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Acetone–butanol–ethanol production from Kraft paper mill sludge by simultaneous saccharification and fermentation

Wenjian Guan [a], Suan Shi [a], Maobing Tu [b,c,*], Yoon Y. Lee [a]

[a] Department of Chemical Engineering, Auburn University, 212 Ross Hall, Auburn, AL 36849, United States
[b] Department of Biomedical, Chemical and Environmental Engineering, University of Cincinnati, 2901 Woodside Drive, Cincinnati, OH 45221, United States
[c] Forest Products Laboratory and Center for Bioenergy and Bioproducts, Auburn University, 520 Devall Drive, Auburn, AL 36849, United States

HIGHLIGHTS

- Pulp mill sludge a great feedstock for ABE production.
- Calcium carbonate in pulp mill sludge plays a positive role in ABE fermentation.
- No detoxification was required for SSF of pulp mill sludge.

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ABSTRACT

Paper mill sludge (PS), a solid waste from pulp and paper industry, was investigated as a feedstock for acetone–butanol–ethanol (ABE) production by simultaneous saccharification and fermentation (SSF). ABE fermentation of paper sludge by *Clostridium acetobutylicum* required partial removal of ash in PS to enhance its enzymatic digestibility. Enzymatic hydrolysis was found to be a rate-limiting step in the SSF. A total of 16.4–18.0 g/L of ABE solvents were produced in the SSF of de-ashed PS with solid loading of 6.3–7.4% and enzyme loading of 10–15 FPU/g-glucan, and the final solvent yield reached 0.27 g/g sugars. No pretreatment and pH control were needed in ABE fermentation of paper sludge, which makes it an attractive feedstock for butanol production. The results suggested utilization of paper sludge should not only consider the benefits of buffering effect of $CaCO_3$ in fermentation, but also take into account its inhibitory effect on enzymatic hydrolysis.

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1. Introduction

Butanol is an advanced biofuel and a versatile platform chemical usable for synthesis of various industrial chemicals (Durre, 2008; Tashiro et al., 2013). Fermentative production of butanol has failed to compete with the chemical synthesis route from propylene since 1960s (Durre, 2008; Jones and Woods, 1986). Recently, bio-based butanol has been proposed as the next generation biofuel due to its properties superior to ethanol (higher energy density, less corrosiveness, and low vapor pressure) (Anbarasan et al., 2012; Durre, 2008; Tashiro et al., 2013).

The bioprocess for butanol production, however, is challenged by low solvent titer, high cost of feedstock and high energy consumption for solvent recovery. Strong end-product inhibition is known to be the major cause for the low solvent titer and low productivity, which in turn raises the cost of product recovery (Anbarasan et al., 2012; Tashiro et al., 2013). High feedstock cost has been cited as the primary reason for cessation of commercial bioprocess in 1960s (Jones and Woods, 1986). Conventional feedstocks for ABE fermentation are food-based sugars, starch and molasses. Although those substrates have good fermentability, their cost is too high to make it a viable bioprocess for butanol production via fermentation. Lignocellulosic biomass has therefore drawn attention as a feedstock for ABE production (Ezeji and Blaschek, 2008) (Ezeji and Blaschek, 2008; Lu et al., 2012; Qureshi et al., 2010a,b).

Paper mill sludge, a waste material generated from pulp and paper plants, is one of such with a great potential as it has a number of attractive features as a raw material. Being a waste material, it carries zero or, in some cases, negative cost (elimination of disposal cost). Sludge from common pulping process (Kraft pulping) has low lignin content. Therefore it does not require pretreatment prior to enzymatic hydrolysis. Pretreatment along with the detox-

* Corresponding author at: Department of Biomedical, Chemical and Environmental Engineering, University of Cincinnati, 2901 Woodside Drive, Cincinnati, OH 45221, United States. Tel.: +1 513 556 2259; fax: +1 513 556 4162.
E-mail address: tumg@uc.edu (M. Tu).

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ification of the post-pretreatment effluent is one of the major cost factors in bioconversion of lignocellulosic materials, especially in the case of ABE production from lignocellulose (Ezeji et al., 2007; Palmqvist and Hahn-Hagerdal, 2000; Qureshi et al., 2010a; Sun and Liu, 2012). Furthermore, the short fibers found in most Kraft mill sludges are readily hydrolyzed by enzymes into fermentable sugars (Lark et al., 1997; Marques et al., 2008). A major challenge for sludge as a feedstock is coping with high ash content originated from filler materials (clay, TiO_2, and $CaCO_3$), which severely impede the enzymatic hydrolysis (Lynd et al., 2001; Nikolov et al., 2000). Ash removal is therefore necessary for efficient bioconversion.

Simultaneous saccharification and fermentation (SSF) has been suggested to be more efficient bioconversion strategy than separate hydrolysis and fermentation (SHF) in ethanol production (Alkasrawi et al., 2003; Kadar et al., 2004). The advantages of the SSF over SHF include low equipment cost and alleviation of product inhibition of glucose and cellobiose on cellulase enzymes. Reduction of equipment and operation is achieved by performing enzymatic hydrolysis and fermentation concurrently in a single process. More importantly, SSF achieves high product yield and productivity keeping glucose level, consequently end-product inhibition on enzymatic hydrolysis low, since the sugar is simultaneously consumed by the microbes (Alkasrawi et al., 2003; Linde et al., 2008). SSF has been investigated to produce butanol from corn stover by *Clostridium beijerinckii* P260, however overliming detoxification of dilute acid pretreated substrates and hydrolysates was required (Qureshi et al., 2014). Shah et al. (1991) identified the effect pretreatment on converting hardwood substrates to butanol in a SSF process, they revealed that both glucose and xylose could be utilized simultaneously by *C. acetobutylicum* ATCC 824 (Shah et al., 1991). Calcium carbonate ($CaCO_3$) has been found to increase butanol yield significantly in a SSF process by enhancing the buffer capacities and the activities of NAD(P)H-dependent butyraldehyde and butanol dehydrogenases (Li et al., 2015). Since paper mill sludge in this study is a pretreated material from Kraft pulping, which contains mainly glucan, xylan and ash ($CaCO_3$), we hypothesize that both glucan and xylan in paper mill sludge can be directly converted into butanol in a SSF process without pH control.

Paper mill sludge has been evaluated as a feedstock for ethanol and lactic acid production, and results have been positive (Budhavaram and Fan, 2009; Kang et al., 2011; Lark et al., 1997; Marques et al., 2008). It is, however, yet to be studied for butanol production. The objective of this study is to investigate the technical feasibility of bioconversion of paper mill sludge into acetone–butanol–ethanol (ABE) through simultaneous saccharification and fermentation (SSF). The main technical issues of our interest are to assess the effects of enzyme loading and solid loading on the SSF and to see how the results from the sludge compared with those of other biomass feedstocks.

2. Methods

2.1. Feedstock, enzyme and microorganism

Recycled Kraft paper mill sludge, collected from Boise Cascade (Jackson, AL), was used as feedstock for ABE production. Cellulase Cellic® C-Tec 2 (Batch No. VCNI0001) was a kind gift from Novozymes, North America (Franklinton, NC). The protein content and specific activity for C-Tec2 were 255 mg protein/mL and 119 FPU/mL. *C. acetobutylicum* ATCC-824™ (Lot NO. 58727357) was purchased from American Type Culture Collection (ATCC). Avicel PH-101 was purchased from Sigma–Aldrich (St. Louis, Mo.). Switchgrass was provided by Ceres Inc. (Thousand Oaks, CA). Switchgrass was pretreated by soaking in 2% (w/w) sodium hydroxide solution at 60 °C for 24 h. The liquid-to-solid ratio in switchgrass pretreatment was 9:1. After pretreatment, the solids were washed with water until the pH reached 6.0 before subjecting to further analysis. The chemical compositions of paper mill sludge and switchgrass were determined according to the NREL standard procedure (NREL/TP-510-42618). The solid composition of NaOH pretreated switchgrass was analyzed to contain 54.1% of glucan, 23.8% of xylan, 10.4% of lignin and 6.2% of ash.

2.2. Ash removal of paper mill sludge

The ash in paper mill sludge was partially removed as previously described (Kang et al., 2011). Briefly, the sludge was first suspended in DI water at 3% (w/w) consistency and blended with bench-top stirrer (IKA® RW16, Germany) for 30 min. It was then dewatered by filtering through a 100-mesh screen. The dewatered sludge was dried at 45 °C until the moisture content reached below 10% for further processing and sample analysis. Multiple de-ashing cycles was applied to reduce the ash content of sludge to a level (6.1% ash) that can achieve acceptable saccharification rate. PS2 indicates the paper sludge de-ashed by two cycles of washing. PS3, PS4, etc. are defined accordingly.

2.3. Culture maintenance and inoculum preparation

The stock of *C. acetobutylicum* was maintained as spores at −20 °C in Elliker broth (BD Difco™) supplemented with 20% (v/v) of glycerol. The spores were revived by inoculating one loop of the spore suspension in the Reinforced Clostridial HiVeg™ Broth (HiMedia Laboratories, India) and anaerobically incubated for 24 h at 36 °C. The inoculum was prepared by transferring 1 mL of actively growing culture into 50 mL of P2 medium supplemented with 10 g/L of glucose in a 100 mL screw-capped Pyrex bottle and anaerobically incubated for 24 h. The P2 medium formula was as follows (g/L): KH_2PO_4, 0.5; K_2HPO_4, 0.5; $MgSO_4{\cdot}7H_2O$, 0.2; $FeSO_4{\cdot}7H_2O$, 0.01; $MnSO_4{\cdot}H_2O$, 0.01; NaCl, 0.01; ammonium acetate, 2.2; yeast extract, 1.0.

2.4. Enzymatic hydrolysis of paper mill sludge

Enzymatic hydrolysis was performed to estimate the digestibility of de-ashed paper mill sludge. The experiment was carried out in batch mode using 125 mL Erlenmeyer flasks for 120 h under 50 °C and 150 rpm. Sodium citrate (0.05 M, pH 4.8) was used as buffer and 0.01% (w/v) of sodium azide was used as biocide to prevent microbial contamination. To assess the effect of ash content on enzymatic hydrolysis, the de-ashed paper mill sludge (4% glucan) was hydrolyzed with cellulases under an enzyme loading of 10 FPU/g glucan.

2.5. ABE fermentation with mixed glucose and xylose as substrates

ABE fermentation with mixed glucose (44.8 g/L) and xylose (16.1 g/L) as substrates was carried out in 125 mL serum bottle with a working volume of 50 mL. P2 medium was used as inorganic mineral fermentation nutrients. The initial pH of the fermentation broth was adjusted to 6.7 with calcium carbonate (5.0 g/L). The fermentation bottle was flushed with nitrogen gas for 5 min and crimp-sealed with rubber stopper. The bottles were loaded with sugars and fermentation medium, autoclaved at 121 °C for 15 min, inoculated with the actively growing seed-culture at 5% (v/v). Fermentation was carried out at 36 °C, 150 rpm, and under strict anaerobic condition. Aliquots of samples were taken with 12-h interval.