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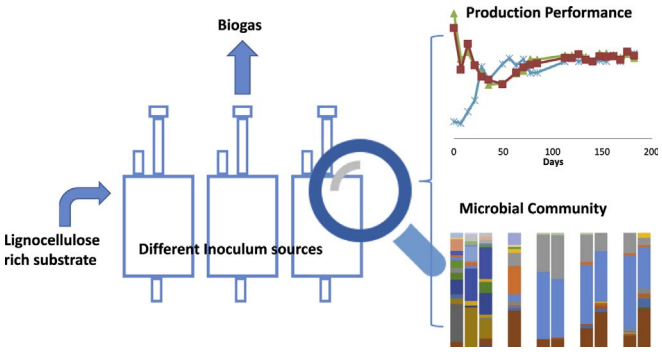
Importance of inoculum source and initial community structure for biogas production from agricultural substrates

Tong Liu[1], Li Sun[1], Bettina Müller, Anna Schnürer*

Department of Molecular Science, Swedish University of Agricultural Science, Uppsala BioCenter, P.O. Box 7025, SE-75007 Uppsala, Sweden

GRAPHICAL ABSTRACT



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ABSTRACT

This study evaluated the importance of inoculum source for start-up and operation of biogas processes. Three different inocula with different community structure were used to initiate six laboratory continuous stirred tank reactor (CSTR) processes operated with a grass manure mixture as substrate. The processes were evaluated by chemical and microbiological analysis, by targeting the overall bacterial community and potential cellulose-degrading bacteria. As expected, the results showed a large difference in community structure in the inocula and in process performance during the first hydraulic retention time (HRT). However, the performance and overall microbial community structure became similar in the reactors over time. An inoculum from a high-ammonia process, characterized by low diversity and low degradation efficiency, took the longest time to reach stability and final methane yield. The overall bacterial community was mainly shaped by the operating conditions but, interestingly, potential cellulose-degrading bacteria seemed mainly to originate from the substrate.

1. Introduction

Biogas is one of the most promising bioenergy alternatives for fossil fuel-based energy. Many biodegradable organic wastes can be used as substrates to produce biogas, which eases the pressure on the environment, waste treatment, and energy supply to cities (Weiland, 2010). Among these substrates, lignocellulosic materials such as agricultural residues are highly interesting due to high abundance and potential for biogas production (Meyer et al., 2017).

Biogas is produced through anaerobic degradation, engaging various microorganisms performing four major degradation steps: hydrolysis, fermentation, acetogenesis, and methanogenesis (Schnürer, 2016). The degree and rate of degradation and the biogas yield depend on the chemical and physical characteristics of the substrate, but also

* Corresponding author.
E-mail address: anna.schnurer@slu.se (A. Schnürer).
[1] Tong Liu and Li Sun contributed equally to this work.

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on the chosen process parameters, such as temperature and retention time, that shape the composition of the different microbial groups and communities active in the process (Schnürer, 2016). When agricultural residues (stalks, straw, husks, cobs, grass, etc.) and manure are used as substrates, the initial hydrolysis step is usually rate-limiting for the whole degradation process, as the lignocellulose in these plant-based materials is difficult for microorganisms to access and utilize (Azman et al., 2015). Many attempts have been made to improve the degree of degradation, e.g., by using different pre-treatment techniques to improve the accessibility of the substrate for microorganisms (Carrere et al., 2016). Bioaugmentation with bacteria with high degradation efficiency for lignocellulosic material has also been evaluated, but with varying results (Poszytek et al., 2016; Tsapekos et al., 2017).

Recent research related to the degradation of lignocellulose in biogas processes has had a strong focus on the microorganisms involved, with the aim of further understanding and improving degradation. These studies have e.g., analyzed the whole bacterial and archaea community by analyzing the 16 rRNA genes (Azman et al., 2015; Sun et al., 2016). However, it is difficult to address community changes in lignocellulose degraders exclusively based on a phylogenetic marker, such as the 16S RNA gene. Thus, some recent studies have tried to target the cellulose-degrading community specifically by various other molecular tools based on functional genes. For example, Pereyra et al. (2010) designed consensus degenerate hybrid oligonucleotide primers to directly target the genes encoding glycoside hydrolase. Use of quantitative PCR (qPCR) has revealed higher abundance of these genes in lignocellulose-fed rather than ethanol-fed bioreactors (Pereyra et al., 2010). Sun et al. (2016) used the same primer sets to investigate the potential cellulolytic bacterial community patterns in 10 full-scale biogas plants through terminal restriction fragment length polymorphism (T-RFLP) combined with clone libraries. The results showed a high correlation between lignocellulose degradation and presence of glycoside hydrolase families 5 and 48, mainly representing bacteria belonging to the phyla Firmicutes and Bacteroidetes. Moreover, stable isotope probing (SIP) combined with fluorescent in situ hybridization (FISH) has revealed that Firmicutes and Bacteroidetes play important roles in cellulose degradation (Li et al., 2009).

In a previous study, we investigated degradation of straw and cellulose during batch cultivation using material from different full-scale biogas plants as the inoculum source (Sun et al., 2016). The results showed similar biogas yields but differences in the degradation rate, as well as a correlation between degradation rate and the composition of the cellulose-degrading community. These results imply that the lignocellulolytic microbial community in the initial inoculum could be an important factor for start-up of a biogas process and later on for regulating the degradation rate of lignocellulose-rich material. The importance of inoculum source for biogas production from different substrates, such as food waste, sewage sludge, crops, microalgae, and manure, has been demonstrated previously (De Vrieze et al., 2015b; Koch et al., 2017; Mahdy et al., 2017). Moreover, a recent survey investigating the importance of the methanogenic population on start-up of a biogas process showed that different sources of inoculum with different methanogenic composition and abundance resulted in different biomethane potential, most likely as a result of differences in ammonium tolerance in the initial community (De Vrieze et al., 2015b). However, most of the above-mentioned studies have investigated the importance of inoculum source using a batch cultivation system and few studies have investigated effects in a continuous digestion system. In addition, the importance of inoculum source for the degradation of lignocellulosic materials has not been specifically addressed.

Thus, the overall aim of the present study was to investigate the importance of the inoculum source for efficient biogas production from lignocellulose-rich material in a continuously operated process, the impact of the initial microbial community structure on the degradation efficiency, and how these were affected by the substrates and operating parameters used. The hypothesis tested was that choosing a suitable inoculum gives an early advantage in biogas production and degradation of lignocellulose. Inocula were taken from three different industrial-scale biogas plants in Sweden showing differences in degradation of straw and cellulose. A manure-grass mixture was used as substrate to operate the laboratory-scale CSTR reactors initiated with the different inocula. The processes were monitored by analysis of different chemical parameters. The microbial community structures were analyzed by Illumina sequencing targeting the 16sRNA genes and by T-RFLP analysis targeting glycoside hydrolase families 5 and 48. The degradation of the substrate and biogas production were also evaluated using batch cultivation, started with the different initial inocula and with inocula retrieved from the laboratory-scale CSTR reactors at the end of the experiment.

2. Materials and methods

2.1. Biogas plants

Inocula for the laboratory-scale continuously stirred tank reactors (CSTR) was taken from three industrial-scale biogas plants in Sweden. One plant (GA) was associated with a wastewater treatment facility and operated with sludge as the sole substrate. The second plant (GB) used stillage from an ethanol production process as the main substrate, while the third (GC) was an agricultural biogas plant using mainly manure and grass silage, but occasionally other substrates. Information about the plants is summarized in Table 1. Operating information on the full-scale plants (GA and GB, designated WWTP03 and CD02, respectively, in Sun et al. 2016), can also be found in our previous publications (Moestedt et al., 2016; Sun et al., 2016).

2.2. Anaerobic reactors

The laboratory-scale CSTR (8L, Dolly Belach) were initiated with inocula from the selected biogas plants. Each inoculum was used for duplicate reactors, giving in total six reactors designated GA1, GA2, GB1, GB2, GC1, and GC2. The reactors were started by filling them with 5 L of the respective inoculum. From day 2, the reactors were fed with a manure-grass mixture 6 days a week, initially with a daily load of 0.6 g volatile solids (VS) per liter. The load was then gradually increased to 2.6 g VS/L/day, with an increase of around 0.5 g VS per week, reaching full load after a total 37 of days of operation. The hydraulic retention time (HRT) was kept at 40 days during the whole period by adjusting the volume by addition of tap water. The chemical composition of the manure-grass mixture used as substrate is summarized in Table 2. The reactors were operated for 191 days, corresponding to 4.8 HRT at full load. All reactors were operated at mesophilic (37 °C) temperature and with a stirring speed of 90 rpm. Total gas production and CO_2 content in the gas were recorded daily and gas and liquid samples were taken every week for analysis of methane, pH, and volatile fatty acids (VFA). Liquid samples (15 and 400 mL) were also taken and frozen at −20 °C for later microbial community structure analysis and for analysis of total nitrogen, carbon, phosphorus, potassium, magnesium, calcium, sodium, sulfur, organic nitrogen, and ammonium nitrogen (NH_4^+-N).

Table 1
Operating parameters of the three industrial-scale biogas plants located in Sweden.

Reactor code	HRT (day)	TM (°C)	Organic loading rate (OLR) (VS g/L/day)	Major substrate
GA	18	38	2.0	Mixed sludge
GB	55	38	2.9	Thin stillage
GC	45	38	2.0	Agricultural waste