Fluidized Bed Design Parameters Affecting Novel Lactic Acid Downstream Processing

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Lactic acid purification was directly done from fermentation utilizing a fluidized bed column refilled with a strong anionic exchange resin. The purpose of this work was to study the influence of two important design parameters, bed-diameter **(D)** and bed-height **(H)**, in the lactic acid binding and elution capacity of the matrix. By changing the settled bed height from 2.5 to 5 cm for each diameter of column analyzed it was possible to obtain an 50% increase in the binding capacity of the resin in all experiments. This fact was attributed to a higher contact time between the culture broth and the anionic resin produced by the increase of back mixing and lactic acid residence time.

Introduction

The science of biotechnology covers the exploitation of microorganisms and cell cultures, which form the major source of high value compounds. The industry today manufactures, on a large scale, compounds that would otherwise have been difficult, if not impossible, to produce in significant quantities for treating many diseases (*1*).

In biotechnology, separation and purification of biomolecules from a large-scale fermentation represents the major manufacturing cost, therefore competitive advantage in commercialization will depend not only on biomolecule production, but also on innovation and optimization of downstream processes (*2*). In recent years considerable advances have been achieved in purification technologies of biomolecules with multiple industry requirements (*3*−*7*). Lactic acid (α-hydroxy-acid) has a great requirement in food industry in sugar confectionery as an additive to make perfectly clear sweets, in bakery products for direct acidification of rye or rye-wheat breads increasing butter stability, and in pickles, jams, jellies, gelatins, and frozen fruit desserts. Production of lactic acid has recently increased due to biodegradable polymers manufacturing for biomedical applications because of their biocompatibility, body absorbability and their reasonable blood compatibility (*8*,*9*).

Recovery process of lactic acid traditionally involves precipitation with calcium hydroxide and separation by filtration of the calcium salt of the acid (*10*). Treatment of the precipitated with sulfuric acid leads to preferential precipitation of $CaSO_4$, which is filtered off. Concentration of lactic acid solution by evaporation and purification by several chromatographic steps are used to achieve the final product specifications.

Other techniques employed are the extraction with organic solvents (*11*−*12*), aqueous two-phase extraction (*13*), and electrodialysis (*14*−*16*).

Afterward, ionic exchange resins have been utilized by (*17*−*19*) obtaining 0.1, 0.18 and 0.2 g LA/g Res (g lactic acid/g resin), respectively. All purification techniques above-mentioned need previously cell removal by filtration or by centrifugation. Those steps mean increases in equipment and energy costs, increase in total process time, and reduction in global yield.

LA (lactic acid) was recovered and purified by using a fluidized bed column refilled with a strong anionic exchange resin: Amberlite IRA-400 achieving 0.126 g LA/g Res (*20*). This methodology is more useful considering the fact that only controlling the ascensional velocity it is enough to washout the biological solids and to bind the lactate to the resin in only one step. A very important advantage that this technique counts with is the use of simply equipments, such as a glass column, an anionic exchange resin, a peristaltic pump, and commercial grade reagents, to carry out the early recovery process of lactic acid from the culture (*21*).

Thus, with the aim to optimize the system configuration, the present work studies the influence of two design parameters, bed-diameter (D) and bed-height (H), on lactic acid binding capacity utilizing fluidized bed columns refilled with anionic exchange resin.

Materials and Methods

Fermentation. LA production was performed at bench scale in a continuous stirred (250 rpm) batch controlled reactor (LH Fermentation Inceltech, France) where the selected microorganism, *Lactobacillus casei* CRL686, was grown at 40 °C. The culture medium (*22*) had the following composition in g/L: yeast extract, 10; $MgSO_4 \cdot 7H_2O$, 0.05; $(NH_4)_2HPO_4$, 2.5; $MnSO_4$, 0.005 and glucose, 40. pH was controlled and maintained at 5.5 by automatic addition of NH_4OH (20%).

The bioreactor was inoculated with a 10% (v/v) inoculum from an overnight flask culture grown in MRS broth. After 48 h of cultivation all glucose was consumed and a final concentration of 45 g/L of LA was obtained.

Microscopical observation of the cells from the culture during the batch fermentation revealed the optimal

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conditions of the pure culture with all the characteristics of *Lactobacillus* as described by Kandler et al., 1986 (*23*) corresponding to regular nonsporing, Gram-positive rods which presented a diameter of rod between 0.5 and 1.1 μm. As particle size and shape depend on the species of *Lactobacilli*, the age and physiological condition of the culture, and the culture medium, no chains were observed in this culture medium as observed in the studies by Bruno-Barcena, 1997.

Pretreatment of Culture Broth. Fermentation broth was previously conditioned in order to remove residual NH_4+ which forms with the lactate ion a slightly dissociated compound that interferes with the binding process of the lactate to the resin matrix. Thus, sequential adjustments to pH 8.0 with 1.25 N NaOH followed of heating at 100 °C during 15 min, were carried out until no NH_4^+ was detected in the culture broth, according to the Berthelot technique (*24*).

Recovery Columns. Two different glass columns, Pharmacia 10/20 (10 mm internal diameter, 20 cm height) and Bio Rad 25/25 (25 mm internal diameter, 25 cm height), were utilized for the fluidized bed experiments. The Pharmacia column was refilled with 1 and 2 g of the anionic exchange resin which had determined two different heights: 2.5 and 5 cm of settled bed, respectively. The Bio Rad column was refilled with 7 and 14 g of the same resin determining 2.5 and 5 cm of settled bed, respectively.

Both columns were provided with flow distributors placed at top and bottom of the recovery columns consisting in a stainless steel screen (500 μm opening) which allowed the passage of cells from the culture broth, but maintaining the resin particles inside the column.

Regeneration of Ion Exchange Resin. An anionic exchange resin Amberlite IRA-400 (Cl^- form) was used for the lactate separation process. The resin was commercially provided (ISO 9002) with chloride ions on its surface, which have high electro negativity as compared to lactate ions. The Cl^- form with a matrix of styrene divinylbenzene copolymer has a specific gravity of 1.063–1.093 and a bulk density of 630–700 g/L, having an effective particle size $\geq$500 μm with a mean particle diameter of 600–800 μm.

The resin with chloride ions had to be replaced with hydroxyl ions for lactate separation. The resin was saturated with 1.25 N NaOH in the fluidized mode and washed with distilled water to remove all the hydroxyl ions not bounded to the resin.

Recovery and Purification Process. After resin conditioning, the unclarified culture broth was fed to the columns in up flow mode by using a variable speed peristaltic pump (Watson Marlow, UK). The anionic exchange resin in the column selectively removes the lactate ions from fermentation broth passing through it. Pharmacia 10/20 column (Amersham Pharmacia Biotech, Sweden) was refilled with 1 g of Amberlite IRA 400; the feeding flow (471 mL/h) was enough to attain in all assays a fluidization of 2-fold of the settled bed volume, allowing lactate ions to bind to the resin, but permitting the biological solids from the culture broth to be washed out (Sosa et al., 2000).

Afterward, the resin was washed with distilled water in order to remove the unadsorbed lactic acid. The columns were conditioned to packed bed mode and 4 N HCl was pumped down flow at 235 mL/h for elution of the lactate bounded. Finally, the resin was again washed with distilled water. Particular care was taken to prevent any air bubbles in the recovery system, as their presence was a common source of bed instability.

Frontal analysis was performed in order to determine the influence of the scaling-up of two design parameters, H and D, on the binding capacity of Amberlite IRA 400. Same operational protocol for all the experiments used an automatic collector device (GradiFrac System, Pharmacia Biotech, Sweden). Fractions of 3 mL of the outlet stream were collected, fraction zero corresponded to the startup of the run, and the sampling was continuous with uniform intervals between fractions. Later on, LA concentration was analyzed in each sample collected.

Same procedure was carried out in the case of Bio Rad 25/25 column, but changes in flow rates were introduced (2935 mL/h) to attain again the condition of fluidization of 2-fold of the settled bed volume and the 4 N HCl was fed at 1467 mL/h during the elution procedure.

Lactic Acid Determination. Lactic acid was determined by HPLC (Beckman, CA) using a Rezex organic acid analysis column (300 × 7.8 mm; Phenomenex, CA) and differential refractometer detector (2142, LKB, Sweden). The injection volume of the sample was 50 μL and the column was eluted with 10 mM H_2SO_4 at a flow rate of 0.6 mL/min. The temperature of the chromatograph column was maintained at 55 °C. The retention time of lactic acid under these conditions was 16.53 min.

Results and Discussion

Conventional biomolecule recovery process unfailingly require a number of complementary steps to perform solid separation prior to the final purification of the desire metabolite and the most frequently physic-chemical operations used in the industry for solids removal are filtration, ultrafiltration, centrifugation and sedimentation.

The novel LA (lactic acid) downstream process described in this work permitted the reduction in the recovery process steps above-mentioned because of the simultaneous solids removal and lactic acid recovery from the culture broth utilizing a unique equipment.

By using an adequate ascensional velocity of 600 cm/h, which is higher than the biological solids terminal velocity, it was possible to maintain the bed fluidization, allowing the lactic acid adsorption to the matrix, the resin beads permanency in the column and biological solids elimination from the column.

Pharmacia 10/20 column was refilled with 1 g of Amberlite IRA-400 reaching a settled bed height of 2.5 cm. The bed was fluidized to 5 cm by feeding 18 mL of the pretreated unclarified culture broth at 471 mL/h. A number of three runs were performed in the same manner and the analysis of the outlet stream indicates that the resin adsorbed in average 0.25 g LA/g Res (g lactic acid per g of resin). Afterward, in the elution procedure it was possible to recover up to 0.12 g LA/g Res. The total LA reported as eluted was composed by the fraction desorbed with hydrochloric acid and the LA contained in the final wash. The volume in which the LA was recovered was near the same of that of the unclarified culture broth fed to the column.

For a new set of assays the Pharmacia 10/20 was refilled with 2 g of resin giving a settled bed height of 5 cm. The flow rate was maintained at 600 cm/h feeding in this case twice the volume fed for the case of 1 g of resin and achieving a final fluidized bed height of 10 cm. Thus, 0.39 g LA/g Res was bounded to the matrix and it was possible to recover 0.34 g LA/g resin in average at the end of the elution procedure.

When utilizing the 2.5 cm diameter column (Bio Rad 25/25) the assays were conducted pouring 7 g of Amber-

Table 1. Summary of Lactic Acid Recovery in Fluidized Bed Columns Using Anionic Exchange Resin: Amberlite IRA-400[a]

column type	settled bed height (cm)	settled bed diameter (cm)	resin (g)	adsorption (gLA/gRes)	elution (gLA/gRes)	eluted (%)
Pharmacia 10/20	2.5	1.0	1.0	0.250	0.12	48.0
Pharmacia 10/20	5.0	1.0	2.0	0.390	0.340	87.2
Bio-Rad 25/25	2.5	2.5	7.0	0.260	0.12	46.2
Bio-Rad 25/25	5.0	2.5	14.0	0.394	0.33	84.0

[a] Influence of the settled-bed height and the settled-bed diameter on the lactic acid recovery process. In all cases, the feeding was carried out in fluidized bed and the elution in packed bed mode. LA = Lactic Acid, Res = resin.

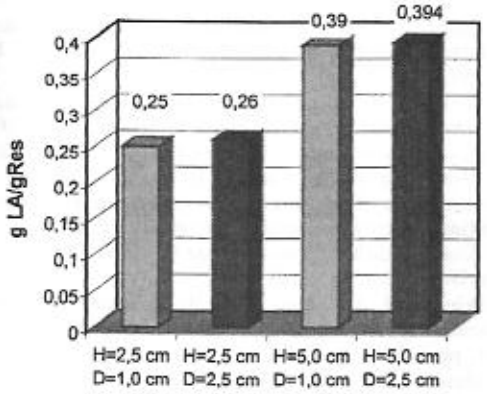

Figure 1. Lactic acid adsorption in Pharmacia 10/20 column (light gray) and in Bio Rad 25/25 (dark gray) column poured with Amberlite IRA-400 and operated in fluidized bed mode at different H y D. The ascensional velocity applied for 2-fold the settled bed was 600 cm/h.

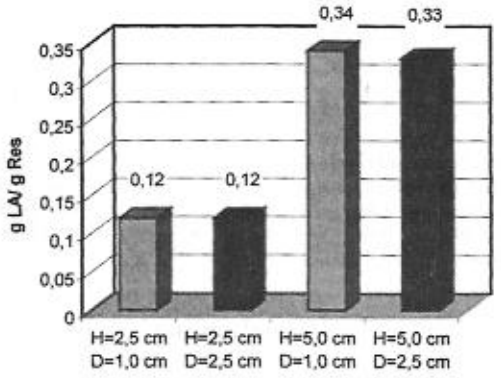

Figure 2. Elution of lactic acid from Amberlite IRA-400 with 4 N HCl. Pharmacia 10/20 column (light gray) and Bio Rad 25/25 column (dark gray) were utilized for the downstream processing modifying H and D parameters in packed bed mode.

lite IRA-400 to conform a settled bed height of 2.5 cm. The feeding was 180 mL of culture broth and the height of the fluidized bed was maintained at twice of the settled bed height, as in all cases studied for the Pharmacia 10/20 column. Three runs were performed in these conditions giving 0.26 g LA/g Res bounded to the resin and the elution resulted in 0.12 g LA/g Res in average. The height of this column was changed to 5 cm by pouring 14 g of resin in the Bio Rad 25/25 column. For these assays, 360 mL of culture broth was treated with the resin resulting 0.394 g LA/g Res bounded to the resin matrix and 0.33 g LA/g Res in average was eluted.

In all cases the lactic acid was recovered using the fluidized bed technique with the resin at 100% of the bed expansion applying an ascensional velocity of 600 cm/h. For elution, the ratio of 4 N HCl to fermentation broth was always equal to 0.5. The volumes of distilled water used for washing the resin after lactate adsorption and lactate elution were the same as that of the fermentation broth volumes fed to the columns.

When the purification process was scaled-up on diameter, from 1 to 2.5 cm, maintaining the height constant, no variations in the resin binding capacity were observed. By changing the settled bed height from 2.5 to 5 cm for each diameter of column analyzed, it was possible to obtain up to 56% increase in the binding capacity of the resin in all experiments as shown in Figure 1.

For lactic acid elution, experimental data showed the same profile as in binding operation, which means that increasing the bed height in both columns, the amount of lactic acid eluted per gram of resin was significantly enhanced up to 180% (Figure 2).

Table 1 shows experimental results as average of three runs for Pharmacia 10/20 column poured with 1 and 2 g of Amberlite IRA-400 and for Bio Rad (25/25) refilled with 7 and 14 g of the same anionic exchange resin. Analysis of the global purification process indicated an improvement from 47% to 86% in average of the total amount of lactic acid recovered when the process was scaled up in height.

The curves of lactate breakthrough were plotted from data obtained from the assays performed in the Pharmacia 10/20 column for 2.5 and 5 cm settled bed height (similar experimental results were drawn from those trials performed in the Bio Rad column). Analysis of each of the 3 mL fractions collected were performed in order to determine gradually the changes of lactate concentration in the outlet stream of the column. Data were expressed as C/Co (%), where C is the LA concentration of each fraction and Co represents the initial concentration of lactic acid in the unclarified culture broth. In Figure 3 the C/Co ratio was plotted versus the volume of adsorbate solution applied to the fluidized beds as the set parameter (3 mL) in the Gradi Frac fraction collecting device. A qualitative analysis of both curves of lactate adsorption onto the resin, indicated that the curves were different in shapes. The curve of lactate breakthrough resulted somewhat sharper when the settled bed was 2.5 cm than in the case of 5.0 cm height, but the resin was saturated earlier in the first case than in the second case mentioned. Dynamic binding capacities of Amberlite IRA-400 were calculated from the curves and Equation 1, revealing that $q = 0{,}26$ g of lactate acid/g of resin for 2.5 cm of settled bed height and $q = 0.39$ g of lactate acid/g of resin for 5.0 cm of settled bed height.

$$q = \frac{V_{1/2}{}^{*}Co}{R} \qquad (1)$$

Where q is the dynamic binding capacity (g LA/g Res), Co is the initial concentration of lactic acid in the unclarified culture broth (g LA/L), $V_{1/2}$ is the volume corresponding to 50% of C/Co (L) and R is the amount of resin poured in the fluidized column (g).

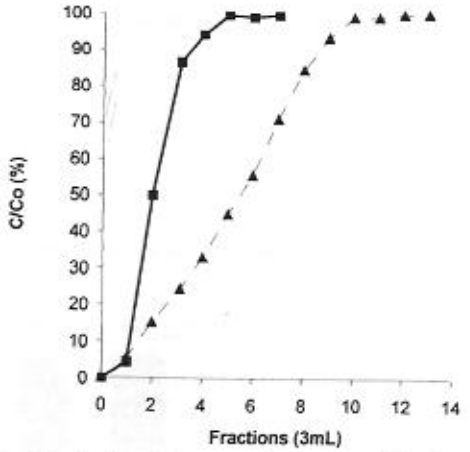

Figure 3. Breakthrough curves for lactic acid adsorption in fluidized bed columns. Inlet lactic acid concentration 45,0 g/L. (■) Lactic acid adsorption breakthrough in Pharmacia 10/20 column refilled with 1 g of resin. (▲) Lactic acid adsorption in Pharmacia 10/20 column poured with 2 g of resin.

Conclusions

The binding capacity of the resin was constant during the lactic acid downstream process when the columns were scaled-up on diameter, keeping constant the bed height.

Substantial increase (50%) in lactic acid adsorption to Amberlite IRA-400 was achieved in the case of scaling-up the H parameter maintaining constant the diameter of the columns.

The same ratio of grams of lactic acid to grams of resin was fed to both columns in all experiments. Thus, the great lactic acid recovery attained was not dependent on the increase of the resin poured to the columns but due to the increase in the hydraulic residence time and back mixing which resulted in a high contact time between lactate ions and resin particles.

There is a further need to study the effect of other parameters on the performance of lactic acid purification process utilizing fluidized bed columns, taking into account the developing of this procedure in larger scale.

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