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Review Paper

Microbial mats for multiple applications in aquaculture and bioremediation

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Abstract

Microbial mats occur in nature as stratified communities of cyanobacteria and bacteria, but they can be cultured on large-scale and manipulated for a variety of functions. They are complex systems, but require few external inputs. The functional uses of mats broadly cover the areas of aquaculture and bioremediation. Preliminary research also points to promising uses in agriculture and energy production. Regarding aquaculture, mats were shown to produce protein, via nitrogen fixation, and were capable of supplying nutrition to tilapia (*Oreochromis niloticus*). Current research is examining the role of mats in the nitrification of nutrient-enriched effluents from aquaculture. Most research has addressed bioremediation, within which two majors categories of contaminants were examined: metals and radionuclides, and organic contaminants. Mats sequester or precipitate metals/radionuclides by surface absorption or by conditioning the surrounding chemical environment, thus bioconcentrating the metal/radionuclide in a small volume. Organic contaminants are degraded and may be completely mineralized. For agriculture mats hold promise as a soil amendment and nitrogen fertilizer. The use of mats in biohydrogen production has been verified, but is in a preliminary phase of development. We propose a comprehensive closed system based on microbial mats for aquaculture and waste management.

Keywords: Aquaculture; Bioremediation; Cyanobacteria; Hydrogen production; Microbial mat; Nitrogen fixation; Purple bacteria

1. Introduction

Microbial mats are laminated, cohesive microbial communities, composed of a consortium of bacteria dominated by photoautotrophic cyanobacteria (also referred to blue–green algae) (Nisbet and Fowler, 1999). Mats generally include anoxygenic photoautotrophs (purple bacteria) and sulfur-reducing bacteria. They are embedded in a negatively charged polymeric matrix of gel. Mats are ubiquitous in nature, commonly found over the sediment surfaces or as floating masses in marine waters (Bebout et al., 1987; Otte et al., 1999; Steppe et al., 1996), hypersaline waters (Hoehler et al., 2001; Nuebel et al., 2001; Pinckney and Paerl, 1997), estuaries (Olendzenski, 1999), fresh waters (Brunberg et al., 2002), hot springs (Hiraishi et al., 1999; Nakagawa and Fukui, 2002; Skirnisdottir et al., 2000), soils (Steppe et al., 1996; Watanabe et al., 2000), deep ocean hydrothermal vents (Lutz et al., 2001; Taylor et al., 1999) and Antarctic ponds and sea ice (Madigan et al., 2000). The 3.5-billion-year survival of mats testifies to their capacity in adapting to and altering hostile environments through cellular and community-mediated activities (Des Marais, 1990; Hoehler et al., 2001). In nature, mats generally attach tightly to soil or submerged sediments, then rise to the surface due to buoyancy exerted by the formation of gases in the mat matrix. Subsequently, another mat begins to form over the vacated sediment surface. These communities can sequester organics and metals from their environment. The accretion and production of nutrients generates self-sufficiency among microbial members as materials are passed along wide-ranging redox gradients within the mats. Metabolizing organic materials can generate, as byproducts, hydrogen, methane (Hoehler et al., 2001) and hydrogen sulfide (Taylor et al., 1999). Ecological success of microbial mats and their broad array of microbial activities suggest that these microbial ecosystems might

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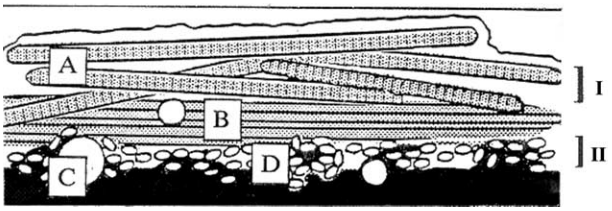

Fig. 1. Schematic of microbial mat: (I) oxic photosynthetic zone (II) anoxic zone. (A) Filamentous cyanobacteria, *Oscillatoria* sp., (B) grass silage, (C) entrapped gas, (D) heterotrophic bacterial colonies, such as *Rhodopseudomonas* sp. and sulfur-reducing bacteria.

be useful to bioremediation of environmental pollutants and biogeneration of useful products.

Microbial mats used for various applications, described in this paper, contain *Oscillatoria* sp. (cyanobacteria), *Rhodopseudomonas* sp. (Mehrabi et al., 2001) and undefined sulfur-reducing bacteria cultured on a matrix of silage. Since mats may contain both nitrogen fixing and photosynthetic bacteria (Pinckney and Paerl, 1997), they tend to be self-sufficient, solar-driven ecosystems having few growth requirements. However, rapid development of mats can be stimulated by adding ensiled grass clippings (silage) to mat inocula in water (Bender et al., 1989a). Mats spontaneously attach to the floating silage and eventually form a durable, leathery mass on the surface. Blades of silage provide not only a physical scaffolding for the developing community but also an initial concentrated supply of preferred nutrients, such as lactic acid, amino acids and a variety of minerals (Bender et al., 1989a). Once established, the rapid movement of metabolic products along gradients of redox and nutrient concentrations mediates the efficient exchange of materials, supporting the productivity of the entire community. Mats generated on silage showed production rates of 14.96 $\mathrm{g\,m^{-2}\,d^{-1}}$ (Bender et al., 1989a). This rate surpasses those of some of the most productive legume field crops. Mature mats showed increases of over 300% in biomass and 100% in protein content over silage feedstock (Bender et al., 1989a). When supplemented with silage, they followed a predictable and reproducible growth pattern, resulting in a final laminated mat community 1–3 mm thick (Fig. 1).

This paper reviews research on the application of microbial mats in aquaculture and bioremediation, and to a lesser extent, in agriculture and energy production. We provide a theoretical model for an integrated approach in the implementation of this microbial consortium, or community. The technique for development and use of these microbial mats is patented (US Patent Nos. 5,522,985 and 5,614,097).

2. Uses of microbial mats: an overview

The mature microbial mat consortium is a durable microbial community that may be applied in a variety of uses related to aquaculture, bioremediation of contaminants, agriculture and energy production. The following is a summary of actual and potential applications of mats, followed by expanded discussion.

2.1. Aquaculture

Microbial mats have been used as a feed for tilapia, *Oreochromis niloticus* (formerly *Tilapia nilotica*) (Bender et al., 1989a; Ekpo and Bender, 1989; Phillips et al., 1994a) and as an effluent filtering system, transforming nitrogenous fish wastes into benign products (Bender et al., 2004; Lee et al., 1996). The related process of rapidly sequestering ammonia from water is described by Goodroad et al. (1995).

2.2. Bioremediation

Mats have been shown to sequester heavy metals (Bender et al., 1989b, 1994a,b,c, 1995a, 1997; Mehrabi et al., 2001; Phillips et al., 1994b, 1995, 1996, 1999; Phillips and Bender, 1995, 1998) and radionuclides (Bender et al., 2000), as well as degrade recalcitrant toxic organic contaminants (Bender et al., 1994d, 1995b; Phillips et al., 1994c; Phillips and Bender, 1995; Murray et al., 1997; O'Niell et al., 2000).

2.3. Agriculture

Cyanobacteria have been tested as an amendment to enhance soil fertilization via nitrogen fixation (Rao and Burns, 1990a,b; Fernandez Valiente et al., 2000). Being photosynthetic, they sequester carbon dioxide, a significant greenhouse gas, whether employed as a fish feed (Bender et al., 1989a) or as soil amendments. The integration of plant culture with mats on the soil surface for carbon dioxide sequestration may provide a constant supply of fixed nitrogen and growth stimulators to maintain the C/N balance for vigorous and continuous growth of trees. In nutrient-poor soils, the ability of trees to sequester carbon dioxide is severely inhibited (Oren et al., 2001).

2.4. Bioenergy

The use of microbial mats in energy production is speculative. Mats produce hydrogen (Hill, 2001) and, individually, various cyanobacteria produce hydrogen (Hansel and Lindblad, 1998; Rao and Hall, 1996; Schulz, 1996; Shah et al., 2001; Tsygankov et al., 1998).

3. Microbial mat uses in aquaculture

3.1. Fish feeds

Feeds must be based on a fish species nutrient requirements. To be cost effective, the following must be taken into consideration: (1) Agricultural byproducts, to be incorporated into the feed, must be available at a reasonable cost. (2) Fish must be able to digest the feed ingredients. (3) Feeds must be stable. (4) Feeds must be readily accepted by fish and efficiently utilized. Microbial mat feed fills all of these requirements (Bender et al., 1989a; Ekpo and Bender, 1989; Phillips et al., 1994a).

Tilapia are known to consume bacteria, green algae, cyanobacteria (El-Sayed and Teshima, 1991) and vascular plants in addition to processing organisms contained in the sediment of aquatic ecosystems (Bowen, 1976). As a top consumer of a nitrogen fixing microbial-based food web, tilapia can convert microbial mat protein into palatable animal protein for human consumption. Ekpo and Bender (1989) confirmed that microbial mats were 81% digestible by tilapia and also 75% digestible by silver carp (*Hypophthalmichthys molitrix*).

In fish culture, the purchase of commercially prepared feeds may account for more than 50% of the total cost of production (El-Sayed and Teshima, 1991) due primarily to the cost of the protein component. For tilapia culture to be an inexpensive aquaculture option in developing countries where much of its culture occurs, inexpensive diets are required. As a fish feed, microbial mats can utilize nearby biomass (grass) for making silage. During fermentation, in which water-soluble carbohydrates promote growth of lactic acid bacteria, the pH is lowered and this inhibits growth of competing microorganisms. Therefore, silage is stable for an indefinite period of time (McDonald, 1981; Woodard et al., 1991). As mats develop on silage, its biomass increases by photosynthesis and nitrogen fixation. Methods of field-scale mat production and a specific pond design for use of microbial mats as fish feed are described by Phillips et al. (1994a) and Bender et al. (1989a). Feeding studies, conducted in laboratory tanks and in field ponds in the Dominican Republic, showed that tilapia grew faster (laboratory data) or at the same rate (field data) when grazing on a ration of microbial mats compared to commercial fish feed. Since mats can be easily cultured in modified fish ponds and demand little management, the cost savings of using this natural feed may be significant, particularly in smaller, subsistence-level aquaculture systems in developing countries (Phillips et al., 1994a).

3.2. Effluent treatment

Based on the ability of mats to rapidly remove concentrated levels of ammonia from water in landfill leachate (Goodroad et al., 1995), a simple trickling filter for a recycled-water aquaculture system was constructed with microbial mats. The concept of using microbial mats to reduce nutrient loading in aquaculture effluent has parallels to the Algal Turf Scrubber^{TM} (Adey and Loveland, 1998; Craig et al., 1996). The ATS^{TM} is based on naturally occurring bacteria, microalgae and filamentous algae. It has been shown to significantly reduce phosphorus in large-scale testing of municipal wastewater (Craig et al., 1996). The focus of this system is on eukaryotic organisms, cyanobacteria *Oscillatoria* and *Calothrix* are known components (Adey and Loveland, 1998).

Laboratory experiments showed that the mats rapidly removed ammonia (from 4.1 to 0.2 mg l^{-1}), presumably by the activity of nitrifying bacteria residing at the mat/substrate interface (Bender et al., 2004; Goodroad et al., 1995; Lee et al., 1996). Photosynthesis in the upper cyanobacteria stratum provided a continuous supply of oxygen for the process. Some ammonia was possibly removed by volatilization and assimilation. Ammonia stabilized at 0.4 mg l^{-1} and oxygen was above 5 mg l^{-1} (though occasionally dropping to 3 mg l^{-1} under dark conditions) with fish densities of either 20 fish at 0.06–0.08 g each or 6 fish at 0.07–0.9 g each in 500 l of water (Phillips et al., 1994a).

In aquaculture applications, it is important to consider several simultaneous activities performed by the mats in relation to nitrogen and carbon management. Production of protein is a function of either nitrogen fixation in nutritionally poor water or of assimilation of combined nitrogen in eutrophied water. The energy-intensive process of nitrogen fixation is driven by photosynthesis resulting in rapid production of protein and carbohydrate essentially synthesized from water, sunlight and carbon dioxide. However, when the surrounding environment contains high concentrations of fixed nitrogen, several options are available to this mixed bacterial consortium. Ammonia is converted to nitrate by nitrifying bacteria, which take up residence under the cyanobacteria (Bender et al., 2004; Goodroad et al., 1995). Some nitrogen is also assimilated by mats, producing a thick, protein-rich product (approximately 26% protein, Bender et al., 1989a). Others have demonstrated that nitrate is removed by three cyanobacterial strains of the genus *Synechocystis* (Hu et al., 2000).

Sequestration of carbohydrates from water is also performed by cyanobacteria and purple bacteria, since they can use various organic materials, including lactic acid in the silage, for cyclic photosynthesis (Fogg et al., 1973; Sasikala et al., 1993). For example, Lee et al. (1996) showed that mats reduced dissolved organic carbon from 125 to 10 mg l^{-1} in an aquaculture system. Eukaryotic green algae and vascular plants have lost this ability. Thus because microbial mats either synthesize proteins and carbohydrates from simple materials, or sequester eutrophying nutrients (nitrogen) from the water and transform them into proteins and carbohydrates, mats can function in both processes: fish feed production and conversion of fish wastes.

4. Microbial mat uses in bioremediation

4.1. Metal and radionuclide removal

Microbial mats sequester heavy metals, metalloids, radionuclides and oxyanions. Although these contaminants have complex and contrasting chemistries, mats display a wide variety of mechanisms for removal (Bender and Phillips, 1994; Phillips and Bender, 1995). These mechanisms take place at the cellular level of the constituent microorganisms and at the community level of the entire consortium. The pilot studies, described as follows, include descriptions of various mechanisms employed by mats in remediation of contaminated water.

In tub-and-sink style bioreactors containing 100 l of water recycled at 5 l min^{-1}, chromium (Cr^{3+}), cadmium (Ca) and lead (Pb), spiked at 10 mg l^{-1}, were nearly completely removed within 24 h (Bender et al., 1997). The average batch removal of all three metals combined was 23 g. In a subsequent field trial, an array of spiked metals were continuously recycled at 1 l min^{-1} through two funnel-and-gate systems (Lehr and Lehr, 2000) with mats immobilized on polyester fiber. One system was exposed to natural lighting and the other was darkened. Each system contained 4000 l into which 1 mg l^{-1} of cesium (Cs), chromium (Cr^{6+}) and strontium (Sr) were spiked four times and barium (Ba), cobalt (Co) and Pb were spiked five times in 167 days. A total of 68.2 g metal was removed from the photosynthetic system (63.5% of the total amount spiked) and 67.4 g was removed from the darkened system (62.7%). In both systems redox ranged from −87.9 to −99.8 and pH from 8.2 to 8.7. Grab samples of microbial mat/polyester fiber substrate showed metal concentrations ranging from 2100 to 1500 mg kg^{-1} for Co to 30 and 85 mg kg^{-1} for Cr^{6+} in the photosynthetic and darkened systems, respectively (Phillips et al., 1999). With a toxicity characteristic leaching procedure (TCLP) for metal retention subjected to a weak acid wash, the effluent showed all Resource Conservation and Recovery Act regulated metals below limits of detection.

In field ponds receiving drainage from an abandoned coal mine in Alabama, after pH neutralization, microbial mats removed manganese contained in the drainage. During 3 years, input manganese, ranging from 4 to 9 mg l^{-1}, was flowed under floating mats, and effluent manganese was 0.05 mg l^{-1}. With a flow of 161 min^{-1}, manganese was removed at a rate of 6.5 g m^{-2} day^{-1}. A crystalline manganous deposit collected at the bottom of the pond was identified by X-ray analysis as manganese calcite. Because pH remains high in the presence of photosynthesizing mats, the possibility of remobilization of manganese was minimal (Phillips et al., 1995; Phillips and Bender, 1998).

In a Colorado precious metal mine drainage containing manganese at 3–34 mg l^{-1} and zinc at 6–43 mg l^{-1}, and a 40 l min^{-1} flow rate, the mean decrease of manganese was 21% and zinc 22%. These metals accumulated on mat up to 12,050 mg kg^{-1} Mn and 30,300 mg kg^{-1} Zn and did not leach from the mat (TCLP) (Phillips et al., 1996; Phillips and Bender, 1998). Other low-concentration metals removed by mats in the drainage included silver, cadmium, copper, chromium, nickel, lead, and iron. The cumulative metal deposit was 190 g kg^{-1} (Phillips and Bender, 1998). A subsequent test used mats attached to a coconut mesh substrate. Total metal removal by mats plus coconut mesh was 60% greater than the coconut mesh control alone (Phillips et al., 1996).

A simple barrel reactor (200-l, darkened) was used to treat water containing 2.4 mg l^{-1} uranium (U^{6+}). In these experiments silica-mat particles (microbial mats immobilized in silica particles) were circulated in the water column. U^{6+} was reduced by 88% in 15 min (Bender et al., 2000). XANES spectra for uranium-contaminated silica-mat particle samples indicated that all of the U^{6+} was reduced to U^{4+}. Sequential batch experiments showed that spent silica-mat particles could be rejuvenated and used for subsequent uranium removal by adding a minimal culture medium (Allen and Arnon, 1955) and allowing a 6-d growth period.

At a Superfund Site with groundwater containing 0.2 mg l^{-1} U^{6+} and 293 mg l^{-1} HCO_3^- (which binds to U^{6+} causing it to function as an anion), over 80% of the dissolved U^{6+} (present mainly as U^{6+}-carbonate) was removed within 15 min by the silica-mat particles. Drying of uranium-contaminated silica-mat particles produced a hard compact product (1% of original weight), which did not leach uranium after mixing in water for 24 h (Bender et al., 2000).

These latter two experiments with mats immobilized on silica gel particles (Bender et al., 2000) represented an effective system for maintaining mats in a water column rather than having contaminated water flowing over a two-dimensional laminar surface.

4.2. Organic degradation

Microbial mats are capable of degrading various organic compounds. Data shows or suggests that these compounds are mineralized (complete digestion to inorganic molecules, such as carbon dioxide). These include petroleum distillates (Phillips et al., 1994c); trichloroethylene (TCE) (Bender et al., 1995b; Phillips and Bender, 1995; O'Niell et al., 2000); tetrachloroethylene (PCE) (O'Niell et al., 2000; Mehrabi et al., 2001); 2,2′-4,4′-5,5′-hexachlorobiphenyl (PCB) (Bender et al., 1995b); octachlorocyclopentadine (chlordane) (Bender et al., 1994d; Murray et al., 1997); 2,4,6-trinitrotoluene (TNT) (Bender and Phillips, 1994; Mondecar et al., 1994; Phillips and Bender, 1995; Ortiz, 1996); 2,4-dinitrotoluene (DNT) (Ortiz, 1996); the pesticides carbofuran, paraquat and prophos, (Murray et al., 1997) and absorbable organochlorine compounds (AOX) as effluents from the pulp and paper kraft mill industry (Bender et al., 1995b). Summarized results for some of the aforementioned are (1) under dark conditions at 90 days, 19% of naphthalene was mineralized, 24% phenanthrene, 21% chrysene and 9% hexadecane (Phillips et al., 1994c); (2) chlordane, in water, was 91% removed after 21 days and no parent compound remained; (3) PCB was 95% removed in the same time period; (4) TCE, formed from the degradation of PCE, was shown to be completely degraded within a minimum of 50 days; (5) AOX was 78% removed after one day and greater than 90% removed after 7 days. Other researchers have shown that cyanobacteria and microbial mats degrade a variety of organic compounds such as the chlorinated aliphatic pesticide, lindane, and 4-chlorobenzoate (Kuritz and Wolk, 1995), various petroleum compounds (Abed et al., 2002) and crude oil (Raghukumar et al., 2001).

An in situ 6-d field trial in a St. Vincent, West Indies banana farm showed increased degradation of carbofuran and a carbofuran isomer when a microbial mat was applied to the soil surface compared to controls without mat. Carbofuran concentration decreased from 30.4 to 2.8 mg kg^{-1} with a microbial mat treatment (82% decrease). Control plot concentrations actually increased from 28.7 to 35.0 mg kg^{-1} (26%). The carbofuran isomer decreased from 1960.0 to 243.3 mg kg^{-1} in 6 days with a microbial mat treatment (75% decrease). Its control plot concentration showed a decrease from 3933.3 to 1733.3 mg kg^{-1} (49% decrease). A 60-day field trial showed enhanced prophos degradation by microbial mats applied to soils. Prophos concentration (5–13 mg kg^{-1}) decreased significantly after four days (0–6 mg kg^{-1}) in the plots with a microbial mat, whereas the prophos concentration increased in the control plots, from 7 to 12 mg kg^{-1} at day 0 to 6–180 mg kg^{-1} at day 4. After 60 days, no prophos was detected in any plot with a microbial mat, whereas all but one control plot had prophos (0–12 mg kg^{-1}) in the soil (Murray et al., 1997).

Mineralization experiments performed with hexachloro-PCB and chlordane showed extensive degradation of the parent compound and metabolites. In a three-week treatment ^{14}C mass balance analysis of the carbon label showed that 17% was recovered as CO_2, 78% as cellular constituents, such as mat protein and 2% as unidentified polar metabolites. No parent compound was recovered (Bender et al., 1995b).

Mat treatment of 2,4,6-TNT, applied to the water column at a concentration of 100 mg l^{-1}, showed 99% removal of TNT in 6 days. Four expected metabolites were detected but their combined concentrations never exceeded 10 mg l^{-1}. Experiments with ^{14}C-labeled TNT showed no appearance of the label as CO_2, which may have been sequestered during photosynthesis (Mondecar et al., 1994).

4.3. Mechanisms of bioremediation

The broad scope of remediations described previously can possibly be accounted for by (1) the presence of a variety of microorganisms that contrast in their anaerobic/aerobic functions (Bender et al., 1994d, 1995b); (2) the distinct zonation within the macrostructure of the mat (Bender and Phillips, 1994; Pinckney and Paerl, 1997); (3) the mediation of the chemistry in the surrounding water column (Phillips et al., 1995; Phillips and Bender, 1998); (4) releases of bioflocculants from the mats (Bender et al., 1994d, 1995a); and (5) filamentous cyanobacteria with negative surface charge (Bender et al., 1994b). The following describes mechanisms that likely relate directly to the removal of specific metals, oxyanions and organic contaminants.

Mat surfaces are highly negatively charged, demonstrating a zeta potential range of −12 to −69 (Bender et al., 1994b). The charges distributed along the filamentous cyanobacteria provide an enormous surface area for binding of positively charged metals (Ahuja et al., 1999; Blanco et al., 1999). Fig. 2 illustrates an electron micrograph of mats after sequestering Pb^{2+} from solution (2A) and the elemental analysis for Pb^{2+} of the same view (2B), showing the deposits of Pb^{2+} along the filaments (dots). Metal deposits, which are congruent with cell morphology, suggest surface binding/ion

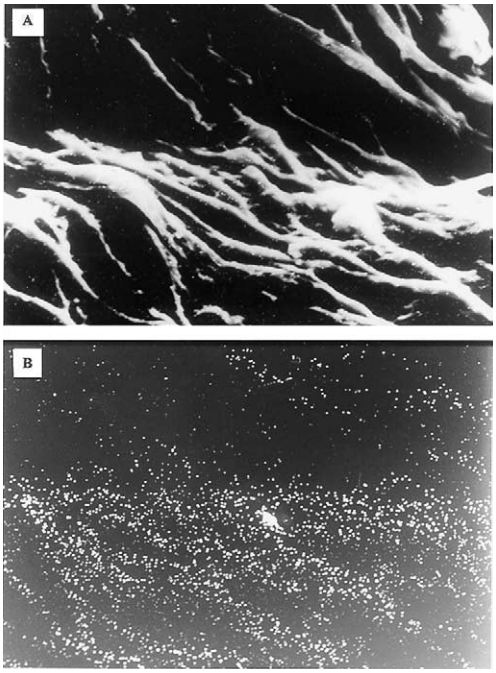

Fig. 2. (A) Filamentous cyanobacteria surface and (B) elemental analysis for lead (Pb^{2+}) of same view (25 mm = 10 μm).

exchange mechanisms. When this is dominant, mats will sorb metals rapidly, then become expended when all binding sites are occupied by metals. Allowing a short growth period partially restores the metal-removal capacity of the spent mats (Bender et al., 1995a).

Metallic species such as U^{6+}, Cr^{6+} and Se^{6+} require reduction for removal from solution. This is accomplished by community-level mechanisms, particularly those in mat anaerobic zones. Metal reduction requires conditions of reduced photo-oxygen, elevated production of reducing components (H_2 or H_2S) and release of reductase enzymes. Under limited light conditions the water splitting, oxygenic photosystem II is not functional, and residual oxygen is rapidly sequestered by aerobes. This results in a reducing environment within and around the mat community. During this period H_2 and H_2S are released and extracellular reductases are produced by *Rhodopseudomonas* (Mehrabi et al., 2001) and other members of the consortium. Uranium becomes reduced from U^{6+} to U^{4+}, precipitating as UO_2 (Bender et al., 2000). Cr^{6+} reduces to Cr^{3+} (Mehrabi et al., 2001), which will precipitate as $Cr(OH)_3$ at slightly elevated pH. Se^{6+} is reduced to elemental Se (Bender et al., 1991). Electron micrographs of Se^{6+} or U^{6+}-exposed mats show that the metals are not associated with cells, but exist as large crystalline deposits in the cellular environment (Fig. 3). In contrast to Fig. 2 in which the metal outlines the surface of the cells, Se deposits as an amorphous clump of elemental selenium within the cell matrix. For manganese, oxic conditions, generated during daylight were most important for its precipitation as an oxide (Phillips et al., 1994b, 1995; Phillips and Bender, 1998).

Another microbial mat remediation mechanism is the synthesis of negatively-charged bioflocculants which are released into the water (Bender et al., 1994a). These carbohydrate molecules bind a number of metals, causing them to deposit at the bottom of the water column. The presence of bioflocculants may account for the capacity of mats to simultaneously remove heavy metals and degrade toxic organics. If bioflocculants rapidly bind metals, enzymes necessary for organic compound degradation are protected.

Because reductive dechlorination is the initial process in the degradation of compounds such as TCE, PCE, TCP, PCB, chlordane and chlorinated effluents from the paper industry (Bender et al., 1995b), maintaining low redox conditions within the mats is also central to their removal. Kuritz and Wolk (1995) also showed that the cyanobacteria species *Anabaena* and *Nostoc* will biodegrade chlorinated organic compounds. Controlling or manipulating diurnal cycles can provide an ideal light/dark environment for complete degradation of this group of contaminants. The highly reducing conditions during the dark results in reductive dechlorination of the compounds, thereby producing metabolites which are more labile to digestion by aerobic heterotrophs. The aerobes in the mats becomes active during the light period when photosynthetic oxygen is high and the remaining organic structures of the dechlorinated molecules will degrade or mineralize.

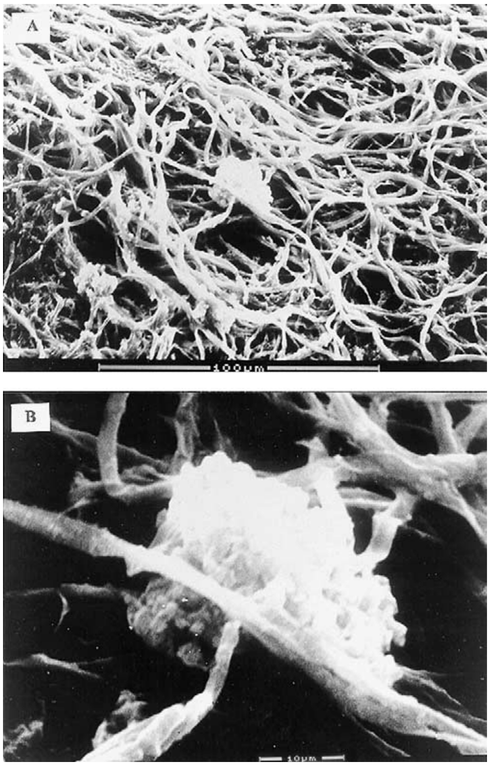

Fig. 3. (A) Filamentous cyanobacteria with deposit of elemental selenium (Se^0) (center)—57 mm = 10 µm. (B) Enlargement of Se deposit—25 mm = 10 µm.

5. Microbial mat uses in agriculture

Mats provide several functions that can be important to sustainable agricultural systems. They excrete bioflocculants, complex polysaccharides (Bender et al., 1994a), which can improve soil aggregation, stimulate the growth of other beneficial soil microorganisms, improve soil water-holding capacity, increase soil organic matter and make phosphates more soluble (Rao and Burns, 1990a). The use of cyanobacteria as a nitrogen fertilizer has been documented, but most work has focused on rice cultivation (De, 1939; Fernandez Valiente et al., 2000; Watanabe, 1951). In upland crops, less research has been conducted. Rao and Burns (1990b) and Reynaud and Metting (1988) examined the role of cyanobacteria as a source of nitrogen for oil-seed rape and winter wheat, respectively. Both showed the need to increase algal biomass in order to make this a viable fertilizer source without commercial fertilizer supplements. Nitrogen fixing studies in mats suggest that a major problem with fixation rate is the limitation of phototsynthetic efficiency (Bebout et al., 1993; Paerl et al., 1991). In practical terms, the energy-intensive process of nitrogen fixation and protein production might be enhanced by adding a low-cost outside source of fixed carbon. Agricultural byproducts (silage) or aquaculture systems (pond filtration systems), where fixed carbon is available, may provide an answer to the biomass production problem in agriculture applications. In terms of silviculture, Oren et al. (2001) showed that rapid forest growth was stimulated by increased concentrations of carbon dioxide in the atmosphere until the soil nitrogen became the limiting factor. Seeding mats in soil as a silviculture practice might remedy this limitation by providing a continuous supply of nitrogen (via nitrogen fixation) necessary for rapid sustained tree growth.

6. Microbial mat uses in biohydrogen production

Capacity for biohydrogen generation via anoxygenic photosynthesis exists in cyanobacteria and purple bacteria components of microbial mats (Lambert and Smith, 1981; Sasikala et al., 1993). Organic compounds, such as lactic acid (in silage) is processed in photosystem I. Because hydrogen production in mats is generally driven by the nitrogen fixing apparatus, the process is far less efficient in terms of ATP requirements than hydrogenase-mediated production. However, Skyring et al. (1989) found that although hydrogenase was present in all mats that they examined, only nitrogenase systems were involved in hydrogen production. The highest H_2 photoproduction rate in natural mats was 6 $\mathrm{nmol\,cm^{-2}\,day^{-1}}$.

In our research, after enrichment with silage, an additional silage wash (by soaking 10 $\mathrm{g\,l^{-1}}$ silage) was added to the medium (Allen and Arnon, 1955) to provide a supply of lactic acid for the photosynthesis system II. Fixed nitrogen in silage (amino acids and other complex nitrogenous compounds) provides cell support without interfering with nitrogenase activity necessary for hydrogen production. The mat system (with silage) produced 27 $\mathrm{\mu mol\,H_2\,l^{-1}\,d^{-1}}$ (Hill, 2001). Although this level of production is higher than natural mats without silage (Skyring et al., 1989) it is too low to be considered a practical energy source. Mats might be developed for higher H_2 production by constructing a synthetic microbial mat consortium containing a broad range of desired bacteria and photobacteria, or by integrating microbial strains which have been specifically developed for hydrogen production by directed evolution, a strategy imitating biological evolution, in order to optimize technological systems (Rechenberg, 1998).

7. Conclusions

Research on bioremediation, bioproduction (aquaculture and agriculture applications) and bioenergy production of microbial mats suggests a theoretical model for an integrated approach in the implementation of this complex consortium. Fig. 4 is a schematic of such an integrated system. Mats are cultured on silage. Silage

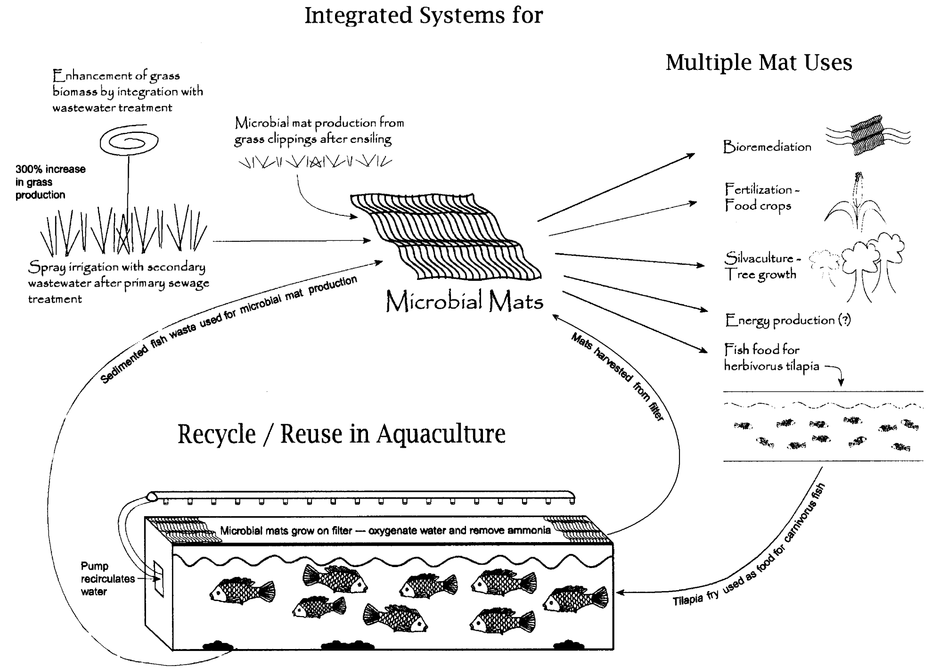

Fig. 4. Conceptual model of an integrated system for aquaculture and bioremediation using microbial mats.

productivity may be enhanced by integrating its production with secondary sewage treatment. The grass will utilize the nutrients, while polishing secondary wastewater as it percolates through grass fields. Resulting mats might be used in several ways including the bioremediation of water contaminated with hazardous metals and organic pollutants. The mat, with its nitrogen content, can be a fertilizer and can restore microbial flora to depleted soils. Integrating mats in silviculture systems can supply fixed nitrogen to managed forests. This is particularly important if silviculture evolves as a strategy to reduce greenhouse gases. Sequester of carbon dioxide will continue only if the trees have a supply of nitrogen to support rapid growth (Oren et al., 2001). Finally, although the utilization of mats for bioenergy production has not been effectively demonstrated, preliminary results (Hill, 2001) show that this application merits further investigation.

In aquaculture, mats convert ammonia and organic materials from fish wastes into mat cellular protein that can be subsequently used as tilapia feed (Phillips et al., 1994a). These prolific fish may be a food source for carnivorous fishes (Bender et al., 2004), resulting in a closed system whereby fish waste is transformed into consumable fish products.

A final consideration that should be taken into account with microbial mats is that highly toxic factors are released by certain species of cyanobacteria. Hughes et al. (1958) extracted a toxin, microcystin, from their *Microcystis* isolate that proved fatal to mice and produced symptoms similar to those which had been described in animals which had ingested waterblooms containing this species of cyanobacteria. Microcystins are known to produce hepatic disease in cultured Atlantic salmon (Andersen et al., 1993). Species of the cyanobacteria genus *Trichodesmium* have been implicated in both fish and invertebrate mortality (Chellam and Alagarswani, 1981 and Suvapepun et al., 1984 in Landsberg, 2002) as well as considered benign (Hawser and Codd, 1992, and O'Niel and Roman, 1992, 1994, and Sellner, 1997 in Landsberg, 2002). Other cyanobacteria species, including species in the following genera, *Aphanizomenon*, *Anabaena* and *Microcystis*, produce toxins (Paerl and Tucker, 1995) as well. Not all species of cyanobacteria produce toxins, yet clearly, identification of the potential to produce toxins is central to the application of mats in useful technologies.

The economic advantages of using natural systems in dealing with various problems of remediation, bioproduction and energy production might best be realized by implementing various integrated systems of waste treatment and biomass production. Exploration of these multi-phase systems might best be accomplished by using a microbial consortium such as a microbial mat with its broad spectrum of capabilities and its highly flexible biological potential.

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