Purification of lactic acid obtained from a fermentative process of cassava syrup using ion exchange resins

Purificación de ácido láctico obtenido a partir de un proceso fermentativo de jarabe de yuca, empleando resinas de intercambio iónico

Joan Quintero[1], Alejandro Acosta[1], Carlos Mejía[1], Rigoberto Ríos[2], Ana María Torres[3]*

[1] Grupo de Biotransformación. Escuela de Microbiología, Universidad de Antioquia, Calle 67 N°. 53-108, bloque 5, oficina 226. Medellín, Colombia.

[2] Grupo de Bioprocesos. Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad de Antioquia. Calle 67 N°. 53-108, bloque 18, oficina 439. Medellín, Colombia.

[3] Grupo de Bioprocesos. Programa de Bioingeniería, Facultad de Ingeniería, Universidad de Antioquia. Calle 67 N°. 53-108, bloque 19, oficina 415. Medellín, Colombia.

Abstract

In this work, the fermentative lactic acid (LA) production and its further separation by ionic exchange resins was evaluated. A *Lactobacillus brevis* strain was used to perform lab scale experiments under anaerobic conditions, using a low nutritional content media with cassava flour as carbon source (HY1). For a fermentation time of 120h in a 7.5-L bioreactor, the LA concentration was 24.3±0.07g LA/L and productivity 0.20 g/L/h, at pH 6.5 and 38°C.

For LA recovery, the Amberlite IRA-400 and IR-120 exchange resins were used. First of all, a LA isothermal adsorption on Amberlite IRA-400, Cl⁻, OH⁻ and HSO_4^{2-} activated form, was performed at 25°C. The Cl⁻ activated resin was tested at pH 5, whereas the OH⁻ activated form was tested at pH 3.5 and 6.3. The highest adsorbate content was 0.59±0.03 g LA/g resin at pH 6.3 when the resin was OH⁻ activated. Following, the breakthrough curves were carried out in an Amberlite IRA-400 packed column at pH 3 and 5, and 0.5

* Autor de correspondencia: teléfono: + 57 + 4 + 219 85 90, correo electrónico: atorres@udea.edu.co (A. Torres)

and 1mL/min; the maximum LA loaded was 0.109±0.005 g AL /g resin at pH 3 and 0.5 mL/min. Finally, the LA recovery was assessed in a system of series of columns packed with Amberlite IRA-400 e IR-120; the LA recovery was 77% and 73%, when the system was set at 0.5mL/min, 25°C, and a feeding at pH 3 and 5, respectively, into the packed columns.

---------- *Keywords:* Lactic acid, *Lactobacillus brevis*, ionic exchange resins, adsorption isotherms

Resumen

En el presente trabajo se evalúo la producción de ácido láctico (AL) vía fermentativa, y su posterior separación mediante un sistema de resinas de intercambio iónico. Para la biosíntesis de AL se usó la cepa *Lactobacillus brevis* la cual fue cultivada bajo condiciones anaeróbicas usando un medio de bajo contenido nutricional a base de hidrolizado de yuca, denominado HY1. Para una cinética de cultivo de 120 h, en un biorreactor de 7,5 L, la más alta concentración de AL encontrada fue 24,3±0,07g AL/L, con una productividad de 0,20 g/L/h, a pH 6,5 y 38ºC.

Para la recuperación del AL se usaron las resinas de intercambio iónico Amberlite IRA-400 e IR-120. Inicialmente se determinó la isoterma de adsorción de AL (25°C) sobre la resina Amberlite IRA-400 activada en su forma Cl$^-$, OH$^-$ y HSO$_4^{2-}$. La forma Cl$^-$ de la resina activada fue evaluada a pH 5, mientras que la forma OH fue evaluada a pH 3,5 y 6,3. El más alto contenido de adsorbato fue 0,59±0,03g AL/g resina at pH 6,3, cuando la resina esta activada en su forma OH$^-$. Seguidamente, se desarrollaron las curvas de ruptura en la resina Amberlite IRA-400 a pH 3 y 5, y 0,5 y 1mL/min de flujo de alimentación. La máxima concentración de AL adsorbida fue 0,109±0,005g AL/g resina a pH 3 y 0,5 mL/min. Finalmente, la recuperación de AL se evaluó en un sistema de columnas en serie empacadas con las resinas Amberlite IRA-400 e IR-120; La recuperación de AL fue 77% y 73%, cuando el sistema se ajustó en 0,5mL/min, 25°C, pH 3 y 5, respectivamente.

---------- *Palabras clave:* Ácido láctico, *Lactobacillus brevis*, resinas de intercambio iónico, isotermas de adsorción

Introduction

Lactic acid (LA) production has been a topic of continuous interest mainly due to its wide variety of applications, not only the acid itself but its derivatives. Among others, LA has been extensively used in food, textile, and pharmaceutical industry [1] and more recently, its demand has dramatically increased for poly-lactic acid (PLA) production, where it is used as a monomer [2, 3]. The LA market is continuously growing; it has been estimated that its worldwide demand ranges from 130000 to 150000 ton/year (approx. U$ 68 million per year) [4].

Even though LA production started 100 years ago, research in this field is very active, mainly, due to new LA applications and the need for lower production and recovery costs that still are indeed high at large scale.

Regarding LA production, 90% comes from microbial fermentation, in contrast to chemical synthesis. The organisms traditionally used in fermentation processes are gram-negative bacteria belonging to the species *Lactobacillus, Carnobacterium, Leuconostc, Tetragenococus, Pediococcus, Streptococcus, Lactococcus, Vagococcus, Esterococcus, Aerococcus* y *Weissellas* [5, 6].

Biotechnological production of LA employs mainly saccharose and glucose as carbon source; however, due to the high-quality sugar's cost, different studies, aim at evaluating cheaper sources such as cassava syrup, are carried out.

Bioprocess studies for higher LA production are commonly performed in erlenmeyer or lab scale batch bioreactors. Environmental conditions are usually set at 30-42ºC, 120-200 rpm, and pH ranging from 5 to 6.8 [7]. In the search of obtaining a high purity product, many studies have been conducted to assess diverse separation techniques, such as reactive extraction [8], membrane separation [9], ionic exchange [10, 11], electrodialysis [12], and reactive distillation [13].

One of the most widely used LA separation and purification techniques is ionic exchange resins since the required equipment is relatively simple and inexpensive [14]; its use is specially recommended when the LA solution has low salt concentration [15]. The process involves cationic exchange resins for Na^+, Ca^{2+}, Mg^{2+} removal, and the anionic type for eliminating chlorine and sulfate. Commercial resins such as Amberlite IRA-420 [16], IRA-400 [17], DOWEX-50W [13] and PVP [18] are currently available. The LA recovery and purification process using ion exchange resins can be described in three different steps: lactate ion adsorption, elution, and lactate conversion to its acid form [11].

In the task of having a viable LA ionic exchange resin purification process at industrial scale, it is required to perform optimization and scaling studies that eventually involve research at lab and pilot plant scale, to observe the effect of diverse environmental conditions on LA yield and recovery. This work aims at studying the ionic exchange resins as a promising alternative for LA recovery and purification. LA was produced using *Lactobacillus brevis*, growing on cassava syrup as the unique carbon source.

Materials and methods

Microorganism, culture medium and lactic acid production

A *Lactobacillus brevis* strain was used. Cryogenic vials with a high nutritional content medium (MRS) [g/L]: glucose: 100, peptone: 10, yeast extract: 10, meat extract: 10, K_2HPO_4: 2, sodium acetate: 5, ammonium citrate: 2, $MgSO_4.7H_2O$: 0.2, $MnSO_4.H_2O$: 0.05 and 30% glycerol, were used for strain conservation at -4°C. The strain was maintained in Petri dishes with MRS medium and 1% agar at 4°C, and subcultured every other month.

Inoculums for bioreactor operation were grown in a low nutritional content medium with cassava flour as the unique carbon source, named HY, whose composition was [g/L]: reducing sugars from enzymatic hydroxylation of cassava flour: 30, yeast extract: 15, KH_2PO_4: 5.6, K_2HPO_4: 4.16; initial pH set at 5.5. Operating conditions for inoculums were set at 38°C, 150 rpm and 24h. The culture medium used for LA production in bioreactor was the HY1 medium which includes [g/L]: reducing sugars from enzymatic hydroxylation of cassava flour: 100, yeast extract: 15, KH_2PO_4: 5.6, K_2HPO_4: 4.16; initial pH was set at 6.5. LA biosynthesis was carried out in a 7.5-L bioreactor, containing 5-L of culture media. (BioFlo/CelliGen 115-New Brunswick). Operating conditions were set at 38ºC, 200rpm for 120h. Inoculum preparation consisted of 490mL of medium HY. For pH control, NaOH 2N y H_3PO_4 8.5% solutions were used. Experiments were performed with one replicate.

LA separation and purification techniques

LA recovery using ion exchange resins Amberlite IRA- 400 and IR-120

For resin activation Anionic Amberlite IRA-400 was converted into its three forms: OH⁻, Cl⁻ and HSO_4^{2-}, following the protocol reported by Moldes et al. [11]. Once activated, resins were exposed to dryness (60°C, 12h). Cationic resin Amberlite IR-120 was converted into its H^+ form as described by Vaccari et al. [19]. Once activated, it was exposed to dryness (60°C, 12h). Each ionic Amberlite IRA-400 resin form (1g), was tested with an LA solution (34g/L) prepared with residual fermentation broth, pH 5. Samples were incubated at 25°C, 150rpm for 12h (this time was set up based on previous experiments, data not shown). Following, LA concentration was determined by HPLC and adsorbed LA was calculated by mass balances. Assays for this and the remaining experiments were performed by triplicate.

Isothermal adsorption for the ionic resin Amberlite IRA-400

For the Cl⁻ form study, 10mL solutions with different LA concentration ranging, from 15 to 250g/L, were prepared using residual fermentation broth. Values for LA concentration range were set up based on previous experimental results (data not shown). After the pH of each solution was set to 5, 1g of resin was added to each solution. Solutions were incubated at 25°C, 150rpm for 12h. The remaining LA in the supernatant was measured by HPLC; the amount of LA adsorbed was determined by mass balances. The same protocol was followed for the case of the resin in its OH⁻ form; in this case, solutions were prepared with LA concentration ranging from 35 to 380g/L, based on previous experimental results (data not shown), and the pH for each solution was set to 3, 5 and 6.3. Assays were performed by triplicate. The pH value of 6.3 was set up based on previous experimental work showing that at

pH 6.5, there was an inflection point suggesting a slightly lower value for the upper experimental bound to be selected (data not shown).

Yield and breakthrough curves in an Amberlite IRA-400 resin

For analyzing the adsorption process behavior in a continuous system, a glass column (1cmx40cm) was packed with 18g of dry resin into its OH⁻ form. Following, an LA solution with a concentration corresponding to that of the fermentation process was passed through until resin saturation. Eluent was continuously sampled to determine LA concentration by a colorimetric enzymatic assay (Roche). Two pH levels (3 and 5) and two flow rates (0.5 and 1 mL/min) were studied by a factorial experimental design 2x2. In an attempt for evaluating the adverse consequence of the remaining culture media components, assays with an aqueous LA solution (0.5mL/min; pH 3) were performed. LA acid solution were prepared using a commercial 88% LA (Carlo Erba)

Eluent selection for lactate ion recovery in an Amberlite IRA-400 resin

Seven grams of resin were saturated with lactate ions coming from a concentrated LA solution, during 12h at 150rpm and 25°C. Next, 10mL solutions of eluents (HCl 0.1N, CH_3OH 10%, H_2SO_4 1M, NaCl 1M, NaOH 1N) were prepared and inoculated with 0.5g of the saturated resin. The solutions were incubated at 150 rpm and 25°C for 12h; afterwards, LA concentration in the resulting solutions was determined by a colorimetric enzymatic assay (Roche).

LA recovery and purification by using the Amberlite IRA-400 and IR-120 resins, arranged in series

A first glass column (1cmx40cm) was packed with 18g of Amberlite IRA-400 resin and fed with a fermentative LA solution at pH and flow rate set up according to the yield and breakthrough assays. The column was washed with distilled

water and fed with NaOH 1N for the purpose of ion lactate recovery. In order to recover the lactate ion as lactic acid, the eluent coming out from this first column was added to a second glass column (1cmx40cm), packed with 20g of the cationic Amberlite IR-120 resin. Distilled water was passed through both columns for 30min; then, the cationic resin was activated by means of addition of a 50mL solution of HCl 1N. Samples were sequentially taken from the second column for LA enzymatic determination. Sulfate was also determined, both, prior to the resin purification and after resin exposure. The feeding process was repeated by passing spent fermentation broth through the first column looking for identifying resin activity lost.

Analytical methods

LA was measured by HPLC using a C-610H column with 7.8mm ID, and 30cm length; H_3PO_4

at 0.1% and 0.5ml/min as the mobile phase, UV detection at 210nm, and 30°C [20]. LA was also determined by a colorimetric enzymatic assay (Roche). Sulfate concentration was evaluated by a turbidimetric method 4500 SO4 E [2].

Results and discussion

Lactic acid production

The genus *Lactobacillus* has been widely known as the major LA producer strain. For the purpose of this work, a *Lactobacillus brevis* strain was used; it can be stated that cassava flour, the main component for the HY1 medium, is an appropriate substrate for LA biosynthesis. In addition to reducing sugars, cassava flour has salts, aminoacids and proteins that might favor bacterial growth and product biosynthesis. Figure 1 shows the obtained profile for LA accumulation over a 120h of cultivation.
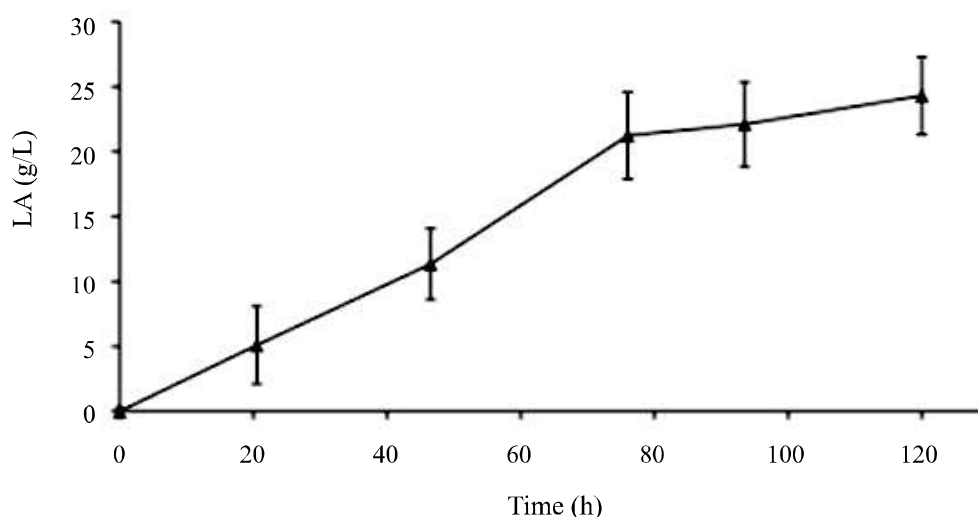
Figure 1 LA accumulation profiles for fermentations with the *Lactobacillus brevis* strain, in a 5-L biorreactor (working volume) with pH control at 6.5

The largest LA accumulation, reached at 120h, was 24.3±3.0 g/L; this value is comparable to most literature reports. For substrates such as glucose, corn flour, sawdust, molasses, whey, among others, LA production ranges from 1 to 100 g/L, using commercial strains such as *Lb. plantarum, Lb. casei, Lb. delbrueckii, Lb. helveticus*, etc [1]. Siebold et al. [21] and Kious et al. [22] did report LA production close to 26.8-27.8 g/L and 13.3-19.6 g/L, respectively, using glucose as the only substrate.

Separation and purification

LA recovery by using ionic exchange resins

Evaluation of the ionic form of the resin Amberlite IRA-400

It was observed that the resin OH⁻ form did show higher LA adsorption capacity rather than the Cl⁻ and HSO_4^{2-} forms (table 1), perhaps due to the fact that the adsorption process in ionic exchange resins is favored towards ions with high valence and low molecular weight (MW); under these circumstances, the chemical potential between the resin active site and the working solution is enlarged [23].

Table 1 LA adsorption in Amberlite IRA–400 resins, activated in different ionic forms

| Resin Form	g LA adsorbed /g resin	LA adsorbed (%)
OH⁻	0.337±0.050	19.39±2.84
Cl⁻	0.107±0.043	6.15±2.48
HSO_4^{2-}	0.004±0.003	0.25±0.19

Since the OH⁻ ion has a lower MW than Cl⁻, it establishes weaker bonds with the resin active sites, thus propitiating its displacement by lactate ion, during adsorption. Moldes et al. [11] found adsorption capacities of 0.10 and 0.13g LA adsorbed/g resin, for the Cl⁻ and OH⁻ form, respectively. These results are comparable to those found in this work.

On the other hand, the high HSO_4^{2-} ion MW, which dissociates in SO_4^{2-} having two charges, leads to the formation of stronger bonds with the resin.

Adsorption isotherms for the anionic Amberlite IRA-400 resin in its Cl⁻ and OH⁻ forms

Ion exchange resin adsorption capacity is usually favored by the ion-of-interest valency number, its size and concentration, activity coefficient as well as the active surface of the exchange resin [24].

The figure 2 shows the acquired adsorption isotherms for the Amberlite IRA–400 resin in its Cl⁻ y OH⁻ forms.

(A)
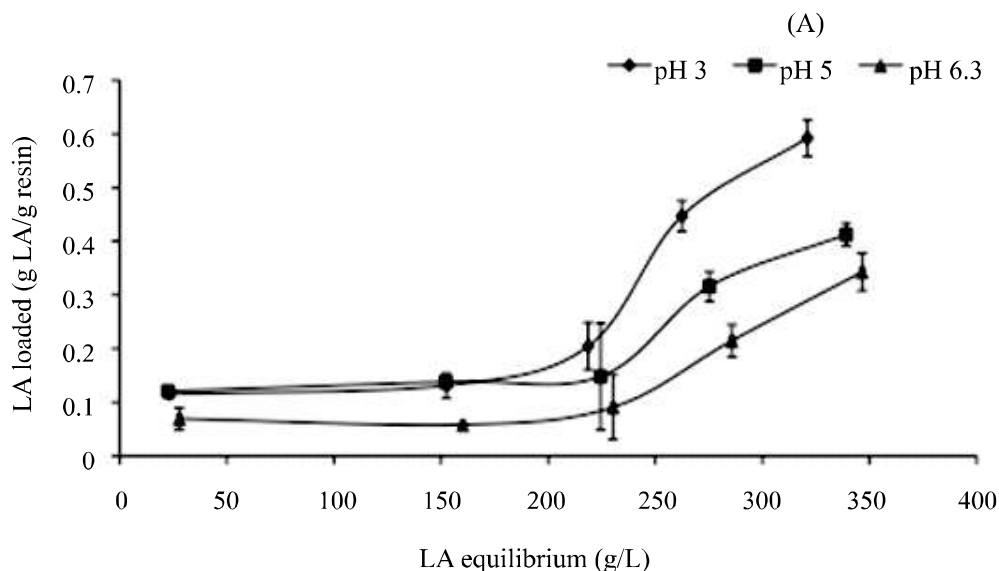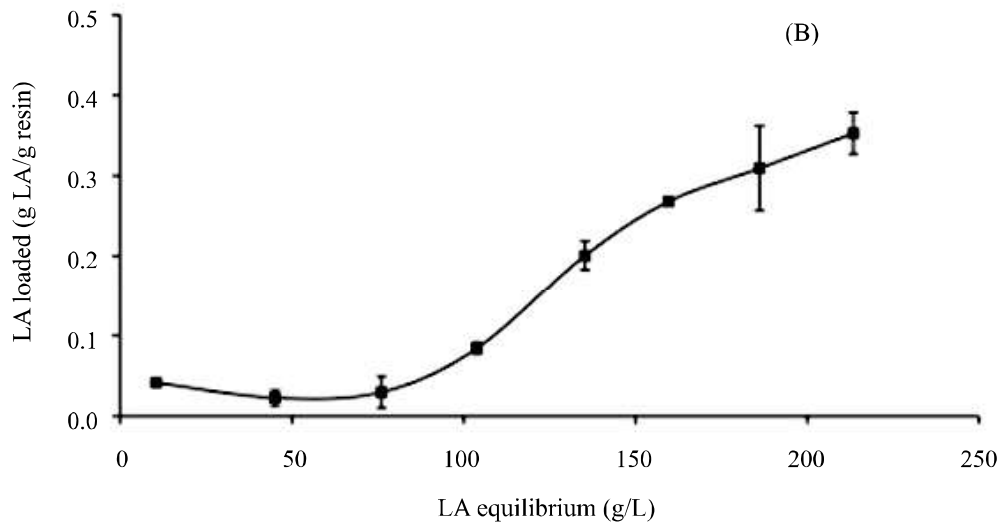
Figure 2 Adsorption isotherms for the Amberlite IRA-400 resin in its OH⁻ form: (A) at pH 3, 5, 6.3; (B) and in its Cl⁻ form at pH 5

For the Amberlite IRA-400 resin, a pH below of the lactic acid pKa value (3.86) has a manifest incidence on lactate adsorption. Larger pH values would provoke reduction in acid dissociation, thus affecting the adsorption process.

As it is observed, by comparing the results, at pH 5, the resin in its OH⁻ activated form has larger lactate ion adsorption capacity (0.41±0.02g LA adsorbed/g resin) than that of the Cl⁻ form at the same pH (0.35±0.03g LA adsorbed /g resin), mainly due to the lower OH⁻ ion molecular weight compared to that of the Cl⁻ ion; this facilitates ion transport from the resin active site. The strength of chemical bonds, formed between the resin active site and the exchange ions, depends on electronegative differences. Due to its smaller atomic ratio, Cl⁻ ions have higher ionization energy, making stronger chemical bonds and causing difficulties in its displacement.

On the other hand, the experimental data for the resin Cl⁻ and OH- form does not follow the Langmuir model, $R^2=0.5894$, $R^2=0.7393$ respectively, but do follow the semi-empirical Boltzmann model, $R^2=0.5894$, $R^2=0.998$ respectively (See table 2, figure 3). The Boltzmann model is known as:

$$q^x = q_F - \left[\frac{(q_F - q_O)}{1 + \exp\left(\dfrac{C - C_O}{\delta_C}\right)} \right] \quad (1)$$

Where,

q^*: Adsorbate per gram of resin in equilibrium with LA, C, in solution [g LA/g resin].

q_F and q_O: Final and initial loading of adsorbate per gram of resin [g LA/g resin].

C_O: LA concentration in the middle point [g/L].

δ_C: Increment value for LA.

Table 2 Adsorption parameters (regression coefficient and statistical parameters) found in the equilibrium study for the Amberlite IRA-400 resin in its OH⁻ at different pH values and Cl⁻ form at pH: 5

	OH⁻ form		*Cl- form*	
	pH=3	*pH=5*	*pH=6.3*	*pH=5*
q_F	0.595	0.408	0.351	0,3475
q_o	0.123	0.130	0.086	0,0242
C_o	245.9	262.7	287.2	134,254
δ_C	19.89	18.03	19.30	20,896
R^2	0.998	0.994	0.934	0,9926
Variance	1 E-4	1E-4	8E-4	2,38E-04
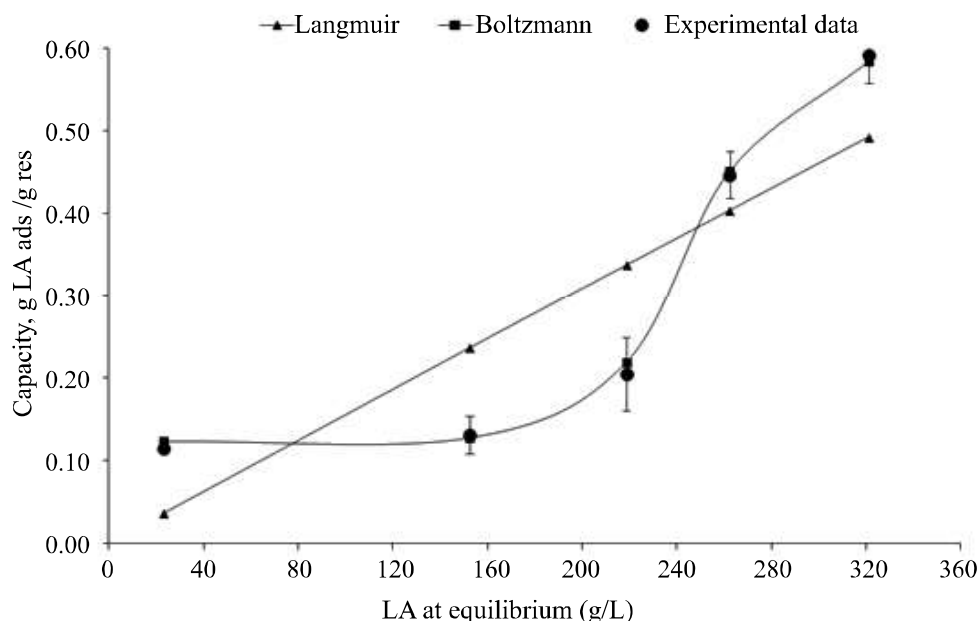
Figure 3 Ajustment of experimental data to the models of Langmuir and Boltzmann for the Amberlite IRA-400 resin, in its OH⁻ form at pH 5 (A least square method was used).

The low experimental data adjustment to the Langmuir model gives insights about the resin surface, and allows one to consider it as no plane and/or homogeneous. The kind of interaction between the resin active sites and lactate ions present in the fermentative solution, are not just simple-site type, but acid-base, hydrogen bonds, and/or hydrophobic interactions [13]. Based on the Chenlo et al. [25] classification, the isotherms created for the lactate adsorption in the Amberlite IRA-400 resin, are type V, which means, they are not suitable for the lactate ion loading, but good for the elusion step in a fixed bed system [26].

Yield and breakthrough curves in an Amberlite IRA-400 resin

Table 3 shows the experimental results for LA adsorption on Amberlite IRA-400 in its OH⁻ form. Both, pH values lower than those for

LA pKa (3.86), and LA solution inlet flow rate reduction, did favor adsorption of lactate ion on the Amberlite resin. This is in agreement with the adsorption isotherms acquired from the batch system experiments. The lower the volumetric flow rate, the higher the residence time, (13.92 ± 0.85 min and 24.24 ± 0.90min, for 1 and 0.5mL/min respectively), thus improving ion exchanging and reducing mass transport problems such as channeling and/or dead volume.

Table 3 LA adsorption yields on Amberlite IRA-400 at 25°C and different flow rates

Experiment	Q (mL/min)	pH	g LA adsorbed /g resin
1	0.5	3	0.109±0.005
2	1	3	0.088±0.011
3	0.5	5	0.095±0.004
4	1	5	0.084±0.003
Standard solution	0.5	3	0.129±0.005

Breakthrough curves have an initial step of maximum adsorption rate until the resin saturation is reached; after that, the concentration of the target ion noticeably increases in the effluent. The closer operation conditions that did render this trend were pH 5 and 0.5 mL/min; the remaining curves showed a progressive increase of LA concentration in the effluent, perhaps because of the competition that might occur between aminoacid and salts in the spent culture media for interacting with the resin active site. Under similar flow and pH conditions a loading of 0.129g LA/g resin was acquired for the LA aqueous solution, 18.35% higher than that of the spent culture medium (figure 4), with statistical significance ($p=0.0029$). It was also proved that there exists statistical significance between the evaluated volumetric flow rates with a p value of 0.0021.
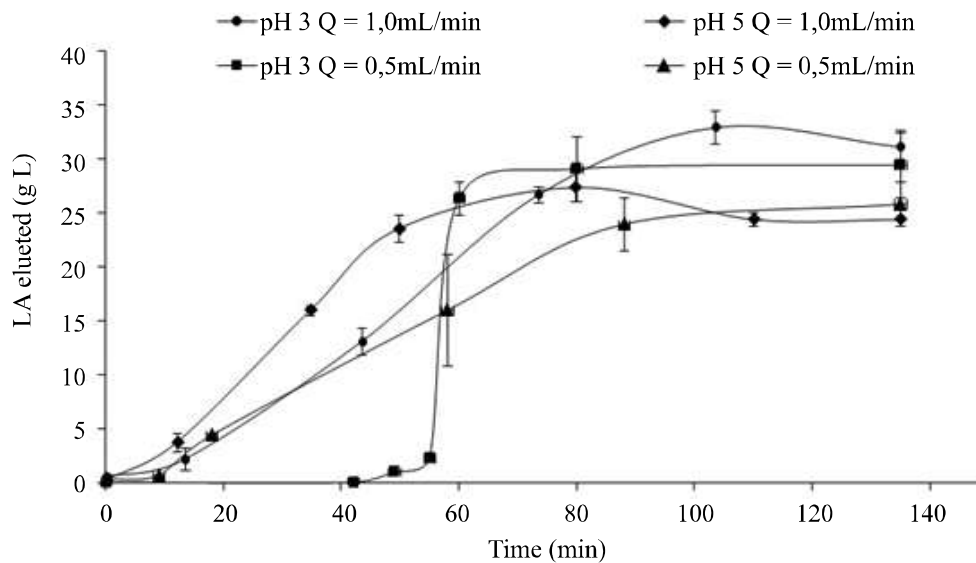
Figure 4 Breakthrough curves for the Amberlite IRA-400 resin in the OH⁻ form at different pH and LA feeding flow

For all glass columns, resin loading reached 95% of that of the batch system, for a solution with a concentration equal to the fermentation media; however, as a result of the dilution effect, it just achieved 18.31% of the batch system maximum capacity, being necessary to concentrate the LA solution before it is fed to the ion exchange columns.

Eluent selection for lactate ions recovery

The resin was loaded with 0.332 g LA/g resin. Table 4 shows the results for lactate ion elution in the anionic resin IRA-400. Though there is no statistical significance (p=0.0571) between the acquired results with NaOH 1N or H_2SO_4

1M, it is recommended to use NaOH in order to attend regulatory commission rules such as that of the Food Chemical Codex (FCC), aim at producing LA with low sulfate concentrations [19]. In addition, due to the sulfate ion bivalence properties, it shows higher affinity for the resin active sites limiting future resin applications. The additional evaluated eluents showed lower lactate ion elution which might be the result of its lower adsorption capacity and dielectric constant; the lower the dielectric constant, the higher the limitations of ion transport in solution. In a further process for LA recovery using a system with resins, IRA-400 and IR-120 resins, arranged in series, NaOH 1N was used as eluent.

Table 4 LA recovery from the Amberlite IRA-400 resin using different eluents

Eluent	Extracted LA (g)	Extraction (%)
HCl 0.1 M	0.1230±0.0025	74.1064±1.5311
CH_3OH 10%	0.0008±0.0003	0.4637±0.1660
H_2SO_4 1M	0.1477±0.0027	89.0006±1.6512
NaCl 1 M	0.1190±0.0061	71.6566±3.6747
NaOH 1M	0.1539±0.0031	92.6901±1.8678

LA recovery in a series of resin - Amberlite IRA-400 and IR-120 - purification system

Table 5 shows the results for LA recovery and purification in a packed system with the ion exchange resins IRA-400 and IR-120, connected in series. Amberlite IRA-400 did not reach saturation due to lack of enough retention time; thus, adsorbed LA concentration was 0.0367 and 0.0160g LA/g resin, for a process at pH 3 and 5, respectively. On the other hand, the washing

steps did yield LA losses close to 5 and 8%, when the feeding solution was set to pH 3 and 5, respectively. The anionic resin showed better regeneration capacity at pH 3. LA losses changed from 14.08% in the first cycle to 21.6% in the second, whereas at pH 5, losses changed from 11.4% to 31.73% in the first and second cycle, respectively; this can be explained considering that, in spite of washing, OH⁻ ions were accumulated favoring the equilibrium reaction towards the complex R-OH generation, instead of lactate ion L-Na⁺ formation.
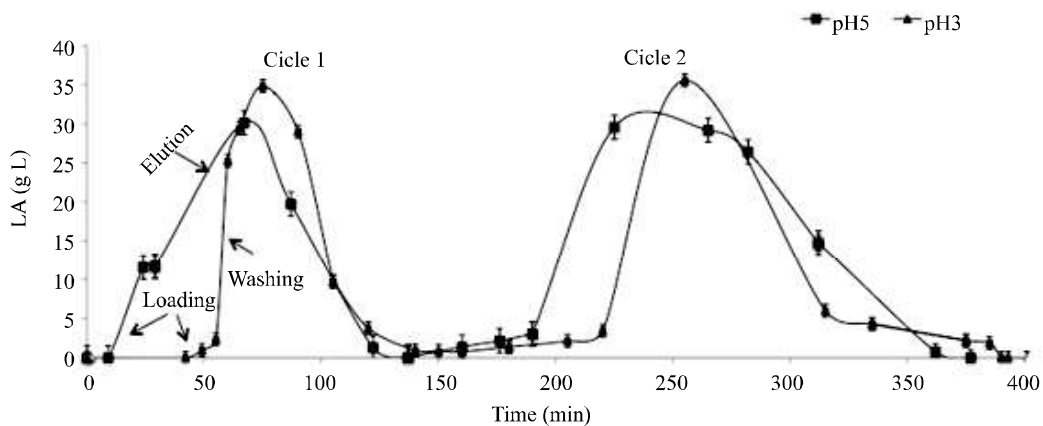
Table 5 LA recovery and purification in a packed system with the ion exchange resins IRA-400 and IR-120, connected in series

STEP	LA loaded or adsorbed (g)		LA recovered or not adsorbed (%)	
	pH 3	**pH 5**	**pH 3**	**pH 5**
CYCLE 1*				
Load	0.6608	0.2884		
Effluent	0.0931	0.0332	14.0874	11.5069
Washing	0.0299	0.0252	4.5225	8.7417
Elution	0.5097	0.2118	77.1354	73.4541
CYCLE 2*				
Load	1.1414	0.6008		
Effluent	0.2430	0.1880	21.2852	31.2909
Washing	0.0647	0.0479	5.6657	7.9724
Elution	0.8199	0.3459	71.8278	57.5789

* Cumulative values

For the sake of clarity, it is needed to emphasize that the reduction in the Amberlite IRA-400 resin adsorption capacity between the first and second cycle, could be the result of a normal active site activity lost due to usage, without resin physical degradation occurrence, as it has been reported by Avila et al. [27], for these types of resin.

The acquired results endorse the better performance of the ion exchange resin working at pH lower than the LA pKa value. At pH 3, the system showed a higher capacity, approximately 50% higher than that at pH 5; in addition, the process profile showed low LA concentrations in the effluent of the loading step (See figure 5), which is in agreement with the results from the breakthrough curve.

Figure 5 LA recovery profiles in packed columns connected in series and having the Amberlite IRA-400 and IR-120 resins. Feeding solutions were set at pH 3 and 5

The LA acid purification using ion exchange resins did appear to be a workable alternative, since up to 77% of the LA in the fermentation media was recovered, a value close to that attained from the salt precipitation treatment. Even though it was not promising to concentrate LA in the eluent solution, it was possible to remove macromolecules such as reducing sugars whose content changed from 27.6 g/L in the fermentation broth to 1 g/L in the eluent solution. After resin treatment, a sulfate reduction close to 99.68% was observed; this calls attention to the importance of using ion exchange resins for sulfate removal (sulfate content prior to resin treatment: 16.61±1.65 g/L and after resin treatment: 0.0525±0.002.7 g/L).

Conclusions

LA biosynthesis was accomplished using alternative low-price nutrient-rich substrates such as cassava flour. This kind of substrate showed high nutrient and reducing sugar content as well as calcium and aminoacids, all of them available for the fermentative process.

On the other hand, LA recovery by means of a fixed packed bed, is enhanced by a reduction in the inlet/feeding volumetric flow rate (set to 0.5 mL/min), perhaps because of the higher residence time, allowing longer time of contact between the resin and LA solution phases.

At low scale, ionic exchange systems are suitable for LA purification since, at a low cost, it allows one to remove macromolecules and possibly ions formed during fermentation; the obtained recovery percentages were close to 77 %.

The reusing of the Amberlite IRA-400 resin allows a recovery of LA in various steps, showing loss of resin activity close to 7% during the first two cycles.

Concentrating the acid before it is actually fed to the ion exchange column would cause reduction in the Amberlite IRA-400 resin capacity. Now, desorbing the acid from the resin at temperature higher than the ambient one, would direct the adsorption equilibrium towards eluent solution, concentrating it and allowing column restoration.

In spite of the good results for LA recovery using ion exchange resins, it is necessary to implement further separation and concentration steps in order to obtain a product that efficiently satisfy the purity requirements for a specific application such as polymerization processes.

Acknowledgments

References

1. S. Cock, A. Rodríguez. "Producción biotecnológica de ácido láctico: estado del arte. Sociedad Mexicana de nutrición y tecnología de alimentos". *Ciencia y tecnología alimentaria*. Vol. 5. 2005. pp. 54-65.

2. S. Ahmad, E. Vasheghani. "In situ separation of lactic acid from fermentation broth using ion exchange resins". *J. Ind. Microbiol. Biotechnol*. Vol. 35. 2008. pp. 1229-1233.

3. T. Harington, Md. Hossain. "Extraction of lactic acid into sunflower oil and its recovery into an aqueous solution". *Desalination*. Vol. 218. 2008. pp. 287-296.

4. Y. Wee, J. Kim, H. Ryu. "Biotechnological production of lactic acid and its recent applications. *Food Technolog. Biotechnol*. Vol. 44. 2006. pp.163-172.

5. P. Sneath. *Bergey's*. Manual of systematic bacteriology. Ed. Williams and Wilkins Baltimore. London, UK. 1984. pp. 175-181.

6. K. Hofvendahl, H. Hhanhägerdal. "Factors affecting the fermentative lactic acid production from renewable resource. *Enzyme and Microbial Technology*. Vol. 26. 2000. pp. 87-107.

7. L. Serna, A. Rodríguez. "Producción Biotecnológica de ácido láctio: Estado del arte". *Cienc tecnol. Aliment*. Vol. 5. 2005. pp. 54-65.

8. M. Järvinen, L. Myllykosk, R. Keiski, J. Sohlo. "Separation of lactic acid from fermented broth by reactive extraction". *Bioseparation*. Vol. 9. 2000. pp.163-166.

9. A. Persson, A. Jönsson, G. Zacchi. "Separation of lactic acid-producing bacteria from fermentation broth using a ceramic microfiltration membrane with constant permeate flow". *Biotechnol. Bioeng*. Vol. 72. 2001. pp. 269-277.

10. A. Sosa, A. Ochoa, N. Perotti. "Modeling of direct recovery of lactic acid from whole broths by ion exchange adsorption". *Bioseparation*. Vol. 9. 2000. pp. 283-289.

11. A. Moldes, J. Alonso, J. Parajó. "Recovery of lactic acid from simultaneous saccharification and fermentation media using anion exchange resins". *Biop. Biosystem Eng*. Vol. 25. 2003. pp. 357-363.

12. K. Lee. "A media design program for lactic acid production coupled with extraction by electrodialysis". *Bioresource Technology*. Vol. 96. 2005. pp. 1505-1510.

13. J. Chol, W. Hong. "Recovery of lactic acid by batch distillation with chemical reactions using ion exchange resin". *Journal of chemical engineering of Japan.* Vol. 32. 1999. pp. 184-189.

14. W. Tong. "Purification of L(+)-lactic acid from fermentation broth with paper sludge as a cellulosic feedstock using weak anion exchanger Amberlite IRA-92". *Bioch. Eng. Journal*. Vol. 18. 2004. pp. 89-96.

15. M. Fernández, R. de la Vega. *Planta de producción de ácido láctico alimentario*. Desarrollo de la ingeniería del proceso y del proyecto industrial. Universidad de Oviedo. 2006. Disponible en: http://aeipro.com/congreso_03/pdf. Consultado en mayo 19 de 2009.

16. Y. González, G. Vaccari, E. Dosi, A. Trilli, M. Rossi, D. Matteuzzi. "Enhanced production of l(+)-lactic acid in chemostat by *Lactobacillus casei* DSM 20011 using ion-exchange resins and cross-flow filtration in a fully automated pilot plant controlled via NIR". *Biotechnol. Bioeng*. Vol. 67. 2000. pp. 147- 156.

17. X. Cao, H. Yun, Y. Koo. "Recovery of (+)-lactic acid by anion exchange resin Amberlite IRA-400". *Biochem. Eng. J*. Vol. 11. 2002. pp. 189-196.

18. Y. Zheng. X. Ding, P. Cen, C. Yang, G. Tsao. "Lactic acid fermentation and adsorption on PVP. *Appl. Biochem. Biotechnol*. Vols. 57/58. 1996. pp. 627-632.

19. G. Vaccari, A. González, L. Anna, E. Dosi, P. Brigidi, D. Matteuzzi. "Fermentative production of L-lactic acid by Lactobacillus casei DSM 20011 and product recovery using ion exchange resins*". Applied Microbiology and Biotechnology*. Vol. 40. 1993. pp. 23-27.

20. M. Hussain, D. Rouch, M. Britz. "Biochemistry of non-starter lactic acid bacteria isolate Lactobacillus casei GCRL163: Production of metabolites by stationary-phase cultures". *International Dairy Journal*. Vol. 19. 2009. pp. 12-21.

21. M. Siebold, P. Frieling, R. Joppien, D. Rindfleisch, K. Schügerl, H. Röper. "Comparison of the production of lactic acid by three different lactobacilli and its recovery by extraction and electrodialysis". *Process biochemistry*. Vol. 30. 1995. pp. 81-95.

22. J. Kious. *Lactobacillus and lactic acid production*. Tesis. Applied biological science branch. Le Tourneau university. Golden, Colorado (USA). 2000. pp. 15-21.

23. F. Dechow. *Separation and Purification Techniques in Biotechnology.* Ed. William Andrew. New Tork, EEUU 1989. pp. 42-58.

24. A. Zerquera, G. Pérez, I. Díaz, S. Delgado, R. de Armas, S. Leyva. "Resinas de intercambio iónico para prolongar la liberación de los fármacos". *Rev. Cubana Farm*. Vol. 34. 2000. pp. 196-206.

25. F. Chenlo, R. Moreira, L. Chaguri, F. Santos. "Isotermas de desorción de pimiento sde padrón (Capsicum annuuml. Var. Longum)". *Ciencia y tecnologia alimentaria*. Vol 5. 2005. pp. 18-24.

26. R. Perry, D. Green. *Adsorption and ion exchange*. Perry's Chemical Engineers` Handbook. 7th ed. Ed. McGraw-Hill. New York (USA). 1997. Vol. 4. pp. 16/12 -16/15

27. J. Avilla. "Lo Esencial acerca del intercambio iónico". *Resintech Inc*. Available in: http://javilla@resintech.com. Accessed 19 may 2009.