Bioresource Technology 198 (2015) 658–663

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Efficient magnesium lactate production with *in situ* product removal by crystallization

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HIGHLIGHTS

- The ISPR process is based upon magnesium lactate crystallization.
- No external seed crystals are needed in crystallization.
- The crystallization is conducted at 42 °C without cooling and re-heating.
- Using ISPR fermentation saves 40% water, 41% inorganic salts and 43% yeast extract.

ARTICLE INFO

Article history:
Received 27 July 2015
Received in revised form 13 September 2015
Accepted 14 September 2015
Available online 26 September 2015

Keywords:
Magnesium lactate
Lactic acid fermentation
Crystallization
Lactobacillus rhamnosus

ABSTRACT

In this paper, attempts were made to develop an *in situ* product removal process for magnesium lactate production based on crystallization. The crystallization was conducted at 42 °C without seed crystal addition. The product concentration, productivity and yield of fermentation coupled with *in situ* product removal (ISPR) reached 143 g L^{-1}, 2.41 g L^{-1} h^{-1} and 94.3%. In four cycles of crystallization, the average reuse rate of fermentation medium and removal rate of product reached 64.0% and 77.7%. At the same time, ISPR fermentation saved 40% water, 41% inorganic salts and 43% yeast extract (YE) as compared to fed-batch fermentation. The process introduces an effective way to reduce the amount of waste water and the raw material cost in magnesium lactate fermentation.

1. Introduction

Lactic acid is a natural organic acid, which can be produced via either chemical synthesis or microbial fermentation (Nakano et al., 2012): 90% of lactic acid is produced by lactic acid bacteria fermentation every year (Datta and Henry, 2006; Gao et al., 2007). To ensure the productivity of lactic acid, a suitable pH value for bacterial growth must be maintained at around 5–7 by addition of pH regulating agent (Hetényi et al., 2011; Hofvendahl and Hahn-Hägerdal, 2000), and the responding metal lactates are produced. However, as an economical magnesium additive in food, drinks, dairy, flour and pharmaceutical industries (Lawless et al., 2003; Soliman and Abdel-Fattah, 2012), magnesium lactate is commonly made from neutralization reaction by purified lactic acid and magnesium base. Few reports studied direct magnesium lactate production by bacterial fermentation, in which magnesium base was added as neutralizing agent. In addition, the relative low solubility of magnesium lactate in the aqueous solution facilitates product separation (Apelblat et al., 2005; Manzurola and Apelblat, 2002). Therefore, efforts should be directed toward developing an ISPR process for magnesium lactate production based on crystallization. In this study, we aimed to investigate the feasibility of a cost-effective ISPR process, in which magnesium lactate crystals were removed continuously to reduce end product inhibition and reuse the fermentation medium.

2. Methods

2.1. Materials, strains and culture media

YE (yeast extract), beef extract and soya peptone were purchased from Aobox Biotechnology Co., Ltd. (Beijing, China). All other chemicals used were of analytical grade and commercially available.

Lactobacillus rhamnosus LA-04-1 (screened by our research group and maintained at the Key Lab of Bioprocess of Beijing, Beijing University of Chemical Technology, China) was used in all experiments. The medium for agar slant and inoculum preparation was as previously described (Wang et al., 2014). The fermentation

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http://dx.doi.org/10.1016/j.biortech.2015.09.058
0960-8524/

Table 1
Comparison of different end product removal methods used in fermentation.

Lactic acid removal technique	Strains	Fermentation mode	Product (calculated as lactic acid)			References
			Concentration ($g L^{-1}$)	Productivity ($g L^{-1} h^{-1}$)	Yield ($g g^{-1}$)	
Electrodialysis with bipolar membranes	*Lactobacillus plantarum* CICC 20768	Batch	92	1.8	0.57	Wang et al. (2012)
Electrodialysis	*Lactobacillus rhamnosus* IFO 3863	Continuous	20	13.2	0.77	Hirata et al. (2005)
Activated carbon absorption	*Saccharomyces cerevisiae* OC-2T T165R	Batch	60	–	0.71	Gao et al. (2011)
Ion exchange resins	Stable mixed-culture (a *Bacillus coagulans* as dominating bacterium)	Repeated fed-batch	–	0.329	0.9	Garrett et al. (2015)
Solvent extraction	*Saccharomyces cerevisiae* OC-2T T165R	Batch	34	1.1	0.36	Gao et al. (2009)
Integrated membrane	*Lactobacillus delbrueckii* NCIM-2025	Continuous	82.7	0.78	0.965	Dey and Pal (2012)
Ultrafiltration membrane	*Bacillus coagulans* IPE22	Repeated batch	56.5	2.35	0.96	Zhang et al. (2014)
Integrated membrane	*Lactobacillus plantarum* NCIM 2912	Continuous	119	1.98	0.96	Sikder et al. (2012)
Rotating fibre bed	*Rhizopus oryzae* NRRL 395	Repeated-batch	127	1.65	1.0	Tay and Yang (2002)
Crystallization of magnesium lactate	*Lactobacillus rhamnosus* LA-04-1	Repeated fed-batch	142	2.41	0.943	This study

medium used in the 5-L fermentor contained 20 $g L^{-1}$ YE, 150 $g L^{-1}$ glucose, 0.01 $g L^{-1}$ NaCl, 0.5 $g L^{-1}$ sodium acetate, 0.2 $g L^{-1}$ triammonium citrate, 0.2 $g L^{-1}$ KH_2PO_4, 0.2 $g L^{-1}$ $MgSO_4{\cdot}7H_2O$ and 0.05 $g L^{-1}$ $MnSO_4{\cdot}7H_2O$. The refilled medium used in 5-L fermentor contained 20 $g L^{-1}$ YE, 420 $g L^{-1}$ glucose and salts as those of fermentation medium. All culture medium were autoclaved at 116 °C for 25 min before used.

2.2. Culture conditions

Stock cultures of strain LA-04-1 were maintained at 4 °C. Inoculum preparation was carried out in 250 ml conical flasks with a working volume of 100 ml, which were incubated at 42 °C and 150 rpm for 24 h on a rotary shaker. The seed culture was then inoculated into 2 L fermentation medium in 5-L fermentor (SGB-5L, Changzhou Sungod Bio-technology & Engineering Equipment Co., Ltd., Jiangsu, China) with an inoculate volume of 10% (v/v). The temperature and stirring speed were 42 °C and 120 rpm, respectively, and MgO (15% w/w) was added as a neutralizer to keep the pH at 6.25. When glucose was exhausted, the refill medium was added by pulse fed-batch method.

2.3. Optimization of crystallization conditions

The optimization of crystallization temperatures was performed in a series of 250 ml conical flasks containing 100 ml broth. Product (calculated as L-lactic acid) concentration of the broth was 140 $g L^{-1}$. The conical flasks were kept undisturbed for 2 h in different temperatures ranging from 25 to 42 °C. Samples of the supernatant were taken to determine the product concentration and three parallel replicates were used in this experiment, with deviations from the mean given as error bars in the figures.

Product (calculated as L-lactic acid) concentration in broth for crystallization was optimized, and a series of fermentation broths containing 110, 120, 130, 140, 150 and 160 $g L^{-1}$ L-lactic acid were used. All broths were kept in 42 °C for 2 h. Product concentrations in the supernatants were determined and three parallel replicates were used in this experiment, with deviations from the mean given as error bars in the figures.

In the optimization of crystallization time, product (calculated as L-lactic acid) concentration in fermentation broth was 140 $g L^{-1}$, and the temperature for crystallization was kept at 42 °C. Samples of the supernatant were taken every 20 min, and the concentrations of product were determined. Three parallel replicates were used in this experiment, and deviations from the mean are given as error bars in the figures.

2.4. Fermentation in 5-L fermentors

The fed-batch fermentation in 5 L fermentor was carried out with 2 L initial fermentation medium. A refill medium, containing 420 $g L^{-1}$ glucose, was added when glucose was exhausted.

In a 5 L fermentor with 2 L initial fermentation medium, the fermentation coupled with ISPR was carried out, and a refill medium containing 420 $g L^{-1}$ glucose was added during the fermentation when glucose was exhausted. When the product (calculated as L-lactic acid) concentration reached 140 $g L^{-1}$, the ISPR process was carried out. Fermentation broth was pumped out for crystallization and precipitation at 42 °C, and 0.5 L broth was left in the fermentor. After crystallization and precipitation, the supernatant was transferred back into the fermentor, and the refill medium was added to replenish glucose and nutrients for the next fermentation. Four ISPR-cycles were carried out and detailed information is shown in Table 1. For each cycle of ISPR, the product removal rate of each cycle was calculated as follows:

$$R_p = \frac{M_{Re}}{C_b \times V_c} \times 100\%, \quad (1)$$

where R_p is the product removal rate of each cycle, M_{Re} is the amount of product removed, C_b is the product (calculated as L-lactic acid) concentration in broth and V_c is the volume of broth for crystallization. And the reuse rate of fermentation medium was calculated as follows:

$$R_m = \frac{V_s + V_r}{V_t} \times 100\%, \quad (2)$$

in which R_m is the reuse rate of fermentation medium, V_s is the volume of supernatant transferred back, V_r is the residual volume of broth in fermentor and V_t is the total volume of the broth after fermentation. The crystallizer was thermally insulated for heat preservation.

2.5. Analytical methods

Product (calculated as L-lactic acid) and glucose were measured by SBA-40C biosensor analyzer (Institute of Biology, Shandong Province Academy of Sciences, Shandong, China). The samples were diluted with distilled water, and were submitted to ultrasonic treatment at 28 kHz for 10 min before determination. L-lactic acid