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Open fermentative production of L-lactic acid using white rice bran by simultaneous saccharification and fermentation

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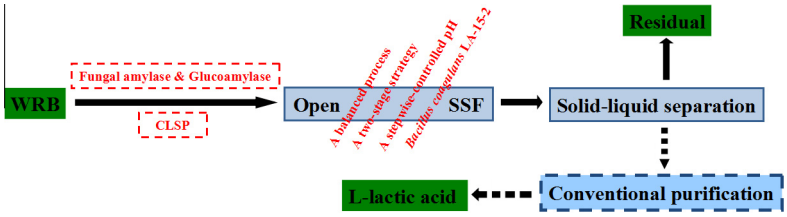
HIGHLIGHTS

- A two-stage strategy was efficiently used for L-lactic acid production from WRB.
- A stepwise controlled pH was proposed for indigenous bacteria suppression.
- A balanced process was obtained under an open SSF process.

GRAPHICAL ABSTRACT



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ABSTRACT

To reduce raw material cost for lactic acid production, white rice bran as an important byproduct in rice milling, was used in L-lactic acid production by open simultaneous saccharification and fermentation (SSF). Although one thermotolerant strain was used at a temperature as high as 50 °C, the open fermentation was still inefficient due to the indigenous thermophilic bacteria from corn steep liquor powder. A stepwise controlled pH was proposed in open SSF process, and no complicated pretreatment or sterilization was needed before fermentation. In batch fermentation, 117 g L^{-1} lactic acid was obtained, and the productivity and yield reached 2.79 g L^{-1} h^{-1} and 98.75%, respectively. These results showed an efficient way to develop high value-added products from white rice bran.

1. Introduction

As a natural organic acid, lactic acid has versatile applications in food, pharmaceutical, textile, leather and chemical industries (Gao et al., 2011; Yun et al., 2003). The high raw material cost in the production of optically pure lactic acid has limited the applications of lactic acid in more fields, especially in polylactic acid (PLA). Recently, great attention has been paid to agro-industrial wastes which can replace the refined and costly raw materials (Ouyang et al., 2013; Sun et al., 2012).

Rice bran (RB) could be classified as yellow rice bran and white rice bran according to the rice milling process. White rice bran (WRB) is the inner layer of RB, and is rich of starch and protein. As oil content in WRB is less than that in yellow rice bran and the rich starch in WRB is not suitable for oil extraction, WRB is commonly separated from yellow rice bran to be used as cattle feed (Lin et al., 2009).

In order to use the nutrients in rice milling byproducts for lactic acid production, Gao et al. (2008) hydrolyzed rice bran by H_2SO_4, and 30 g L^{-1} rice bran provided a productivity equal to that of 8 g L^{-1} yeast extract (YE). Li et al. (2012) used the hydrolysate of WRB and corn steep liquor as carbon and nutrient sources in L-lactic acid fermentation, and a high productivity (3.73 g L^{-1} h^{-1}) was obtained in batch fermentation. Defatted rice bran (DRB)

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was also used as the sole substrate for L-lactic acid fermentation in previous study, and a productivity of 3.63 g L^{-1} h^{-1} in batch fermentation was obtained (Wang et al., 2014). These results showed the use of rice bran or its derivates could provide a solution for reducing substrate cost in lactic acid fermentation. However, it is quite difficult to sterilize solid materials such as rice bran or its derivates, and much heat energy is required for complete sterilization before inoculation. In recent years, non-sterilized fermentation with thermotolerant *Bacillus* for lactic acid production have received great interest by researchers (Ma et al., 2014; Ouyang et al., 2013; Zhao et al., 2010). Among these thermophilic bacteria, *Bacillus coagulans* has advantageous characteristics of growing and fermenting at temperature range from 50 to 60 °C, high optical purity of product and simple nutrition requirement (Budhavaram and Fan, 2009; Hakoda et al., 2009; Zhou et al., 2013). However, few reports have studied open fermentative production of L-lactic acid from WRB using *B. coagulans*. To simplify lactic acid fermentation process and further reduce the cost of lactic acid production, L-lactic acid production from WRB by open SSF was studied in this paper.

2. Methods

2.1. Microorganisms

The *B. coagulans* LA-15-2, which was derived from ATCC 7050, was screened by evolutionary engineering and used for L-lactic acid production in this study. *B. coagulans* Z1 was screened from corn steep liquor powder (CSLP) at 50 °C, and it was considered as the only indigenous bacterium which affected the open fermentation in this study.

2.2. Materials and culture media

White rice bran was kindly provided by WAN YUAN OIL CO., Ltd. (Heilong Jiang, China). The components of WRB used in this study were as follows: protein (9.31 ± 0.120%), lipid (8.43 ± 0.117%), starch (36.45 ± 0.732%), ash (6.45 ± 0.596%), moisture (13.8 ± 0.106%) and crude fiber (8.42 ± 0.130%). Yeast extract was purchased from Aobox Biotechnology Co., Ltd. (Beijing, China). And CSLP was provided by Beijing Mannafeed International Group (Beijing, China). Fungal amylase used in this work was derived from *Aspergillus oryzae* (Heshibi Bio-Products Co., Ltd. Gansu, China). And glucoamylase was produced by *Aspergillus niger* (Longda Bio-products Co., Ltd. Shandong, China). The specific activities of fungal amylase and glucoamylase were 10,000 U g^{-1} and 120,000 U ml^{-1}, respectively, according to the manufacturers' data. And all other chemicals used were reagent grade.

The medium for agar slant and inoculum preparation was as previously described (Wang et al., 2014). Aerobic growth medium, which containing 18 g L^{-1} glucose and 15 g L^{-1} CSLP, was used for microorganism culture. Anaerobic fermentation medium consisted of WRB, fungal amylase and glucoamylase on the basis of aerobic growth medium. WRB was added into aerobic growth medium with a concentration of 50% (w/v). Fungal amylase and glucoamylase were added separately at a ratio of 1 g to l kg WRB. Then the fermentation enters into anaerobic stage.

2.3. Optimization of WRB concentration in saccharification

The optimal pH and temperature of the fungal amylase are 5.0 and 60 °C according to the manufacturers suggested protocol. Those of the glucoamylase are 6.0 and 60 °C. To examine the effect of WRB concentration on saccharification, 2 g L^{-1} of NaN_3 was added into the mixture to suppress the growth of indigenous microorganisms in WRB. 25 g of WRB powder was suspended in each 250 ml screw bottle, and the ratio of WRB to water (w/v) ranged from 1:2 to 1:8. Fungal amylase and glucoamylase were added separately at a ratio of 1 g to l kg WRB. The pH of saccharification was 6.25. A rotary shaker at 50 °C and 180 rpm was used. And the saccharification time was 45 h.

2.4. Microorganism cultivation conditions

B. coagulans LA-15-2 was maintained on Modified De Man-Rogosa-Sharpe (mMRS) agar slant. After incubated at 50 °C for 24 h, the full grown slant was stored at 4 °C. And the stock culture was transferred to fresh mMRS agar slants monthly. In seed culture preparation, the cells were transferred to 100 ml liquid mMRS medium containing 40 g L^{-1} glucose in a 250 ml Erlenmeyer flask and were incubated for 20 h at 50 °C and 150 rpm on a rotary Shaker. The seed culture was then inoculated into 900 ml aerobic growth medium in 5-L fermenter (SGB-5L, Changzhou Sungod Bio-technology & Engineering Equipment Co., Ltd. Jiangsu, China) for aerobic cell growth. The inoculation rate was 10% (v/v).

2.5. Aerobic growth and anaerobic fermentation

A two-stage strategy containing aerobic growth and anaerobic fermentation was used in this study. The whole process was conducted without sterilization, and the temperature was kept at 50 °C. In aerobic growth stage, the aeration rate and agitation speed were kept at 2 vvm and 400 rpm, respectively, and pH was maintained at 6.50 which was optimal for *B. coagulans* LA-15-2. In the anaerobic fermentation stage, WRB was added into the aerobic growth medium containing active *B. coagulans* LA-15-2 cells. At the same time, fungal amylase and glucoamylase were added separately at a ratio of 1 g to l kg WRB. The agitation speed was kept at 260 rpm without aeration, and the pH was automatically adjusted by 33% $Ca(OH)_2$.

In order to study the efficiency of open fermentation relying solely on temperature at 50 °C, L-lactic acid production under sterilized conditions was conducted as a contrast to that under unsterilized conditions. 1 L aerobic growth medium mentioned above was sterilized at 116 °C for 25 min, and a cell density of 14.0 (OD_{620}) was obtained after aerobic growth. The mixture was centrifuged at 3500g for 15 min. And the sediment was transferred to anaerobic fermentation medium under sterile conditions. The anaerobic fermentation medium containing 15 g CSLP, 500 g WRB and 1 kg tap water was sterilized at 116 °C for 25 min. Fungal amylase and glucoamylase used in the experiment was dissolved in 20 ml deionized water, and the mixture was filtered through a 0.22-μm-pore-size membrane (Millipore) before addition.

To evaluate the effect of pH which ranged from 5.60 to 6.20 on anaerobic fermentation, a middle-degree solid loading of WRB was used to shorten fermentation period, and 250 g WRB was mixed with aerobic growth medium with a ratio of 1:4 (w/v) in 5-L fermentor. However, in L-lactic acid fermentation after pH optimization, high solid loading was required to increase the lactic acid titer, and 500 g WRB was added as carbon source to 1 L aerobic growth medium in anaerobic fermentation stage. Because of rich salts in CSLP, no inorganic salts were added. In this study, fungal amylase and glucoamylase were adopted to saccharify the starch in WRB, avoiding the damage to amino acids and vitamins caused by acid hydrolysis (Gao et al., 2008).

2.6. Analytical methods

L-Lactic acid and glucose were measured by SBA-40C biosensor analyzer (Institute of Biology, Shandong Province Academy of Sciences, Shandong, China), and the fermentation broth was