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Periodic peristalsis increasing acetone–butanol–ethanol productivity during simultaneous saccharification and fermentation of steam-exploded corn straw

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The acetone–butanol–ethanol (ABE) fermentation of lignocellulose at high solids content has recently attracted extensive attention. However, the productivity of high solids ABE fermentation of lignocellulose is typically low in traditional processes due to the lack of efficient intensifying methods. In the present study, periodic peristalsis, a novel intensifying method, was applied to improve ABE production by the simultaneous saccharification and fermentation (SSF) of steam-exploded corn straw using *Clostridium acetobutylicum* ATCC824. The ABE concentration and the ABE productivity of SSF at a solids content of 17.5% (w/w) with periodic peristalsis were 17.1 g/L and 0.20 g/(L h), respectively, which were higher than those obtained under static conditions (15.2 g/L and 0.14 g/(L h)). The initial sugar conversion rate over the first 12 h with periodic peristalsis was 4.67 g/(L h) at 10 FPU/g cellulase dosage and 15% (w/w) solids content, an increase of 49.7% compared with the static conditions. With periodic peristalsis, the period of batch fermentation was shortened from 108 h to 84 h. The optimal operating regime was a low frequency (6 h^{-1}) of periodic peristalsis in the acid-production phase (0–48 h) of SSF. Therefore, periodic peristalsis should be an effective intensifying method to increase the productivity of ABE fermentation at high solids content.

[**Key words:** Acetone–butanol–ethanol fermentation; Periodic peristalsis; Lignocellulose bioconversion; Simultaneous saccharification and fermentation; High solids content; Steam explosion; Corn straw]

Biobutanol produced from lignocellulose is a promising alternative to fossil-based resources (1,2). Butanol has excellent fuel characteristics, including a higher caloric value and a lower volatility, compared with ethanol. Butanol can be used in internal combustion engines and transported in existing pipe lines. Butanol is also less corrosive than ethanol (3,4).

Butanol can be produced in the acetone–butanol–ethanol (ABE) fermentation, with these products being produced in a ratio of 3:6:1. Potentially, lignocellulose could be used as a sustainable source of mixed sugars for ABE fermentation (5,6). In fact, simultaneous saccharification and fermentation (SSF) of lignocellulose could be used to combine hydrolysis and fermentation into a single operation, which is performed in a single reactor. SSF is more likely to be commercially viable than separate hydrolysis and fermentation, due to its higher yield and lower investment in equipment (7). However, unlike ethanol production from lignocellulose by the simultaneous saccharification and fermentation (SSF) process, which is a mature technology, SSF has not been fully adapted for butanol production. This is because butanol producing strains *Clostridium acetobutylicum* and *C. beijerinckii* have low tolerance to butanol and fluid shear stress (8–11).

The acetone–butanol–ethanol (ABE) fermentation is typically operated with gas stripping, which not only relieves inhibition by reducing the butanol concentration but also improves mass transfer within the medium (12,13). However, gas stripping is not particularly effective in SSF processes with high contents of lignocellulose as substrates. These substrates have low densities and complex porous structures. Therefore, when the SSF process is performed at a high solids content (i.e., ≥15%, w/w), and the solid particles and the cellulase solution are difficult to mix and hence the mass transfer efficiency decreased (14). Furthermore, once carbon dioxide is generated in the fermentation process, a complicated gas–liquid–solid multiphase medium is formed. This multiphase medium is hard to liquefy, leading to a high apparent viscosity, poor mass and heat transfer efficiencies, low sugar yields, and thus, low butanol yields (15). High-power mechanical stirring can overcome these problems, but high fluid shear stress will damage microbial cells, leading to a consequent decrease in cell activity and solvent yield (16,17). Nevertheless, conversions at high solids content potentially offer many advantages over those at low or moderate solids content, including increased sugar and butanol concentrations and decreased operating costs, energy costs and water consumption (14). Thus, it is essential to develop a novel intensifying method for acetone–butanol–ethanol (ABE) production by SSF at high solids content.

Recently, researchers have developed several novel reactors for the bioconversion of lignocellulose at high solids content and have mainly focused on enzymatic hydrolysis and the SSF process for ethanol production. For example, Jørgensen et al. (18) used a gravimetric-mixing reactor for the liquefaction of lignocellulose at up to 40% (w/w) solids content, while Zhang et al. (15) used a novel

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helical impeller in a bioreactor for the SSF of steam-exploded corn straw at 35% (w/w) solids content. The helical impeller provided a better performance with respect to the saccharification yield, ethanol titer, and energy cost compared with a Rushton impeller. However, these strategies have not been applied to the SSF processes for butanol production because of high levels of dissolved oxygen and high-strength fluid shear stress.

Therefore, an efficient SSF process for butanol production at high solids content is necessary to increase the sugar conversion rate and shorten the liquefaction time while minimizing damages to microbial cells. In this study, periodic peristalsis, a novel intensifying method, was used to improve the SSF performance for ABE production from steam-exploded corn straw. The SSF processes by different intensifying methods including periodic peristalsis, stirred tank, and static conditions were compared. The SSF performance using different durations, frequencies of periodic peristalsis and solid contents were then investigated to optimize this new periodic peristalsis strategy.

MATERIALS AND METHODS

Steam explosion of corn straw Corn straw was obtained from the experimental plot of the Chinese Academy of Agricultural Sciences in Beijing, China. The corn straw was manually cut into 5-cm long pieces. The chopped corn straw (10 kg, about 45% water content) was placed in a 4.5-L steam explosion reactor (Weihai Automatic Control Co. Ltd., Weihai, China) and was treated at 1.5 MPa for 5 min with saturated steam (19). The steam-exploded corn straw was washed thrice with three volumes of distilled water, dried naturally and sieved to retain the materials from 14 mesh to 50 mesh.

The main chemical compositions, such as cellulose, hemicelluloses, lignin and ash, of steam-exploded corn straw were determined using a modified sequential gravimetric method (20). The contents of cellulose, hemicellulose, lignin and ash were 53.5%, 13.4%, 30.8%, and 2.1% (w/w) on a dry weight basis, respectively. All chemicals used in this study were purchased from Beijing Chemicals Factory, Beijing, China.

Enzymatic hydrolysis of steam-exploded corn straw All enzymatic hydrolysis experiments were performed in a 500 g reaction system. Briefly, 75 g steam-exploded corn straw was added to the cellulase solution to a final 15% (w/w) solids content. The cellulase solution included Cellic CTec 2 cellulase (Novozymes Biotechnology Co., Ltd., China) with 50 mmol/L Na-citrate buffer (pH 4.8). The protein content of Cellic CTec 2 cellulase was 181.2 mg/mL. The filter paper and β-glucosidase activities were 100 FPU/mL (0.55 FPU/mg) and 1017 IU/mL (5.61 IU/mg), respectively, as determined according to the following methods. The β-glucosidase activity (1 IU corresponding to conversion of 1 μM substrate min^{-1}) was assayed according to Kubicek et al. (21) with some slight modifications. *p*-Nitrophenyl-β-D-glucoside was used as a substrate and was dissolved in 1 mL Na-citrate buffer. One milliliter of properly diluted enzyme solution was added, and the solution was incubated for 10 min at 50°C. The reaction was terminated by adding 2 mL of 1 M Na_2CO_3. Finally, the solution was diluted for measurements. β-Glucosidase cleaves this substrate, forming *p*-nitrophenyl, which has an absorbance maximum at 412 nm. The filter paper activity was assayed based on the method of Ghose et al. (22). The substrate was a 50 mg Whatman No. 1 filter paper strip (1.0 × 6.0 cm, Whatman, Maidstone, UK), which was hydrolyzed in the enzyme solution under a properly diluted Na-citrate buffer. The resulting solution was incubated for 60 min at 50°C. The product, a reducing sugar, was detected by the DNS method using a spectrometer (UV2550, Shimadzu, Japan) at 540 nm absorbance.

Four systems (Cases 1–4) were utilized for enzymatic hydrolysis. In Case 1, the bioreactor was a 1.0-L disposable polyethylene bag that had been previously sterilized at 121°C for 15 min and intensified by periodic peristalsis using a Texture Analyzer TAXT2i (Stable Micro Systems, Surrey, England). The periodic peristalsis system was designed according to the principle of solid digestion in the rumen. Through vertical stretch movements, the metal probe (6.5-cm diameter) of the instrument generated a peristaltic pressure maximum of 0.15 MPa (gauge pressure) with a frequency of 12 h^{-1} for the first 48 h. In case 2, a 1.0-L stirred tank reactor with Rushton impellers was operated at 30 rpm for the first 48 h. In Cases 3 and 4, the enzymatic hydrolyses were conducted under static conditions. The dosages of cellulase in cases 1, 2 and 3 were 10 FPU/g solid (dry matter, DM), whereas the dosage in case 4 was 20 FPU/g DM. The four systems were incubated at 37°C for 96 h. Each case condition was carried out in triplicate.

The initial sugar conversion rate and sugar conversion were calculated as follows,

$$r_{cvs} = \frac{c_{cvs}}{t} \quad (1)$$

$$\alpha_{cvs} = \left(1 - \frac{w_{res}}{w_{all}}\right) \times 100\% \quad (2)$$

where c_{cvs} is the sugar concentration in g/L, r_{cvs} is the initial sugar conversion rate in g/(L h), α_{cvs} is the sugar conversion, w_{all} is the cellulose content of steam-exploded corn straw and w_{res} is the residue cellulose content. r_{cvs} was calculated based on data collected over the first 12 h and α_{cvs} was calculated based on 48 h of hydrolysis.

Preparation of *C. acetobutylicum* ATCC 824 *C. acetobutylicum* ATCC 824 was purchased from the China General Microbiological Culture Collection Center. A culture was grown to an OD_{600} of 0.8–1.0 and then aliquots were stored at −80°C as 20% (v/v) glycerol stocks. After removed from the freezer, the strain was heat-shocked at 70°C for 2 min, then inoculated into a glass tube (3-cm diameter; 15-cm height) containing 25 mL of 7% (v/v) corn mash as the seed activation culture, which was incubated for 36 h at 37°C (23).

The seed culture was developed in P2 medium with 10% (v/v) seed activation culture (3) and incubated at 37°C for 20–24 h until an OD_{600} of 0.5 was reached, after which the culture was diluted by 15 times.

Simultaneous saccharification and fermentation of steam-exploded corn straw The SSF medium contained steam-exploded corn straw, cellulase and supplementary medium. The total weight of the steam-exploded corn straw and supplementary medium was 500 g. The supplementary medium contained the following components: 3.68 g/L $(NH_4)_2SO_4$, 1.77 g/L KH_2PO_4, 2.94 g/L K_2HPO_4, 2.00 g/L $Ca(OH)_2$, 10 mg/L *p*-amino benzoic acid and 10 mg/L biotin (24). In all experiments, the medium was adjusted to pH 6.5 and then sterilized at 121°C for 15 min. All experiments were carried out in an anaerobic incubator at 37°C (YQX-II, Xinmiao, China), which was purged with 99.9% N_2 to ensure 100% anaerobic conditions during the whole process.

Experiment 1: SSFs were conducted using different intensifying methods. The SSF process with periodic peristalsis involved 12 h^{-1} of 0.15 MPa vertical pressure for 0–48 h. SSF with the stirred tank was conducted at 30 rpm for 0–48 h. The static SSF process was the control group. For all systems, the solids content was kept constant at 15% (w/w), and the enzyme dosage was 10.0 FPU/g DM. Finally, 10% (v/v) of the actively growing culture was inoculated into each SSF system.

Experiment 2: Periodic peristalsis was applied for different durations of 0–24 h, 0–48 h, 0–72 h and 0–84 h in different treatments at a peristaltic frequency of 12 h^{-1}. The other conditions were the same as those of Experiment 1.

Experiment 3: The peristaltic frequency was varied at 3, 6, 12and 20 h^{-1} in different treatments. The other conditions were the same as those of Experiment 1.

Experiment 4: SSFs were conducted with different solid contents of 12.5, 15.0, 17.5 and 20.0% with a frequency by periodic peristalsis of 12 h^{-1} for 0–48 h.

Sugar consumption rate, ABE productivity and yield were calculated as follows:

$$r_{csp} = \frac{(w_{all} - w_{res}) \times 1.1 - c_{res}v}{vt_{fp}} \quad (3)$$

$$r_{ABE} = \frac{c_{ABE}}{t_{fp}} \quad (4)$$

$$y_{ABE} = \frac{c_{ABE}v}{(w_{all} - w_{res}) \times 1.1 - c_{res}v} \quad (5)$$

where r_{csp} is the sugar conversion rate in g/(L h), r_{ABE} is the ABE productivity in g/(L h), y_{ABE} is the ABE yield, c_{ABE} is the total ABE concentration in g/L, c_{res} is the residue sugars concentration in g/L, v is the total volume of the fermentation medium in L, and t_{fp} is the fermentation period in h.

Samples were periodically removed for determination of product, residual sugar and cell density. The batch experiments were carried out three times and three parallel samples were harvested each time for analysis.

Measurements of sugar, fermented products and biomass The concentrations of glucose and xylose were determined using a high-performance liquid chromatograph (HPLC, Agilent HPLC 1200, America) equipped with a refractive index detector. Samples were loaded on an Aminex HPX-87H column (6.2 × 250 mm, 5 μm) maintained at 65°C and eluted with 0.5 mM H_2SO_4 solution at a flow rate of 0.6 mL/min. The detector was operated at 35°C (19).

ABE and acids (acetic acid and butyric acid) were measured with a gas chromatograph (7890A, Agilent, USA) equipped with a flame ionization detector and an innowax capillary column (30-m length, 0.25-mm internal diameter, 19095N-113, Agilent Technologies, Beijing, China). The oven temperature was held at 85°C for 4.5 min, and then increased to 170°C at 20°C/min. The final temperature was held for 2.5 min. The injector and detector were maintained at 250°C. N_2 was used as the carrier gas and iso-butanol was used as the internal standard (23).

The microbial density was determined by microscopic counting. The suspension was placed in a Petroff–Hausser counting chamber (model 3900, Hausser Scientific, Horsham, PA, USA). Cells and endospores were observed at 400× magnification using a phase contrast microscope (Nikon Eclipse 80i, AG Heinze Co, Lake Forest, CA, USA) mounted with a digital camera (TK-C9201EC, JVC, Japan) (25). The cell is fusiform and the endospore is oval with strong refraction. To obtain statistically

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