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Applications of immobilized algae

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Abstract

Applications of immobilized microalgae in environmental aquatic research have been recently increased. Current uses of immobilized microalgae include removing of undesired substances (nutrients, metals and organic pollutants) from media, culturing for metabolite production and culture collection handling. Toxicity of substances and effluents can be measured by novel immobilization techniques and the use of living microalgae as biosensors in electronic devices designed, demonstrated to be a very promising topic. Recent research pointed out the advantages of mixed algal–bacterial and co-immobilized systems in water treatment plants. Application of immobilized systems in the production of non-contaminant energy (as Hydrogen obtained from algal cultures) is also an important topic to be explored. The future prospects of this area of algal immobilization are considered.

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Introduction

The use of microalgae in biotechnology has been increased in recent years, these organisms being implicated in food, cosmetic, aquaculture and pharmaceutical industries (Borowitzka & Borowitzka, 1988), but small size of single cells implies a problem in the application of biotechnological processes to those organisms. Cell immobilization techniques have been developed in order to solve those problems. Although in the initial stage most of the research work on immobilization dealt with systems designed for the release of products, synthesized by enzymes or multi-enzyme complexes, a more recent development focuses on the immobilization of complete cells or cell agglomerates (Becker, 1995). 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). Frequent uses of immobilized algal cells are the nutrients, metal and organic pollutant removal from aquatic media, culturing for metabolite production, improvement of culture collections handling, measurement of toxicity, obtaining of energy (via Hydrogen or electricity) and co-immobilization system production for different purposes.

Removal of Nutrients

Immobilized algae have been investigated for their potential use for the uptake of nitrogen and phosphorus. The algal cells immobilized in carrageenan and alginate beads had the efficiency to remove nitrogen and phosphorus from wastewater as the free suspended cells (Tam & Wong, 2000). Immobilized *Scenedesmus* was found to be capable of removing 90% of the ammonium within four hours and 100% of phosphate within two hours from a typical effluent (Chevalier & de la Noue, 1985), suggesting possible uses in the tertiary treatment of wastewaters. Travieso et al. (1996) used immobilized *Chlorella vulgaris* for secondary wastewater treatment in a laboratory scale for 6 months.Their conclusion was immobilized cells of *Chlorella vulgaris* could be used for sewage treatment; the down-flow fluidized column appears to be the better reactor for sewage treatment. Sawayama *et al.,* (1998) used *Phormidium laminosum* immobilized on hollow fibres to remove nitrate and phosphate ions from water. Kobbai *et al*.,

(2000) applied the immobilization technique for two green fresh water microalgae *Scenedesmus obliquus* and *Chlorella vulgaris* for wastewater treatment. They reported 86 and 81% phosphorus removal and 100 and 98.4% ammonia removal in the two reactors after 7 days of treatment. Perez *et al.,* (2004) used immobilized *Scenedesmus intermedius* and *Nannochloris sp*. for phosphorus and nitrogen uptake from wastewater.

Removal of Metals

Immobilized algal systems have been tested by many workers for their efficiency in heavy metals removal (Mallik, 2002). Immobilization of biomass also provides protection to cells from metal toxicity (Bozeman et al., 1989). Size of immobilized bead is a crucial factor for use of immobilized biomass in bio-sorption process (Mehta and Gaur, 2005). It is recommended that beads should be in the size range between 0.7 and 1.5 mm, corresponding to the size of commercial resins meant for removing metal ions (Volesky, 2001). Significant uptake of Co, Zn and Mn was also recorded for *Chlorella salina* cells immobilized in alginate (Garnham *et al*., 1992). Travieso *et al*. (2002) designed a bio-alga reactor, which consisted of a pilot scale model that was operated with synthetic wastewater with an initial concentration of 3000 μG/l of cobalt ion. *Scenedesmus obliquus* was immobilized in the reactor, which was operated in a batch mode. They recorded that the maximum removal of cobalt ion of 94.5% was reached after 10 days. The unicellular green microalga, *Chlorella sorokiniana* was immobilized on loofa sponge and successfully used as a new bio-sorption system for the removal of lead (II) ions from aqueous solutions (Akhtar *et al*., 2004).

Moreno-Garrido *et al*. (2005) used the marine microalga *Tetraselmis chui*, to perform a short term heavy metal accumulation experiment. Beads of calcium alginate containing the algal cells were exposed to 820 μG L^{-1} Cu and 870 μG L^{-1} Cd during a 24 hour period and reported that practically all Cu was removed by the beads. Martinez *et al*. (2006) developed a method for mercury speciation in water using columns packed with *Chlorella vulgaris* immobilized on silica gel. This method was applied to the analysis of tap, sea and wastewater samples. Binding of metals to algal surface occurs in living and non-living algae (Greene and Bedell, 1990), cell surface area being a major parameter in the uptake of metals by microalgae (Khoshmanesh *et al*., 1997). Dead cells can be very efficient in accumulating metals (Donmez *et al*., 1999). In addition, non-living biomass can be immobilized in a granular or polymeric matrix and used in conventional ion exchange equipment (Volesky, 1990). The advantages of using inactive or dead biomass over ion exchange resins can include lower cost, improved selectivity for specific metal interest, easy regeneration and in some cases higher capacity (Volesky, 1990). Packed-bed columns containing immobilized cells seem to be very efficient in the removal of metals from aquatic media (Moreno-Garrido *et al.,* 2002). Tan *et al*. (2002) investigated the bio-sorption of copper by inactivated biomass of the brown seaweed *Sargassum baccularia*, immobilized into polyvinyl alcohol gel beads. Noble metals can also be accumulated by algae and selectively eluted from them (Guo *et al.*, 2000). Gee and Dudeney (1987) described the adsorption of gold from a dissolved metal mixture by *C. vulgaris and Spirulina platensis* immobilized in Ca-alginate. Kishore et al.,2006, carried out biosorption studies for removal of chromium using immobilized marine microalgae *Isochrysis galbana* in Ca-alginate.

Removal of organic pollutants

Aksu and Tezer (2005) studied the biosorption of three reactive dyes onto dead biomass of *C. vulgaris*, concluding that dye sorption was highly dependent on pH. Few works describing algal capacity for degrading oil have been published. Chaillan *et al.* (2006) described the appearance of cyanobacterial mats (*Phormidium animale*) in petroleum-polluted site. Radwan *et al.*, 2002 confirmed that the macroalgae covered with biofilm-forming bacteria can be able to degrade oil (Radwan *et al*., 2002). *Chlorella sorokiniana* in aggregation with bacteria was able to successfully remove salicylate from a photobioreactor in an experiment described by Munoz *et al*. (2006). Hydrocarbons, nevertheless, have been reported to be degraded by Ca-alginate immobilized colorless *Prototheca zopfii* (Yamaguchi *et al*., 1999).

Culturing for metabolite production

Microalgae has been used as biocatalysts in biotransformation and de novo biosynthesis (Ignacio 2008). Biotransformation of phenylpropanoid compounds to HPLC-detectable vanilla flavour metabolites by free and Ca-alginate immobilized cells of Haematococcus pluvialis was described by Tripathi *et al*. (2002). Immobilized cells showed higher vanillin accumulation than free cells. Santos-Rosa *et al.* (1989) described transformation of nitrite into ammonium in a medium containing L-methionine-D, L-sulphoximine and the inhibitor of glutamine synthetase,) by the use of a Ba-alginate immobilized mutant strain of *Chlamydomonas reindhartii*. Production of polysaccharides by the unicellular red algae *Porphyridium cruentum* immobilized in polyurethane foams was studied by Thepenier *et al*. (1985). At the beginning of the tests, no oxygen evolution was observed in immobilized cultures, due to high degree of cellular destruction, although after many flushing rinses, survival colonies began to grow again. When culture reaches stationary phase, production of polysaccharides was noticeable.

Culture collection handling

Long-term storage of microalgae culture is a very interesting topic, saving human and economic resources.The physiological activities of Ca alginate entrapped *Scenedesmus quadricauda* cells were maintenaned by Chen (2001), upto three years by storage in darkness at 4ºC without culture medium, with the starving symptom of pyrenoid disappearance.After replacing long-term stored beads in fresh media, the number of immobilized coenobia increased by 40 times in four weeks. Chen, (2003) repeated the experience with *Isochrysis galbana*, achieving good results after one year of storage. Joo *et al*., (2001) immobilized four microalgal species viz., Dunaliella bardawil, Chlorella minutissima, Pavlova lutheri and Haematococcus pluvialis in Ca-alginate beads and achieved higher concentration of encapsulated cells. Nowak *et al*., (2005) designed a system based in a 96-well plate where a membrane filter, which immobilizes algal strains, constitutes the bottom of each well. This allows culturing of several microalgal strains with less effort, time and money.

Toxicity Measurement

Microalgae have been found to be sensitive organisms to different pollutants (Leon *et al*., 2001) in toxicity bioassays, possibly due to their high surface/volume ratio. Bozeman *et al.* (1989), in a pioneering work, compared the toxicity of seven pollutants such as Cd, Cu, Hydrothol, Glyphosate, pentachlorophenol, Paraquat and sodium dodecyl sulphate to free and immobilized cells of the green microalga *Selenastrum capricornutum* , suggesting the possibility of the use of immobilized systems in toxicity experiments. Several studies on toxicity of metals on algae confirmed the deleterious effect of metals to biological macromolecules (Tripathi *et al*., 2004). Toxicity assessment of a number of pesticides to immobilized cultures of the green alga *Selenastrum capricornumtum* was adopted by Abdel-Hamid, (1996). Naessens *et al*. (2000) constructed a new biosensor for the detection of some herbicides viz., diuron, atrazine and simazine) based on kinetic measurements of chlorophyll-a fluorescence in immobilized *Chlorella vulgaris* cells. Slijkerman *et al*. (2005) studied the use of an in situ assay with immobilized *Chlorella vulgaris* as an indicator of effects on ecosystem functioning with regard to primary production. They used the herbicide linuron to induce direct effects on primary producers. Luan *et al*. (2006) studied the removal and degradation of tributyltin (TBT) contamination levels by alginate immobilized *Chlorella vulgaris* beads.Their results suggested that the alginate immobilized alga *C.vulgaris* was able to continuously detoxify TBT into DBTand MBT for six consecutive cycles. Awasthi and Rai (2005) used immobilized *Scenedesmus quadricauda* to assess the toxicity of nickel, zinc and cadmium on nitrate uptake.

Production of energy (electricity or hydrogen)

Bioproduction of hydrogen has demonstrated to be environmental friendly and less energy consumer when compared with thermochemical and electrochemical processes (Kapdan and Kargi, 2006). Some algae are able to produce hydrogen under stress conditions (Melis, 2002) such as the deprivation of sulphur-nutrients in green algae. Lack of sulphur prevents the protein synthesis and algae cannot perform the turnover of specific photosystem-II reaction centre protein. Then, photochemical activity of PSII declines in the absence of sulphur and oxygen is released to the media at lower rates than O_2 consumption. Dante (2005) used C. reindhartii for the same experiment. Co-firing of microalgal biomass with coal is another power-generating procedure. CO_2 produced in power-plant fuel gas can be used to improve microalgal growth. Microalgal biomass obtained in this way is burned again in the same power plant (Kadam, 2002). This could be a suitable way of reducing the high amounts of carbon dioxide produced globally.

Co-immobilized systems

Efforts have been made in the field of co-immobilization (Nagase *et al.*, 2006). De-Bashan et al. (2004) co-immobilized *Chlorella* sp.with a microalgae growth-promoting bacterium (*Azospirillum brasiliense*) in Ca-alginate beads. This bacterium is not able to remove nutrients from wastewaters, but enhances growth of immobilized algae. Co-immobilized biological system removed higher percentages of nutrients from wastewaters (100% of ammonium, 15% nitrate and 36% of phosphorus) when compared with immobilized algal cells without bacteria (75% ammonium, 6% nitrate and 19% phosphorus) (Ignacio, 2008). Munoz and Guieysse (2006) reviewed the interactions between algae and bacteria in processes designed for the treatment of hazardous contaminants. Production of oxygen by algae improves degradation of substances that must be degraded aerobically. Consumption of CO_2 and extracellular matter production by algae can enhance bacterial growth rate, as well as CO_2 and growth promoter substances production by bacteria can enhance microalgal growth.

Future prospects

The algal immobilization is still an open area for various researches in different fields. The technology of immobilized algae proved to be very efficient in remediation, heavy metals removal and toxicity assessment. One of the most promising areas of research is using this technology to reduce environmental pollutions through bio-sorption and biodegradation of many harmful compounds. Immobilization techniques can be used in molecular biology, as plasmid stability in immobilized cells has been reported (Cassidy *et al*., 1996). Risk of unwanted mutations is reduced when cells are immobilized (Codd, 1987).Genetic manipulation of immobilized cyanobacteria (hydrogenase negative gene) could also improve hydrogen generation processes (Das and Veziroglu, 2001). Immobilized microalgae have been recently used as a tool for water quality control in fish culture. The use of microalgae in the design of biosensors is a very recent and interesting topic in biotechnology (Chouteau *et al.*, 2004). Combinations of solar energy and algal immobilization technologies can be successfully used in industrial processes (Mallick, 2002).

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