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Improving lactic acid productivity from wheat straw hydrolysates by membrane integrated repeated batch fermentation under non-sterilized conditions

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HIGHLIGHTS

- LA was produced from wheat straw hydrolysates under non-sterilized conditions.
- Membrane integrated repeated batch fermentation (MIRB) could increase LA productivity.
- With MIRB, the simultaneous fermentation of hexose and pentose sugars was realized.
- LA productivity of 2.35 g/L/h was obtained from wheat straw hydrolysates by MIRB.

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ABSTRACT

Bacillus coagulans IPE22 was used to produce lactic acid (LA) from mixed sugar and wheat straw hydrolysates, respectively. All fermentations were conducted under non-sterilized conditions and sodium hydroxide was used as neutralizing agent to avoid the production of insoluble $CaSO_4$. In order to eliminate the sequential utilization of mixed sugar and feedback inhibition during batch fermentation, membrane integrated repeated batch fermentation (MIRB) was used to improve LA productivity. With MIRB, a high cell density was obtained and the simultaneous fermentation of glucose, xylose and arabinose was successfully realized. The separation of LA from broth by membrane in batch fermentation also decreased feedback inhibition. MIRB was carried out using wheat straw hydrolysates (29.72 g/L glucose, 24.69 g/L xylose and 5.14 g/L arabinose) as carbon source, LA productivity was increased significantly from 1.01 g/L/h (batch 1) to 2.35 g/L/h (batch 6) by the repeated batch fermentation.

1. Introduction

Lactic acid (LA) is an important commodity chemical for various applications in food, pharmaceutical and the cosmetic industry (Datta and Henry, 2006). It can also serve as a precursor for producing poly lactic acid, which is a promising biodegradable, biocompatible and environment-friendly alternative to plastics derived from petrochemicals (Lasprilla et al., 2012). LA can be produced in two ways: chemical synthesis and fermentation. The fermentation process is attractive because of its advantages of using renewable carbohydrates and producing optically pure LA (John et al., 2009). Abundant and renewable lignocellulosic materials are regarded as suitable feedstocks for LA production (Abdel-Rahman et al., 2013a). Lignocellulose is composed of cellulose, hemicellulose and lignin, which can be hydrolyzed to a mixture of hexose and pentose sugars. Therefore, a strain capable of fermenting all the lignocellulose released sugars is essential for the economical production of LA (Kim et al., 2010a).

Production of LA using lignocellulosic hydrolysates by *Bacillus coagulans* has drawn much attention due to its strong ability to ferment both hexose and pentose sugars (Ou et al., 2011; Ye et al., 2013a; Zhou et al., 2013). Moreover, the fermentation could be operated under non-sterilized conditions by virtue of the strain's thermophilic feature (Ouyang et al., 2012b; Ye et al., 2013b; Zhao et al., 2010; Zhou et al., 2013). Non-sterilized fermentation is

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energy-efficient, cost-effective and labor-saving. Despite having these advantages, production of LA by *B. coagulans* still has several challenges remaining to be resolved. As reported by Walton et al. (2010) and Ou et al. (2011), some *B. coagulans* strains could not simultaneously ferment hexose and pentose, and these mixed sugar could only be sequentially metabolized due to carbon catabolite repression (CCR) (Kim et al., 2010a), which would decrease the efficiency of fermentation. In addition, feedback inhibition during the fermentation could limit the LA production (Ou et al., 2011). To solve these problems, method of genetic modification is undoubtedly the primary strategy to be considered, while optimization of fermentation strategy is also an alternative.

It is well known that the repeated batch fermentation is a feasible method for improving LA productivity due to the reduction in fermentation time and the skip of inoculums preparation (Abdel-Rahman et al., 2013b; John et al., 2007). During the repeated batch fermentation, cells could be reused by centrifugation (Abdel-Rahman et al., 2013b; Kim et al., 2010b; Zhao et al., 2010) or cell immobilization (Rosenberg et al., 2005; Shi et al., 2012). Usually, cell reuse by centrifugation could not realize automatically continuous operation. And the immobilized cell bioreactor may be suffered from productivity loss due to the limited mass transfer and the accumulation of dead cells (Shi et al., 2012). Membrane techniques have many advantages in separation of cells from fermentation broth, such as energy-efficient, low damage to cells and easy to scale up for industrial production (Pal et al., 2009; Zhao et al., 2010). The strategy of integrating membrane module with fermentor, termed as membrane integrated repeated batch fermentation (MIRB), could efficiently achieve cell-recycled repeated batch. Thus, a lot of efforts have been made to improve the productivity of LA by MIRB (Kim et al., 2006; Oh et al., 2003; Wee et al., 2006) and most of the studies concerned the MIRB using single sugar as carbon source. To the best of our knowledge, there has been no report regarding LA production from sugar mixture of hexose and pentose using MIRB, especially from lignocellulosic hydrolysates.

In conventional LA production by fermentation, calcium hydroxide or calcium carbonate is normally used to neutralize the produced LA, thus resulting in the production of calcium-LA. The salt of calcium lactate has to be acidified with H_2SO_4, thus resulting in the production of insoluble $CaSO_4$ in LA extraction and purification steps. Gypsum poses serious environmental problem in waste treatment during large-scale LA production. Fermentation using sodium hydroxide as neutralizing agent could avoid the above problem (Qin et al., 2010). Another advantage of fermentation without calcium ion is that the potential of membrane fouling can be significantly decreased, and thus making the MIRB technology more practical in industrial LA production (Pal et al., 2009).

Recently, a thermophilic LA producing bacterium, *B. coagulans* IPE22, was isolated and characterized in our lab, and this strain showed remarkable capability to ferment pentose, hexose and cellobiose and was highly resistant to inhibitors from lignocellulosic hydrolysates (Zhang et al., 2014). The objective of the present work is to evaluate the performance of MIRB in LA production from wheat straw hydrolysates by *B. coagulans* IPE22, aiming to eliminate the sequential utilization of mixed sugar and feedback inhibition for efficient LA production. All the fermentations were operated under non-sterilized conditions and sodium hydroxide was used as neutralizing agent to avoid the production of insoluble $CaSO_4$. Firstly, the effect of initial concentration of sugar mixture on the production of LA was investigated. Then, MIRB was employed to produce LA from both mixed sugar and wheat straw hydrolysates. Fermentative parameters in terms of sugar consumption, cell mass accumulation and viable cells were monitored and the mechanisms were discussed.

2. Methods

2.1. Microorganisms and medium

B. coagulans IPE22 was used in this study. A modified De Man-Rogosa-Sharpe (mMRS) medium was used for seed culture and fermentation. Medium of mMRS contained 10 g/L peptone, 10 g/L beef extract, 5 g/L yeast extract, 2 g/L dipotassium phosphate, 0.2 g/L magnesium sulfate heptahydrate, 0.05 g/L manganese sulfate tetrahydrate and different carbon sources. The type and concentration of carbon sources varied in different fermentation experiments.

2.2. Preparation of wheat straw hydrolysates

Wheat straw was cleaned, chopped, and then pretreated by 2% (w/v) sulfuric acid at a 10% (w/v) loading. The mixture was treated at 121 °C for 90 min, and the obtained slurry was filtrated to achieve liquid and solid fractions. Both liquid and solid fractions were collected and defined as dilute acid hydrolysates and water insoluble solids (WIS), respectively. The WIS (containing 59.96% cellulose) was hydrolyzed by commercial cellulase (Sunson Group Ningxia Enzyme Preparation Plant, China) at a 10% (w/v) solid loading. Enzyme loading was 20 FPU (filter paper activity units)/g cellulose. The enzymatic hydrolysis was carried out in a 10 L jar fermentor (GUJS-10, Zhenjiang Dongfang Bioengineering Equipment Company, China) at 50 °C, pH 5.0 and 200 rpm. Enzymatic hydrolysates were obtained by filtration with filter paper (No. 43, Whatman, UK). Finally, dilute acid hydrolysates and enzymatic hydrolysates were mixed and concentrated by vacuum evaporation at 45 °C. The wheat straw hydrolysates after concentration normally contained 59.55 g/L mixed sugar (29.72 g/L glucose, 24.69 g/L xylose and 5.14 g/L arabinose).

2.3. Batch fermentations

To prepare seed culture, the strain of *B. coagulans* IPE22 was grown on agar mMRS plate for 2 days at 45 °C. The cells were transferred to liquid mMRS media containing 10 g/L glucose and cultured for 6 h at 50 °C. This culture was used to provide 5% (v/v) inocula for fermentation. The optimal fermentation condition for *B. coagulans* IPE22 to produce LA was 52 °C and pH 6.0, as described by Zhang et al. (2014). So, batch fermentations were carried out in fermentor at 52 °C, pH 6.0 and 100 rpm. Sodium hydroxide solution of 400 g/L was automatically added to maintain the pH value by a peristaltic pump. Samples were taken with specific time intervals to determine cell mass, residual sugars and products.

2.4. Membrane integrated repeated batch fermentation (MIRB)

Repeated batch fermentation was performed in a membrane integrated bioreactor, the schematic diagram of which was shown in Fig. 1. The fermentor was coupled with a membrane module. Fermentation broth was transferred to the membrane module by a diaphragm pump. A polyacrylonitrile (PAN) ultrafiltration membrane with a nominal molecular weight cut-off of 20 kDa was used in the experiments. The effective area of the used ultrafiltration membrane was 0.18 m^2. Fermentation was carried out in the fermentor with a working volume of 8 L, and the culture condition was same with that of batch fermentation (Section 2.3). When the sugars in the culture broth were depleted, the integrated membrane module was started to filter fermentation broth. Eighty percent (v/v) of the culture broth (6.4 L) was removed as the permeate liquid. Then 1.6 L broth with concentrated cells was obtained, and