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Production of succinic acid in basket and mobile bed bioreactors — Comparative analysis of substrate mass transfer aspects☆

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ABSTRACT

The glucose mass transfer in the biosynthesis of succinic acid with immobilized *Actinobacillus succinogenes* cells has been comparatively analyzed for a bioreactor with mobile bed *vs.* a stationary basket bioreactor. The process has been considered to occur under substrate and product inhibitory effects. The results indicated that the bioreactor with mobile bed is more efficient for biocatalyst particles with a diameter over 3 mm, while the basket bioreactor is more efficient for smaller biocatalyst particles and basket bed thickness below 5 mm. The performances of both configurations of immobilized *A. succinogenes* cell beds were found to be superior to the column packed bed bioreactor.

1. Introduction

Succinic acid has numerous applications in chemical industry (reagents, synthetic resins, biodegradable polymers, electroplating, green solvents, inks), agriculture (pesticides, growth regulators, and stimulators), and pharmaceutical and food industries (amino acids, antibiotics, vitamins, surfactants, additives) [1–3]. At industrial scale, this acid is produced by chemical synthesis using butane *via* maleic anhydride, but this technology raises important problems concerning the environmental protection [1,3]. Depending on the product required purity, this technology cost could reach 6.3 EUROs·kg^{-1} succinic acid, the contribution of raw material to the final cost varying between 16% and 24% [1,3].

The "white biotechnology", concept promoted since 2007, sustains the priority of the use of renewable sources for chemical production by low-expensive and eco-friendly biotechnologies [4]. These premises lead to the increasing of the interest in producing succinic acid by fermentative low-cost technologies. Therefore, a large number of microorganisms have been tested as potential producers of succinic acid (Table 1).

These strains can convert various carbon sources (glucose, saccharose, molasses, glycerol, starch, cellulosic hydrolysates or milling by-products) under anaerobic conditions into succinic acid and secondary acids (formic, acetic, pyruvic acids). However, either due to the low productivity, or to the non-Newtonian rheology and complex composition of the final broth, only the strains *Actinobacillus succinogenes* and *Actinobacillus succiniproducens* have been considered as important producers with potential application at larger scale [8,10]. Among these two strains, *A. succinogenes* allows reaching higher concentration of succinic acid during mixed-acid fermentation [11].

Most of the fermentations for succinic acid production have been carried out using free *A. succinogenes* cells, the processes being affected by the substrate and product inhibition phenomena [8,10]. Although the utilization of immobilized microorganisms or enzymes offers the advantages of the increase of the thermal, chemical, and to the shear forces resistance of the biocatalysts, as well as the diminution or avoidance of

Table 1
Microorganisms used for succinic acid biosynthesis [4–9]

Type of microorganism	Strain
Bacteria	*Veillonella parvula*, *Selenomonas ruminatum*, *Succiniclasticus ruminis*, *Corynebacterium glutamicum*, *Enterococcus fecalis*, *Actinobacillus succinogenes*, *Actinobacillus succiniproducens*, *Mannheimia succiniproducens*, *Escherichia coli*
Yeast	*Saccharomyces cerevisiae*
Fungus	*Aspergillus niger*, *Aspergillus fumigatus*, *Byssochlamys nivea*, *Lentinus degener*, *Paecilomyces varioti*, *Penicillium viniferum*

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the substrate inhibition processes, easier recovery of the biocatalysts from the final broths, and, consequently, the increase of number of the biosynthesis cycles re-using the same particles of biocatalysts, there are only few reports in literature concerning the succinic acid fermentation with immobilized *A. succinogenes* cells [10].

Generally, the bioreactors with immobilized cells or enzymes can be designed as column, stirred, gas-lift or membrane bioreactors, being operated in batch, continuous or semicontinuous systems, with fixed, mobile/stirred, expanded or fluidized bed. The bioreactors with fixed bed of biocatalysts are widely preferred. However, these equipments have some major disadvantages [11,12]. Thus, due to the laminar flow inside the bed, the rates of mass and heat transfer are low and the backmixing or reverse flow phenomenon could be induced. Furthermore, the solid particles from effluent can clog the biocatalyst bed, the effect consisting on the reducing of the flow rate inside the bed and on the possibility to inactivating the biocatalysts. On the other hand, the formation of the preferential flow channels inside the bed leads to the deviation from the plug flow and to the inefficient conversion of the substrate.

For the above reasons, the previous studies on succinic acid fermentation with immobilized *A. succinogenes* cells have been carried out in a bioreactor with mobile bed of biocatalysts and in a stationary basket bioreactor [10,13]. Due to the bioreactor with mobile bed of immobilized bacterial cells constructive and operational characteristics, which are similar to the well-known stirred bioreactors, higher rates of heat and mass transfer have been reached. In the same time, the biocatalyst physical integrity could be affected by the shear forces, this leading to the reduction of the number of successive fermentation cycles [10].

The design of the bioreactor of basket type is derived from the bioreactors with fixed beds, the biocatalyst particles being fixed in an annular cylindrical or conic bed, which is either static and placed around the stirrer, or rotary [13–17]. This bioreactor avoids both the disadvantages of the bioreactors with fixed beds and the flooding/deposition or the mechanical disruption of the biocatalysts particles, phenomena encountered in the bioreactors with mobile beds. As the consequence of the combination between the perfect mixed flow around the basket and the plug flow inside the biocatalysts bed, the hydrodynamics of the medium around the basket exhibits an important influence on the transfer processes involved in the substrate conversion.

The previous studies on fermentations with yeast or bacterial cells immobilized in alginate indicated that their utilization in systems with stirred bed or basket bed of biocatalysts can represent viable alternatives to the processes involving free cells [10,13]. By selecting the optimum operating regime of the two types of bioreactors, the activity and physical integrity of the immobilized cells remained unaffected for many fermentation cycles, even if the fermentation is carried out under substrate inhibition conditions.

In this context, on the basis of the previous results on glucose mass transfer into the liquid phase and inside the particles of immobilized *A. succinogenes* cells [10,13], as well as on its consumption in succinic acid fermentation, the aim of this work is to establish the influence of bioreactor design and operating conditions on the efficiencies of transfer and conversion processes. In this purpose, by assuming that the glucose consumption respects the kinetic model including the substrate and products inhibitions, the rates of external and internal diffusions of the substrate, and their influences on substrate conversion have been comparatively analyzed for two types of bioreactors, with mobile bed and, respectively, basket bed of biocatalysts.

2. Materials and Methods

2.1. Bioreactors

The experiments have been carried out in batch system in two types of bioreactors: a mobile bed bioreactor and a stationary basket bioreactor, both with immobilized *A. succinogenes* cells.

The bioreactor with mobile bed of biocatalysts was a 10 L (8 L working volume) laboratory stirred bioreactor (Biostat A, B. Braun Biotech International), with computer-controlled and recorded parameters [18]. The mixing equipment consisted on two pitched bladed turbines of 64 mm diameter and three baffles. The inferior impeller has been placed at 64 mm from the bioreactor bottom. The superior impeller was placed on the same shaft at a distance of 32 mm from the inferior one. The rotation speed was maintained at 250 $r \cdot min^{-1}$, this value avoiding the "cave" formation at the broth surface, solid phase deposition at the bioreactor bottom and mechanical disruption of the biocatalysts particles. According to the previous results, these impellers combination and rotation speeds were found to be the optimum ones for the investigated fermentation system [19]. Any mechanical damage of the biocatalyst due to the shear forces was recorded during the experiments.

The stationary basket bioreactor was designed by modifying the above presented stirred bioreactor. In this case, the bioreactor was provided with a cylindrical bed of basket type having the inner diameter of 100 mm, height of 100 mm and the bed thickness of 10 mm (Fig. 1). The basket was made by plastic mesh and placed centered around the stirrer, at 100 mm from the bioreactor bottom. The mixing system consisted on two Rushton turbines on the same shaft, the superior one placed outside the basket and the other inside the basket at its inferior extremity [13]. The impeller rotation speed was of 250 $r \cdot min^{-1}$.

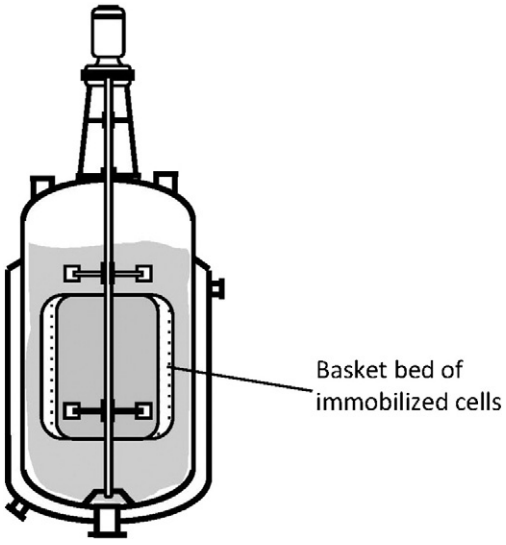

Fig. 1. Experimental stationary basket bioreactor.

2.2. Strain, growth conditions, and cell immobilization

In both cases, the medium composition was (per liter): glucose 30 g, yeast extract 5 g, $NaH_2PO_4 \cdot H_2O$ 1.16 g, Na_2HPO_4 0.31 g, NaCl 1.0 g, $MgCl_2 \cdot 6H_2O$ 0.2 g, $CaCl_2 \cdot 2H_2O$ 0.2 g, vitamin B_{12} 1 μg, biotin 20 μg, folic acid 20 μg, thiamine 50 μg, riboflavin 50 μg, niacin 50 μg, pantothenate 50 μg, *p*-aminobenzoate 50 μg, lipoic acid 50 μg, vitamin B_6 100 μg, $MgCO_3$ 30 g, silicone antifoam 1 ml [20]. The fermentation temperature was 37 °C.

A. succinogenes ATCC 55617 cells immobilized in alginate have been used in the experiments. The microorganism was provided by the American Type Culture Collection and was preserved at −70 °C. The inoculum has been prepared by incubating *A. succinogenes* at 30 °C in 100 ml Duran bottles each containing 50 ml of trypticase soya broth. The bottles were stirred at 100 $r \cdot min^{-1}$ on a rotary shaker for 48 h.

The immobilization has been carried out by bacterial cells inclusion into the alginate matrix, respecting the method given in literature [21]. The biocatalysts were prepared separately for each bioreactor in