

In situ separation of lactic acid from fermentation broth using ion exchange resins

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Received: 9 March 2008 / Accepted: 29 July 2008 / Published online: 20 August 2008
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Abstract Lactic acid fermentation is an end product inhibited reaction. In situ separation of lactic acid from fermentation broth using ion exchange resins was investigated and compared with conventional fermentation system. Amberlite resin (IRA-400, Cl^-) was used to separate lactic acid from fermentation broth and pH was controlled online with an automatic pH controller. The effect of process variables on lactic acid production by *Lactobacillus casei* in whey permeate was studied. The maximum productivity was obtained at $\text{pH} = 6.1$, $T = 37^\circ\text{C}$ and impeller speed = 200 rpm. The maximum concentration of lactic acid at optimum condition was found to be 37.4 g/L after 38 h of fermentation using in situ separation system. The productivity of in situ separation system was five times increased in comparison with conventional system.

Keywords Whey · Lactic acid · In situ separation · Ion-exchange resins · *Lactobacillus casei*

Introduction

Whey is a by-product of cheese production with high COD (chemical oxygen demand) content (50 Kg O_2 per ton permeate) and cannot be processed by sewage treatment without expense [10]. Making dairy powder from whey is

possible, but it needs special equipment that makes it economically unfavorable [1].

Whey contains all the lactose of milk (4–5%), proteins, vitamins and mineral salts. It is generally a low-priced source of nutrient for the fermentation processes [1, 17]. One possibility for reducing the COD is the fermentative conversion of the lactose to lactic acid [10]. Lactic acid is commercially produced by fermentation of whey with lactic acid bacteria [5]. It is used widely in numerous fields of application such as food, medicine, and pharmaceutical, and in other industrial applications and its demand could rise rapidly in near future [14]. L(+)-lactic acid has been considered a potential feedstock for biodegradable lactide polymers (poly lactic acid), e.g., for use in packaging [2, 3, 12]. Poly L(+)-lactic acid is a biodegradable and biocompatible polymer and used in medicine especially for controlled release drug delivery and tissue engineering [11].

The end product inhibition by lactic acid causes several problems, among which low lactate formation rate and its recovery from fermentation broth are the most important ones [15, 16]. Therefore, an integrated approach with separation of product has gained a lot of interest in this fermentation [23].

Solvent extraction of lactic acid from fermentation broth is one way for reducing the concentration of lactic acid [6]. The solvent extraction technique is not preferred because it causes several physical, chemical and biochemical problems on the catalytic activity of the cells, e.g., interference with the aseptic condition and contamination of fermentation system [24]. The membrane extraction method has advantages over conventional solvent extraction and may be applied as the mechanism in separation, purification, pollutant removal and recovery processes [18]. A cellulose triacetate (CTA) membrane containing quaternary ammonium salt was used for lactate separation from fermentation

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broth [13]. A satisfactory recovery of lactic acid from both aqueous solution and actually fermented broth was accomplished; signifying the great potential of integrating the membrane extraction with fermentation process [21]. Continuous extraction of lactic acid from a simulated aqueous stream was achieved using Alamine 336 in 2-octanol, contained in a hollow-fiber membrane extractor. In this process, the extractant was simultaneously regenerated by stripping with NaOH in a second membrane extractor, and the final product is a concentrated lactate salt solution [8].

The restriction imposed by lactic acid on its fermentation has been avoided by an extractive fermentation technique employing ion exchange resins with automatic control of pH [22].

Lactobacillus casei subsp. *casei* is a homo-fermentative lactic acid bacterium which yielded L(+) lactic acid exclusively [24]. This type of lactic acid isomer has some special medical uses, e.g., production of biodegradable polymers that have applications in making surgery threads and currently costs ten times more than D(−) form [7].

The objective of the present work was to study the effect of process variables on production of L(+) lactic acid using in situ separation technique.

Materials and methods

Bacterial strain and media

Lactobacillus casei sbsps. *casei* PTCC 1293, provided by “Razi Institute, Tehran, Iran” was used for lactic acid production.

The medium for seed culture contained per liter: peptone 10 g, meat extract 5 g, yeast extract 5 g, Glucose 20 g, potassium hydrogen phosphate 2 g, Tween80 1 g, Di-ammonium hydrogen citrate 2 g, sodium acetate 5 g, magnesium sulfate 0.1 g, manganese sulfate 0.01 g [19].

A volume of 100 mL of the sterile medium was inoculated with the microorganism and after 24 h of incubation at 37 °C, it was used as seed culture for the fermentation system. The microbial density of seed culture at the time of inoculation was about 1.8 g/L. Whey from Pegah milk factory (Tehran, Iran) was used as fermentation medium. Lactose content of the whey was 44 g/L. Since the whey contains vitamins, protein and minerals, it considered as a self sufficient medium for fermentation [4].

Fermentor

A 2-L balloon shaped glass jar with 1.6 L working volume was used as fermentor. As illustrated in Fig. 1, it was connected to a column packed with ion-exchange resin. A filter was provided to prevent microbes entering the ion-

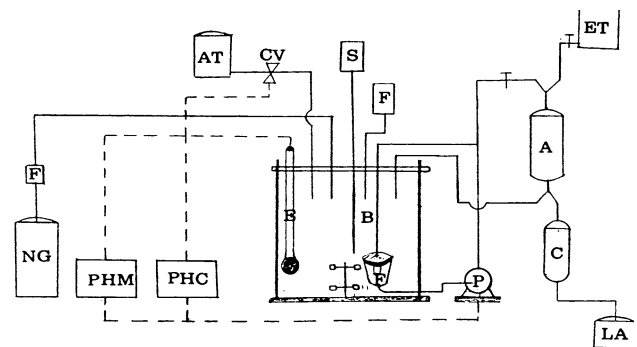


Fig. 1 Schematic diagram of extractive lactic acid fermentation. A anion exchanger column, B fermentor, C cation exchanger column, E electrode of pH meter, F filter, P pump, S site for sampling, AT alkali solution tank, CV control valve, ET eluant solution, LA Lactic acid (product), NG N₂ gas, PHC pH controller equipment, PHM pH meter

exchange column. A magnetic stirrer was used to stir the fermentation broth. Anaerobic condition was maintained by passing N₂ gas through the broth in the vessel and the head-space of the fermentor.

Columns of ion-exchange resins

Amberlit resin (IRA-400, Cl[−] Rohmand Haas Company, USA) was used to separate lactic acid from the fermentation broth. As shown in Fig. 1, fermentation broth passed through an ion-exchange column that had been hydroxylated previously with a solution of sodium hydroxide (2.5 N). Lactate ions exchanged with hydroxide ions and were separated from the fermentation broth. After saturation, the column was eluted with a solution of sodium hydroxide (1.5 N). Sodium lactate, resulted from the elution, entered the second column packed with Amberlite (IR 120, H⁺). In the second column, sodium ions exchanged with hydrogen ions and lactic acid exited from the column. The second column after saturation was regenerated with a solution of hydrochloric acid (1.5 N).

Preparation of proteolytic solution

A liquid medium containing 2 g/L yeast extract, 5 g/L peptone and 5 g/L sodium chloride was inoculated with the species *Bacillus subtilis* PTCC 1204 and incubated in a shaker incubator at a speed of 60 rpm at 30 °C for 24 h. This solution was used for hydrolysis of whey proteins.

Fermentation and product separation

For each experiment, 1 L of whey was poured into the fermentor and supplemented with the proteolytic solution (1% V/V) while pH of the solution was adjusted to 5.2. After 5 h of stirring at 100 rpm (for protein hydrolysis), the fermentor

tor content was sterilized at 121 °C for 15 min. After cooling, the fermentor was inoculated with the seed culture of *Lactobacillus casei* subsp. *casei* (10%V/V). The extractive fermentation was carried out at different pH, temperatures and impeller revolutions to determine the optimum conditions at which the rest of experiment was conducted.

The fermentor was connected to the columns of ion exchange resins (anionic and cationic) and incubated at 37 °C. Constant stirring (200 rpm) was maintained during the fermentation.

As fermentation proceeded, the pH of medium decreased gradually due to lactic acid production. When it reached below 6.0, the controller switched on the recycle pump and allowed the broth to pass through the anion exchange resin at a flow rate of three bed volumes per hour (3 BV/h). The anion exchanger resin selectively removes the lactate ions. The effluent of the column was recycled back to the fermentor and pH of the medium was increased. The pump stopped when the pH reached 6.2. Therefore the pH of fermentation medium was kept constant at 6.1 ± 0.1 using an automatic pH controller. The fermentation process was terminated after 38 h. Sodium lactate was eluted by NaOH (1.5 N) from the anion-exchange column with a rate of three bed volume per hour (3 BV/h). Sodium lactate entered into the cation-exchange column where lactic acid was produced and was then collected for analysis. This latter column was regenerated with (1.5 N) HCl (1.5 BV/h).

Analytical methods

Lactic acid concentration was determined by an HPLC equipped with ODS-PLO column (Waters-associates, USA). A mixture of water-methanol (1:1) with the flow rate of 1 mL/min was used as the carrier phase. The system was equipped with a UV detector. Concentration of lactic acid was detected at 275 nm.

Concentration of lactose was determined using the method of Somogi–Nelson [20].

Results and discussions

The batch fermentation of lactic acid was carried out in 250 mL shake flasks for 120 h at various temperature (32, 35, 37, and 40 °C), pH (4.5, 5, 6, and 7) and impeller revolutions (100, 150, 200, and 300) by classical method to optimize these variables. Figure 2 shows the effect of temperature on lactate fermentation. Lactic acid production was attained at 40 °C.

Despite the high production of lactic acid at 40 °C, the productivity of extractive fermentation decreased due to the low capacity of resin to adsorb lactate ions at high temperature. Therefore the extractive fermentation is very much

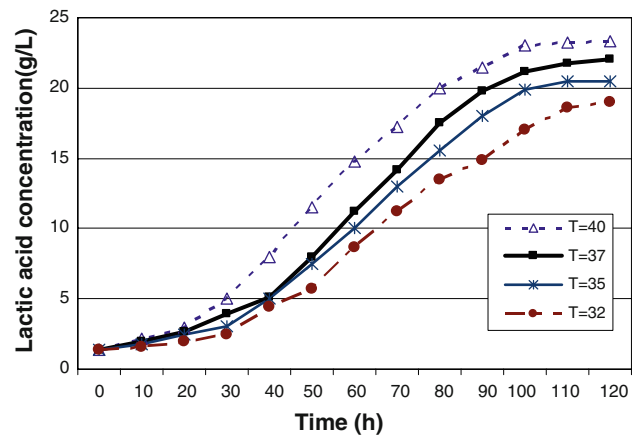


Fig. 2 The effect of temperature on lactic acid production by batch fermentation

dependent on the temperature of operation and 37 °C is the optimum temperature of extractive fermentation.

Figure 3 shows the effect of pH on lactate fermentation. This result indicates that lactic acid production decreased at high acidic condition as expected due to inhibitory effect on lactic acid bacteria. Furthermore, at extremely low pH values the lactic acid is mainly in undissociated form. Ion exchange resins extract dissociated acids from the aqueous phase by forming lactate ion.

Figure 4 shows the effect of impeller speed on lactate fermentation. It is well known that the stirring of the medium is required for homogeneity and increase of mass transfer coefficient. Lactic acid production increased with increasing stirring rate, but only slightly. Reduced lactic acid production at a higher revolution might be due to a lower activity of bacteria under high shear stress. Therefore the impeller speed was adjusted to 200 rpm in extractive fermentation.

The performance of batch and extractive fermentation for lactic acid production is compared in Table 1. Furthermore Figure 5 shows the concentrations of lactic acid

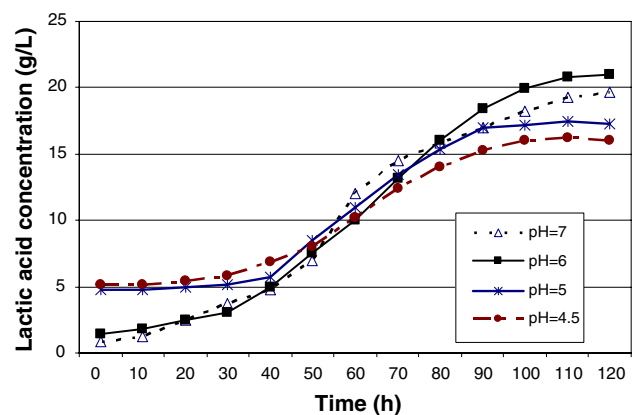


Fig. 3 The effect of pH on lactic acid production by batch fermentation

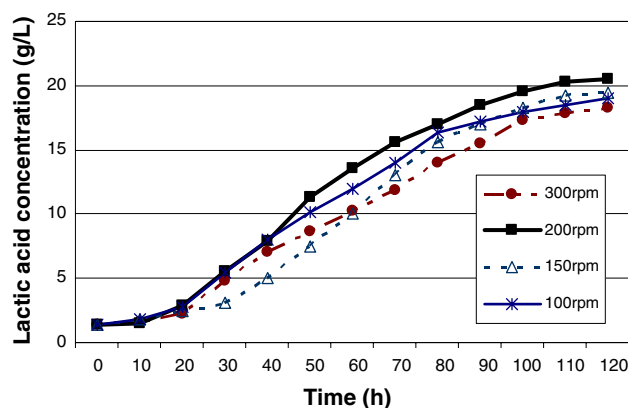


Fig. 4 The effect of impeller speed on lactic acid production by batch fermentation

Table 1 Comparison of batch and extractive fermentation for lactic acid production

Parameter	Batch fermentation	Extractive fermentation
Time (h)	120	38
Lactose conversion%	76	99.3
Yield (P^a/S^b)	0.5	0.85
Productivity (g/L per hour)	0.183	0.984

^a Product (lactic acid)

^b Substrate (lactose)

production and lactose consumption under optimum conditions (pH 6.1, 37 °C, 200 rpm). In extractive fermentation, after 38 h 99.3% of the lactose content of the whey was depleted. The concentration of lactic acid at this stage was 37.4 g/L. It is worth to mention that in a recent study [5], the amount of lactic acid produced by extractive fermentation using ion exchange resins was 32.7 g/L. This result is comparable to the production of 37.4 g/L lactic acid in the present study. Jarvinen et al. [9] studied separation of lactic acid from fermentation broth with combined liquid–liquid and reactive extraction using 80 g/L glucose as substrate. On addition of ion exchange resin to lactate solution, 45.6 g/L lactic acid was produced.

It can be seen from this figure that the concentration of lactic acid in the conventional fermentation system (Batch) after 38 h was only 6 g/L, and after 5 days, the concentration increased to the maximum value of 22 g/L. But in extractive fermentation (in situ separation), this maximum value was 37.4 g/L within 38 h. It means that the yield of lactic acid production increased by 60% in extractive fermentation compared to conventional fermentation, while the fermentation time decreased considerably.

The measurement of COD (chemical oxygen demand) of whey before and after the extractive fermentation showed a 65% decrease in the COD content of the whey. Extractive

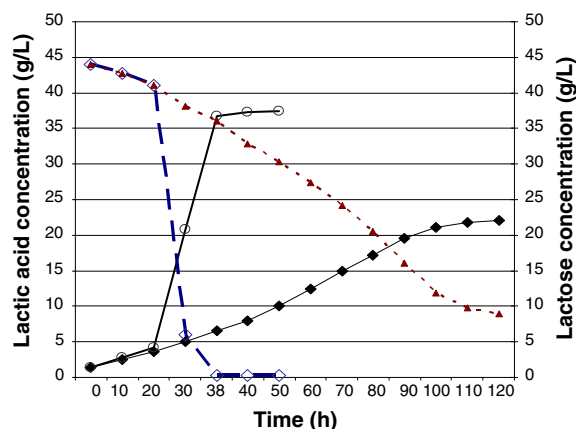


Fig. 5 Lactic acid production and lactose consumption in batch and extractive fermentation

Fig. 5 Lactic acid production and lactose consumption in batch and extractive fermentation

fermentation of whey reduced its COD content considerably and produced lactic acid with high productivity, which is of prime importance from environmental and economical points of view. Polarimetric experiments gave the value of +2.6 for the product. This value is for the L(+) isomer of lactic acid (Sigma, 1997; Merck Index, 1998).

Conclusions

Due to sterile-conditions requirement and product inhibitory effects, in situ separation of lactic acid from fermentation broth using ion exchange resin at controlled pH is a useful technique. L(+) lactic acid yield by extractive fermentation in comparison with non-extractive process was considerably increased while the time of maximum yield decreased. As a result productivity of the extractive fermentation process was about 4.3 times higher than that of the conventional process for L(+) lactic acid production from cheese whey.

Acknowledgment We thank Razi institute for providing *Lactobacillus casei* and *Bacillus subtilis* strains.

References

- Ataei SA, Sh Dolatshahi (2005) Isolation and determination of the most appropriate microbial strain and fermentation condition for single cell protein production from whey. *J Res Health Sci* 5:54–58
- Barrera DA (1993) Synthesis and characterization of a novel biodegradable polymer Poly (lactic acid-Co-lysine). *DAI-B* 54(6):3096–3099
- Dunn RL, English JP, Strobel JD, Cowsar DR, Tice TR (1988) Preparation and evaluation of lactide/glycolide co polymers for

- drug delivery. In: Migliaresi C (ed) *Polymers in medicine III*. Elsevier, Amsterdam, pp 149–159
4. Faith WL (1993) *Encyclopedia of chemical technology*. Interscience Pub, Dublin, pp 576–577
 5. Gonzalez MI, Alvarez S, Riera F, Alvarez R (2007) Economic evaluation of an integrated process for lactic acid production from ultrafiltered whey. *J Food Eng* 80:553–561
 6. Hatzini KDG, Wang HY (1992) Extractive fermentation systems for organic acids production. *Can J Chem Eng* 70:543–552
 7. Holland SJ, Tighe BJ, Gould PL (1986) Polymers for biodegradable medical devices I. The potential of polymers as controlled macromolecular release system. *J Control release* 4:155–160
 8. Huang H, Yang ST, Ramey DE (2004) A hollow-fiber membrane extraction process for recovery and separation of lactic acid from aqueous solution. *Appl Biochem Biotechnol* 114:671–688
 9. Jarvinen M, Mylikoski L, Keiski R, Sohlo J (2000) Separation of lactic acid from fermentation broth by reactive extraction. *Bioseparation* 9:163–166
 10. Krischke W, Schroder M, Trosch W (1991) Continuous production of L-lactic acid from whey permeate by immobilized *Lactobacillus casei* subsp. *casei*. *Appl Microbiol Biotechnol* 34:573–578
 11. Lewis D (1990) *Biodegradable polymers and drug delivery systems*. Dekker, Newyork, pp 203–208
 12. Lipinsky ES, sinclair RG (1986) Is lactic acid a commodity chemical? *Chem Eng Prog* 82:26–32
 13. Matsumoto M, Kondo K (1998) Fermentation of lactic acid combined with solvent extraction or membrane separation containing a mobile carrier. *Recent Res Dev Ferment Bioeng* 1:241–252
 14. Nolan-Itu Pty Ltd (2002) Prepared in association with Excel Pals Australia. *Biodegradable plastic Developments and Environmental*
 15. Pirt SJ (1985) Effect of chemical inhibition and activation of growth. *Principles of microbe and cell cultivation*. Blackwell Scientific Publications, Oxford, pp 170–185
 16. Roucourt A, Girard D, Prigent Y (1989) Continuous lactic acid fermentation with cell recycled by ultrafiltration and lactate separation by electrodialysis: model identification. *Appl Environ Microbiol* 30:528–534
 17. Roukas T, Kotzekidou P (1991) Production of lactic acid from deprotonized whey by Coimmobilized *Lactobacillus casei* and *Lactococcus* lactic cells. *Enzyme Microb Technol* 13:33–38
 18. Thang VH, Koschuh W (2004) Desalination of high salt content mixture by two-stage electrodialysis as the first step of separation valuable substances from grass silage. *Desalination* 162:343–353
 19. The American type culture collection (ATCC) (1993)
 20. Thimmaiah SR (2004) *Standard methods of biochemical analysis*. Kalyani publishers, New Delhi, India, pp 49–57
 21. Tong Y, Hirata M, Takanashi H, Hano T, Kubota F, Goto M, Nakashio F, Matsumoto M (1998) Extraction of lactic acid from fermented broth with microporous hollow fiber membranes. *J Memb Sci* 143:81–91
 22. Vaccari G, Gonzalez-vara A, Gampi AL, Dosi E, Brigid P, Matteuzzi D (1993) Fermentative Production of L Lactic acid by *Lactobacillus casei* DSM 20011 and product recovery using ion exchange resin. *Appl Microbiol Biotechnol* 40:23–27
 23. Wang HY (1983) Integrating biochemical separation and purification steps in fermentation Process. *Ann NY Acad Sci* 413:313–321
 24. Wardel JM, King CJ (1978) Solvent equilibria for extraction of carboxylic acid from water. *J Chem Eng Data* 23:144–150