Bioresource Technology 102 (2011) 4028–4035

Contents lists available at ScienceDirect

Bioresource Technology

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

Performance and microbial community analysis of two-stage process with extreme thermophilic hydrogen and thermophilic methane production from hydrolysate in UASB reactors

Prawit Kongjan [a], Sompong O-Thong [a,b], Irini Angelidaki [a,*]

[a] Department of Environmental Engineering, Technical University of Denmark, DK-2800 Lyngby, Denmark
[b] Department of Biology, Faculty of Science, Thaksin University, Phathalung 93110, Thailand

ARTICLE INFO

Article history:
Received 22 June 2010
Received in revised form 1 December 2010
Accepted 1 December 2010
Available online 7 December 2010

Keywords:
Hemicelluloses
Two-stage anaerobic digestion
Hydrogen and methane
UASB reactor

ABSTRACT

The two-stage process for extreme thermophilic hydrogen and thermophilic methane production from wheat straw hydrolysate was investigated in up-flow anaerobic sludge bed (UASB) reactors. Specific hydrogen and methane yields of 89 ml-H_2/g-VS (190 ml-H_2/g-sugars) and 307 ml-CH_4/g-VS, respectively were achieved simultaneously with the overall VS removal efficiency of 81% by operating with total hydraulic retention time (HRT) of 4 days . The energy conversion efficiency was dramatically increased from only 7.5% in the hydrogen stage to 87.5% of the potential energy from hydrolysate, corresponding to total energy of 13.4 kJ/g-VS. Dominant hydrogen-producing bacteria in the H_2-UASB reactor were *Thermoanaerobacter wiegelii*, *Caldanaerobacter subteraneus*, and *Caloramator fervidus.* Meanwhile, the CH_4-UASB reactor was dominated with methanogens of *Methanosarcina mazei* and *Methanothermobacter defluvii*. The results from this study suggest the two stage anaerobic process can be effectively used for energy recovery and for stabilization of hydrolysate at anaerobic conditions.

1. Introduction

As a substantial unexploited source of mixed sugars (hexose and pentose), lignocellulosic biomass has a potential for the production of ethanol, methane, and hydrogen bio-fuels by applying fermentation or anaerobic digestion. These energy carriers have the potential to reduce fossil fuels for transportation and for heat and power production. Additionally, bio-fuel production from lignocelluloses could decouple the production of food and energy, increase the value of the world's production from agriculture and forestry, reduce green house gas (GHG) emissions, and certainly provide a more stable and secure supply of energy (Larsen et al., 2008).

In order to utilize sugars consisted in lignocelluloses for bio-fuels production, pre-treatment is required to break down their dense structure and improve digestibility. At commercial scale, hydrothermal pre-treatment is an efficient and potential way of pre-treating lignocelluloses. During the hydrothermal pre-treatment, the cellulose (C_6 sugars) and lignin are recovered in a solid fraction, while hemicelluloses (C_5 sugars) and degradation products are contained mainly in a liquid fraction called hydrolysate. Fermentation of the C_6 sugars present in the solid fraction by using wild *Saccharomyces cerevisiae* for bio-ethanol production has been demonstrated successfully, but utilization of hydrolysate (C_5 sugars) is currently inefficient and uneconomical for bio-ethanol production (Larsen et al., 2008). Alternatively, hemicelluloses hydrolysate can be converted to hydrogen by using mixed extreme thermophiles in the UASB reactor with a high yield of 203 ml-H_2/g-sugars (Kongjan and Angelidaki, 2010). Moreover, soluble products such as volatile fatty acids, lactic acids, and alcohols generated simultaneously during the fermentative hydrogen production from hydrolysate could serve as ideal substrate for methane production by a subsequent anaerobic digestion (Kaparaju et al., 2009b). Methane production is, therefore, considered as suitable additional step to hydrogen production.

Two-stage anaerobic digestion for bio-fuels (hydrogen and methane) production and organic waste treatment has now emerged as an attractive and promising process. In this process, two separate reactors are connected in series, separating acidogenesis and methanogenesis, are operated at different temperatures, acidity and hydraulic retention time (HRT) appropriate for individual optimization of growth of the different communities of micro-organisms in each reactor (Demirel and Yenigun, 2002). Acidogenic bacteria convert organic substrates mainly into hydrogen, organic acids, solvents and CO_2 in the first stage under slightly acidic (pH ~ 5.0–6.0) conditions, and typically shorter HRTs of 1–3 days. Methane producing micro-organisms in the second stage, on the

* Corresponding author. Present address: Department of Science, Faculty of Science and Technology, Prince of Songkla University (Pattani), Muang, Pattani 94000, Thailand. Tel.: +45 4525 1429; fax: +45 4593 2850.
E-mail address: ria@env.dtu.dk (I. Angelidaki).

0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.12.009

other hand, have a lower growth rate, requiring a longer HRT (10–20 days) at a more neutral acidity (pH ~ 7.0–8.0) for converting organic acids and solvents contained in the effluent from the first reactor to methane (Antonopoulou et al., 2008; Liu et al., 2006). More importantly, controlling the acidification in the first reactor could prevent the overloading and/or inhibition of methanogenesis in the second reactor and consequently increase the stability of the whole process.

UASB reactors are extensively used in anaerobic digestion for treating various dilute liquid wastes consisting typically of 1–3% total solid concentration with high organic loading rates and short HRTs because they can retain high biomass concentrations with high specific activity in the form of granular sludge (Lepistö and Rintala, 1999; Batstone et al., 2004). It has also been shown that UASB reactors are less sensitive to fluctuations of environmental parameters (e.g. acidity and HRT) than a continuously stirred tank reactor (CSTR), resulting in process better stability (Gavala et al., 2006; Kotsopoulos et al., 2006). Hydrogen producing UASB reactors can risk developing methanogens because cells are immobilised and are not washed out from the UASB reactor, even at flow rates much higher than the maximum growth rate of the methanogens. In such immobilised-type reactors, a combination of operating at extreme thermophilic temperatures and a slightly acidic environment at 5.0–6.0 pH can be applied to effectively eliminate methane production (Kongjan and Angelidaki, 2010; Kotsopoulos et al., 2006). Combination of the advantages of the two stage process with the advantages of UASB reactors, for treatment of carbohydrate rich substrates would give the possibility to efficiently produce hydrogen and methane in a two step process at a high rate and fully utilise the energy contained in the organic matter.

Application of UASB reactors in a two stage process of hemicelloses hydrolysate, a by-product for second generation ethanol processing has not been tried to date. Therefore, in this study, we have investigated a two steps process in UASB reactors, operated in series, with a hydrogen producing reactor at 70 °C, and subsequent methane reactor at 55 °C, from diluted hydrolysate. Microbial communities of selected mixed cultures of two-stage process were assessments by PCR-DGGE based on 16S rDNA.

2. Methods

2.1. Wheat straw hydrolysate

Hydrolysate was kindly provided from Department of Biosystems, Risø DTU (Denmark), generated by the hydrothermal pretreatment of wheat straw in a three step reactor system. The hydrothermal pre-treatment of wheat straw resulted in a solid fraction containing the mainly the cellulose and lignin; and a liquid fraction so-called hydrolysate, mainly containing hemicelluloses (Kaparaju et al., 2009b). Hydrolysate used in this study, had a total solids (TS) and volatile solids (VS) content of 3.3% and 4.4%, respectively, and was consisting of 12.6 g/l pentoses and 2.9 g/l hexoses. More detailed composition of the hydrolysate can be found in Kongjan et al. (2010).

2.2. Experimental set-up and operation

Continuous hydrogen and methane production were carried out in 2 UASB reactors with a 220-ml working volume each. The reactors were maintained at 70 and 55 °C for H_2 UASB reactor (R1) and CH_4 UASB reactor (R2), respectively, by circulating hot water inside a water jacket surrounding each UASB reactor. A hydrogen UASB reactor fed with 25% (v/v) hydrolysate at 1 day HRT in our previous investigation (Kongjan and Angelidaki, 2010) was used. For avoiding clogging in the tubes (i.d.3.2 mm) the influent to reactor contained hydrolysate diluted with water (6:4 v/v) and basic anaerobic (BA) medium (1:1 v/v), corresponding to 30% (v/v) hydrolysate and an organic loading rate (OLR) of 9.9 g-VS/d·l. BA medium containing nutrients and bicarbonate buffer was as previously (Angelidaki et al., 1990) and was amended with 1 g/l of yeast extract. After reaching steady state characterized by a constant gas production rate (±5%), the R1 effluent was collected (Table 1) was kept at −20 °C for further use in the R2 reactor.

The R2 reactor was filled with 40 ml of methonogenic granules from a potato-processing wastewater treatment plant (Kruiningen, Netherlands) and 60 ml of digested manure from a thermophilic pilot scale plant (Lyngby, Denmark), for providing a good diverse thermophilic inoculum for methane production. Subsequently 120 ml of a mixture consisting of R1 effluent and BA medium (1:1 v/v) was then manually added into the reactor. The R2 reactor was then purged with nitrogen gas for 10 min to establish anaerobic conditions. The homogenised effluent from R1 was initially fed into the methane reactor at 5.2 days HRT. The reactor OLR was afterwards increased stepwise by decreasing the HRT to 4.4, 3.0, 1.8, 1.0 and 0.5 days, corresponding to the organic loading rates of 1.6, 1.9, 2.7, 4.5, 8.2, and 15.7 g-$VS_{R1\text{-}eff}$/d l, respectively. Each level of loading was kept, after steady state was achieved, for 2 HRTs. The process was considered to be in steady state when the variation of biogas production was less than 5% (Kaparaju et al., 2009a).

2.3. Monitoring and analysis

The composition of the biogases produced (H_2, CH_4 and CO_2) were daily monitored, and liquid samples were taken daily for further analysis of volatile fatty acids (VFAs), alcohols, lactate, formate, total sugars, total solid (TS) and volatile solid (VS). Hydrogen, methane and CO_2 were determined by gas chromatography (GC) (MicroLab, Arhus, Denmark) equipped with a thermal conductivity detector (TCD) (Kotsopoulos et al., 2006). The VFAs and alcohol were measured using a GC (GC-2010 Shimadzu, Kyoto, Japan) equipped with a flame ionization detector (FID) and Zebron (ZB) – FFAP column (dimensions 30 m × 0.53 mm) (Kongjan et al., 2010). Lactate and formate were analyzed by suppressed ion exclusion chromatography equipped with a high performance liquid chromatography (HPLC) pump (L2100 Hitachi), a HPLC autosampler (L2200 Hitachi), a suppressor (Dionex AMMS-IEC2), and a column (ICE-AS1) at 9 × 250 mm (Kongjan et al., 2009). Sugars were analyzed by HPLC equipped with refractive index (RI) detector, and toxic compounds of furfural and HMF were determined by a HPLC fitted with an ultraviolet (UV) detector (Kaparaju et al., 2009b).

2.4. Microbial community analysis

Polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used to study microbial community structure in the two-stage process. Liquid samples were collected from bioreactors under steady state conditions. Genomic DNA extractions as well as PCR-DGGE for bacteria were made as previous described (Kongjan et al., 2010). PCR-DGGE for *archaea*, genomic DNA was used as a template for PCR reactions with a primer pair specific Arch21f and Arch958r (Lane, 1991) in order to detect archaea populations in methanogenic reactor. Genomic DNA from *Caldicellulosiruptor saccharolyticus* and *Sulfolobus islandicus* were used as negative and positive controls for archaea – PCR, respectively. First, archaea – PCR products were used as a template for nested PCR reactions with a primer pair specific for archaea (PARCH519R and PARCH340F with 40 bp GC clamp at the 5′ end (Keyser et al., 2006) were used to amplify the 200 bp fragment of the V_3 region. The amplicons were used as DNA template to incorporate a GC clamp in the DNA fragment prior to DGGE