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Integrated lignocellulosic bioprocess for co-production of ethanol and xylitol from sugarcane bagasse

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ABSTRACT

Economics of cellulosic ethanol production could be supported by high-value chemicals co-production, e.g. xylitol, which has high market value in food and pharmaceutical industries. Herein, we present an integrated sugarcane bagasse processing for the co-production of ethanol and xylitol from cellulose and hemicellulose, respectively. Process integration comprised pretreatment followed by separation of hemicellulosic hydrolysate and pretreated cellulose, in combination with enzymatic hydrolysis and fermentation. *Saccharomyces cerevisiae* was used for ethanol production in a simultaneous saccharification and fermentation process of pretreated cellulose, and *Candida tropicalis* was selected for xylitol fermentation of hemicellulosic hydrolysate. Batch process parameters were systematically optimized resulting in 7.5 FPU/g optimal enzyme load, 0.03 g/L and 0.035 g/g optimal inoculum size, 89 h^{-1} optimal k_La and 54 g/g optimal sugar-to-nitrogen ratio. Finally, fed-batch strategy was accomplished yielding maximum concentrations of ethanol by *S. cerevisiae* and xylitol by *C. tropicalis* at 56.1 g/L and 24.0 g/L, respectively. Of the total glucose and xylose available in bagasse, the product yield of 0.44 g/g (ethanol) and 0.50 g/g (xylitol) were reached. Economic analysis revealed that the incorporation of xylitol with ethanol in an integrated biorefinery together with fed-batch strategy for high-titer product could improve profitability by 2.3-folds compared to base case of cellulosic ethanol production. Hence, this integrated biorefinery for co-production could be attractive to current cellulosic ethanol for advancing economic feasibility.

1. Introduction

Recent biorefinery technologies have focused on process integration for the production of different valuable coproducts from lignocellulosic biomass in order to reduce the overall processing cost. Specifically, the profitability of a low-value biofuel such as ethanol (estimated selling price at 0.7 USD/l) from lignocellulose could be supported by integrated production of high-value chemical co-products that could contribute to the overall economy of the process (Morales-Rodriguez et al., 2016; Rueda et al., 2016). Alternatively, xylose available in lignocellulosic hydrolysate can be fermented into xylitol, a five-carbon sugar alcohol used as a sweetening agent. The global market of xylitol in 2015 was estimated at > 700 million USD/year in food and pharmaceutical industries (www.grandviewresearch.com/industry-analysis/xylitol-market). The production of xylitol from xylose in hemicellulose should offer better alternative to the fermentation of xylose to ethanol as xylitol has higher market value than ethanol. Hence, the co-production of xylitol with ethanol in an integrated biorefinery would create economic benefits making the overall lignocellulose-based process more cost effective.

Many xylose-assimilating *Candida* sp. are known to be able to reduce xylose to xylitol with relatively high yield (Rafiqul and Sakinah, 2013). Among xylitol-producing yeasts, *C. tropicalis* is commonly used due to its high inhibitor-tolerance with capability to grow in number of hemicellulosic hydrolysate and efficient xylitol production with an NADH-NADPH dual cofactor dependent xylose reductase for more flexible redox regeneration (Ping et al., 2013; Jia et al., 2016). For ethanol production, *Saccharomyces cerevisiae* is the workhorse yeast used for fermenting glucose from lignocellulosic biomass to ethanol. Nevertheless, there are few reports about an integrated process for co-producing ethanol and xylitol from lignocellulose. In addition, the product titers achieved in the co-production process remained relatively low at < 30 g/L for ethanol and < 20 g/L for xylitol (Mattam et al., 2016; Dasgupta et al., 2017).

Previous works have used single or mixed cultures to co-produce ethanol and xylitol. Cheng et al. (2014) utilized the xylose-fermenting *C. tropicalis* for ethanol and xylitol co-production from corn cob. However, low product titers were obtained due to the repression of

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xylose utilization and xylitol production by glucose. Other attempts to overcome glucose repressioneffects were carried out using mixed cultures of *S. cerevisiae* and *C. tropicalis* in which the fast utilization of glucose to ethanol by *S. cerevisiae* should maintain low concentration of glucose hence permit xylose to be converted to xylitol by *C. tropicalis* (Cunha-Pereira et al., 2011; Castanon-Rodríguez et al., 2015). However, *C. tropicalis* has low ethanol tolerance in which its viability was decreased to 50% when the concentration of ethanol accumulated above 30 g/L (Castanon-Rodríguez et al., 2015), preventing mixed culture from achieving high titers of both ethanol and xylitol. In addition, the rapid growth and fermentation of *S. cerevisiae* may also cause nutritional deficiencies that may in turn decrease the fermentative activity of *C. tropicalis*. Furthermore, optimal culture conditions for ethanol and xylitol fermentation are often different. Aerated condition is typically favorable for xylitol while limited aeration or anaerobic one is preferential for ethanol (Venkateswar Rao et al., 2016). Without strict control of dissolved oxygen in the mixed culture, oxygen deficiency often occurred due to the rapid glucose catabolism by *S. cerevisiae* thereby limiting a conversion of xylose to xylitol by *C. tropicalis*. Hence, for better process strategy and control, an efficient co-production of ethanol and xylitol was accomplished in separate cultures of *S. cerevisiae* and *C. tropicalis* in this study.

Several process parameters are known to influent microbial growth and fermentation efficiency. In simultaneous saccharification and fermentation (SSF) process, enzyme load and yeast cell inoculum could affect fermentation performance (Koppram and Olsson, 2014). The factors affecting xylitol fermentation included aeration rate, inoculum concentration and culture media (carbon, nitrogen, etc.) (Vallejos et al., 2016). Proper control of these parameters is of great importance for achieving high yield, titer and productivity. Since several process parameters can affect the co-production performance, it is important to systematically optimize these parameters in order to maximizing both products. Experimental statistical design is a commonly used tool for systematic multi-parameter process optimization (Khajeeram et al., 2017).

As a result, the present work aimed to demonstrate high-titer ethanol and xylitol co-production in an integrated ligno-biorefinery using *S. cerevisiae* and *C. tropicalis*. The co-production was accomplished via a separate bioconversion of cellulose and hemicellulose of sugarcane bagasse to ethanol and xylitol, respectively. The cultures of *S. cerevisiae* for ethanol production in pretreated cellulose SSF and *C. tropicalis* for xylitol production in hemicellulosic hydrolysate fermentation were systematically optimized by Design of Experiment (DoE). Together with optimal process conditions, a robust fed-batch strategy was implemented to further improve product titers. Economic impact of the integrated process was also evaluated. This ethanol/xylitol co-production process could offer a cost-effective route for better economic viability and sustainability of lignocellulosic biofuel industry.

2. Materials and methods

2.1. Strains and media

Saccharomyces cerevisiae (S288C derivative) and *Candida tropicalis* (BCC, Thailand Bioresource Research Center) used for ethanol and xylitol production, respectively, were routinely maintained on YPGX agar (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 20 g/L xylose, 25 g/L agar). Seed cultures were prepared in YPGX media at 30 °C, 200 rpm for overnight prior to use. All chemicals were purchased from Sigma-Aldrich Chemical Co, USA.

2.2. Feedstock preparation and processing

Sugarcane bagasse with its composition of approximately 40% cellulose and 25% hemicellulose was collected, dried at 80 °C for 24 h, physically processed using a SM2000 cutting mill (Retsch, Germany) and sieved through a 0.25–1 mm mesh. Pretreatment of milled sugarcane bagasse was performed at 20% (w/v) solid load, 2.5% (w/v) sulfuric acid at 121 °C for 30 min to hydrolyze hemicellulose (Canilha et al., 2011). This pretreatment condition was chosen as it permits an efficient separation of cellulose (in solid stream) and hemicellulose (in liquid stream). Biomass slurry after pretreatment was washed and separated by filtration into pretreated solid fraction and liquid hemicellulosic hydrolysate fraction. Pretreated solid was dried at 80 °C for 24 h and then used in SSF for ethanol production, while liquid hydrolysate was partially concentrated threefold by evaporation at 70 °C and used for xylitol fermentation. Composition of hydrolysate before and after concentration is provided in Supplementary S1. Concentrated hemicellulosic hydrolysate was eventually used as feed media during fed-batch operation. pH of the pretreated solid and liquid hydrolysate was adjusted to 6–6.5 using KOH and $Ca(OH)_2$ prior to use in ethanol SSF and xylitol fermentation, respectively.

2.3. Batch and fed-batch xylitol fermentation

Batch experiments for optimizing xylitol production were carried out in 500 mL Erlenmeyer flask at 30 °C with no pH control. Media used in batch and fed-batch cultures was hemicellulosic hydrolysate supplemented with 1.7 g/L yeast nitrogen base without amino acids and ammonium sulfate and various concentration of $(NH_4)_2SO_4$. The ratio of sugar-to-nitrogen in batch media was varied between 1–100 g-sugar/g-nitrogen by adjusting the amount of $(NH_4)_2SO_4$ supplemented to hemicellulosic hydrolysate. The composition of optimal batch and fed-batch hydrolysate media is provided in Supplementary S1. Oxygen transfer coefficient (k_La, h^{-1}) in the 100 mL batch culture was varied through changing agitation (100–410 rpm). The value of k_La for each agitation rate was determined based on an empirical correlation previously reported for shake flask operation ($k_La = 16.61 \times n^{1.09} \times \left(\frac{A}{V}\right)^{0.87}$, Raynoso-Cereceda et al., 2016). Variables n and A/V represent orbital shaking speed and superficial area per filling volume, respectively. Inoculum size was optimized in the range of 0.01–0.05 g/L. Optimal batch fermentation conditions to maximize xylitol titer were systematically determined by experimental design optimization tool and were experimentally validated (see Result Section 3). To determine fermentation kinetics, samples were taken every 12 h for residual sugars, yeast cell concentration and products analyses. Batch fermentation lasted 48 h while fed-batch started after the 36 h batch process and lasted for 96 h. In fed-batch, equal pulse feeding of fed-batch media each time at 25% of total batch volume was conducted every 12 h. Agitation was increased along with increasing culture volume during fed-batch in order to maintain constant oxygen transfer.

2.4. Simultaneous saccharification and fermentation for ethanol production

Batch and fed-batch SSF were carried out in 500 mL Erlenmeyer flask at 35 °C, 300 rpm shaking rate and no pH control. The batch SSF optimization was operated in 48-h containing 10% (w/w) pretreated solid bagasse, 5 g/L $(NH_4)_2SO_4$, 1.7 g/L yeast nitrogen base, various initial yeast cell concentrations and cellulase loads. Commercial cellulase Cellic C-TEC2 (Novozymes, Denmark) was used. Enzyme and yeast cell dosages within the range of 2.5–12.5 filter paper units (FPU)/g-bagasse and 0.005–0.06 g-cell/g-bagasse, respectively, were simultaneously optimized using experimental design tool to maximize ethanol titer (see Result Section 3). For higher ethanol titer, fed-batch SSF under optimal enzyme and yeast cell inoculum was initiated after 24-h batch with an addition of pretreated solid based on the pulse-feed profile described in Unrean et al. (2016). Samples were collected every 12 h for residual sugars, yeast cells and products analyses to determine SSF kinetics. Fig. 1 depicts integrated process diagram describing sugarcane bagasse processing steps for co-production of ethanol and xylitol.