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Resin Selection and Single-Step Production and Recovery of Lactic Acid from Pretreated Wood

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Abstract

Four ion-exchange resins (Amberlite IRA 900, IRA 400, IRA 96, and IRA 67) were employed for lactic acid recovery from simultaneous saccharification and fermentation (SSF) media. The best resins (Amberlite IRA 900 and IRA 400) were assayed for capacity, regenerant consumption, percentage of lactic acid recovery, and product concentration. Almost quantitative lactic acid recoveries at constant capacities were achieved in four sequential loading/regeneration cycles. A strong-base resin (Amberlite IRA 400) was selected for intermittent lactic acid separation in a typical SSF process, in which pretreated wood was saccharified by cellulases in the presence of *Lactobacillus delbrueckii*. The dynamics of lactic acid generation and lactic acid recovery were established.

Index Entries: Ion-exchange resin; lactic acid; *lactobacillus delbrueckii*; separation; simultaneous saccharification and fermentation.

Introduction

Lactic acid is widely employed in both food and chemical industries. Even though it can be obtained by chemical synthesis, the biotechnologic route has became the most important one for lactic acid production owing to the consumer's preference for products of natural origin *(1)*. The biotechnologic production of lactic acid is based on the fermentation of sugars such as sucrose, glucose, and lactose by bacteria or fungi *(2–6)*.

Alternatively, lactic acid can be directly produced from cellulosic materials by simultaneous saccharification and fermentation (SSF). This

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technology, first employed for ethanol production *(7)*, is suitable because the inhibition of enzymatic activity caused by lactic acid is lower than that caused by sugars coming from enzymatic hydrolysis, and because the operational conditions (composition of media, pH, and temperature) are compatible for saccharification and fermentation *(8)*.

Takagi *(8)* proposed that SSF can be used for lactic acid production based on the limited inhibition caused by this compound on cellulases from *Trichoderma reesei*. This idea was applied to the bioconversion of pure cellulose and newspaper in a further study *(9)*. Previous works *(10,11)* dealt with the SSF conversion of pretreated wood using single or multiple substrate additions. In these studies, it was found that cellobiose (an intermediate product of cellulose saccharification) was directly metabolized by *Lactobacillus delbrueckii*, keeping a low cellobiose concentration in the fermentation medium. In comparison with separate saccharification and fermentation, the SSF processing of wood resulted in higher concentrations, yields, and productivities *(10,12)*.

The final product concentration and the product-to-enzyme ratio in SSF processing can be increased by multistep feeding, because the enzyme can be used until exhaustion and the stirring problems caused by high substrate concentrations are avoided. However, cellulase inhibition becomes important when these enzymes are kept in media containing moderate lactic acid concentrations during long periods *(8)*. On the other hand, it has been proved that moderate lactic acid concentrations (mainly the undissociated form) cause an important inhibitor effect on microorganisms *(13)*. Other investigators *(14,15)* observed that the lactic acid removal during fermentation of glucose or sucrose improved the results obtained in a single batch.

In multistep feeding SSF, enzymes and microorganisms remain for several days in the presence of moderate lactic acid concentrations (30–50 g/L) *(11)*. On the basis of the discussed ideas, lactic acid recovery during the SSF should result in a higher productivity.

This work deals with the experimental assessment of coupling an ion-exchange stage for intermittent recovery of lactic acid during an SSF process by means of an automatized system. An ion-exchange resin in hydroxyl form (necessary for neutralizing lactic acid) was selected and used in lactic acid recovery. With the proposed operational scheme, the pH was kept near its optimum value without the addition of external alkali. Multistep feeding was employed to maximize the product-to-enzyme ratio, leading to a final fermentation broth containing enzymes susceptible to being reutilized.

Materials and Methods

Preparation of Resin

The anion-exchange resins Amberlite IRA 900, IRA 400, IRA 96, and IRA 67 employed were obtained from Rohm and Hass. Table 1 presents their main characteristics. Resins were washed and converted into their

Table 1
Characteristics of Resins

Resin	Matrix	Active group	Supplier's form	Basicity	Lactic acid capacity (meq/g)	pH range
Amberlite IRA 900	Macroreticular	Quaternary ammonium	Cl^-	Strong	4.2	0–14
Amberlite IRA 400	Gel	Quaternary ammonium	Cl^-	Strong	3.8	0–14
Amberlite IRA 96	Macroreticular	Polyamine	Free base	Weak	4.7	0–7
Amberlite IRA 67	Gel	Polyamine	Free base	Weak	5.6	0–7

OH^- form. Because of their own nature, Amberlite IRA 67 and IRA 96 could not be obtained in OH^- form (both resins remain in free base form at pH > 9.0) *(16)*.

Preparation of Aqueous Solutions of Lactic Acid

Samples of preset concentrations were heated near the boiling point to hydrolyze the lactic anhydride, cooled, and stored at 4°C. Lactic acid was determined by high-performance liquid chromatography (HPLC) before use (see Analytical Methods).

Effect of Temperature

An aqueous solution (20 mL) containing 37 g of lactic acid/L was contacted with 2 g of dry resin in 50-mL Erlenmeyer flasks placed in an orbital shaker (150 rpm, 25 and 45°C). The initial pH was kept in the range of 4.80–4.85 by alkali addition, and the flasks were shaken for 24 h (long enough to reach equilibrium). Then samples of supernatants were withdrawn and analyzed for lactic acid by HPLC (see Analytical Methods).

Equilibrium and Selectivity

Dry resin (2 g) and 20 mL of either aqueous lactic acid solutions (with or without cellobiose and glucose) or SSF media were contacted in 50-mL flasks at 45°C. The initial pH was fixed at 4.85 (the optimum value for SSF). When equilibrium was reached, samples of supernatants were withdrawn and analyzed for their contents in lactic acid, glucose, and cellobiose by HPLC.

Fixed-Bed Operation

A glass column (6-cm length, 1.2-cm id) was charged with 2.90 g of resin, filled with water, vacuum-drained to determine the interstitial volume, and refilled with distilled water. Then, a solution containing 25 g of lactic acid/L was pumped through the column at a flow rate of 3.5 mL/min.

Fractions of the effluent were collected and analyzed for lactic acid. The resin was considered to be saturated when the lactic acid concentration in the effluent reached the same value as the feed. The interstitial solution was removed by pumping distilled water until the lactic acid concentration of the effluent was <0.2 g/L. Lactic acid was recovered by pumping 1 *N* HCl through the column. Samples of the effluent were collected and analyzed for lactic acid until the outlet concentration was <0.1 g/L. Before a new utilization, the resins were washed with distilled water to remove the interstitial HCl and converted into their OH^- form or free base form by pumping 1 *N* NaOH, and a new washing step was carried out.

SSF with Coupled Lactic Acid Recovery

SSF of processed *Eucalyptus* wood *(11)* was carried out in a 2-L bioreactor with a 1.0-L working volume. A medium (containing water and processed wood at a liquor-to-solid ratio of 30:1, 5 g of yeast extract/L, 10 g of peptone/L , 5 g of sodium acetate/L, 2 g of sodium citrate/L, 2 g of K_2HPO_4/L, 1 mL of Tween-80/L, 0.58 g of $MgSO_4 \cdot 7H_2O$/L, 0.12 g of $MnSO_4 \cdot H_2O$/L, and 0.05 g of $FeSO_4 \cdot 7H_2O$/L) was sterilized in an autoclave. After temperature regulation, an inoculum of *L. delbrueckii* NRRL-B445 (10% [v/v]) and the amount of cellulases from *T. reseei* needed to achieve an enzyme:substrate ratio of 28 filter paper units/g of solid were added to the medium. Temperature was kept at 45°C. A new addition of substrate (in the same amount employed initially) was carried out after 10 h. A column charged with 166 g of selected resin in hydroxyl form was attached to the bioreactor. During fermentation, a device governed by the pH controller of the fermentor was used to pump the culture medium through the column containing the ion-exchange resin (Fig. 1). At preset times, the whole system was stopped and a sample of the fermentation medium was withdrawn and analyzed for lactic acid, glucose, and cellobiose, whereas the ion-exchange resin was regenerated and the lactic acid content of the effluent was also determined by HPLC. The ratio between the amount of lactic acid retained by the resin and the amount of free lactic acid remaining in the fermentation medium was defined as the recovery factor (RF).

Analytical Methods

Lactic acid and sugars were determined by HPLC using a Hewlett Packard chromatograph fitted with an RI detector and an ION-300 column (mobile phase = 0.003 *M* H_2SO_4).

Results and Discussion

Effect of Temperature

As has been shown in previous studies *(10)*, the optimum temperature for lactic acid production by SSF is 45°C. During the SSF process with intermittent product recovery, a stream of medium has to be withdrawn

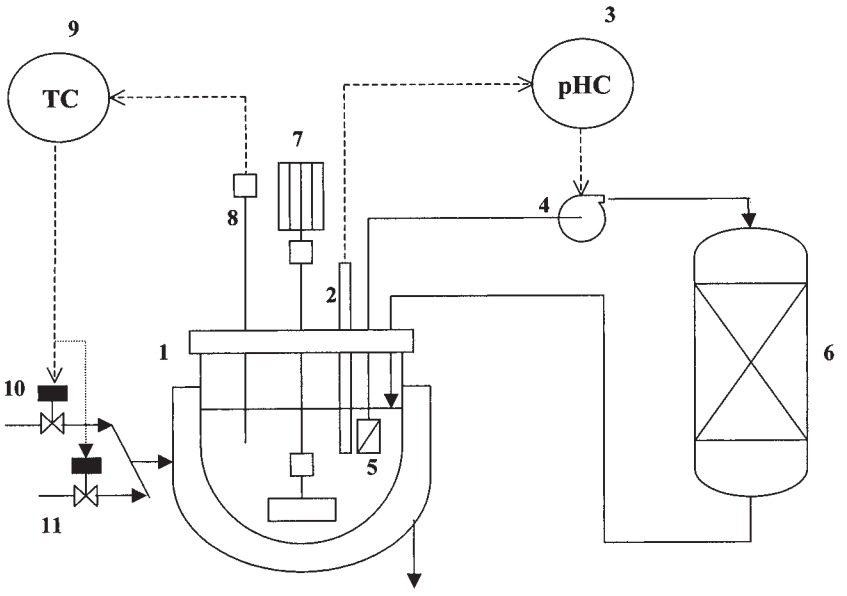

Fig. 1. Principle of experimental device employed.

and passed through the column containing the resin. The resin capacities were determined at 25 and 45°C using aqueous solutions of commercial lactic acid to assess the possible benefits derived from cooling the SSF media before lactic acid separation.

Figure 2 shows the results obtained. Since the equilibrium capacities of the resins were almost not influenced by temperature in the studied range, it can be concluded that the recovery of lactic acid can be carried out at 45°C, the optimum temperature for SSF.

Ion-Exchange Isotherms

The equilibrium capacity is one of the key parameters to be considered in the selection of an ion-exchange resin for a given objective. The experimental determination of resin capacities was carried out under the optimum operational conditions for SSF (45°C, initial pH of 4.85). Figure 3 shows the equilibrium isotherms for the resins considered. Strong-base resins showed the highest capacities, which is in agreement with data in the literature *(16)*. Two simultaneous phenomena happen when strong-base resins are used in OH^- form *(17)*: ion exchange (hydroxyl ions are exchanged with lactate ions because of their different affinity), and neutralization reactions with the hydronium ions present in the medium.

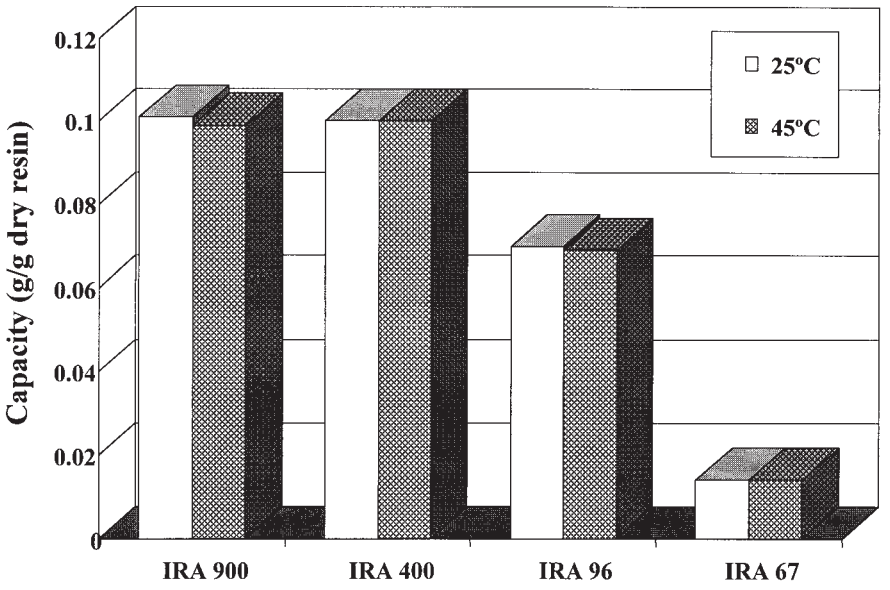

Fig. 2. Influence of temperature on resin capacity (initial concentration = 37 g of lactic acid/L; initial pH = 4.85).

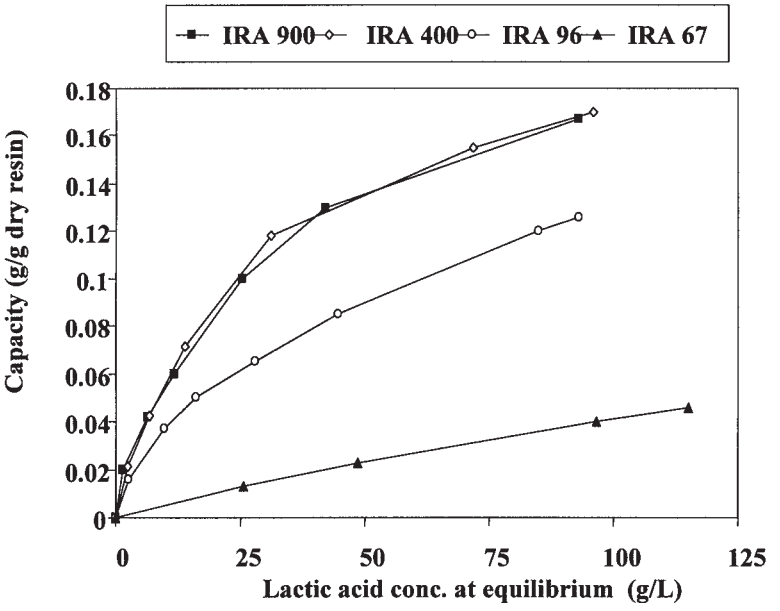

Fig. 3. Ion-exchange isotherms (temperature = 45°C, initial pH = 4.85).

Both effects tend to increase the pH of media (*see* Fig. 3), leading to increased (lactate)/(molecular lactic acid) ratios owing to the displacement of the equilibrium. By contrast, when a weak-base resin in free-base form is treated with NaOH, the resin remains equal, the only possible mechanism being

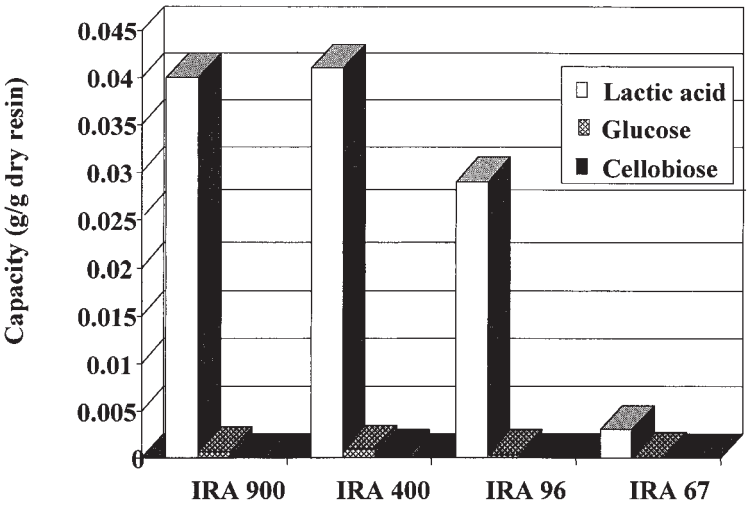

Fig. 4. Capacities of resins for lactic acid, glucose, and cellobiose determined at 45°C (initial concentration = 10 g of lactic acid/L, 10 g of glucose/L, and 10 g of cellobiose/L).

the adsorption of the molecular form of lactic acid (which is minor at pH 4.85). This difference in behavior between strong- and weak-base resins could explain their different capacities. On the other hand, the higher capacity shown by Amberlite IRA 96 in comparison with Amberlite IRA 67 can be explained on the basis of the physicochemical characteristics of both resins: the higher porosity of Amberlite IRA 96 (a macroporous resin with high porosity) allows an enhanced access of the chemical species to the inner surface.

Selectivity

Since glucose and cellobiose are present in the initial stages of a typical SSF process, a set of experiments was carried out to determine whether the resins can bind sugars, causing decreased fermentation yields. In these experiments, the resins were contacted with solutions containing commercial glucose, cellobiose, and lactic acid, and the system was allowed to reach equilibrium. The experimental results shown in Fig. 4 proved that the sorption of sugars was negligible, even when their concentrations in the medium were higher than the ones expected in SSF operation.

Additional experiments were conducted to assess the possible differences in lactic acid recovery either from fermentation broths or from synthetic solutions. As can be seen in Fig. 5, the resin capacities determined for operation with fermentation medium decreased by 6–14% in comparison with the ones obtained with synthetic solutions. This finding can be explained by the possible exchange of ions different from lactate (e.g., sulfate, phosphate, citrate, and acetate) when the resin was contacted with fermentation medium, as well as by the possible adsorption in the polymeric matrix of other molecular components of medium (including nutri-

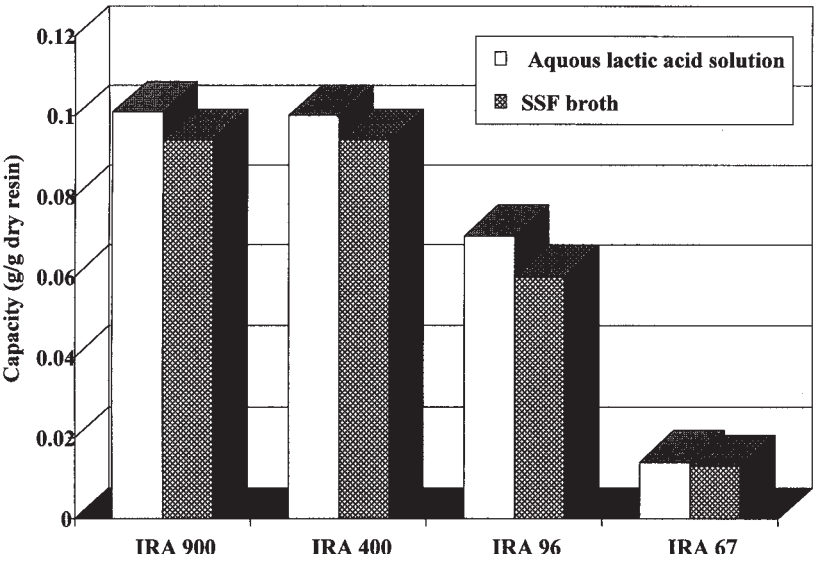

Fig. 5. Capacities of resins determined at 45°C for commercial aqueous lactic solutions and for SSF medium containing 37 g of lactic acid/L (initial pH = 4.85).

ents and enzymes). A related situation was studied by other investigators *(18)*, who found that several compounds present in the fermentation medium (including salts and other components) were first retained by the resin during the normal operation and then recovered during the regeneration step.

Fixed-Bed Operation

Based on the results of capacity and selectivity we have described, the two strong-base resins were selected for further fixed-bed operation using solutions of synthetic lactic acid. In this stage of the work, four loading-regeneration cycles were carried out. The experimental variables determined were resin capacity, percentage of lactic acid recovery, regenerant consumption, and product concentration in the effluent. The results, summarized in Table 2, show that the average lactic acid capacity of both resins was similar (0.273 g/g) and remained fairly constant after four loading-regeneration cycles, but that Amberlite IRA 400 required a lower amount of regenerant and allowed a higher lactic acid concentration in the effluent during desorption (8.2–11.3 g/L). The resin capacities determined for fixed-bed operation were significantly higher than the ones found for batch operation (0.1 g/g), as expected from and in agreement with data in the literature *(17)*. This behavior is ascribed to the fact that the OH^- ions remain in solution in batch experiments, whereas they are continuously eluted in column operation.

Almost quantitative lactic acid recovery (97.5–100%) was achieved in the regeneration of both resins, a result confirming the potentiality of the resins selected for this purpose.

Table 2
Results on Lactic Acid Recovery Achieved with Solutions in Fixed-Bed Operation

	Amberlite IRA 900			
	Cycle 1	Cycle 2	Cycle 3	Cycle 4
q_{FB} (g/g)[a]	0.278	0.276	0.271	0.265
Regenerant volume (L)	0.115	0.103	0.110	0.109
Lactic acid concentration (g/L)[b]	7.02	7.85	7.05	7.09
Recovery (%)	97.5	99.6	99.6	97.9
	Amberlite IRA 400			
q_{FB} (g/g)[a]	0.279	0.276	0.268	0.269
Regenerant volume (L)	0.087	0.070	0.092	0.096
Lactic acid concentration (g/L)[b]	9.2	11.3	8.4	8.2
Recovery (%)	98.3	98.5	99.6	100

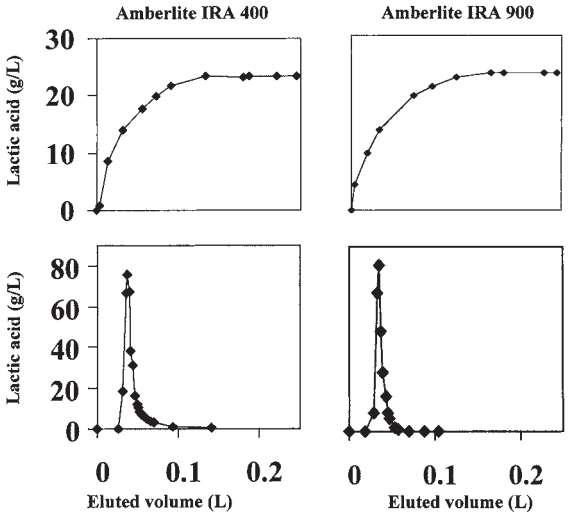

Fig 6. Profiles of lactic acid elution during loading and regeneration of resins IRA 400 and IRA 900 with aqueous solutions of lactic acid (concentration = 24 g/L).

Figure 6 shows the profile of lactic acid concentration in the eluent during a loading and a typical HCl-based regeneration step for the resins IRA 400 and IRA 900, respectively.

Sequential loading-regeneration cycles were carried out using fermentation medium containing the same concentration of lactic acid previously used in experiments with synthetic solutions. The results in Table 3 show that the lactic acid capacity of IRA 400 remained constant after successive cycles of loading and regeneration, but that its absolute value decreased in comparison with the ones determined using synthetic lactic acid solutions (Table 2).

Table 3
Results on Lactic Acid Recovery Achieved
with SSF Broth in Fixed-Bed Operation with Amberlite IRA 400

	Cycle 1	Cycle 2	Cycle 3	Cycle 4
q_{FB} (g/g)[a]	0.142	0.143	0.138	0.140
Regenerant volume (L)	0.075	0.070	0.068	0.089
Lactic acid concentration (g/L)[b]	5.07	5.23	5.37	4.65
Recovery (%)	99.2	99.6	98.9	98.2

[a]Resin capacity in fixed-bed operation.
[b]Lactic acid concentration in effluent from column.

Coupled Production and Recovery of Lactic Acid

On the basis of the results discussed, Amberlite IRA 400 was selected to perform the coupled production and recovery of lactic acid. For this purpose, the culture medium was automatically pumped through a column containing this resin when the pH of the medium decreased owing to the increased concentration of lactic acid. The effluent (with increased pH owing to the neutralization caused by the hydroxyl ions exchanged with lactate) was recycled to the fermentor (*see* Fig. 1), increasing the pH of the fermentation medium. Pumping was automatically controlled to keep the pH in the range of 4.85–4.80; the normal fermentation (leading to acidification of the medium) was allowed to proceed until the lower pH limit was reached, and then a new neutralization cycle started. To obtain reliable data, separate fermentation-recovery runs were carried out for selected reaction times, and lactic acid was measured from the fermentation broth and liquors from resin regeneration. This information was used to calculate an RF measuring the relative proportions of lactic acid exchanged with the resin and remaining in liquid phase.

This operational procedure caused an increase in lactic acid concentration in the fermentation medium from 1.38 up to about 7.4 g/L (Fig. 7), with the latter corresponding to the steady-state concentration allowed by the control system. Enzymes are not significantly inhibited by this concentration of the end product.

Comparatively low concentrations of glucose (0–0.58 g/L) and cellobiose (1.6–2.5 g/L) were determined in the assay, both values being below the threshold leading to inhibition of cellulases and cellobiase. No concentration peaks of glucose and cellobiose were observed at short reaction times using the experimental procedure proposed in this work.

Figure 7 also shows the time course of the RF. Considering the autoionization reaction of lactic acid, it can be inferred that the stationary RF value (3.75) would increase by selecting a pH higher than 4.85, but this strategy would result in decreased enzymatic activities.

The overall lactic acid productivity can be calculated from both the lactic acid concentrations and the RF values shown in Fig. 7. The corre-

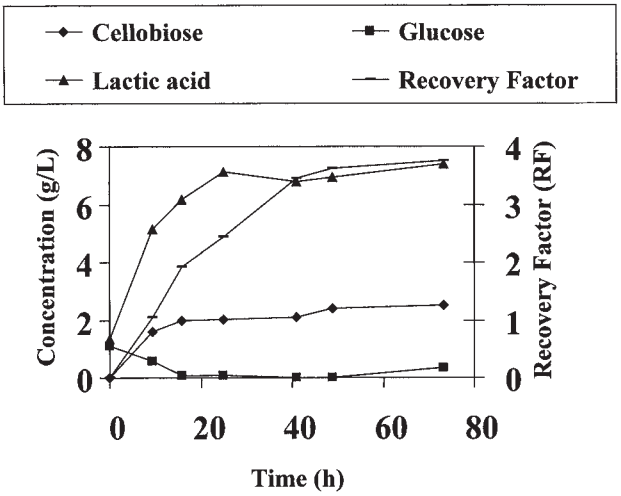

Fig. 7. Time course of SSF experiments with coupled lactic acid recovery by ion exchange.

sponding results showed that remarkable lactic acid productivity (1.18 g/[L·h]) was achieved in the first 20 h of operation, with a good balance between the reaction rates of hydrolysis and fermentation (as is proved by the low glucose concentrations achieved in the fermentation medium). This productivity is higher than the one (0.91 g/[L·h]) obtained for the same conditions of fermentation and saccharification without lactic acid recovery *(11)*. The observed increase in productivity is ascribed to the limited product concentration in the medium, which enhanced both saccharification and fermentation. After this initial period, the productivity decreased (to reach 0.74 g/[L·h] after 40 h) owing to the lower availability of cellulose, with possible side effects related to adsorption of nutrients or enzymes in the solid phase. According to the literature *(15)*, no harmful effects of resin on the microbial metabolism are expected. The overall productivity in prolonged operation was similar to the data reported for SSF operation *(11)*. Note that lactic acid was the final product of the studied process, whereas alternative processing schemes led to sodium lactate *(19)*.

The overall operation was carried out with moderate concentrations of the carbon source and nutrients, there was no fortification of medium with further nutrient addition, and both temperature and pH were fixed in compromise values regarding both fermentation and saccharification. This strategy compares favorably in terms of productivity, enzyme loading, and cost of the raw material with other alternatives studied for the same purpose, such as the SSF of commercial cellulose with simultaneous recovery by liquid-liquid extraction *(19)*.

On the other hand, the productivities are near the ones reported by Srivastava et al. *(15)* (0.889–1.665 g/[L·h]) for the fermentation of sucrose to lactic acid with coupled recovery of the product by ion exchange. It must

be considered that in that case, they started from a disaccharide in comparison with the polymeric substrate (cellulose) employed in our study.

Conclusion

Four anion resins in OH^- form were tested for lactic acid recovery from SSF medium. The resin capacities were almost not influenced by temperature at 25 and 45°C. Amberlite IRA 400 and Amberlite IRA 900 showed the highest capacities for lactic acid with negligible sorption of glucose and cellobiose.

The resin capacities determined for fixed-bed operation were significantly higher than the ones found for batch operation. This behavior is ascribed to the fact that the OH^- ions remain in solution in batch experiments, whereas they are continuously eluted in column operation. The capacity of the resins tested remained constant after successive cycles of loading and regeneration with almost quantitative lactic acid recovery.

In addition, our study shows the feasibility of coupling SSF and ion exchange in a single step of production and recovery of lactic acid from pretreated wood without the addition of external alkali to the fermentation medium. Remarkable productivity of lactic acid (1.18 g/[L·h]) was achieved during the first 20 h of operation.

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