Bioresource Technology 194 (2015) 7–13

Contents lists available at ScienceDirect

Bioresource Technology

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

The effect of a combined biological and thermo-mechanical pretreatment of wheat straw on energy yields in coupled ethanol and methane generation

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HIGHLIGHTS

- Integrated storage and pretreatment using *Schefferomyces stipitis* for inoculation.
- Steam explosion pretreatment of wheat straw at 180, 200 and 220 °C.
- Combined ethanol and methane utilisation scenario and balancing of overall energy yields.
- Increasing severity supporting conversion of cellulose to ethanol.
- Combined ethanol/methane production equal to exclusive AD considering energy yields.

ARTICLE INFO

Article history:
Received 19 April 2015
Received in revised form 19 June 2015
Accepted 20 June 2015
Available online 30 June 2015

Keywords:
Lignocellulose
Pretreatment
Effects
Methane
Ethanol

ABSTRACT

Ethanol and biogas are energy carriers that could contribute to a future energy system independent of fossil fuels. Straw is a favorable bioenergy substrate as it does not compete with food or feed production. As straw is very resistant to microbial degradation, it requires a pretreatment to insure efficient conversion to ethanol and/or methane. This study investigates the effect of combining biological pretreatment and steam explosion on ethanol and methane yields in order to improve the coupled generation process. Results show that the temperature of the steam explosion pretreatment has a particularly strong effect on possible ethanol yields, whereas combination with the biological pretreatment showed no difference in overall energy yield. The highest overall energy output was found to be 10.86 MJ kg VS^{-1} using a combined biological and steam explosion pretreatment at a temperature of 200 °C.

1. Introduction

The production of renewable energy from lignocellulosic feedstocks has recently garnered substantial interest as a replacement for fossil-derived energy. Not only could this source of renewable energy help in the transition away from fossil fuels, but it does not compete with food and feed production to the same extent as ethanol or biogas production from dedicated energy crops such as maize.

One of the most interesting feedstocks for lignocellulose-derived fuels is cereal straw (Chen et al., 2007). Straw is co-produced with cereal grain and has a broad field of application in agriculture. One of these applications is as a raw material for bioethanol production, as, for instance, demonstrated by the Inbicon-pilot plant in Denmark (Larsen et al., 2008). However, the moisture content of straw must be below 18% to prevent mould growth, regardless

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http://dx.doi.org/10.1016/j.biortech.2015.06.093
0960-8524/

of its subsequent application. Field storage is commonly employed as a method of drying straw. If air humidity is too high, the low moisture content required for storage cannot be reached. For instance, the potential of straw production in Sweden is about one million tons, however, due to frequent high humidity, only a small proportion can be utilized (Nilsson, 2000).

Due to its lignocellulosic structure, the biomass is recalcitrant to biological conversion and hence requires a pretreatment prior to further processing (Taherzadeh and Karimi, 2008). Most pretreatment technologies presently in use are energy intensive or require chemicals, which are derived from fossil resources and may also require large amounts of energy for their production (Alvira et al., 2010). As such, the ratio between inputs necessary to process the feedstock and outputs (in terms of liquid or gaseous fuels) is crucial to economic and ecological viability. Decreasing inputs while increasing outputs has the potential to make the pretreatment process more competitive than conventional energy production.

Steam explosion is a commonly used pretreatment technology, typically carried out at temperatures of 160–260 °C and retention times of several minutes (Sun and Cheng, 2002). The feasibility of using steam explosion for the pretreatment of this feedstock has already been shown. Bauer et al. (2009) found an increase in methane yield from wheat straw pretreated with steam explosion ranging from 275 l_N kg VS^{-1} to 331 l_N kg VS^{-1} (+20%) at a temperature of 180 °C and a retention time of 15 min. Dererie et al. (2011) investigated the effect of steam explosion on the combined ethanol and methane yield of oat straw and found an overall energy output of 9.5 MJ kg DM^{-1} when using a 190 °C pretreatment temperature and a 10 min retention time. The amount of thermal energy that is required for the steam explosion pretreatment is very high; hence it is crucial to have a source of waste heat that is large enough to provide enough thermal energy for the pretreatment. Moreover, due to the harsh thermochemical pretreatment, compounds are formed that inhibit the subsequent fermentation processes. Acetic acid, furfural, hydroxy-methyl furfural (HMF) and phenolic compounds derived from hemicellulose and lignin can inhibit the yeasts from ethanol fermentation (Klinke et al., 2004; Larsson et al., 1999; Parawira and Tekere, 2011). Furfural and HMF inhibit the growth of yeast cells but are themselves degraded to furfuryl alcohol and furoic acid (Boyer et al., 1992; Taherzadeh et al., 2000). The inhibitory effect of those compounds on the consortium that is responsible for anaerobic digestion is not yet clear.

For the operation of a biomass-based fuel-generation plant to be economical, it is necessary to guarantee a good degree of capacity utilization. As wheat straw is harvested only once a year it is crucial to store it properly. As mentioned above, in some colder climatic regions it is a problem to remove water from the straw by drying it on the field. Microorganisms that are able prevent mould growth may offer one possibility to conveniently store the wheat straw. Moreover, Passoth et al. (2013) showed that the yeast *Scheffersomyces stipitis* is also capable of increasing ethanol yields of conserved wheat straw, likely due to its ability to partially degrade hemicellulose.

A combination of a low energy biological pretreatment combined with steam explosion could reduce the required thermal energy input while delivering the same energy output in ethanol and methane. Reducing the required temperature level could also lead to the reduced formation of furfural and HMF, which are formed during the degradation of hemicellulose (Larsson et al., 1999). Although separation (Qi et al., 2011) and biotransformation (Boopathy, 2009) are discussed as strategies to overcome inhibition caused by reaction products (resulting from thermophysical pretreatment), a reduction in their formation is considered to be the more economically efficient option.

The objective of this study was to investigate the effect of a combined biological (ISP) and thermochemical (steam explosion) pretreatment on ethanol and methane yields of wheat straw. As the concept of combined ethanol and methane production is regarded as a promising production system for fuels in the future, the residues from ethanol fermentation were also investigated in anaerobic batch experiments.

The goal of this study is to obtain the overall energy yield of combined ethanol and methane generation. This means that, after biomass is pretreated, ethanol is produced. The fermentation residues resulting from ethanol fermentation subsequently serve as a substrate for anaerobic digestion for the generation of methane.

2. Methods

This section explains the experimental setup for the biological and steam explosion pretreatments as well as the determination of methane and ethanol yields in detail. The experiments and analysis of sample material was performed strictly in order to determine the overall energy yield of coupled methane and ethanol generation.

2.1. Biomass

The biomass used in the trials was wheat straw that had been grown in 2012 in Lower Austria. Prior to the experiments it was ground to a particle size of <5 mm using a cutting mill (Retsch SM100). The dry matter content of the untreated sample was 92.52%. The content of volatile solids was 94.62%.

2.2. Strains and culture media

S. stipitis CBS 5774 was used for the ISP experiments and *Saccharomyces cerevisiae* J672 was used for simultaneous saccharification and fermentation. Both yeasts were from the strain collection at the Dept. of Microbiology, SLU, Uppsala. *S. cerevisiae* (Blomqvist et al., 2010) was subcultured on YPD-agar (yeast extract 10 g L^{-1}, bacteriological peptone 20 g L^{-1}, glucose 20 g L^{-1}, bacteriological agar 16 g L^{-1}) and *S. stipitis* on YM-agar (yeast extract 3 g L^{-1}, malt extract 3 g L^{-1}, bacteriological peptone 5 g L^{-1} and glucose 10 g L^{-1}, bacteriological agar 16 g L^{-1}). For the pre-cultures, *S. stipitis* was grown in YM (yeast extract 3 g L^{-1}, malt extract 3 g L^{-1}, bacteriological peptone 5 g L^{-1} and glucose 10 g L^{-1}) and *S. cerevisiae* in YPD (yeast extract 10 g L^{-1}, bacteriological peptone 20 g L^{-1}, glucose 20 g L^{-1}, bacteriological agar 16 g L^{-1}).Integrated storage and pretreatment

The inoculation of the wheat straw samples with *S. stipitis* (CBS 5774) was performed at the Department of Microbiology, SLU, Uppsala, Sweden. The experiment was carried out as described by Passoth et al. (2013). For the pre-culture, *S. stipitis* was grown in a 300 ml shake flask containing 100 ml YM-medium at a rotary table for 24 h at 25 °C. The optical density (OD_{600}) was measured and the volume of cell suspension needed to reach a starting OD of 0.1 was harvested. The cell suspension was subsequently centrifuged for 10 min at 4000*g* and washed with sterile saline (NaCl 9 g L^{-1}), centrifuged again, resuspended in 1 ml saline and inoculated in 300 m YM in a 1 L shake flask. The main culture was incubated at 25 °C for 20 h at a rotary table. Cells were counted in a Bürker chamber, washed and then resuspended in sterile deionized water. Before inoculation of the wheat straw with the yeast cells, water was added to reach a final water content of 30% in the wheat straw. Samples were subsequently inoculated with yeast cells to reach a ratio of about 10^5 cells per g DM of wheat straw. Wheat straw samples were then stored in plastic bags for 42 days at 4 °C until the steam explosion pretreatment.