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Immobilized microalgae for removing pollutants: Review of practical aspects

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ABSTRACT

This review analyzes the state-of-the-art of a specific niche in biological wastewater treatment that uses immobilized eukaryotic microalgae (and several prokaryotic photosynthetic cyanobacteria), with emphasis on removing nutrients with the support of microalgae growth-promoting bacteria. Removal of other pollutants by this technology, such as heavy metals and industrial pollutants, and technical aspects related to this specific subfield of wastewater treatment are also presented. We present a general perspective of the field with most known examples from common literature, emphasizing a practical point of view in this technologically oriented topic. The potential venues of future research in this field are outlined and a critical assessment of the failures, limitations, and future of immobilized microalgae for removal of pollutants is presented.

1. Introduction

Large-scale production of wastewater is an inevitable consequence of all contemporary societies. Most wastewaters are usually hazardous to human populations and the environment and must be treated prior to disposal into streams, lakes, seas, and land surfaces. Secondary treatments of domestic and agro-industrial wastewater still release large amounts of phosphorus and nitrogen. These nutrients are directly responsible for eutrophication of rivers, lakes, and seas (Lau et al., 1997; Trepanier et al., 2002) and disposal of partially treated wastewaters produces a constant threat to dwindling freshwater resources on a global scale (Montaigne and Essick, 2002).

Prior to discharging wastewater into water bodies, removing most nutrients is usually obligatory, even though it is not performed in many cases, especially in developing countries. The wastewater treatment industry presently uses several methods to remove phosphorus and nitrogen (Dueñas et al., 2003) and other pollutants. Some are used in large-scale treatment facilities and a few are experimental projects and used on a small-scale basis (from a process-engineering viewpoint, see Stratful et al., 1999; Van Loosdrecht et al., 1997).

Immobilization of microalgae, as part of a global trend of immobilizing microorganisms in an assortment of matrices, is used for a wide variety of biotechnological applications that started over 40 years ago. More recently two excellent reviews on physiology of immobilized microalgae, strategies to enhance nutrient and heavy metal removal, and various biotechnological applications of immobilized microalgae (Lebeau and Robert, 2006; Moreno-Garrido, 2008) and one on the general use of bacteria and algae for treating hazardous contaminants (Muñoz and Guieysse, 2006) were published. They briefly mention, with a few examples, the practical topics covered in this review. This review expands on these topics, bringing together scattered information and analyzes, in detail, the state-of-the-art of a specific niche in biological wastewater treatment (also known as biofiltration; Cohen, 2001; Gadd, 2009) using immobilized microalgae (and some photosynthetic cyanobacteria). Special emphasis was given for removing nutrients with the aid of microalgae growth-promoting bacteria. We present a general perspective of the field with most examples found in the literature. Several studies published earlier than 2000 and reviewed in two previous reviews on the topic (Cohen, 2001; Mallick, 2002) are only briefly presented and discussed. The potential venues of future research in this field are outlined and a critical assessment of the future of immobilized microalgae for removing pollutants is presented. The general objective is to assist new and established researchers with current knowledge of this field and its challenges.

2. The concept

An immobilized cell is defined as a living cell that, by natural or artificial means, is prevented from moving independently from its original location to all parts of an aqueous phase of a system (Tampion and Tampion, 1987). The underlying concept is that

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immobilized microalgae in matrices, biological or inert, may assist the required biotechnological benefits from the mass culture of the microalgae, either a specific metabolite or removal of pollutants. This concept evolved from the basic nature of its components, the microalgae and the immobilizing matrix.

Microalgae are mostly suspension-type microorganisms and very efficient solar energy converters that can produce massive blooms. For decades, they have demonstrated that they can produce a great variety of useful secondary metabolites (Lebeau and Robert, 2006; Moreno-Garrido, 2008) and are potentially useful as treating agents for wastewater. Presently, the commercial impact of microalgae on the wastewater industry, the subject of this review, is minor (de-Bashan and Bashan, 2004), as it was 16 years ago (Chaumont, 1993). One of the major practical limitations of the two microalgal cultivation systems used today, shallow open ponds, where culture is circulated by a paddle-wheel and photoreactors system (made of transparent tubes, sleeves, or containers with a natural or artificial light source) is the harvesting of the produced biomass from the treated water. An efficient removal system is *the key* for recycling of wastewater. The use of industrial filtration and centrifugation is not cost effective for wastewater treatment. One of the methods suggested for the harvesting problem is immobilization of microalgae in polymers. Although immobilization of microalgae has been known for a long time in the secondary metabolite industry, historically, this research field emerged slightly more than 20 years ago with the pioneering studies of de la Noüe and his collaborators from Canada who introduced the concept to wastewater treatment (Chevalier and de la Noüe, 1985a,b; de la Noüe and de Pauw, 1988; de la Noüe et al., 1990).

Six different immobilization types have been defined: covalent coupling, affinity immobilization, adsorption, confinement in liquid–liquid emulsion, capture behind semi-permeable membrane, and entrapment in polymers (Mallick, 2002). These types of immobilation can be grouped as "passive" (using the natural tendency of microorganisms to attach to surfaces – natural or synthetic – and grow on them) and "active" (flocculant agents, chemical attachment, and gel encapsulation) (Cohen, 2001; Moreno-Garrido, 2008).

Most of the general immobilization techniques for microorganisms can be easily modified and applied to microalgae, adding a design factor that these are photosynthetic microorganisms that require light. The most commonly used method is immobilization (a.k.a. entrapment and encapsulation) in polymers, a technique that is also being considered for microbial inoculants in agriculture (Bashan, 1998). In polymeric immobilization systems, similar to other biofiltration systems, there is physical separation between the microorganisms and the treated wastewater. The microorganisms are immobilized (trapped) alive within the polymer because its pores are smaller than the microorganisms, while the fluid flows through it and sustains their metabolism and eventual growth (Cohen, 2001).

Immobilization of microalgae for wastewater treatment is based on the principle of keeping the living cells within a gel matrix metabolically active as long as possible, during which time they have very limited mobility. On rare occasions, a dead mass of microalgae can also be used (see below). After absorption of the contaminants by the microalgae, the cleaner waters diffuse out of the polymers and are collected and reused and the process is repeated for several cycles. Consequently, many polymers can fulfill this requirement. The major issues using any of them are (1) efficiency of the system to remove pollutants; (2) cost of the polymer; (3) cost of the immobilization process. The immobilization method has some major advantages (1) concentrates high biomass that can be used as a byproduct; (2) avoids filtration of the treated wastewater, which can be used as is; (3) has high resistance to toxic compounds within the treated wastewater; (4) can immobilize more than one microorganism (discussed later); (5) is simple to apply by nonprofessionals.

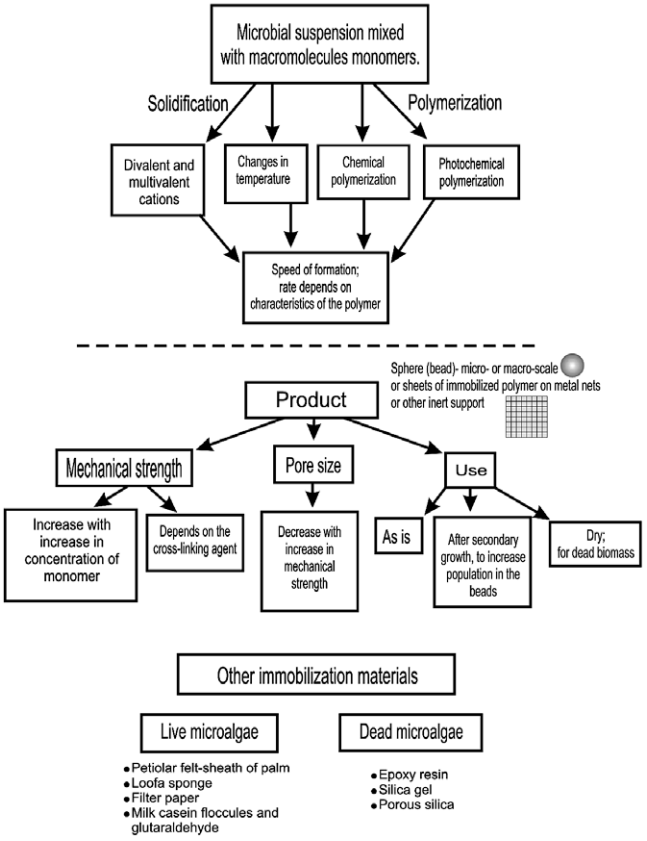

Fig. 1. Flow chart for procedures for immobilization of microalgae in polymers.

Several synthetic (acrylamide, polyurethane, polyvinyl, resins) and natural polymer derivatives of algal polysaccharides (alginate, carrageenan, agar, agarose), and chitosan, an amino polysaccharide derived from chitin, has been experimentally used. Regardless of the polymers used, the material must be hydrophilic, allowing wastewater to diffuse into the bead. The most commonly used polymers are the natural polymers alginate and carrageenan (Bashan, 1998; Moreno-Garrido, 2008; Murano, 1998), even though natural polymers are less stable in wastewater than synthetic polymers. Some natural polymers can dissolve in highly contaminated wastewater, while synthetic polymers do not. Also, natural polymers are more vulnerable to environmental degradation by microbes. However, diffusivity is higher in natural polymers and they are less hazardous to produce (Leenen et al., 1996). Useful guidelines for selecting a polymer, once the application is clear, was provided by Leenen et al. (1996).

In general terms, most immobilizations share the same production protocol, regardless of the polymer that is used (Fig. 1). The microbial suspension is mixed with macromolecule monomers of the polymer. Then, the mixture is solidified to produce a polymeric gel of various shapes. This is done by linking the monomers to each other to form a polymer with as little interaction with the living microorganisms as possible, preferably leaving the microbes inside the matrix intact. Polymerization can be achieved by several physical and chemical treatments, largely depending on the polymer's characteristics. Solidification can be done by linking the monomers with di- and multi-valent cations, as done with alginate. The rate of solidification can be increased or decreased by changing the temperature and different chemical and photochemical reactions (Cohen, 2001). This can be a very fast process, as in the case of alginate (Bashan, 1986) or slower for other polymers (Cohen, 2001).

Generally, the mechanical strength of the final polymer increases with the increase in concentration of the monomers and the cross-linking agents used. With increase in strength, the pore size of the polymer decreases. Spherical beads, the most common form for application, are made by slowly dropping the mixture of monomer and microorganisms through a small orifice, such as a syringe or specific equipment designed for that purpose (discussed below). Once produced, the beads can be used directly or with secondary multiplication in a growth medium to increase the number of microorganisms within the bead (Bashan, 1986; de-Bashan et al., 2004). Beads also can be dried, as for agricultural inoculants (Bashan et al., 2002) or when used with dead biomass (described later). Although drying is not practical for microalgae, bacteria entrapped in dry alginate beads could remain viable after storage for 13 years (Bashan and Gonzalez, 1999).

3. General effects on microalgae from immobilization in polymers

Immobilization or encapsulation of microorganisms in polymers exerts a significant stress on the microorganisms because of chemical forces and interactions between the immobilization matrix and the cell wall. Confinement in a limited space also affects the metabolism of the microorganisms. The most notable effect, detected decades ago in immobilized bacteria, is reduction of the immobilized population, compared to the population before polymerization (Bashan, 1986). This minor deficiency can be overcome simply by allowing the cells a second period of growth within the matrix. Other physiological deficiencies were recently reviewed (Moreno-Garrido, 2008).

In most cases, immobilization is beneficial for the entrapped microorganisms. Apart from direct positive effects, such as avoiding grazing by aggressive zooplankton (Faafeng et al., 1994) and reduction in competition for nutrients with other microbial species, several improvements in the metabolism, function, and behavior of the microalgae were recorded. Immobilization in alginate beads of the hydrocarbon-rich microalgae, *Botryococcus braunii* and *Botryococcus protuberans*, yielded a significant increase in chlorophyll, carotenoids, dry weight, and lipids during the stationary and resting growth phases, compared to free-living cells. Photosynthesis in both species was enhanced, relative to free cells; senescence was significantly delayed (Singh, 2003). Immobilization in chitosan protected the cell walls of *Synechococcus* sp. against NaOH toxicity. Immobilized cells showed better growth than free cell cultures (Aguilar-May et al., 2007).

Several biotechnological applications of microalgae are beneficiaries of immobilization. Immobilization in alginate of the wall-less marine microalga *Dunaliella tertiolecta* in hypersaline medium produce significant amounts of glycerol (Grizeau and Navarro, 1986). Similarly, immobilization of *Dunaliella salina* in agar–agar significantly improved production of glycerol, in comparison with free-living cells (Thakur and Kumar, 1999b). Immobilization of the marine diatom *Haslea ostrearia* in a tubular agar gel layer increase synthesis of marennin, a blue-green pigment involved in commercial culturing of oysters (Lebeau et al., 2000). Conversion of the alkaloid codeine to morphine within the growth medium, but without accumulation of morphine by the cells of the cyanobacteria *Spirulina platensis*, increases slightly by immobilization in alginate (Rao et al., 1999). Immobilization of the nitrogen-fixing cyanobacteria *Anabaena azollae* in polyurethane foam increase normal ammonia production by the cyanobacteria. Foam-immobilized cyanobacteria treated with fungicides stimulate nitrogenase activity and increase ammonia production at significantly higher rates. Inoculation of rice plants in the field with this formulation significantly increases ammonia that the cyanobacteria excrete into the flood water of the rice fields, increases chlorophyll content of the plants, and eventually increases rice grain and straw yields (Kannaiyan et al., 1997). Several more cases of improvement in performance by immobilization were described (for review: Cohen, 2001; Moreno-Garrido, 2008).

4. Removal of nutrient pollutants from wastewater by microalgae

Nitrogen is biologically removed from wastewater in two major ways. (1) Uptake of nitrogenous compounds by microorganisms and larger organisms growing in wastewater in an assimilative way creates a biomass that concentrates the nitrogen and leaves the water with less nitrogen. (2) Oxidation of ammonium to nitrate, nitrite, and NO eventually forms gaseous nitrogen that is evaporated to the atmosphere. The more difficult removal from wastewater is phosphorus.

Today, main commercial processes for removing phosphorus from wastewater effluents are chemical precipitation with iron, alum, or lime (Donnert and Salecker, 1999; Penetra et al., 1999), and to a lesser extent, biological removal (de-Bashan and Bashan, 2004; Stratful et al., 1999). Occasionally, auto-precipitation of phosphorus (like struvite) occurs under special conditions and composition of the wastewater (Van Der Houwen and Valsami-Jones, 2001). Removal of phosphates (PO_4^{3-}) can also be achieved by air stripping CO_2 from anaerobic effluents (Kalyuzhnyi et al., 2003).

In all cases, phosphorus is removed by converting phosphorus ions in wastewater into a solid fraction. This fraction can be an insoluble salt precipitate, a microbial mass in an activated sludge, or a plant biomass in constructed wetlands. These approaches do not recycle phosphorus as a truly sustainable product because it is removed with various other waste products, some of which are toxic. Non-solubilized phosphates are either buried at landfills after incineration of the organic matter or used as sludge fertilizer, if the treatment facility eliminates human pathogens and toxic compounds.

Biological removal of nutrients involves bacterial and microalgal processes. The only commercial biological process is the Enhanced Biological Phosphorus Removal (EBPR) process by bacteria, reviewed in detail by de-Bashan and Bashan (2004). In general, removal of phosphate by microorganisms, compared to nitrogen, was found to be far slower, less efficient, and frequently with lower percentage of removal (usually <30% of total phosphorus in solution), excluding the EBPR process, which is efficient, but more complicated (Aslan and Kapdan, 2006). Also, a gradual decline in efficiency from the first to later removal cycles is commonly observed. These are the Achilles' heel of these systems.

Removal of nutrients by suspension of free-living microalgae is the predecessor of the immobilization process; the theme of this review will therefore briefly mention studies on microalgae in suspension from the last decade. For earlier exploratory studies, see de la Noüe and De Pauw (1988). Unicellular microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus* are capable of removing up to 55% of the phosphates from dairy and pig farming wastewaters in Colombia (Gonzalez et al., 1997). Another strain of *Scenedesmus*, grown in artificial wastewater, removed >50% of the phosphates (Voltolina et al., 1999, 2004, 2005). The cyanobacterium *S. platensis* efficiently removed nitrates, ammonia, and phosphates from synthetic wastewater (Ogbonna et al., 2000). Production of starch generates wastewater with a unique C:N:P ratio (24:0.14:1) that supports good growth of *S. platensis*. Reductions in phosphate levels of the digested effluent was >99% (Phang et al., 2000). Recently, L-glutamic acid was shown to have an effect on the growth and removal of ammonium by *C. vulgaris* from an ammonium solution.

Higher levels of L-glutamic acid, compared to a control without L-glutamic acid, negatively affected growth of *C. vulgaris*, but enhanced removal of ammonium; after 24 h of incubation in the presence of L-glutamic acid, 99% of the ammonium was removed, whereas removal by the control culture was only ~70%. Exposure to light provided higher initial removal of ammonium, but this effect disappeared after 24 h, with no effect on the pH of the solutions or ammonium removal. Adsorption of ammonium ions on the surface of dead *C. vulgaris* cells was low, ~11% (Khan and Yoshida, 2008).

Apart from the straightforward studies mentioned above that used single-species cultures, the idea that a combination of more than one microorganism is better than a single microorganism is gaining acceptance in agriculture and forestry (Bashan and de-Bashan, 2005) and is starting to appear in studies of removing nutrients from wastewater. For example, when grown as a monoculture, neither *Rhodobactor sphaeroides* nor *Chlorella sorokiniana* could simultaneously remove acetate, propionate, ammonia, nitrate, and phosphate from synthetic wastewater, while a mixed culture accomplished this (Ogbonna et al., 2000). A microalga (*C. vulgaris*) and a macrophyte (*Lemna minuscula*) could be applied in tandem for biological treatment of recalcitrant anaerobic industrial effluent that otherwise prevented growth of any macrophyte. First, the *C. vulgaris* reduced ammonium ions (72%), phosphorus (28%), and COD (61%). Consequently, *L. minuscula* was able to grow in the treated wastewater, precipitate the microalgal cells by shading the culture, and reduced organic matter and color. However, *L. minuscula* did not significantly improve further nutrient removal (Valderrama et al., 2002).

All of these studies employed microalgae in a suspension. Application is severely hampered by the difficulties of harvesting or disposing of the enormous microalgal population developed in the water after treatment. This involves the high cost and time-consuming filtration and centrifugation, which are not applicable techniques for a wastewater industry dealing with enormous effluents. The idea of entrapping microalgae in spherical gels for easy removal by sedimentation after wastewater treatment gained significant momentum in the last decade. Initially, it was the main reason for immobilization. So far, immobilization appears to be one of the best techniques and is cost effective for separating microalgae from their culture medium in tertiary wastewater treatment with algae (Olguín, 2003).

4.1. Removal of nutrients by microalgae immobilized in polymers

As explained above, microalgae are immobilized in various polymers for different biotechnological purposes, such as morphology studies, production of fine chemicals, energy production, and wastewater treatment (Lebeau and Robert, 2006; Moreno-Garrido, 2008). Immobilization is especifically important in wastewater treament because it solves the inherent problem of biomass produced by suspended microalgae in the wastewater, as explained above (de la Noüe and De Pauw, 1988; Travieso et al., 1992; Valderrama et al., 2002).

Many examples on the capacity of immobilized microalgae and cyanobacteria to remove nutrients from wastewater and therefore serve as an agent for tertiary wastewater treatment are available in common peer-reviewed literature. This is summarized in Table 1. Many more are available as industrial and personal messages on the internet. Several of the more significant studies are described in more details.

Chlorella vulgaris, immobilized in carrageenan and alginate was used to treat primary domestic wastewater. Although algal cells in both kinds of polymer beads grew more slowly than suspended cells, the immobilized cells were more metabolically active. Over 95% of ammonium and 99% of phosphates were removed from the wastewater in 3 days. This was much more efficient than suspended cells that removed only 50% of N and P in the same time frame (Lau et al., 1997). In a later study by the same authors, uptake of nutrients by algae and adsorption on alginate gels were shown to be the major mechanisms involved in removing ammonium and phosphates (Tam and Wong, 2000). Two decades ago, chitosan-*Phormidium* sp. aggregates were tested as potential biological tertiary treatment to remove nitrogen and phosphorus from a secondary effluent in Canada. Removal of about 95% of inorganic nitrogen was attained after 4–6 h and phosphorus levels were reduced by 87% after 24 h. Chitosan alone was responsible for about 60% of orthophosphate removal, but no reduction of nitrogen by the polymer itself was observed. Disappearance of orthophosphate in the presence of the polymer was attributed to its co-precipitation with calcium released from the chitosan by abrasion. The presence of the microalgae protected the chitosan from abrasion and *Phormidium* directly assimilated the orthophosphate and inorganic nitrogen, thus reducing their levels in the effluent (de la Noüe and Proulx, 1988a,b). *Scenedesmus* spp. cells, immobilized in chitosan, showed high viability after the immobilization process. One of the immobilized strains of *Scenedesmus* sp. had a higher growth rate than its free-living counterpart. Immobilized cells accomplished a 70% nitrate and 94% phosphate removal within 12 h of incubation while free-living cells removed 20% of the nitrates and 30% of the phosphates within 36 h of treatment. However, similar to the previous study, blank chitosan beads were responsible for removing up to 20% nitrate and 60% phosphate (Fierro et al., 2008). Immobilized cells of *Dunaliella salina* show a better uptake capacity of nitrate, ammonium, and phosphate than free-living cells (Thakur and Kumar, 1999b). The cyanobacterium *Phormidium laminosum*, immobilized on polymer foams, was demonstrated to have potential value for removing nitrates in a continuous-flow system (Garbisu et al., 1991). *Spirulina maxima* immobilized in polymers enhanced removal of ammonium from swine waste. More than 90% of ammonium was removed (Cañizares et al., 1993). Immobilization of *Chorella vulgaris*, *C. kessleri*, and *Scenedesmus quadricauda* in several polymers was used to remove nutrients from raw sewage and pretreated cattle manure. Here, immobilization with alginate pellets of *C. vulgaris* and *C. kessleri* performed best with raw sewage under natural light, but not with pretreated cattle manure because the dark color of the latter substrate probably had a negative effect on the photosynthetic microorganisms (Travieso et al., 1996). However, for final treatment of cattle manure, immobilization of microalgae in polyurethane was more successful for removing ammonia and orthophosphate (Cordoba et al., 1995). Removing nutrients can be accomplished at high temperatures in immobilized systems. Removal of nitrate and phosphate ions from secondarily treated sewage with the thermophilic cyanobacterium *Phormidium laminosum*, immobilized on hollow cellulose fibers in a tubular photobioreactor at 43 °C was achieved (Sawayama et al., 1998). Similarly, removal of ammonium from wastewater at high temperatures was recently demonstrated for a heat and intense sunlight-tolerant strain of *C. sorokiniana*, after an acclimation period (de-Bashan et al., 2008c).

Small technical parameters are often of utmost importance for success in this technologically oriented field. The physical shape of the immobilization system's beads or screens should be considered. The capacity of *Scenedesmus bicellularis* to treat municipal wastewater was compared under different conditions: free cells with air bubbling; cells immobilized in alginate beads, and cells immobilized on alginate screens, all conditioned in synthetic culture medium depleted in N and P at relatively low temperatures of 18 ± 2 °C. This work demonstrates that, by using immobilization on screens, removal of nutrients from wastewater was higher than with conventional biological tertiary wastewater treatments, such as free cells or bead-shaped alginate particles (Kaya et al., 1995).

Table 1
Removal of nitrogen and phosphorus by immobilized microalgae.

Pollutant	Immobilizing material	Microalgae species	Reference
Nitrogen	Alginate	*Anabaena* sp.; *Anabaena doliolum*; *Clorella vulgaris*; *C. sorokiniana*; *Chlamydomonas reinhardtii*; *Isochrysis galbana*; *Scenedesmus obliquus*	Chen (2003), de-Bashan et al. (2002a,b, 2004, 2008), Hernandez et al. (2009), Jeanfils et al. (1993), Jeanfils and Thomas (1986), Lee et al. (1995), Mallick and Rai (1993) and Vilchez and Vega (1994, 1995)
	Polyvinyl foam	*Phormidium uncinatum*	Gil and Serra (1993)
	Polyurethane and polyvinyl foam	*Phormidium laminosum*; *S. obliquus*	Garbisu et al. (1991) and Urrutia et al. (1995)
	Filter paper	*Trentepholia aurea*	Abe et al. (2003)
Phosphorus	Alginate	*Chlorella vulgaris*; *C. sorokiniana*; *C. emersonii*	Hernandez et al. (2006, 2009), Robinson (1995), Robinson et al. (1988, 1989) and Robinson and Wilkinson (1994)
Nitrogen + phosphorus	Alginate	*Anabaena doliolum*; *Chlamydomonas reinhardtii*; *Chlorella vulgaris*; *C. sorokiniana*; *C. kessleri*; *C. emersonii*; *Chlorosarcinopsis* sp.; *Dunaliella salina*; *Euglena* sp.; *Macrochloris* sp.; *Nannochloris* sp.; *Palmellopsis gelatinosa*; *Scenedesmus bijugatus*; *S. intermedius*; *S. quadricauda*; *S. bicellularis*; *Selenastrum capricornutum*	Abdel Hameed (2007), de-Bashan et al. (2002b, 2004), Faafeng et al. (1994), Garbayo et al. (1996), Jimenez-Perez et al. (2004), Kaya et al. (1995, 1996), Kaya and Picard (1995), Lau et al. (1997), Mallick and Rai (1993, 1994), Megharaj et al. (1992), Pérez-Martínez et al. (in press), Rai and Mallick (1992), Robinson et al. (1989), Tam and Wong (2000), Tam et al. (1994), Thakur and Kumar (1999a), Travieso et al. (1992, 1996) and Zhang et al. (2008)
	Carrageenan	*Anabaena doliolum*; *Chlorella vulgaris*; *C. kessleri*; *Scenedesmus obliquus*; *S. quadricauda; S. acutus*; *Spirulina maxima*	Cañizares et al. (1993, 1994), Chevalier and de la Noüe (1985a,b), Lau et al. (1997, 1998a,b), Mallick and Rai (1994) and Travieso et al. (1996)
	Chitosan	*Anabaena doliolum*; *Chlorella vulgaris*; *Phormidium* sp.; *Scenedesmus bicellularis*; *Scenedesmus* sp.; *S. obliquus*; *Synechococcus elongates*	Aguilar-May and Sánchez-Saavedra (2009), de la Noüe and Proulx (1988a,b), Fierro et al. (2008), Kaya and Picard (1996) and Mallick and Rai (1994)
	Polyurethane	*Chlorella vulgaris*; *C. kessleri*; *Scenedesmus quadricauda*	and Travieso et al. (1996)
	Polyvinyl foam and polyvinyl acetate-sulfate	*Chlorella pyrenoidosa*; *Phormidium laminosum*	Garbisu et al. (1992, 1993) and Huang et al. (2003)
	Polystyrene	*Chlorella vulgaris*; *C. kessleri*; *Scenedesmus quadricauda*	Travieso et al. (1996)
	Cellulose fibres	*Phormidium laminosum*	Sawayama et al. (1998)
	Micro- and macro-porous fibrous tissue	*Chlorella vulgaris*; *Scenedesmus rubescens*	Shi et al. (2007)

Hyperconcentrated microalgae cultures (up to 3.3 g DW L^{-1}) were immobilized in κ-carrageenan beads. It appears that entrapped algae are able to efficiently remove nitrogen and phosphorus from urban secondary effluent and be considered a tertiary wastewater treatment (Chevalier and de la Noüe, 1985b). The influence of pH value on removing nitrate and phosphate by immobilized *Chlorella pyrenoidosa* in polyvinyl acetate (PVA)-sulfate showed that the jointly immobilized system was somewhat adaptable to the environment; the microalgae reproduced rapidly inside a PVA gel carrier under pH ranging from 5 to 10. Phosphate-removing efficiency of the immobilized system was distinctly affected by pH, but not the removal of nitrate. The immobilized system had higher removal efficiency in the water when close to neutral pH and efficiency to remove nitrate reached 80%; meanwhile, the highest efficiency for removing phosphate was 88%, but decreased over time to 56% (Huang et al., 2003). The matrix itself has an indirect effect on the efficiency of the immobilized microorganisms. *Anabaena doliolum* and *C. vulgaris* immobilized on chitosan were more efficient at removing NO_3^-, NO_2^-, and PO_4^{3-} from wastewaters than cells immobilized on agar, alginate, carrageenan, or free cells. Carrageenan-immobilized cells, however, were better at removing NH_4^+. The capacity of PO_4^{3-} uptake was significantly increased in cells starved of PO_4^{3-} for 24 h (Mallick and Rai, 1994). The effect of gel characteristics on immobilized *S. bicellularis* was tested in high and low viscosity chitosan and gels supplemented with Japanese konjac flour to enhance the stability of hardened gels during tertiary treatment of wastewaters that contained high concentrations of phosphate salts that disrupt alginate hardened by calcium. Immobilization in high viscosity chitosan gels showed a more significant chemical stability than low viscosity chitosan or mixed gels. Sodium pyrophosphate was the best chelating agent for cross-linking and did not affect the diffusion of inorganic nutrients through the hardened gels or the microalgal growth inside the network of chitosan-immobilized cells. After more than 1 month of incubation of the microalgae in chitosan beads soaked daily in a fresh Na_3PO_4 solution, the stability of hardened gels was not affected and no significant release of entrapped cells to the medium was observed. This system worked well for removing nutrients. After the second uptake, the elimination of NH_4^+ and PO_4^{3-} reached 100% within 3 h and maintained its capacity up to the fourth uptake (Kaya and Picard, 1996). Finally, convenience of handling is also a consideration. *Scenedesmus obliquus* cells were immobilized by adsorption in commercially available, preformed polyurethane and polyvinyl foams and by entrapment using a urethane pre-polymer. Adsorption on polymeric foams was a convenient immobilization method, mainly when cells were initially adsorbed in a nitrogen-free medium. Nitrogen starvation of cells enhanced their adsorption to the polymeric foams. The net O_2 evolution activity and nitrate-removing capacity of free-living and polyvinyl-adsorbed cells were similar, indicating that immobilization did not significantly alter the physiology of microalgae. Nitrogen starvation greatly increased the nitrogen uptake rate of immobilized cells. This system was suggested for biological treatment of nitrogen-polluted drinking waters contaminated with nitrogenous fertilizers (Urrutia et al., 1995).

It is not always more efficient to increase the concentration of microalgae in the bead. Relatively low concentrations of alginate-immobilized *Anabaena doliolum* and *C. vulgaris* (0.1 g DW L^{-1}) were

the most efficient for nitrate and ammonium removal in the pH 6–8 range, although uptake of these nutrients was higher in beads with concentrated algae at diminished efficiency (Mallick and Rai, 1993). Recently, different cells in alginate beads, bead sizes, and amount of beads per volume of effluent were tested for nitrogen and phosphorus uptake from wastewaters. Increased concentration of cells in beads did not enhance efficiency, instead causing leakage problems. Also, increasing the number of beads per volume of effluent reduced light penetration, enhanced self-shading effects, and the beads settled to the bottom of the reactor. As a whole, this study removed, as most studies demonstrated, most of the ammonium and ~95% of the phosphorus (Abdel Hameed, 2007). However, high rates of phosphorus removal are far less common in these systems (Hernandez et al., 2006). Furthemore, immobilization did not always enhance removal of nutrients. Immobilization of *C. vulgaris* in carrageenan only had a positive effect on chlorophyll synthesis by the microalgae, although the immobilized cells had assimilated a similar amount of nitrate as free cells (Lau et al., 1998a,b). Similarly, cells of the cyanobacterium *Phormidium uncinatum* were immobilized by adsorption into polyvinyl foam pieces. After 2 months of immobilization, similar net O_2 exchange activity and similar nitrate uptake capacity was measured in immobilized and free-living cells (Gil and Serra, 1993). Rates of phosphorus uptake demonstrated by immobilized *Chlorella emersonii* in alginate were found to be much lower than those of non-immobilized cells. Uptake was dependent upon stocking density in the matrix, cell precultural conditions, and cell viability, but not on cell growth (Robinson et al., 1988). Uptake of nitrate was studied with free-living and alginate-immobilized *C. vulgaris.* Maximum uptake rates were very similar for free-living and immobilized microalgae; however, at different concentrations immobilization modified the rate of nitrate uptake (Jeanfils et al., 1993).

In sum, evaluating the bulk of experimental evidence of the last two decades, it seems that in most cases, removal of nitrogen is favorable in immobilizing systems. Biological removal of phosphorus, although sometimes enhanced in immobilized system, still presents a challenge for the industry. Biological removals are affected by technical and environmental parameters that, if researched and defined precisely, can evolve into an acceptable and practical "greener" technology for wastewater treatment.

4.2. Removal of nutrients by microalgae immobilized in polymers with microalgae growth-promoting bacteria

The most unusual combination of microalgae and bacteria suggested so far for wastewater treatment is to use plant growth-promoting bacteria (PGPB), used in agriculture (Bashan and de-Bashan, 2005), to enhance the growth and nutrient removal capacity of microalgae. The bacterial species of choice so far belongs to the genus *Azospirillum* and is widely used as an inoculant to promote the growth and yield of numerous crop plants, mainly by affecting hormonal metabolism and mineral absorption of the plants (Bashan et al., 2004). The underlying hypothesis assumes that the bacteria will enhance the performance of unicellular plants, that is, microalgae, and that the single-cell plant will respond to bacterial inoculation like higher plants (de-Bashan and Bashan, 2008).

Immobilization of *C. vulgaris* and *C. sorokiniana* with *Azospirillum brasilense* in small alginate beads significantly enhanced all the growth parameters of the microalgae, including the general population, colony size, biomass, and in some strains, cell size (de-Bashan and Bashan, 2008; de-Bashan et al., 2002a; Gonzalez and Bashan, 2000). Furthermore, these artificial combinations, thus far not found in natural habitats, profoundly changed many cytological, physiological, and biochemical pathways and metabolites within the microalgal cells, such as photosynthetic pigments, lipid content, and the variety of fatty acids (de-Bashan and Bashan, 2008; de-Bashan et al., 2002a, 2005; Gonzalez-Bashan et al., 2000; Lebsky et al., 2001). Duo immobilization under semi-continuous synthetic wastewater cultivating conditions, significantly increased removal of ammonium and soluble phosphate ions, compared to microalgae immobilized alone (de-Bashan et al., 2002b; de-Bashan and Bashan, 2003; Yabur et al., 2007). Recently, these combinations were successful in reducing ammonium and phosphate levels of municipal wastewater (de-Bashan et al., 2004; Hernandez et al., 2006). Consequently, *A. brasilense* a PGPB has been called a "microalgae growth-promoting bacteria" or MGPB (de-Bashan et al., 2002a,b, 2004, 2005). Immobilization of the diazotrophic PGPB *Bacillus pumilus* from arid region soils with *C. vulgaris* did not enhance the capacity of the microalgae to remove nitrogen and phosphorus from the medium, as a culture. However, when the capacity of cells was evaluated, each cell could remove more nutrients (Hernandez et al., 2009).

A combination form of treatment, where microalgae and bacteria were immobilized separately in synthetic capron fibers and later mixed in a highly contaminated treatment pond with phenols, oil spills, and heavy metals, showed that a microalgae–bacteria consortium was formed. This consortium, composed of two *Chlorella* sp., *Scenedesmus obliquus*, several strains of *Stichococcus* sp., and *Phormidium* sp. and the bacterial species *Rhodococcus* sp. and *Kibdelosporangium aridum*, greatly reduced the level of pollutants in the pond (Safonova et al., 2004). However, not all associated bacteria are beneficial for a synergistic interaction. In an agro-industrial wastewater pond, a naturally occurring unicellular microalga, *C. vulgaris*, was closely associated with the N_2-fixing bacterium *Phyllobacterium myrsinacearum*. When the two microorganisms were artificially combined and immobilized in alginate beads, sharing the same internal bead cavities, production of five microalgal pigments increased, but there was no effect on the number of cells or the microalgal biomass. The association, reduced the ability of *C. vulgaris* to remove ammonium ions and phosphorus from wastewater (Gonzalez-Bashan et al., 2000). When this associative bacteria was compared to the known PGPB, *A. brasilense,* initially in both cases, most of the small cavities within the beads were colonized by microcolonies of only one microorganism, either bacteria or microalgae, regardless of the bacterial species used for a mixed culture with the microalga. Subsequently, the bacterial and microalgal microcolonies merged to form large, mixed colonies within the cavities. At this stage, the effect of the bacterial association with the microalga differed, depending on the bacterium present. Though the microalga entered a senescence phase in the presence of *P. myrsinacearum*, it remained in a growth phase in the presence of *A. brasilense*. This suggests that there are commensal interactions between the microalga and the two plant associative bacteria, and that, with time, the bacterial species determines whether the outcome for the microalga is senescence or continuous multiplication (Lebsky et al., 2001).

The mechanisms by which bacteria affect growth and nutrient metabolism of microalgae have only recently been investigated. General analysis of numerous experiments show that immobilization of microalgae with *A. brasilense* could result in two independent phenomena. These phenomena are directly affected by the nature of the nitrogen compound, the pH, and the presence of carbon in the substrate. First, growth of the microalgal population increased without an increase in the capacity of the single cells to take up nitrogen, or second, the capacity of cells to take up nitrogen increased without an increase of the total microalgal population. These phenomena were dependent on the population density of the microalgae, which was in turn affected by cultivation factors. This supports the conclusion that the size of the microalgal population controls the uptake of nitrogen in *C. vulgaris* cells—the

higher the population, the less nitrogen each cell takes up (de-Bashan et al., 2005).

The cellular mechanisms are probably related to (1) enhancement of enzymes involved in nitrogen metabolism of the microalgae, such as glutamate dehydrogenase and glutamine synthetase (de-Bashan et al., 2008b) and (2) the capacity of the PGPB to produce phytohormones that increase microalgae growth. Mutants of the PGPB that did not produce sufficient phytohormone indole-3 acetic acid also failed to affect growth and metabolism of the microalgae (de-Bashan et al., 2008a). Starvation also plays a key role in this association by having a synergistic effect on absorption of phosphorus from wastewater and merits consideration in designing future biological treatments of wastewater. Growth and absorption of phosphorus by *C. sorokiniana*, immobilized with *A. brasilense*, were significantly enhanced after a starvation period of 3 days in saline solution. The best phosphorus removal treatment from domestic wastewater was with tandem treatments of wastewater treatment with starved macroalgae that were immobilized and followed by replacement of this culture after one cycle of phosphorus removal with a new, similarly starved culture. This treatment with two cultures was capable of removing up to 72% of the phosphorus in the wastewater. There was a direct correlation between the initial load of phosphorus in the domestic wastewater and the efficiency of removal, being highest at higher phosphorus loads in jointly immobilized cultures. Further, the results showed that the negative effects on growth of starving microalgae were mitigated by the application of the MGPB *A. brasilense* Cd (Hernandez et al., 2006).

In summary, immobilization of microalgae with MGPB is a new and simple way to affect metabolic performance of microalgae (Fig. 2). Based on the data published so far regarding removal of nutrients from wastewater, this approach may lead to a different technology in wastewater treatment.

5. Removal of metals with microalgae immobilized in polymers

Industrials are constantly creating wastewater rich in heavy metals or derivatives of heavy metals like organo-metal compounds. Awareness of the problem has created worldwide concern and brought stronger regulations to control polluting industries. In many rapidly developing countries, these regulations are not enforced, which creates a potential environmental nightmare. Industrial discharges containing heavy metals remain in sediments and are slowly released into the water body to create a long-term source of sustainable pollution (de la Noüe and De Pawn, 1988).

The most commonly used processes for metal removal, and sometimes metal recovery, are chemicals added to the wastewater to precipitate the metals or ion-exchange resins for binding or flocculating similar to industrial processes used for removing phosphorus from wastewater. These strategies change the pollutant from an aquatic phase to a solid phase, which creates materials that usually end in landfills, legally or illegally (de-Bashan and Bashan, 2004). Less frequent but far costlier methods include adsorption by activated carbon, electrodialysis, or reverse osmosis. These are primarily used for drinking water because of cost.

Less information is available on biological processes. Initially, it was proposed that bioremoval, use of biological systems for removing metal ions from polluted waters, has the potential to achieve greater performance at lower cost than conventional wastewater treatment technologies (Wilde and Benemann, 1993). For example, using sulfate-reducing bacteria to remove heavy metals by the production of metal-sulfide precipitates (White et al., 1997). Removal of heavy metals from industrial effluents and domestic wastewater are one of the main foci for microalgal use in biotechnology employing high-rate algal ponds (Oswald, 1988). A patented Algal Turf Scrubber (Craggs et al., 1996), using suspended biomass of common green algae (Adey et al., 1996; Toumi et al., 2000), was also proposed. Selected strains of microalgae, purposefully cultivated and processed for specific bioremoval applications, have the potential to provide significant improvements in world-wide problems of metal pollution.

The use of metabolically active immobilized microalgae is an especially attractive option in applications where extremely low levels of residual metal ions is necessary (Wilde and Benemann, 1993), for detoxification processes, and for metal recovery (Greene and Bedell, 1990). This is feasible because a large part of the metal bound to live or dead cellular surfaces, the major binding sites of microalgae and immobilizing matrices can be desorbed by acid treatment and later recovered (Dönmez and Aksu, 2002). The initial biosorption by microalgae that is independent on light, temperature, or a metabolic inhibitor, is followed by a slower accumulation phase that depends on cellular metabolisms (Moreno-Garrido et al., 1998, 2002). Although live and dead cells absorb metals, dead biomass absorbs less (Moreno-Garrido et al., 1998). A short review on heavy metal detoxification by mostly free-living microalgae, with special in-depth emphasis on those involving the metallothionein peptides or phytochelatins was published recently

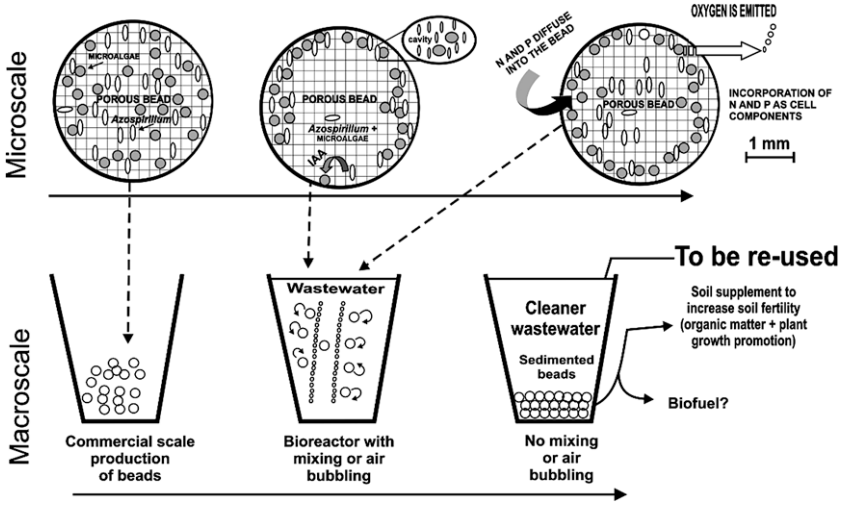

Fig. 2. Experimental model for using immobilized microalgae and microalgae growth-promoting bacteria for wastewater treatment. Drawing not to scale.

(Perales-Vela et al., 2006); therefore this topic will be be discussed very briefly.

Although traces of heavy metals are essential as co-factors for many enzymatic activities in microalgae, as in most organisms, higher concentrations of heavy metals are toxic. Nonetheless, some microalgae can absorb large quantities of heavy metals from wastewater and store them in different cytoplasmatic structures without toxic consequences. They use trace amounts of essential metals for growth and metabolically ignore the non-essential heavy metals. Microalgae have an affinity for polyvalent metals, leading to their application as cleaning agents in water and wastewater containing dissolved metallic ions. For removing metals, the microalgae of choice were usually species of *Chlorella* and *Scenedesmus.* For example, a strain of *Chlorella* can live in a cadmium-rich suspension and remove up to 65% of this pollutant (Matsunaga et al., 1999); the cyanobacterium *Anacystis nidulans* can remove most of the chromium from a solution (Khattar et al., 2007); and the genus *Scenedesmus* is capable of removing uranium (Zhang et al., 1997), copper, cadmium (Terry and Stone, 2002), and zinc (Aksu et al., 1998; Cañizares-Villanueva et al., 2001; Travieso et al., 1999). However, a major reservation should be mentioned; most studies on tolerance and removal of heavy metals by microalgae were done under *in vitro* conditions in batch culture and needs to be demonstrated *in situ* before any recommendation for their use can be made.

Many cases of removing metals and other pollutants that involve immobilized microalgae and cyanobacteria have been published in recent years and are listed in Table 2. The most notable cases are illustrated in more detail in the followings section, arranged by the specific pollutant.

5.1. Cadmium

Cadmium is a common, toxic pollutant because it is part of many industrial and domestic applications, such as batteries, pigments, coatings, electro-plating, stabilizer in plastics, and in alloys with low melting temperatures. Removal of cadmium is a top priority in many developed countries. Immobilization of the cyanobacterium *Spirulina maxima* with the microalgae *Tetraselmis chuii*, both with a high cadmium uptake capacity, was done by adherence of the microbial cells to the surface of the brown seaweed *Sargassum* sp. (a good cadmium biosorbent material) and the green seaweed *Ulva* sp. (a poor cadmium biosorbent material). "Immobilization" (adhesion to surfaces) of the microorganisms on the external surfaces of the seaweeds was done by cultivating the microorganisms with the seaweeds. This simple procedure enhanced the cadmium biosorption (da Costa and de França, 1996). When immobilized in alginate, *T. chuii* was capable of removing about 20% of the cadmium from marine seawater (Moreno-Garrido et al., 2005) and *Spirulina platensis* was efficient at removing cadmium, as well (Rangasayatorn et al., 2004). *Scenedesmus acutus* and *C. vulgaris*, immobilized in polyurethane foam or κ-carrageenan gel showed tolerance for cadmium, chromium, and zinc concentrations above the normal concentration of these ions in industrial wastewaters (Travieso et al., 1999). Removal of cadmium from cadmium-zinc mixtures was higher using immobilized *C. vulgaris* than with suspended cells (Cañizares-Villanueva et al., 2000). A vegetable sponge served as an immobilizing matrix for *C. sorokiniana* to remove cadmium (Akhtar et al., 2003; Saeed and Iqbal, 2006). Regarding the immense problem of cadmium pollution, bioremoval is still a minuscule technique in wastewater treatment.

5.2. Chromium

Chromium is used primarily in metallurgy, automotive paint, tannins of leather, and catalyst industries and is not usually considered a health hazard in its metal forms. However, chromium(VI) compounds are toxic, especially to the eyes and are carcinogenic. Levels of toxicity in drinking water are low, hence the motivation for cleanup. The potential of alginate-immobilized *A. doliolum* and *C. vulgaris* to remove chromium depends on cell density and on the pH of the growth medium; higher density was correlated with higher uptake, although intermediate cell density was the most efficient for removing the metal. Neutral pH was also optimal for uptake of chromium, with decreasing effectiveness when pH increases and decreases (Mallick and Rai, 1993). Wastewater from the plating industry, rich in chromium, is a potential hazard for people and the environment. The efficiency of removing chromium by *S. obliquus* was 12–27%, depending on the metal species. Immobilization increased removal of chromium to 95% (Pellon et al., 2003). Similarly, immobilization of *Anacytis nidulans* in agar increased chromium absorption (Khattar et al., 1999). Similar to cadmium, very little is known about the *in situ* application potential of immobilized microalgae.

5.3. Cobalt

Cobalt is used for alloys, magnets, paints, and catalyst and, like nickel, is essential for life in minute quantities, but carcinogenic in higher doses. Because this metal is very useful, it attracted very limited cleanup activities. In a pilot program, *S. obliquus* was immobilized in a slowly moving rotary biofilm reactor that was run with synthetic wastewater with heavy loads of cobalt for 20 years. A maximum removal of cobalt ion of 94.5% was reached after 10 days (Travieso et al., 2002). Significant accumulation of cobalt, zinc, and magnesium was measured in *C. salina* cells immobilized in alginate (Granham et al., 1992).

5.4. Copper

Copper is an essential component of most living organisms. Additionally, it is extensively used in numerous industrial activities; it has some derivatives that are mildly poisonous. Its main danger to the environment results from the huge quantities accumulating in every habitat. *C. vulgaris* that was immobilized in alginate was evaluated for it capacity to remove copper and nickel during hydraulic retention of 30 min. Over 97% of copper and 91% of nickel were removed from the wastewater. The consistency of the rate of removing metal after 10 metal loadings and regeneration cycles of the system suggest that immobilized microalgae can treat over 400 times the bead's volume of contaminated wastewater (Lau et al., 1998c). When there are two metals in the solution this can affect adsorption of both metals by the alginate-entrapped *C. vulgaris.* In individual dilute metal solutions of copper and nickel, almost complete removal of the test metals was achieved within 1 h. The presence of a second metal inhibited the sorption of the primary metal by free, as well as immobilized cells with inhibition to nickel sorption from the presence of copper being stronger than inhibition of copper sorption by nickel. The total metal (nickel + copper) sorbed from the binary metal solution by free or immobilized cells always remained lower than the total sorption of individual metals from their respective single metal solutions. This suggests competition between nickel and copper for the common binding sites on *Chlorella* and that the microalgae has a greater affinity for copper than for nickel (Mehta and Gaur, 2001). The efficiency of removing copper from a solution was also studied. The amount of copper that was removed was directly related to density of the *C. vulgaris* cells; higher density led to higher rates of sorption of copper, but the specific amount of uptake of copper by the microalgae was reduced. Hence, the amount of algal biomass used for removing metal is an important factor that should be optimized. Metal-saturated beads could be regenerated and

Table 2
Removal of heavy metals and other pollutants by immobilized microalgae, macroalgae and cyanobacteria.

Pollutant	Immobilizing material	Microalgae species	Reference
Cadmium	Alginate	*Chlorella homosphaera*; *C. vulgaris*; *Chlamydomonas reinhardtii*; *Oscillatoria* sp.; *Tetraselmis chuii*	Bayramoğlu et al. (2006), Cañizares-Villanueva et al. (2000), da Costa and Leite (1991), Katircioğlu et al. (2008) and Moreno-Garrido et al. (2005)
	κ-Carrageenan	*Chlorella vulgaris*; *Scenedesmus acutus*	Travieso et al. (1999)
	Polyurethane foam	*Chlorella vulgaris*; *Scenedesmus acutus*	Travieso et al. (1999)
	Surface of *Sargassum* sp.	*Spirulina maxima*; *Tetraselmis chuii*	da Costa and de França (1996)
	Surface of *Ulva* sp.	*Spirulina maxima*; *Tetraselmis chuii*	da Costa and de França (1996)
	Silica gel	*Spirulina platensis*	Rangasayatorn et al. (2004)
	Luffa cylindrica sponge	*Chlorella sorokiniana*; *Synechococcus* sp.	Akhtar et al. (2003) and Saeed and Iqbal (2006)
Cadmium and lead	Milk casein + glutaraldehyde	*Heterosigma akashiwo*	Seki and Suzuki (2002)
Caesium	Alginate	*Chlorella salina*	Avery et al. (1993)
Chromium	Alginate; κ-carrageenan	*Anabaena doliolum*; *Chlorella vulgaris*; *C. miniata*; *Scenedesmus acutus*; *S. obliquus*; *Spirulina platensis*	Gokhale et al. (in press), Mallick and Rai (1993), Pellon et al. (2003), Tam et al. (2009) and Travieso et al. (1999)
	Agar	*Anacytis nidulans*	Khattar et al. (1999)
	Polyurethane foam	*Chlorella vulgaris*; *Scenedesmus acutus*	Travieso et al. (1999)
	Luffa cylindrica sponge	*Chlorella sorokiniana*	Akhtar et al. (2008)
Cobalt	Alginate	*Chlorella salina*	Granham et al. (1992)
	Polyurethane	*Scenedesmus obliquus*	Travieso et al. (2002)
Copper	Alginate	*Anabaena doliolum*; *Chlorella vulgaris*; *C. miniata*; *Nannochloropsis gaditana*; *Scenedesmus quadricauda*; *Tetraselmis chuii*	Bayramoğlu and Arica (2009), Lau et al. (1998c), Mehta and Gaur (2001), Moreno-Garrido et al. (2002, 2005), Rai and Mallick (1992) and Tam et al. (2009)
	Polyvinyl alcohol	*Sargassum baccularia*	Chu and Hashim (2001)
Gold	Alginate	*Chlorella homosphaera*	da Costa and Leite (1991)
Ferous	Alginate	*Anabaena doliolum*; *Chlorella vulgaris*	Rai and Mallick (1992)
Lead	Alginate	*Chlorella vulgaris*; *Chlamydomonas reinhardtii*	Abdel Hameed (2006) and Bayramoğlu et al. (2006)
	Luffa cylindrica sponge	*Chlorella sorokiniana*	Akhtar et al. (2004a,b)
Manganese	Alginate	*Chlorella salina*	Granham et al. (1992)
Mercury	Silica gel	*Chlorella vulgaris*	Tajes-Martinez et al. (2006)
	Alginate	*Chlorella emersonii*; *Chlamydomonas reinhardtii*	Bayramoğlu et al. (2006) and Wilkinson et al. (1990)
	Agarose	*Chlorella emersonii*	Wilkinson et al. (1990)
Nickel	Alginate	*Anabaena doliolum*; *Chlorella vulgaris*; *C. miniata*; *Scenedesmus quadricauda*	Bayramoğlu and Arica (2009), Al-Rub et al. (2004), Lau et al. (1998c), Mallick and Rai (1993, 1994), Mehta and Gaur (2001) and Tam et al. (2009)
	Loofa sponges	*Chlorella sorokiniana*	Akhtar et al. (2004a,b)
Zinc	Alginate	*Chlorella homosphaera*; *C. miniata*; *C. salina*; *Nannochloropsis gaditana*; *Scenedesmus quadricauda*	Bayramoğlu and Arica (2009), da Costa and Leite (1991), Granham et al. (1992), Moreno-Garrido et al. (2002) and Tam et al. (2009)
	κ-Carrageenan	*Chlorella vulgaris*; *Scenedesmus acutus*	Travieso et al. (1999)
	Polyurethane foam	*Chlorella vulgaris*; *Scenedesmus acutus*	Travieso et al. (1999)
Uranium	Polyacrylamide	*Chlorella regularis*	Nakajima et al. (1982)
Mixture of Cu, Fe,Ni, Zn	Polysulphone and epoxy resin	*Phormidium laminosum*	Blanco et al. (1999)
Other pollutants			
Hydrocarbons	Polyurethane foam	*Prototheca zopfii*	Ueno et al. (2006, 2008) and Yamaguchi et al. (1999)
	Alginate	*Selenastrum capricornutum*	Tam et al. (2009)
Organotin compounds (biocides)	Alginate	*Chlorella emersonii*; *C. vulgaris*	Luan et al. (2006), Tam et al. (2009) and Zhang et al. (1998)
LAS (surfactant)	Alginate	*Phaeodactylum tricornutum*	Moreno-Garrido et al. (2007)
Mix of phenols, oil spill and heavy metals	Capron fibers (synthetic)	*Chlorella* sp.; *Phormidium* sp.; *Scenedesmus obliquus*; *Stichococcus* sp.	Safonova et al. (2004)

reused (Tam et al., 1998). When immobilized in alginate, the microalgae *T. chuii* was capable of removing all copper from marine seawater (Moreno-Garrido et al., 2005).

Because copper has high value, recycling is an important consideration when devising a method for removal from solution. The characteristics of desorption of copper from the biomass of the immobilized marine macroalga, *Sargassum baccularia*, were studied using HCl as the eluent. The extent and rate of desorption were directly affected by the pH of the eluent. Nearly 91% of the copper initially adsorbed by the macroalga was released back into an HCl solution at pH 1.0. Increasing the pH decreased desorption and increased the required time for desorption. The immobilized beads of seaweed biomass could be regenerated with an HCl solution to form multiple cycles of copper biosorption–desorption (Chu and Hashim, 2001). Although copper recovery generated more studies than other metals, practical information about its cleanup is still limited.

5.5. Gold

Recovery of precious metals from any environment can be an obviously profitable venture, yet only one study documented

absorption of gold by immobilized microalgae, where alginate-immobilized *Chlorella homosphaera* was capable of absorbing 90% of the initial amount of gold. The alginate, by itself, was responsible for uptake of 40% of the gold by the microalgae (da Costa and Leite, 1991).

5.6. Ferrous iron

As with copper, iron does not produce a significant environmental hazard, despite its endless uses. Alginate immobilized and free cells of *A. doliolum* and *C. vulgaris* showed higher uptake rates of copper and iron, suggesting that immobilization offers some protection against metal toxicity. Compared with free cells, immobilized cells showed greater efficiency for removing iron, even after three cycles, although there was a gradually decrease in efficiency in the second and third cycles (Rai and Mallick, 1992).

5.7. Lead

Lead is a toxic metal, but is an extremely valuable component in numerous industrial applications, such as car batteries, glazed ceramics, and bullets. It causes serious damage to the nervous system (especially in young children) and causes blood and brain disorders. Half of the lead produced is from recycling and, therefore, using immobilized microalgae has the same objectives. *C. sorokiniana* immobilized on loofa sponge (*Luffa cylindrica*) was successfully used for removing lead ions from aqueous solutions. The biosorption of lead ions by the biomass increased as the initial concentration of lead ions in the medium increased. The process is very rapid, with 96% of adsorption occurring within the first 5 min and equilibrium reached after 15 min. The biosorption capacity was pH dependent; maximum adsorption occurred when the solution was pH 5 (Akhtar et al., 2004b).

Involvement of the matrix in removing pollutants without the microalgae is significant. Alginate beads immobilized with *C. vulgaris* were more effective and suitable than free cells that yielded inconsistent levels of removed and recovered lead. Consistently high levels of lead removal (>90%) and recovery (~100%) were achieved by this immobilized microalga. However, adsorption was mainly caused by the alginate matrix with only a minor contribution by the microalgae (Abdel Hameed, 2006).

5.8. Mercury

Mercury is used in many electrical and electronic applications; however, the main source of pollution is from coal-fired power generating facilities, gold and cement production, and smelters. As such, the environment has a constant supply of polluting mercury. Mercury is harmless in an insoluble form, such as mercuric sulfide, but it is extremely poisonous in soluble forms, such as mercuric chloride and organic compounds. Therefore, several studies investigated removal by immobilized microalgae.

In matrix immobilizing systems, removal of mercury from solution is a combination of three phases involving (1) mercury accumulation by the cells; (2) volatilization by the immobilization matrix; (3) volatilization by the growing cells. A comparison of accumulation and volatilization of mercury by non-immobilized and immobilized cultures of *Chlorella emersonii* show that reduction of the mercury concentration in the growth medium by non-immobilized cells was highly dependent on the density of the inoculum, while reduction of the concentration of the mercury by immobilized cells was rapid at all inoculum densities. Accumulation of mercury by immobilized cells was significantly greater than by non-immobilized cells. Volatilization of mercury by non-immobilized cell systems was greatest at higher inoculum densities, whereas more mercury was volatilized from immobilized cell cultures at lower inoculum densities and was greatest with alginate beads not containing microalgae. Accumulation and volatilization of mercury by microorganism-free immobilization matrices revealed that agarose volatilized far less mercury than alginate or agar. This volatilization phenomenon is probably governed by chemical interactions of mercury with the matrices (Wilkinson et al., 1990).

When *Chlamydomonas reinhardtii* is immobilized in alginate, enhanced absorption of mercury, cadmium, and lead ions from aqueous solutions occurs with optimum removal at pH 5–6 with no effect by temperature (Bayramoğlu et al., 2006). Removal of organic and inorganic mercury from different waters was evaluated by using columns packed with *C. vulgaris* immobilized on silica gel. The procedure tested removal of mercury from samples of spiked tapwater, seawater, and wastewater; high recovery of both mercury species was achieved, but this worked only for inorganic pollutants in wastewater and unfiltered seawater (Tajes-Martinez et al., 2006).

5.9. Nickel

Industrial uses of nickel include stainless steel, magnets, coinage, plating, and special alloys with copper, chromium, aluminum, lead, cobalt, silver, gold, as well as providing a green tint to glass. Nickel also plays numerous essential roles in the biology of microorganisms and plants, but some nickel compounds, including nickel sulfide fumes and dust are possibly carcinogenic. It has high value and therefore, after removal, the product is recycled. Removing nickel with immobilized *A. doliolum* and *C. vulgaris* was not different and was affected by the same factor of cell density and pH as removing chromium by the microalgae mentioned earlier (Mallick and Rai, 1993). Blank alginate beads with suspended living and dead cells of *C. vulgaris* and immobilized cells for the removal of nickel ions from aqueous solutions showed that immobilization enhanced the sorption of nickel. Repetitive use of the sorbent was achieved. Increasing the initial pH or initial nickel ion concentration produced increased nickel absorption in all treatments (Al-Rub et al., 2004). Immobilization of *C. sorokiniana* in loofa sponges recovered larger amount of nickel compared to free-living microalgae. Almost all the metal can be recovered by acid washing the loofa (Akhtar et al., 2004a).

5.10. Uranium

Nuclear power plants are the main use of uranium, but the major application of uranium and the main source of environmental contamination is the use of high-density, depleted uranium in penetrating shells by the military. Although uranium is extremely valuable and is probably recycled by the power industry, this information is not publically available. Only one old study of *Chlorela* sp., which was immobilized in polyacrylamide gel, was shown to recover uranium. The adsorption of uranium by the immobilized cells was not affected by pH 4–9, indicating that uranium adsorption becomes independent of pH after immobilization. The amount of uranium adsorbed by immobilized cells increased linearly with temperature, suggesting that adsorption of uranium by the immobilized cells is an endothermic reaction. In freshwater and seawater, the immobilized cells can recover uranium and almost all adsorbed uranium can be desorbed. Thus, the immobilized *Chlorella* cells can be used repeatedly in adsorption–desorption processes (Nakajima et al., 1982).

5.11. Zinc

Zinc is the fourth most common industrial metal, trailing iron, aluminum, and copper. Zinc is used to galvanize steel to avoid

corrosion of many alloys, in casting in automobiles and batteries. Even though zinc is an essential element for sustaining all life, too much zinc can be harmful. Excessive absorption of zinc can also suppress copper and iron absorption. The free zinc ion in solution is highly toxic to plants, invertebrates, and even vertebrate fish. Interestingly, zinc has received minimal attention related to removal. Biosorption of wide ranges of cadmium and zinc, alone or in combination, was demonstrated by alginate-immobilized *C. homosphaera* cells. In all cases, the removed metal was nearly 100%. When these metals were combined, the rate of absorption decreased (da Costa and Leite, 1991).

From a literature search, there are apparently many studies on removing metal by immobilized microalgae, but the number of studies for each metal element is very limited and, in most cases, the information collected so far is only embryonic in nature and cannot be used for applicative conclusions.

6. Removing industrial pollutants with microalgae immobilized in polymers

In spite of worldwide pollution from uncontrolled and unregulated industrial effluents, especially in rapidly developing countries, there are only a few reports concerning removing industrial pollutants with microalgal procedures (Aksu, 2005) and even less using immobilized microalgae.

6.1. Biocides; organotin compounds

Biosorption and degradation of alginate-immobilized *C. emersonii* were studied for its impact on residues of industrial butyltin compounds, one of the most hazardous compounds to human health, which are used in organotin biocides (wood and textile preservatives), fungicides, pesticides, and anti-fouling paint on ships. Immobilization of *C. emersonii* have higher respiratory and growth rates, degrade tri-, di-, and mono-butyltin chlorides faster, and lower accumulation of the biocide than free cells in aquatic solutions (Zhang et al., 1998).

Biosorption and biodegradation by *C. vulgaris* immobilized in alginate removes tributyltin (TBT), a toxic and persistent contaminant, that is an active ingredients in biocides. More than 90% of TBT was rapidly removed within 1 day by both immobilized microalgae and blank beads, irrespective of the TBT concentrations and the number of cycles used, which demonstrates that initial removal was mainly by biosorption and the alginate matrix provided many binding sites. For immobilized *C. vulgaris*, TBT was mostly adsorbed onto the alginate matrix with some on algal cell walls; less than 10% of the TBT accumulated inside cells. TBT was biodegraded by immobilized beads at the end of the six cycles. The amounts of less toxic debutylated products, such as dibutyltin (DBT) and monobutyltin (MBT) in medium increased gradually with treatment cycles and with concentrations of TBT. Accumulation of DBT and MBT, similar to TBT, within the cells was relatively small compared to that in the medium and the alginate matrix. These results suggest that *C. vulgaris* immobilized in alginate continuously detoxify TBT into DBT and MBT over six consecutive cycles, even at the highest TBT contamination level (Luan et al., 2006).

6.2. Hydrocarbons

Several species of microalgae can degrade hydrocarbons (for review: Semple et al., 1999). However, the heterotrophic, colorless microalga *Prototheca zopfii* is the only microalga reported so far with the capacity to degrade hydrocarbon in an immobilized state (Suzuki et al., 1998; Yamaguchi et al., 1999,). Immobilization of a thermo-tolerant strain of *P. zopfii* and a nonthermo-tolerant strain in polyurethane foam was tested on a mixed hydrocarbon substrate (MHS) containing aliphatic and polycyclic aromatic hydrocarbons (PAH). The thermo-tolerant strain degraded MHS at 35 °C, while the nonthermo-tolerant strain had no effect at temperatures higher than 30 °C. Immobilization of *P. zopfii* resulted in a shortened lag time for growth-associated biodegradation of *n*-alkanes (paraffins) in MHS (Ueno et al., 2006). The same immobilized system was used to measure degradation of mixed hydrocarbon substrates composed of *n*-alkanes and polycyclic aromatic hydrocarbons (PAH) in five successive cycles of batch cultivation. Immobilized *P. zopfii* degraded *n*-alkanes almost completely, but it barely degraded PAH. PAH accumulated in the matrix. This phenomenon is completely different from what occurs with free-living cells. There, *P. zopfii* reduces concentrations of both *n*-alkanes and PAH. However, accumulation of PAH in the immobilization matrix did not impair the performance of the immobilized microalga to use *n*-alkanes. These results suggest that immobilized cells in polyurethane foam can repeatedly retrieve PAH from oil-polluted waters after biodegradation of *n*-alkanes by microalgae (Ueno et al., 2008). A naturally immobilized cyanobacteria mat made of *Phormidium animale* was capable of biodegrading crude oil, but degradation was probably done by other microorganisms associated with the mat, rather than the cyanobacteria that served only as a matrix (Chaillan et al., 2006).

6.3. Surfactants

The marine diatom *Phaeodactylum tricornutum* immobilized in alginate was exposed to two sediments containing the surfactant linear alkylbenzene sulphonate (LAS), a common surfactant in detergents that normally biodegrades quickly into water and carbon dioxide; hence, almost harmless to most aquatic environments. Possible toxic responses were compared for free and immobilized microalgae. Although there was a direct relation between LAS content in sediment and inhibition, immobilized microalgae suffered less inhibition than free cells (Moreno-Garrido et al., 2007).

As in the case of removing metals, current studies only demonstrate the capacity of immobilized microalgae to remove industrial pollutants, such as the algae-silica preparation called algaSORB® (http://www.p2pays.org/ref/19/18762.pdf, accessed 11 March 2009), but the actual potential for application has yet to be demonstrated.

7. Technical aspects of immobilization of microalgae

Immobilization and especially immobilization of two microorganisms is a highly technical field and success or failure can be partly determined by small technical aspects or the technology chosen for cleanup.

7.1. Alternative polymers and matrices

Alginates (polymers made of different proportions and sequences of mannuronic and guluronic acids extracted from brown algae) are the polymers of choice in most systems of immobilization because they are easy to handle, nontoxic to humans, the environment, and the entrapped microorganisms, legally safe for human use, available in large quantities, and inexpensive. From a physiological perspective, a major advantage of alginate is that immobilized cells do not suffer extreme changes in physicochemical condition during the procedure of immobilization and the gel is transparent and permeable. However, other natural polymers, such as carrageenan (a collective name for polysaccharides

extracted from red algae) and agar derivatives have been investigated, as well.

Agar–agar, agarose, carrageenan, and calcium alginate were used for immobilization of *Dunaliella salina* cells. Agar–agar performed most effectively (Thakur and Kumar, 1999b), but it is not cost effective. Chitosan (a linear amino polysaccharide of β-D-glucosamine obtained mainly from the chitin of crustaceans) was used to remove nutrients from wastewater, as described above (Kaya and Picard, 1996). A detailed study of the interaction of *C. vulgaris* with the carrageenan gel matrix showed that the growth of the microalgae had a 1 day lag when compared with a free cell system. The rate of chlorophyll synthesis in immobilized cells was double that of free cells, compensating for the screening effect of the gel. The depletion of nitrate and phosphate from the medium was similar to the activity of free cells. In sum, carrageenan gel had no adverse effect on growth and physiology of the immobilized microalga and can be used as an alternative gel matrix for immobilizing microalgae (Lau et al., 1998a,b). The use of the cheap, stable, and easily available petiolar felt-sheath of palm as a novel matrix for immobilizing the microalga *Porphyridium cruentum* was proposed, but not tested, for removing pollutants. The immobilized cells, compared to free cells, had significantly higher biomass and polysaccharide production after 27 days of cultivation. Immobilized cells were successfully maintained through 12 successive batch cultures over 96 days (Iqbal and Zafar, 1997). Cells of the aerial microalga *Trentepohlia aurea*, immobilized on filter paper, created a biofilm. When immersed in medium containing ammonium, nitrate, and nitrite, the microalga reduced the level of nitrogenous compounds slowly but steadily over 40 days (Abe et al., 2003). A combination of milk casein floccules and glutaraldehyde was used to remove cadmium and lead from seawater by the microalgae *Heterosigma akashiwo* (Seki and Suzuki, 2002). Immobilization of *C. sorokiniana* on natural loofa sponge (*Luffa cylindrica*) was successfully used as a new biosorption system for removing lead from aqueous solutions (Akhtar et al., 2004a,b).

Sometimes, one can use matrices that do not usually sustain microbial life (synthethic polymers, resins, minerals) by immobilizing dead microalgae in them to remove pollutants. Entrapped dead biomass of the cynobacterium *Phormidium laminosum* in epoxy resin (its two pre-polymer components are toxic for living cells, but together produce an inert gel) accumulated copper, iron, nickel, and zinc from culture medium and the rate of accumulation was directly related to the biomass entrapped (Blanco et al., 1999). Immobilization of dead *S. platensis* in silica gels adsorbed cadmium similar to alginate-entrapped cells (Rangasayatorn et al., 2004). Recently, an immobilization technique in transparent porous silica matrices was developed for *C. vulgaris* to avoid generation of by-products that are detrimental to the microalgae when entrapped in silica. So far, it has not been used for wastewater treatment (Hanh and Canh, 2007).

Not all immobilizations yield positive results. Immobilization of the hydrocarbon-rich microalga *Botryococcus braunii* in 11 polyurethane pre-polymers was highly toxic to this microalga in several foams of polyurethane. Nonetheless, large portions of microalgal population survive immobilization. However, prolonged incubation revealed long-term toxicity. Photosynthetesis and hydrocarbon production by the entrapped microorganisms were drastically reduced relative to the free controls (Bailliez et al., 1998).

7.2. Avoiding gel disruption and grazing by phytoplankton

Contaminants and local microorganisms in wastewater may interact with the matrix and cause its disruption. The susceptibility of alginate matrices to cation chelating agents and to anti-gelling cations, which can cause bead disruption or dissolution, is a major limitation for *in situ* exposure in polluted marine systems or wastewater treatment ponds. For example; the green microalga *Selenastrum capricornutum* was immobilized in alginate beads to avoid being grazed by zooplankton in a small stream; however, the alginate matrix was markedly degraded by microorganisms when incubated in polluted streams for more than 2 weeks (Faafeng et al., 1994). A solution to this problem was recently suggested; different concentrations of alginate, different hardening cations and different sources of alginate isolated from macroalgae were evaluated. Beads prepared with 4.9% of the macroalgae *Laminaria hyperborea* alginate and a 4% strontium solution as the hardening cation were found to be the most stable and the most suitable for growing *Phaeodactylem tricornutum* in *in situ* marine systems (Moreira et al., 2006a,b).

7.3. Other systems of immobilization

Several methods of immobilization were proposed and reviewed, mostly in the 1990s (Chen, 2001; Mallick, 2002), but no notable development has occurred in this niche in recent times. The twin-layer system has been used as an alternative to immobilization within a gel bead. In the twin-layer system, microalgae are immobilized by adhesion on a wet, microporous, ultrathin substrate (substrate layer). Subtending the substrate layer is a second layer consisting of a macroporous fibrous tissue (source layer) that provides the growth medium. Twin-layers effectively separate microalgae from the bulk of their growth medium, yet allow diffusion of nutrients. In this system, microalgae remain fully immobilized, which compares favorably with gel entrapment methods. *C. vulgaris* and *Scenedesmus rubescens* entrapped in this system removed nitrogen and phosphorus from wastewater (Shi et al., 2007).

7.4. Enhancing storage capacity

Management of a microalgal collection is labor intensive, expensive, and time consuming (Moreno-Garrido, 2008). Immobilization was found to be a practical means to reduce the burden. Entrapment of the marine diatom *Haslea ostrearia* (oyster feed) in calcium alginate beads was performed successfully. After storage for almost 2 months, viable and cultivable cells were recovered from beads by dissolving the alginate matrix (Lebeau et al., 1998). This success was recently repeated. To preserve the size and shape of the marine diatom during long-term storage, the cells were immobilized in alginate beads and stored at 4 °C under reduced light for up to 4 months. Immobilization strongly slowed down the decrease in size of the population with time and allowed the storage of concentrated and calibrated inocula that could be inoculated directly into liquid culture medium, without needing to dissolve the beads (Gaudin et al., 2006). The marine microalga *Isochrysis galbana* immobilized in alginate can be used for long-term storage of algal stock and easily applied to clam cultures. The entrapped cells were alive and maintained their physiological activities after 1 year of storage in absolute darkness at 4 °C, without a liquid medium, and even increased its population density. The immobilized culture was applied to feed and control of water quality in clam cultures that led to marked reduction in ammonium concentrations (Chen, 2003). In a recent study, 12 species of the benthic diatom *Haliotis diversicolor* were used as feed in hatcheries for small abalone and entrapped in alginate beads for long-term storage. The immobilized diatoms remained alive and held their capacity to multiply after one year of storage in absolute darkness at 4 °C without liquid media. These diatoms were subsequently applied successfully as feed to cultivate postlarval *H. diversicolor* (Chen, 2007). It is unknown whether any microalgal collection adopted this practice.

7.5. High-density cultivation

Immobilization for culturing microalgae in alginate beads was an effective method for high-density cultivation. *Dunaliella bardawil* and *Haematococcus pluvialis* were immobilized independently in Ca-alginate beads and cultivated in batch culture under aerobic conditions. The thickness of the bead and the supply of CO_2 did not affect the growth of the immobilized *D. bardawil*. The concentration of cells of immobilized microalgae within the bead was five times greater than the microalgae population as free cells (Joo et al., 2001).

7.6. Immobilization (encapsulation) equipment

Most experimental immobilizations are done manually, using large syringes for dripping monomers into solidifying solutions. The main limitation of this simple technology is the slow rate of bead production because it involves dropping the viscous polymer solution (mostly alginate) through a small opening in a large syringe, which is a tiring procedure for lab personnel. An automatic procedure was developed to overcome the basic problem of excessive time and effort to produce beads. This tool produces beads (spheres) in any quantity for experimental use with little effort. It is basically an assembly with a peristaltic pump, tubing, syringe, and sources of monomers and microorganisms (Fig. 3B) (http://bashanfoundation.org/beads/macrobead.html, accessed 23 August 2009). For large scale application in wastewater treatment, machines that produce larger quantities of beads are required, such as the one proposed by Hunik and Tramper (1993) for κ-carrageenan. Several prototypes were proposed for this purpose in the last decade. Earlier prototype equipment for mass production of beads is summarized by Cassidy et al. (1996).

A multi-nozzle encapsulation/immobilization system to produce uniform beads of alginate was developed but not reported, so far, for removal of pollutants (Brandenberger and Widmer, 1998). A completely sterilizable encapsulation device, designed on the basis of the laminar jet break-up technique, was developed for cell immobilization with different types of alginate. The encapsulator has a capacity for continuous production of beads from 250 μm to 1 mm in diameter, with a size distribution below 5%, at a flow rate of 1–15 ml min^{-1}. A modification of the device, to incorporate an electrostatic potential between the alginate droplets and an internal electrode, results in enhanced monodispersity with no adverse effects on cell viability. The maximum cell loading capacity of the beads directly depends on the nozzle diameter, as well as the cells used. For example; for the yeast *Phaffia rhodozyma*, it is possible to generate 700-μm alginate beads with an initial cell concentration of 100×10^6 cells ml^{-1} of alginate, whereas only 1×10^6 cells ml^{-1} could be entrapped within 400-μm beads. The beads remain stable in the presence of acetic acid, hydrochloric acid, water, basic water, and sodium ions. The latter stability applies when the ratio of sodium to calcium ions is less than 1:5. Complexing agents, like sodium citrate, yield rapid solubilization of the beads by removing calcium. The presence of cells does not affect the mechanical resistance of the beads. Finally, the mechanical resistance of alginate beads can be doubled by treatment with 5–10 kDa chitosan, resulting in reduced leaching of cells (Serp et al., 2000). An equipment design for microbead production (100–200 μm) for agricultural and environmental inoculants with bacteria can be used for microalgae immobilization. Microbial suspensions are placed in Erlenmeyer flasks or a beaker that is attached to the microbead-producing device and pressurized at 10–15 psi with a compressor. The suspension was drawn through a partial vacuum from the Erlenmeyer flask and then forced to pass through a capillary tube (222-mm diameter) that creates a fine spray of miniature droplets. The mist is sprayed into a stainless steel pan that is slowly rotating and contains a solidifying agent. Microbeads form instantly as the droplets contact the solidifying solution. After curing for 30 min in the solution, rinsing, and filtering the access solution, the microbeads are ready (Fig. 3C) (Bashan et al., 2002 and http://bashanfoundation.org/bead.html, accessed 23 August 2009). A very simple device for production of large quantities of larger beads (2–4 mm) was also developed. The device is essentially an open showerhead box with an array of 64 specially shaped apertures formed in the bottom plate. Liquid alginate is poured into the box and beads will form under gravity flow. The liquid drips into a receptacle containing a hardening solution. The showerhead satisfies the following set of conditions: no pressurized air is needed; beads form under the pull of gravity; beads are a suitably small diameter; bead diameters are consistent; rate of formation is rapid; apparatus can be autoclaved for sterilization, and adequate amounts of alginate solution can be processed at one time. The device produces 0.5 l of alginate beads in about 5 min. Some larger devices with the same nozzle design can produce larger quantities of beads (Fig. 3A) (de-Bashan et al., 2004).

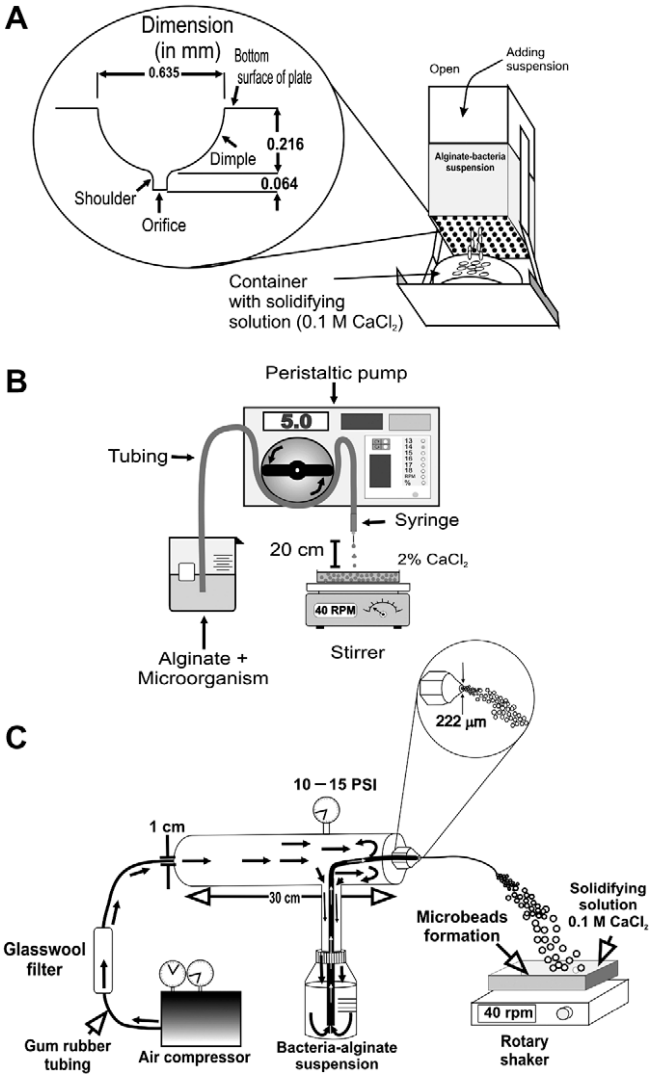

Fig. 3. Equipment for producing (A) large quantities of alginate macrobeads (modification of a device published by de-Bashan et al. in Water Research (2004) 38, 466–474); (B) automated production of laboratory scale macrobeads (with permission of the author); (C) a device for production of alginate microbeads (modification of a devise published by Bashan et al. (2002) in Biology and Fertility of Soils 35, 359–368).

8. Concluding remarks

Although the use of microorganisms in wastewater treatment is commonplace, so far, there is no new technology to emerge from decades of research to *intentionally* use specific microorganisms for removing nutrients. Several proposals, including immobilization of microorganisms in polysaccharide gels, including microalgae and combinations of several microorganisms for simultaneous treatment of the wastewater, have the best potential for future commercial use. So far, in terms of wastewater treatment, it is difficult to differentiate between the role of microalgae and other microorganisms, mostly bacteria. A large number of microbial isolates are available for testing, which blur the boundaries between the two groups. However, crossover technologies that were developed for bacteria, for example, can be extended to microalgae after considering the differences in metabolic function and physiology of the two groups.

There are two main drawbacks for employing microalgae in wastewater schemes. (1) There is limited knowledge of the technical details of biological treatment systems that result in relatively higher cost compared to traditional chemical treatments that are more straightforward and cheaper (i.e., mixing a chemical with the wastewater and collecting the sediment). Considering the amount of wastewater to be treated, any slight increase in cost of operation makes the implementation of new technology a difficult sell to wastewater plants. (2) The relatively low proportion of removal of some contaminants, especially phosphorus, and the longer retention time in the treatment plants (days compared to hours), raise the cost and increase the reluctance of engineers to get involved in systems that, by nature, are not very precise and depend on many unpredictable environmental parameters. All this makes biological treatment of wastewater, especially with microalgae, a niche technology. At best, under current operational procedures, this technology might be an auxiliary technology, to be combined with other biological technologies as alternatives to traditional chemical technologies.

Currently, there are major advantages to 'greener' technologies. The public, even in more affluent developing countries, is constantly demanding green technologies for most aspects of civil life. The common decontamination technologies, wastewater included, that produce more, or a different kind, of secondary pollution (like precipitation of phosphorus in wastewater by metal salts that are disposed of as a toxic waste in landfill) have a negative public perception. With public opinion on its side, it appears that immobilization technology for microalgae is a prime candidate as a green technology.

From a scientific standpoint, this technology has many advantages. (1) Its main advantage is that it controls and protects the dominant and always useful microorganism within the polymer itself. The selected microorganism, with usually superb ability to treat wastewater, can be maintained within the polymer without competition from other microorganisms present in the wastewater. Many excellent isolates for wastewater treatment, especially genetically engineered ones, are not necessarily environmentally competitive when applied directly to the wastewater, resulting in frequent treatment failures when applied in suspension. (2) It is possible to mix in one bioreactor different microalgal immobilization systems using different microorganisms to simultaneously treat several contaminants in the wastewater. This will be useful, especially for recalcitrant compounds that require specialized microalgae for degrading the pollutant. (3) The immobilized microorganism has better plasmid stability within the polymeric matrices, allowing successful use of genetically modified microalage designed for specific cleaning purposes and avoiding the common failure of such genetically modified microorganisms in environmental systems.

From a practical view, microalgal systems use solar energy and need relatively small amounts of other inputs for operation. They are relatively easy to handle on a large scale because they have been used by compound producing industries for a very long time. These systems produce no health hazards, are environmental friendly (promoting the image of the company that uses them), produce no secondary pollution, and their end products can be converted to additional by-products (like fertilizers or biofuel) that may further reduce costs. The compactness of these systems produces less sludge and is smaller and simpler to maintain than large fluidized beds. A comparison between attached growth systems in beds and immobilization technology in polymers for wastewater treatment was summarized by Cohen (2001), and is as valid today, as it was a decade ago. It is assumed that immobilization technology of microalgae will find its greatest usefulness when (1) several different contaminants need to be treated simultaneously; (2) when the contaminants in the wastewater do not change much in daily operations, as is common in domestic wastewater; or (3) when complex degrading processes are needed that require specialized microalgae.

There are still a number of technical aspects of this technology that could be developed, such as improvement of the polymers themselves to create "alloys" of organic polymers by mixing different polymer types to improve diffusion of effluents, development of less stressing immobilization procedures for microalgae, and optimizing selection of the proper polymer for specific applications by understanding the attachment properties of the microoraganisms to the matrix surfaces and the changes in metabolism of the microalgae that it induces. Solving these shortcomings will enhance the future potential of immobilized microalgae in commercial wastewater treatment facilities.

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