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Optimized feeding schemes of simultaneous saccharification and fermentation process for high lactic acid titer from sugarcane bagasse

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ABSTRACT

A simultaneous saccharification and fermentation (SSF) process of pretreated sugarcane bagasse by *Lactobacillus pentosus* was designed and optimized for lactic acid (LA) production. Different feeding schemes in SSF process were studied for their effects on LA production efficiency. In addition to feeding optimization, adaptation of seed cultivation in hydrolysates was necessary to achieve high LA yield and productivity. LA yield and productivity was enhanced by 1.43 folds with cell feeding in comparison to initial cell addition in SSF. Likewise, pulse enzyme feeding was preferred condition for high LA production efficiency. A combinatorial feeding of pretreated bagasse, enzyme and cell enables a maximum LA titer of 72.75 g/L by *L. pentosus* with 0.61 g/g LA yield and 1.01 g/L-hr LA productivity. The SSF process with optimizing feeding scheme of pretreated biomass, enzyme and cell could provide lignocellulose-based process platform for achieving high LA titer meeting techno-economic requirements.

1. Introduction

Lactic acid (LA), a versatile block-building chemical, is commonly used as the monomer of biodegradable and biocompatible plastic polylactic acid (PLA) which provides a sustainable alternative to petroleum-derived plastics (Abdel-Rahman et al., 2013). Currently, corn and cassava were commercially used as substrate for LA fermentation in which the cost of feedstock accounts for more than 50% of the overall production cost (Yadav et al., 2011). It is, therefore, essential to select for a low-cost feedstock alternatives for corn and cassava. Lignocellulose such as agricultural residues is abundant, renewable, low-cost, and offers a favorable alternative as a feedstock for biofuel and biochemical production (Kawaguchi et al., 2016). Hence, the development of efficient high-titer LA process from low-cost lignocellulosic feedstock could make the PLA production cost-effective as the biomass feedstock is cheaper than either corn or cassava and has no competing food value.

Lignocellulosic material, for example sugarcane bagasse, contains 35–40% cellulose and 25–30% hemicellulose, which can be hydrolyzed into hexoses (D-glucose, D-mannose, D-galactose) and pentoses (D-xylose, D-arabinose) for fermentation (Bezerra and Ragauskas, 2016). From economic perspective, a strain capable of fermenting sugar mixtures of hexoses and pentoses derived from lignocellulosic material is needed. *Lactobacillus pentosus* is LAB that is capable of fermenting both hexoses and pentoses making it a promising candidate for lignocellulose-based lactic acid fermentation (Yoshida et al., 2011). As a result, we examined in this study the application of *L. pentosus* for sugarcane bagasse-to-LA conversion in order to achieve process cost efficiency and sustainability. The conversion of lignocellulosic sugars to LA often results in low yield and productivity due to the presence of inhibitory compounds such as weak acids and aldehydes, generated during the pretreatment step (Van der Pol et al., 2016a,b; Zhang et al., 2016). Weak acids e.g. acetic acid is known to inhibit cell proliferation by intracellular anion accumulation causing ATP depletion and inducing oxidative stress. Aldehydes e.g. furfural or HMF inactivates cell replication by disturbing cellular redox balance and inhibiting several enzymes in central metabolism (Caspeta et al., 2015). In order to improve the cell's fermentation capacity in sugarcane bagasse hydrolysates, cell adaptation method during seed cultivation step has been investigated.

Besides overcoming inhibitor repression, an efficient process for high-titer LA from lignocellulosic biomass is essential. Simultaneous saccharification and fermentation (SSF) for conversion lignocellulosic feedstock to bioproducts offers several advantages including minimized end-product inhibition of hydrolysis enzyme caused by glucose and cellobiose accumulation, and reduced capital cost by reducing the number of reactors needed in the process. These led to an improved

Abbreviations: SSF, Simultaneous saccharification and fermentation; WIS, Water insoluble solid; LA, Lactic acid; S_O, Initial addition of pretreated bagasse; S_F, Pulse feeding of pretreated bagasse; E_O, Initial addition of enzyme; E_F, Pulse feeding of enzyme; C_O, Initial addition of cell; C_F, Pulse feeding of cell

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product yield, productivity and reduced production cost in SSF process compared to other process configurations (Choudhary et al., 2016; Dong et al., 2016; Kawaguchi et al., 2015). LA production via SSF is expected to enhance yield and productivity as the sugars released following enzymatic hydrolysis is rapidly converted to LA by *L. pentosus* during SSF. High-solid SSF should be favorable for LA production since high solid loading can yield high-titer LA thereby reducing cost during downstream processing.

Operation at high water insoluble solid (WIS) content can be achieved in fed-batch SSF as the substrate could then be continuously fed and degraded in the process, reducing the viscosity of the fermentation medium and mixing or mass transfer limitation problems compared with batch process. The concentration of accumulated inhibitory compounds would also be lower in fed-batch than in batch process, as all the substrate is not added at once, thus reducing the inhibitory effects of these compounds on the cell. Previous studies have shown that high solid substrate operation could be possible in SSF configuration which would then lead to high titer of ethanol at the end process (Li et al., 2014; Nguyen et al., 2017). We have also previously demonstrated an optimized fed-batch SSF at high-solid loading for the production of lignocellulosic ethanol meeting techno-economic requirements (Unrean et al., 2016). Hence, to further demonstrate lignocellulosic SSF bioconversion for high-titer bioproducts which is key for sustainable bioeconomy, we implemented in this study a high-solid, fed-batch SSF for the production of LA from sugarcane bagasse by *L. pentosus*. Specifically, feeding strategy options for pretreated bagasse, enzyme and cell in SSF were explored for their influences on lactic acid fermentation efficiency. The optimal feeding schemes to maximize LA production efficiency in SSF process was determined. This high-solid SSF yielding high titer, yield and productivity of LA offers a process strategy that could move lignocellulose-based LA production process towards economic feasibility for industrialization.

2. Materials and methods

2.1. Raw material

Sugarcane bagasse was collected, physically processed using a SM2000 cutting mill (Retsch, Haan, Germany) and sieved through a 0.25–1 mm mesh. Pretreated bagasse used throughout SSF experiments was prepared via stream pretreatment with 0.5% (w/v) H_2SO_4 at 121 °C for 30 min. Biomass slurry after pretreatment was washed and separated by filtration into a pretreated solid fraction and a liquid hydrolysate fraction. The pretreated solid was used for SSF experiment, while the liquid hydrolysates was used for seed cultivation. Sugar composition in pretreated bagasse was 0.37 g-glucose and 0.23 g-xylose per gram bagasse, while the hydrolysates composed of 5–10 g/L glucose, 10–15 g/L xylose, 0.5–1 g/L acetic acid and 0.1–0.2 g/l furfural. Water insoluble solid (WIS) content in the pretreated solid was determined by washing the pretreated bagasse with excess deionized water before drying at 80 °C followed by weighing.

2.2. Strain and media

Lactobacillus pentosus (TBRC, Thailand Bioresource Research Center) was the organism used throughout all experiments. The strain was routinely maintained on MRS agar (Sigma-Aldrich, USA). Seed cultivation use as inoculum in SSF was produced in aerobic batch culture containing 5% molasses media (for non-adapted cell) or 5% molasses media mixed with bagasse hydrolysates (for adapted cell). The culture media contained 5% (v/v) molasses, 0.75 g/L $(NH_4)_2SO_4$, 0.35 g/L KH_2PO_4, 0.07 g/L $MgSO_4{\cdot}7H_2O$, 5 g/L yeast extract, 0.1 M potassium phosphate buffer pH 6. For adapted cell, water used for preparing culture media was replaced with bagasse hydrolysates. Cultivation conditions were 1% (v/v) inoculum, initial pH 6-6.5, 37 °C and 200 rpm for 48 h. Seed culture was harvested by centrifugation, concentrated in the same culture media before being used in SSF.

Table 1
Feed profiles of pretreated bagasse, enzyme and cell in shake-flask SSF, carried out at 22% accumulated WIS, 12.5 FPU/g-WIS enzyme dosage and 0.02 g-cell/g-WIS cell loading.

Time (hrs)	Initially added enzyme/cell (E_OC_O)			Initially added enzyme/feed cell (E_OC_F)		
	Pretreated bagasse (g)	Enzyme load (FPU)	Cells load (g)	Pretreated bagasse (g)	Enzymes load (FPU)	Cells load (g)
0	4.43	253	0.42	4.43	253	0.04
24	30.80			30.80		0.18
48	36.28			36.28		0.20
Time (hrs)	Initially added cell/feed enzyme (E_FC_O)			Feed enzyme/feed cell (E_FC_F)		
	Pretreated bagasse (g)	Enzymes load (FPU)	Cells load (g)	Pretreated bagasse (g)	Enzymes load (FPU)	Cells load (g)
0	4.43	16	0.42	4.43	16	0.04
24	30.80	109		30.80	109	0.18
48	36.28	128		36.28	128	0.20

2.3. Simultaneous saccharification and fermentation in shake flask

Batch simultaneous saccharification and fermentation (SSF) was carried out in a 250 mL Erlenmeyer flask containing pretreated bagasse at the 10% WIS content, 0.75 g/L $(NH_4)_2SO_4$, 0.35 g/L KH_2PO_4, 0.07 g/L $MgSO_4{\cdot}7H_2O$, 5 g/L yeast extract, 0.1 M potassium phosphate buffer pH 6, enzyme and microbial cell. SSF was operated at 12.5 FPU/g-WIS enzyme dosage and 0.02 g-cell/g-WIS cell load. Commercial enzyme Cellic C-TEC2 (Novozyme, DK) capable of hydrolyzing both cellulose and hemicellulose was used. The culture was conducted at 37 °C, agitation rate at 200 rpm, initial pH 6-6.5 adjusted using 4 M NaOH with no pH control during the cultivation. Fed-batch SSF was carried out the same as batch for 24 h followed by pulse feeding of pretreated solid, enzyme and cell every 24 h as described in Results and discussion section. The pulse feeding of pretreated solid was designed previously by Unrean et al. (2016). Final WIS content accumulation during fed-batch SSF was 22%. Feeding profiles implemented during fed-batch SSF was summarized in Table 1. Owing to the addition of solid, enzyme and cell, the volume changed along the fed-batch process. To maintain the same volume under different feeding schemes, water equal to the amount of enzyme or cell solution added was used for the experiment with no cell and/or enzyme feeding. Samples were taken every 12 h for analysis of residual sugars, fermentative products and fed-batch SSF lasted 72 h. All experiments were performed in duplicate. SSF process with optimal feeding schemes was later scale-up in bioreactor for higher LA titer.

2.4. Simultaneous saccharification and fermentation in bioreactor

Fed-batch simultaneous saccharification and fermentation (SSF) in bioreactor was started as 1L batch at an initial 7% WIS for 24 h in 2.5L Labfors 5 BioEtOH (Infors HT, Switzerland). During fed-batch, pretreated bagasse, enzyme and cell were added pulse-wise every 24 h to reach a final solid loading of 30% WIS content. Batch and fed-batch SSF in bioreactor was carried out at 12.5 FPU/g-WIS enzyme dosage and 0.02 g-cell/g-WIS cell load and was conducted at 37 °C, agitation rate at 150 rpm, pH 6–6.5 controlled using 4 M NaOH for 96 h. Samples were collected every 12 h, and analyzed for sugars and fermentative products.