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# Immobilized Microalgae in Biotechnology

**S. G. Vasilieva, E. S. Lobakova, A. A. Lukyanov, and A. E. Solovchenko**

*Department of Bioengineering, Faculty of Biology, Moscow State University, Moscow, 119234 Russia*

*e-mail: vankat2009@mail.ru*

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**Abstract**—This paper considers immobilization of oxygenic phototrophic microorganisms, cyanobacteria and eukaryotic microalgae, in natural and artificial experimental systems. This review emphasizes that microbial immobilization, for example, as a part of biofilms, is a strategy widespread in nature ensuring the survival of cells. Accordingly, artificially immobilized cells of oxygenic phototrophic microorganisms can be considered as a special group of biomimetic (bioinspired) materials. Particular attention is paid to the influence of different immobilization methods on the physiological state of cells of cyanobacteria and microalgae, their resistance to stresses, and the productivity of immobilized cultures. This review analyzes the advantages and disadvantages of modern immobilization methods and currently used carriers. It also considers the possibility of using immobilized cultures of oxygenic phototrophic microorganisms in various areas of biotechnology, such as the production of biomass and metabolites, biomass harvesting, and purification of water areas and sewage from heavy metals, excess nutrients, and organic pollutants.

*Keywords:* immobilization, microalgae, cyanobacteria, biotechnology, biofilms, review

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## INTRODUCTION

In natural conditions, many oxygenic phototrophic microorganisms, including cyanobacteria and eukaryotic microalgae, exist in the form of communities (associations) with heterotrophic and other microalgae. Morphologically, such associations may be aggregates, clusters, flakes, or granules, that is, attached communities or communities suspended in an aqueous medium [1], in which the cells are enclosed in a matrix of extracellular biopolymers. Therefore, immobilization of microalgal cells can be regarded as a universal form of their existence, in which microorganisms immobilized in a biopolymer matrix function as a consistently acting multicellular organism [1].

Currently, there is intensive development of biotechnology based on immobilized microalgal cultures. Cells attached to the surface and (or) various carriers are widely used to produce biomass and metabolites and for removal of excess nutrients and heavy metals from wastewater [3]. Advantages of immobilized cells compared with cell suspensions include simplification of biomass harvesting and increased cell resistance to unfavorable factors (temperature, acidity, and toxic compounds).

This review is designed to organize data on the immobilization methods, carriers used, effects of immobilization on cellular physiology, as well as advantages and disadvantages of using microalgal immobilized cultures in various biotechnological processes in comparison with free cultures.

### *Natural Biofilms as a Prototype of an Immobilized Culture of Microalgae*

Formation of stable algobacterial associations in nature is due to the fact that microalgae are the centers of formation of sustainable production systems. Their central role is defined by the presence of highly organized surface structures (mucous capsules, covers, and colonial mucus), as well as the ability to release various organic compounds that support the growth and physiological activity of components of the emerging community. In associations, cells of microorganisms integrated into the extracellular matrix have limited mobility and are concentrated in a limited volume, that is, are immobilized [4].

Components of the communities with microalgae have different types of bonds—trophic, spatial, and protective—and the regulation of their stability is based on intercellular communication. Examples of such communities are stromatolites and modern cyanobacterial microbial mats, the most ancient (3.5 billion years) evolutionarily successful form of life [5]. Current literature broadly denotes such associations (communities) of microorganisms by the term biofilms [6].

### *Biofilm Formation as the Main Survival Strategy of Microorganisms under Natural Conditions*

Approximately 99% of all prokaryotes exist in the form of biofilms whose formation is a complex, strictly

regulated biological process. Microorganisms integrated into the biofilm are protected against adverse physical, chemical, and biological factors of the environment: extreme temperatures, dehydration, ultraviolet radiation, nutritional deficiency, and toxic agents, getting the opportunity to exist in relatively constant conditions [7]. Formation of biofilm communities is the basic survival strategy of microorganisms under natural conditions.

Communication between microorganisms in biofilms is carried out through chemical signals that regulate gene expression in microorganisms forming the biofilm, allowing the cells to control their own structure, morphogenesis, and adaptation [8]. The biopolymer matrix is composed mainly of polysaccharides and proteins (up to 85% in total), which form polyanionic hydrogel matrices, as well as minor amounts of nucleic acids and lipids [6].

Biofilms implement the basic principle of the evolutionary development of microorganisms, the principle of cooperative existence [9], when life products of one species serve as nutrients for another species, and microorganisms of the same or different species interact through special signaling systems [10].

An example of natural immobilization is microalgal colonization of the surface of transparent gel-like structures of animals living in the photic zone: hydroids, mollusks, nematodes, etc. *In hospite*, microalgae provide nutrients to animals, participate in the synthesis of protective mucus, chemical defense compounds, mineralization of external coatings, and protective pigmentation of the animal. In turn, animals provide a habitat for microalgae, protection from adverse environmental factors, and, most importantly, deliver microalgal cells to light [11].

It can be assumed that immobilization of microalgae in artificial systems will increase the cell resistance to stresses and provide advantages compared to the use of suspension cultures.

### *Microalgal Immobilization Methods*

Immobilization is the process of attachment of the cells to the carrier or their inclusion into the volume of the latter [12]. Making the cells part of polymer beads allows achieving higher specific concentration of the attached cells compared to immobilization on the surface. In addition, the cells enclosed into the volume of the polymer are protected from contamination by foreign microorganisms [13].

Terms of immobilization and carriers should ensure minimum damage to the cells and inhibit diffusion. Most standard methods of immobilization of microorganisms are potentially useful for microalgae if their cells receive a sufficient amount of light.

### *Immobilization Carriers*

Carriers for immobilization of microorganisms are divided into natural and synthetic. Examples of natural carriers are insoluble materials to which cells adhere in vivo (wood, wool, or minerals). The advantages of natural carriers include hydrophilicity, biocompatibility, and ease of recycling, while shortcomings include low stability and high cost. Carriers for immobilization of microorganisms often consist in substrates of loofah fruits, sphagnum, peat, glass, plastic, wood, natural polysaccharides (agar-agar, cellulose, alginate, carrageenan, chitosan), and synthetic polymers (polyacrylamide, polyurethane, polyvinyl chloride, polypropylene, polysulfone, epoxy resin) [14, 15].

The ideal carrier for microalgal cells should not inhibit their ability to live nor prevent the mass transfer and block the light. The carrier should have high mechanical, chemical, and biological stability and processability. Furthermore, it should be inexpensive, securely hold the cells, and have a high hydrophilicity (without which reactions in an aqueous medium are not possible).

### *Immobilization Methods*

Currently, there are two groups of immobilization methods: passive and active. Passive immobilization is based on the natural ability of microorganisms to attach to solid or gel-like carriers [16]. This is the simplest method of immobilization of microbial cells that does not cause cellular stress and simulates the process of cell attachment in nature.

In contrast, active methods of immobilization do not depend on the natural ability of microalgal cells to attach to any surface and include two main approaches:

(1) Covalent bonding of the cells with the surface of the carrier via cross-linking agents, such as glutaraldehyde.

(2) Inclusion of the microorganism cells into the mass of the carrier, for example, inclusion into alginate beads [16].

*Passive immobilisation*. Natural microalgal cell attachment to solid and gel-like surfaces is caused by chemical (covalent) and physical (ionic, electrostatic, hydrophobic) mechanisms [13]. Immobilization is implemented through the use of synthetic and natural materials, such as processed loofah fruits [17]: they are quite porous, biodegradable, nontoxic, and cheap. Travieso et al. [18] used 1 $cm^3$ polyurethane foam cubes as a carrier for *Scenedesmus quadricauda* cells in wastewater treatment. The same authors proposed a bioreactor design with a rotating drum made of polyurethane foam for biocapture of heavy metals from wastewater. Passive methods of immobilization are also used with such carriers as glass, plastic, and wood,

especially in environmental, ecotoxicological and biotechnological research [19, 21].

*Active immobilization*. In covalent cell binding, both synthetic and natural materials are used, such as chitin or chitosan. However, this type of active immobilization involves the use of toxic bifunctional reagents (dialdehydes, diisocyanates), so it is more suitable for attachment of dead cells [16].

For immobilization in the carrier volume, natural polymers, such as agarose and agaropectin, are often used. The advantages of agar include nontoxicity, low melting point, and the ability to form mechanically strong gels even at low concentrations [22]; thus, the use of cell immobilization in agar became widespread [23].

Microalgal immobilization using calcium alginate pellets is also one of the most widely used methods at present [3]. Growth of microalgal cells as part of pellets is not limited by light intensity [23], and they are not toxic for microalgal cells [24–26], but such carriers are partially destroyed in seawater and sewage water. Carrageenans are also widely used now despite their lesser stability in aqueous media compared to alginates.

### *Effect of Immobilization on Microalgal Cells*

The ability of microalgal cells to attach to the surface of various carriers is largely determined by the age, the state of culture, and the composition of the culture medium. This capability is maximal in the cells in the exponential growth phase, while it is usually decreased in the stationary phase [27].

Immobilized microalgal cells showed a marked increase in the pigment content, as well as changes in the amount and composition of lipids and fatty acids compared to free-living cells [14, 28]. Thus, the chlorophyll content in cells of *Chlorella vulgaris* immobilized in carrageenan gel is two times higher than in suspension culture [28], and cells of *Botryococcus braunii* and *B. protuberan* within alginate beads have a high content of chlorophylls, carotenoids, and lipids compared with free cells [29]. Joint immobilization of microalgae *Chlorella* spp. and bacteria *Azospirillum brasilense* also results in the increased content of pigments and lipids in microalgal cells [30].

Immobilization can be a powerful stressor for microalgal cells, leading to a reduction in the number of living cells. This reduction may be compensated later if the immobilized cells do not lose the ability to divide [14]. It was found that cells of *Skeletonema costaum* and *Heterocapsa* sp. inside alginate beads do not divide, whereas the growth rate of other microalgae is not different from that in the suspension [31].

In some cases, such as in *Chlorella minutissima*, *Pavlova lutheri*, *Haematococcus pluvialis*, and *Dunaliella bardawil*, fixed in 2% carboxymethylcellulose gel, immobilization stimulates the growth of microalgal cultures [32]. Opposite effects are possible due to the toxicity of the polymers and the compounds used for immobilization of cells [33, 34].

As noted above, immobilized cells become more resistant to changes in pH, temperature, and ionic strength of the medium [13]. Thus, immobilization of cells of *Synechococcus* sp. in chitosan increases the resistance of cells to NaOH [35]. There is also an increase in cell resistance to various toxic substances. For example, the toxic effect of Ni and Cr ions on the cells of nitrogen-fixing cyanobacteria *Aulosira fertilissima* is significantly reduced when they are immobilized in alginate beads [36].

Immobilization also affects the metabolic activity of cells: *Dunaliella salina* cells immobilized in agar synthesized more glycerol as compared with free cells [37], and marine diatom *Haslea ostrearia* cells immobilized in agarose gel increased production of marenin used for feeding oysters [38].

There is evidence of significant changes in the shape and increased sizes of immobilized cells [39], trichomes, and colonies [2].

The effect of immobilization on the photosynthetic activity of microalgal cells is ambiguous [39–41] and depends on changes in effective cell illumination. In case of insufficient or excessive illumination of immobilized cells, the photosynthesis rate drops, but the carrier may protect cells from photodamage by dissipating excess light. Photosynthesis can also be limited by the lack of $CO^2$. In this case, it is efficient to carry out joint immobilization of microalgal and heterotrophic microorganisms that supply microalgae with carbon dioxide during respiration [16].

### *Use of Immobilized Microalgae in Biotechnology*

Currently, immobilized microalgal cells are widely used in biotechnology to produce biomass, valuable metabolites, and biohydrogen; to clean water areas and wastewater from heavy metals, biogenic elements, and organic compounds; and also as biosensors to measure the degree of water pollution [2, 4, 31]. One of the challenges of biotechnology using microalgae is to search for optimal ways of biomass harvesting. Currently used approaches (filtration, centrifugation, flocculation) are energy-intensive and time-consuming [42]. The use of immobilized cultures makes it possible to simplify and reduce the cost of biomass harvesting. Other areas of application are covered more in the following sections.

### *Production of Biomass and Valuable Metabolites*

Immobilized microalgal cells, for example, *Porphyridium cruentum*, are used to obtain sulfated polysaccharides [43]. Cells of cyanobacterium *Aphanocapsa* MN-11 immobilized in alginate beads coated with light-scattering optical fiber excrete substantial amounts of sulfated polysaccharides [44]. There is a

description of the preparation of alkaloid codeine from morphine by cyanobacterium *Spirulina platensis* immobilized in alginate [45]. Cells of nitrogen-fixing cyanobacteria *Anabaena azollae* immobilized in polyurethane pellets were cultured in a photobioreactor to obtain $NH_3$. The possibility of using the specified culture immobilized on various carriers as a bio-fertilizer in rice fields has been studied [46].

### *Production of Biohydrogen*

There is a growing interest in renewable energy sources, such as biohydrogen produced by microalgal cells. Some microalgae are capable of releasing hydrogen in light under stress conditions (for example, in the absence of sulfur compounds in the culture medium). Lack of sulfur compounds blocks synthesis of proteins of the photosynthetic apparatus, leading to reduced activity of the second photosystem, hydrogenase synthesis induction, and hydrogen release [47]. Presently, the *Chlamydomonas reindhartii* culture is the most promising and studied candidate for commercial biohydrogen production [48, 49]. In cyanobacteria, most promising is the light-dependent hydrogen production by heterocyst species synthesizing $H_2$ as a by-product of their nitrogenase activity. It is essential that nitrogenase in heterocysts is protected from the inhibitory effect of oxygen [48]. It is shown [50] that *Anabaena* N-7363 cells immobilized in 2% carrageenan gel produce 2.4 times more hydrogen (up to 3.24 mmol per hour per 1 g of dry gel) as compared with free cells. Immobilization of *C. reinhardtii* cells in alginate beads decelerates hydrogenase inactivation by oxygen since the alginate layer limits oxygen supply into the beads. As a result, the cell produces more hydrogen as compared with free cells [48]. In *C. reinhardtii* cells immobilized on glass fibers, the period of active hydrogen production lengthens, while the rate of hydrogen production in free and immobilized cells is the same [49].

### *Removal of Biogenic Elements from Wastewater*

Biological treatment using microalgae seems to be one of the most promising biotechnologies for purification of wastewater (including sewage from farms), which makes it possible to effectively and economically dispose of wastewater with minimal damage to the environment [51]. Cultivation of microalgae in wastewater containing biogenic elements, such as nitrogen and phosphorus, makes it possible to combine purification and biomass production [2]. One of the most promising ways of recycling of microalgal biomass enriched with bio-available forms of nitrogen and phosphorus is the production of fertilizers.

For effective removal of biogenic elements, it is now proposed to use microalgae enclosed in natural and synthetic polymer gels [2] or immobilized on the surface of various polymeric materials [52]. The increasing efficiency of removal of biogenic elements from wastewater using immobilized microalgal cells may be due to not only the increase in their photosynthetic activity but also adsorption of biogenic ions. For example, the matrix of carrageenan adsorbs ammonium cations, while chitosan adsorbs anions (phosphate, nitrate, and nitrite) [27]. It is shown that *C. vulgaris* cells immobilized in alginate beads are able to use up to 80% of ammonium and 70% of phosphorus from wastewater [53]. Immobilized on the polyurethane and polyvinyl carrier, the culture of microalgae *Phormidium laminosum* is also successfully used to remove nitrate from the medium [34]. Cells of cyanobacterium *Phormidium* sp. immobilized on the surface of chitosan (adsorbing 60% of orthophosphate from the cultivation medium within 4–6 h) remove up to 95% of inorganic nitrogen and 87% of phosphates within 24 hours [54].

When using microalgal cells encapsulated in polymer beads, it should be noted that, at low concentrations of biogenic elements, the rate of their supply into the beads reduces greatly. For example, it was demonstrated in [55] that *C. reindhartii* immobilized in calcium alginate do not accumulate nitrates if their concentration in the medium is below 0.14 mmol/L, while the suspension culture of these microalgae removes nitrates completely.

Despite the limited growth of the culture in the beads as compared with the suspension, the metabolic activity of the immobilized cells may be greater, thereby increasing the completeness and the rate of wastewater purification. Thus, within 3 days, more than 95% of ammonium and 99% of phosphate was used by immobilized *C. vulgaris* cells, which is two times higher than that for free cells [28].

The use of thermophilic cultures can improve the effectiveness of bioremediation if the temperature of water under treatment is more than 30°C. Thus, cyanobacterium *Phormidium laminosum* [56] immobilized on porous cellulosic fibers in tube bioreactors efficiently removed biogenic elements at the temperature of 43°C.

### *Biocapture of Heavy Metals*

Currently, the problem of environmental pollution with heavy metals (HM) is becoming increasingly important. Microalgal cells can accumulate many elements, including heavy metals, in high concentrations, so they are widely used for cleaning wastewater from heavy metals [57].

The process of adsorption of metal ions on the surface of microalgal cells involves their binding by the cell wall and/or cytoplasmic membrane, as well as capsule substances and extracellular compounds. The efficiency of biosorption of metals by microalgal cells directly depends on the total cell surface area, therefore immobilization of cultures on the surface of vari-

ous carriers, which makes it possible to concentrate them in a small volume, in most cases leads to a significant increase of biocapture of heavy metals [58]. Currently, heavy metals are biocaptured using mainly microalgal cultures immobilized on natural media (carrageenan, alginates, chitosan, and agarose) and chemical polymers (polyacrylamide, polypropylene, polysulfone, and various copolymers). In [59], seaweed *Sargassum* sp. and *Ulva* sp. were used for immobilization of *Tetraselmis chuii* and *Spirulina maxima* cells.

Dead microalgal cells also very effectively accumulate heavy metal ions. In [33], biomass of cyanobacterium *Phormidium laminosum* immobilized on polysulfone and epoxy resin is used to concentrate ions of Cu, Fe, Ni, and Zn. It is shown that the amount of adsorbed metal increases with the increasing amount of biomass and heavy metal concentration in aqueous media. It is noted that the efficiency of heavy metal biocapture is not reduced after ten cycles of adsorption–desorption. Copper ions are selectively adsorbed by alginates [60]. In [61], polystyrene sulfonate (NaPSS) was added to alginate gel to improve the adsorbing capacity with respect to copper, but the introduction of cyanobacterium *Microcystis* sp. cells to alginates contributed to a significant increase in the amount of adsorbed copper.

A series of papers [17, 62] studied the process of biocapture of Ni, Cd, Cr, and Pb ions from aqueous media by cells of *C. sorokiniana* immobilized on a loofah carrier. The maximum adsorption capacity with respect to Cd and Ni is 192 mg and 71 mg per 1 g of immobilized microalgal biomass, respectively. The greatest amount of lead is adsorbed at pH 5, and the heavy metal adsorption efficiency is 96% after 5 min.

The authors of [63] describe the use of microalgae *Chlamydomonas reinhardtii* immobilized in alginate beads for biocapture of Hg, Cd, and Pb from aqueous media. At pH 5.0–6.0, biosorption of Hg, Cd, and Pb was 89.5, 66.5, and 253.3 mg/g of dry weight, respectively. In [64], biocapture of uranium from samples of salty and fresh water was carried out using microalgae *Chlorella* spp. immobilized in polyacrylamide gel. It was shown that microalgal cells can be used in multiple cycles of adsorption after desorption of metal ions.

Currently, some species of microalgae are used for separation and concentration of Pd, Pt, Pb, Cu, Cd, and Au [31]. The authors of [65] show selective biosorption of palladium and platinum from highly acidic environments (pH < 2) by *C. vulgaris* cells immobilized on anion exchange resin Cellex-T.

## FINDINGS

Thus, the results of numerous experimental works summarized in this report make it possible to conclude that immobilized cultures of microalgae can be applied for commercial cultivation of these organisms. Immobilization simplifies biomass harvesting, contributes to resistance of cultures against stresses, and simplifies development of hardware for cultivation. All of this generally leads to higher productivity of cultures, and to the increase in the efficiency of wastewater treatment in the case of environmental biotechnology. Right now, for the use of immobilized cultures, special biofilm photobioreactors (PBI) are developed, which are oriented for microalgae culturing in order to obtain biomass and valuable metabolites, as well as for wastewater bioremediation. In conclusion, it should be emphasized that the successful use of immobilized microalgae cultures in real technology critically depends on the choice of the carrier and the cell immobilization method.

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