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Efficient calcium lactate production by fermentation coupled with crystallization-based *in situ* product removal

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HIGHLIGHTS

- Fermentation with *in situ* product removal (ISPR) was used to produce calcium lactate.
- The ISPR technique used in this study was based on calcium lactate crystallization.
- Fermentation with ISPR produced 74.4% more L-lactic acid than with fed-batch method.
- Fermentation with the ISPR technique resulted in 1.7 times higher productivity.

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ABSTRACT

Lactic acid is a platform chemical with various industrial applications, and its derivative, calcium lactate, is an important food additive. Fermentation coupled with *in situ* product removal (ISPR) can provide more outputs with high productivity. The method used in this study was based on calcium lactate crystallization. Three cycles of crystallization were performed during the fermentation course using a *Bacillus coagulans* strain H-1. As compared to fed-batch fermentation, this method showed 1.7 times higher average productivity considering seed culture, with 74.4% more L-lactic acid produced in the fermentation with ISPR. Thus, fermentation coupled with crystallization-based ISPR may be a biotechnological alternative that provides an efficient system for production of calcium lactate or lactic acid.

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1. Introduction

Lactic acid is a promising platform chemical, which has various industrial applications such as in the food, textile, cosmetic, and pharmaceutical industries. Besides its utility as green feedstock, lactic acid can be used to produce a variety of derivatives, such as poly lactic acid (PLA), acrylic acid, pyruvic acid, 1,2-propanediol, and lactate ester (Gao et al., 2011a). Lactic acid has attracted considerable attention because of its role in the production of PLA that is a promising biodegradable and biocompatible material. The global demand for lactic acid increases by 5–8% every year (Abdel-Rahman et al., 2013). Calcium lactate, the end product in lactic acid fermentation when calcium carbonate or calcium hydroxide is used as the neutralizing agent, is an important food additive. It can be used as a nutritional supplement or firming agent (Martin-Diana et al., 2007; Sheikh et al., 1987).

Microbial fermentation can produce optically pure L-lactic acid (Abdel-Rahman et al., 2013; Gao et al., 2011a). To date, various microbial strains have been used in L-lactic acid production. Recent studies show that strains of *Bacillus coagulans* are excellent L-lactic acid producers with advantages, such as the ability to perform open fermentation, the highly optically pure product, and the utilization of raw substrates (Qin et al., 2009; Wang et al., 2010, 2013; Xu and Xu, 2014). Recently, the properties of *B. coagulans* strains such as high substrate efficiency and excellent environmental tolerance have also been clarified by genomic analysis (Su and Xu, 2014).

On the industrial scale, batch and fed-batch fermentations are widely used for lactic acid production (Abdel-Rahman et al., 2013). In order to increase productivity per batch, end product inhibition during fermentation is an inevitable challenge. Fermentation coupled with an *in situ* product removal (ISPR) technique, a type of fermentation integrated with downstream processing, could provide a way to reduce end product inhibition by separating the product from the broth during fermentation. Thus, higher productivity can be achieved (Urbanus et al., 2010). In previous studies, efforts were made to produce lactic acid using fermentation with an end product removal process. Several product removal techniques such as solvent extraction, ion

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exchange, electrodialysis, activated carbon absorption, and fluidized bed bioreactors have been developed (Gao et al., 2009, 2011b; Patel et al., 2008; Seyed and Ebrahim, 2008; Wang et al., 2012). Most of these techniques can efficiently remove the product during fermentation. However, drawbacks of these techniques limit their application. Solvent extraction includes a back-extraction process for the recovery of lactic acid from the extractants, which complicates the process. Moreover, the extractants are commonly cytotoxic (Wasewar et al., 2004; Zhou et al., 2011). Ion exchange techniques may cause substrate loss, which decreases the yield. Furthermore, ion-extraction resins need to be regenerated for continuous extraction (Seyed and Ebrahim, 2008). Electrodialysis requires specialized equipment and also causes substrate loss (Gao et al., 2004). Activated carbon absorption is sensitive to pH changes and an additional step of desorption is required to release lactic acid from activated carbon (Gao et al., 2011b). As for the fluidized bed bioreactor, the design and development of equipment are key issues (Patel et al., 2008). Therefore, development of an efficient and economical technique for end product removal such as lactic acid fermentation with an ISPR technique is required. Previous studies show that calcium lactate could be efficiently produced during lactic acid fermentation using calcium carbonate as a neutralizer, and could be easily removed by crystallization (Chemaly et al., 1999; Nakano et al., 2012). However, an efficient fermentation method coupled with the ISPR technique based on calcium lactate crystallization has not yet been reported.

In the present study, we used crystalline precipitation of calcium lactate as the product removal method during L-lactic acid fermentation (Fig. 1), in order to develop an efficient and economical method of fermentation for production of L-lactic acid. The conditions for calcium lactate crystallization and the fermentation with ISPR based on crystallization in a 5-L bioreactor were studied.

2. Methods

2.1. Chemicals

Soy peptide was purchased from Comwin Pharm-culture Co., Ltd. (Beijing, China). Yeast extract was purchased from Angel Yeast Co., Ltd. (Hubei, China). Cottonseed protein was purchased from Aoboxing Universeen Bio-Tech Co., Ltd. (Beijing, China). All other chemicals were of analytical grade and commercially available.

2.2. Microorganism and culture conditions

B. coagulans H-1 used in this study was isolated from soil by our group. Strain H-1 (CCTCC M 2013105) is a filamentous homofermentative L-lactic acid producer, preserved at the China Center for Type Culture Collection (CCTCC) (Xu et al., 2013). The stock culture of strain H-1 was maintained on deMan-Rogosa-Sharpe (MRS) agar slants at 4 °C. The seed medium for seed culture contained 10 g/L yeast extract, 5 g/L soy peptide, 100 g/L glucose, and 60 g/L $CaCO_3$ in Erlenmeyer flasks. The medium was autoclaved at 115 °C for 15 min. Seed cultures were amplified with an inoculum volume of 10% (v/v). When cell density (OD_{600}) of the seed culture in flasks reached 5, a 350 mL seed was inoculated into a 5-L bioreactor containing 2.5 L seed medium for further preparation at 52 °C and 80 rpm for 16 h. Seed cultivation in the 5 L bioreactor was considered complete when OD_{600} reached 8. The pH was maintained at 6.2 by 25% (w/v) $Ca(OH)_2$. The fermentation medium used in the 5-L bioreactors contained 12.6 g/L yeast extract, 1.2 g/L soy peptide, 3 g/L cottonseed protein, 1 g/L $NaNO_3$, 1 g/L NH_4Cl, and 200 g/L glucose (Qin et al., 2009).

2.3. Optimization of seed crystal quantity

Calcium lactate pentahydrate in particulate form was used as seed crystal in this study, to improve calcium lactate crystallization by directly adding it into the broth. To optimize the quantity of seed crystal to be added, the experiment was carried out with a series of 100 mL beakers containing 60 mL fermentation broth. The broth contained 120 g/L L-lactic acid. After the broth was cooled to room temperature (about 23 °C), seed crystals (particles of calcium lactate pentahydrate) were added in a series of 1–9% (w/v) of the broth in each beaker. After 3 h with no disturbance, the crystals of calcium lactate formed in the beakers were separated and weighed on a balance. Three parallel replicates were used in this experiment.

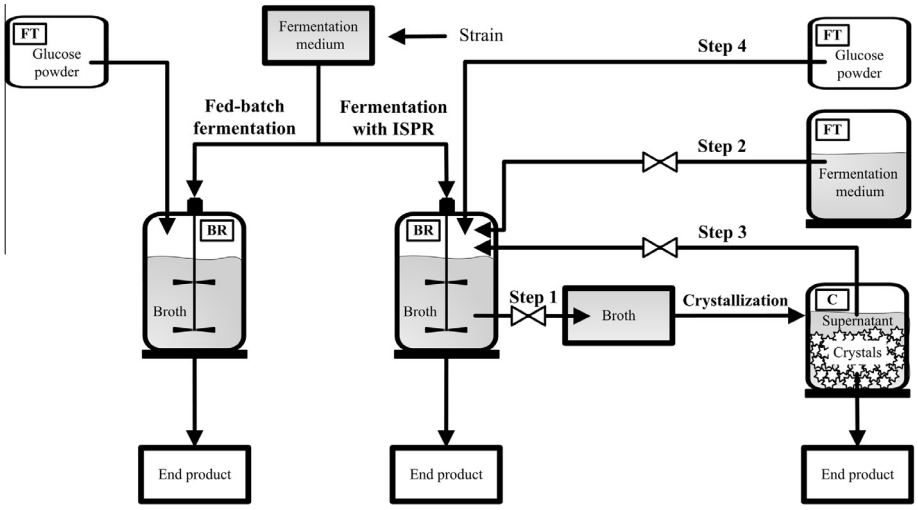

Fig. 1. Schematic diagram of the experiments. FT: feed tank. BR: bioreactor. C: crystallizer. Fed-batch fermentation was carried out by adding glucose powder when residual glucose was exhausted in the bioreactor. Fermentation coupled with calcium lactate crystallization was carried out as follows: crystallization was carried out in the crystallizer when residual glucose was exhausted in the bioreactor. Part of the broth was delivered to the crystallizer for calcium lactate crystallization (Step 1). Fresh broth was then added into the bioreactor (Step 2). After crystallization, supernatant of the broth in the crystallizer was fed back into the bioreactor (Step 3). Glucose powder was fed into the bioreactor when residual glucose was exhausted after the completion of all cycles of crystallization (Step 4).

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