High-titer and productivity of L-(+)-lactic acid using exponential fed-batch fermentation with *Bacillus coagulans* arr4, a new thermotolerant bacterial strain

Luciana Fontes Coelho¹ · Susan Michelz Beitel¹ · Daiane Cristina Sass¹ · Paulo Marcelo Avila Neto¹ · Jonas Contiero^{1,2}

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Abstract

Bacillus coagulans arr4 is a thermotolerant microorganism with great biotechnological potential for L-(+)-lactic acid production from granulated sugar and yeast extract. The highest L-(+)-lactic acid production was obtained with Ca(OH)₂. The maximum production of L-(+)-lactic acid (206.81 g/L) was observed in exponential feeding using granulated sugar solution (900 g/L) and yeast extract (1%) at 50 °C, pH 6.5, and initial granulated sugar concentration of 100 g/L at 39 h. 5.3 g/L h productivity and 97% yield were observed, and no sugar remained. Comparing the simple batch with exponential fed-batch fermentation, the L(+) lactic acid production was improved in 133.22% and dry cell weight was improved in 83.29%, using granulated sugar and yeast extract. This study presents the highest productivity of lactic acid ever observed in the literature, on the fermentation of thermotolerant *Bacillus* sp. as well as an innovative and high-efficiency purification technology, using low-cost substances as Celite and charcoal. The recovery of lactic acid was 86%, with 100% protein removal, and the fermentation medium (brown color) became a colorless solution.

Keywords *Bacillus* sp. · L(+) lactic acid · Fed-batch · Thermotolerance · Purification

Introduction

Lactic acid is used in pharmaceutical industries such as anti-acne solutions and moisturizers. Calcium lactate is used as a therapy for calcium deficiency and acts as a dental caries scavenger (Narayanan 2004). In the food industry, it can be used as a flavoring agent, acidulant, and emulsified, and its derivatives are used to manufacture breads, sweets, and candy, among others (Naveena et al. 2005). In addition, lactic acid inhibits sporulation of bacteria in processed foods. In the chemical industry, lactic acid can be used as organic

solvents, 1,2-propanediol, and acrylic acid (Hofvendahl and Hahn-Hägerdal 2000).

Currently, polylactic acid (PLA) is one of the most advantageous renewable and biodegradable plastics (Sakai et al. 2004), with many applications, mainly due to its high chemical resistance, which is interesting for manufacturing films and fibers, as well as food packaging and various plastic utensils, and can replace products made from raw-material petroleum, highlighting the utility of this chemical (Datta et al. 1995).

Using pure lactic acid isomers, high-strength polymers can be produced, which can have important applications in medicine. These polymers have the important characteristic of being bioresorbable and may be used in tissue regeneration, sutures, fracture fixations, bone replacement, cartilage repair, meniscus repair, ligament fixation, and implants. In addition, screws, implants, pins, staples, and plates have been produced from lactic acid polymers for orthopedic and oral surgery applications in humans and animals (Sakata et al. 2004).

The advantages in producing lactic acid by fermentation comparing with chemical synthesis are the lesser electrical energy consumption, use of renewable and cheap

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✉ Jonas Contiero
jconti@rc.unesp.br

¹ Department of Biochemistry and Microbiology, Biological Sciences Institute, São Paulo State University (UNESP), Rio Claro, SP, Brazil

² São Paulo State University (UNESP), Bioenergy Research Institute (IPBEN), Rio Claro, SP, Brazil

substrates, as well as the possibility of obtaining pure isomers of lactic acid, while a racemic mixture is typically formed using chemical synthesis (Mussatto et al. 2008).

Alternative substrate sources used for lactic acid production are mixed restaurant food waste (Pleissner et al. 2016), whey (Bernardo et al. 2016), molasses (Coelho et al. 2011b), sugarcane juice (Coelho et al. 2011a), cassava wastewater (Coelho et al. 2010), broken rice (Nunes et al. 2017), peanut flour (Beitel et al. 2016), coffee mucilage (Neu et al. 2016), renewable lignocellulosic biomass (Zhang et al. 2016), and paper sludge (Takano and Hoshino 2016).

In general, lactic acid is produced by mesophilic bacteria, which increases the risk of contamination of fermentation. This challenge could be solved by the use of thermophilic microorganisms, which can allow for fermentation without the need for sterile conditions (Qin et al. 2009). Thus, the use of thermophilic microorganisms in lactic acid production could be an effective alternative because it does not require sterile conditions, as supported by work with *Bacillus coagulans* (Pleissner et al. 2016), *Geobacillus stearothermophilus* (Smerilli et al. 2015), *Bacillus licheniformis* (Wang et al. 2011), and *Bacillus* sp. (Qin et al. 2010).

According to Kranenburg et al. (2013), high temperature in industrial-scale fermentation has some advantages, such as higher enantiomeric purity, because of the lower risk of contamination, as well as faster reactions. Some species of Bacilli, such as *Bacillus coagulans*, are facultative anaerobes, which allow fermentation under anaerobic conditions, or at least under low partial pressure of oxygen, which is desirable for industrial-scale processes because it allows for relatively inexpensive equipment and processing. Furthermore, the nutrient requirements of these bacteria are less demanding than those of *Lactobacillus* species.

It has not been found in the literature thermophilic bacteria able to tolerate high concentration of substrates (more than 160 g/L of sugar). One strategy would be fed-batch fermentation that some authors have investigated, Ou et al. (2011) got 182.2 g/L of L-lactic acid using pulse fed batch. Meng et al. (2012) observed 225 g/L of L(+) lactic acid using multi-pulse fed-batch fermentation with *Bacillus* sp. WL-S20, a yield of 99.3%, and they got 180 g/L of L(+) lactic acid and 98.6% of yield with single-pulse fed-batch. High L(+) lactic acid production (172.5 g/L) was also observed by Qin et al. (2009), using fed-batch fermentation, with *Bacillus* sp. 2–6. However, in all of these studies, although production has been high, productivity is still considered low, less than 3.5 g/L h.

This way, the development of a thermophilic microorganism that produces high concentration of lactic acid, with high productivity, using an inexpensive substrate fermentation and purification processes that guarantee a good production with low cost is necessary.

Thus, the objectives were to increase the production of L-(+)-lactic acid using a new thermophilic strain of *Bacillus coagulans* arr4 through the optimization of fermentation parameters and the use of fed-batch fermentation, and to efficiently purify the lactic acid produced in the fermentation broth.

Materials and methods

Microorganism

The microorganism used in these experiments, *Bacillus coagulans* arr4, was isolated from *Ruta graveolens* rhizosphere, collected from a residential garden in the state of São Paulo-SP, Brazil, and presented as an excellent L-(+)-lactic acid producer, is characterized as rod-shaped, Gram-positive, spore-forming, thermotolerant and homofermentative, producing 99.11% of L(+) lactic acid and 0.88% of D(−) lactic acid. This strain was maintained in GYP medium with 15% of glycerol at − 80 °C. The GYP medium was described by Fujita et al. (2010) and contains the following composition (g/L): glucose (20.0), yeast extract (10.0), peptone (10.0), sodium acetate (10.0), and 5 mL/L of saline solution. The salt solution consists of (g/L): MnSO₄ (2.0), MgSO₄ (40.0), NaCl (2.0), and FeSO₄·7H₂O (2.0). On solid medium was added 20.0 g/L of Agar.

Inoculum

Bacterial reactivation of *Bacillus coagulans* arr4, stored in glycerol stocks at − 80 °C, was performed by two successive subcultures, transferring 10% of the grown microorganism to new flask contained GYP media. Each subculture was performed after 24 h in an incubator without shaking at 35 °C. The inoculum was then standardized to an optical density (OD_{600nm}) of 2.0, and 10% of the workload was inoculated in all experiments.

Effect of pH and neutralizing agents

Experiments to evaluate the effect of neutralizing agents and differences in pH on the production of L-(+)-lactic acid were performed in a fermenter (Model Multifors 2, Infors HT, Switzerland), consisting of six vessels with 500-mL capacity, with independent control of pH, temperature, agitation, and aeration. The agitation and temperature were set to 100 rpm and 42 °C, respectively.

The production medium contained 100 g/L of granulated sugar, 30 g/L of yeast extract, and 5 mL/L of the salts in GYP medium. Thirty milliliter of inoculum was added to 270 mL of production medium. The following seven neutralizing agents were evaluated: 50 g/L CaCO₃ alone,

400 g/L sodium hydroxide (NaOH) + 50 g/L CaCO_3 , 200 g/L of calcium hydroxide (Ca(OH)_2) + 50 g/L CaCO_3 , 10% ammonium hydroxide (NH_4OH) + 50 g/L CaCO_3 , 400 g/L sodium hydroxide (NaOH), 200 g/L of calcium hydroxide [Ca(OH)_2], and 10% ammonium hydroxide (NH_4OH).

NaOH, Ca(OH)_2 , and NH_4OH were prepared and placed in separate containers coupled to a peristaltic pump to be intermittently added in the fermentation medium to keep the pH at 6.0 ± 0.1 throughout the fermentation period, and CaCO_3 was added to the medium in one portion directly in the medium of production before beginning the fermentation. To evaluate the best pH, the same conditions mentioned above were used, and ammonium hydroxide (10%) was used. In these experiments, the pH values evaluated were 5.0, 5.5, 6.0, 6.5, and 7.0.

Exponential fed batch

All fermentations (batch and fed batch) were performed in a fermenter (Model Infors HT, Switzerland) with 13-L capacity and independent control of pH, agitation, and aeration. The temperature and agitation were 50 °C and 100 rpm, respectively, and the pH was kept constant at 6.5 by adding a solution of 400 g/L of Ca(OH)_2 . The initial working volume was 4.5 L. To maintain anaerobic conditions within the bioreactor jar, during fermentation, 0.5 mL/min of nitrogen flow was added for 12 h.

For both fermentations, the initial culture medium has 109 g/L of granulated sugar, 30 g/L of yeast extract, and 5 mL/L of salts of GYP medium.

The exponential fed batch takes into account the specific growth rate of microorganism providing the substrate at an appropriate rate. The feed rate (F) was calculated as previously reported by Ding, Tan (37), based on Eq. (1), using Iris 6 software:

$$F = \frac{\mu}{Y_{x/s}(S_i - S)} V_0 X_0 \exp(\mu t), \quad (1)$$

where V_0 = initial volume (4.5 L), X_0 = biomass at the beginning of fermentation (0.23 g/L), X = biomass at the start of feed (4.8 g/L), and $Y_{x/s}$ = yield of the cell/substrate (0.11 g/g). The yield $Y_{x/s}$ was calculated between the beginning of the fermentation and the beginning of the feed. $Y_{x/s}$ = (biomass at the beginning of feed – biomass at the beginning of fermentation) / (substrate at the beginning of fermentation – substrate at the beginning of feed). S_i is the substrate concentration in the feed solution (900 g/L). S is the substrate concentration in the reactor at the beginning of the feed (67.6 g/L). μ is the specific growth rate (0.23 h⁻¹). t is time (h) feeding (variable throughout the fermentation).

The feed solution contained 900 g/L of granulated sugar and 1% of yeast extract. The feed started at 10 h of

fermentation and lasted 6 h and 30 min. Nine hundred thirty milliliter of this feed solution was added into the reactor.

Fermentation started with around 109 g/L of sucrose, as this was the maximum sugar concentration that does not cause inhibition to the microorganism, according to preliminary studies. And the rest of the sugar was added gradually according to the need of the microorganism.

Samples were collected every 6 h.

Purification and recovery of L-(+)-lactic acid

The fermentation medium (94 mL) containing 206.81 g/L of L-(+)-lactic acid was diluted and acidified to pH 5.0 and was filtered on a cellulose acetate ultrafiltration membrane, and the volume of the solution was 146 ml with a concentration of 133.44 g/L of L-(+)-lactic acid and 0.91 g/L of protein. Then, the 146 ml medium was vacuum filtered on a sintered plate funnel with a layer of Celite® (Sigma-Aldrich), and a layer of powdered activated carbon (Sigma-Aldrich) was each added approximately 1.00 cm thick followed by washing with water, this procedure was repeated twice. Then, the pH 5 solution was passed through an Amberlite IRA 120 cation-exchange column (hydrogen forms, Sigma-Aldrich), after passing through the ion exchange column the solution had pH 3. Finally, solution was filtered using a cellulose acetate membrane (pore diameter 0.2 µm), and this final solution was used for the determination of lactic acid and proteins.

Purification and recovery efficiency calculation was made using the formula below:

- L-(+)-lactic acid recovery efficiency (%) = (final L-(+)-lactic acid mass / initial L-(+)-lactic acid mass) × 100
- Protein removal efficiency (%) = [(final protein mass / initial protein mass) × 100] – 100

Analytical methodology

Samples (1 mL) were centrifuged for 15 min, and the supernatant was used to lactic acid and sugar determination, and the precipitate was washed twice with distilled water and used to biomass quantification. After washing, the precipitate was suspended to the initial sample volume and diluted appropriately for spectrophotometer reading at 600 nm. Dry cell weight was determined by a calibration curve ($y = 0.539x - 0.0159$) with associated absorbance and cell concentration (g/L). For fermentations, where Ca(OH)_2 was used as the pH controller, the samples were diluted with 0.3 N HCl to remove Ca^{2+} and to liberate lactic acid prior to centrifugation.

For lactic acid and sugar quantification, a high-performance liquid chromatograph (HPLC) (Shimadzu, Prominence series) equipped with an ultra-violet detector at

210 nm and refractive index detector was used. The column used was Rezex ROA (300×7.8 mm) from Phenomenex, eluted with 0.005 N H₂SO₄ as the mobile phase with a flow of 0.6 mL/min and injection volume of 5 µL, temperature of oven set at 65 °C.

To determine the optical purity of lactic acid, the same HPLC was used equipped with an ultra-violet detector at 256 nm. The column used was a Chirex 3126 phenomenex (150×4.6 mm) eluted with 1 mM of CuSO₄ as the mobile phase with a flow of 1 mL/min and injection volume of 5 µL, temperature of oven set at 26 °C.

The protein concentration was analyzed by the Lowry method (Peterson 1977).

Results and discussion

pH on L-(+)-lactic acid production

For this study, the following five pH values were assessed: 5.0, 5.5, 6.0, 6.5, and 7.0. Table 1 shows the results at 12 h of fermentation, the highest production (68.43 g/L) and productivity (5.7 g/L h) were obtained at pH 6.5. Meng et al. (2012) observed the best L-lactic acid concentration at pH 9.0 when using *Bacillus* sp. WL-S20; the values were, respectively, 37 and 100%. Qin et al. (2010) used a thermophilic *Bacillus* mutant for lactic acid production observed in pH 6.0, a total of 106.0 g/L, and productivity of 3.53 g/L h during 30 h of fermentation.

As shown in Fig. 1, the lowest production and the highest concentration of residual sugar were observed at pH 5.0, 5.5, and 7.0. At pH 6.5, the highest production was obtained and all the sugar had been metabolized in 18 h.

Neutralizing agents in the production of L-(+)-lactic acid

As shown in Table 2 and Fig. 2a, b, at 12 h of fermentation, the highest production of L-(+)-lactic acid (85.12 g/L), as well as, the highest productivity (7.09 g/L h) was obtained when the pH was maintained just with Ca(OH)₂. When the

Fig. 1 Production of L-(+)-lactic acid and residual sucrose at pH values 7.0, 6.5, 6.0, 5.5, and 5.0, with an initial sucrose concentration of 90 g/L. Empty symbols: sucrose and Filled symbols: Lactic acid

pH was maintained by NaOH, NH₄OH, or just by CaCO₃, the production and productivity were lower and there was a greater concentration of residual sugar at the end of fermentation.

The production of lactic acid in the experiments using Ca(OH)₂ was 10–15% higher compared to the other neutralizing agents. In addition, productivity in the experiments with Ca(OH)₂ was 75–100% higher at 18 h, and sucrose had been completely metabolized (Fig. 2a). Liu et al. (2014) also reported that Ca(OH)₂ was the best pH controller, enabling improvement in productivity, which was three times better than KOH and NH₄OH.

Ye et al. (2013) got 100 g/L of lactic acid from xylose using *Bacillus coagulans* C106 and NaOH as neutralizing agent. When these authors used Ca(OH)₂ and 120 and 154 g/L of xylose, they observed 5.7 and 4.8 g/L h of lactic acid productivities, respectively, in batch fermentation. Lactic acid production was improved using fed-batch fermentation and Ca(OH)₂ with pH controller, after 54.5 h xylose was

Table 1 Comparison between different pH values at 12 h of fermentation

pH	Production (g/L)	Productivity (g/L h)	Residual sucrose (g/L)
5.0	15.41	1.28	72.28
5.5	30.46	2.54	54.68
6.0	40.91	3.4	43.24
6.5	68.43	5.7	6.23
7.0	48.16	4.0	34.12

Table 2 Comparison of neutralizing agents at 12 h of fermentation

Neutralizing agent	Production (g/L)	Productivity (g/L h)	Residual sucrose (g/L)
NaOH	57.52	4.79	48.80
NaOH + CaCO ₃	64.32	5.36	36.40
NH ₄ OH	56.40	4.70	45.28
NH ₄ OH + CaCO ₃	66.56	5.55	32.24
Ca(OH) ₂	85.12	7.09	0.96
Ca(OH) ₂ + CaCO ₃	65.52	5.46	25.44
CaCO ₃	43.07	3.59	56.19

Fig. 2 Production of L-(+)-lactic acid (**a**) and residual sucrose (**b**) from different neutralizing agents, CaCO_3 , NaOH , NH_4OH , Ca(OH)_2 , $\text{CaCO}_3 + \text{NaOH}$, $\text{NH}_4\text{OH} + \text{CaCO}_3$, and $\text{Ca(OH)}_2 + \text{CaCO}_3$. Initial concentration of 110 g/L sucrose

completely consumed, the lactic acid production, yield, and productivity were 216 g/L, 95%, and 4.0 g/L h, respectively. Yen et al. (2010) have observed, using *Rhizopus oryzae*, on sweet potato starch, that although lactic acid production was better with CaCO_3 (43.3 g/L) than with NaHCO_3 (35.5 g/L), this last one is the most indicated, because the recovery and purification is simpler and the cost is lower.

Nakano et al. (2012), using *Lactobacillus delbrueckii* on broken rice, observed maximum productivities of lactic acid (3.59 g/L h), when using Ca(OH)_2 comparing with NaOH , Ca(OH)_2 , and NH_4OH as neutralizing agents in simultaneous fermentation and saccharification.

The highest lactic acid production was observed with Ca(OH)_2 that must have occurred due to the lower osmotic pressure in production medium. Two molecules of lactic acid can be neutralized using just one molecule of Ca(OH)_2 due to the +2 charge in Ca^{2+} ; however, only one molecule of lactic acid can be neutralized using one molecule of NaOH or NH_4OH , this way, less Ca(OH)_2 will be necessary, which leads to less osmotic pressure. As a result, the cellular biomass and lactic acid production are higher (Nakano et al. 2012). Moreover, L-(+)-calcium lactate has a solubility of about 80 g/L at 37 °C (Cao et al. 2001). However, ammonium lactate, sodium lactate, and potassium lactate have

Table 3 Comparison of batch and exponential fed-batch methods

Parameters	Batch	Exponential fed batch ^a
L-(+)-Lactic acid (g/L)	88.52	206.45
Productivity (g/L/h)	5.03	5.29
Yield (%)	98	97
Cellular biomass (g/L)	7.54	13.82
Residual sucrose (g/L)	0	0
Fermentation time (h)	18 h	39 h
Initial volume (L)	4.50	4.50
Final volume (L)	4.94	6.226

^aFeed solution sucrose (900 g/L) and yeast extract (1%)

solubility greater than 100 g/L. If all of these salts are soluble, an increase in osmotic pressure will be observed, which will cause lower cell growth and lower productivity. For these reasons, we chose Ca(OH)_2 in the batch and fed-batch fermentations on a larger scale.

Exponential fed batch

A comparison between the batch method and exponential fed-batch method is shown in Table 3. In the exponential fed-batch method, there were increases of 133.22% in the production of L-(+)-lactic acid and 83.29% in the cellular biomass, which corroborates with Ding and Tan (2006) who observed that exponential feeding was the most efficient method, increasing lactic acid production in 56.5% compared to the batch process, using *L. casei* as microorganism and glucose and yeast extract as substrates. In the exponential feed, softwares are used, with specific formulas, which are setting with determined data of the kinetic fermentation values. These programs are able to adjust the feed flow as requested, taking into account the conversion rates, as well as the speed at which they occur.

Fed-batch fermentation is an alternative to avoid the inhibition by excess of substrate, as well as catabolic repression. It is a way to eliminate the inhibition by the substrate, resulting in high productivity, higher concentration of the product, and improvements in the cellular growth rate (Bernardo et al. 2016).

As shown in Fig. 3a, the logarithmic growth phase occurs from 6 to 12 h of batch fermentation, but in the fed-batch fermentation, this exponential log phase is extended up to 21 h (Fig. 3b). The feed started in the middle of the logarithmic growth phase after 10 h of fermentation, and it is possible to observe that the growth of the microorganism increases rapidly during the 6 h and 30 min of feed and proceeds in rapid growth up to 21 h of fermentation. After 24 h of fermentation, a decrease in the growth of the

Fig. 3 Kinetic production of L-(+)-lactic acid, and sugar consumption of the microorganism biomass in fermentation batch (a) and exponential fed batch (b). The fermentation was conducted in a 13-L bioreactor with an initial volume of 4.5 L at 50 °C agitated at 100 rpm. The pH was maintained at 6.5 by the automatic addition of

400 g/L $\text{Ca}(\text{OH})_2$ using a peristaltic pump. The feed solution (900 g of sucrose + 1% yeast extract) was pumped into the bioreactor using a computer coupled to the peristaltic pump and used in the exponential fed-batch method. Open circle: biomass, filled square: sucrose, and filled triangle: L-(+) lactic acid

microorganism is observed, probably occurred autolysis of the cells. One explanation for this would be due to the high concentrations of lactic acid. Some genes of microorganisms can be repressed or induced depending on the stressful environment which occurs during fermentation (Serrazanetti et al. 2011). The main challenge of lactic acid production by *Bacillus* is the growth rate inhibition by the final product accumulation and acid stress (Poudel et al. 2015). According to Nancib et al. (2015), the specific growth rate decreased with the increase in lactate concentration.

Although, in the exponential fed-batch method, the maximum cell growth (18.13 g/L) and productivity (7.32 g/L h) were achieved after 24 h of fermentation, the L-(+)-lactic acid production (175.77 g/L) was lower comparing with the L-(+) lactic acid production (206.81 g/L), and no residual sugar was observed in the time of 39 h.

The concentration of sugar added was considered suitable. The exponential feeding method proved to be efficient to increase the production of L-(+)-lactic acid, productivity, as well as the growth of cells. In both batches, the sugar was completely consumed, showing that there was no substrate inhibition. It is known that lactic acid production and productivity decrease due to high concentrations of product and/or substrate (Abdel-Rahman et al. 2013). This inhibition is explained by Kotzamanidis et al. (2002) and is due to the

reduction of water activity when the substrate concentration is above a critical value, causing cell plasmolysis.

L-(+)-Lactic acid purification

The mean values obtained in duplicate experiments are shown in Table 4.

As shown in Table 4, the purification as well as the recovery of lactic acid was efficient. Eighty-six percent of L-(+)-lactic acid was recovered from the fermentation medium with an optic purity of 99.11% L(+). The proteins were efficiently removed (100%), and the fermentation medium (brown color) became a colorless solution. Geanta et al. (2013) obtained 79.34% of lactic acid recover using Micellar-Enhanced UltraFiltration (MEUF) and pretreated beet

Table 4 Results of the purification experiments of the fermentation medium

Parameters	Initial solution	Final solution
Lactic acid (g/L)	133.44	74.20
Protein (g/L)	0.91	0.00
Volume (mL)	146	226
Recovered lactic acid (%)	—	86
Protein removal (%)	—	100

molasses with activated charcoal. A weak anion-exchange resin was analyzed by Tong et al. (2004) in the lactic acid purification, and they reached a yield up to 82.6% and purity up to 92.2%. Chen et al. (2012) applied a process with solvent butyl alcohol for L-lactic acid purification and obtained a purity of 91.6% and yield of 61.73%. Observing the values found by these authors, it can be said that the value of 86% is close to these and the most important is that there is no residue of protein and sugar.

The lactic acid production methodology presented in this manuscript is based on a culture medium containing crystal sugar and yeast extract, which is more expensive than agro industrial residues. Otherwise, they are considered as pure substrates comparing with waste, which facilitates and reduces costs with the downstream process and scale-up, since these substrates are easy to purify and easily available. In addition, they do not show large variations in the composition of nutrients between the lots received over time, compared to the residues, facilitating the standardization of the production. The use of yeast extract as a source of nitrogen and $\text{Ca}(\text{OH})_2$ as a pH control agent provides a higher productivity, as well as a significantly higher production of lactic acid, comparing with other sources of nitrogen (corn steep water, yeast autolysate) and other pH controllers (NaOH , KOH , NH_4OH). However, a purification using a fermentation broth that used $\text{Ca}(\text{OH})_2$ as a pH control agent may be more complex, due to the difficulty of calcium separation, as well as the generation of large amounts of CaSO_2 (gypsum). More future studies are needed to reduce the amount of neutralizers used in the lactic acid production process.

Conclusions

Bacillus coagulans arr4 proved to be a thermotolerant microorganism with great biotechnological potential for L-(+)-lactic acid production from granulated sugar and yeast extract. $\text{Ca}(\text{OH})_2$ was considered the best neutralizing agent, because it provided the highest L-(+)-lactic acid production. The exponential fed-batch fermentation method was effective, and the sugar concentration in the feed solution and the feed time were adequate to increase yield, production, productivity of L-(+)-lactic acid, and growth of the cells. The maximum production of L-(+)-lactic acid (206.81 g/L) was observed at 39 h of fermentation, with 97% yield, high productivity (5.29 g/L h), and no residual sugar, demonstrating a lack of inhibition by the substrate and final product, which is beneficial in reducing the cost of product recovery. The purification and recovery of L-(+)-lactic acid produced were achieved by filtration on Celite® and activated carbon and use of a cation-exchange column. This method was efficient in getting a good recovery and purity of L-(+)-lactic acid.

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