Waste Management xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Waste Management

journal homepage: www.elsevier.com/locate/wasman

Efficient production of ethanol from waste paper and the biochemical methane potential of stillage eluted from ethanol fermentation

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ARTICLE INFO

Article history:
Received 7 July 2015
Revised 3 November 2015
Accepted 30 November 2015
Available online xxxx

Keywords:
Ethanol
Presaccharification
Simultaneous saccharification and fermentation (SSF)
Methane fermentation

ABSTRACT

Waste paper can serve as a feedstock for ethanol production due to being rich in cellulose and not requiring energy-intensive thermophysical pretreatment. In this study, an efficient process was developed to convert waste paper to ethanol. To accelerate enzymatic saccharification, pH of waste paper slurry was adjusted to 4.5–5.0 with H_2SO_4. Presaccharification and simultaneous saccharification and fermentation (PSSF) with enzyme loading of 40 FPU/g waste paper achieved an ethanol yield of 91.8% and productivity of 0.53 g/(L h) with an ethanol concentration of 32 g/L. Fed-batch PSSF was used to decrease enzyme loading to 13 FPU/g waste paper by feeding two separate batches of waste paper slurry. Feeding with 20% w/w waste paper slurry increased ethanol concentration to 41.8 g/L while ethanol yield decreased to 83.8%. To improve the ethanol yield, presaccharification was done prior to feeding and resulted in a higher ethanol concentration of 45.3 g/L, a yield of 90.8%, and productivity of 0.54 g/(L h). Ethanol fermentation recovered 33.2% of the energy in waste paper as ethanol. The biochemical methane potential of the stillage eluted from ethanol fermentation was 270.5 mL/g VTS and 73.0% of the energy in the stillage was recovered as methane. Integrating ethanol fermentation with methane fermentation, recovered a total of 80.4% of the energy in waste paper as ethanol and methane.

1. Introduction

Waste paper is one of the most widely recycled materials. It can theoretically be recycled 6–7 times but is typically recycled 2.4 times on average worldwide (Zhang et al., 2015). Annually, more than 400 million tons of waste paper is generated (Shi et al., 2009). Management of downgraded waste paper, such as in landfills and by incineration, is regarded as environmentally unfriendly because of the generation of greenhouse gases and contaminative leachate (Rahman et al., 2014; Zhang et al., 2015). For the better recycling of waste paper, it would be beneficial to develop various techniques to convert waste paper to additional valuable products such as ethanol (Dubey et al., 2012; Elliston et al., 2013; Sangkharak, 2011; Wang et al., 2011), methane (Baba et al., 2013; Yuan et al., 2012), hydrogen (Pendyala et al., 2013), and carbonaceous materials (Hassanzadeh et al., 2015; Wei et al., 2014).

In 2004, the Chinese government formulated the Middle and Long-Term Development Plan of Renewable Energy in which ethanol from nonfood material was expected to expand to 10 million tons per year by 2020 (Tao et al., 2011). Waste paper, derived from cellulosic biomass, consists of 40–80% cellulose, 5–15% hemicellulose, and negligible lignin (Ioelovich, 2014). More than 90 million tons of waste paper are produced every year in China and can potentially serve as feedstock of ethanol to contribute to the renewable energy goal. Comparing with the other lignocellulosic biomass like agricultural residue, waste paper used as the feedstock for the production of ethanol has the following advantages: (1) energy-intense thermophysical pretreatments are not required to enhance enzymatic saccharification because the pretreatments have been done in papermaking (Elliston et al., 2013); (2) waste paper is abundant, cheap, and renewable (Brummer

Abbreviations: SSF, simultaneous saccharification and fermentation; PSSF, presaccharification and simultaneous saccharification and fermentation; BMP, biochemical methane potential; FPA, filter paper activity; NREL, National Renewable Energy Laboratory; CMCase, carboxymethyl cellulase; CMC-Na, sodium carboxymethyl cellulose; BG, β-glucosidase; ES, enzymatic saccharification; YP, yeast extract and peptone; YPD, yeast extract, peptone, and dextrose; VTS, volatile total solid; TS, total solid; VFA, volatile fatty acid; LHV, lower heating value.

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http://dx.doi.org/10.1016/j.wasman.2015.11.051
0956-053X/

et al., 2014b; Hassanzadeh et al., 2015); and (3) there is already a well-established national supply chain (Elliston et al., 2014; Pivnenko et al., 2015). Therefore, there has been increasing interest in research on ethanol production from waste paper. However, in most of the published literature, product (ethanol) concentrations ranged from 3.7 g/L to 38.8 g/L (Elliston et al., 2013; Prasetyo and Park, 2013). Huang and Percival Zhang (2011) reported that the energy for ethanol separation greatly increased when the ethanol concentration was below 4% w/w, which was a critical value for ethanol separation. Although Elliston et al. (2013) developed a process to increase the ethanol concentration to as high as 92.8 g/L, it required a long fermentation time of 408 h and the ethanol yield was only 65.5%.

Waste paper contains approximately 10% xylan. Genetically engineered *Saccharomyces cerevisiae* strains are required to ferment xylose to ethanol, but it is still far away from industrial application. Moreover, it is difficult to economically convert the xylan with relative low content in waste paper to value-added product except methane. Our previous studies have revealed that it was beneficial to treat stillage from ethanol production (hereafter called the stillage) by using methane fermentation (Koike et al., 2009; Takaki et al., 2015; Tang et al., 2008).

In this study, to reduce the cost of ethanol fermentation and distillation, we aimed to produce ethanol from waste paper with an ethanol concentration of over 40 g/L and a yield of over 90% within 72 h. First, the enzymatic saccharification of waste paper was optimized. Then, simultaneous saccharification and fermentation (SSF) in the batch mode was used to effectively convert waste paper to ethanol and SSF in fed-batch mode was used to decrease the enzyme loading. In addition, to recover the energy after ethanol fermentation, the biochemical methane potential (BMP) of the stillage was evaluated.

2. Materials and methods

2.1. Waste paper, cellulase, and yeast strains

Shredded copy papers were used as material and were kindly provided by Hitachi Zosen Corporation in April 2009 (waste paper 1) and January 2015 (waste paper 2) (Table 1). The cellulase Cellic CTec2 (hereafter called CTec2) was kindly provided by Novozymes, Denmark. Yeast strain *S. cerevisiae* KF-7, generated by protoplast fusion between the flocculating yeast strain *S. cerevisiae* IR-2 and the thermotolerant yeast strain *S. cerevisiae* EP-1, was used for fermentation (Kida et al., 1992).

2.2. Enzyme assays

2.2.1. Filter paper activity (FPA)

FPA was measured according to the method recommended by the National Renewable Energy Laboratory (NREL), USA (Adney and Baker, 2008).

2.2.2. Carboxymethyl cellulase (CMCase) activity

To measure CMCase activity, 0.625 g of sodium carboxymethyl cellulose (CMC-Na) (Wako Pure Chemicals, Tokyo, Japan) was dissolved in 100 mL of 0.05 mol/L acetate buffer (pH 5.0) and used as the carbon source. Four milliliters of the CMC-Na solution was added to a test tube and preheated at 40 °C for 10 min. Enzyme solution (1 mL) was added and the mixture was incubated at 40 °C for 30 min. The reaction was stopped by adding 1 mL of Somogyi copper reagent (Wako Pure Chemicals, Tokyo, Japan). The test tube was tightly stoppered using an aluminum cap and incubated in boiling water for 30 min. After cooling in an ice bath, 1 mL of Nelson reagent (Wako Pure Chemicals, Tokyo, Japan) was added and distilled water was added to make up a constant volume of 25 mL. The mixture was incubated at room temperature for 15–20 min and the optical density was measured at 660 nm (V-530, Jasco, Tokyo, Japan). One unit of CMCase activity (CUN) was defined as the amount of enzyme required to release 1 μmol of glucose per minute under the conditions described above.

2.2.3. β-glucosidase (BG) activity

To measure BG activity, 1.0 g of cellobiose (Wako Pure Chemicals, Tokyo, Japan) was dissolved in 100 mL of 0.05 mol/L acetate buffer (pH 5.0) as the carbon source. One milliliter of the cellobiose solution was mixed with 1 mL of 0.05 mol/L acetate buffer in a test tube and preheated at 50 °C for 10 min. One milliliter of the enzyme solution was added and the mixture was incubated at 50 °C for 30 min. The test tube was covered with an aluminum cap and incubated in boiling water for 30 min to stop the reaction. The generated glucose was measured using F-kit D-glucose (J.K. International, Tokyo, Japan). One unit of BG activity (U) was defined as the amount of enzyme required to release 1 μmol of glucose per minute under the conditions described above.

2.3. Enzymatic saccharification (ES)

Waste paper slurries (15% w/w) was prepared by mixing 15 g dry based waste paper with 75 mL water. The mixture was adjusted to pH 4.5–5.0 using 20% w/w H_2SO_4 solution and was milled using a mixer (BLA-2001, Nihonseiki Kaisha Ltd., Tokyo,

Table 1
Components of waste paper material, stillage eluted from ethanol fermentation, inoculum for BMP, and sludge after BMP test.

Components	Material		Stillage	Inoculum for BMP	Sludge after BMP test	
	Waste paper 1	Waste paper 2			Inoculum	Stillage
Glucan (%, dry)	58.8	43.3 ± 2.0	NM[c]	NM	NM	NM
Xylan (%, dry)	11.2	7.0 ± 0.7	NM	NM	NM	NM
Lignin (%, dry)	1.0	1.0	NM	NM	NM	NM
Ash (%, dry)	8.8	9.1 ± 0.5	14.0 ± 3.5	27.7 ± 0.0	30.9 ± 0.0	35.4 ± 0.4
C (%, dry)	40.9	38.0 ± 0.6	37.8 ± 0.3	NM	NM	NM
H (%, dry)	5.6	5.1 ± 0.1	4.8 ± 0.0	NM	NM	NM
N (%, dry)	0	0.3 ± 0.1	1.7 ± 0.0	NM	NM	NM
S (%, dry)	0	0.1 ± 0.0	0.2 ± 0.0	NM	NM	NM
O (%, dry)	44.7	47.4 ± 0.6	41.5 ± 0.3	NM	NM	NM
TS[a] (g/kg, wet)	981.0	971.8 ± 1.6	82.8 ± 0.7	31.0 ± 0.1	24.6 ± 0.1	31.3 ± 0.7
VTS[b] (g/kg, wet)	894.7	883.5 ± 5.0	71.2 ± 3.5	22.4 ± 0.0	17.0 ± 0.1	20.2 ± 0.6

[a] TS, total solid.
[b] VTS, volatile total solid.
[c] NM, not measured.

Please cite this article in press as: Nishimura, H., et al. Efficient production of ethanol from waste paper and the biochemical methane potential of stillage eluted from ethanol fermentation. Waste Management (2015), http://dx.doi.org/10.1016/j.wasman.2015.11.051