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ABSTRACT

This study focused on the integration of lactic acid adsorption and desorption onto commercially available resins in batch reactors with consequent scale-up in fixed-bed reactors. Amberlite® IRA-900, IRA-400, IRA-96 and IRA-67 were used as adsorbents for lactic acid recovery from solutions that mimic the fermentation broth of *Thermotoga neapolitana*. The best resins were further tested in adsorption–desorption batch experiments, over 13 cycles, with IRA-67 showing an average removal efficiency of 97%. IRA-67 was tested in fixed-bed reactor experiments and an average desorption efficiency of 68% was achieved using three bed volumes of 0.5 M NaOH as the desorbing agent.

Introduction

Increasing fossil fuel prices along with a major attention on environmental issues and sustainable development based on renewable resources push the transition from a traditional petrochemical-based to a biotechnological-based production of valuable chemicals such as lactic acid.[1,2] Lactic acid (2-hydroxypropionic acid) is a common carboxylic acid that can occur in two separated stereoisomers, *i.e.* L (+) and D (−), or in a racemic mixture. Although this compound is conventionally produced by chemical synthesis, about 90% of the annual worldwide lactic acid production directly derives from the fermentation of carbohydrates, such as glucose, sucrose, lactose and starch-[3,4]. In order to reduce the overall cost of biotechnological lactic acid production, it is important to optimize the fermentation phase in terms of feeding conditions, pH, temperature, mixing conditions, nutrient supply, type and initial substrate concentration[5,6]. Depending on the specific lactic acid-producing microorganisms involved, many hexose or pentose sugars can be utilized for lactic acid production by fermentation from different food waste materials, *e.g.* molasses[3], milk whey[7] or date juice[8]. Moreover, starchy and lignocellulosic materials, *e.g.* starches from potato[9], rice[10], wheat and pineapple[11], *Eucalyptus* wood[12], wheat straw[13] and corncobs[14], were found as proper and cheap starting substrates for lactic acid production.

The L (+) form is preferred by industry due to the presence of L-lactate dehydrogenase inside the human body[15] that enables its application in four main categories: food, cosmetic, pharmaceutical and chemical applications[16]. Recently, the global lactic acid consumption has considerably increased due to its relevant application as a monomer in the production of biodegradable polylactic acid (PLA), which is a well-known sustainable bioplastic material[17]. Noticeably, high purity lactic acid for high crystallinity PLA production is required[18].

The recovery and purification of lactic acid from dilute and complex aqueous solutions, such as fermentation broths, is still a challenging operation in terms of efficiency and costs[19]. Indeed, the recovery and purification steps account for about 50% of the overall lactic acid production costs[20]. Nowadays, different downstream processing techniques for lactic acid recovery are available[21,22], including calcium lactate crystallization, adsorption, ion exchange, reactive extraction, membrane extraction, reverse osmosis, conventional or bipolar membrane electrodialysis, esterification and reactive distillation. These purification

techniques for lactic acid recovery from fermentation broths can be classified as *in situ* and downstream extraction processes[23]. In the case of downstream extraction, the main issue relies on the production of other compounds, such as gypsum, produced during the additional washing step prior to obtaining pure lactic acid. For *in situ* extraction, it is important to avoid any toxicity effects on the fermentative microorganisms that can negatively affect the fermentation process and increase the cost of lactic acid production. A recent review[24] provides an extensive overview of the state of the art of the more promising production and isolation processes related to lactic acid production. As an example, membrane technologies for *in situ* separation of lactic acid, such as microfiltration, ultrafiltration, nanofiltration, and electrodialysis are one of the most efficient and environmental friendly purification methods[25]. By using sequential or multi-step membrane reactors, high concentrations of high purity lactic acid (*i.e.* 250 g L^{-1} and 95% purity) can be obtained from the fermentation of a wide range of raw materials[26,27]. As the long-time operation of the membranes can be affected by fouling, the selection of an appropriate separation module is a crucial step for membrane technology applications[28].

Due to the high selectivity, low energy required and ease of regeneration, sorption technologies are a competitive separation technique. The adsorption capacity and removal efficiency are influenced by various factors such as properties of the adsorbent (*e.g.* matrix, structure, functional group and porosity), adsorbate, *i.e.* lactic acid (polarity, molecular weight, structure and solute concentration), and operating conditions (*e.g.* contact time, pH, temperature and mixing conditions)-[29,30]. Adsorption can be highly selective for the targeted chemical by varying the functional groups and support matrix of the resins employed as adsorbent. Moreover, they can be directly used in the fermenter for *in situ* recovery, as the resins are non-toxic to microorganisms[31]. The complexity of the lactic acid containing fermentation medium can be a limiting factor as the resin capacity can decrease by 30–35% when ions other than lactate (*e.g.* sulphate, phosphate, citrate, acetate, nutrients or enzymes) are present in the fermentation broth.[4].

The sorption of lactic acid on polymeric anionic resins can be ascribed to two different phenomena that occur simultaneously depending on the operating conditions: adsorption and ion exchange[4]. Adsorption mainly occurs at pH values lower than the pKa (3.86), where lactic acid is in its non-dissociated molecular form, while ion exchange occurs at higher pH values as lactate is in the charged ionic form. In this context,

the adsorption by polymeric anionic resins is promising for lactic acid recovery from biological processes[32–34]. In a recent study[35], the authors reported that IRA-400 is suitable for *in situ* lactic acid recovery from the spent broth of the hyperthermophilic bacterium *Thermotoga neapolitana* that produces hydrogen together with a relatively low yield of this organic acid (<5 g L^{-1}) by dark fermentation and capnophilic lactic fermentation (CLF)[35–39].

Based on these previous results, the aim of this study was to test the adsorption ability of four anionic exchange resins (Amberlite® IRA-900, IRA-400, IRA-96 and IRA-67) in batch conditions that mimic the concentrations of lactic acid in the *T. neapolitana* fermentation broth. The lactic acid adsorption performance of the best strong (IRA-400) and weak (IRA-96) anionic resins were further tested by varying the contact time, the eluent concentration and its volume in the desorbing step. Low concentration solutions of HCl and NaOH were also tested with the strong and the weak resins, respectively. Finally, a continuous fixed-bed reactor (FBR) was operated for lactic acid adsorption at different flow rates in order to investigate the stability of IRA-96 in repeated adsorption–desorption cycles.

Materials and methods

Model *T. neapolitana* fermentation broth

A synthetic *T. neapolitana* fermentation broth was prepared from an analytical grade lactic acid stock solution (88% purity) and demineralized water, as described in Prhadan et al. [40]. Due to the high complexity of the spent fermentation broth[38], this study focused on simpler solutions constituted by lactic acid at concentrations close to those achievable by *T. neapolitana* growth on sugars. The broth was heated near the boiling point to both mimic the culture conditions (80°C and above) and completely hydrolyse the lactic anhydride present, reaching a final concentration of 4.5 g L^{-1}. All the samples were stored at 4°C and their lactic acid concentration was checked prior to use. Broth pH was adjusted to 6.5 in order to mimic the medium acidification due to CO_2 sparging during CLF[35,39,41].

Resin selection

Four anionic exchange resins were tested for lactic acid recovery from a model *T. neapolitana* fermentation broth: (i) Amberlite® IRA-900 (Cl⁻) referred as IRA-900, (ii) Amberlite® IRA-400 (Cl⁻) referred as IRA-400, (iii) Amberlite® IRA-96 (free base) referred as IRA-96

and (iv) Amberlite® IRA-67 (free base) referred as IRA-67. The main characteristics of these resins are reported in Table 1. The analytical grade anionic resins were purchased from Sigma-Aldrich (Milan, Italy).

Resin characterization

The surface morphology of the anionic exchange resins was investigated using a JSM-6010LA, Jeol (Tokyo, Japan) scanning electron microscope (SEM). Figure 1a-d shows the initial conditions of the fresh anionic resins. Before the observations, all the samples were sputter-coated with gold with an auto-fine coater (JFC-1300, Jeol, Tokyo, Japan). The coater is a complementary equipment to the SEM and provides necessary preparation of non-metallic non-conductive samples for use in SEM.

The specific number of active sites of the tested anionic resins was determined by potentiometric acid-base titration using an automatic titrator (848 Titrino Plus, Metrohm AG, Herisau, Switzerland) as described by Lützenkirchen et al.[42]. This technique is based on the differences between titrations of resin samples and blanks using HCl as the titrant. The solid samples (or blanks) and titrant were preliminary flushed with a low nitrogen flow overnight and for 1 h, respectively. During the potentiometric acid-base titration, 50 μL of HCl was added to the samples through a burette tip, once every 5 min until a pre-set end-point (same pH or same added volume of titrant in blank and sample titrations) was reached. Samples were continuously mixed using a magnetic stirrer (801 Stirrer, Metrohm AG, Herisau, Switzerland) in the presence of continuously sparging nitrogen gas. In all the phases, nitrogen purging was done to prevent CO_2 uptake from ambient air and related reduction–oxidation reactions that can affect the pH measurements.

Resin pre-treatment

When specified, a resin preparation procedure was adopted to enhance the performance of the resin investigated. The strong anionic resins (IRA-900 and IRA-400) were pre-treated with a specific sequential washing procedure as outlined by Moldes et al.[4]: (i) 1 N HCl solution, (ii) demineralized water, (iii) 1 N NaOH solution, (iv) demineralized water, (v) 1 N HCl solution and thereafter with (vi) demineralized water until pH 7.0 was reached. According to the same study[4], weak anionic resins (IRA-96 and IRA-67) were pre-treated with the following washing steps: (i) 1 NaOH solution, (ii) demineralized water, (iii) 1 N HCl solution, (iv) demineralized water, (v) 1 N NaOH solution and then with (vi) demineralized water until pH 7.0 was reached.

Batch experiments

In preliminary batch experiments, the following tests were carried out: (i) initial pH and pre-treatment of the anionic resins, (ii) initial lactic acid concentration and working temperature, (iii) kinetic behaviour and contact time, and (iv) combined adsorption–desorption cycles. These studies were carried out by placing the test bottles either on an orbital shaker (INNOVA 2100, New Brunswick Scientific, Edison, NJ) or a shaking thermostatic water bath (GFL1083, Burgwedel, Germany) at a speed of 180 rpm for up to 4 h, as recommended in Dethe et al.[43]. Serum bottles (30 mL) with screw caps containing 0.5 g of anionic resin (10% w/v) and 5.0 mL of bulk solution were used for all the batch adsorption studies, unless stated otherwise. All the batch adsorption experiments were performed in duplicate by employing sacrificial serum bottles for each data point.

The initial pH (2.0–6.5) of the samples mimicking T. neapolitana fermentation broth was adjusted by either adding 3.0 M NaOH or 1 M HCl. The behaviour of the resins was compared using fresh (untreated) or pre-treated resins as described in the previous section. The effects of temperature (30°C and 80°C) and initial lactic acid concentration (1.4, 2.3, 4.5, 9.0, 13.5 and 18.0 g L^{-1}) were investigated on the pre-treated strong and weak anionic resins. The adsorption performances for these experiments were analysed at a fixed pH: 5.0 for IRA-400 and 900 and 2.0 for IRA-67 and 96.

Kinetic batch experiments were carried out at the most effective conditions, i.e. 30°C and an initial pH of 5.0 and 2.0 for, respectively, the weak and strong resins. Samples were withdrawn at regular time intervals (10, 20, 40, 60 min and 24 h) for lactic acid measurements.

Combined adsorption–desorption batch studies were performed at 30°C using 2 g of fresh anionic resins IRA-400 or IRA-67 (40% w/v) contacted with 5 mL

Table 1. Physico-chemical properties of the strongly basic (IRA-900 and IRA-400) and weakly basic (IRA-96 and IRA-67) anionic resins.

| Resin	Matrix	Functional group (+)	Form	(diameter)	Moisture content (%)
IRA-900	Styrene-divinylbenzene (macroporous)	Benzyltriethyl-ammonium	Cl⁻	650–820 μm	58–64
IRA-400	Styrene-divinylbenzene (gel)	Quaternary ammonium	Cl⁻	600–750 μm	40–47
IRA-96	Styrene-divinylbenzene (macroporous)	Tertiary amine (at least 85%)	Free base	550–750 μm	57–63
IRA-67	Acrylic (gel)	Polyamine	Free base	500–750 μm	~60
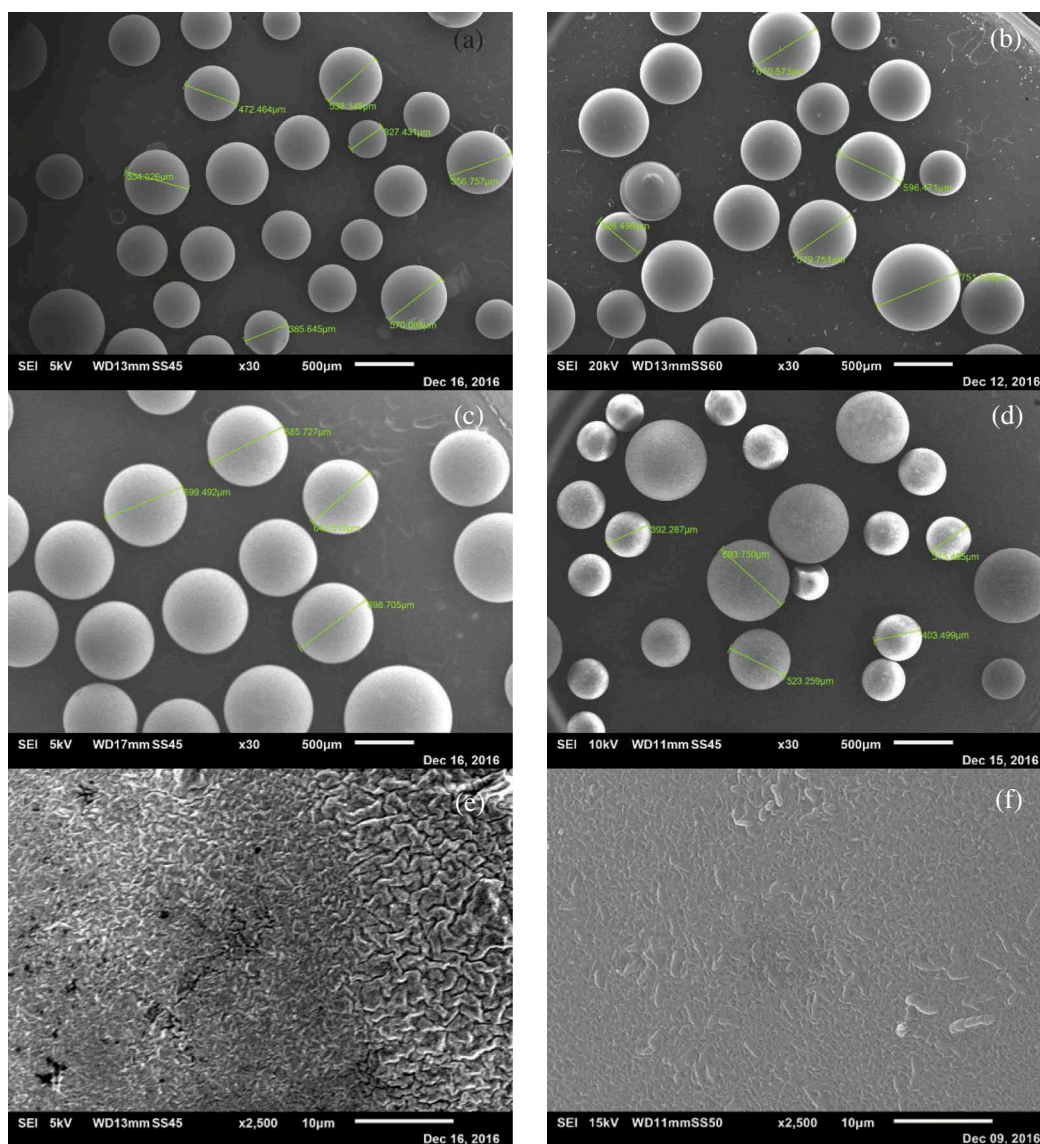
Figure 1. Low magnification SEM images of the fresh anionic resins: (a) IRA-900, (b) IRA-400, (c) IRA-96, and (d) IRA-67 and high magnification SEM micrographs of IRA-67 (e) before, and (f) after six adsorption–desorption cycles during column experiments.

fermentation broth. The model *T. neapolitana* fermentation broth with an initial lactic acid concentration of 4.5 g L^{-1} was periodically added at the start of each 10-min cycle. The initial pH was adjusted to 5.0 (for IRA-400) or 2.0 (for IRA-67) and each cycle was made up of the following sequential stages: (i) pre-washing with demineralized water, (ii) adsorption, (iii) desorption (HCl and NaOH for, respectively, the strong and the weak resin), (iv) washing with demineralized water and (v) final adjustment until pH 7.0 was reached. For the first 5 cycles, the desorption phase was performed using 2 mL of 1 M NaOH or 1 M HCl solutions, while the concentration and volume of the eluents were reduced to 0.5 M and 1 mL, respectively, for the remaining cycles. According to the batch kinetic studies, each of the 13 adsorption and desorption cycles was carried out at a contact time of 10 min.

Fed-batch experiments

The fed-batch experiments were carried out in a temperature-controlled (30°C) environment on an orbital shaker at 180 rpm as described in the previous section. Serum bottles (30 mL) with screw caps were filled with 0.5 g of fresh anionic resins IRA-400 and IRA-67 (10% w/v), and made in contact with 5 mL of model solutions having an initial lactic acid concentration of 4.5 g L^{-1}. The initial pH was adjusted to 5.0 (for IRA-400) or 2.0 (for IRA-67) by adding 3.0 M NaOH and 1 M HCl, respectively.

The constant working volume of the fed-batch system was maintained by withdrawing a certain volume of sample, and it was compensated by the addition of the same volume of fresh model broth. In particular,

the exhausted fermentation broth (4 mL) was withdrawn every hour with the subsequent addition of fresh fermentation broth (4 mL). This procedure was repeated for 6 h. These experiments were designed to get preliminary information on the adsorption of lactic acid using anionic resins in a FBR.

Column experiments in continuous FBR

A glass column (i.d. 2.6 cm, o.d. 2.9 cm, length 17 9 cm) was loaded with 10 g fresh resin IRA-67 (bed height 3.5 cm, bed volume BV 18.6 cm^3). A glass wool layer, previously washed with demineralized water, was placed between the bottom of the column and the resin. The acidified samples mimicking *T. neapolitana* fermentation broth (pH 2.0) as well as demineralized water were fed continuously to the FBR in downflow mode (avoiding bed fluidization) using a peristaltic pump (7528–30 Masterflex, Cole-Parmer, Vernon Hills, IL) fitted with silicone tubing (3 × 5 mm). An additional peristaltic pump (7528–30 Masterflex, Cole-Parmer, Vernon Hills, IL) was used to control the outlet flow rate.

An initial set of column experiments was carried out to investigate the effect of the flow rate variation on the adsorption of lactic acid. In this phase, the critical loading rate (LA$_{clr}$), which represents the volumetric loading rate at which the lactic acid removal efficiency decreases, was experimentally evaluated.

The effect of the flow rate variation was explored during the second set of experiments, in which five adsorption–desorption cycles with five different flow rates (2.5, 3.5, 5.0, 8.0 and 10.0 mL min^{-1}) were tested. Finally, several column experiments were carried out with six adsorption–desorption cycles by applying an adsorption phase of 5 h at a flow rate of 3.5 mL min^{-1} to further check the stability of the IRA-67 resin for lactic acid recovery from the fermentation broth.

Analytical methods

Lactic acid concentrations were determined in duplicate by ionic chromatography analysis using an ICS-1100 (Dionex, Thermo Fisher Scientific Inc., Waltham, MA) as described by Pradhan et al.[40]. The mobile phase was 1.7 mM NaHCO$_3$ and 1.8 mM Na$_2$CO$_3$ with a flow rate of 0.5 mL min^{-1}. Prior to analysis, the pH of all the samples was adjusted in the range of 5.0–8.0 by using 0.1 M NaOH or 0.05 M HCl. The pH was measured by a E510 pH-meter (Metrohm AG, Herisau, Switzerland) provided with a multi-parameter probe and calibrated with commercial buffers.

Results

Batch tests for lactic acid adsorption

Depending on the initial pH used, the lactic acid removal efficiency for the two strong anionic resins tested (IRA-900 and IRA 400) showed a similar trend (Fig. 2). Both resins were effective at a pH above the pKa (3.86) of lactic acid. At pH 5.0 (Fig. 2c), the highest lactic acid removal efficiency was 43.6% (w/p – with pre-treatment or pre-treated) and 46.8% (n/p – not pre-treated or fresh) for IRA-900 and 49.2% (pre-treated) and 50.0% (fresh) for IRA-400, respectively. The lactic acid removal efficiency did not change significantly for pH > 5.0 and the effect of the pre-treatment was negligible for both strong anionic resins (Fig. 2). On the other hand, the weak anionic resins (IRA-96 and IRA-67) showed high selectivity at a pH below the lactic acid pKa, reaching the highest removal efficiency for the pre-treated resins (99.2% for IRA-96 and 99.3% for IRA-67) at pH 2.0 (Fig. 2a).

From Fig. 3 it is evident that the lactic acid removal efficiency sharply decreased from 67.7% to 27.2% as soon as the initial lactic acid concentration increased from 1.4 to 18.0 g L^{-1} for IRA-900 at 30°C. Besides, IRA-400 showed a significant decrease (from 71.1% to 31.6%) in the lactic acid removal efficiency at the same temperature. Conversely, the weak anionic resins showed minimal changes in the removal efficiencies irrespective of the initial lactic acid concentrations. Specifically, IRA-67 exhibited lactic acid removal efficiencies exceeding 99% for a wide range of initial lactic acid concentrations (Fig. 3). The lactic acid removal efficiency decreased in all experiments carried out at 80°C, except for IRA-67, which maintained a lactic acid removal efficiency >90% for an initial lactate concentration ranging from 1.4 to 13.5 g L^{-1}.

To evaluate the effect of contact time on the lactic acid removal efficiency, the most effective pre-treated weak (IRA-67) and strong (IRA-400) anionic resins were further tested at 30°C and at pH 5.0 and 2.0, respectively, in order to ascertain the adsorption behaviour as a function of time with different initial lactic acid concentrations. The results showed that the residual lactic acid concentration significantly decreased after 10 min and reached a constant value after 20 min of operation irrespective of the initial lactic acid concentration (Fig. 4).

Adsorption–desorption experiments

The stability of the fresh anionic resins IRA-400 and IRA-67 (40% *w/v*) for lactic acid recovery was assessed over 13 adsorption–desorption cycles using the optimal
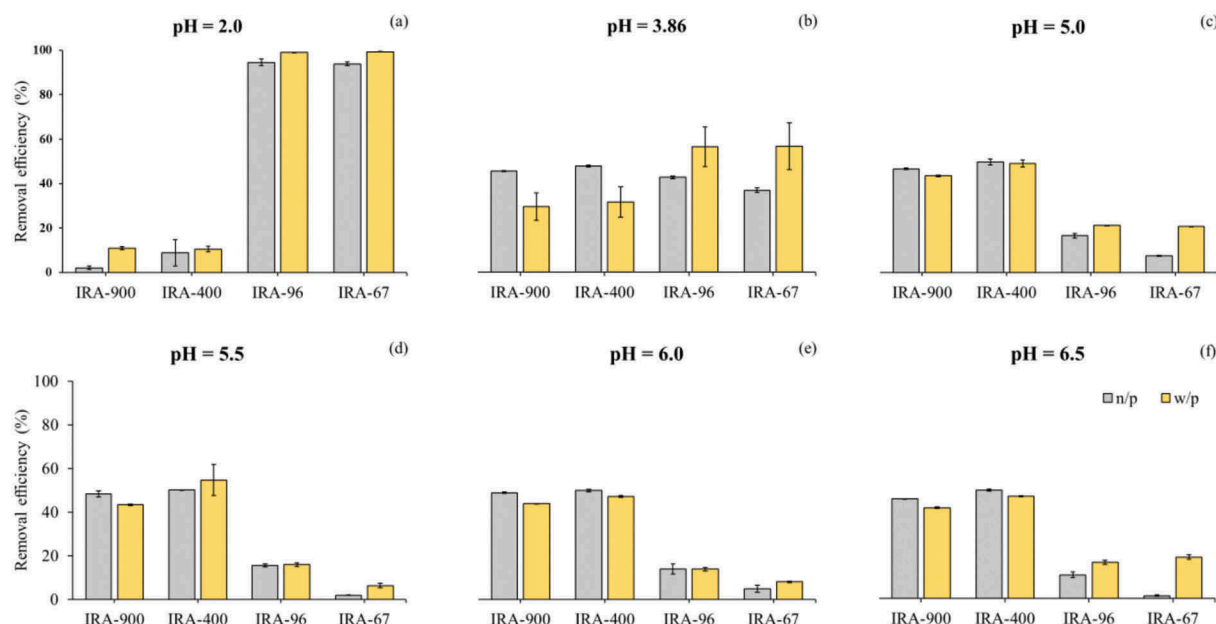
Figure 2. Effect of pH on the lactic acid removal efficiency for four anionic resins (IRA-900, IRA-400, IRA-96 and IRA-67): (a) 2.0, (b) 3.86, (c) 5.0, (d) 5.5, (e) 6.0 and (f) 6.5. n/p = not pre-treated, w/p = with pre-treatment.
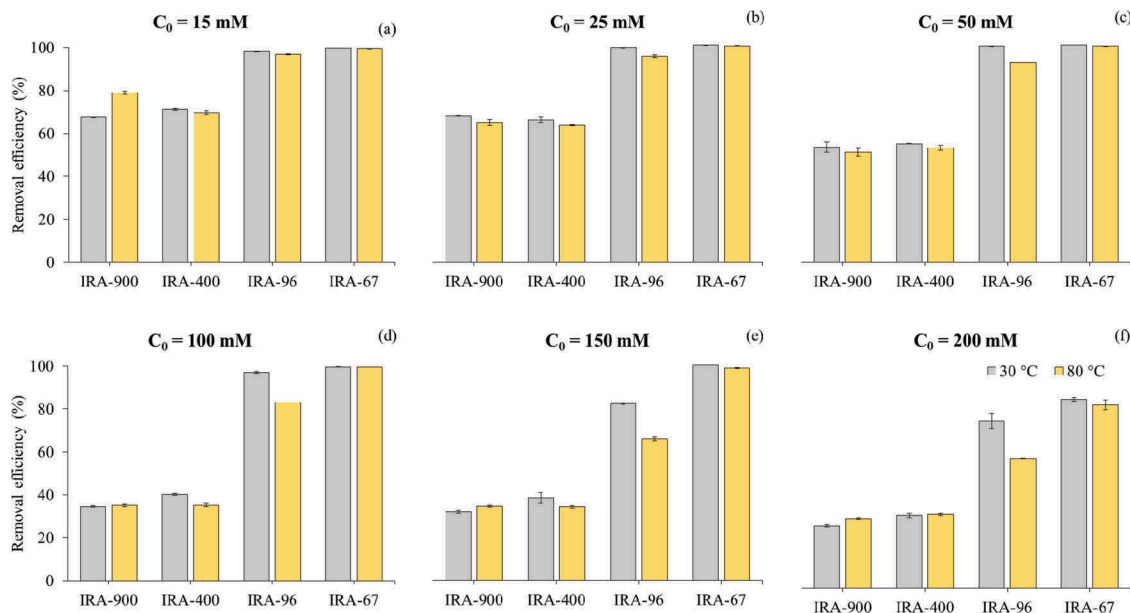

Figure 3. Effects of temperature and different initial lactic acid concentration on lactic acid removal efficiency for four pre-treated anionic resins (IRA-900 and IRA-400 at pH 5.0 and IRA-96 and IRA-67 at pH 2.0): (a) 1.4 g L^{-1}, (b) 2.3 g L^{-1}, (c) 4.5 g L^{-1}, (d) 9.0 g L^{-1}, (e) 13.5 g L^{-1} and (f) 18.0 g L^{-1}.

adsorption contact time achieved in the previous batch experiments. The lactic acid removal efficiency for IRA-67 was >99% until the ninth cycle and thereafter it steadily decreased to 93% during the last cycle (Fig. 5a). In the case of IRA-400 resin, the lactic acid removal performance slightly decreased steadily during the cycles, with the lactic acid removal efficiency decreasing from 80% to 69% (Fig. 5a).

Compared to IRA-67, IRA-400 attained a lower desorption efficiency, ranging from 33% to 47% using 2 mL of 1 M HCl and from 38% to 41% using 1 mL of 0.5 M NaOH as the regenerant. On the other hand, the desorption efficiency of IRA-67 varied between 55% and 65% using 2 mL of 1 M NaOH, and 52% and 65% using 1 mL of 0.5 M NaOH, respectively (Fig. 5b). During the desorption
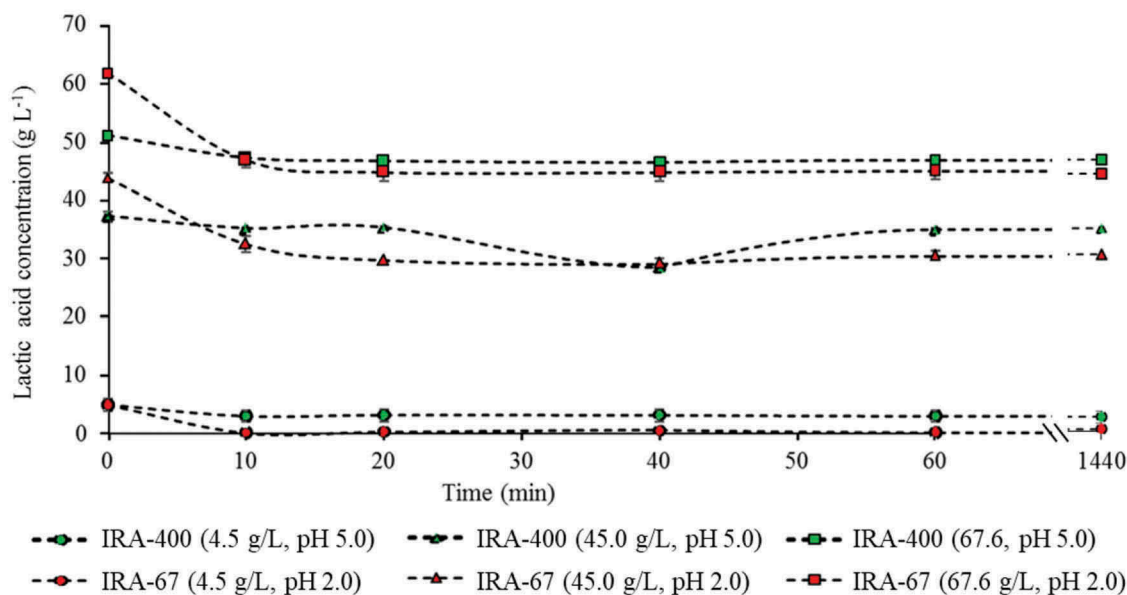
Figure 4. Effect of contact time on residual lactic acid concentration in batch adsorption experiments (0–24 h) using the pre-treated anionic resins IRA-400 (pH 5.0) and IRA-67 (pH 2.0) with three different initial lactic acid concentrations (4.5, 45 and 67.6 g L^{-1}), at 30°C.

step, a lower concentration of the regenerating solutions was used in order to employ a lower amount of chemical without decreasing the lactic acid concentration in the eluate. Despite incomplete regeneration and desorption of lactic acid within the different cycles, IRA-400 and IRA-67 showed stable removal efficiencies. Apparently, resin saturation was not reached during the adsorption phase, allowing additional sorption of lactic acid at the remaining active sites.

Fed-batch experiments

The results from fed-batch experiments highlighted decreasing trends for the lactic acid removal efficiency with time for both resins tested (Fig. 6). IRA-67 showed a lactic acid removal efficiency >99.8% within the first 2 h of operation. The removal efficiency decreased to 75% at the third hour and successively achieved the final value of 12.5% after 6 h. Differently, the removal efficiency for IRA-400 sharply decreased to 50% after 1 h of operation, gradually reaching a constant value of 12.5% after 6 h (Fig. 6).

Continuous FBR operation

Throughout the first experimental set, the lactic acid removal efficiency was >85% during the first 2 h of operation of the FBR at a flow rate ranging between 2.5

and 3.5 mL min^{-1} (Fig. 7a). Later, it sharply decreased, reaching a constant value of 20% at the end of the experiment, at a flow rate of 10 mL min^{-1}. In this preliminary phase, the critical loading rate (LA$_{clr}$) was evaluated for the FBR by plotting the lactic acid volumetric loading rate (LA$_{vlr}$) on the x-axis and the lactic acid volumetric removal rate (LA$_{vrr}$) on the y-axis. The FBR was found to have a LA$_{clr}$ of 63.1 g L^{-1} h^{-1} after 2 h of operation (Fig. 7b), at which the experimental removal efficiency (blue line) deviates from the theoretical 100% removal efficiency line (black line). The latter derives from the theoretical mass balance equation in which all the lactic acid present in the influent is continuously adsorbed by the resin. The desorption phase was carried out using 3 BV of 0.5 M NaOH. The lactic acid desorption efficiency was found to be 45.2%, while the lactic acid concentration in the eluent was >26.5 g L^{-1} for each BV, reaching a value of 36.5 (± 1.5) g L^{-1} with the third BV (Fig. 7c).

After identifying the LA$_{clr}$, the results from the second experimental set showed that the resin IRA-67 maintained the lactic acid removal efficiency >90% for 2 h at the lowest flow rate tested (first cycle at 2.5 mL min^{-1}) and its performance decreased as the operation time and flow rate increased. After 5 h, the lactic acid removal efficiencies reached a constant value ranging between 10% and 15% for all the flow rates tested, with the exception of the highest one. Breakthrough occurred in less than 30 min, except
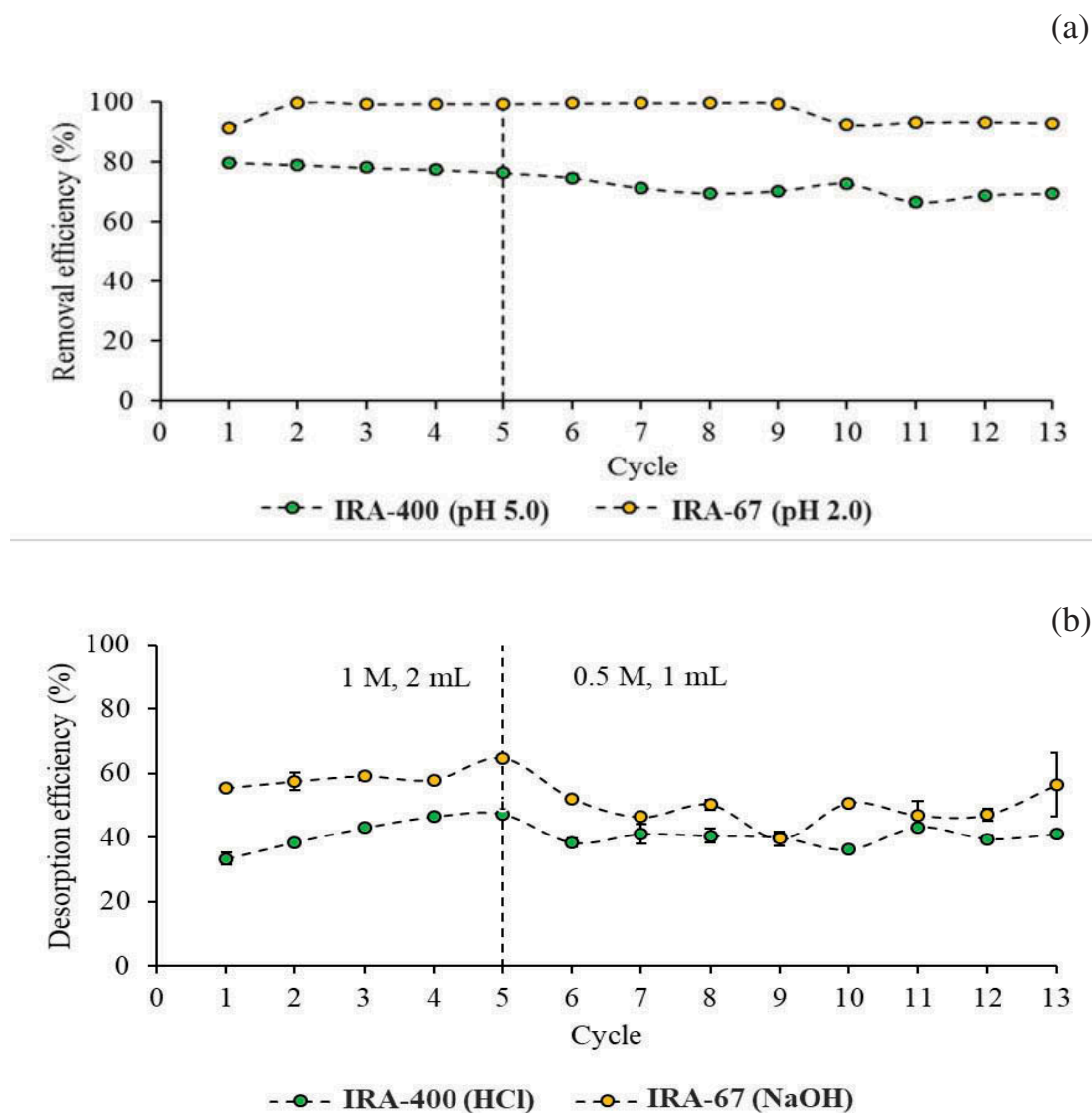
(a)

(b)

Figure 5. Lactic acid removal (a) and desorption efficiency (b) at varying eluent conditions over 13 adsorption–desorption cycles for un-treated IRA-400 (pH 5.0) and IRA-67 (pH 2.0) at 30°C.

for the highest flow rate where it occurred between 30 and 60 min (Fig. 8a). The bed was completely saturated during the last test after an operation time of 2 h (Fig. 9). Afterwards, the experimental breakthrough curves showed decreasing pH of the effluent towards the influent value as the effluent lactic acid concentration steadily increased to values similar to the influent concentration (Fig. 9).

The mass balance on lactic acid during the adsorption phase together with other characteristic parameters (*i.e.* removal efficiency, resin capacity and desorption capacity) are reported in Table 2. The adsorption capacity was similar for the first 3 cycles (2.5, 3.5 and 5 mL min^{-1}), with an unexpected increase after 3 h for the fourth cycle at 8 mL min^{-1}. The uptake of lactic acid reached the

minimum value in the last cycle, which was mainly affected by an incomplete desorption during the fourth cycle. The decrease in the amount of lactic acid desorbed until the fourth cycle may be explained by a negative effect of extended desorption and its consequent decrease in the regeneration efficiency of the active sites. As shown in Fig. 1e and 1f, the external adsorbate layer was easily removed while the internal adsorbate layer was not cleaned after several adsorption–desorption cycles during column experiments.

The stability of IRA-67 in recovering lactic acid from a model *T. neapolitana* fermentation broth was finally demonstrated over six adsorption–desorption cycles at a flow rate of 3.5 mL min^{-1}. The results of the six cycles are summarized in Table 3. The removal efficiency
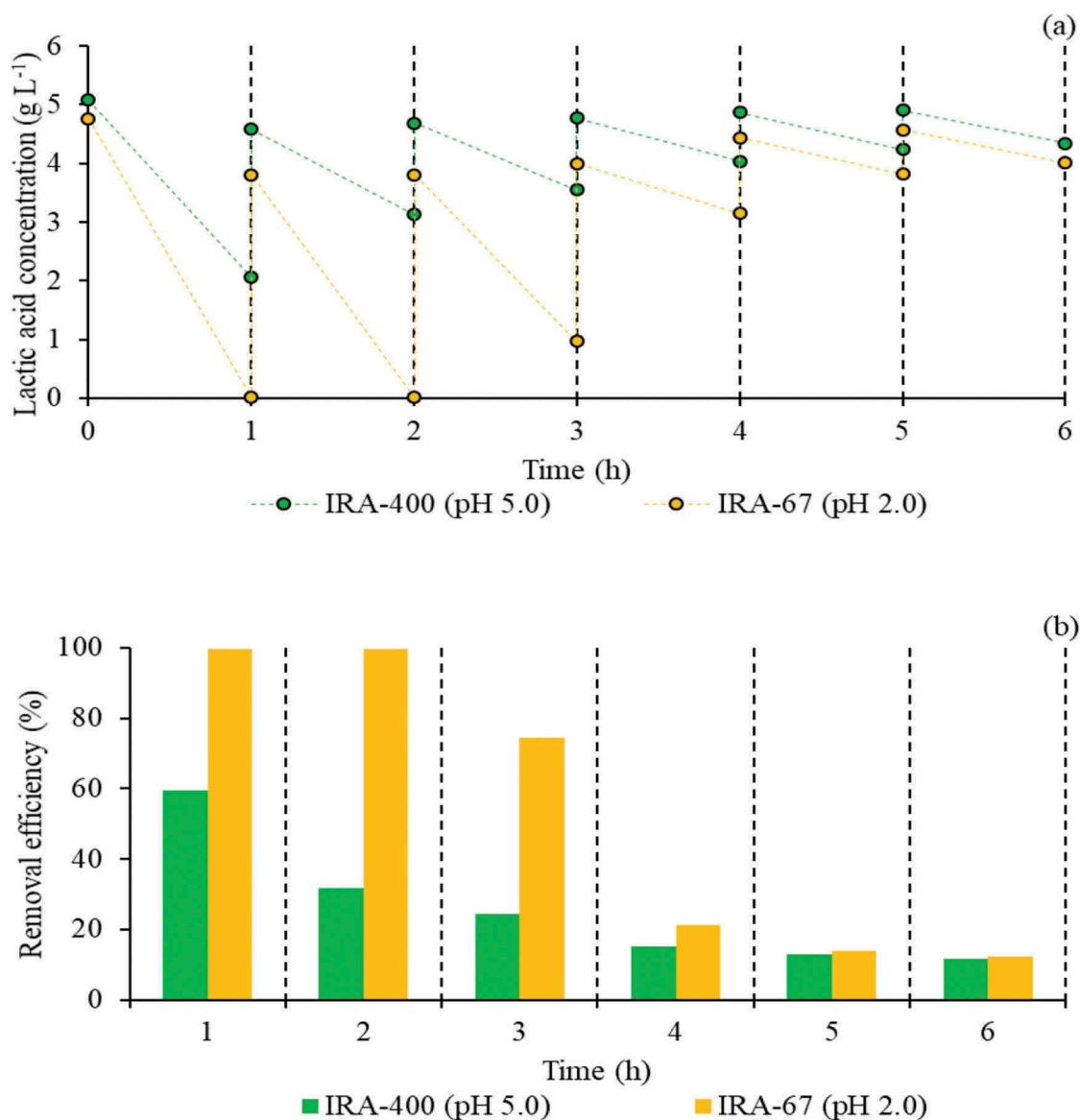
Figure 6. Time course profiles of lactic acid concentration (a) and removal efficiency (b) in fed-batch experiments (0–6 h) for untreated IRA-400 and IRA-67 at 30°C.

decreased from 66.3% to 47.2% between the first two cycles (Fig. 8b), whereas it slightly increased to 56.0% for the fifth cycle and then it reached the lowest value in the last cycle (46.1%). The outlet lactic acid concentration gradually increased up to the feeding value and the pH of the outlet samples decreased until 2.0. Moreover, as the process continued, the breakthrough time decreased from 120 (first cycle) to 90 min (second and fifth cycle), reaching a minimum value of 60 min during the last cycle (Fig. 10). The resin IRA-67 was not saturated during the first cycle and showed fluctuations with operating time to reach saturation (more than 210 min) for the remaining cycles. The desorption efficiency sharply increased from 64.7% up to 89.2%

during the first two cycles and thereafter it decreased to 56.5% during the last cycle. The use of 0.5 M NaOH (3 BV) allowed to achieve a lactic acid concentration in the eluate exceeding 24.5 g L^{-1}, except for the last cycle (19.3 g L^{-1}).

Discussion

Batch experiments to assess in situ and down-stream applications

This study showed that anionic resins can be successfully applied for lactic acid recovery from dark and capnophilic fermentation broths by using the FBR technology. In
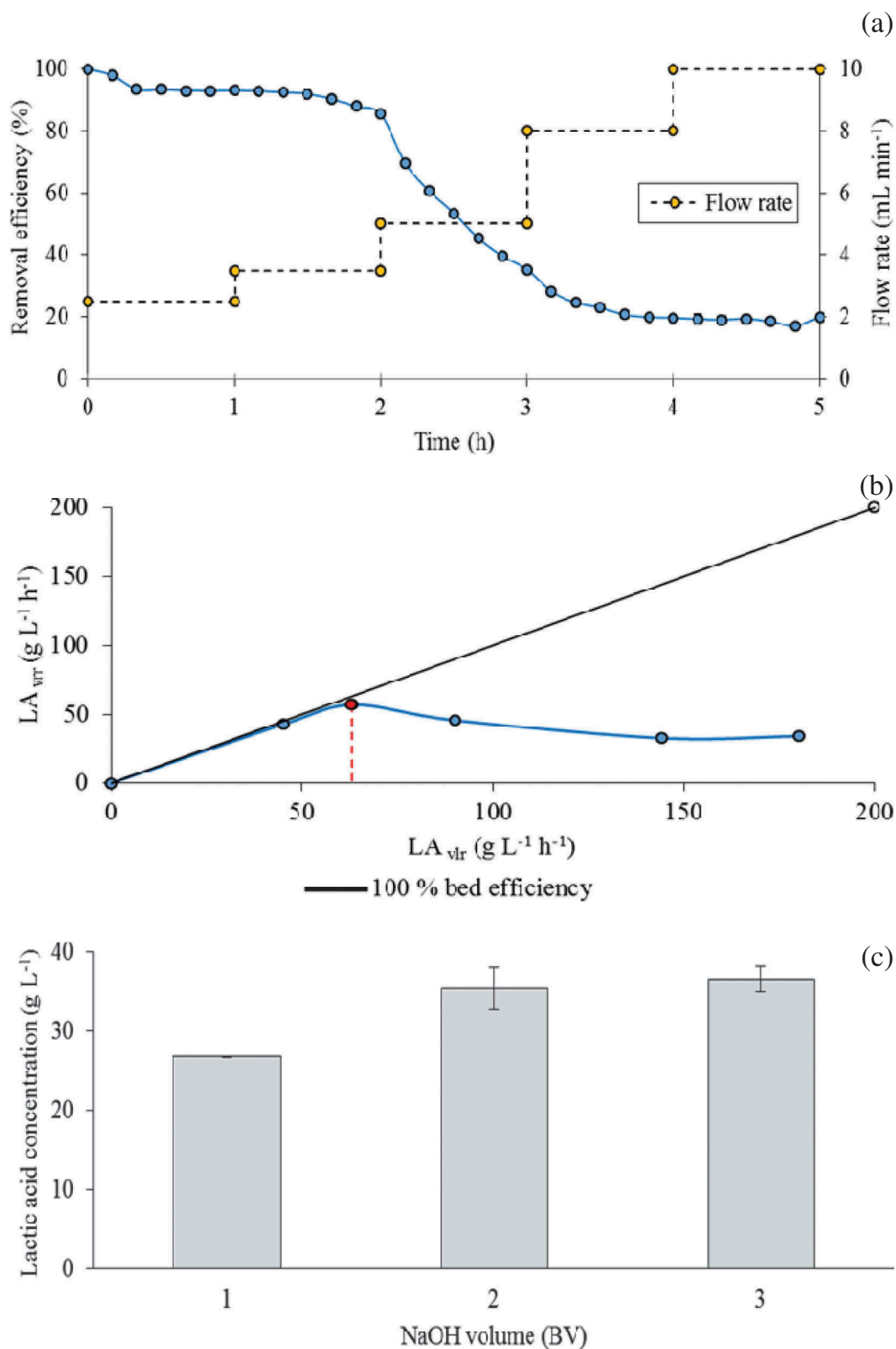
Figure 7. Column experiments with step increase in flow rate once every hour (2.5, 3.5, 5.0, 8.0 and 10.0 mL min^{-1}) (a), graphical determination of the critical lactic acid loading rate (b), and desorbed lactic acid during the regeneration phase with 3 BV of 0.5 M NaOH at 25°C and an initial lactic acid concentration of 4.5 g L^{-1} (c).
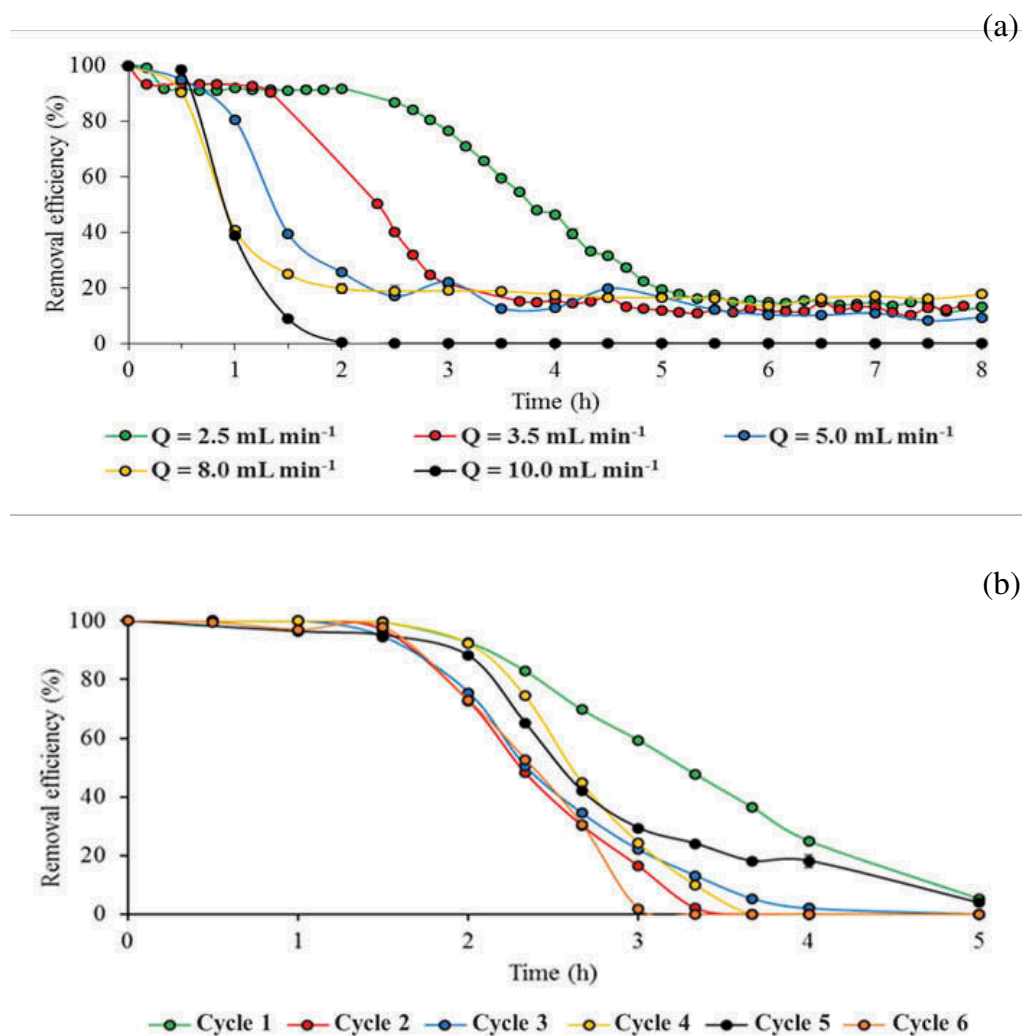
Figure 8. Column experiments (0–8 h) with different flow rates (2.5, 3.5, 5.0, 8.0 and 10.0 mL min^{-1}) (a) and column experiments (0–5 h) with adsorption–desorption cycles at a constant flow rate of 3.5 mL min^{-1} (b) at 25°C and an initial lactic acid concentration of 4.5 g L^{-1}.

particular, the weak resin showed better performance in terms of lactic acid recovery during column experiments than in batch and semi-batch configurations.

The first set of batch experiments highlighted the higher uptake of lactic acid by the tertiary amine based resins, *i.e.* IRA-96, than by the strong base resins with quaternary functional groups. Indeed, IRA-96 and IRA-67 showed most of their adsorption capacity up to the lactic acid pKa, revealing a low efficiency up to neutral pH due to the accessibility for the molecular form of lactic acid to the polyamine or tertiary amine functional groups[44,45]. In comparison, the strong resins IRA-900 and IRA-400 showed a high selectivity at a pH above the lactic acid pKa. This might be due to the presence of quaternary ammonium as functional groups, which interact with negatively charged lactate but have a relatively poor ability to absorb lactic acid in the non-

dissociated form[45]. According to previous studies investigating the adsorption of lactic acid from highly concentrated lactic acid fermentation broths[46], the weak anionic resins were highly effective for lactic acid recovery at a pH below the pKa of lactic acid, while the strong anionic resins reached their highest removal efficiency at higher pH values.

This study showed that the weak anionic resins IRA-96 and IRA-67 cannot be employed for *in situ* lactic acid recovery as they operate properly at a pH lower than the optimal pH (5.0–7.0) for lactic acid fermentation[3]. Indeed, there are some applications of weak resins, *i.e.* IRA-67, for *in situ* extraction of lactic acid from dark fermentation broths[30], but the uncontrolled pH during the biological process usually leads to high acid accumulation rather than H$_2$ production[47]. In fermentation processes devoted to the maximization of H$_2$
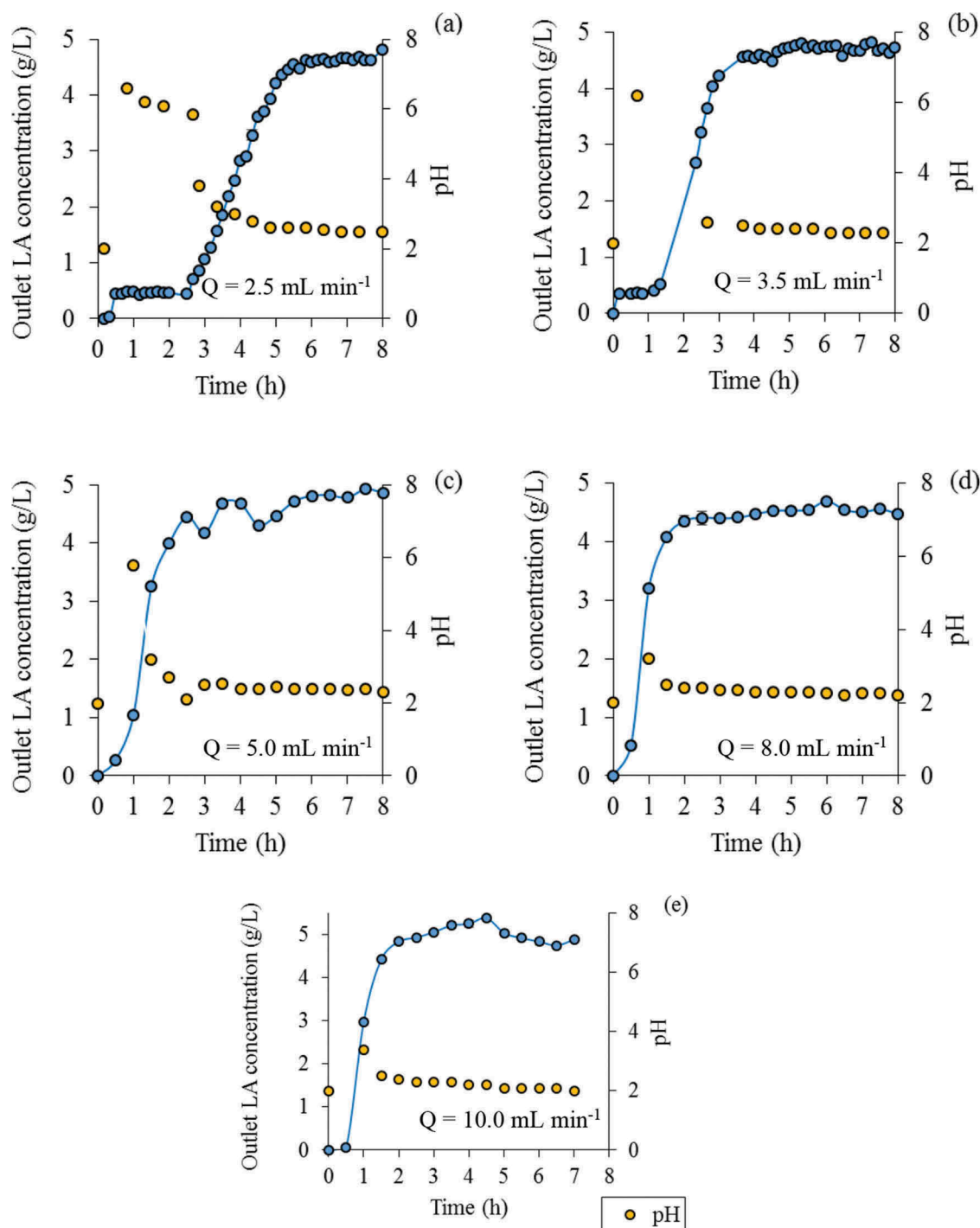
Figure 9. Experimental breakthrough curves and pH trend during five adsorption–desorption cycles in column experiments at 25°C and initial lactic acid concentration of 4.5 g L^{-1}: (a) 2.5 mL min^{-1}, (b) 3.5 mL min^{-1}, (c) 5.0 mL min^{-1}, (d) 8.0 mL min^{-1} and (e) 10 mL min^{-1} (0–8 h).

Table 2. Lactic acid desorption from IRA-67 during five adsorption–desorption cycles in column experiments using 0.5 M NaOH as eluent (3 BV) at 25°C.

Cycle	Flow rate (mL min^{-1})	Flow rate (BV min^{-1})	Cumulative lactic acid desorbed (g)	Resin capacity q_{FB} (g g^{-1})	Desorption efficiency (%)	Lactic acid concentration (g L^{-1})
1	2.5	0.134	1.80 (±0.1)	0.405	54.4 (±0.4)	32.3 (±0.9)
2	3.5	0.188	1.65 (±0.1)	0.322	51.2 (±1.2)	29.6 (±0.9)
3	5.0	0.268	1.67 (±0.1)	0.361	46.3 (±0.8)	30.0 (±0.6)
4	8.0	0.430	1.19 (±0.1)	0.549	21.7 (±0.2)	21.3 (±1.0)
5	10.0	0.537	1.46 (±0.1)	0.286	51.1 (±0.6)	26.2 (±0.6)

Table 3. Lactic acid desorption from IRA-67 during six adsorption–desorption cycles in column experiments using 0.5 M NaOH as eluent (3 BV) at 25°C.

Cycle	Cumulative lactic acid desorbed (g)	Resin capacity q_{FB} (g g^{-1})	Desorption efficiency (%)	Lactic acid concentration (g L^{-1})
1	1.7 (±0.1)	0.256	64.7 (±0.3)	29.7 (±0.4)
2	1.5 (±0.1)	0.174	89.2 (±0.4)	27.8 (±0.4)
3	1.4 (±0.1)	0.189	73.0 (±1.2)	24.8 (±0.4)
4	1.4 (±0.1)	0.197	73.6 (±0.9)	26.0 (±0.3)
5	1.4 (±0.1)	0.232	59.1 (±0.7)	24.6 (±0.3)
6	1.1 (±0.1)	0.191	56.5 (±2.2)	19.3 (±0.8)

production, higher pH values are usually required and the application of a weak resin is not appropriate. Recently, an interesting result was reported by Pradhan et al.[40] by comparing the strong anionic resin IRA-400 with granular activated carbon (GAC) for lactic acid recovery. At 80°C, the resin IRA-400 showed a better performance than GAC, confirming its promising application for *in situ* lactic acid recovery from *T. neapolitana* fermentation broth. The better performance of the strong anionic resin than the granular activated carbon was also verified by other studies[48]. The possibility to use IRA-400 for *in situ* lactic acid recovery from high strength lactic acid (>50 g L^{-1}) fermentation broths was also shown by Quintero et al.[49].

The adsorption of lactic acid by the four resins tested was differently affected by the temperature (Fig. 3). The adsorption by ion exchange resins is an exothermic process with negative heat of adsorption[40,50] and high operational temperatures can cause irreversible changes in the physio-chemical properties of the resins due to disruption of the chemical bonds between the functional groups and the polymeric matrix[51]. Gao et al.[52] indicated that the lactic acid adsorption capacity decreased by ~20% for IRA-67 (10% *w/v* adsorbent dose) when the temperature increased from 20 to 50°C with an initial lactic acid concentration of 42.5 g L^{-1}. The authors suggested that the lactic acid diffusion coefficient is a fundamental parameter to define the adsorption behaviour of anionic resins in the presence of high (>70 g L^{-1}) or low (<50 g L^{-1}) lactic acid concentrations in the fermentation broth. Moldes et al.[12] reported that the lactic acid adsorption capacities of IRA-900, IRA-400, IRA-96 and IRA-67 (40% *w/v* adsorbent dose) were not influenced by temperature within the range of 25–45°C for an initial lactic acid concentration of 37 g L^{-1}. Similarly, in this study, temperature had a negligible influence on the tested resins, except for IRA-96 which showed a decrease of its maximum adsorption capacity (~22%) when operating at 80°C. This difference is probably related to the loss of the weakly bounded tertiary amine groups from the polymeric matrix due to the temperature increase-[53]. On the other hand, IRA-400 confirmed its thermal stability (Fig. 3) as also shown in a previous study[40].

The results obtained by varying the contact time and the lactic acid concentration in the fermentation broth (Fig. 4), within the third batch assessment, are comparable to those presented by Moldes et al.[4] and Rampai et al.[54] in the case of high lactic acid concentrations in the influent. A similar behaviour of the resin was observed in this study for a low lactic acid concentration (4.5 g L^{-1}). Both anionic resins (IRA-400 and IRA-67) showed a common kinetic sorption pattern characterized by an initial rapid decrease in lactic acid concentration followed by a steady-state value. Indeed, the active sites are initially largely available, while the number of occupied sites and the intensity of the repulsive forces between adsorbate molecules increased during the experiment.

IRA-67 showed a higher selectivity and stability throughout the 13 adsorption–desorption cycles than IRA-400 (Fig. 5b). Eluent selection is a very important parameter for the desorption of carboxylic acids from cationic resins. Commonly, different aliphatic alcohols (*e.g.* methanol and ethanol), aliphatic ketones (*e.g.* acetone and methyl ethyl ketone), salts (*e.g.* NaCl and NH$_4$OH), acids (*e.g.* HCl and H$_2$SO$_4$) or bases (*e.g.* NaOH) have been used. The main challenge is the reduction of the costs related to the chemicals employed during the desorbing step. Evangelista and Nikolov[20] employed a mixture of methanol and 5% NH$_4$OH solution for desorbing lactic acid from weakly basic ion exchange resins Dowex MWA-1, Amberlite IRA-35 and Riedel-de-Haen VI-15. Lactic acid was completely desorbed from Riedel-de-Haen VI-15 ion exchange resin using 6.8 BV of methanol. Gao et al.[55] used acetone due to its low volatility and easy recovery from GAC. In particular, the lactic acid recovery rate significantly increased to ~80% with increasing amount of acetone. Quintero et al.[49] observed NaOH (1 M) as the best desorbing agent (92.7% ± 1.9 extraction efficiency) among the different eluents (0.1 M HCl, 10% methanol, 1 M H$_2$SO$_4$ and 1 M NaCl) using the strong anionic resin Amberlite IRA-400.

Da Silva et al.[50] developed a multi-stage desorption process for propionic acid (with 1, 3, 4, 5 and 6 tanks) using ethanol and n-propanol as eluents to reduce the amount of solvent and maximize the amount of adsorbate. Cao et al.[56] investigated the elution of lactic acid adsorbed on Amberlite IRA-400 at different pH using several eluents. At pH 5.0, the highest elution recovery of 97.5% was obtained with 2.5 M H$_2$SO$_4$. However, 1.0 M H$_2$SO$_4$ also showed the high elution recovery of
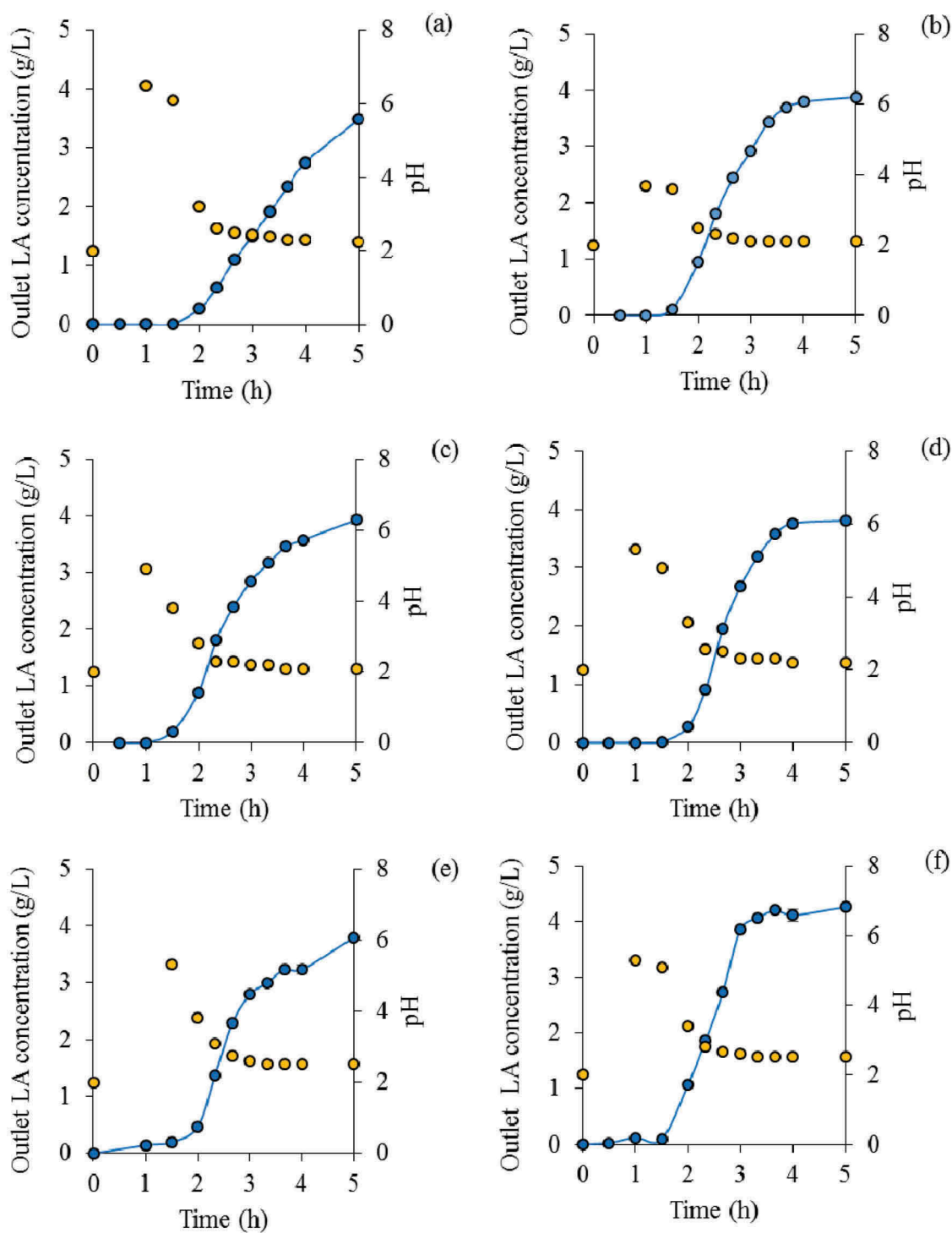
Figure 10. Experimental breakthrough curves and pH trend during six adsorption–desorption cycles (a–f) in column experiments at a flow rate of 3.5 mL min^{-1} at 25°C and an initial lactic acid concentration of 4.5 g L^{-1}.

93.6%. On the other hand, the elution of lactic acid was easily performed using water as eluent when the adsorption was performed at pH 2.0 (desorption efficiency 73.4%).

In the present study, HCl and NaOH were employed as eluents for desorption of the adsorbed lactic acid onto IRA-400 and IRA-67, respectively. HCl is a common eluent for regeneration of the resins in Cl$^-$ form that can be easily separated from lactic acid by selective evaporation[49]. Conversely, NaOH is a non-volatile and low-cost chemical, widely employed for the regeneration of resin in OH$^-$ form in other studies[34,57].

Adsorption capacity in fed-batch applications

The scaling-up of the process proceeded with semi-continuous experiments showing a higher selectivity and stability for IRA-67 compared to IRA-400 (Fig. 6a). This result was influenced by their different adsorption capacities (134.2 mg g^{-1} for IRA-67 and 70.1 mg g^{-1} for IRA-400), evaluated after an operation time of 6 h (Fig. 6). In this study, it is important to notice that the adsorption capacities in fed-batch conditions were found to be higher than those evaluated during the batch experiments. This might be due to the partial removal of the exchanged anions (Cl^{-} and OH^{-}) from the liquid solution in semi-continuous conditions.

The role of chemicals for the desorption step in the fixed-bed reactor

The FBR experiments showed an expected overall decrease of the lactic acid removal efficiency during the adsorption phase due to the higher volumetric loading rate (Fig. 7a) and irreversible lactic acid adsorption (Fig. 1f). During the experimental evaluation of the LA$_{clr}$, IRA-67 was not saturated during the column operation and the pH of the effluent decreased up to 2.0 (Fig. 7b). Moldes et al.[4] tested IRA-67 under similar operational conditions using variable volumes (from 64 to 106 mL) of 1 N HCl for the regeneration of the resin. Moreover, the lactic acid concentrations in the eluent were lower (10.4–17.2 g L^{-1}) than those obtained in the present work (36.5 g L^{-1}; Fig. 7c) with 3 BV of NaOH (0.5 M).

The FBR adsorption capacity of IRA-67 (more than 200 mg g^{-1}) was significantly higher than those observed in batch experiments (35.9–45.2 mg g^{-1}) due to the different adsorption conditions (Fig. 8), revealing the applicability of the FBR for recovering low concentrations (4.5 g L^{-1}) of lactic acid present in fermentation broths. As remarked in a previous study[12], the OH^{-} ions are continuously eluted during column operation, while they remain in solution in batch operation. Figure 8b shows that the amount of lactic acid removed from the resin during the desorption step slightly decreased over the cycles, leading to a gradual decrease of the accumulation performance of lactic acid from the resin. This was probably due to the non-reversible fouling observed for IRA-67 (Fig. 1e and 1f).

John et al.[58] used IRA-67 in Cl^{-} form for lactic acid recovery from aqueous solutions using several cassava bagasse-based fermentation media in a FBR. Column operation was based on sequential steps and the desorption stage was carried out by employing 100 mL of eluent, reaching comparable lactic acid concentrations in the eluate: 17.6 g L^{-1} for real cassava bagasse broth versus 17.8 g L^{-1} for aqueous lactic acid solution with an average lactic acid recovery of 97%[58]. Similarly, Moldes et al.[4] developed a combined adsorption–desorption recovery system from both aqueous lactic acid solutions and simultaneous saccharification and fermentation (SSF) media onto IRA-67 in Cl^{-} form in a FBR. They employed a variable volume of 1 M HCl for lactic acid desorption (86 mL with aqueous lactic acid solution and 70 mL with SSF media) with related lactic acid concentrations in the eluate of 13.3 and 15.9 g L^{-1}, respectively. Moreover, they obtained an average lactic acid recovery of 99.4%.

The lower lactic acid recovery found in the present study (42.9%) can be ascribed to the fact that the adsorption phase was not followed by a washing step to remove the interstitial solution containing lactic acid and to complete the regeneration of the column (Fig. 8b). Indeed, the use of a stronger eluent (1 N HCl), powered by a preliminary washing with demineralized water, enabled to wash out ions differently from the batch operation where lactate remained in the solution affecting pH and the equilibrium conditions. However, the presence of an additional washing step leads to an increase of the overall time and complexity of the cycle for scale-up applications. Contextually, the use of a larger quantity of eluent with a high HCl concentration determines an increase in the costs of desorption as well as a decrease in the lactic acid concentration in the eluate.

For future research, it will be interesting to test different eluents (e.g. NH$_4$OH, methanol or H$_2$SO$_4$) at different concentrations in continuous experiments, making a comparison based on the amount of the eluent required, lactic acid concentration, desorption efficiency and regeneration stability. In addition, the purity of lactic acid is a key parameter that needs to be further investigated. Using NaOH for the desorption stage in column experiments, a dissolved sodium lactate salt is produced, which implies additional costs for the final lactic acid recovery. Recently, Bishai et al.[15] proposed a two-step separation procedure with the application of (i) an anionic and (ii) a cationic resin to purify lactic acid from the sodium ions.

It is important to take into account the expected decrease in the recovery efficiency that can occur when treating real fermentation media, due to the presence of other competitive anions and the adsorption of external components such as nutrients and enzymes. Moreover, in situ and downstream processes should also be studied with the development of mathematical models for such complex mechanisms[59]. For instance, the mathematical description of the biological processes[60,61] could be combined with the adsorption

isotherms[62] to elucidate the interaction between the fermentation process and the adsorption stage.

Conclusions

This work assessed the capacity, efficiency and regeneration potential of strong (IRA-900 and IRA-400) and weak (IRA-96 and IRA-67) anionic resins for lactic acid recovery from solutions that mimic the conditions of *T. neapolitana* fermentation broth. The strong anionic resins (IRA-900 and IRA-400) showed a higher lactic acid removal efficiency and capacity at the pH above the pKa of lactic acid (3.86). For the weak anionic resins (IRA-96 and IRA-67), the highest lactic acid recovery was achieved at a pH below the pKa of lactic acid, irrespective of the resin pre-treatment. Lactic acid recovery was successfully carried out at 80°C, which is the optimum temperature for *T. neapolitana* fermentation. The ideal contact time of 10 min was suitable for lactic acid adsorption onto both anionic resins IRA-400 and IRA-67, the latter exhibiting the highest lactic acid recovery (>91%) and stability over 13 batch adsorption–desorption cycles. During column operation, the lactic acid removal efficiency for IRA-67 was highly influenced by the inlet flow rate, especially when the critical loading rate ($63.1 \, \text{g L}^{-1} \, \text{h}^{-1}$) was exceeded. High concentrations of lactic acid ($\sim 30 \, \text{g L}^{-1}$) in the eluent were found using 3 BV of NaOH as the regenerant during the desorption step. The practical application of having an integrated adsorption–desorption cycle in the adsorption process has been highlighted for FBR operation in order to recover lactic acid from fermentation broths. Further work should test the ion exchange resins for lactic acid recovery from a real *T. neapolitana* fermentation broth and to maximize the H_2 production by this novel fermentation route at the pilot scale.

Acknowledgements

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References

[1] Yeshanew, M.M.; Frunzo, L.; Luongo, V.; Pirozzi, F.; Lens, P.N.L.; Esposito, G. (2016) Start-up of an anaerobic fluidized bed reactor treating synthetic carbohydrate rich wastewater. *Journal of Environmental Management*, 184: 456. doi:10.1016/j.jenvman.2016.10.001

[2] Luongo, V.; Ghimire, A.; Frunzo, L.; Fabbricino, M.; Pirozzi, F.; Esposito, G. (2017) Photofermentative production of hydrogen and poly-β-hydroxybutyrate from dark fermentation products. *Bioresource Technology*, 228: 171. doi:10.1016/j.biortech.2016.12.079

[3] Hofvendahl, K.; Hahn-Hägerdal, B. (2000) Factors affecting the fermentative lactic acid production from renewable resources. *Enzyme and Microbial Technology*, 26(2–4): 87.

[4] Moldes, A.B.; Alonso, J.L.; Parajó, J.C. (2003) Recovery of lactic acid from simultaneous saccharification and fermentation media using anion exchange resins. *Bioprocess Biosystems Engineering*, 25(6): 357. doi:10.1007/s00449-002-0316-7

[5] Martinez, F.A.C.; Balciunas, E.M.; Salgado, J.M.; González, J.M.D.; Converti, A.; Oliveira, R.P.D.S. (2013) Lactic acid properties, applications and production: A review. *Trends in Food Sciences & Technology*, 30(1): 70. doi:10.1016/j.tifs.2012.11.007

[6] Ghimire, A.; Luongo, V.; Frunzo, L.; Pirozzi, F.; Lens, P.N.L.; Esposito, G. (2017) Continuous biohydrogen production by thermophilic dark fermentation of cheese whey: use of buffalo manure as buffering agent. *International Journal of Hydrogen Energy*, 42 (8): 4861. doi:10.1016/j.ijhydene.2016.11.185

[7] Göksungur, Y.; Gündüz, M.; Harsa, Ş. (2005) Optimization of lactic acid production from whey by *L casei* NRRL B-441 immobilized in chitosan stabilized Ca-alginate beads. *Journal of Chemical Technology & Biotechnology International Research in Process, Environmental & Clean Technology*, 80(11): 1282. doi:10.1002/jctb.1326

[8] Nancib, N.; Nancib, A.; Boudjelal, A.; Benslimane, C.; Blanchard, F.; Boudrant, J. (2001) The effect of supplementation by different nitrogen sources on the production of lactic acid from date juice by *Lactobacillus casei* subsp. *Rhamnosus. Bioresource Technology*, 78(2): 149. doi:10.1016/S0960-8524(01)00009-8

[9] Huang, L.P.; Jin, B.; Lant, P.; Zhou, J. (2005) Simultaneous saccharification and fermentation of potato starch wastewater to lactic acid by *Rhizopus oryzae* and *Rhizopus Arrhizus. Biochemical Engineering Journal*, 23 (3): 265. doi:10.1016/j.bej.2005.01.009

[10] Fukushima, K.; Sogo, K.; Miura, S.; Kimura, Y. (2004) Production of D-lactic acid by bacterial fermentation of rice starch. *Macromolecular Bioscience*, 4(11): 1021. doi:10.1002/mabi.200300044

[11] Jin, B.; Yin, P.; Ma, Y.; Zhao, L. (2005) Production of lactic acid and fungal biomass by *Rhizopus* fungi from food processing waste streams. *Journal of Industrial Microbiology & Biotechnology*, 32(11–12): 678. doi:10.1007/s10295-005-0045-4

[12] Moldes, A.B.; Alonso, J.L.; Parajó, J.C. (2001) Resin selection and single-step production and recovery of lactic

acid from pretreated wood. *Applied Biochemistry and Biotechnology*, 95(2): 69. doi:10.1385/ABAB:95:2:069

[13] Garde, A.; Jonsson, G.; Schmidt, A.S.; Ahring, B.K. (2002) Lactic acid production from wheat straw hemicellulose hydrolysate by *Lactobacillus pentosus* and *Lactobacillus Brevis. Bioresource Technolog*y, 81(3): 217. doi:10.1016/S0960-8524(01)00135-3

[14] Luo, J.; Xia, L.; Lin, J.; Cen, P. (1997) Kinetics of simultaneous saccharification and lactic acid fermentation processes. *Biotechnology Progress*, 13(6): 762. doi:10.1021/bp970100h

[15] Bishai, M.; De, S.; Adhikari, B.; Banerjee, R. (2015) A platform technology of recovery of lactic acid from a fermentation broth of novel substrate *Zizyphus oenophlia. 3 Biotech*, 5(4): 455. doi:10.1007/s13205-014-0240-y

[16] Wee, Y.J.; Jin-Nam, K.; Hwa-Won, R. (2006) *Biotechnological Production of Lactic Acid and Its Recent Application. Food Technology and Biotechnology. Faculty of Food Technology and Biotechnology*. University of Zagreb: Croatia.

[17] Datta, R.; Henry, M. (2006) Lactic acid: recent advances in products, processes and technologies – a review. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, 81(7): 1119. doi:10.1002/jctb.1486

[18] Lunt, J.;. (1998) Large-scale production, properties and commercial applications of polylactic acid polymers. *Polymer Degradation and Stability*, 59(1–3): 145. doi:10.1016/S0141-3910(97)00148-1

[19] Milcent, S.; Carrère, H. (2001) Clarification of lactic acid fermentation broths. *Separation and Purification Technolog*y, 22–23: 393. doi:10.1016/S1383-5866(00)00124-6

[20] Evangelista, R.L.; Nikolov, Z.L. (1996) Recovery and purification of lactic acid from fermentation broth by adsorption. *Applied Biochemistry and Biotechnology*, 57 (1): 471. doi:10.1007/BF02941727

[21] Joglekar, H.G.; Rahman, I.; Babu, S.; Kulkarni, B.D.; Joshi, A. (2006) Comparative assessment of downstream processing options for lactic acid. *Separation and Purification Technolog*y, 52(1): 1. doi:10.1016/j.seppur.2006.03.015

[22] Wasewar, K.L.;. (2005) Separation of lactic acid: recent advances. *Chemical and Biochemical Engineering Quarterly*, 19(2): 159.

[23] Abdel-Rahman, M.A.; Tashiro, Y.; Sonomoto, K. (2013) Recent advances in lactic acid production by microbial fermentation processes. *Biotechnology Advances*, 31(6): 877. doi:10.1016/j.biotechadv.2013.04.002

[24] Jantasee, S.; Kienberger, M.; Mungma, N.; Siebenhofer, M. (2017) Potential and assessment of lactic acid production and isolation – a review. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, 92: 2885. doi:10.1002/jctb.5237

[25] Pal, P.; Sikder, J.; Roy, S.; Giorno, L. (2009) Process intensification in lactic acid production: A review of membrane based processes. *Chemical Engineering and Processing: Process Intensification*, 48(11–12): 1549. doi:10.1016/j.cep.2009.09.003

[26] Dey, P.; Pal, P. (2012) Direct production of L(+) lactic acid in a continuous and fully membrane-integrated hybrid reactor system under non-neutralizing conditions. *Journal of Membane Sciences*, 389: 355. doi:10.1016/j.memsci.2011.10.051

[27] Pal, P.; Dey, P. (2013) Process intensification in lactic acid production by three stage membrane integrated hybrid reactor system. *Chemical Engineering and Processing: Process Intensification*, 64: 1. doi:10.1016/j.cep.2012.12.006

[28] Sikder, J.; Roy, M.; Dey, P.; Pal, P. (2012) Techno-economic analysis of a membrane-integrated bioreactor system for production of lactic acid from sugarcane juice. *Biochemical Engineering Journal*, 63: 81. doi:10.1016/j.bej.2011.11.004

[29] Uslu, H. (2009) Adsorption equilibria of formic acid by weakly basic adsorbent Amberlite IRA-67: equilibrium, kinetics, thermodynamic. *Chemical Engineering Journal*, 155(1–2): 320. doi:10.1016/j.cej.2009.06.040

[30] Yousuf, A.; Bonk, F.; Bastidas-Oyanedel, J.R.; Schmidt, J. E. (2016) Recovery of carboxylic acids produced during dark fermentation of food waste by adsorption on Amberlite IRA-67 and activated carbon. *Bioresource Technology*, 217: 137. doi:10.1016/j.biortech.2016.02.035

[31] Zhang, K.; Zhang, L.; Yang, S.T. (2014) Fumaric acid recovery and purification from fermentation broth by activated carbon adsorption followed with desorption by acetone. *Industrial & Engineering Chemistry Research*, 53(32): 12802. doi:10.1021/ie501559f

[32] Nakano, K.; Kataoka, H.; Matsumura, M. (1996) High density culture of *Propionibacterium freudenreichii* coupled with propionic acid removal system with activated charcoal. *Journal of Fermentation and Bioengineering*, 81(1): 37. doi:10.1016/0922-338X(96)83117-5

[33] Aljundi, I.H.; Belovich, J.M.; Talu, O. (2005) Adsorption of lactic acid from fermentation broth and aqueous solutions on Zeolite molecular sieves. *Chemical Engineering Science*, 60(18): 5004. doi:10.1016/j.ces.2005.04.034

[34] Efe, C.; van der Wielen, L.A.M.; Straathof, A.J.J. (2010) High silica zeolites as an alternative to weak base adsorbents in succinic acid recovery. *Industrial & Engineering Chemistry Research*, 49(4): 1837. doi:10.1021/ie901110b

[35] Pradhan, N.; Dipasquale, L.; D'Ippolito, G.; Panico, A.; Lens, P.N.L.; Esposito, G.; Fontana, A. (2017) Hydrogen and lactic acid synthesis by the wild-type and a laboratory strain of the hyperthermophilic bacterium *Thermotoga neapolitana* DSMZ 4359T under capnophilic lactic fermentation conditions. *International Journal of Hydrogen Energy*, 42(25): 16023. doi:10.1016/j.ijhydene.2017.05.052

[36] Raya-Tonetti, G.; Perotti, N. (1998) Expanded bed affinity chromatography using streamline-cibacron red 3ba for simultaneous purification of alcohol dehydrogenase and glucose-6-phosphate dehydrogenase from unclarified yeast homogenate. *Latin American Applied Research*, 28: 89.

[37] Pradhan, N.; Dipasquale, L.; D'Ippolito, G.; Panico, A.; Lens, P.N.L.; Esposito, G.; Fontana, A. (2015) Hydrogen production by the thermophilic bacterium *Thermotoga Neapolitana. International Journal of Molecular Sciences*, 16(6): 12578. doi:10.3390/ijms160612578

[38] d'Ippolito, G.; Dipasquale, L.; Vella, F.M.; Romano, I.; Gambacorta, A.; Cutignano, A.; Fontana, A. (2010) Hydrogen metabolism in the extreme thermophile *Thermotoga Neapolitana*. *International Journal of Hydrogen Energy*, 35(6): 2290. doi:10.1016/j.ijhydene.2009.12.044

[39] Dipasquale, L.; d'Ippolito, G.; Fontana, A. (2014) Capnophilic lactic fermentation and hydrogen synthesis by *Thermotoga neapolitana*: an unexpected deviation from the dark fermentation model. *International Journal of Hydrogen Energy*, 39(10): 4857. doi:10.1016/j.ijhydene.2013.12.183

[40] Pradhan, N.; Rene, E.; Lens, P.; Dipasquale, L.; D'Ippolito, G.; Fontana, A.; Panico, A.; Esposito, G. (2017) Adsorption behaviour of lactic acid on granular activated carbon and anionic resins: thermodynamics, isotherms and kinetic studies. *Energies*, 10(5): 665. doi:10.3390/en10050665

[41] d'Ippolito, G.; Dipasquale, L.; Fontana, A. (2014) Recycling of carbon dioxide and acetate as lactic acid by the hydrogen-producing bacterium *Thermotoga Neapolitana*. *ChemSusChem*, 7(9): 2678. doi:10.1002/cssc.201402155

[42] Lützenkirchen, J.; Preočanin, T.; Kovačević, D.; Tomišić, V.; Lövgren, L.; Kallay, N. (2012) Potentiometric titrations as a tool for surface charge determination. *Croatica Chemica Acta*, 85(4): 391. doi:10.5562/cca2062

[43] Dethe, M.J.; Marathe, K.V.; Gaikar, V.G. (2006) Adsorption of lactic acid on weak base polymeric resins. *Separation Science and Technology*, 41(13): 2947. doi:10.1080/01496390600851384

[44] Gluszcz, P.; Jamroz, T.; Sencio, B.; Ledakowicz, S. (2004) Equilibrium and dynamic investigations of organic acids adsorption onto ion-exchange resins. *Bioprocess and Biosystems Engineering*, 26(3): 185. doi:10.1007/s00449-003-0348-7

[45] Helfferich, F.G.;. (1995) *Ion Exchange*. Dover Publications: New York.

[46] Rincon, J.; Fuertes, J.; Rodriguez, J.F.; Rodriguez, L.; Monteagudo, J.M. (1997) Selection of a cation exchange resin to produce lactic acid solutions from whey fermentation broths. *Solvent Extraction and Ion Exchange*, 15(2): 329. doi:10.1080/07366299708934481

[47] Ghimire, A.; Valentino, S.; Frunzo, L.; Trably, E.; Escudié, R.; Pirozzi, F.; Lens, P.N.L.; Esposito, G. (2015) Biohydrogen production from food waste by coupling semi-continuous dark-photofermentation and residue post-treatment to anaerobic digestion: a synergy for energy recovery. *International Journal of Hydrogen Energy*, 40(46): 16045. doi:10.1016/j.ijhydene.2015.09.117

[48] Bayazit, S.S.; İNci, I.; Uslu, H. (2011) Adsorption of lactic acid from model fermentation broth onto activated carbon and Amberlite IRA-67. *Journal of Chemical & Engineering Data*, 56(5): 1751. doi:10.1021/je1006345

[49] Quintero, J.; Acosta, A.; Mejía, C.; Ríos, R.; Torres, A.M. (2012) Purification of lactic acid obtained from a fermentative process of cassava syrup using ion exchange resins. *Revista Facultad De Ingeniería. Universidad De Antioquia*, 65: 139.

[50] Da Silva, A.H.; Miranda, E.A. (2013) Adsorption/desorption of organic acids onto different adsorbents for their recovery from fermentation broths. *Journal of Chemical & Engineering Data*, 58(6): 1454. doi:10.1021/je3008759

[51] Patel, M.; Bassi, A.S.; Zhu, J.J.-X.; Gomaa, H. (2008) Investigation of a dual-particle liquid-solid circulating fluidized bed bioreactor for extractive fermentation of lactic acid. *Biotechnology Progress*, 24(4): 821. doi:10.1002/btpr.6

[52] Gao, Q.; Liu, F.; Zhang, T.; Zhang, J.; Jia, S.; Yu, C.; Jiang, K.; Gao, N. (2010) The role of lactic acid adsorption by ion exchange chromatography. *PLoS One*, 5(11): e13948. doi:10.1371/journal.pone.0013948

[53] Davison, B.H.; Thompson, J.E. (1992) Simultaneous fermentation and separation of lactic acid in a biparticle fluidized-bed bioreactor. *Applied Biochemistry and Biotechnology*, 34(1): 431.

[54] Rampai, T.; Thitiprasert, S.; Boonkong, W.; Kodama, K.; Tolieng, V.; Thongchul, N.; Thongchul, N. (2016) Improved lactic acid productivity by simultaneous recovery during fermentation using resin exchanger. *Asia-Pacific Journal of Sciences and Technology*, 21(2): 193.

[55] Gao, M.T.; Shimamura, T.; Ishida, N.; Takahashi, H. (2011) PH-uncontrolled lactic acid fermentation with activated carbon as an adsorbent. *Enzyme and Microbial Technology*, 48(6): 526. doi:10.1016/j.enzmictec.2010.12.006

[56] Cao, X.; Yun, H.S.; Koo, Y.M. (2002) Recovery of L-(+)-lactic acid by anion exchange resin Amberlite IRA-400. *Biochemical Engineering Journal*, 11(2): 189. doi:10.1016/S1369-703X(02)00024-4

[57] Chen, C.C.; Ju, L.-K. (1998) Adsorption characteristics of polyvinylpyridine and activated carbon for lactic acid recovery from fermentation of *Lactobacillus Delbrueckii*. *Separation Science and Technology*, 33(10): 1423. doi:10.1080/01496399808545058

[58] John, R.P.; Nampoothiri, K.M.; Pandey, A. (2008) L(+)-lactic acid recovery from cassava bagasse based fermented medium using anion exchange resins. *Brazilian Archives of Biology and Technology*, 51(6): 1241. doi:10.1590/S1516-89132008000600020

[59] Sodsai, W.; Sookkumnerd, T. (2013) Modeling of lactic acid adsorption isotherm by anion exchange resin Amberlite IRA-96. *Current Applied Science and Technology*, 13(2): 82.

[60] Pradhan, N.; Dipasquale, L.; d'Ippolito, G.; Fontana, A.; Panico, A.; Pirozzi, F.; Lens, P.N.L.; Esposito, G. (2016) Model development and experimental validation of capnophilic lactic fermentation and hydrogen synthesis by *Thermotoga neapolitana*. *Water Research*, 99: 225. doi:10.1016/j.watres.2016.04.063

[61] Mattei, M.R.; D'Acunto, B.; Esposito, G.; Frunzo, L.; Pirozzi, F. (2015) Mathematical modeling of competition and coexistence of sulfate-reducing bacteria, acetogens, and methanogens in multispecies biofilms. *Desalination and Water Treatment*, 55(3): 740. doi:10.1080/19443994.2014.937764

[62] D'Acunto, B.; Esposito, G.; Frunzo, L.; Mattei, M.R.; Pirozzi, F. (2015) Mathematical modeling of heavy metal biosorption in multispecies biofilms. *Journal of Environmental Engineering*, 142(9): 1.