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Improvement of (*R*,*R*)-2,3-butanediol production from corn stover hydrolysate by cell recycling continuous fermentation

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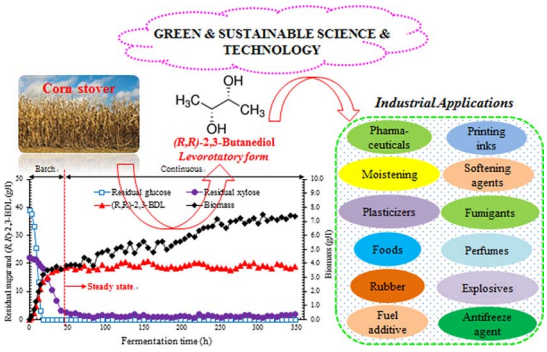
GRAPHICAL ABSTRACT

(*R*,*R*)-2,3-butanediol production from corn stover hydrolysate by cell-recycling continuous fermentation using a nonpathogenic strain of *P. polymyxa*.



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ABSTRACT

To achieve industrial (*R*,*R*)-2,3-butanediol ((*R*,*R*)-2,3-BDL) production from various lignocellulosic biomass, it is essential to develop an efficient fermentation process to overcome the challenge of carbon catabolite repression (CCR). The present study comprehensively investigated the effects of different glucose/xylose mixtures and fermentation strategies on (*R*,*R*)-2,3-BDL production using a nonpathogenic strain of *Paenibacillus polymyxa*. In both batch and fed-batch mode, we observed slow sugar utilization and a lower productivity. By employing cell-recycling continuous fermentation system for (*R*,*R*)-2,3-BDL production from mixed sugar, the volumetric productivity was significantly increased, by an average of 2-fold higher compared to alternative fermentation modes. Moreover, the (*R*,*R*)-2,3-BDL titer, volumetric productivity and yield achieved 18.80 g/l, 1.13 g/l/h and 0.313 g/g when corn stover hydrolysates (CSH)was used as substrate. The results suggested that the reported fermentation system could efficiently eliminate CCR to improve (*R*,*R*)-2,3-BDL productivity. Thus, it had great potential for the industrial-scale (*R*,*R*)-2,3-BDL production from lignocellulosic hydrolysates.

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1. Introduction

2,3-butanediol (2,3-BDL), is an essential platform chemical that has been widely used in the manufacture of pharmaceuticals, foods, fine chemical and cosmetics [1–3]. 2,3-BDL has three stereoisomers in terms of (*R,R*)-, (*S,S*)-, and *meso*-forms, with each stereoisomer having a different industrial application due to their distinct physiochemical properties [4–6]. 2,3-BDL can be produced through chemical synthesis from crude oil. Due to the issues of energy crisis and environmental pollution, microbial fermentation from renewable bioresources, as a promising alternative method for economic production of 2,3-BDL has gained much attention in recent years [7].

A variety of microorganisms have been reported to be capable of converting carbohydrates to 2,3-BDL, including genera *Enterobacter*, *Klebsiella*, *Serratia*, *Bacillus* and *Paenibacillus* [2,8]. In addition to bacteria, *Saccharomyces cerevisiae* has been engineered to produce 2,3-BDL [7,9]. However, the majority of BDL producers that are commonly used in 2,3-BDL fermentation are pathogenic microorganisms (risk group 2 according to German legislation), making their application in industrial-scale fermentation infeasible [1,10]. Therefore, screening for new risk group 1 microorganism with a high yield of 2,3-BDL production is urgent. Among the potential candidates reported, *Paenibacillus polymyxa* is considered to have great potential for industrial 2,3-BDL fermentation due to its nonpathogenic property [4,11–13]. Furthermore, unlike most of the 2,3-BDL producing microbes who normally generate a mixture of two stereoisomers, *P. polymyxa* is one of the few microorganisms that can produce only the *levo*-isomer of 2,3-BDL (*R,R*-BDL) with an optical purity up to 98% [13]. Pure (*R,R*)-2,3-BDL has applicability in chiral chemicals synthesis and is mainly used in the rubber industry, plasticizer and also as anti-freeze agent for its characteristics of low freezing point [14]. Besides, *P. polymyxa* can ferment both C5 and C6 sugars, which is desirable for the economic production of (*R,R*)-2,3-BDL when using lignocellulosic biomass as substrate [11,15,16].

Lignocellulosic biomass is widely regarded as an attractive source for the fermentative production of bio-chemicals, such as bioethanol, lactic acid and biogas due to its inexpensive, abundant and renewable [17,18]. The hydrolysis of lignocellulose yields mixed sugars containing mainly hexoses and pentoses with different ratios, depending both on the type of lignocellulosic biomass and the pretreatment and saccharification method used [19]. At present, the majority of studies aimed at understanding 2,3-BDL fermentation from lignocellulosic biomass focus only on utilization of the hexose fraction, which results in the substrates accounting for greater than half of the total production cost, and accordingly hinder the 2,3-BDL production [20]. To achieve cost-effective production, conversion of both the hexose and pentose sugars into 2,3-BDL is important. One challenge associated with the fermentation of the mixed sugar to bio-chemicals is the carbon catabolite repression (CCR) or glucose effect i.e., sugars were sequentially utilized by microorganism, the xylose consumption commences after the glucose depletion, which commonly leads to the incomplete fermentation with low 2,3-BDL yield and productivity [19,21]. To overcome the CCR effect, considerable efforts have focused on the strain improvement using metabolic engineering strategy. In addition, optimization of the fermentation strategy is recognized as a simple and efficient means to eliminate CCR and improve 2,3-BDL titer and productivity. However, public information regarding (*R,R*)-2,3-BDL production based on lignocellulosic feedstocks remains uncommon. Hence, there is an urgent need to devise an efficient process for (*R,R*)-2,3-BDL fermentation from lignocellulosic hydrolysates.

In the current study, we evaluated the feasibility of (*R,R*)-2,3-BDL production from corn stover hydrolysate using a nonpathogenic strain of *P. polymyxa* ATCC 12321. First, the sugar utilization and (*R,R*)-2,3-BDL production profile of *P. polymyxa* in mixture with different glucose/xylose ratio was systematically and comprehensively investigated in batch, fed-batch and continuous fermentation mode. To eliminate the CCR effect and further improve volumetric productivity, a cell recycling continuous fermentation system was proposed for (*R,R*)-2,3-BDL production. In addition, the optimum dilution rate at each different mixed sugar ratio was examined.

2. Materials and methods

2.1. Microorganism and medium

Paenibacillus polymyxa (formerly *Bacillus polymyxa*) ATCC 12321 was employed throughout this study. The stock culture was maintained at −80 °C in protective medium containing 60% glycerol until further use. The seed culture medium contained the following ingredients (g/l): 20.0 glucose, 3.0 yeast extract, 3.0 malt extract, 5.0 peptone, natural pH. The model fermentation medium contained (g/l): 60.0 reducing sugar, 13.0 yeast extract, 5.8 $(NH_4)_2SO_4$, 1.75 KH_2PO_4, 9.2 K_2HPO_4, 2.9 $(NH_4)_2HPO_4$, 0.2 $MgSO_4{\cdot}7H_2O$, 0.009 $CaCl_2{\cdot}2H_2O$, 0.05 $FeSO_4{\cdot}7H_2O$, 0.013 $MnSO_4{\cdot}5H_2O$, 0.001 $ZnSO_4{\cdot}7H_2O$, 0.05 Na_2EDTA [22].

2.2. Preparation of corn stover hydrolysate (CSH)

The corn stover used in the experiments was harvested from an agricultural area near Zhuanghe (Dalian, China). Corn stover hydrolysate (CSH) was prepared according to the method described in our previous study [23]. Following evaporation, the composition of the CSH was (g/l): glucose, 39.13; xylose, 20.05 and arabinose, 2.46.

2.3. Fermentation

2.3.1. Batch fermentation

For pre-seed inoculum, 1 ml glycerol stock was inoculated into 50 ml of the seed culture medium and incubated at 37 °C for 24 h with agitation at 250 rpm. Next, 1 ml refreshed cells was transferred into Erlenmeyer flask containing 100 ml of seed culture medium and then incubated at 37 °C for 24 h. Afterwards, the seed inoculum was transferred into bioreactors (10%, v/v).

Batch fermentations were conducted to examine the sole and mixed sugar utilization profile of *P. polymyxa* ATCC 12321. Fermentation was conducted in a BIOSTAT® Bplus 2-l bioreactor (Sartorius, Germany) with a starting working volume of 1 l. In the experiment of sole sugar, an initial glucose and xylose concentration of 60 g/l was used. In the case of mixed sugar, different ratios of the glucose/xylose mixture in model medium were prepared as follows (g/l): G50X10, G40X20, G20X40 and G10X50. All the fermentation runs were performed at 37 °C, 500 rpm, with an airflow of 0.2 l/min. During the cultivation, the pH was automatically controlled at 6.5 using 5 N NaOH and H_3PO_4. Besides, Antifoam SI (0.01% v/v, Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used to avoid foaming during the experiment.

2.3.2. Fed-batch fermentation

The fed-batch fermentation was performed using different ratios of glucose/xylose mixtures (G50X10, G40X20, G20X40 and G20X40) under the same conditions as the batch mode. When the residual sugar concentration dropped to 20 g/l, a glucose and xylose mixture (600 g/l) was intermittently fed into the fermenter in order to maintain the total sugar concentration at a concentration between 20 g/l and 60 g/l. Antifoam was controlled by periodically adding Wako Antifoam SI.

2.3.3. Continuous fermentation

The continuous fermentation was performed following the method we previously reported with minor modifications [17]. Briefly, a hollow-fibre microfiltration module (MICROZA PSP 103, Asahi Kasei, Tokyo, Japan) was integrated with a 2-l fermenter for cell recycling. The filtration area, fibre diameter, and pore size of the module was 0.17 m^2, 0.7 mm and 0.1 μm, respectively. The continuous culture was initially operated in batch mode at 37 °C, 500 rpm, and airflow of 0.2 l/