Bioreactors with immobilized biocatalysts

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Abstract

The spectacular applications of the immobilized biocatalysts determined the design and construction of some proper bioreactors, specific or derived from the "classical" ones. Although the most of these bioreactors are used at pilot scale only, their offer is generous and perfect compatible with the new trend on "green chemistry".

Therefore, in this paper the main types of bioreactors with immobilized enzymes or cells (column, stirred, gaslift or membrane bioreactors, with fixed, mobile/stirred, expanded or fluidized bed) are briefly discussed from the viewpoint of their construction and applications, by analyzing comparatively their advantages/disadvantages vs. the conventional bioreactors.

Keywords: bioreactor, immobilized cell, immobilized enzyme, biocatalyst, fixed bed, mobile bed, fluidized bed.

Introduction

The bioreactor is assimilated with the heart of the biotechnological process, being the equipment in which the substrates are converted to the desired products under the microorganisms, cells or enzymes action. This comparison is due to the fact that the bioreactor "aspirates" the nutritive media and the biocatalysts through the upstream routes, "pumping off" the biosynthetic products through the downstream routes [1].

Numerous types of bioreactors are currently used at laboratory or industrial scale. Although the bioreactors with immobilized biocatalysts are derived from the "classical" bioreactors and, therefore, their constructive and functionally characteristics are rather similar with the second ones, the place of bioreactors with immobilized biocatalysts is privileged. The top position is the result of the advantages offered by the use of the immobilized microorganisms, cells or enzymes, namely as: the increase of the thermal, chemical and to the shear forces resistance of the biocatalysts, the increase of the number of the repeated biosynthesis cycles using the same particles of biocatalysts, the easier recovery of the biocatalysts from the final broths, the diminution or avoidance of the inhibition processes [1-4].

The immobilization techniques are various and have been exhaustively described in the literature (adsorption, ionic binding, covalent binding, cross-linking, matrix entrapment, membrane confinement, combined methods) [2,5-10]. The bioreactors using immobilized biocatalyst can be designed as column, stirred, gaslift or membrane bioreactors. They are operated in batch, continuous or semicontinuous systems, with fixed, mobile/stirred, expanded or fluidized bed [1].

In this context, the aim of the paper is to briefly review the recent literature on the main types of the bioreactors with immobilized biocatalysts, from the viewpoint of their construction and applications, by analyzing their advantages/disadvantages comparatively to the other types of bioreactors.

BIOREACTORS WITH FIXED BED OF BIOCATALYSTS

In the last decade, the bioreactors with fixed bed of biocatalysts (packed-bed bioreactors) became some of the most used type of bioreactors, because of their low costs of exploitation and maintenance, easiness of scaling-up and of automatic controlling, generation of significant lower shear forces, and, consequently, avoidance of the breakage of biocatalysts particles [1,11-13]. The packed-bed bioreactors are used for wastewater treatment [1,12,14,15], biosorption from wastewater of different metallic ions [16], solvents and fuels [13,17], pharmaceuticals [18] and fine chemicals production [19-21].

Sarti et al. (2001) designed a packed-bed tubular bioreactor for wastewater treatment with the biocatalyst bed consisting on the anaerobic activated sludge entrapped into polyurethane foam [12]. The bioreactor is placed horizontally, the ratio between its length and diameter being of 20. The formed gas is collected through a perforated tube placed on the bioreactor length. The efficiency of this bioreactor is comparable with the classical basin with activated sludge, but its working volume is significant lower and the process parameters can be more precisely controlled and regulated.

The ethanol has been obtained from molasses by yeasts cells entrapped into alginate matrix, and could be used in food/beverage industry or as biofuel. In this purpose, a bioreactor of column type having the immobilized yeasts on a sieve plate has been used [13]. The fermentation can be carried out at higher sugar concentration and at lower pH-value than the process using free yeasts cells. The optimum alginate particles diameter was found to be between 2 and 2.4 mm.

The biocatalyst could be disposed around the stirrer in a fixed bed with cylindrical geometry. This type of bioreactor is known as "basket bioreactor" and is derived from the "catalytic basket reactor", described for the first time by Carberry in 1964 [22]. In the case of ethanol production, the stirred basket bioreactor offers many advantages compared with the conventional stirred bioreactor: it can be operated in batch, fed-batch or continuous flow systems, the productivity is for 4 - 6 times higher (12 g/l.h), more accurate control of the temperature and pH, the stability of biocatalysts allows to repeating the fermentation cycles for over 35 times [17]. The application of this bioreactor could be extended to the treatment of wastewater, especially for recovering the heavy metals from mine drainage.

BIOREACTORS WITH MOBILE/STIRRED BED OF BIOCATALYSTS

There are some of the most studied and applied bioreactors, owing to their very similar constructive and operational characteristics to those of the classical stirred bioreactors. The main difference between the constructions of the two types of bioreactors consists on the presence at the bottom of the former ones of a sieve which avoids the biocatalysts particles washout. The models describing the flow or the heat and mass transfer in stirred bioreactors, as well as their design and optimization can be easily adapted for the stirred-bed bioreactors. But, these models are valid only for the continuous phase from the bioreactor. Due to the internal diffusion of the substrate or product into the biocatalyst particle, the mechanism and kinetic of the processes occurring into the solid phase become more complex than in the homogeneous systems, thus new models having to be established for the biocatalyst phase [1,5].

The performances of the fermentation processes that are carried out in the bioreactors with mobile bed of biocatalysts are influenced especially by the size of the particles [5], geometrical characteristics of the vessel [23,24], mixing [25-27], concentration of enzymes/cells into the particles [24,28,29], feed strategy [25,30,31].

These bioreactors have been used for production of pharmaceuticals [1,21], chemicals [32], solvents [33,34], whereas the current studies are mainly focused on the treatment of industrial or municipal wastewater [23,24,27-30].

BIOREACTORS WITH FLUIDIZED BED OF BIOCATALYSTS

The bioreactors with fluidized bed are of vertically column type, their construction being rather simple (Figure 1).

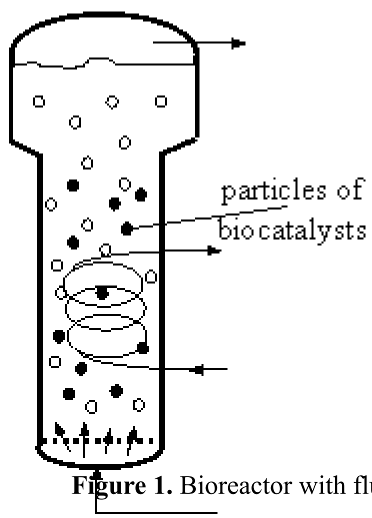

Figure 1. Bioreactor with fluidized bed.

The gas or the liquid (media) are introduced under the layer of biocatalysts with a velocity at least equal with the minimum fluidization velocity. But, the circulation velocity of the heterogeneous phase biocatalysts – media has to be lower than that needed for solid phase pulling-out (the loss of biocatalysts is avoided by enlarging the section of the bioreactor top).

The main advantages of these bioreactors are as follows [1,35]:

- intense mixing of the media, thus leading to high rates of mass transfer and of biosynthesis;
- high rate of heat transfer, therefore lower surfaces needed for heating/cooling;
- the possibility to easily control and regulate the process.

On the other hand, the fluidization induces some negative phenomena: the mechanical lysis of biocatalysts, due to the high shear forces or to the collision between the particles, the non-uniform distribution of the residence time, high consumption of energy [1].

This type of bioreactors are used for enzymatic conversion of agricultural or food residues or wastewater treatment. Therefore, Trivedi et al. (2005) have been obtained phenolic polymers using *peroxidase* immobilized into alginate matrix, in a bioreactor of 4 m height [36]. The bioreactor has been operated in continuous regime, the overall conversion reached over 60% and the activity of biocatalysts remained at a rather constant level for a long time period (the initial decrease in the biocatalysts activity was due to the deposition of the polymer layer on the particles surface) (Figure 2).

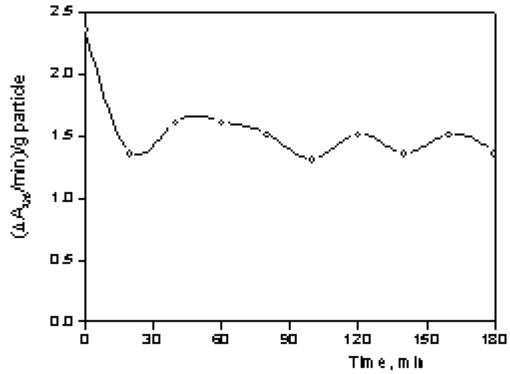

Figure 2. The activity of immobilized *peroxidase* measured at the bioreactor outlet.

Berensmeier et al. (2004) studied the chemical, thermal and to the shear forces stability of the *dextransucrase* correlated with the immobilization method [37]. The experiments have been carried out for the enzymatic hydrolysis of the polysaccharides to dextrins in a fluidized bed reactor.

GASLIFT BIOREACTORS WITH IMMOBILIZED BIOCATALYSTS

The applications of these bioreactors have been extended at industrial scale for antibiotics and vitamins biosynthesis [1,38], bier production [39] or wastewater treatment [1,5]. The broth circulation and, consequently, the mixing, are induced by the gradient of density existing between the gassed/aerated and non-gassed/non-aerated regions into the bioreactor.

Among the gaslift bioreactors, those having the internal circulation of the media are considered the most adequate for the immobilized biocatalysts. These bioreactors could be individually used, or disposed in a cascade system. Thus, Jovetic et al. (2006) performed the desacylation of the glycopeptide antibiotic A40926 in a cascade of three airlift bioreactors with immobilized *Actinoplanes teichomyceticus* cells [38] (Figure 3).

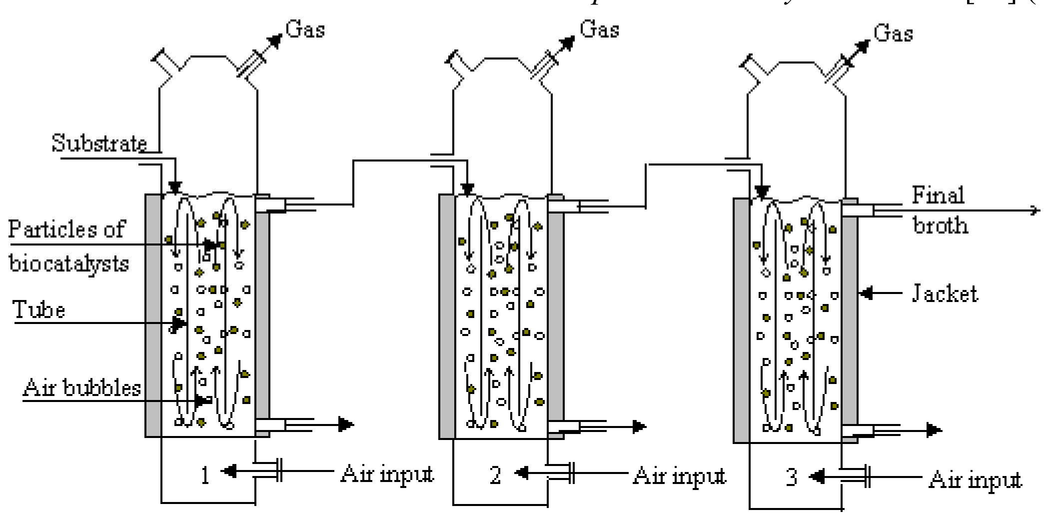

Figure 3. The cascade of the airlift bioreactors used for antibiotic desacylation.

The circulation of the broths can be described by the plug-flow model. The antibiotic conversion was higher than that reached in the single airlift bioreactor or in the conventional stirred bioreactor, being of 95-99%.

MEMBRANE AND HOLLOW FIBER BIOREACTORS WITH IMMOBILIZED BIOCATALYSTS

Initially, the membrane/hollow fiber bioreactors have been used for separating the compounds with different molecular weight from broths by means of ultrafiltration [1]. These bioreactors are useful for enzymatic transformations, because it avoids the free enzymes washout. More recently, the membrane and hollow fiber have been tested for biocatalysts immobilization, either by adsorption into the membrane pores or by delimitation the space in which the biocatalysts exert their activity [5].

Compared with the microporous membranes, the pores blockage by biocatalysts is significantly diminished in the case of hollow fiber, due to the circulation of the media inside the fiber (Figure 4).

The first information in literature concerning the possibility to immobilize the enzymes or cells into the hollow fiber has been given by Rony in 1971 [40]. Its studies have been then continued and developed by Breslau (1981) [41], Deeslie and Cheryan (since 1981) [42,43], Chang et al. (since 1987) [44,45], Berke et al. (1988) [46], Lee and Hong (1988) [47], Shao et al. (1989) [48], Williams et al. (1989) [49], Yuan et al. (1990) [50], Shi (1993) [51], Ulibarri and Hall (1997) [52], Resende et el. (2002) [53], Wenten and Widiasa (2002) [54] for enzymatic oxidation, hydrolysis or separation of amino acids racemic mixtures.

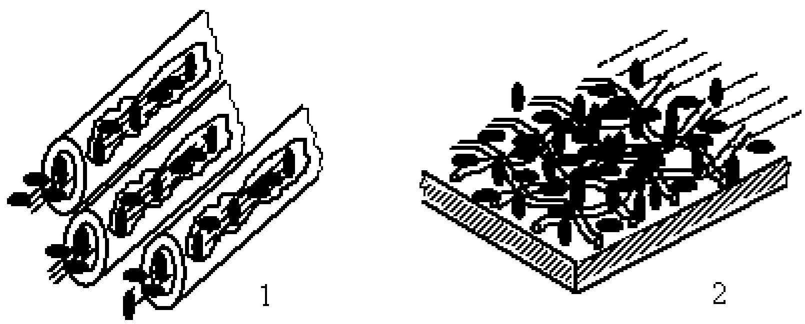

Figure 4. Media circulation inside the hollow fiber (1) and at the microporous membranes surface (2).

Due to the low rate of mass transfer through pores, the industrial applications of hollow fiber bioreactors are very limited, but they were successfully transposed at pilot scale. Thus, Wenten and Widiasa (2002) obtained 6-aminopenicillanic acid by enzymatic hydrolysis of Penicillin G with *penicillinacylase* immobilized into the hollow fiber [54]. The immobilization degree of enzyme was over 90% and its stability was considerably higher than in the case of immobilization on microporous membrane. Because of its lower molecule size, only 6-aminopenicillanic acid diffuses through membrane (Figure 5).

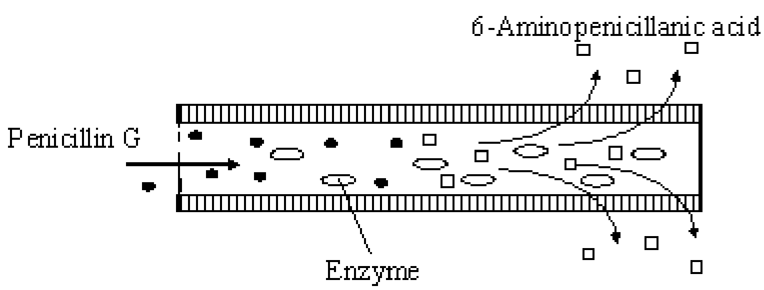

Figure 5. Penicillin G hydrolysis with enzymes confined into hollow fiber.

Besides the enzymes, cells or microorganisms can be confined into the hollow fiber of polymers or ceramics and used for starch hydrolysis [59], proteins hydrolysis [49,50], ATP regeneration [53], aspartic acid production [54], residues conversion [55].

Conclusions

The immobilization makes it possible for biocatalysts to be repeatedly or continuously used without significant loss in their activity. The spectacular applications and the considerably potential of the immobilized biocatalysts determined the design and construction of proper bioreactors, specific or derived from the "classical" ones.

This field of biochemical engineering and biotechnology is just at beginning, but its resources seeming to be unlimited, all the more as it sustain the concept of "green chemistry".

References

1. D. CAŞCAVAL, C. ONISCU, A.-I. GALACTION, *Inginerie biochimică şi biotehnologie. 2. Bioreactoare*, InterGlobal, Iaşi, 2002, pp. 15 - 17.
2. R.H. WIJFFELS, R.M. BUITELAAR, C. BUCKE, J. TRAMPER, *Immobilized Cells: Basics & Applications. Progress in Biotechnology, vol. 11*, Editorial Elsevier, Amsterdam, 1996.
3. W. TISHER, F. WEDEKIND, H.D. FESSNER, *Immobilized Enzymes: Methods and Applications. Biocatalysis. Topics in Current Chemistry*, 95-126 (1999).
4. M.B. ANGELOVA, S.B. PASHOVA, C. SLOKOSKA, *Enzyme Microb. Technol*., **26**, 544-549 (2000).
5. W. HARTMEIER, *Immobilized biocatalysts*, Springer-Verlag, Berlin, 1988.
6. K. NAKAMURA, K. INOUE, K. USHIO, S. OKA, A. OHNO, *J. Org. Chem*., **53**, 2589-2592 (1988).
7. T. KOMETANI, E. KITATSUJI, R. MATSUNO, *Chem. Lett*., 1465-1468 (1989).
8. K. NAKAMURA, Y. KAWAI, A. OHNO, *Tetrahedron Lett.,* **31**, 267-268 (1990).
9. J.V. SINISTERRA, H. DALTON, R.H. WIJFFELS, R.M. BUITELARR, C. BUCKE, J. TRAMPER, *Immobilized Cells: Basic & Applications*, Elsevier Science B.V., London, 1996, pp. 416-423.
10. F. MOLINARI, C. BERTOLINI, F. ARAGOZZINI, *Biocatal. Biotransform*., **16**, 87-93 (1997).
11. A. BODALO-SANTOYO, J.L. GOMEZ-CARRASCO, E. GOMEZ-GOMEZ, J. BASTIDA-RODRIGUEZ, M.F. MAXIMO-MARTIN, A.M. HIDALGO-MONTESINOS, *J. Chem. Technol. Biotechnol.*, **74**, 403-408 (1999).
12. A. SARTI, L.G. TAVARES-VIEIRA, E. FORESTI, M. ZAIAT, *Bioresource Technol.*, **78**, 231-238 (2001).
13. Y. GOKSUNGUR, N. ZORLU, *Turk J. Biol*., **25**, 265-275 (2001).
14. M. ZAIAT, J.A. DOMINGUES-RODRIGUES, E. FORESTI, *Process Biochem.*, **35**, 943-949 (2000).
15. T. GU, M.J. SYU, *Biotechnol. Prog.*, **20**, 1460-1466 (2004).
16. Y. GOKSUNGUR, S. ÜREN, U. GUVENC, *Turk J. Biol.*, **27**, 23-29 (2003).
17. A. GAMARRA, C. CUEVAS, G. LESCANO, *J. Ferm. Tehnol*., **64**, 25-28 (1986).
18. W.W. XI, J.H. XU, *Process Biochem*., **40**, 2161-2166 (2005).
19. I. CHIBATA, T. TOSA, *Ann. Rev. Biophys. Bioeng.*, **10**, 197-216 (1981).
20. I. CHIBATA, T. TOSA, T. SATO, *Adv. Biotechnol. Processes*, **1**, 203-222 (1983).
21. G.B. BORGLUM, J.J. MARSHALL, *Appl. Biochem. Biotechnol.,* **9**, 117-130 (1984).
22. J. CARBERRY, *J. Ind. Eng. Chem*., **56**, 39-46 (1964).
23. R.R. DAGUE, C.E. HABBEN, S.R. PIDAPARTI, *Water Sci. Technol.*, **26**, 2429-2432 (1992).
24. S. SUNG, R.R. DAGUE, *Water Environ. Res.*, **67**, 294-301 (1995).
25. L.T. ANGENENT, R.R. DAGUE, *A laboratory-scale comparison of the UASB and ASBR processes,* 50th Purdue Industrial Waste Conference Proceedings, 365-377 (1995).
26. R. ZHANG, Y. YIN, S. SUNG, R.R. DAGUE, *Anaerobic treatment of swine waste by the anaerobic sequencing batch reactor*, 51st Purdue Industrial Waste Conference Proceedings, Ann Arbor Press, Chelsea, Michigan, 315-320, (1996).
27. A.G. BRITO, A.C. RODRIGUES, F.L. MELO, *Water Sci. Technol.*, **35**, 93-198 (1997).
28. L. FERNANDES, K.J. KENNEDY Z. NING, *Water Res*., **27**, 1619-1628 (1993).
29. H. TIMUR, I. ÖSTURK, *Water Res.*, **33**, 3225-3230 (1999).
30. M. BAGLEY, T.S. BRODKORB, *Water Environ. Res*., **71**, 1320-1332 (1999).
31. S.M. RATUSZNEI, J.A. DOMINGUES-RODRIGUES, E.F. MORALES DE CAMARGO, R. RIBEIRO, M. ZAIAT, *Bioresource Technol.*, **87**, 203-209 (2003).
32. J.F. KENNEDY, J.M.S. CABRAL, in *Applied biochemistry and bioengineering* (I. Chibata, L.B. Wingard eds.), vol. 4, Academic Press, New York, 1983, pp. 53-151.
33. P. LINKO, Y.Y. LINKO, *Crit. Rev. Biotechnol.*, **1**, 289-338 (1984).
34. J.H.T. LUONG, M.C. TSENG, *Appl. Microbiol. Biotechnol.*, **19**, 207-216 (1984).
35. G.F. ANDREWS, J. PRZEZDZIECKI, *Biotechnol. Bioeng.*, **28**, 8-2-810 (1986).
36. U.J. TRIVEDI, A.S. BASSI, J. ZHU, *Process Biochem.*, **40**, 2146-2149 (2005).
37. S. BERENSMEIER, M. ERGEZINGER, M. BOHNET, K. BUCHOLZ, *J. Biotechnol.*, **114**, 255-267 (2004).
38. S. JOVETIC, F. MARINELLI, J. TRAMPER, *Enz. Microb. Technol.*, **38**, 34-39 (2006).
39. T. BRANYIK, D.P. SILVA, A.A. VICENTE, R. LEHNERT, J.B. AALMEIDA, E SILVA, P. DOSTALEK, J.A. TEIXEIRA, *J. Ind. Microbiol. Biotechnol.*, **33**, 1010-1018 (2006).
40. P.R. RONY, *Biotechnol. Bioeng.*, **13**, 431-447 (1971).
41. B.R. BRESLAU, U.S. Patent 4,226,026 (1981).
42. W.D. DEESLIE, M. CHERYAN*, Biotechnol. Bioeng.*, **23**, 2257-2277 (1981).

43. W.D. DEESLIE, M. CHERYAN, *Biotechnol. Bioeng.*, **24**, 69-82 (1982).
44. H.N. CHANG, Y.S. KYUNG, B.H. CHUNG, *Biotechnol. Bioeng.*, **31**, 552-557 (1987).
45. B.H. CHUNG, H.N. CHANG, *Biotechnol. Bioeng.*, **32**, 205 -212 (1988).
46. W. BERKE, H.J. SCHUZ, C. WANDREY, M. MORR, G. DENDA, M.R. KULA, *Biotechnol. Bioeng.*, **32**, 130-139 (1988).
47. C.K. LEE, J. HONG, *Biotechnol. Bioeng*., **32**, 647-654 (1988).
48. X. SHAO, Y. FENG, S. HU, R. GOVIND, *AIChE Symposium Series*, **85**, 85-92 (1989).
49. T.E. WILLIAMS, G. CATAPANO, E. KLEIN, R.A. WARD, *AIChE Symposium Series*, **85**, l-9 (1989).
50. Q. YUAN, S. MA, T. QU, C.N. Patent 90105071.7 (1990).
51. G. SHI, X. YU, Q. YUAN, *Int. Chem. Eng.*, **33**, 691-697 (1993).
52. R.L. ULIBARRI, G.M. HALL, Enz. *.Microb. Technol.*, **21**, 398-404 (1997).
53. M.M. RESENDE, S.M. RATUSZNEI, R.C. GIORDANO, *Process Biochem*., **37**, 1297-1303 (2002).
54. I.G. WENTEN, I.N. WIDIASA, *Desalination*, **149**, 279-285 (2002).