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Short communication

Fermentative production of L-lactic acid from hydrolysate of wheat bran by *Lactobacillus rhamnosus*

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ABSTRACT

To reduce the nutrient cost of L-lactic acid production, wheat bran was chosen as a nutrient source. Various pretreatment processes were investigated, and 80 °C for 20 h by acid-hydrolysis was a suitable method considering the energy consumption. Pretreated wheat bran showed a better performance than that without treatment, especially for L-lactic acid yield (0.99 g/g). Moreover, when 25 g/l wheat bran hydrolysate was combined with 30 g/l corn steep liquor, the L-lactic acid fermentation efficiency (yield 0.99 g/g, productivity 3.75 g/l/h) was even higher than that of the control with 15 g/l yeast extract (yield 0.95 g/g, productivity 2.46 g/l/h). Although much more wheat bran hydrolysate and corn steep liquor were used than yeast extract in total amount, the cost of nitrogen in fermentation was estimated to be ¥1286/t L-lactic acid (25 g/l wheat bran hydrolysate and 30 g/l corn steep liquor), which is only 11% of the ¥11471/t L-lactic acid (15 g/l yeast extract) in control test. Therefore, nutrients of wheat bran hydrolysate and corn steep liquor could be employed to substitute yeast extract for L-lactic acid production.

1. Introduction

Lactic acid and its derivatives are widely used in food, pharmaceutical, leather, and textile industries. Recently, one of the most promising applications is to be used as a starting material for biodegradable plastics. Lactic acid can be produced by chemical synthesis or microbial fermentation. Specific stereo isomeric form can be obtained by microbial fermentation, while chemical synthesis results in racemic mixture of lactic acid. Currently, approximately 90% of lactic acid was produced by lactic acid bacteria fermentation [1–3].

Yeast extract (YE) was a usual nutrient for both microbial growth and lactic acid production since most lactic acid bacteria required a wide range of growth factors including amino acids, vitamins, specific minerals, fatty acids, purines, and pyrimidines [4]. However, the high cost of YE was a limitation for its application in industrial process [3]. Economic analysis for lactic acid production showed that the cost of YE contributed over 30% to the total production cost, which implied an urgent need for a cheaper alternative [4,5]. Great efforts have been made to search other cheaper sources to achieve a partial or complete substitution of YE. Various nitrogen sources for lactic acid fermentation (YE, malt sprouts, peptones, grass extract, corn steep liquor (CSL), casein hydrolysate, distiller's waste, ammonium phosphates, and urea) were studied by Hujanen and Linko [6–7]. Yoo et al. also reported the potential of soybean hydrolysate with addition of five B-group vitamins in lactic acid production, and it could result in a complete utilization of 100 g/l glucose with half (5 g/l) of the usual level of YE. However, YE could not be completely substituted in their results [8]. Kwon et al. reported that 15 g/l YE could be successfully replaced with 19.3 g/l soytone supplemented by vitamins, which resulted in a 125 g/l lactic acid from 150 g/l glucose by batch cultivation. The productivity and L-lactic acid yield were 2.27 g/l/h and 92%, respectively [7]. Altaf et al. reported that 96% lactic acid yield could be obtained when red lentil flour and bake yeast cells were substituted for peptone and YE in solid state fermentation with wheat bran employed as support and substrate [9].

Wheat bran (WB), rich in proteins, oil, nutrients, and calories, is one of the major by-products of wheat production. Since wheat is one of the most important foods for human especially in China, about 109.29 million tons of wheat has been produced in China in 2007 (National Bureau of Statistics of China, 2009). This huge amount of wheat could also result in commensurate amount of by-products, such as wheat bran, which accounts for about 3% of the milled wheat. Therefore, about 3.28 million tons wheat bran was produced in China in 2007, which could be thought as a potential resource.

The present work studied the possibility that wheat bran hydrolysate (WBH) combined with corn steep liquor to completely

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substitute for YE in L-lactic acid production. Moreover, the pretreatment conditions of WB were optimized.

2. Materials and methods

2.1. Bacterial strain and media

The *Lactobacillus rhamnosus* LA-04-1 (screened by this research group and maintained at the Key Lab of Bioprocess of Beijing, Beijing University of Chemical Technology, P.R. China) was used in all experiments. It was maintained on semisynthetic medium consisting of (in g/l): 20 glucose, 5 YE, 10 soya peptone, 10 beef extract, 5 NaCl, 10 sodium acetate, 2 triammonium citrate, 0.2 $MgSO_4{\cdot}7H_2O$, 0.05 $MnSO_4{\cdot}7H_2O$, and 15 agar. The stock cultures were maintained at 4 °C. The medium for cell growth or inoculum preparation contained the following (in g/l): 40 glucose, 20 YE, salts (0.01 NaCl, 0.5 sodium acetate, 0.2 triammonium citrate, 0.2 KH_2PO_4, 0.2 $MgSO_4{\cdot}7H_2O$, 0.05 $MnSO_4{\cdot}7H_2O$), and 16 $CaCO_3$.

Two types of media were used for fermentations. The control medium contained (in g/l): 100 glucose, 15 YE and salts as above. Experiments media contained (in g/l): 100 glucose, salts as above and different nitrogen resource.

2.2. Cultivation conditions

Seeds were cultivated at 42 °C and 150 rpm in 250 ml flasks containing 100 ml of seed medium. L-lactic acid was neutralized by an equivalent amount of $CaCO_3$.

Flask fermentations were carried out in 250 ml flasks containing 100 ml of different media with 75 g/l glucose. L-lactic acid and glucose were observed at 72 h after inoculation. Fermentations were performed in a 5 l fermentor (Zhenjiang, China) with an initial broth volume of 2 L. The temperature was maintained at 42 °C and the rotation speed was 150 rpm, and the pH was controlled at 6.25 by the addition of 35% (w/w) calcium hydroxide solution.

2.3. The process on treatment of WBH

2.3.1. Method A

WB was mixed with water to make the ratio of WB (dry weight)/water 1:4, and the pH of the WB slurry was adjusted to 1 by the addition of 6 M sulfuric acid. The slurry was hydrolyzed at 121 °C for 20, 30, 40 and 50 min, respectively, centrifuged at 8000 rpm for 15 min. The supernatant was used as a nutrient source for the production of L-lactic acid [10].

2.3.2. Method B

The mixture of WB and water with the ratio of WB (dry weight)/water 1:4 was pretreated at 121 °C for 20, 30, 40 and 50 min, respectively. The suspension was centrifuged at 8000 rpm for 15 min. The precipitate was further acid-hydrolyzed by the same procedure as method A. Two kinds of supernatants were mixed and used as nutrients for L-lactic acid production [10].

2.3.3. Methods 4# and 5#

100 g WB was transferred into a 2 l stirred vessel containing 400 ml water equipped with a temperature controller. Extract was performed at 100 °C (4#) and 121 °C (5#) for 20 min. Then the suspension was centrifuged at 8000 rpm for 15 min. The supernatants were used as nutrients source for production of L-lactic acid.

2.3.4. Methods 1#, 2# and 3#

100 g WB was transferred into a 2 l stirred vessel containing 400 ml 1.5% H_2SO_4 equipped with a temperature controller. Hydrolysis was performed at 70 °C (1#), 80 °C (2#) and 90 °C (3#) for 20 h.

2.4. Analytical methods

The fermentation broth (5 ml) was centrifuged at 8000 rpm for 10 min, and the supernatant liquor was diluted before determination. Glucose and L-lactic acid were measured by a SBA-40C biosensor analyzer (Institute of Biology, Shandong Province Academy of Sciences, P.R. China) [11].

For determination of the percentage of dry matter, the samples were weighted before and after dried in air at 105 °C for at least 12 h. Ash percentage was calculated by reweighting the samples after maintaining at 550 °C for 6 h.

Total nitrogen content was determined by the classic Kjeldahl method. The concentration of α-amino nitrogen was determined using the ortho-phtaldialdehyde (OPA) method. The OPA reagent was prepared as follows: 9.53 g disodium tetraborate (borax) and 5 g sodium dodecyl sulfate (SDS) (w/v) were dissolved in 489 ml MilliQ H_2O. Fresh reagents were prepared before each assay by adding 40 mg OPA dissolved in 1 ml methanol and 100 μl β-mercaptoethanol for every 49 ml of borax–SDS-solution. Serine and cysteine were dissolved in water at the concentrations of 0.05 and 0.005 M as standards. The 1740 μl OPA reagent was mixed with 60 μl of diluted hydrolysate or standards. The mixture were shaken briefly in a shaker and incubated at room temperature for exactly 20 min before being read at 340 nm in a spectrophotometer (Unico UV2000, China). The values obtained from the standards were used for calculation of free α-amino nitrogen [4].

For analysis of amino acids, the supernatant obtained by centrifugation and filtration of the sample was analyzed at Feed Research Institute (Chinese Academy of Agriculture Sciences, China). The appropriately diluted supernatant was injected into the cation separation column (LCA K06/Na 1.6 mm × 150 mm; Sykam GmbH, Eresing, Germany) and analyzed with the Sykam S433D amino acid analyzer.

3. Results and discussion

3.1. Chemical composition of the nitrogen resources

The chemical compositions of the nitrogen sources are presented in Table 1. The total nitrogen and α-amino nitrogen contents of YE were higher than those of others. WB and WBH had the same total nitrogen content. However, α-amino nitrogen content of WBH was higher than that of WB due to the hydrolysis procedure. The higher ratio of α-amino nitrogen to total nitrogen content (AN/TN) in WBH indicated a higher degree of hydrolysis, and a generally shorter average peptide length [12]. The amino acid compositions of the various nutrients are shown in Table 2, which indicated generally low amino acids contents in WBH, while YE and CSL had high amino acids contents.

3.2. Effect of wheat bran and yeast extract on L-lactic acid production

Fermentation was carried out to investigate the influence of the addition of WB on fermentation performance. As shown in Fig. 1, WB concentration had significant effect on the production and yield of L-lactic acid. In all cases, glucose was consumed completely, and the final concentration of L-lactic acid was close to each other. However, when WB concentrations were added from 25 to 56 g/l, productivity of L-lactic acid was increased significantly. The L-lactic acid yield and productivity were 94% and 2.05 g/l/h with the addition of 56 g/l WB, which were lower than those of the control with 15 g/l YE (95% and 2.46 g/l/h); however, they were higher than those of 25 g/l WB (80% and 0.89 g/l/h).