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Biotechnological potential of immobilized algae for wastewater N, P and metal removal: A review

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Abstract

This presentation comprises a review on the use of immobilized algae for wastewater nitrogen, phosphorus and metal removal purposes. Details of the use of immobilized algae, the techniques of immobilization and the effects of immobilization on cell function are included. Particularly relevant in their use for heavy metal removal from wastewaters; upon enriching the biomass in metal, can be recoverd, thereby providing economic advantages. The use of immobilized microalgae in these processes is very adequate and offers significant advantages in bioreactors. The future of this area of algal cell biotechnology is considered.

Introduction

William J. Oswald and his 'photosynthetic wastewater treatment' are not new for the environmental scientists who are engaged in wastewater management programme. The cultivation of algae in wastewater offers the combined advantages of treating the wastewaters and simultaneously producing algal biomass, which can further be exploited for protein complements and food additives (for aquaculture, animal and human feed), energy such as biogas and fuels, agriculture (fertilizers and soil conditioners), pharmaceuticals, cosmetics and other valuable chemicals. In comparison to the entirely heterotrophic systems like activated sludge plants, the primary attraction of algal ponds lies in the low-grade technology and in saving of energy, since photosynthetic oxygen production can replace mechanical aeration.

One of the major and practical limitations in algal treatment systems is harvesting or separation of algal biomass from the treated water discharge. An efficient removal of algal biomass is essential for recycling of the wastewater. Numerous efforts have been devoted to develop a suitable technology for harvesting microalgae ranging from simple sand filtration to energy-intensive centrifugation (Richmond & Becker 1986; Mohn 1988; Oswald 1988). Autoflocculation, i.e., self-aggregation by stopping aeration followed by decantation, particularly for cyanobacteria, has also been practised. In this context, immobilization of algal cells for wastewater treatment has been proposed for circumventing the harvest problem as well as retaining the high-value algal biomass for further processing (de la Noüe & de Pauw 1988). Application of immobilization technology to algal wastewater treatment provides more flexibility in the reactor design when compared with conventional suspension systems. Moreover, accelerated reaction rates due to increased cell density, increased cell wall permeability, no washout of cells and better operational stability are the additional advantages of immobilized cells over their free-living counterparts (Brouers *et al.* 1989).

An immobilized cell is defined as a cell that by natural or artificial means is prevented from moving independently of its neighbours to all parts of the aqueous phase of the system under study (Tampion & Tampion 1987). Conventional algal wastewater treatment systems can be viewed as two-component systems consisting of algae and the wastewater which includes interactions between the algae and the wastewater components such as species selection, effect of algal density, prestarvation of algae, acclimation

Table 1. Basic requirements of an useful immobilized algal system and properties of an ideal matrix for immobilization.

Requirements of an useful immobilized algal system	Properties of an ideal matrix for immobilization
Retention of viability	Non-toxicity
Ability to photosynthesize	Phototransparency
High density of cells	Stability in growth medium
Continued productivity	Retention of biomass
Low leakage of cells from matrix	Resistance to disruption by cell growth

and also the nature of wastewater. In addition, it also includes the effect of physical factors such as light intensity, temperature, pH, etc. The incorporation of immobilization technology into algal wastewater treatment systems, however, introduces a third component, the gel matrix, to the systems. The interaction between gel matrix and algae, i.e. how the immobilization affects the algal cells, includes morphological changes, growth characteristics, and the metabolic activities of algae. Table 1 summarizes the principal requirements of a useful and efficient immobilized algal system and the properties of an ideal matrix for immobilization.

Immobilization techniques

In principle, six different types of immobilization methods can be distinguished i.e covalent coupling, affinity immobilization, adsorption, confinement in liquid-liquid emulsion, capture behind semipermeable membrane and entrapment. But one should bear in mind that the following sub grouping of methods is by no means absolute, since in some cases it may be difficult to classify a certain method as belonging to a specific group.

Covalent coupling is the most popular method for immobilizing enzymes, but in the case of cells rather few systems using this technique have been reported (Mattiasson 1983). The main disadvantage of this process is that the living cells, in principle, are characterized by their ability to divide even in immobilized state. Covalently bound preparations would then be expected to leak. The rather few reports in this area are therefore focused on the use of dead cells, or at least cells that are to be utilized for single-step catalytic conversions only.

Affinity immobilization is based on the principle of affinity chromatography. Affinity immobilization involves no drastic reactions and no real exposure to chemicals except for the adsorbent material. However, in case of cell, the support used for affinity immobilization must contain some structures capable of interacting with structures on the cell surfaces. This method is very mild and thus is especially useful for handling labile structures. In contrast, the third one, i.e., adsorption in principle is a reversible process. This means that the support may be recovered after the catalyst is denatured. This has been successfully adopted in enzyme processes. An important difference between adsorbing enzymes and cells is that the latter are bound via multipoint attachment and therefore adhere much more strongly to the sorbent. This though leads to a more efficient adsorption, but also to a more difficult desorption process.

The fourth principle, i.e., confinement in a liquid-liquid emulsion demonstrates that if aqueous solutions of two different water-soluble polymers are mixed with each other the mixture will often be turbid. When left for a while phase separation will occur. The composition of the phase system as well as the chemical nature of the substances to be partitioned determine to which phase a biological structure will partition. Phase systems partition materials according to their surface properties. Small, soluble uncharged molecules are distributed evenly throughout the system, whereas partitioning of larger particles such as a cell and its organelles often results in an enrichment both at the interface, and also in one of the two bulk phases (Mattiasson & Hahn-Hägerdal 1983).

Cells can also be retained by semipermeable membranes that isolate the organisms from the bulk liquid. The cells can be immobilized into the membrane, a technique frequently used for the fabrication of biosensors. The membrane allows the molecular transport of soluble materials to and from the immobilized cells while confining and protecting the enclosed organisms. Hollow-fibre bioreactors, with the organisms confined to one side of the porous fibre and the soluble

substrate and products on the other side, seem to be the most practically used one. However, growth must be controlled to prevent an excessive build-up of biomass since it could cause pressure that would rupture the membrane (Blanch *et al.* 1984).

Entrapment is by far the most frequently used method in laboratory experiments and there are some examples of industrial process based on entrapped cells. Entrapment methods are based on the confinement of the cells in a three-dimensional gel lattice. The cells are free within their compartments and the pores in the material allow substrates and products to diffuse to and from the cells. Several synthetic (acrylamide, polyurethane, polyvinyl, etc.) and natural polymers (collagen, agar, agarose, cellulose, alginate, carrageenan, etc.) are used for this purpose. However, for algal immobilization the most frequently used natural gels are alginate and carrageenan.

The gel is generally formed into useful biocatalyst beads by first adding the cells as a suspension to an aqueous solution of the gelling material. This material is then formed into droplets by forcing it drop wise through a nozzle or orifice to an interacting salt solution. The droplets are subsequently stabilized into biocatalyst beads with entrapped organisms *via* polymerization or other types of cross-linking. For example, alginate droplets can be stabilized with divalent ions such as Ca^{2+} and carrageenan droplets are typically cross-linked with K^{+}.

Techniques permitting the large-scale production of biocatalyst beads by forced flow of the gelling material through multiple nozzles are being developed and it has also been shown that the imposition of vibrational energy onto the bead formation process will allow the production of monodispersed beads (Hulst *et al.* 1985). Recently, Brandenberger & Widmer (1998) developed a new multinozzle system with 13 nozzles for the encapsulation and immobilization of micro-organisms, enzymes or cells. Figure 1 presents a schematic model of the multinozzle system. Based on the laminar jet break-up, monodispersed beads of calcium alginate are produced under sterile and reproducible conditions in the range of 0.2–1.0 mm. An *in situ* cleaning of the nozzles is implemented in order to guarantee several batch process cycles and a productivity of up to 5 l h^{-1}. Beads were analysed and showed that the relative difference of the mean diameter of different nozzles was less than 0.3%, thus suggesting its potential applications in the field of food technology, pharmaceuticals and biotechnology.

Immobilized algae

The first report involving the study on immobilized algae was published in 1966 (Park *et al.* 1966), where they used chemically fixed *Chlorella* cells for the measurement of the Hill reaction. Subsequently Hillier & Park (1969) also demonstrated that glutaraldehyde-immobilized *Anacystis nidulans, Porphyridium cruentum* and *Chlorella pyrenoidosa* were able to perform light-dependent O_2 production in the presence of suitable electron acceptors. However, Prof. de la Noüe and his co-workers, University of Laval, Quebec, Canada, could be considered as the pioneers in introducing algal immobilization technology into wastewater nitrogen and phosphorus removal studies. Indeed, since 1990, 50% of all reports dealing with immobilized algae involve their use in wastewater treatment. Several reports are also available on the use of immoblized algae for metal removal, particularly for the recovery of precious metals. However, before going on to elaborate the use of immobilized cells for the removal of N and P or wastewater treatment I would like to discuss briefly the effect of immobilization on algal physiology and biochemistry.

Effects of immobilization on algae

Growth and morphology

Though growth rates of immobilized cells are generally found to be lower than those of the corresponding free cell cultures (Bailliez *et al.* 1985; Robinson *et al.* 1985), a corresponding study conducted by Chevalier & de la Noüe (1985a) demonstrated an opposite trend. Figure 2 shows two typical curves for *Scenedesmus obliquus* in the free and immobilized states. The lag phase was longer for immobilized algae but thereafter the curve was similar to that of the free cells. The maximum growth rate (μmax) observed during the exponential phase was essentially the same for immobilized and free algal cells. Similar observation was also recorded for *Chlorella vulgaris* immobilized in alginate and carrageenan (Lau *et al.* 1998). Rai & Mallick (1992), however, reported a higher final yield for alginate-immobilized *Anabaena* and *Chlorella* after 15 days in the growth medium as compared to their free-living counterparts.

Studies using microscopes tend to show that immobilization has little effect upon the morphology of the algal cells (Musgrave *et al.* 1983). Immobilized

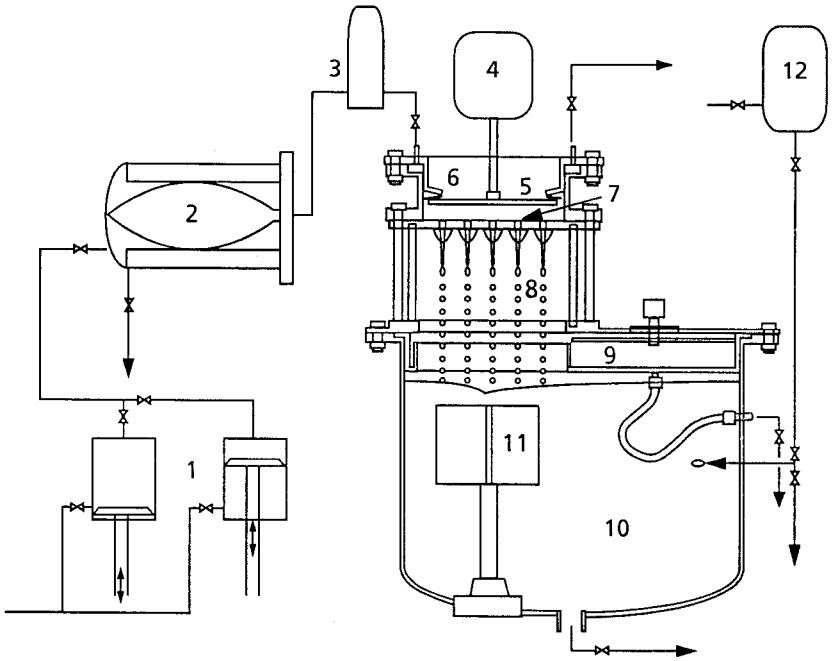

Fig. 1. Schematic representation of the multinozzle system (1: double piston pump; 2: sterile barrier; 3: damper; 4: vibrator; 5: membrane of pulsation chamber; 6: concentric split; 7: pulsation chamber; 8: nozzle plate; 9: bypass system; 10: reaction vessel; 11: stirrer and 12: input hardening solution). Source: Brandenberger & Widmer (1998).

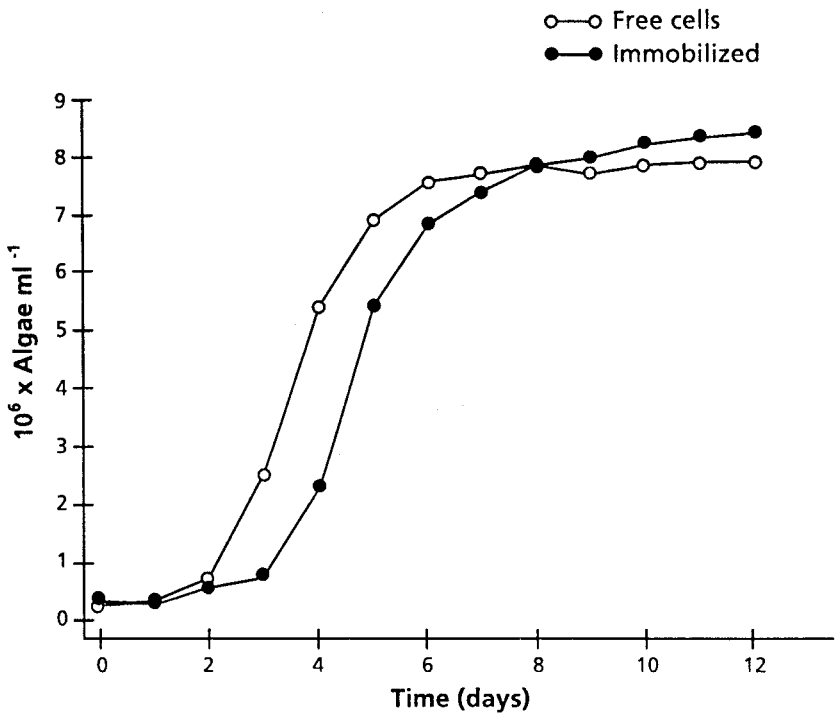

Fig. 2. Growth behaviour of *Scenedesmus obliquus* under free and immobilized condition (Chevalier & de la Noue, 1985a).

colonies of *Botryococcus* however, were found to be more regular in shape and to have mean diameters 2.5 times greater than those of free cell cultures (Bailliez *et al.* 1985). Also, calcium-alginate-entrapped *Chlorella*, usually a unicellular organism, tend to form small colonies (8–30 cells) when released from the immobilized gel (Trevan & Mak 1988).

Metabolism and productivity

Studies on photosynthetic oxygen evolution by immobilized algae have directly compared the activities of immobilized and free cells (Jeanfils & Collard 1983; Robinson *et al.* 1986; Bailliez *et al.* 1986). In *Chlorella* no difference in oxygen evolution between free and immobilized cells was observed under a range of light intensities (Robinson *et al.* 1985). In an another study Bailliez *et al.* (1988) found that oxygen evolution was greater in the immobilized state, suggesting a fundamental change of metabolism. Immobilization enhances the stability of protein-chlorophyll complexes in *Euglena* (Tamponnet *et al.* 1985) and *Botryococcus* (Bailliez *et al.* 1986). In the former case, the immobilized cells retain 90% of their chlorophyll after 3 months of incubation, whereas free cells displayed pheophytinization only after 7 days. There have been few studies on the respiratory rate of immobilized algae. Robinson *et al.* (1985) found the mean respiratory rate of entrapped *Chlorella* was lower than that of free cells. It was reasoned that only a proportion of the immobilized cells in the beads were metabolically active.

It is unclear whether immobilization has any general effect on algal cell productivity. Enhanced hydrocarbon production by *Botryococcus* was reported by Bailliez *et al.* (1985). Likewise hydrogen evolution from the filamentous alga *Anabaena* was reported to increase threefold following immobilization (Kayano *et al.* 1981; Brouers & Hall 1986). Ammonia production by *Mastigocladus laminosus* was greatly increased when the cells were immobilized in polyvinyl blocks (Brouers & Hall 1986). Santos-Rosa *et al.* (1989) also demonstrated that *Chlamydomonas reinhardtii* cells immobilized in Ba-alginate provide a stable and effective system for photoproducing ammonia. Glycolate production in alginate-entrapped cells was shown to be doubled than that in free-living cells (Vilchez *et al.* 1991). Leon & Galvan (1995) studied the production of glycerol in *Chlamydomonas reinhardtii* cells immobilized in Ca-alginate. The immobilized cells registered a production rate of 7 g l^{-1} in comparison to 4 g l^{-1} by their free-living counterparts. Other studies demonstrated a decrease in productivity with immobilization. For example, polysaccharide production by *Porphyridium* was reduced by 65% and keto-acid production by *Anacystis* and *Chlorella* was reduced by 70–90% (Wilkstrom *et al.* 1982) following immobilization.

Removal of N and P

Since the report of Chevalier & de la Noüe (1985a) on carrageenan-immobilized *Scenedesmus obliquus* for the tertiary treatment of wastewater, studies on the application of immobilized microalgae for wastewater nutrient removal have begun. Although their study demonstrated a similar uptake rate in case of PO_4^{3-}-P both for the free and entrapped cells, the curves for NH_4^+-N removal showed a higher uptake rate for the latter. In a parallel study these workers analysed the efficiency of immobilized *S. quadricauda* for the removal of N and P from wastewater in three repeated cycles (Chevalier & de la Noüe 1985b). In general, removal of phosphate was found to be a much slower process than that observed for nitrogen. Moreover, a gradual decline in efficiency from the first to subsequent cycles was also observed. Table 2 presents a detail report on the removal of nitrogen and phosphorus by immobilized algal cells. In 1986, Jeanfils & Thomas with alginate-immobilized *Scenedesmus obliquus* demonstrated that nitrite uptake efficiency was not affected by immobilization, except that a longer lag phase was observed in immobilized cells than the free ones. Megharaj *et al.* (1992) reported that alginate-immobilized *Chlorella vulgaris* was more efficient in removing both the nutrients (N and P) from wastewater than *Scenedesmus bijugatus*. Exponentially growing cells of both the algae effected a greater uptake of phosphorus, whereas the age of the cultures was found to have no direct impact on the removal of nitrogen. Batch culture studies of phosphate uptake by free cells and alginate-entrapped *Chlorella emersonii* have shown that exponentially growing free cells remove phosphate from the medium five times as rapidly as cells in the late stationary phase. When cells of different ages are immobilized in Ca-alginate and packed in a small-scale packed-bed reactor the effects of culture age are sufficient to produced significant differences in reactor performance (Robinson 1995).

Rai & Mallick (1992) demonstrated a higher uptake rate for both N and P by immobilized *Chlorella*

Table 2. Summary of literature on nitrogen and phosphorus removal by immobilized algae.

Algal genera	Type of treatment	Immobilizing matrix	Reference
Scenedesmus obliquus	batch	carrageenan	Chevalier & de la Noüe (1985a)
Scenedesmus quadricauda & S. acutus	batch & repeated batch	carrageenan	Chevalier & de la Noüe (1985b)
Senedesmus obliquus	batch	alginate	**Jeanfils and Thomas (1986)
Phormidium sp.	batch & semicontinuous	chitosan	de la Noüe & Proulx (1988a,b)
Chlorella emersonii	batch	alginate	*Robinson *et al.* (1988)
Chlorella emersonii	batch & PBR	alginate	*Robinson *et al.* (1989)
Phomidium laminosum	PBR	polyurethane foam	Garbisu *et al.* (1991)
Phomidium laminosum	batch	polyvinyl foam	Garbisu *et al.* (1992)
Chlorella vulgaris & Scenedesmus bijugatus	PBR	alginate	Megharaj *et al.* (1992)
Anabaena doliolum & Chlorella vulgaris	batch & repeated batch	alginate	Rai & Mallick (1992)
Chlorella vulgaris & Chorella kessleri	FBR	alginate	Travieso *et al.* (1992)
Anabaena doliolum & Chlorella vulgaris	batch	alginate	Mallick & Rai (1993)
Phormidium laminosum	PBR & FBR	polyvinyl foam	*Garbisu *et al.* (1993)
Phormidium uncinatum	PBR	polyvinyl foam	**Gil & Serra (1993)
Spirulina maxima	FBR	carrageenan	Canizares *et al.* (1993, 1994)
Anabaena doliolum & Chlorella vulgaris	batch	alginate, carrageenan, agar & chitosan	Mallick & Rai (1994)
Chlorella vulgaris	FBR	alginate	Tam *et al.* (1994)
Chlorella emersonii	PBR	alginate & agarose	*Robinson & Wilkinson (1994)
Chlamydomonas reinhardtii	batch	alginate	**Vilchez & Vega (1994)
Chlorella vulgaris & Scenedesmus quadricauda	PBR	alginate & polyurethane	Cordoba *et al.*(1995)
Chlamydomonas reinhardtii	batch & ALR	alginate	**Vilchez and Vega (1995)
Anabaena CH_3	batch & semicontinuous	alginate	**Lee *et al.* (1995)
Scenedesmus obliquus	batch & PBR	polyurethane & polyvinyl foam	**Urrutia *et al.* (1995)
Scenedesmus bicellularis	repeated batch	alginate	Kaya *et al.* (1995)
Scenedesmus bicellularis	repeated batch	alginate	Kaya & Picard (1995)
Chlorella emersonii	batch & PBR	alginate	*Robinson (1995)
Scenedesmus bicellularis	repeated batch	alginate	Kaya *et al.* (1996)
Scenedesmus bicellularis	repeated batch	chitosan	Kaya & Picard (1996)
Chlamydomonas reinhardtii	batch & FBR	alginate	Garbayo *et al.* (1996)
Chlorella vulgaris *Chlorella kessleri* *Scenedesmus qudricauda*	PBR	carrageenan, alginate, polyurethane & polystyrene	Travieso *et al.* (1996)
Chlorella vulgaris	batch	carrageenan & alginate	Lau *et al.* (1997)
Phormidium laminosum	HFR	cellulose	Sawayama *et al.* (1998)
Chlorella vulgaris	batch	carrageenan	Lau *et al.* (1998)
Chlorella emersonii	batch & PBR	alginate	Robinson (1998)
Dunaliella salina	batch	alginate	Thakur & Kumar (1999)
Chorella vulgaris	PBR	alginate	Tam & Wong (2000)

**studies specially for nitrogen, *studies specially for phosphorus and the data without any star show studies for both nitrogen and phosphorus removal.

and *Anabaena* than their free-living counterparts. Similar results were also observed in case of immobilized *Spirulina maxima* grown in swine waste. In subsequent studies Mallick & Rai (1993, 1994) also observed that immobilized algae with a cell density of 0.1 g dry wt l^{-1} was the most efficient for nutrient and metal removal in a pH range of 6.0 to 8.0, and chitosan could be a promising algal support for wastewater detoxification. Vilchez & Vega (1994) reported that alginate-entrapped *Chlamydomonas reinhardtii* cells provide a stable and functional system for removing nitrogenous contaminants from wastewater. These workers also studied the nitrite uptake from wastewater as an initial step required to establish optimal conditions for the potential use of the system in bioreactors. A group of parameters such as matrix concentration, cell loading, temperature and pH were also considered in order to determine the best working conditions for the immobilized cells. In the case of *C. reinhardtii* cells an alginate concentration of 3% is adequate for minimizing substrate diffusion problems, thereby allowing the beads to attain a physical consistency. Their results showed that when an initial cell loading of about 30–40 μg chlorophyll g^{-1} gel was used for immobilization of *Chlamydomonas reinhardtii* the resulting cell beads showed an optimum nitrite uptake rate of 14 μmol nitrite mg^{-1} Chl. h^{-1} at 30 °C and a pH of 7.5. Removal of nitrate

from wastewater by foam-immobilized *Phormidium laminosum* in batch and continuous flow reactors has been reported by Garbisu *et al.* (1991, 1992). The system was strictly dependent on the availability of light and CO_2, and is of a potential value for biological nitrogen removal because the cells showed high photosynthetic activity after 50 days in the reactor and the nitrate uptake rate was maintained for two months. Nitrogen starvation greatly increased the N uptake rate of immobilized *Scenedesmus obliquus* (Urrutia *et al.* 1995).

In 1995 Kaya & Picard developed a novel immobilized algal system for wastewater biotreatment. In this new process an unicellular green microalga *Scenedesmus bicellularis* was isolated from the secondary decantation tank and grown in a synthetic medium for 12 days. The cells were then concentrated by centrifugation and immobilized on alginate screens. The screens were inserted into a photochamber saturated at 100% relative humidity and a photoperiod of 16 h with an illumination of 150 μE m^{-2} s^{-1} (Figure 3). After 48 h of nutrient starvation, the immobilized cells were used for the removal of ammonium and orthophosphate from a synthetic secondary wastewater effluent in a flexiglass reactor. In a subsequent study Kaya *et al.* (1996) demonstrated that intermittent CO_2 enrichment during nutrient deprivation accelerates tertiary wastewater treatment. Kaya & Picard (1996) conducted an experiment with immobilized *Scenedesmus bicellularis* using high and low viscosity chitosan and konjac flour to enhance the stability of hardened gels during tertiary treatment of wastewaters containing high concentration of phosphate (1M). The disruption due to chemical interactions of pure high and low vicosity chitosan or mixed gels with konjac flour was examined daily by microscopic counting of the total cells released in the medium during seven weeks. A removal of ammonium and phosphate was monitored during 4 nutrient starvation removal cycles. A complete cycle consisted of starving immobilized cells for 48 h in air saturated at 100% relative humidity, followed by removal of nutrient from the wastewater. The immobilization obtained from 2% (m/v) high viscosity chitosan gels showed a more significant chemical stability in medium with 1 M Na_3PO_4 that contained viable cells than low viscosity or mixed gels. Sodium pyrophosphate was the best chelating agent used for cross-linking and did not affect the diffusion of inorganic nutrients through the hardened gels or the micro algal growth inside the network of chitosan immobilized cells. During the first and second uptakes, immobilized *Scenedesmus bicellularis* cells removed more than 95% of NH_4^+-N. After starting the third uptake, a significant decrease in ammonium was observed. In contrast, the removal of phosphate was significantly affected by the phosphorus present in the chelating agents. However, after the third starvation-incubation cycles, a rapid exhaustion of phosphorus was observed with a short retention time, i.e., the removal of 97% was obtained for a 75 min incubation time. It is important to note here that after more than one month incubation of chitosan gel beads containing *Scenedesmus bicellularis* cells that had been soaked daily in a fresh 1M phosphate solution, the stability of hardened gels was not affected and no significant released of entrapped cells was observed in the medium.

The removal of nitrate and phosphate from wastewater with the help of the thermophilic cyanobacterium, *Phormidium laminosum*, immobilized on cellulose hollow fibres in a tubular photobioreactor at 43 °C was studied by continuously supplying dilute growth medium for 7 days and then secondarily treated sewage for 12 days (Sawayama *et al.* 1998). The removal of nitrogenous and phosphate ions from secondary-treated-sewage were 0.24 and 0.11 mmol day^{-1} l^{-1}, respectively under the same condition. Thakur & Kumar (1999) also demonstrated a high N and P removal efficiency of the salt-tolerant microalga *Dunaliella salina* immobilized in Ca-alginate beads. Recent study of Tam & Wong (2000) with Ca-alginate-immobilized *Chlorella* cells packed in a transparent PVC column also showed a significantly higher nitrate removal rate.

Metal removal

Besides N and P another important contaminant in wastewater is metal. Nowadays the most commonly used processes for metal removal are addition of chemicals for metal precipitation or exchange of resins to bind them. Other less frequently used methods include activated carbon adsorption, electrodialysis and reverse osmosis. One of the main interests for microalgae in biotechnology is focused on their use for heavy metal and radionuclide removal from effluents and wastewater. In parallel to detoxification, it is also possible to recover valuable elements such as gold and silver after appropriate treatment of the loaded algal biomass (Brierley *et al.* 1986).

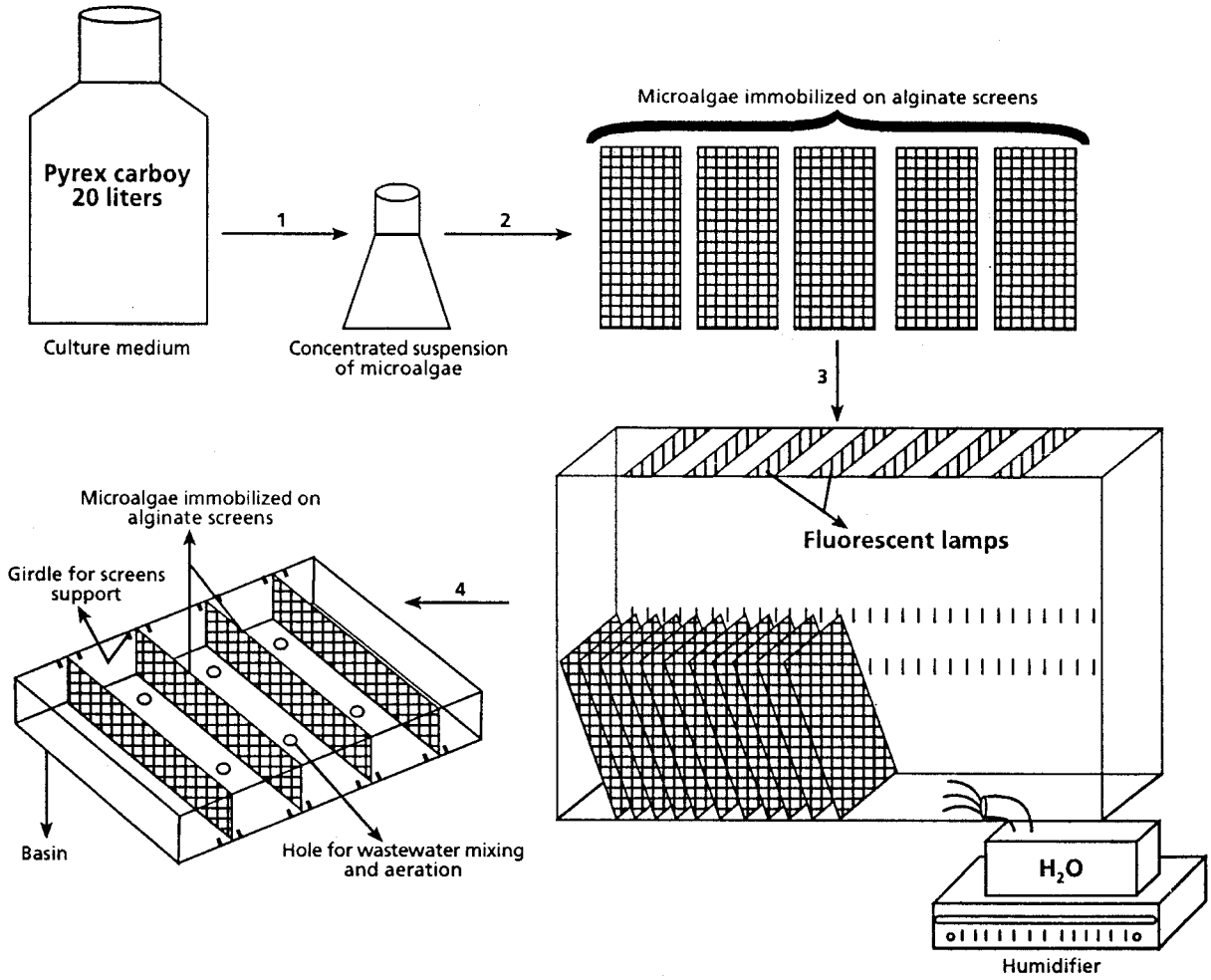

Fig. 3. Schematic description of wastewater treatment by microalgae immobilized on screens and starved in air, saturated at 100% relative humidity (1: centrifugation, 2: immobilization, 3: starvation in air saturated at 100% relative humidity and 4: incubation procedure in wastewater). Source : Kaya & Picard (1995).

Accumulation of mercury by free and immobilized algal systems (*Chlorella emersonii*) was studied by Wilkinson *et al.* (1990). About 90% recovery was recorded after 12 days (initial concentration of mercury, 1 mg l^{-1} with an initial cell loading of 10^6 cells $bead^{-1}$) and the immobilized cell systems was found to accumulate more mercury than free cell systems. Based on this property of algae, Darnall and his colleagues from New Mexico State University patented one biosorption column, known as 'AlgaSORB' (Darnall 1991). In this process algae are packed in a column-shaped matrix of solid silica gel. The process of packing the algae in the solid matrix kills the microorganisms. Nevertheless, their cell walls still provide a plentiful source of binding sites, which can hook heavy metal ions from the solution. The nature of these binding sites is the key to the novelty and potential of 'AlgaSORB'.

Chlorella homosphaera cells immobilized in alginate supply a good system to remove Cd, Zn and Au from wastewater (da Costa & Leite 1991). Significant accumulation of Co, Zn and Mn was also recorded for *Cholrella salina* cells immobilized in alginate (Granham *et al.* 1992). Avery *et al.* (1993) reported that these cells may remove 70% of Cs from liquid medium after 15 h of continuous process in which the initial metal concentration was 6.5 mg l^{-1}. Studies conducted by Rai & Mallick (1992) and Mallick & Rai (1993, 1994) also demonstrated a greater potential of immobilized *Chlorella* and *Anabaena* in accumulating heavy metal ions, such as Cu, Ni and Fe.

The removal of mercury from aqueous solution by a packed-bed reactor (PBR) containing *Chorella emersonii* entrapped in alginate and agarose gels was studied (Robinson & Wilkinson 1994). Reactors were constructed from chromatography columns packed with 200 gel particles of 4–6 mm in diameter. The effects of variation in cell stocking density, influent mercury concentration and flow rate on mercury removal were investigated. Phosphate uptake rate was also evaluated to give an indication of cell viability. It was suggested that levels of mercury volatilization could be reduced by using agarose rather than alginate as the immobilizing matrix. Volesky & Prasetyo (1994), however, used a new biosorbent material derived from the marine brown alga, *Ascophyllum nodosum*, in a packed-bed flow through column. Removal of Cd was found to reach 99.98% from an effluent containing 10 mg Cd l^{-1}. A laboratory scale algal column reactor with the green microalga *Chlorella vulgaris* was packed with 75 ml alginate-algal beads and was used to treat 30 mg l^{-1} Cu and Ni with a hydraulic retention time (HRT) of 30 min (Lau *et al.* 1998). At the end of loading 4 l metal solution, over 97% of Cu and 91% of Ni were removed from the wastewater. Up-flow was preferred to down-flow in maintaining a constant flow rate. The consistency in the metal removal performance by the algal column over 10 treatment and regeneration cycles suggested that agal beads can treat Cu and Ni bearing wastewater over 400 times of its own volume.

Tam *et al.* (1998) studied the efficiency of alginate-immobilized *Chlorella vulgaris* for removing copper from the solution. The amount of copper removal was related to cell densities, more copper was removed with high cell densities. However, increase in cell density led to a lower specific uptake of copper by algal cells, suggesting the amount of algal biomass employed for metal treatment is an important parameter and must be optimized. This study also demonstrated that the immobilized beads had a large capacity to retain copper but at pH lower than 2 adsorbs metals would be disrobed, suggesting the metal saturated beads could be regenerated and reuse. Travieso *et al.* (1999) studied metal removal efficiency of *Scenedesmus acutus* and *Chlorella vulgaris* immobilized in polyurethane foam and k-carrageenan gel in fluidized- and packed-bed reactors. Immobilized cells are found tolerant to Cd, Cr and Zn than free cells, thus implying their great possibility for wastewater treatment processes.

Recently, Rai and coworkers, Banaras Hindu University, Varanasi, India, have gathered interesting information on the metal removal efficiency of the bloom-forming cyanobacterium *Microcystis. Microcystis* is an abundantly occurring nuisance cyanobacterium in many eutrophic ponds and reservoirs of India and other tropical countries. This cyanobactrium occurs in a naturally-immobilized state due to presence of a capsule or slime layer around the cell. The structure of the capsule was studied by Nakagawa *et al.* (1986) and Plude *et al.* (1991). Attempts have been made by Rai and his group to acertain if this cyanobacterium could be used in column packing for large-scale application and, if so, whether it would be affected by environmental variables while alive or dead. Biosorption was found to be influenced by pH and temperature (Pradhan *et al.* 1998). Interestingly, however, heat-killed, formaldehyde-treated, and metabolically-inactive (DCMU-treated) cells had the same biosorption potential as the metabolically-active cells (Parker *et al.* 1998). This suggests that even the dead biomass could be equally useful for metal removal. Application of mathematical models (Langmuir, Freunnlich and BET isotherms) demonstrated a monolayer binding for some metals (Fe and Cu) and multilayer binding for others (Ni and Cd; Singh *et al.* 2000). This group is currently working on the feasibility of packing column with dried dead algal biomass for large scale application of *Microcystis* as an 'Algasorb' for metal removal.

Bioreactor concept

Much of the past research on immobilized cells has concentrated on immobilization techniques and the characterization of immobilizing systems. Since specific applications are becoming the centre of attention, bioreactor concepts have gained increased importance. A survey of the literature showed mainly five types of bioreactors are being examined by various workers. We will briefly discuss below the major advantages and constraints of each system.

Fluidized-bed bioreactors (FBR)

Bioprocessing concepts that require relatively short residence times are effectively used in fluidized-bed systems. In general small biocatalyst particles must be used to ensure fluidization, and they must be sufficiently stable to withstand significant shear forces

for long periods of time. Travieso *et al.* (1992) designed a fluidized-bed reactor with a flexiglass column of 1 l effective capacity with an internal diameter of 5.3 cm. The columns were filled with pellets of immobilized microalgae of 5 mm diameter. The height of the support inside the column was 24 cm and the medium hydraulic retention time was 8 h. The fluidized effect was obtained by up-flow aeration at a rate of 10.8 l/min. Immobilized *Chlorella vulgaris* was found more efficient than *Chlorella kessleri* in the wastewater depuration process. Garbisu *et al.* (1993) studied phosphate removal efficiency of foam-immobilized *Phormidium laminosum* both in batch and continuous flow fluidized-bed bioreactors. In their study three different types of fluidized-beds were used. They were designed as funnel-shaped, column-type beds and beds in Erlenmeyer flasks. The bioreactors were continuously illuminated with cool white fluorescent lamps at an irradiance of 100 μmol photon m^{-2} s^{-1} and were operated at 45 °C by submerging the lower part of the bioreactor in a thermostatically controlled waterbath. Pre-boiled, washed and dried 5 mm foam cubes were placed in algal suspension and immobilization by adsorption was continued over 2 months. Once the foam cubes were fully colonized, they were introduced into the bioreactors. Although cyanobacteria immobilized on the polymer foams did not show high phosphate uptake efficiencies, the simultaneous removal of nitrate and phosphate from wastewater following nitrogen starvation appeared to be a promising possibility. Canizares *et al.* (1993) also studied the behaviour of immobilized *Spirulina maxima* in fluidized-bed conditions for the removal of nutrients from swine wastes. At dilutions of 25 and 50% swine waste, more than 90% ammonium-nitrogen removal was obtained. However, fluidized-bed reactors resulted in a heterogeneous system which is unsuitable for most laboratory standardization.

Packed-bed bioreactors (PBR)

Packed-bed reactors are being studied for immobilized cellular processes more than any other bioreactor configuration. In general, such systems are appropriate when relatively long retention times are required and external biomass build-up is minimal. There have been some innovations in the design and operation of such bioreactor concepts, including the use of horizontal packed bed and multiple column sequences. Robinson *et al.* (1989) designed small-scale packed-bed reactors with Phramacia K9/30 chromatography columns. Columns of 30 cm long with 0.9 cm internal diameter were sterilized with 70% ethanol and rinsed thoroughly in sterile distilled water, and packed with 400 algal (*Chorella emersonii*) beads immobilized in Ca-alginate. These columns were operated at room temperature for the removal of phosphorus. Santos-Rosa *et al.* (1989), Megharaj *et al.* (1992), Garbisu *et al.* (1993), Cordoba *et al.* (1995) and Robinson (1998) also studied the removal of nutrients in packed-bed reactors. Gil & Serra (1993) prepared a lab-scale photobioreactor packed with polyvinyl foam pieces colonized *in situ* by cells of *Phormidium uncinatum*. Under the best working conditions, nearly 90% of nitrate supplied in the influents (50 mg l^{-1}) was removed by the cells having a residence time of 3–4 h. Most recently, Tam & Wong (2000) also studied the removal of nitrate and phosphate in packed-bed reactors made up of PVC columns with five algal bead concentrations ranging from 4 to 20 beads ml^{-1}. A complete removal of NH_4^+-N (initial concentration 30 mg l^{-1}) and around 95% reduction in PO_4^{3-}-P (initial concentration 5.5 mg l^{-1}) was achieved within 24 h of treatment in bioreactors having a bead concentration of 12 beads ml^{-1}. In addition, NH_4^+-N could be lost via ammonia volatilization while PO_4^{3-}-P was removed by precipitation as an alkaline pH was recorded in immobilized microalgal treatment system. However, packed-bed reactors suffer from the difficulties of poor light penetration, mixing and gaseous flux.

Parallel-plate bioreactors (PPR)

Parallel Plate reactors are by definition capacitively coupled, they are either bottom or top powered. Etching in a top powered reactor is referred to as 'plasma mode' etching and etching a bottom-powered reactor it is referred to as 'Reactive Ion Etching'. This is a bit of a misnomer, because both systems are generically 'plasma etchers'. The first fully automated Parallel Plate Reactor was the 'Reinburg' reactor, which was developed at Texas Instruments in 1972. There are many variants of this design, but they are all basically a bottom powered electrode system, large enough to accommodate twenty-five 100-mm wafers. In 1979, Tegal Corporation introduced the first fully automated, single wafer parallel plate system used in production lines. Because of superior process results that single wafer systems offer, almost all production etch systems are of this configuration. Parallel-plate reactors

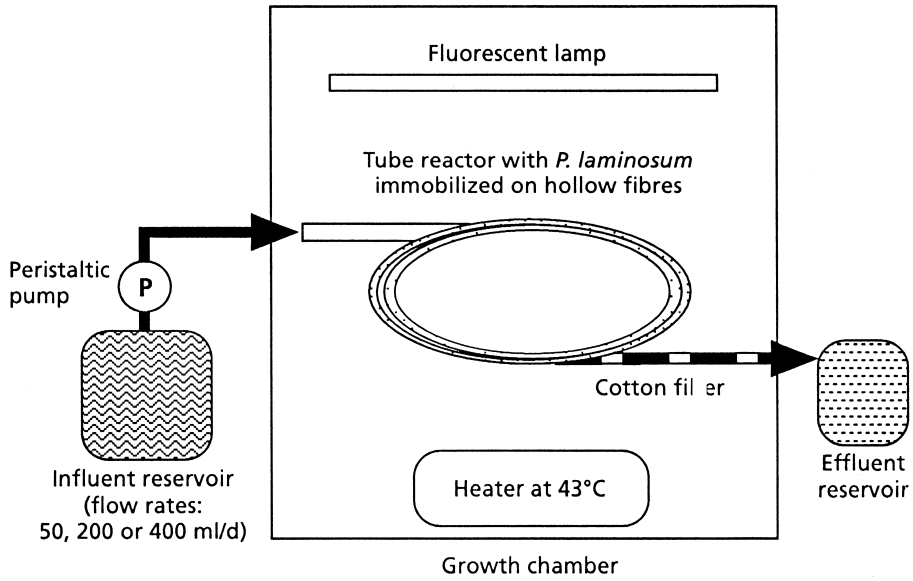

Fig. 4. Schematic diagram of the hollow-fibre photobioreactor. Source: Sawayama *et al.* (1998).

are suitable for the effective utilization of sunlight, but are difficult to construct.

Air-lift bioreactors (ALR)

These are a relatively new type of fermenter offering a homogeneous system, very well suited for laboratory optimization. In this system the content is pneumatically stirred by a stream of air or other gases. Furthermore, this stream also has the important function of promoting the exchange between the gas phase and the medium, and the flow will depend on the geometry of the system. Vilchez & Vega (1995) studied the biological viability and nitrite uptake by *Chlamydomonas reinhardtii* cells entrapped in calcium alginate beads in discontinuous and continuous airlift reactors. In the discontinuous flow reactor system a maximum nitrite uptake rate of 90 μmol h^{-1} was obtained for a cell loading of 90 μg chlorophyll g^{-1} gel after 8 days, whereas the rate was 120 μmol h^{-1} in a continuous flow reactor and could be maintained for at least 21 days. These data suggest that the continuous flow system is preferable to the discontinuous mode. The main disadvantage of this system is that it is unable to provide the high cell stocking densities of packed-bed reactor, which makes it of less value for use in large-scale commercial processes.

Hollow-fibre bioreactor (HFR)

One of the major problems with the use of various gel materials in bioreactor systems is the inherent instability of such structures. A study conducted by Robinson (1998) with small-scale packed-bed reactors showed difficulties in maintaining the integrity of alginate gels beyond a few weeks. In addition, such polysaccharide-based matrices are very much prone to microbial attack when placed in natural environment. Although agarose gel-based reactors showed comparatively more stability than others the gel-based immobilized techniques are not suitable for such large-scale practical operations. Robinson (1998) proposed hollow-fibre reactors to overcome these difficulties.

Hollow-fibre cartridges are commercially available in a variety of sizes. Robinson (1998) prepared the hollow-fibre reactors with 50 cylindrical tubes of polysulfone bundled and sealed in a transparent cartridge of 20 cm in length. Each fibre is of 1.1 mm internal diameter and the fibre wall is perforated by numerous pores. Such a cartridge could be operated with agal cells contained within the lumen of each fibre and the nutrients pumped in through the shell space or *vice versa*. Preliminary results showed that phosphate uptake rate declined within a matter of hours, not due to any lack of activity but as a result of biomass settling within the reactor. However, when cells were suspended in a solution of 1% Na-alginate settlement rates were significantly slow. These reactors attained

a steady-state removal rate quickly which was found to remain constant for several weeks. Such bioreactors are physically stable and can be cleaned and re-stocked with new biomass when the removal rate falls below an acceptable standard.

Sawayama *et al.* (1998) also designed a hollow-fibre photobioreactor with PVC tubing on a laboratory scale with the thermophilic cyanobacterium *Phormidium laminosum*. The photobioreactor comprised a transparent PVC tubing containing cellulose hollow fibres, a peristaltic pump, a heater and two cool white fluorescent lamps (Figure 4). Before inoculation, the tube bioreactor was sterilized with 1% sodium hypochlorite solution and washed with distilled water. Cells were immobilized in hollow fibres and placed in the PVC tubing. Immobilization was accomplished by the addition of cell culture to the reactor using the peristaltic pump. Two days after cell inoculation the secondarily treated sewage was passed through the photobioreactor at 43 °C at a flow rate of 50 ml day^{-1} with continuous fluorescent light of 60 μM photon s^{-1} min^{-1}. Samples were collected after a 24 h period and analyses for N and P were done. The removal rates of N and P ions by this hollow-fibre reactor were 3.36 mg N day^{-1} l−1 reactor and 3.30 mg P day^{-1} l^{-1} reactor respectively as compared to 8.88 mg N day^{-1} l^{-1} and 1.47 mg P day^{-1} l^{-1} in the case of the chitosan-immobilized *Phormidium*. This hollow-fibre immobilization presents a better system for P removal than the chitosan immobilization.

The way ahead

From this review it is quite clear that a combination of solar energy utilization and algal immobilization technologies can be advantageously applied in the development of photoactivated systems for the treatment of wastewater including recovery of precious metals, and production of valuable algal products. However, there is an indication that immobilization affects the cell's behaviour, but many of the observations, particularly with respect to productivity are contradictory. It is therefore, there is a need to increase understanding on the effects of immobilization on algal cell physiology and biochemistry.

The leakage problem is one of the key concerns in cell immobilization since it obviates the primary purpose of delimiting viable cells in a confined matrix. Hallow-fiber cartridges are seems to be good enough to solve this problem. When metal recovery is of economic interest, destructive recovery may be accomplished by treatment of biomass with strong acids. Attention has to be foccussed towards non-destructive desorption from loaded biomass and regeneration of biomass for reuse.

A wastewater purification system using thermophilic cyanobacteria has advantages since contamination can be avoided because of their ability to tolerate high temperature and they can be treated at high temperatures such as 45 °C. Furthermore, investigations on the optimization of operating conditions including temperature, light, CO_2 supply, control of cell leakage, use of the inner space of the hollow fibres, increased life span, capacity and reuse of reactor, and utilization of the cells produced are necessary to make this system a practical reality. In the meantime, it will certainly be wise for human societies to reduce their output of wastes by examining critically the various production/ consumption systems those lead to waste generation.

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References

Avery SV, Codd GA, Gadd GM. 1993 Salt-stimulation of caesium accumulation in the euryhaline green microalga, *Chlorella salina*: Potential relevance to the development of a biological Cs-removal process. *J Gen Microbiol* **139**, 2239–2244.

Bailliez C, Largeau C, Casadevall E. 1985 Growth and hydrocarbon production of *Botryococcus braunii* immobilized in calcium alginate gel. *Appl Microbiol Biotechnol* **23**, 99–105.

Bailliez C, Largeau C, Berkaloff C, Casadevall E. 1986 Immobilization of *Botryococcus braunii* in alginate: influence on chlorophyll content, photosynthetic activity and degeneration during batch cultures. *Appl Microbiol Biotechnol* **23**, 361–366.

Bailliez C, Largeau C, Casadevall E, Yang LW, Berkaloff C. 1988 Photosynthesis, growth and hydrogen production of *Botryococcus braunii* immobilized by entrapment and adsorption in polyurethane foams. *Appl Microbiol Biotechnol* **29**, 141–147.

Blanch HW, Vickroy TB, Wilke CR. 1984 Growth of procaryotic cells in hallow-fiber reactors. *Ann NY Acad Sci* **434**, 373–381.

Brandenberger H, Widmer F. 1998 A new multinozzle encapsulation/immobilization system to produce uniform beads of alginate. *J Biotechnol* **63**, 73–80.

Brierley JA, Brierley CL, Goyak GM. 1986 AMT-BIOCLAIM: a new wastewater treatment and metal recovery technology. In: Lawrences RW, ed. Proc. of the 6th Int. Symp. *Biohydrometallurgy*. 291–304.

Brouers M, Hall DO. 1986 Ammonia and hydrogen production by immobilized cyanobacteria. *J Biotechnol* **3**, 307–321.

Brouers M, Dejong H, Shi DJ, Hall DO. 1989 Immobilized cells: An appraisal of the methods and applications of cell immobilization techniques. In: Cresswell, RC, Rees, TAV and Shah, N, eds. *Algae and Cyanobacterial Biotechnology*. New York: Longman Scientific and Technical Pub; 272–290.

Canizares RO, Dominguez AR, Rivas L, Montes MC, Travieso L, Benitez F.1993 Free and immobilized cultures of *Spirulina maxima* of swine waste treatment. *Biotechnol Lett* **15**, 321–326.

Canizares RO, Rivas L, Montes C, Dominguez AR, Travieso L, Benitez F. 1994 Aerated swine-wastewater treatment with k-carrageenan-immobilized *Spirulina maxima. Bioresource Technol* **47**, 89–91.

Chevalier P, de la Noüe J. 1985a Wastewater nutrient removal with microalgae immobilized in carrageenan. *Enzyme Microb Technol* **7**, 621–624.

Chevalier P, de la Noüe J. 1985b Efficiency of immobilized hyperconcentrated algae for ammonium and orthophosphorus removal from wastewater. *Biotechnol Lett* **7**, 395–400.

Cordoba LT, Hernadez EPS, Weiland P. 1995 Final treatment for cattle manure using immobilized microalgae. I. Study of the support media. *Res Conserv Recycl* **13**, 167–175.

da Costa ACA, Leite SFG. 1991 Metals biosorption by sodium alginate immobilized *Chlorella homosphaera* cells. *Biotechnol Lett* **13**, 559–562.

Darnall DW. 1991 Removal and recovery of heavy metal ions from wastewaters using a new biosorbents; AlgaSORB. In: Freeman HM and Sferra PR, eds. *Innovative Hazardous Waste Treatment Technology Series. Biological Processes 3.* Lancaster, Pennsylvania: Tech. Pub. Co.; 65–72.

de la Noüe J, de Pauw N. 1988 The potential of microalgal biotechnology: A review of production and uses of microalgae. *Biotechnol Adv* **6**, 725–770.

de la Noüe J, Proulx D. 1988a Biological tertiary treatment of urban wastewaters with chitosan-immobilized *Phormidium. Appl Microbiol Biotechnol* **29**, 292–297.

de la Noüe J, Proulx D. 1988b Tertiary treatment of urban wastewaters by chitosan-immobilized *Phormidium* sp. In: Stadler T, Mollion J, Verdus M-C, eds. *Algal Biotechnology*. London: Elsevier; 159–168.

Garbayo I, Braban C, Lobato MV, Vilchez C. 1996 Nitrate uptake by immobilized growing *Chlamydomonas reinhardtii* cells. In: Wijffels RH, Buitelaar RM, Bucke C, Tramper J, eds. *Immobilized Cells: Basics and Applications.* Elsevier Science BV; 410–415.

Garbisu C, Gil JM, Bazin MJ, Hall, DO, Serra L. 1991 Removal of nitrate from water by foam-immobilized *Phormidium laminosum* in batch and continuous-flow bioreactors. *J Appl Phycol* **3**, 221–234.

Garbisu C, Hall DO, Serra JL. 1992 Nitrate and nitrite uptake by free-living and immobilized N-starved cells of *Phormidium laminosum. J Appl Phycol* **4**, 139–148.

Garbisu C, Hall DO, Serra JL. 1993 Removal of phosphate by foam-immobilized *Phormidium laminosum. J Chem Technol Biotechnol* **57**, 181–189.

Gil JM, Serra JL. 1993 Nitrate removal by immobilized cells of *Phormidium uncinatum* in batch culture and a continuous-flow photobioreactor. *Appl Microbiol Biotechnol* **39**, 782–787.

Granham GW, Codd GA, Gadd GM. 1992 Accumulation of cobalt, zinc and manganese by the esturine green microalga *Chlorella salina* immobilized in alginate microbeads. *Environ Sci Tech* **26**, 1764–1770.

Hiller UW, Park RB. 1969 Photosynthetic light reactions in chemically fixed *Anacystis nidulans, Chlorella pyrenoidosa and Phormidium cruentum. Physiol Plant* **44**, 535–539.

Hulst AC, Tramper J, van't Riet K, Weesterbeek JMM. 1985 A new technique for the production of immobilized biocatalyst in large quantities. *Biotechnol Bioeng* **27**, 870–876.

Jeanfils J, Collard F. 1983 Effect of immobilized *S. obliquus* cells in a matrix in oxygen evolution and fluorescence properties. *Eur J Appl Microbiol Biotechnol* **17**, 254–257.

Jeanfils J, Thomas D. 1986 Culture and nitrite uptake in immobilized *Scenedesmus obliquus. Appl Microbiol Biotechnol* **24**, 417–422.

Kaya VM, Picard G. 1995 The viability of *Scenedesmus bicellularis* cells immobilized on alginate screens following nutrient stravation in air at 100% relative humidity. *Biotechnol Bioeng* **46**, 459–464.

Kaya VM, Picard G. 1996 Stability of chitosan gels as entrapment matrix of viable *Scenedesmus bicellularis* cells immobilized on screens for tertiary treatment of wastewater. *Bioresource Technol* **56**, 147–155.

Kaya VM, de la Noüe J, Picard G. 1995 A comparative study of four systems for tertiary wastewater treatment by *Scenedesmus bicellularis*: New technology for immobilization. *J Appl Phycol* **7**, 85-95.

Kaya VM, Goulet J, de la Noüe J, Picard G. 1996 Effect of intermittent CO_2 enrichment during nutrient starvation on tertiary treatment of wastewater by alginate-immobilized *Scenedesmus bicellularis. Enzyme Microb Technol* **18**, 550–554.

Kayno H, Karube I, Matsunaga T, Suzuki S, Nakayama O. 1981 A photochemical fuel cell system using *Anabaena* N-7363. *Eur J Microbiol Biotechnol* **12**, 1–5.

Lau PS, Tam NFY, Wong YS. 1997 Wastewater nutrients (N and P) removal by carrageenan and alginate immobilized *Chlorella vulgaris. Environ Technol* **18**, 945–951.

Lau PS, Tam NFY, Wong YS. 1998 Effect of carrageenan immobilization on the physiological activities of *Chlorella vulgaris. Bioresource Technol* **63**, 115–121.

Lee CM, Lu C, Lu WM, Chen PC. 1995 Removal of nitrogenous compounds from wastewaters using immobilized cyanobacteria *Anabaena* CH_3. *Environ Technol* **16**, 701–713.

Leon R, Galvan F. 1995 Glycerol photoproduction by free and calcium-entrapped cells of *Chlamydomonas reinhardtii. J Biotechnol* **42**, 61–67.

Mallick N, Rai LC. 1993 Influence of culture density, pH, organic acids and divalent cations on the removal of nutrients and metals by immobilized *Anabaena doliolum* and *Chlorella vulgaris. World J Microbiol Biotechnol* **9**, 196–201.

Mallick N, Rai LC. 1994 Removal of inorganic ions from wastewater by immobilized microalgae. *World J Microbiol Biotechnol* **10**, 439–443.

Mattiasson B. 1983 *Immobilized Cells and Organells*. Vol. I. Boca Raton: CRC Press; 143 pp.

Mattiasson B, Hahn-Hägerdal B. 1983 Utilization of aqueous two-phase systems for generating soluble immobilized preparations of biocatalyst. In: Mattiasson B, ed. *Immobilized Cells and Organells*. Boca Raton: CRC Press; 121–134.

Megharaj M, Pearson HW, Venkateswarlu K. 1992 Removal of nitrogen and phosphorus by immobilized cells of *Chlorella vulgaris* and *Scenedesmus bijugatus* isolated from soil. *Enzyme Microb Technol* **14**, 656–658.

Mohn FH. 1988 Harvesting of microalgal biomass. In: Borowitzka MA, Borowitzka LJ, eds. *Micro-Algal Biotechnology.* NY: Cambridge University Press; 395–414.

Musgrave SC, Kerby NY, Codd GA, Stewart WDP. 1983 Structural features of calcium alginate entrapped cyanobacteria modified for ammonia production. *Eur J Appl Microbiol Biotechnol* **17**, 133–136.

Nakagawa M, Takamura Y, Yagi O. 1986 Isolation and characterization of slime from a cyanobacterium, *Microcystis aeruginosa* K-3A. *Agric Biol Chem* **1**, 329–337.

Oswald WJ. 1988 Micro-algae and waste-water treatment. In: Borowitzka MA, Borowitzka LJ, eds. *Micro-Algal Biotechnology.* NY: Cambridge University Press; 305–328.

Park RB, Kelly J, Drury S, Sauer K. 1966 The Hill reaction of chloroplasts isolated from glutaryldehyde-fixed spinach leaves. *Proc Natl Acad Sci USA* **55**, 1056–1062.

Parker DL, Rai LC, Mallick N, Rai PK, Kumar HD. 1998 Effect of cellular metabolism and viability on metal ion accumulaltion by cultured biomass from a bloom of the cyanobacterium *Microcystis aeruginosa. Appl Environ Microbiol* **64**, 1545–1547.

Plude. JL, Parker DL, Schommer OJ, Timmerman RJ, Hagstrom SA, Joers JM, Hnasko R. 1991 Chemical charactrization of polysaccharides from the slime layer of the cyanobacterium *Microcystis flosaquae* C3-40. *Appl Environ Microbiol* **57**, 1696–1700.

Pradhan S, Singh S, Rai LC, Parker DL. 1998 Evaluation of metal biosorption efficiency of laboratory-grown *Microcystis* under various environmental conditions. *J Microbiol Biotechnol* **8**, 53–60.

Rai LC, Mallick N. 1992 Removal and assessment of toxicity of Cu and Fe to *Anabaena doliolum* and *Chlorella vulgaris* using free and immobilized cells. *World J Microbiol Biotechnol* **8**, 110–114.

Richmond A, Becker EW. 1986 Technological aspects of mass cultivation – A general outline. In: Richmond A, ed. *Handbook of Microalgal Mass Culture.* Boca Raton: CRC Press Inc.; 245–263.

Robinson PK. 1995 Effect of pre-immobilization conditions on phosphate uptake by immobilized *Chlorella. Biotechnol Lett* **17**, 659–662.

Robinson PK. 1998 Immobilized algal technology for wastewater treatment purposes. In: Wong Y-S, Tam NFY, eds. *Wastewater Treatment with Algae.* Berlin: Springer-Verlag & Landes Bioscience; 1–16.

Robinson PK, Wilkinson SC. 1994 Removal of aqueous mercury and phosphate by gel-entrapped *Chlorella* in packed-bed reactors. *Enzyme Microb Technol* **16**, 802–807.

Robinson PK, Dainty AL, Goulding KH, Simpkins I, Trevan MD. 1985 Physiology of alginate-immobilized *Cholrella. Enzyme Microb Technol* **7**, 212–216.

Robinson PK, Goulding KH, Mak AL, Trevan MD. 1986 Factors affecting the growth characteristics of alginate-entrapped *Chlorella. Enzyme Microb Technol* **8**, 729–733.

Robinson PK, Reeve JO, Goulding KH. 1988 Kinetics of phosphorus uptake by immobilized *Chlorella. Biotechnol Lett* **10**, 17–20.

Robinson PK, Reeve JO, Goulding KH. 1989 Phosphorus uptake kinetics of immobilized *Chlorella* in batch and continuous-flow culture. *Enzyme Microb Technol* **11**, 590–596.

Santos-Rosa F, Galvan F, Vega JM. 1989 Photoproduction of ammonium by *Chlamydomonas reinhardtii* cells immobilized in barium alginate: A reactor feasibility study. *Appl Microbiol Biotechnol* **32**, 285–290.

Sawayama S, Rao KK, Hall DO. 1998 Nitrate and phosphate removal from water by *Phormidium laminosum* immobilized on hallow fibres in a photobioreactor. *Appl Microbiol Biotechnol* **49**, 463–468.

Singh S, Pradhan S, Rai LC. 2000 Metal removal from single and multimetallic systems by different materials as evaluated by differential pulse anodic stripping voltametry. *Process Biochem* **36**, 175–182.

Tam NFY, Wong YS. 2000 Effect of immobilized microalgal bead concentrations on wastewater nutrient removal. *Environ Pollution* **107**, 145–151.

Tam NFY, Lau PS, Wong YS. 1994 Wastewater inorganic N and P removal by immobilized *Chlorella vulgaris. Water Sci Technol* **30**, 369–374.

Tam NFY, Wong YS, Simpson CG. 1998 Removal of copper by free and immobilized microalga, *Chlorella vulgaris*. In: Wong Y-S, Tam NFY, eds. *Wastewater Treatment with Algae.* Berlin: Springer-Verlag & Landes Bioscience; 17–35.

Tampion J, Tampion MD. 1987 *Immobilized Cells: Principles and Applications*. Cambridge UK: Cambridge University Press; 257 pp.

Tamponnet C, Costantino F, Barbontin J-N, Calvayrac R. 1985 Cytological and physiological behaviour of *Euglena gracilis* cells entrapped in calcium alginate gel. *Physiol Plant* **63**, 277–283.

Thakur A, Kumar, HD. 1999 Nitrate, ammonium and phosphate uptake by the immobilized cells of *Dunaliella salina. Bull Environ Contam Toxicol* **62**, 70–78.

Travieso L, Benitez F, Dupeiron R. 1992 Sewage treatment using immobilized microalgae. *Bioresource Technol* **40**, 183–187.

Travieso L, Benitez F, Weiland P, Sanchez E, Dupeyron R, Dominguez AR. 1996 Experiments on immobilization of microalgae for nutrient removal in wastewater treatments. *Bioresource Technol* **55**, 181–186.

Travieso L, Canizares RO, Borja R, Benitez F, Dominuez AR., Dupeyron R, Valiente YV. 1999 Heavy metal removal by microalgae. *Bull Environ Contam Toxicol* **62**, 144–151.

Trevan MD, Mak AL. 1988 Immobilized algae and their potential for use as biocatalysts. *Trends Biotechnol* **6**, 68–73.

Urrutia I, Serra JL, Lama MJ. 1995 Nitrate removal from water by *Senedesmus obliquus* immobilized in polymeric foams. *Enzyme Microb Technol* **17**, 200–205.

Vilchez C, Vega JM. 1994 Nitrate uptake by *Chlamydomonas reinhardtii* cells immobilized in calcium alginate. *Appl Microbiol Biotechnol* **41**, 137–141.

Vilchez C, Vega JM. 1995 Nitrate uptake by immobilized *Chlamydomonas reinhardtii* cells growing in airlift reactors. *Enzyme Microb Technol* **17**, 386–390.

Vilchez C, Galvan F, Vega JM. 1991 Glycolate photoproduction by free and alginate-entrapped cells of *Chlamydomonas reinhardtii. Appl Microbiol Biotechnol* **35**, 716–719.

Volesky B, Prasetyo I. 1994 Cadmium removal in a biosorption column. *Biotechnol Bioeng* **43**, 1010–1015.

Wilkinson SC, Goulding KH, Robinson PK. 1990 Mercury removal by immobilized algae in batch culture sytems. *J Appl Phycol* **2**, 223–230.

Wilkstrom P, Swajcer E, Brodelius P, Nilsson P, Mosbach K. 1982 Formation of α-keto acids from amino acids using immobilized bacteria and algae. *Biotechnol Lett* **4**, 153–158.