from the mats or in the case of grasses or horticultural plants, the plants can be transplanted. Harvested biomass can be used to make compost for use as a soil amendment, or potentially for use in making biofuel.

Both past and ongoing research has shown that floating vegetated mats can be used to grow biomass and remove nutrients from wastewater. Completed experiments on both moderately and severely contaminated swine lagoon wastewater have shown that species can be found to grow in such waters. With the moderately contaminated swine lagoon wastewater we were able to grow and produce cattail and maidencane. With the severely contaminated swine lagoon wastewater we grew

two varieties of bermuda grass and a wild millet. Preliminary research using floating mats on aquaculture wastewater showed that iris grew best. The aquaculture wastewater had much lower levels of N and P than the wastewater from the swine lagoons.

Future use of floating mat technology will depend on research to match plant species to water quality, determine which species produce the most biomass and remove the most nutrients, development of methods for using the biomass such as composting, using as a bioenergy feedstock, or else directly transplanting material, development of engineering techniques to completely mechanize the process, and economic analyses of all facets of this emerging technology.

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