Journal of Cleaner Production 39 (2013) 273–279

Contents lists available at SciVerse ScienceDirect

Journal of Cleaner Production

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

Cassava pulp enzymatic hydrolysis process as a preliminary step in bio-alcohols production from waste starchy resources

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ARTICLE INFO

Article history:
Received 7 July 2011
Received in revised form
30 July 2012
Accepted 30 July 2012
Available online 7 August 2012

Keywords:
Cassava pulp
Clostridium butyricum
Saccharomyces cerevisiae
ABE productions
Enzymatic hydrolysis
Cassava wastewater

ABSTRACT

Tapioca starch factories generate a large amount of wastewater and solid waste which take several steps of waste treatment to manage. The solid waste in particular contains a high level of starchy-lignocellulosic biomass, especially cassava pulp (CP). The wastewater, above mentioned, contains also residual cassava constituents and, usually are named cassava wastewater (CWW). These residual resources are potentially promising substrates for the production of biofuels (renewable energy carriers), such as products resulted from acetone-butanol-ethanol (ABE) fermentation process. The conventional combination steps between acid hydrolysis and enzymatic hydrolysis of starchy-lignocellulosic substrate, before fermentation process, generate complicated problems such as acid contaminated waste and spending of a long time (several hours) for hydrolysis reaction process. To address these problems, cassava pulp (CP) alone or supplemented with cassava starch wastewater (CWW) was used as a model feedstock (raw material) and an adapted one step enzymatic hydrolysis was created. This one step enzymatic hydrolysis process shows a shortened optimum treatment time (2 h) and yielded a reducing sugar level that was equal to that previously reported for the two stage combination between of acid hydrolysis and enzymatic hydrolysis. After adapted one step enzymatic hydrolysis of the starch the reducing sugar solution was fermented using either *Saccharomyces cerivisiae* TISTR5339 or *Clostridium butyricum* TISTR1032 for ethanol and ABE production, respectively. The ethanolic fermentation (by using *S. cerevisiae*) of CP (67 g/L) in sterilized wastewater solution, yielded a bioproduct mixture having a content consisting (beside other components) from 8.8 g/L of ethanol, but this ethanol concentration was increased to 12.9 g/L with the replacement of the water by CWW. Fermentation of the saccharified CP alone or with CWW with *C. butyricum* yielded a total ABE production of 9.65 g/L and 10.24 g/L, respectively, but the ethanolic production was reduced from 9 g/L (93% of the solvent composition) to 1.64 g/L (16% solvent composition) by the addition of the CWW, with butanol as the major product (53.2%; 2.5 g/L).

1. Introduction

Nowadays, due to the limited and non-renewable levels of petroleum resources, research has focused upon the search for alternative renewable energy sources (Dürre, 2007). Currently, there are two different kinds of bioalcohol which attractive in vehicle factory, bioethanol and biobutanol, have been shown to be promising alternatives to gasoline. These alcohols give high octane number especially butanol which has superior characteristics. Butanol energy content is as well as gasoline and much higher than that in ethanol for 30 percent. Butanol can be stored safer since its volatility is less than that of gasoline and an even lesser volatility than that of ethanol (see their Reid vapor pressures) for 6.7 folds. Also octane number is not much higher than petroleum oil. For this reason, it can be blended with gasoline with quality as same as gasoline. In the same way, gasoline can be compensated by ethanol in 85 percent maximum. So on, butanol can be varied ratio to mix

Abbreviations: ABE, acetone-butanol-ethanol; CP, cassava pulp; CSW, cassava solid waste; CWW, cassava wastewater; YMB, yeast malt broth.

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http://dx.doi.org/10.1016/j.jclepro.2012.07.055

with gasoline more flexible. There are several routes for producing bioethanol and ABE from agricultural feedstocks (Cascone, 2008; Champagne, 2008; Deverell et al., 2009; Gholamhassan et al., 2009).

In theory, second generation (as in derived from lignocellulosic substrates that are typically not food) bioethanol can reduce the food vs. fuel conflict since it can be produced from non-food crops or, especially, from food crop waste residues such as corn stover and rice straw (Kim and Dale, 2004). These lignocellulosic wastes are a good source of carbon. Ideally, second generation bioethanol production should have a low raw material costs, a higher yield per tonne of biomass, and be based upon an abundant non-food raw material. Nevertheless, there are many problems with second-generation bioethanol production, such as the raw material collection, pretreatment, and, especially the enzyme cost and recovery of the ethanol from the low concentrations formed in the fermentor. Accordingly, second-generation bioethanol production is at present still not financially feasible and cannot reduce the current dependency on fossil fuels. Starchy-lignocellulosic biomasses from agricultural wastes are an alternative source of materials for third generation biofuels. Their conversion to alternative petroleum-replacing fuels can be applied with a low carbon-emission, such as those from acetone-butanol-ethanol (ABE) or ethanol fermentation.

Therefore, this study intends to combine the advantages of both primary and secondary generation bioethanol production, and develop a commercially viable bioethanol and biobutanol production process using the waste material from the Thai food and agricultural industry. Thailand is a major exporter of tapioca, which comprises 14–18.1% of the total export value of the country after rice and sugar. Thailand has 92 tapioca processing plants with a total production capacity of native and modified starch of about 16,910 and 4350 tonne (metric ton or Mg)/day, respectively, from cassava roots (Chavalparit and Ongwandee, 2009). However, processing one tonne of cassava roots generates around 3 m^3 of cassava wastewater (CWW) and 150 kg of cassava solid waste (CSW) (15% (w/w) of raw material) (Hien et al., 1999). Cassava roots are currently produced an average of around 25–30 million tonnes of per year, with for example 26,411,233 tonnes in 2007 (Cardona et al., 2010), this generates around 3–5 million tonnes of CSW and roughly 75–90 $\times$ 10^6 m^3 of CWW annually from the cassava starch industry. Despite having reasonably high starch contents, the CSW and cassava pulp (CP) are often left to rot in an open field, which later becomes an environmental problem (Avancini et al., 2007). In general, the conversion of cassava roots to starch generates about 15–20% (w/w) CSW which includes CP and cassava root pulp, and around 10–30 m^3 of acidic CWW pH 4.5–5.0 per tonne of tapioca starch (Ouephanit et al., 2011). These environmental problems can't be solved by any single available technology but rather requires an interdisciplinary approach and applications, especially biorefinery sciences. The use of CSW and CP for ABE or bioethanol production is an interesting raw material to attempt to combine first- and second-generation bioethanol production technology at a reasonable cost in an environmentally improving or friendly manner. Indeed, due to their waste nature, CSW and CP are sold at a relatively low price when compared to cassava roots, although this may in part reflect the current lack of demand for CSW, which would change if used for biofuel production. Regardless, the problems of the substrate pretreatment processes and unsatisfactory ethanol yields are still challenging. In addition, substrate hydrolysis is one of the important key steps to get higher levels of fermentable sugars for ethanol fermentation. Process of initial starch processing requires the liquefaction of starch at a high temperature which then generates a high energy consumption, that usually amounts to about 30–40% of all the energy needed for ethanol production. In addition, Szymanowsky and Grajek (2011) reported that the one-step method by simultaneous hydrolysis and fermentation is a possible energy-efficient solution to reduce the cost. Thus, this study reports basic data on the utilization of CP and CWW from a starch factory as the feedstock and water source to reduce the hydrolysis process reaction time and the fresh water consumption in bioethanol/ABE processing. In addition, the substrate hydrolysis processes were adapted to be a single step enzyme hydrolysis, which improves environmental aspects of the cassava processing factory by reducing the CWW and CP treatment requirements.

2. Materials and methods

2.1. Sampling preparation and content analysis

CP and the CWW were collected from the Sanguan Wongse tapioca starch factory in the Nakorn Ratchasima province, Thailand, and samples of each were dried and ground before being sent to the Institute of Food Research and Product Development of Kasetsart University (Thailand) for content analysis. The starch content analysis was performed using High Performance Liquid Chromatography (Biorad Aminex HPX-87 H column with corresponding guard column) and the T-CM-064 method based on AOAC 950. 55 (2000). Cellulose, hemicelluloses, lignin and ash levels were analyzed using the detergent analysis method (Goering and Van Soest, 1970). The total ash, and the fraction of this that was acid insoluble, was determined as described in AOAC (2000).

2.2. Liquefaction and saccharification

2.2.1. Hydrolysis process

Liquozyme® SC DS (alpha-amylase 240 KNU/g), Spirizyme® Fuel (gluco-amylase 750 AUG/g), Novozyme® NS 50012 (multienzyme complex with 100 fungal β-glucanase Units/g) and Novozyme® NS 50013 were purchased from EAC (Thailand) Co. Ltd., a representative of Novozyme (Denmark).

CP and CWW were sterilized (121 °C, 1.5 mPa) in an autoclave (Tomy, Japan) for 15 min. Liquozyme® SC DS, Spirizyme® Fuel, Novozyme® NS 50012 and Novozyme® NS 50013 were added at different time intervals to 67 g/L of CP, based on the two-step liquefaction and saccharification method of Zhao et al. (2009), with then some modifications for the one-step liquefaction and saccharification process. Liquozyme® SC DS, Spirizyme® Fuel, Novozyme® NS 50012 and Novozyme® NS 50013 were used at the maximum manufacturer's recommended enzyme dosage of 0.01–0.2% (w/w), 0.046–0.066% (w/w), 0.05–0.4% (w/w) and 0.2–0.8% (w/w), respectively.

To optimize the pH, the slurry of 6.67% (w/w) CP in distilled water was adjusted pH to one of six pH values between 4 and 6.5 (4 samples for Novozyme® NS 50012: pH 4, 4.5, 5, 5.5 and 5 samples for Novozyme® NS 50013: pH 4.5, 5, 5.5, 6.0 and 6.5). Novozyme® NS 50012 or Novozyme® NS 50013 were then added at 0.4% (w/w) and 0.8% (w/w), respectively, and incubated at the optimum temperature (50 °C) for each enzyme for 24 h. Reaction were incubated at the indicated temperature with rotary shaking at 200 rpm for the indicated times, with 1 mL samples being taken at 1, 4, 6, 9, 12 and 24 h after initiation of the incubation, respectively. The reducing sugar content produced was analyzed in terms of concentration by the DNS method (Miller et al., 1960), and corrected for the sample removal at each time point. The experimental conditions, in terms of the pH, incubation time and temperature and order of enzyme addition for the four modified liquefaction and saccharification steps are described in Table 1.