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PNEUMATIC BIOREACTORS WITH IMMOBILIZED BIOCATALYSTS: AN OVERVIEW

BY

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Abstract. The air-lift bioreactors, which are the most attractive type of pneumatic bioreactors, have been widely used in the last decades, due to their numerous advantages compared to the stirred ones: low energy consumption, simple design and exploitation, easily maintaining of the medium sterility due to the lack of the moving elements, superior values of heat and mass transfer rates, and, especially, the possibility of growing microorganisms sensitive to high shear stress.

At the same time, the use of immobilized biocatalysts in chemical bioprocesses became a prevalent working-method, owing to the higher thermal and mechanical stability of biocatalysts, easier recovery of the immobilized microorganisms, high specificity of the biochemical reaction, and low overall cost of the biochemical technology.

This work reviews the main applications of the pneumatic bioreactors with immobilized biocatalysts, as well as the characteristics and performances of bioprocesses carried out in air-lift bioreactors catalyzed by biocatalysts entrapped on various supports by different immobilization techniques.

Key words: air-lift bioreactor, immobilized biocatalysts, hydrodynamics, oxygen mass transfer.

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1. Introduction

Recently, the use of pneumatic bioreactors became a common working technique for many biochemical processes, due to their benefits compared to the stirred–tank bioreactors: low demand of energy for mixing and aeration, low operational cost, the maintaining of the medium sterility owing to the absence of the moving parts, as well as its great potential for cultivating animal and plant cells sensitive to higher shear stress.

The air-lift bioreactors (ALBs) are an attractive type of pneumatic bioreactors and are widely used for different bioprocesses, like antibiotics and vitamins biosynthesis, protein production on substrate of oil fractions, etc. In the pneumatic bioreactors, the difference between the medium density and that characteristic to the liquid-air dispersion represents the driving force for mixing and medium circulation (Oniscu *et al*., 2002). Depending on their structure, the ALBs could be classified into internal (Figs. 1 *a* and *b*) and external-loop ALBs (Fig. 1 *c*), characterized by medium circulation through a cyclic-pattern defined by a baffle or a draft tube, or by an external tube connecting the riser with the downcomer. Regardless of the ALB geometrical configuration, the bubbles motion and buoyancy induce the existence of four different regions of medium circulation into the ALBs: the riser, the downcomer, the bottom and the top gas separator areas (Chisti & Moo-Young, 1993).

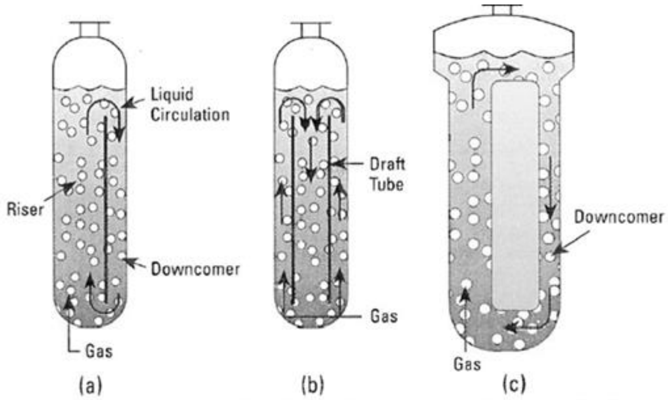

Fig. 1 − Medium circulation into ALBs, with internal (*a*, *b*) and external loop (*c*).

The most important parameters that induce the medium circulation into the ALBs are the liquid circulation velocity and gas hold-up, which are controlled by the geometrical characteristics of the bioreactor, liquid height, and operational conditions, namely liquid velocity, gas bubble size, and superficial tension of liquid phase (Ebrahimifakhar *et al*., 2011).

2. Applications of Pneumatic Bioreactors with Immobilized Biocatalysts

The influence of mean bubble size represented the main subject for many research studies, most of them using photographic techniques. Cerri and his group (2010) revealed that the gas hold-up is the main parameter that controls the efficiency of the oxygen mass transfer and should be taken into consideration for the scale-up of this type of bioreactors (Cerri *et al*., 2010).

In order to quantifying better the influence of electrolytes addition on oxygen mass transfer coefficient (k_La), Al Taweel and coworkers (2013) studied separately its effect on the liquid mass transfer coefficient (k_L), and on the specific interfacial area (a), respectively, in a multibubble-sparged air-lift reactor. Their results are in agreement with those previously reported in literature, according to which the electrolytes addition exhibits a negative influence on oxygen mass transfer coefficient. However, its negative effect could be attenuated by using an ultrasonic sparger generating microbubbles, with the positive effect on decreasing the surface tension and increasing the interfacial area between the aqueous and gaseous phases (Al Taweel *et al*., 2013).

Moraveji and coworkers (2012) observed a positive effect of surfactants addition (SDS, HCTBr, and Tween 40) on oxygen transfer rate, the value of the oxygen mass transfer coefficient being calculated from the slope of the straight line described by the eq. (1):

$$\ln\frac{C^{*}-C_{L}}{C^{*}-C_{0}}=k_{L}at \qquad (1)$$

where C^* is the saturation concentration of dissolved oxygen at the operating temperature, C_0 − the initial concentration of dissolved oxygen, and C_L − the measured instantaneous concentration at time t (Moraveji *et al*., 2012).

Eq. (1) represents a simplified form of eq. (2), proposed by Garcia-Ochoa and Gomez (2009) for larger values of time response of the oxygen electrode (t_E) (Garcia-Ochoa & Gomez, 2009):

$$\frac{C^{*}-C_{L}}{C^{*}-C_{0}}=\left(\frac{e^{-tk_{L}a}}{t_{E}}-k_{L}ae^{-\left(\frac{t}{t_{E}}\right)}\right)\frac{1}{1-t_{E}k_{L}a} \qquad (2)$$

In their paper, Liu and coworkers (2013) discussed the efficiency of *laccase* production by *Pycnoporus spp*. SYBC-L3 in a 65 L air-lift fermentor. After 6 days of cultivation, the activity of this enzyme reached its maximum value

(72 000 U/L), as well as long shelf-life at room temperature and great thermal stability at high temperature (Liu *et al.*, 2013).

In order to establish the influence of sparger configuration on hydrodynamics and mass transfer in ALBs, Luo *et al.* (2011) studied three different types of spargers (Fig. 2), for both homogeneous and heterogeneous flow regimes. The authors observed a more pronounced influence of sparger configuration on hydrodynamic parameters in the heterogeneous flow regime, as well as the increased efficiency of the sparger with four-orifice nozzles, in comparison to that recorded for the O-ring distributor and two-orifice nozzles sparger (Luo *et al.*, 2011).

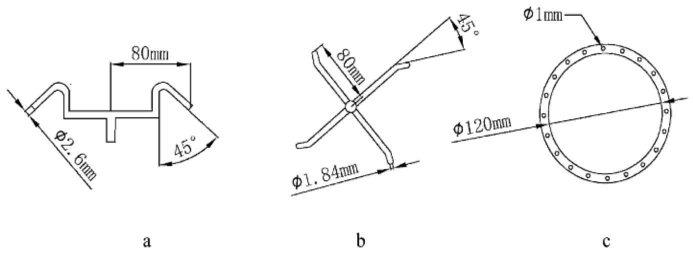

Fig. 2 − Sparger types: *a* − 2 orifice nozzles; *b* − 4 orifice nozzles; *c* − O-ring distributor.

The studies concerning the effect of aliphatic alcohols addition on hydrodynamics and mass transfer in ALBs performed by Gharib and coworkers (2013) revealed that the oxygen mass transfer coefficient increases with the increase of alcohols concentration and content of carbon atoms from their molecule. The air sparged into bioreactors with a content of maximum 1% alcohol (methanol, ethanol, n-propanol, and n-butanol) led to a more important improvement of mass transfer rate in the external-loop ALB, compared to the effect registered for the internal-loop ALB (Gharib *et al.*, 2013).

Ntihuga and his group (2013) used two different configurations of the ALBs for continuous bio-ethanol production. They compared the performances of a Blenke cascade (Fig. 3) and a plate heat exchanger bioreactor type, from the point of view of mixing efficiency, easiness of maintaining the aseptic conditions, and overall cost of the fermentation (Ntihuga *et al.*, 2013). The cascade Blenke configuration (Fig. 3 *a*) is the one obtained by changing the mixing almost completely axial of a common air-lift bioreactor by a more complex one, in which metallic inserts of ring-like (Fig. 3 *b*) and discoidal (Fig. 3 *c*) shapes create eddies in the fluid, enhancing the flow velocity (Fig. 3 *d*) and mixing intensity.

The alcoholic fermentation has been carried out on a combined substrate of glucose and maltose, at the room temperature, initially in a batch mode and afterwards in a continuous one. The results suggested a more intense mixing and a higher productivity for the Blenke cascade configuration (Q_p = 3.07 g/L·h *vs.* Q_p = 2.31 g/L·h) compared to the plate heat exchanger (Ntihuga *et al.*, 2013).

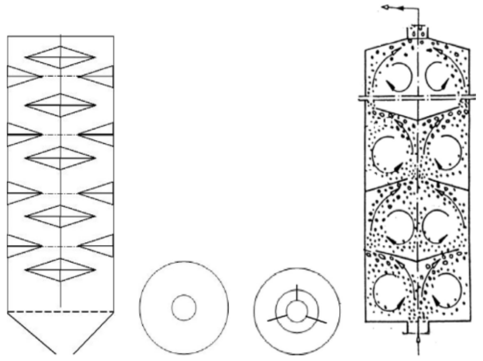

Fig. 3 − Blenke cascade bioreactor configuration: *a* − Cascade configuration, *b* − Donut inserts, *c* − Disc inserts, *d* − Flow behavior.

Hama and coworkers (2007) analyzed the biodiesel-fuel production in a packed-bed reactor using *lipase*-producing *Rhizopus oryzae* cells immobilized on polyurethane foam. The authors used a 20 L ALB for the batch cultivation and obtained higher value of yield in methyl-ester (more than 90%), recorded for the first 10 cycles of biosynthesis (Hama *et al.*, 2007).

In their paper, Paji´c-Lijakovi´c *et al.* (2007) established a mathematical equation quantifying the yeast cells growth in microbeads of Na alginate in an ALB. The authors concluded that yeast cells growth is affected especially by the steric hindrance, the internal diffusion exhibiting only a negligible effect on medium hydrodynamics. Moreover, after reaching the maximum value, the growth rate of cells decreased drastically and then suddenly increased under the same optimal experimental conditions. This complex phenomenon was attributed to the cells growth inside the immobilization support, with the accumulation of energy within the matrix alginate and its further disintegration (Paji´c-Lijakovi´c *et al.*, 2007).

Jovetic *et al*. (2006) experimented for 56 days the process of enzymatic deacylation of glycopeptide antibiotic A40926. The biochemical process has been carried out in a cascade of three perfectly mixed ALB, using *Actinoplanes teichomyceticus* cells immobilized in 2% Ca alginate. The mathematical model based on the Michaelis-Menten kinetics takes into account the simultaneous diffusion and conversion of the substrate inside the biocatalyst particle, with the progressive decrease of its concentration inside the alginate matrix (Jovetic *et al.*, 2006).

Milivojevic and coworkers (2007) studied the mixing efficiency in an external-loop ALB for both laminar (homogeneous) and heterogeneous flow regime, by means of slip velocity (Milivojevic *et al.*, 2007). The authors quantified the effects of the geometrical configuration and main operational parameters of bioreactor on slip velocity (eq. (3)):

$$V_S = \frac{V_g}{\varepsilon} - \frac{V_L}{1-\varepsilon} \tag{3}$$

where in V_g and V_L are the superficial velocities of the gaseous and liquid phase, V_s is the slip velocity, and ε is the gas hold-up (Milivojevic *et al.*, 2007).

According to the authors conclusions, the mixing efficiency and mass transfer rate could be enhanced by the slip velocity increasing, if the following conditions are respected:

− higher values of gas superficial velocity in the heterogeneous regime;
− the minimum ratio of 4 between the downcomer and riser cross-flow areas;
− the minimum bioreactor diameter of 0.14 m (Milivojevic *et al.*, 2007).

Kilonzo and coworkers (2010) studied the medium hydrodynamics of an inverse internal-loop airlift-driven fibrous-bed bioreactor of 21 L working volume, maintaining the bottom clearance at the value of 0.059 m. The air previously filtered through glass wool was introduced by a sparger of stainless steel provided with 24 orifice nozzles placed equidistantly. To quantify the liquid circulation rate, the authors used the tracers method, measuring the time needed to for the pH to reach a constant value after the addition of 10 mL of HCl 8 N. Finally, the authors established mathematical equations describing the liquid mixing time and gas hold-up for both riser and downcomer regions (Kilonzo *et al.*, 2010).

Bezbradica *et al.* (2007) performed the beer fermentation with immobilized biocatalysts by using a non-invasive method of yeast cells immobilization. *S. cerevisiae* cells have been cultivated on sterile medium and subsequently immobilized on PVA particles. The batch fermentation has been carried out in an internal air-loop ALB and the nitrogen sparging the medium was introduced by means of a glass sparger placed at the base of the bioreactor. The PVA used for yeast cells immobilization was found to be a very appropriate one, because the immobilized biocatalysts possess a very high mechanical stability, also after 30 days of their continuous utilization (Bezbradica *et al.*, 2007).

Kunamneni and coauthors (2007) studied the process of *cyclodextrin glucanotransferase* production in an internal air-loop bioreactor provided with draft tube. The authors used *Bacillus spp.* cells immobilized in Na alginate. Compared to the stirred bioreactors, the ALBs were more suitable for cultivating these cells, which are sensitive to the mechanical damage. The air-lift bioreactor with concentric draft tube was operated both in batch mode and continuous one. The results indicated the higher productivity with continuous fermentation in the presence of immobilized cells compared to the free ones (13.65 U/mL·h *vs.* 6.89 U/mL·h) (Kunamneni *et al.*, 2007).

Benyahia and coworkers (2005) reported studies on the kinetics and mass transfer for the process of nitrification catalyzed by free and immobilized cells of

Nitrosomonas in Ca alginate. For their investigations, the authors used a simulated wastewater with a high content of ammonia.

In order to ensure a homogeneous flow regime, as well as to avoid the deposition of immobilized biocatalysts at the bioreactor bottom, the authors used a configuration of U-shape of an internal-loop ALR. For counteracting the negative effect of external diffusion, the mixing intensity had to be maintaining at low level (Fig. 4). Although the oxygen mass transfer and consumption respect a first order kinetics, the effective diffusion coefficient D_e obtained by authors had a lower value (8.7 10^{-9} m^2/s) than those from literature, mainly due to the biomass supplementary presence and the different conditions for cultivation (Benyahia *et al.*, 2005).

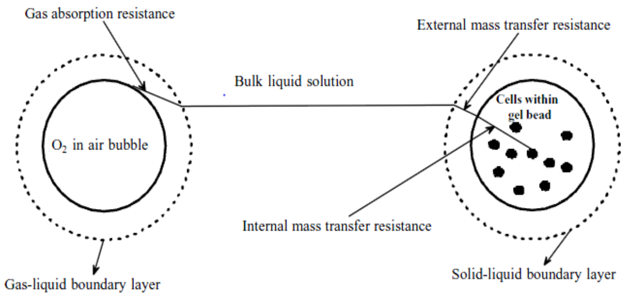

Fig. 4 − Oxygen transfer resistances in the nitrification system.

Behin and coworkers (2013) used digital image processing technique for measuring the residence time distribution in an ALB of 2.4 L working volume, with air and tap water as gaseous and liquid phase, respectively. The obtained data for three different values of gas superficial velocities (of 0.5, 1, and 1.5 cm/s, respectively) were compared with those recorded by means of the conventional, colorimetric method, the advantages of the new method resulting from the possibility of obtaining on-line the most accurate results for residence time distribution (Behin *et al.*, 2013).

4. Conclusions

The utilization of air-lift bioreactors with biocatalysts immobilized by different techniques on various supports represents a very attractive topic for the worldwide researchers, and remains one of the most promising alternatives for biochemical processes.

At present, the main challenge for the specialists in the biochemical engineering and biotechnology is to find the optimum specific design and operational parameters of these bioreactors for a given biotechnological process.

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BIOREACTOARE PNEUMATICE CU BIOCATALIZATORI IMOBILIZAŢI

(Rezumat)

Bioreactoarele pneumatice au cunoscut o dezvoltare apreciabilă în ultimii ani, datorită avantajelor majore pe care le prezintă în comparaţie cu bioreactoarele cu amestecare mecanică: construcţia simplă şi operarea facilă, costurile reduse de energie pentru amestecarea mediului, menţinerea uşoară a sterilităţii, datorită absenţei elementelor rotative aflate în contact cu exteriorul (axul agitatorului), valorile ridicate ale proceselor de transfer de masă şi căldură, dar, mai ales, posibilitatea cultivării microorganismelor sensibile la forţele de forfecare. Bioreactoarele pneumatice sunt utilizate în diferite procese de biosinteză (obţinerea pe diferite substraturi a proteinelor microbiene, obţinerea antibioticelor, a vitaminelor etc.), principiul de funcţionare al acestora bazându-se pe inducerea circulaţiei mediului ca efect al diferenţei de densitate între regiunea în care există doar mediul şi cea în care există dispersia gaz-mediu. De asemenea, utilizarea preparatelor enzimatice imobilizate la transformarea substratelor a devenit o practică de lucru curentă, datorită numeroaselor avantaje pe care le prezintă: posibilitatea recuperării şi a reutilizării biocatalizatorilor imobilizaţi, creşterea stabilităţii termice şi mecanice a enzimelor imobilizate, înalta specificitate a reacţiei biochimice etc. Pe baza informaţiilor din literatura de specialitate, lucrarea de faţă oferă o privire de ansamblu asupra principalelor aplicaţii ale bioreactoarelor pneumatice cu biocatalizatori imobilizaţi, precum şi caracteristicile şi performanţele bioproceselor realizate în astfel de bioreactoare.