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Biomethanation from enzymatically hydrolyzed brewer's spent grain: Impact of rapid increase in loadings

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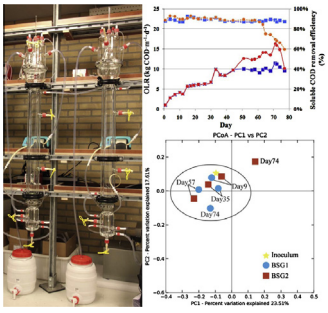
HIGHLIGHTS

- Enzymatically hydrolyzed brewer's spent grain was digested by EGSBs.
- A rapid increase in OLR can have negative influence on the reactor performance.
- Obvious changes of microbial communities appeared after the rapid increase in OLR.

GRAPHICAL ABSTRACT



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ABSTRACT

Enzymatically hydrolyzed brewer's spent grain (BSG) was digested in two expanded granular sludge beds (EGSBs, named BSG1 and BSG2, respectively). Both reactors were operated with the same organic loading rate (OLR) from 1 to 10 kg COD m^{-3} d^{-1} during the first 45 days. Hereafter a rapid OLR increase was applied to BSG2 from 10 to 16 kg COD m^{-3} d^{-1} within three weeks, while the OLR of BSG1 was increased by less than 2 kg COD m^{-3} d^{-1} in the same period. Results showed that a 30% decrease in COD removal and 70% decrease in methane yield appeared in BSG2 after the rapid OLR increase, and volatile fatty acid (VFA) accumulated more than thirty times compared to BSG1. The biomass structure deteriorated and 15% of the biomass was lost from the BSG2 reactor. 454-PyroTag and qPCR analysis revealed a rapid growth of acidifiers (i.e., *Bacteroides*) and a unique microbial community in BSG2 following the rapid increase in OLR.

1. Introduction

Brewer's spent grain (BSG) is a by-product of the beer brewing process that mainly consists of carbohydrates, proteins and lignin, which is a potential bio-resource for reuse or biogas production (Beldman et al., 1987; Bochmann et al., 2007). As a cost- and energy-saving method, anaerobic digestion has been widely applied for the treatment of wastewater and waste solids with various types of reactors (Abbasi et al., 2012; Appels et al., 2011; Li et al., 2011). However, anaerobic digestion processes can be rate limited by the hydrolysis stage (Zhang et al., 2014), due to the lack of sufficient and specific extracellular enzymes that target the solids (van Lier et al., 2008) and/or the weak accessibility of these

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enzymes to polymeric particles (Zhang et al., 2007). Therefore, an enzymatic pre-hydrolysis step could be advantageous for increasing the rate of anaerobic digestion of agro-materials (DeMartini et al., 2013; Yu et al., 2014) by hydrolyzing particulates into small-molecules and simple-structured substrates for subsequent fermentation and methanogenesis (Alvira et al., 2010; Cateto et al., 2011; Yoshida et al., 2009).

A complete process of anaerobic digestion is achieved by a series of biochemical processes, including hydrolysis, acidogenesis, acetogenesis and methanogenesis (Batstone et al., 2002). Because of different efficiencies among the functional bacteria or archaea that carry out these processes, the balance of each step's rate is of crucial importance to a system (Lv et al., 2013). For example, generally, the acidogenesis step is faster than the methanogenesis step, leading to that an anaerobic system is prone to be "souring" in case of over loading of organic matter or inhibition (van Lier et al., 2008). Although the tracing of biogas production and microbial communities before/after overloading has been done in several studies (Blume et al., 2010; Chen et al., 2012; Sundh et al., 2003), there is still a lack of understanding about how a system could perform and how the microbial populations would dynamic in response to a rapid increase in organic loading rate (OLR).

In this study, enzymatically hydrolyzed BSG was digested in duplicate EGSB reactors under mesophilic conditions. The purpose was to study the influence of such highly concentrated hydrolysates on reactor performance. Particular attention was paid to the impact of a rapid increase in OLR on the organic conversion process, granular morphology and microbial community through comprehensive measurements and analysis.

2. Methods

2.1. Enzymatically hydrolyzed brewer's spent grain

The raw BSG used in this study was collected from a brewery plant in The Netherlands. It was a wet slurry, mainly consisting of protein, lipids, lignin and carbohydrates, and an inert ash fraction. Multi-step enzymatic hydrolysis was applied to hydrolyze the raw BSG in a stainless steel tank that had a working volume of 1.5 m^3. Three types of enzymes, including Protease (Delvolase®), cellulase (Filtrase® NL) and hemicellulase (Bakezyme® ARA10.000), were supplied by DSM (Delft, The Netherlands). Several steps in processing raw BSG were followed: Firstly, a thermochemical pretreatment was applied under the conditions of pH 10.7, 90 °C for 4 h in order to improve the solubilization yield of organic matters which were released from solid fraction of raw BSG. Then enzymatic hydrolysis was applied under the optimum conditions of each enzyme. Delvolase® was applied in the first place under 60 °C and pH 8. After 4 h, Filtrase® NL and Bakezyme® ARA10.000 were added together to break intricate 3D-networks of cellulose and hemicellulose under 50 °C and pH 4.5. Twenty hours later, the pH of slurry was neutralized and then the slurry was filtrated by a multifilament cloth (Sefar Tetex, Switzerland) to get the liquid fraction for the subsequent AD reactors. The pH was adjusted by adding NaOH or HCl solutions. The filtered liquid was used as influent substrate for the EGSBs and is addressed as "hydrolysates" in this study. The main components of the BSG hydrolysate is given in Table 1.

Table 1
Components of the raw BSG hydrolysates* used in this study.

Component	Value (g/kg)	Component	Value (g/kg)
Dry matter	74	Ammonia nitrogen	0.2
Arabinose	3.3	Sodium	4.9
Glucose	4.6	Phosphorus	0.5
Xylose	6.9	Sulfur	0.3
COD	86	Calcium	0.2
Total nitrogen	3.5	Magnesium	0.1

* The raw BSG hydrolysates here represented the final product after the enzymatically hydrolysis. The hydrolysates were diluted with tap water before feeding the EGSB reactors in order to achieve the targeted organic loading rates.

2.2. Setup, inoculum and operation

Two same glass-made EGSB reactors (Fig. S1) with a working volume of 3.8 L were used. Three ports, i.e., top, middle and bottom, distributed along the height of each reactor for sampling. The temperature in the reactor was maintained by a water jacket at 34–35 °C. An effluent recirculation was used to supply a constant upflow velocity of 8 m h^{-1}. The biogas was led through alkali liquor to strip CO_2 and H_2S whereafter the amount was measured by a gas counter (MGC-1 PMMA, Ritter, Germany). The temperature, ORP and pH were on-line monitored by sensors (Mettler Toledo, Switzerland). Anaerobic granules from a full-scale UASB treating potato processing wastewater (Stavenhagen, Germany) were used to inoculate both reactors with the same amount after fully mixed.

The OLR increase was reached by lowering the dilution factor of the raw BSG hydrolysates with tap water. The total COD (tCOD) concentration of actual influent was 4.3 g L^{-1} at OLR 1 kg COD m^{-3} d^{-1} for both reactors and stepwise increased to 28.6 g L^{-1} at OLR 11.5 kg COD m^{-3} d^{-1} for BSG1 and 40.6 g L^{-1} at OLR 16.2 kg COD m^{-3} d^{-1} for BSG2, respectively. The hydraulic retention time (HRT) decreased during this period from 4.3 to 2.5 days.

2.3. Analysis

The samples for soluble COD (sCOD) measurement were filtered through 0.45 micrometer fiber glass filters. COD was measured by testing kits (MERCK, Germany) and spectrophotometer (Spectroquat TR420/NOVA60, MERCK, Germany). TSS, VSS, TS, VS were analyzed according to the Standard Methods (APHA, 2005). The methane content in biogas was measured with a Varian 3800 gas chromatograph equipped with a flame ionization detector (FID). The concentration of volatile fatty acid (VFA) were measured by a Focus GC (Thermo Electron Corporation, Waltham, MA, USA) connected with FID. The methane yield was calculated according to the following equation:

$$\text{Methane yield } (\text{g COD}_{CH_4} \cdot \text{g COD}_{fed}^{-1}) = \text{COD}_{CH_4}/\text{tCOD}_{fed}$$

where COD_{CH_4} (g COD_{CH_4} d^{-1}) represents the calculated COD normalized by methane; $tCOD_{fed}$ (g COD_{CH_4} d^{-1}) is the total amount of COD received by the EGSBs per day.

2.4. Biomass preparation for molecular biology analysis

The inoculum and granules in the EGSBs were sampled and prepared for 454-PyroTag analysis and qPCR test. The fresh granular samples were collected on the 9th, 35th, 57th and 75th day. All the granules were sampled from the middle part (Fig. S1) of the EGSBs except for the Day 57 samples that were collected from the top, middle and bottom part of BSG1 and BSG2.

The biomass samples were firstly washed by 1 × PBS and then centrifuged under 7000 × G for 7 min. The supernatant was removed and the pellet was washed by 1 × PBS again and centrifuged under 17,000 × G for 20 min. The supernatant was removed and the pellet was stored for less than one month at −25 °C until DNA extraction. DNA extraction was performed using a microbial DNA isolation kit (UltraClean, MoBio Laboratories, Inc., CA, USA) following the instruction of the product. The quality of