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Modeling of direct recovery of lactic acid from whole broths by ion exchange adsorption

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Abstract

Lactic acid fermentation process with *L. casei* CRL 686 was performed. The static adsorption isotherm over a strong anionic exchange resin, Amberlite™ IRA-400 was measured, and the static binding capacity parameters were quantified. Early recovery of lactic acid from this lactate producer from unclarified culture broth was performed in a liquid solid fluidized bed, with the resin as the solid adsorbent, and the dynamic adsorption capacity was calculated. Good agreement was found between static and dynamic binding capacity values. The fluidized bed height was twice the settled bed height and the overall process was controlled by the liquid solid mass transfer. This operation was also simulated by continuously well stirred tanks arranged in series and superficial solid deactivation as in a gas solid catalytic reactor. The deactivation process takes into account liquid channeling and agglomerations of solid induced by the viscosity of the broth and also by the cells during the adsorption. These patterns were also verified by experimental observations, and are in agreement with the results found in the literature. The breakthrough data together with others from previous works were satisfactorily fitted until the 90% dimensionless concentration was reached for both culture broths. The model could be used in future studies on predictions about the liquid solid fluidized bed behavior and other different operating conditions.

Introduction

Both the food and polymer industries use lactic acid sourced from a racemic mixture obtained from chemical synthesis. In contrast, the biological production provide a high concentration of one of the isomers (L+) giving better quality products, but the low concentration of the lactic acid produced by bioconversion generally results in a high cost of recovery and purification (Córdoba et al., 1996). Moreover, the rise in the global process efficiency and the development and process evaluation of early recovery of biomolecules is a key factor to make competitive the downstream scale up of biotechnological processes.

The biological systems are commonly aqueous by nature and strongly influenced by ionic interactions. In order to perform an early and direct recovery of lactic acid it seems reasonable that the ionic exchange process plays a major role in the downstream separation process, which also gives lower separation costs. Data related to these processes is scarce because of a large number of industrial patents on this subject (Asenjo, 1990).

During the fermentation process for lactic acid it is desirable to avoid product inhibition, which diminishes fermentation productivity, and to establish a simultaneous efficient recovery. The development and optimization of fermentation and simultaneous recovery of lactic acid processes are strongly dependent on accurate, real-time control of chemical and physical process variables (González-Vara et al., 2000). Planas et al. (1999) mentioned the most widely used processes for recovery of lactic acid, alternative techniques and presented a novel aqueous two phase system (ATPS) with the same purpose.

Another strategy was recently improved by Córdoba et al. (1999) for lactic acid recovery from unclarified culture in a simple fluidized bed. Amberlite™

IRA-400, a strong anionic exchange resin, was used for recovering lactic acid from a fermented broth. A fermentation process with *Lactobacillus casei* ADNOX (LA1) over an immobilized bed reactor was performed. The static and dynamic adsorption capacities were evaluated. The effect of different broth conditioning cycles – heating time and pH values – and different contact times were also studied (Córdoba et al., 1999).

Thus, the reported adsorption values for lactic acid were almost four hundred percent higher than previous works (Córdoba et al., 1996). The laboratory results obtained were satisfactory, encouraging a follow up with this early separation technique in order to achieve scale-up.

In this sense, the aim of the present work was to obtain lactic acid with *Lactobacillus casei* CRL 686 (LA2) in a batch reactor, followed by the characterization of the static adsorption of lactate over the Amberlite^{TM} IRA-400 ion exchange resin. Afterwards, to present a model for the extraction of lactic acid in a single purification step, using the strong anionic exchange resin in a fluidized bed column. The breakthrough experimental data from own and previous work was also fitted.

Materials and methods

The unclarified culture broth was obtained from a batch reactor. *Lactobacillus casei* CRL 686 (LA2) lactate producer was used, the procedure is detailed below.

Fermentation

Lactobacillus casei CRL686 was grown in a medium containing the following composition in g/l: yeast extract, 10; $MgSO_4 \cdot 7H_2O$, 0.05; $(NH_4)_2\ HPO_4$, 2.5; $MnSO_4$, 0.005 and glucose, 40. pH was maintained at 5.5 by adding NH_4OH (20%) and the temperature was controlled at 40 °C. After 48 h of cultivation in a batch reactor, a final concentration of 43.9 g/l of lactic acid was obtained in the broth.

Pretreatment of culture broth

In order to remove residual NH_4^+, the fermentation culture broth was conditioned by sequential adjustments to pH 8.0 with 1.25 N NaOH followed by heating at 100 °C during 15 min, until no NH_4^+ was detected.

Recovery columns

Pharmacia 10/20 column (10 mm internal diameter and 20 cm height) filled with 2 g of the anionic exchange resin was used. Two adapters were necessary to obtain the desired bed heights for fluidized and packed beds.

Regeneration of ion exchange resin

To conditioning the resin to the OH^- form, 15 ml of 1.25 N NaOH was pumped to the column in upflow mode. The resin was later taken up to neutrality with distilled water pumped in upflow mode.

Recovery and purification process

Once the resin was conditioned, the unclarified culture broth was fed to the column in upflow mode by using a variable speed peristaltic pump to attain a fluidization of two fold the settled bed volume. Afterwards, the resin was washed with 40 ml of distilled water. For elution, 20 ml of 4.0 N HCl was pumped downflow to the packed bed column. Finally, the resin was washed with 40 ml of distilled water. In both cases, culture broth was fed to the column until the outlet concentration was the same as the inlet one.

3 ml fractions of unclarified culture broth were collected at the outlet of the column. The dynamic binding capacity was analyzed in those fractions.

Lactic acid determination

In all experiments lactic acid determination was done by an enzymatic and colorimetric reaction using a lactate reagent provided by SIGMA (Cat. No. 735-10).

Adsorption isotherms

Amberlite^{TM} IRA-400 characterization was performed by preparing solutions of the unclarified culture broth at different concentrations (10%, 20%, 40%, 60%, 80% and 100%) diluted with distilled water; 10 ml of those solutions were mixed with 0.5 g of resin (dry weight) in glass flasks; a period of 60 min was required until adsorption equilibrium was reached.

Results and discussion

Static adsorption of LA2 on the resin was made calculating the maximum capacity (Q_{max}) and K_D coefficients, based on the Langmuir equation. The batch

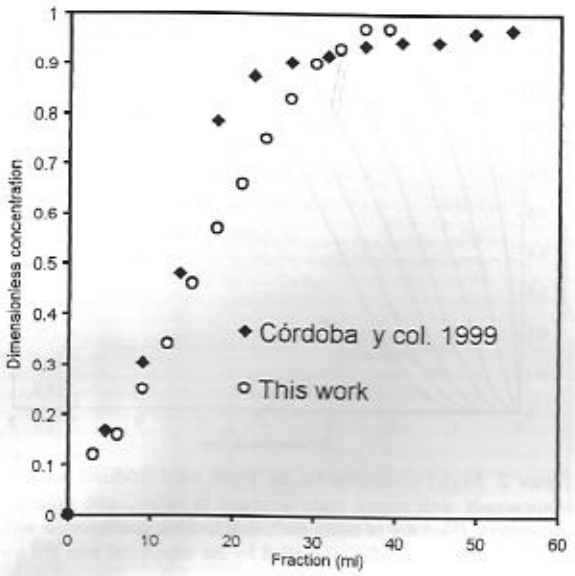

Figure 1. Breakthrough curves for *Lactobacillus casei* ADNOX (LA1, ♦ u = 720 cm hr^{-1}, C_o = 53.6 g l^{-1}) and *Lactobacillus casei* CRL 686 (LA2, ○ u = 600 cm hr^{-1}, C_o = 43.9 g l^{-1}).

adsorption experiments gave a Q_{max} of 0.342 g LA/g resin while the K_D coefficient value was 0.0207. In previous works Córdoba et al. (1999) working with different source of lactic acid (LA1) found that Q_{max} = 0.125 g LA/g resin and a K_D value of 0.00097.

Lactic acid from fermentation was early recovered in a fluidized bed column filled with Amberlite™ IRA-400. Once the culture broth was conditioned for ammonium elimination, it was analyzed for frontal analysis in order to evaluate the dynamic binding capacity of the resin. Breakthrough curves of LA1 and LA2 are shown in Figure 1.

The resulting dynamic binding capacity was 0.35 g LA/g resin for unclarified culture broth. A value of 0.36 g LA/g resin was previously found by Córdoba et al. (1999). Similar dynamic binding capacities were achieved in both cases, as was expected.

Although dynamic binding capacities obtained by frontal analysis in both cases were almost the same, some differences could be appreciated between the static binding capacities evaluated from adsorption isotherms. From the comparison between the dynamic and static (Q_{max}) binding capacity values calculated in this work, more similar values are found with respect to those encountered by Córdoba et al. (1999). Differences on the batch adsorption isotherms procedure could have been responsible for the not similar Q_{max} values, suggesting that a lower mass of resin to solution volume ratio could be better for reaching to the equilibrium condition.

Particle bulk density (ρ_b) value of 0.64 g ml^{-1} was taken (Perry and Chilton, 1982). The superficial velocity, u, were 12 cm min^{-1} and 10 cm min^{-1} for the Pharmacia columns: 10/40 and 10/20, respectively.

The evaluation of the separation factor, S (Perry and Chilton, 1982), was done in order to establish the determinant step of the whole process. The corresponding equation for S is the following:

$$S = \left(1 + \frac{C_o}{K_d}\right)^{-1} \quad (1)$$

where C_o (g l^{-1}) is the lactic acid concentration in the culture broth and K_d (g l^{-1}) the inverse of the equilibrium constant. LA concentration value of 53.6 and 43.9 g l^{-1} for both LA1 (Córdoba et al. 1999) and LA2 were employed, respectively. For the LA1 and LA2 producers, K_d values are 0.000977 g LA ml^{-1} (Córdoba et al. 1999) and 0.0207 g LA ml^{-1}, respectively. Then, a S value proximate to zero were encountered in both processes and the whole process was governed by the mass transfer.

The solid liquid fluidized bed model was written on the basis of the literature encountered hydrodynamic behavior for the solid and liquid phases and the operating conditions attained in the present study. Changes in the quality of fluidization are also of major importance whenever a fluidization process is scaled up (Harrison et al., 1961). Agglomeration of solid and channeling effect were observed specially when, particle diameter, d_p < 0.1 cm. As an example, in the present work d_p=0.12 cm. These agglomerations resulted from the interaction between neighborhood particles when a viscous liquid was present (Carlos and Richardson, 1968). They could be explained because the cross sectional area rise proportionally with porosity and velocity profiles through the column (Fan et al., 1985). Inhomogeneities in the solid distribution near the column distributor were also mentioned by Khan and Richardson (1995) and Di Felice (1995). The latter author published a table with a large number of experimental research articles in which this phenomenon was verified.

Finally, the turbulence at the exit of the distributor originated by the liquid could enhance agglomerations of solids, which were experimentally visualized by ascendant patterns in the center and descendent patterns near the wall. Bascoul et al. (1988) gave evidence for this behavior, particularly for short columns, independently whether an inert fluidized or fixed bed

was put before the adsorption column. A more pronounced agglomeration effect was observed experimentally along the column until the culture broth cells left the fluidized bed.

Development of the model equations were based on two major articles. First, liquid axial mixing in the adsorption column was represented by N well stirred vessels, according to Buijs and Wesselingh (1980). Because the ratio between the column diameter, D (cm^{-1}) and particle diameter d_p (cm) was greater than 4, radial dispersion was not considered. Finally, a deactivation process was assumed over the superficial area of the resin, in analogy with fouling in catalysis (Carberry, 1980), so as to represent the experimental inhomogenieties on the solid patterns and a less disposable superficial area for the biomolecules adsorption process. The assumptions were resumed in the following statements:

(1) Axial liquid mixing is represented by the number of agitated vessels N.
(2) Well stirred mixing solids in each tank.
(3) Adsorption equilibrium is favorable ($S \rightarrow 0$).
(4) The whole process is governed by the liquid solid mass transfer.
(5) Inhomogenieties on the solid flux pattern and accessible superficial area to biomolecules was taken into account by a superficial area deactivation coefficient (α), in a similar way as in catalytic gas solid reactions.

According to Belter (1988), the expression for the lactic acid (LA) mass balance in each well stirred vessel was

ENTRANCE = EXIT + ACCUMULATION + ADSORPTION ON THE RESIN

Thus, by considering constant the volumetric flow rate Q, (ml min^{-1}), porosity, ϵ, the feed concentration C_o, (g l^{-1}), and the mass transfer coefficient the mathematical equations were

$$QC_o = QC_1 + \epsilon V_1 \frac{\delta C_1}{\delta t} + (k_l a_p) e^{(-\alpha t)} V_1 C_1 \quad j = 1 \tag{2}$$

$$QC_{j-1} = QC_j \epsilon V_j \frac{\delta C_j}{\delta t} + (k_l a_p) e^{(-\alpha t)} V_j C_j \quad j = 2, \ldots N \tag{3}$$

where V_j (j=1, 2, ... N) is the tank volume (ml), $k_l a_p$ is the liquid solid mass transfer coefficient (min^{-1}) and α is the superficial deactivation coefficient (min^{-1}).

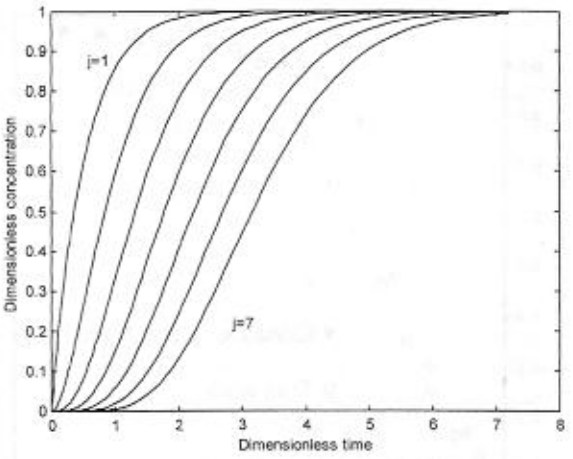

Figure 2. Model simulation for the liquid solid fluidized bed as a continuously well stirred tanks arranged in series, with arbitrary parameters. (Number of tanks N=7, deactivation coefficient α = 5, liquid solid mass transfer multiplied by the superficial area $k_l a_p$ = 5.)

The set of differential ordinary non linear dimensionless equations were solved with the help of a Runge–Kutta routine. The corresponding expressions are the following:

$$\frac{\delta y_1}{\delta \theta} = 1 - \left[1 + (k_l a_p) e^{-\alpha t} \left(\frac{L_f}{Nu}\right)\right] y_1 \tag{4}$$

$$\frac{\delta y_j}{\delta \theta} = y_{j-1} - \left[1 + (k_l a_p) e^{-\alpha t} \left(\frac{L_f}{Nu}\right)\right] y_j \quad j = 2, \ldots, N \tag{5}$$

where $y_j = C_j/C_o$ is the dimensionless exit concentration of lactic acid from the vessel j and L_f is the fluidized bed height (cm); u is the superficial velocity (cm min^{-1}). The initial conditions are given by $y_j = 0 \; \forall \; j$. A dimensionless time, θ, was defined and the equation is the following:

$$\theta = \frac{t}{\left(\frac{\epsilon L_f}{N_u}\right)} \tag{6}$$

Simulation results for the dimensionless concentration C_j/C_o (solid lines) versus the dimensionless time is presented in Figure 2 with arbitrary parameters. The dimensionless concentration curve at the exit of the adsorption column (tank j = 7) is called the breakthrough curve, because it is comparable with the dimensionless concentration obtained from the collected fractions in the experimental runs. The more sigmoidal curve is represented by a high dynamic binding capacity. In these sense, the graph clearly showed that the major number of serial agitated tanks

Table 1. Experimental liquid solid fluidized bed model parameters for *Lactobacillus casei* ADNOX (LA1) and *Lactobacillus casei* CRL 686 (LA2)

Parameter	
Fluidized bed length, L_f (cm)	10
Mass transfer coefficient, k_l (cm s^{-1})	0.156
Superficial area, a_p (cm^2 cm^{-3})	120.11
Fluidized bed porosity, ϵ	0.65

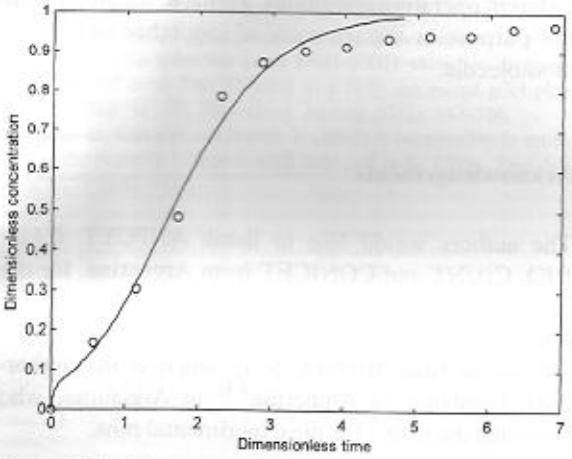

Figure 3. Experimental *Lactobacillus casei* ADNOX data (LA1 open symbol) versus numerical model (solid line). Fitted parameters: number of tanks N = 1 and α=2.5.

(N) the more adsorption capacity was reached for a given α value.

Table 1 summarizes the fluidized bed height, L_f, the mass transfer coefficient k_l (cm min^{-1}) (Arters et al., 1988), the superficial area a_p (cm^2 cm^{-3}) (Ramachandran and Chaudari, 1983) and porosity ϵ (Hartman et al., 1992).

Experimental data were fitted adjusting the deactivation coefficient, α, and the number of well stirred

Table 2. Fitted parameters for the breakthrough experimental data for *Lactobacillus casei* ADNOX (LA1) and *Lactobacillus casei* CRL 686 (LA2)

Parameter	LA1	LA2
Inlet Lactic Acid, C_o (g l^{-1})	53.1	43.9
Superficial velocity, u (cm min^{-1})	12	10
Deactivation coefficient, α (min^{-1})	2.5	1.8
Residue	$2.5\ 10^{-2}$	$4.5\ 10^{-3}$
Culture broth volume (ml)	54	39

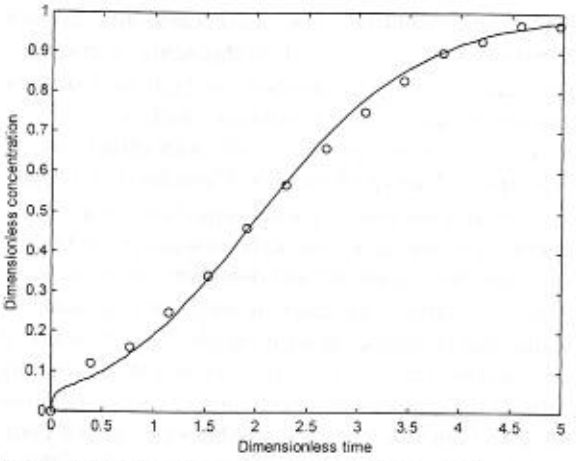

Figure 4. Experimental *Lactobacillus casei* CRL 686 data (LA2 open symbol) versus numerical model (solid line). Fitted parameters: number of tanks N = 1 and deactivation coefficient α = 1.8.

vessel, N. The residue function, R, was minimized and the corresponding experimental data (open symbol) versus simulation curves (solid line) are presented in Figures 3 and 4. The experimental data were satisfactory fitted, up to 90% of the maximum dimensionless concentration value for the two experimental data set. Table 2 resumes the different α coefficient and residue values found, in both cases the number of agitated tanks was N=1. These results were also confirmed by the solid particles pattern seen in the column through the experimental runs. The corresponding expression for the residue is

$$R = (y_i - y_{exp})^2 \; i = 1, 2, \ldots, M \qquad (7)$$

The same number of agitated tanks (N = 1) were estimated. An average residence time for the solid is defined by Bascoul et al. (1993) as the corresponding to twice the individual solid particle circulation time through the column. Following them, an estimation of this value was done for our system. This time was compared with the corresponding to the liquid solid mass transfer. Because the later is higher than the former our system could be represented by a completely stirred tank, which can explain the unitary value of N.

Two different values of the deactivation coefficient (α) were found for both processes. A proportional relationship between superficial velocity, u, and the deactivation coefficient, α, was encountered, following the expected trends pointed out in the literature (Davidson et al., 1961; Carlos and Richardson, 1968; Khan and Richardson, 1985; Di Felice, 1995). This

relationship could be explained because two distinct distributors were employed. In this sense, a lower superficial velocity was attained for high hole density and low pressure drop distributor (Asif et al., 1992), as was used in the present work, with respect to the experimental runs performed by Córdoba et al. (1999).

A well defined solid particle circulation was experimentally verified, and had to be upward in the middle and near the distributor, and downward near the wall. The descending aggregates of solid moving near the wall could be responsible for the observed channeling and agglomerations. The viscosity and the ascending cells of the culture broth could also be responsible of the effects mentioned above. Otherwise, such a complex behavior was found well represented by the α parameter. At the top of the fluidized bed no marked tendency was found. These remarks were according to previous works published in the literature Latif and Richardson (1972).

Conclusions

Laboratory experiments for the fermentation process and the adsorption of *L. casei* CRL 686 were performed. The recovery of lactic acid was achieved in a Pharmacia 10/20 column filled with a strong anionic exchange resin Amberlite^{TM} IRA-400, operated as a liquid solid fluidized bed, and the breakthrough data was obtained. From the comparison between dynamic and static binding capacity adsorption values with previous works, a closer agreement was found for present results. Differences with previous articles were pointed out, suggesting that more precise static adsorption values were obtained with the technique presented in this work.

Whether the whole process was governed by the liquid solid mass transfer and the absence of residual NH_4^+ is true, modeling the adsorption of lactic acid in fluidized liquid solid bed filled with a strong ionic exchange resin was well represented by a continuously well stirred tanks arranged in series.

The mathematical dimensionless model for the recovery of lactic acid in a liquid solid fluidized bed, found from two different sources, was in agreement with preliminary experimental data. The exit concentration within a mass transfer controlled process was encountered to be a function of a deactivation process on the superficial area (a_p) , liquid solid mass transfer coefficient (k_l), fluidized bed height (L_f), porosity (ϵ), superficial velocity (u) and liquid axial mixing (N). Only two parameters were needed: the number of continuously well stirred tanks arranged in series (N), which is a measure of the liquid axial mixing, and the superficial area deactivation coefficient (α). Experimental data from two different sources of lactic acid were successfully fitted with this model.

These approximations on the modeling of a liquid solid fluidized bed governed by the liquid solid mass transfer could be useful in the future for preliminary predictions on the performance of the column with different operating conditions. Perhaps, for either control purpose or the recovery of any other interesting biomolecule.

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