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An economical approach for D-lactic acid production utilizing unpolished rice from aging paddy as major nutrient source

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ABSTRACT

In order to reduce the raw material cost of D-lactic acid fermentation, the unpolished rice from aging paddy was used as major nutrient source in this study. The unpolished rice saccharificate, wheat bran powder and yeast extract were employed as carbon source, nitrogen source and growth factors, respectively. Response surface methodology (RSM) was applied to optimize the dosages of medium compositions. As a result, when the fermentation was carried out under the optimal conditions for wheat bran powder (29.10 g/l) and yeast extract (2.50 g/l), the D-lactic acid yield reached 731.50 g/kg unpolished rice with a volumetric production rate of 1.50 g/(l h). In comparison with fresh corn and polished rice, the D-lactic acid yield increased by 5.79% and 8.71%, and the raw material cost decreased by 65% and 52%, respectively, when the unpolished rice was used as a major nutrient source. These results might provide a reference for the industrial production of D-lactic acid.

1. Introduction

Lactic acid (LA), a useful organic acid, is widely used in the food, pharmaceutical, leather and textile industries. Its most promising application is being used as a major raw material for the manufacture of poly(lactic acid) (PLA). As a kind of biodegradable polymer, PLA might become a potential environmentally friendly substitute of non-biodegradable plastics derived from petrochemicals (Akerberg and Zacchi, 2000). There are three types of lactic acid: optically active L-lactic acid, D-lactic acid and racemic DL-lactic acid. Recently, it was reported that an equimolar blend of poly(L-lactic acid) and poly(D-lactic acid) generated a racemic crystal called stereo-complex poly(lactic acid) which was more heat-resistant than the poly(L-lactic acid) homo-polymer due to the high melting temperature (Sawai et al., 2007). This finding made D-lactic acid more and more important.

D-Lactic acid was primarily produced from a variety of feedstocks by fermentation using lactic acid bacteria (Hofvendahl and Hahn-Hägerdal, 2000; Fukushima et al., 2004). A major concern in D-lactic acid fermentation was to reduce the cost of raw materials which accounted for more than 34% of total production-cost (Akerberg and Zacchi, 2000). Utilization of cheap carbon sources was considered as an effective approach. Though many starchy materials from agriculture such as corn, rice and rice starch were used as carbon sources in many studies, the media costs were still high in relation to synthetic media (Fukushima et al., 2004; Lee, 2007). The utilization of cellulosic wastes such as cardboard and corn cobs as substrates for lactic acid fermentation by simultaneous saccharification and fermentation (SSF) was considered a promising approach (Rivas et al., 2004). However, there were many technical problems, for instance, the enzymes of cellulose hydrolysis were inhibited by the intermediate product (cellobiose), and the lactic acid biosynthesis was inhibited by the final product (lactic acid). Many investigations had been carried out to relieve the inhibitions, for example, in situ product removal technology was applied during the SSF process, which need large electric energy or high-level equipment (Li et al., 2004; Tanaka et al., 2006; Romani et al., 2008). Therefore, for the industrial production of D-lactic acid, it was quite necessary to provide cheap carbon sources which could be easily utilized by lactic acid bacteria, and to obtain the optimal conditions of fermentation with higher yield and production rate.

Unpolished rice is a type of rice that had paddy hull removed during the processing but not the bran layer. Besides abundant starch, unpolished rice also contains greater amounts of dietary fibers, proteins, vitamins and minerals than polished rice (Das et al., 2008). Unpolished rice manufactured from fresh paddy is a healthy food. In China, an agricultural country that produces paddy in large volumes, a considerable amount of aging paddy is rejected for use as foodstuff, due to its less tasty flavor in comparison to fresh paddy. This aging paddy is mostly used in the feedstuff industry, which does not bring equivalent profit (Heerink et al., 2007; Liang et al., 2008). Thus, this cheap farm product containing abundant nutrients was used as a major nutrient source for D-lactic acid production in this study.

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The objectives of this study were as follows: (i) to produce D-lactic acid by *Lactobacillus delbrueckii* HG 106 utilizing unpolished rice from aging paddy, and obtain the unpolished rice dregs, a byproduct of D-lactic acid production; (ii) to screen out an inexpensive nitrogen source from agricultural waste, and optimize the dosage of medium compositions by response surface methodology (RSM) and (iii) to compare fermentative effect using the different raw materials such as unpolished rice, fresh corn and polished rice.

2. Methods

2.1. Samples of unpolished rice, polished rice and corn

The samples of aging paddy and fresh paddy were harvested from Hubei Province of China in August 2004 and July 2007, respectively. The unpolished rice was manufactured from the aging paddy by removing the paddy hull. The polished rice was manufactured from the fresh paddy by removing the paddy hull and bran layer. The sample of fresh corn was harvested from Hebei Province of China in October 2007.

2.2. Microorganism, culture media and culture conditions

Lactobacillus delbrueckii HG 106, a highly optical purity D-lactic acid producing strain (content of D-form lactic acid is more than 97.5%) used in this study, was provided by Guangshui Chemical Company of Hubei Province of China. MRS medium was used as the culture medium of seed activation. The media used for screening nitrogen sources contained 2 g/l of yeast extract and 20 g/l of nitrogen sources such as corn steep liquor, soybean meal powder, wheat bran powder, and the treated products. Here, the nitrogen sources were limited at a lower level, namely the nitrogen sources were not superfluous. The media used for studying the effect of yeast extract on D-lactic acid fermentation contained 20 g/l of wheat bran powder, 0, 2 and 4 g/l of yeast extract, respectively. Other fermentation media's compositions were shown further. The initial reducing sugar concentrations of unpolished rice saccharificate were controlled at 100 g/l in all fermentations. All the media were sterilized at 121 °C for 15 min. Flask experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml of medium at 45 °C, with the shaking speed 150 rpm. A 5 l fermentor (Biostat Er B. Braun) was employed for the ampliative fermentation with an initial culture volume of 3 l. The agitation speed and culture temperature were controlled at 150 rpm and 45 °C, respectively. The culture pH of shaking flask and fermentor were automatically controlled by the addition of 5% (w/v) sterilized $CaCO_3$ before fermentations. Five percent (v/v) activated inoculums incubated in MRS medium were used in all fermentations.

2.3. Preparation of the hydrolysate of wheat bran and soybean meal

The wheat bran and soybean meal were comminuted into powder and sieved through a screen with 0.074 mm aperture. Then, the powder was added into water in a ratio of 3:10 (w/w). After the initial pH was regulated to 1.0 by the addition of 3 M H_2SO_4 and hydrolyzed at 121 °C for 20 min, the slurry was cooled to room temperature and filtered (Gao et al., 2007). The filtrates were readjusted to pH 7.0 as nitrogen sources of D-lactic acid production.

2.4. Preparation of the unpolished rice saccharificate and rice dregs

The unpolished rice was comminuted into powder and sieved through a screen with 0.074 mm aperture. Then, the rice powder was mixed with tap water in a ratio of 1:2 (w/w). $CaCl_2$ was added into the slurry with a final concentration of 0.1 M. After the pH of the rice slurry was adjusted to 6.0, the α-amylase (20,000 U/ml, Wuxi Jieneng Bioengineering Company, China) was added into the rice slurry with a dosage of 12 U/g unpolished rice. The starch liquefaction was carried out at 95 °C for 60 min. When the starch liquefaction was completed, the solution was cooled to below 60 °C. After the pH of this hydrolysate was readjusted to 4.5, the amyloglucosidase (100,000 U/ml, Wuxi Jieneng Bioengineering Company, China) was added into the hydrolysate with a dosage of 120 U/g unpolished rice. The saccharification was carried out at 60 °C for 32 h. When the unpolished rice hydrolysate was entirely transformed into saccharificate, the mixture was heated at 100 °C for 10 min to make the enzymes inactivated. After the mixture was cooled to room temperature, the rice dregs were removed from the mixture by centrifugation at 8000g for 10 min (Yun et al., 2004). Finally, the pH of the unpolished rice saccharificate was adjusted to 7.0 and the reducing sugar concentration was adjusted to 100 g/l. This unpolished rice saccharificate was used as the carbon source for D-lactic acid fermentation.

2.5. Optimization of the dosage of wheat bran powder and yeast extract

Response surface methodology (RSM) and central composite design (CCD) were applied to optimize the dosage of fermentation medium compositions (Chauhan et al., 2006; John et al., 2007). The software Design-Expert 7.0.0 (Stat-Ease Inc., USA) was used for experimental design, data analysis and quadratic model building. For statistical calculation, independent variables were coded as

$$x_i = (X_i - X_0)/\Delta X_i \quad (1)$$

where x_i represents the coded values for X_i (i = 1, 2, 3, 4, etc.), X_i is the experimental value of variable, X_0 is the mid-point of X_i, and ΔX_i is the step change in X_i.

For predicting the optimal point, a second-order polynomial equation was fitted to correlate the relationship between variables and response. The equation is

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_i^2 + \sum_{i}^{k} \sum_{j}^{k} b_{ij} x_i x_j \quad (2)$$

where y is predicted response, x_i and x_j ($i \leqslant j$) are coded variables, b_0, b_i, b_{ii}, b_{ij} are regression coefficients calculated from the experimental data by second-order multiple regression, and k is the number of factors.

The experimental data were statistically analyzed using the Fischer's statistical test for analysis of variance (ANOVA). The fitted polynomial equation was then expressed in the form of three-dimensional surface plots to illustrate the relationship between the responses and the experimental levels of each of the variables utilized in this study.

2.6. Analytical methods

The samples of unpolished rice, saccharificate and dregs were analyzed after pre-treatment (Das et al., 2008). The amount of reducing sugar was determined by the 3,5 dinitro salicylic acid method (Miller, 1959). The amino acids were measured by amino acid analyzer (Beckman-6300) referring to GB/T 5009.124-2003 of China. The amount of vitamins was determined by HPLC system using a C18 column with guard column holder (Kromasil 5 μm 250 × 4.6 mm, Agilent 1200) (Das et al., 2008). The color of fermentation broth was measured by spectrophotometer at an absorbency of 420 nm (OD_{420}). Unit price (RMB/kg) of raw material used in this study was present price in China. Incubation period was defined as the time (h) that the concentration of D-lactic acid reached the maximum during the process of the fermentation.