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PROBLEMS AND PROSPECTS

Immobilization of Microbial Cells for Biotechnological Production: Modern Solutions and Promising Technologies

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Abstract—A review of modern works on the creation of biotechnological processes using cells immobilized on different carriers is presented. General material requirements are given for for immobilization mainly performed by absorption and mechanical fixation methods. The results of studies on cell immobilization are considered, and an analysis of the materials and methods used is given. Some potential and active applications of systems with immobilized cells for biotechnological productions are described. A review of possible variants of technological solutions for bioreactors loaded by carriers with immobilized cells is also presented. It was demonstrated that the use of such a load allows constructive bioreactor solutions of significantly more efficient.

Keywords: bioreactor, biotechnology, cell immobilization, nanocellulose, synthetic peptides, enzymatic catalysis

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At present, industrial biotechnology continues to be actively developed. New microorganism strains are being created to obtain new products or more efficient strains of already produced biosynthetic products, and different enzymes are being used. In addition, recombinant strains with enzymes immobilized on the cell wall as cellular catalysts are beginning to be used [1]. Thus, studies in the field of biodiesel production with the use of such strains, for example, are being conducted [2].

To date, the enzymes immobilized on any carrier are used in the industrial processes. This direction has been widely developed, and a number of carriers (for example, alginate, ion-exchange resins, etc.) became the industrial standard [3]. However, the use of similar carriers to immobilize intact cells that perform biosynthesis of the required products or are involved in biosynthesis of the products as cellular catalysts is also topical at present [3, 4]. This problem is more complex, since, in the case of cells, we are talking about the immobilization of a complex, physicochemical system, the interaction of which with the environment is extremely diverse.

In the present work, the main pathways of cell immobilization, immobilization materials, and approaches to the theoretical analysis of immobilization processes are considered.

Cell immobilization can provide the following advantages in the case of the biosynthesis of useful products: the possibility of creating a flow system with multiple circulation of the substrate; the possibility of the permanent removal of reaction products; the possibility of simplifying biomass separation after completion of the process; and advanced possibilities of using gaseous substrates via the hollow fiber system.

The choice of technology for immobilized cell use is primarily determined by the strain, its productivity, and used substrates [5].

When immobilized cells are used as catalysts, their immobilization can also provide the following advantages: the possibility of creating more compact bioreactors (including flow reactors); a possibly permanent supply of reacting substances and the possible removal of products; the possibility of multiple circulation of substances through the reactor.

Currently, immobilized cells are widely used in the production of different alcohols (including ethanol) with use of recombinant *Saccharomyces cerevisiae* strains [6–9]. With the immobilized cells, it is also possible to synthesize different enzymes, such as lipases (*Aspergillus niger*, *Rhizopus chinensis*, *Candida rugosa*), pectinases (*Aspergillus niger*), proteases

Abbreviations: CL, cultural liquid.

(*Bacillius subtilis*), amylases (*E. coli*), etc. [10–16]. The possibility of obtaining a number of antibiotics, including erythromycin (*Saccharopolyspora erythraea*), neomycin (*Streptomyces marinensis*), patulin (*Penicillium urticae*), oxytetracycline (*Streptomyces rimosus*), cephalosporin (*Streptomyces clavaligerus*), was demonstrated; data on the synthesis of bacitracin, nikkomycin, and candicidin by immobilized cells are available [17–21]. The obtainment of different organic acids (such as lactic, citric, and acetic acids) by means of immobilized cells is possible [22, 23]. Special attention is paid to the use of immobilized cells to purify waste waters [24–29].

In the case of microorganism immobilization for biotechnological microorganisms, there are the following material requirements [5]: stability under dynamic conditions with the flow of working medium and increased pressure; chemical stability, which provides enzymes stability and/or microorganisms survival; the possibility of creating reparation granules with a certain volume (obtaining of monodisperse powders is desirable); the possibility of scaling immobilization processes and of an absence of factors that can result in the inactivation of enzymes and/or immobilized cells; material availability in large amounts at low cost; the possibility of safe utilization (belonging to a category of safe waste products).

The first requirement is directly associated with the method of immobilization (more precisely, those physicochemical interactions that are established between the carrier and the cell). The force of these interactions determines preparation stability in the conditions of the flow of working media and cultural liquids. The stability of the cell carrier granules themselves under the effect of hydrodynamic forces in the flow reactor is important.

The chemical stability of the material should provide permanent cell fixation on the surface at all stages of the process. It is also desirable that the material can be used for the maximal possible number of cycles during the immobilization of different microorganism species [5, 30]. During the creation of flow reactors, the granulometric composition of the loaded preparation significantly effects the hydrodynamics of the entire system and, accordingly, the energy costs associated with pumping of the introduced substances.

To date, cell immobilization is widely used in medicine [31]; however, there are completely different material requirements as compared with biotechnological productions in this area [5, 31].

Thus, the characteristics of the immobilization material and the cell fixation method are important parameters during the realization of biotechnological processes by means of immobilized cells in a bioreactor.

EXISTING METHODS OF MICROORGANISM IMMOBILIZATION

To date, it is rather difficult to describe the processes of microorganism immobilization with different carriers and to take into account the influence of all theoretically possible factors. In general, the main variants of the interaction process between cells and the carrier during immobilization can be divided into three large groups [4, 5]:

In chemical binding, a chemical interaction occurs between the cell wall compounds and the carrier substance, thus generating connections that prevent cell separation from the carrier.

Cell adsorption is due to intermolecular and/or surface interactions (such as, for example, hydrophobic/hydrophilic interactions).

With mechanical fixation in the layer or structure of any material, no chemical interactions between the cell wall and the material occur; mechanical obstacles for cell dissociation from the carrier are created.

The second and the third approaches became the most widespread due to their simplicity and relative universality. In the present review, data that mai concerning the adsorption and mechanical cell immobilization are analyzed. Some of the carriers were successfully used to immobilize different microorganisms [1, 4, 5].

Binding in calcium alginate is one of the most spread methods of *mechanical fixation* of both free enzymes and unicellular microorganisms [30–32]. The method is based on the generation of flaky structures of calcium alginate. The flakes physically capture cells in the process of their generation if present in the medium. The process passes in the presence of sodium alginate and calcium chloride in water medium at normal thermodynamic parameters. In general, the reaction is as follows:

$$(C_{12}H_{14}O_{12}Na_2)_n + nCaC_2 \rightleftharpoons (C_{12}H_{14}O_{12}Ca)_n + 2nNaCl_2. \quad (1)$$

The simplicity of its realization and the use of relatively inexpensive and safe reagents are advantages of the method. The method was used for *Saccharomyces cerevisiae* immobilization in the production of alcohol drinks [32] and the purification of waste waters with the use of *Yarrowia lipolytica W29* [30]. The method was also used to obtain pharmaceutical preparations (including different antibiotics) with the use of *Saccharopolyspora erythraea* cells (erythromycin) and members of the *Streptomyces* genus (actinomycin D, cephalosporin, and nikkomycin) [1].

In the work [8], the authors made a comparison of the productivity of ethanol production processes when using different carriers. In the present work (during permanent ethanol production with the use of mutant

Saccharomyces cerevisiae AS 2.11900 strain cells immobilized on polyvinyl alcohol), the maximal fermentation efficiency was 83.26%, the maximal ethanol yield was 0.44 g/g of biomass, the ethanol concentration was 65.81 g/L, and the the productivity was 2.74 g/L/h.

The simplicity of realization, the low cost of the used reagents, and the stability of cell attachment to the carrier in nonaggressive media can be considered advantages of this method. However, the method also has disadvantages, including [1, 30] the complexity of using living cells (which pass into the medium during division) and the possibility for some part of the biomass of appearing in the envelope from calcium alginate during fixation and thus to be inactivated.

Cell adsorption on the carrier in water media usually occurs due to the action of electrostatic forces and hydrophobic interactions [33]. This type of fixation is used for cell immobilization on porous materials, fibromaterial, and hydrogels. The material characteristics also play a significant role in preparation stability [34], especially the porosity and the pore size itself. Pores provide a high specific surface for microorganism fixation, and their size should be sufficient both to place microorganisms and to provide the required media inflow (in order for the cell to carry out its functions). The size of fibers or material elements also affects the pair potential of hydrophobic interaction, which can be generally characterized as [35]:

$$\Delta G \approx -20\sigma, \quad (2)$$

where ΔG is the pair potential of hydrophobic interaction (kJ/mole) and σ is the size of the carrier elements.

To date, many different materials for cell immobilization were studied due to the indicated interactions [36, 37]. It was demonstrated that cell fixation by means of adsorption was rather stable [36, 38].

However, the stability of this type of immobilization sharply decreases when nonaqueous media are used. This is largely caused by a change in the character of electrostatic and hydrophobic (solvophobic) intermolecular interactions in nonaqueous solutions. The process of interesterification during biodiesel production can serve as an example [39]. It is a reaction between alcohol and triglycerides with the production of fatty acid ethers and glycerin. Methanol, which is given with an excess as compared with oil, is more frequently used [40]. Hydrophobic interactions are absent when a nonaqueous medium is used, and it negatively affects the stability of cell fixation on the carrier.

In addition, it is necessary to take into account the polarization of the cell wall compounds and the carrier material itself in the case of immobilization by such a method in aqueous media and electrolyte solutions [41], as well as a change in the screening character of electrostatic intermolecular interactions depending on the concentration and type of ions in the used solution [42–44].

An increase in absorption stability is possible due to preliminary processing of the material [36, 45] with its use as reagents for the processing of polyethyleneimine [36] and glutaraldehyde, together with polyethyleneimine [45].

The kinetics of the absorption and desorption processes was analyzed in general in the work [46]. The authors represented in total the processes of adsorption and desorption by means of a simple reaction:

$$N + E \leftrightarrow NE, \quad (3)$$

where N is the amount of cells in the solution; E is the amount of potential sites for the cell fixation; and NE is the number of sites occupied by cells.

In equation (3), the direct process characterizes the adsorption; the reverse shows desorption. Thus, the adsorption process rate can be in general written as

$$-d[N]/dt = k_a[N][E] - k_d[NE], \quad (4)$$

where t is the time of the process; k_a is the absorption process rate constant; and k_d is the desorption process rate constant.

Solving equation (4) with a number of assumptions, the authors [46] obtained the following dependence for the change in the number of cells in the solution in the process of adsorption:

$$\ln(N/N_0) = -k_a E_0 t, \quad (5)$$

where N_0 is the initial number of cells in the solution and E_0 is the number of potential sites on the carrier before the beginning of cell adsorption.

This equation describes the results of the experiment with a good correlation [46]; thus, it can be used to estimate cell adsorption on the carrier. A more detailed analysis should be carried out with main cell wall properties of the specific microorganism being taking into account [47, 48].

Adsorption as a method of immobilization is increasingly applied in different biotechnological processes. In particular, it was demonstrated during a study of enzymatic ethanol production with *Saccharomyces cerevisiae* yeast cells immobilized on bagasse obtained from the *Anacardium occidentale* fruits (cashew apple bagasse (CAB)) that the productivity of immobilized cells (approximately 2.58–2.99 g/L/h) and the ethanol concentration (approximately 20.63–23.92 g/L on average) were somewhat higher than with the use of free cells [9].

The possibility of α-amylase production using the *Bacillus subtilis* cells was studied in [10]. Free cells and cells immobilized in polyvinyl alcohol and sodium glycinate were compared. As a result, it was established that the specific CL activity during immobilization on polyvinyl alcohol was 11% higher than on sodium glycinate and 28% higher than with free cells.

Very interesting results on the productivity of immobilized cells were seen in [22]. The productivity of the *Rhizopus oryzae* NRRL 395 cells (immobilized on polyurethane) by lactic acid was 55% higher under optimal conditions as compared with the cell suspension (93.2 and 60.0 g/L, respectively).

In [23], the authors considered the questions of immobilization immediately on several inexpensive carriers. Porous delignified cellulose (or tubular cellulose, TC) of Indian mango (*Mangifera indica*) and shorea (*Shorea robusta*) wood, and rice husk, as well as TC/calcium alginate/polylactide composites, were used as carriers to immobilize *Lactobacillus delbrueckii* subsp. *bulgaricus* DSMZ 20081 during fermentation of the lactic acid of serum cheese. Such carriers made it possible to shorten the time of fermentation, to increase the lactic acid yield of (g/g) and productivity (g/L/h) as compared with free cells by 38–46%.

Fibromaterials of natural origin (such as silk thread) also demonstrate sufficient fixation stability [49, 50] and, at the same time, are nontoxic, biodegradable, and have a high porosity [51, 52].

Anionic polyurethane may be a very promising material for *Saccharomyces cerevisiae* yeast cell immobilization [53]. The authors used immobilized cells with an activity close to the activity of free cells for ethanol production; however, the working pH range decreased. At the same time, the temperature optimum (40°C) remained unchanged. The increase in the ethanol concentration occurred more slowly for immobilized cells than for free cells; however, larger concentrations were achieved. With free cells, the ethanol concentration stabilized 8 h after fermentation at a level of approximately 15 g/L, while it was only 7 g/L for those immobilized at the same moment. The ethanol concentration reached approximately 30 g/L 5 h later with the use of immobilized cells.

In [54], the bagasse was used as a basis for *Candida tropicalis* PHB5 immobilization with the purpose of phenol biodegradation. The efficiency of yeast absorption on the bagasse was 87.74%. The maximal levels of phenol destruction reached 9.599, 1.665, and 2.248 g/g/h for free cells immobilized on calcium alginate and sugar cane bagasse, respectively. It should be noted that bagasse is a very cheap and available material in countries where sugar cane growing is actively developed.

Activated carbon, zeolite, diatomite, and agar balls were used as a substrate for the immobilization of *Actinobacillus succinogenes* cells in order to obtain succinic acid [55]. Cells immobilized on diatomite substrate synthesized 6.7 g/L product; at the same time, incomplete glucose consumption was observed. This phenomenon could arise due to the low holding capacity of the diatomic substrate. The yield of succinic acid during the cultivation of immobilized *A. succinogenes* cells on activated carbon and free cells was the same (10.4 g/L). The largest yield of succinic acid was observed during the cultivation of *Actinobacillus succinogenes* bacteria (enclosed in the agar balls) and was 12.4 g/L.

In [56], glycerin (waste of biodiesel production) was used for bioconversion into 1,3-propanediol via *Klebsiella pneumoniae* BLh-1 cells immobilized on calcium alginate. The greatest possible productivity of immobilized cells was observed at a size of calcium alginate balls of 3.4 mm and was 1.85 g/L/h upon 1-hour cultivation as compared with 1.22 g/L/h upon 16-h cultivation of the free cell suspension.

Bioremediation is an interesting direction for the use of immobilized microorganisms. In [57], marine *Acinetobacter* sp. HC8-3S bacteria immobilized on cotton fibers were used for the destruction of raw oil. The authors registered an approximately 30% increase in the degree of carbohydrate destruction when using immobilized bacteria. Fixed microorganisms are also used for the removal of heavy metals. Thus, the possibility of using of the *S. cerevisiae* for these purposes has already been demonstrated [32, 58–60]. In [61], hydroxyapatite was selected as a carrier for these cells. The immobilization method itself consisted of yeast cell cultivation in a medium to which hydroxyapatite tablets were initially added; the system demonstrated its efficiency with the removal of heavy metals.

BIOREACTORS FOR WORKING WITH IMMOBILIZED CELLS

As already mentioned above, the appearance of carriers for cell immobilization provides a basis for the creation of special bioreactors that can be more compact than traditional ones [3, 5]. Cell immobilization by an adsorption method on fibromaterials opens new possibilities in the field of cultivation using gaseous media [62, 63]. In particular, technologies based on the supply of gases in the cultivation medium through hollow fibers (on the surface of which the cells grow as films) are being developed (Fig. 1).

Bioreactors based on hollow fibers are mainly used to cultivate cell lines such as hybridomas, Chinese hamster ovary cells, and human kidney embryonic cells. Polysulfone and cellulose derivatives were used as material [64]. However, such bioreactors are also currently applied when microorganisms are used. Thus, in [65] asymmetric hollow fiber membranes were used to immobilize actively growing *Escherichia coli* C600(pBR322) culture (the producer of beta-lactamase, the concentration of which usually reached more than 10^{12} cells/mL during cultivation in a macroporous matrix and which developed a tissue-like mass in the available free volume.

At present, a bioreactor construction of, which includes the immobilization of membranes from hollow fibers, has already been developed [66–69]. In one of the performance variants, a microporous layer containing a biofilm of the cultivated organism and an

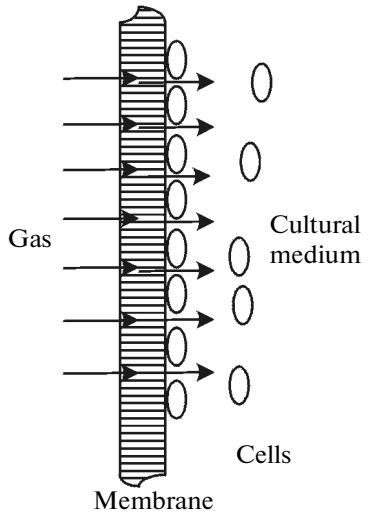

Fig. 1. Scheme of bioreactor functioning of with hollow fiber membrane.

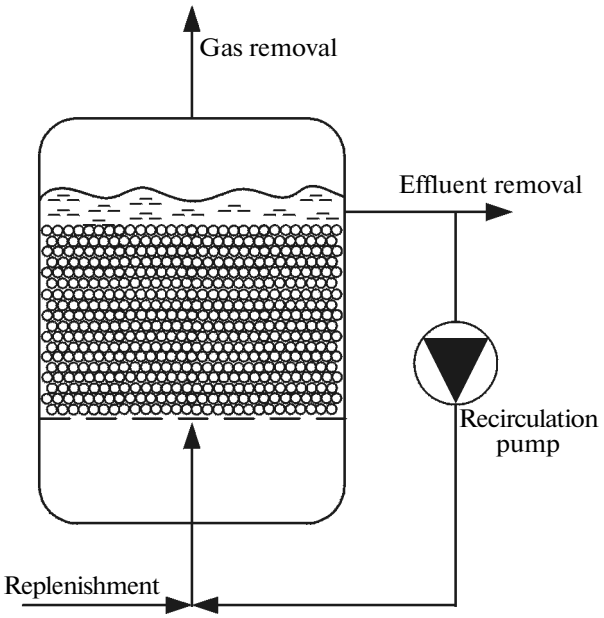

Fig. 2. Scheme of bioreactor with close-packed layer of cell carrier.

external liquid-impermeable layer allowed gas to pass through the liquid phase. This solution enables gas to directly contact with the cultivated organism (preventing the need for a primary gas supply to the liquid medium) [68].

The use of hollow fiber membranes in bioreactors also made it possible to realize solutions by a biotechnological method upon the production of products such as ethanol from synthesis gas [70]. In addition to ethanol, isopropanol, butanol, and acetic acid are considered promising substances for biosynthesis using the synthesis gas [71]. *Clostridium ljungdahlii*, *C. autoethanogenum*, *C. carboxidivorans*, *C. ragsdalei*, and *Alkalibaculum bacchi* are potential microorganisms for performing these processes [71–73].

At present, several companies are specialized in the biotechnological production of different products based on synthesis gas. INEOS Bio, Coskata, and Lanza Tech are pioneers in this area. INEOS Bio was created in 2008 as a branch of the INEOS company. It is reported that a pilot plant produces 100 ethanol gallons from each ton of dry raw material when using isolated and registered *Clostridium ljungdahlii* microorganism as a biocatalyst. In 2011, INEOS Bio started construction of the first industrial plant in Florida. The planned plant will be able to produce 8 million gallons of ethanol per year with a power of 300 tons a day. The second plant was built in 2013 in Great Britain [74]. The Coskata company based in 2006 in United States uses the registered *Clostridium coskatii* bacterium for ethanol production [75]. A demonstrational plant is located in Madison and has operated since October 2009; synthesis gas is produced there from woody biomass and solid municipal wastes by a plasma gasification process developed by the Westinghouse Plasma Corporation [74]. LanzaTech was based in New Zealand in 2005; the company's commercialization path is based on the use of synthesis gas and waste gases from industrial enterprises, which are rich in CO, to produce ethanol and 2,3-butanediol with use of the registered *C. autoethanogenum* strain [76].

A bioreactor based on microporous polypropylene hollow fibers was used for the accumulation of *Streptomyces aurefaciens* biomass (ATCC 12416c) and tetracycline production. The cell concentration in the free intertubular space was 10^{11}/mL, and the productivity by tetracycline was 5.5 mg/mL/h [77].

A bioreactor with polypropylene hollow membranes was also used for *S. cerevisiae* ATCC 4126 cultivation in order to obtain alcohol. The total reactor volume was 3.5 mL, the cell concentration was 3.5×10^9 /mL, and the productivity by alcohol was 17 g/L/h on the second day of cultivation [78].

A bioreactor with a close-packed load layer is an interesting variant of immobilized cell use [4] (Fig. 2). Granules with immobilized cells act as such a load. However, different complex media containing organic substances can be supplied to the bioreactor as an inflow depending on the technological process [79]. Thus, for example, alcohol and oil are supplied during the production of biodiesel; it is rather difficult to mix them, and it is therefore extremely difficult to provide even movement of the front of reactions by the load height or complete flow of all necessary processes with substrate passage through the reactor. Gas generation (which is typical for some biotechnological processes) is one more problem for such reactors [80]. Gas diffusion and bubble generation result in mixing of the load layer and damage the evenness of the substrate distribution by the front of the filtering. This leads to the

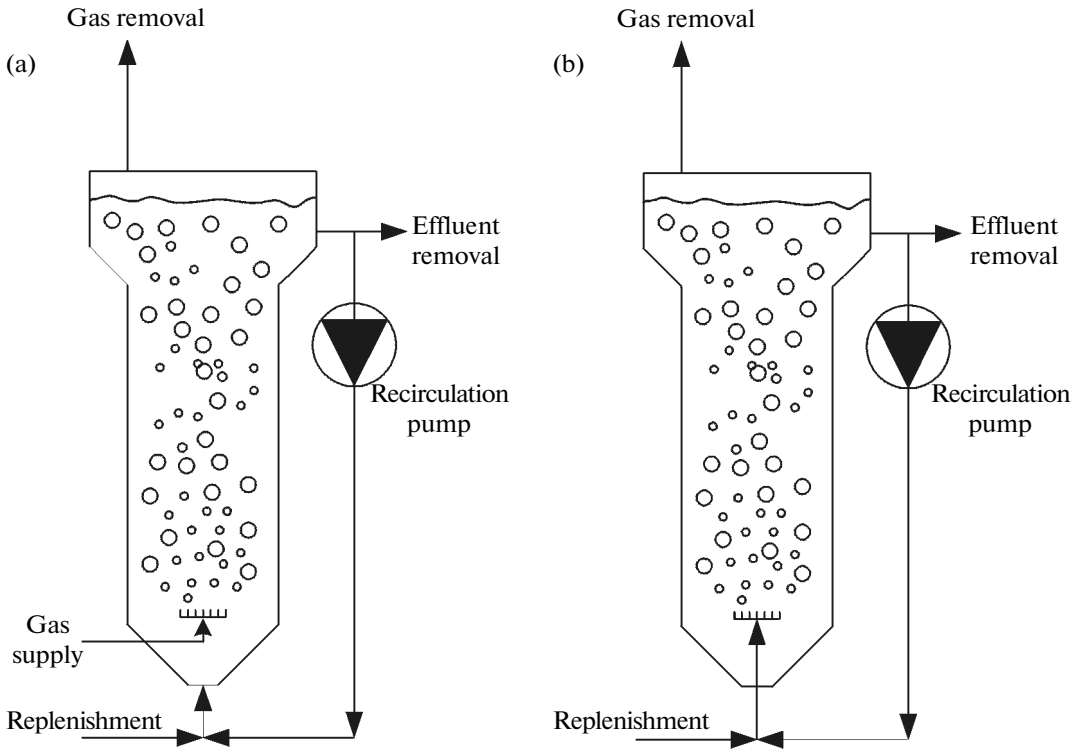

Fig. 3. Scheme of "boiling layer" bioreactor: (a) using gas under pressure; (b) using working medium.

fact that the supplied substrate can be incompletely used during one passage through the reactor, and recirculation in the solution may be necessary. It should be also noted that this bioreactor is a system that works under the pressure created in the flow of introduced media to overcome the hydraulic resistance of the load layer. Thus, in this case it is necessary to pay attention to the resistance of the selected biocatalyst in such complicated dynamic conditions. However, bioreactors with a close-packed layer of the cell carrier can be much more compact as compared with other constructions of the same productivity due to the high contact area between the cells and media (upon an efficient hydraulic regime of working) [63].

The maintenance of granules in so called "boiling layer" is one more variant of providing maximal contact between the load and substrate [4] (Fig. 3). Either gas (see Fig. 3a) or substrate (see Fig. 3b) is supplied in this work regime in the lower part of the bioreactor depending on the biotechnological process under pressure. In the first case, the load granules are in the suspended state due to the intensive diffusion of gas bubbles and the hydraulic flow of the working medium upwards; in the second case, it is exclusively due to the liquid flow. Theoretically, using the flow of both liquid and gas to create a "boiling layer" is more efficient [81]; however, there are a number of factors that must be taken into account when choosing the method of "boiling layer" creation: the weight, size, and form of the load particles; the hydrophobicity of the load particles when water media are used, since this parameter determines the degree of particle contact with gas bubbles and, consequently, the rate of their emersion; the hydraulic characteristics of the working medium (dynamic viscosity, density, etc.); and the chemical composition of the medium, particularly, the presence of organic compounds that show hydrophobic properties, since such compounds can be coupled with air bubbles and intensively float to the surface.

Analysis of the indicated factors should help to select a technological regime in which the load would be located in the suspended state and, at the same time, no flotation effect would be observed. During flotation, the load particles can float to the surface together with bubbles, thus significantly decreasing the contact between the cells and substrate. To date, this phenomenon is rather well studied [82]; therefore, determination of the flotation parameters can be conducted by relying on established regularities [82, 83].

Although bioreactors with a "boiling layer" are inferior to bioreactors with a close-packed load by the ratio of the load volume to the reactor volume, they allow the efficient contact of cells with the medium and (which is important) efficient medium mixing. However, serious attention must be paid to the limitations indicated above (those existing for this type of reactors), and the fact that the use of gases results in high energy costs must be taken into account.

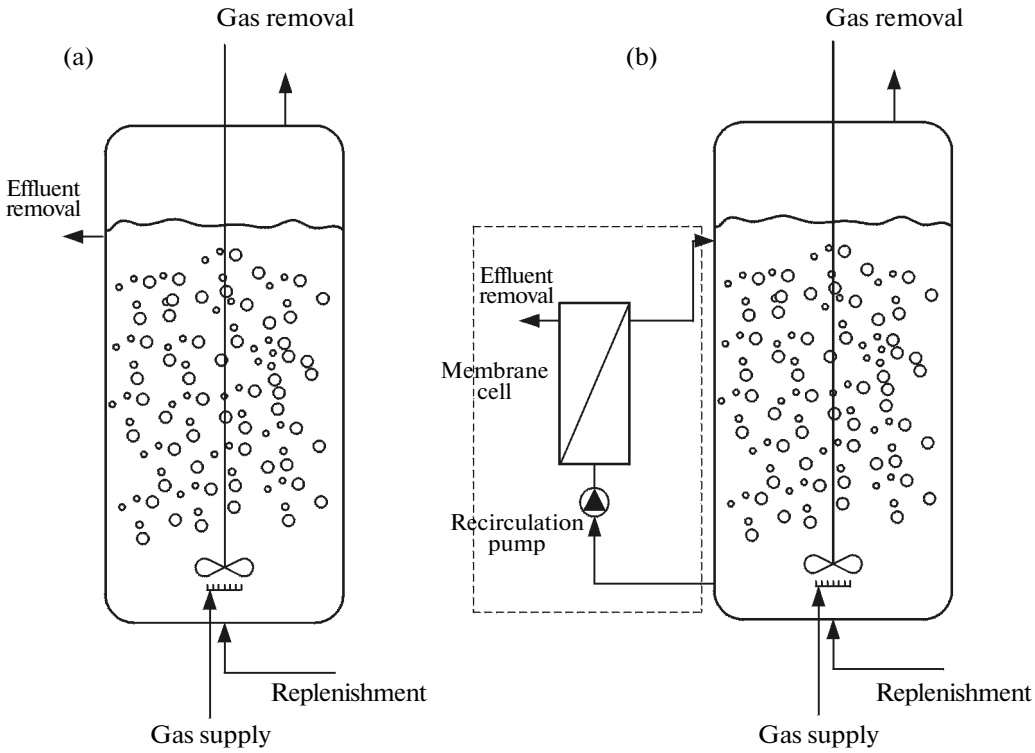

Fig. 4. Scheme of bioreactor with mechanical mixing: (a) without membrane filtration of effluent; (b) with membrane filtration.

The use of more traditional bioreactors with mechanical mixing (for example, by means of an overhead mixer) also deserves attention (Fig. 4).

Bioreactors with mechanical mixing are characterized by the largest volume per unit of loaded material; however, their advantage consists in the simplicity of exploitation. The possible problem with the load washout from effluent is solved either by organization of the effluent settling or by a membrane filter [4] (see Fig. 4b).

As seen from the data presented in the review, different materials and methods of microorganism immobilization are being considered at present. At the same time, immobilized microorganisms are used both for the biosynthesis of various products and as cellular catalysts. The criteria for selecting the immobilization method include not only an increase in productivity (for example, as in works [22] and [23]) but also an increase in the manufacturability of the process itself. The latter is understood as supplying microorganisms with nutrients, as well as, for example, simplification of the procedure for product purification from the biomass. Thus, it is difficult to separate the cellular mass from glycerin during biodiesel production (using the cellular catalyst) without such energy-intensive methods as centrifugation. In such cases, cell immobilization will allow decreased losses of biomass and, correspondingly, to decrease the costs of growing the new one and the precipitate separation. In light of the above, a question arises: in what cases it is necessary to use immobilized cells in biotechnological production? It is obvious that the possibility of immobilization is primarily determined by the microorganism strain used, its properties, and the characteristics of the process for which it was developed.

Based on the information given in the review, it is possible to indicate a number of common criteria that can help to determine whether it is necessary to start research on the immobilization of new strains:

1. Process type. Immobilization is more promising when a cellular catalyst is used (that is, when substances, the reaction between which is catalyzed by enzymes adsorbed on the cells, enter into the bioreactor). The cells themselves in this case do not produce the required substances. In this case, immobilization makes is possible to use one of the reactor types presented in the review or to make the system flow at sufficient reaction rates (see Fig. 2). In addition, the use of these reactors can decrease the geometric sizes of the whole technological line and, correspondingly, the workshop size [63]. For biosynthesis of the required substances by cells, such advantages are not obvious and require studies that can clearly demonstrate that immobilization does not provide a serious increase in productivity. For example, if a highly efficient strain (which provides good productivity in traditional bioreactors) is developed, it is probably not necessary

to concentrate on conducting serious studies regarding its mobilization.

2. Product type. If we are talking about large-capacity production of inexpensive product, a decrease in bioreactor size due to successful microorganism immobilization can result in a decrease in the material capacity of the entire technological line and, ideally, a decrease in capital costs [3, 4].

3. Materials and methods of immobilization. They can not be completely universal for all processes. Some approaches and carriers can be widely applied in different processes (for example, alginate). At the same time, as was mentioned above, immobilization methods are inefficient for nonaqueous media due to hydrophobic interactions.

4. Complexity of CL division. It is appropriate to consider the possibility of immobilization if it is difficult to separate biomass from the product after classical cultivation.

5. Substrates. When gaseous substrates are used, it is extremely important for them to provide an efficient supply of the cells. For these purposes, the use of hollow fiber systems is a very efficient solution [66–69, 74–76].

Attention should be paid to the fact that new materials are currently appearing that can be used to solve the considered problems. Thus, for example, nanocellulose is already being discussed as a potential cell carrier for biotechnology [84–86]. Depending on the production method, this material can show hydrophobic properties up to strongly hydrophobic [87, 88]. Hydrogels with different properties can be also obtained from nanocellulose [89, 90]. In addition, this material is biocompatible [91, 92] and perfectly interacts with different polysaccharides [93].

Synthetic peptides have interesting properties as carriers [94–97]. These materials have a wide spectrum of physicochemical properties that can be varied depending on their primary amino acid sequence [95, 96]. However, their cost is still too high for use as a material to immobilize commercially valuable microorganisms [94]. A decrease in their cost, which results from an increase in production for the needs of other branches, may make it possible in the future to use them in the applications considered in this article.

As is possible to see from the presented review, the problem of cell immobilization is generally topical for many biotechnological processes. Such approaches are the most interesting in the creation of systems with biocatalysts, when cells with enzymes immobilized on the cell wall are in turn fixed on the carrier. However, as was demonstrated in the review, immobilization in some cases also allowed an increase in the efficiency of biosynthetic processes. The possibility of creating compact bioreactors with low material consumption is an important factor; other things being equal, this makes it possible to decrease capital costs for the organization of production.

It should be also noted that the appearance of new, relatively inexpensive materials can significantly broaden the possibilities for systems with immobilized cells in biotechnological processes.

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