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# Adaptation of continuous biogas reactors operating under wet fermentation conditions to dry conditions with corn stover as substrate

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## ABSTRACT

Corn stover (CS) is the agricultural by-product of maize cultivation. Due to its high abundance and high energy content it is a promising substrate for the bioenergy sector. However, it is currently neglected in industrial scale biogas plants, because of its slow decomposition and hydrophobic character.

To assess the maximum biomethane potential of CS, long-term batch fermentations were carried out with various substrate concentrations and particle sizes for 72 days. In separate experiments we adapted the biogas producing microbial community in wet fermentation arrangement first to the lignocellulosic substrate, in Continuous Stirred Tank Reactor (CSTR), then subsequently, by continuously elevating the feed-in concentration, to dry conditions in solid state fermenters (SS-AD).

In the batch tests, the <10 mm fraction of the grinded and sieved CS was amenable for biogasification, but it required 10% more time to produce 90% of the total biomethane yield than the <2 mm sized fraction, although in the total yields there was no significant difference between the two size ranges.

We also observed that increasing amount of substrate added to the fermentation lowered the specific methane yield.

In the CSTR experiment, the daily substrate loading was gradually increased from 1 to 2  $g_{vs}/L/day$  until the system produced signs of overloading.

Then the biomass was transferred to SS-AD reactors and the adaptation process was studied. Although the specific methane yields were lower in the SS-AD arrangement (177 mL  $CH_4/g_{vs}$  in CSTR vs. 105 mL in SS-AD), the benefits of process operational parameters, i.e. lower energy consumption, smaller reactor volume, digestate amount generated and simpler configuration, may compensate the somewhat lower yield.

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### 1. Introduction

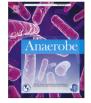
As global climate change becomes more and more extensive, the problems associated with it also become ever more of a great consequence [1]. Due to humanity's severe impact on the global climate, environment and biosphere, planet Earth is proposed to be entering the Anthropocene epoch [2]. Shifting the economy from a

traditional fossil fuel based to a more sustainable one in order to reduce carbon emissions is a major aspect of perhaps humanity's most important challenge that is to stop global warming and the environmental issues resulting from it [3].

Biomass-based energy carriers can play a central role in this effort, as they are nearly GHG-neutral [4]; and they gain more and more attention, with a current estimated global total final energy share of 14% [5]. Second-generation biofuels are to be produced from lignocellulosic biomass [6] and thus are not in conflict with crops grown for food or feed. Maize is cultivated in large quantities for nutritional purpose; its agricultural by-product is corn stover (CS). In China 300 million tons is produced annually [7], and half of the CS is abandoned and often burnt in the open field [8]. Around

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28 million tons of CS are generated annually in Europe [9] and more than 216 million is yielded annually in the USA [10]. Due to its high abundance and high energy content CS is a promising substrate for the bioenergy sector [11]. Methane production from biological wastes, like CS, through anaerobic digestion (AD) is growing worldwide and is considered to be ideal in many ways because of its economic and environmental benefits [12]. However, CS has currently very limited use in industrial scale biogas plants, because lignocellulosic substrates cannot be very effectively digested anaerobically [13]. An additional problem associated with corn stover fermentation is the formation of floating layers in CSTR reactors, which makes stirring difficult and inhibits the formation of biogas [8]. Untreated CS is therefore considered as a poor substrate in industrial CSTR systems [14].

Liquid or wet AD (CW-AD) operates at a total solids (TS) content of less than 15% while solid-state AD (SS-AD) is generally conducted at a TS content of 15% or higher [15] and is considered ideal for feedstock such as agricultural and municipal solid waste due to their low moisture content [16]. SS-AD reactors have simpler configuration [17], and it is a cheaper technique, mainly due to its lower energy and less water supply requirements [18]. The numerous advantages of SS-AD over CW-AD also include smaller reactor volume for the same organic loading; fewer moving parts; lower energy input from heating and mixing; and usually higher volumetric biomethane productivity (Vp) [14]. Furthermore, floating and crust formation problems are not present in SS-AD [19]. There are challenges, however, in SS-AD fermentation of lignocellulosic substrates as well, mainly due to the relatively low methane vield, slow methane generation and potential process instability [20]. Facing these challenges can make SS-AD a better solution for the degradation of the recalcitrant lignocellulosic substrate cost-effectively.

In this work we first tested the effect of mechanical pretreatment on CS in batch reactors to improve the efficacy of AD. In a separate set of experiments the aim of our semi-continuous fermentation experiments was to achieve efficient AD of raw CS and follow the activity of the microbial consortia from wet conditions to dry conditions with dried and milled CS as substrate. To achieve this, 5 L laboratory-scale CSTRs were employed. During the semi-continuous fermentation, the daily substrate quantity was gradually increased to 2 gvs/L/day - until the reactors started to show signs of upcoming system failure. When the total solid content and the VOA/TIC values in the fermentors appeared to be too high, the substrate feeding were kept at this constant value, then it was stopped. After some resting period, the biomass was transferred to solid-state AD reactors (SS-AD), was supplemented with additional CS and the adaptation process and the fermentation parameters were examined. The experiments revealed some challenges to be considered upon transition from CSTR to SS-AD operational mode. In addition, the consecutive CSTR and SS-AD fermentations may offer a novel strategy for biogas production from lignocellulosic substrates.

#### 2. Materials and methods

#### 2.1. Substrate specification

Corn stover (CS) was dried at room temperature, milled and sieved to a maximum particle size of either <2 or <10 mm, with an electric grinder (Retsch SM 100, Haan, Germany). The total solids (TS) and volatile solids (VS) values of the substrate were determined. The TS content was measured after drying the biomass at 105 °C until the mass remained constant. The VS values were calculated after all the organic mass of the substrate was oxidized by heating the biomass to 550 °C for 1 h.

Carbon and nitrogen contents of the substrate was measured with a Vario Analyzer Vario MAX CN (Elementar Group, Hanau, Germany). The equipment operates using the principle of catalytic tube combustion under an O<sub>2</sub> supply at high temperatures (combustion temperature: 900 °C, post-combustion temperature: 900 °C, reduction temperature: 830 °C, column temperature: 250 °C). The components were separated from each other with the aid of specific adsorption columns (containing Sicapent (Merck, Billerica, USA), in C/N mode) and determined in succession with a thermal conductivity detector. Helium served as carrier and flushing gas.

The fiber composition of the substrate on a dry weight basis was measured with a FIWE 3 Fiber Analyzer (VELP Scientifica) according to the Van Soest method [21]. The measured values are indicated in Table 1.

#### 2.2. Inoculation sludge

A fresh sample from an industrial scale mesophilic biogas plant, fed with pig slurry and maize silage mix (Zöldforrás Biogas Plant, Szeged, Hungary) was obtained, filtered through a 2 mm mesh and was used as an inoculum in the experiments.

#### 2.3. Fermentation configurations

All AD experiments were carried out under mesophilic conditions at 37 °C; in every case, methane concentration of the produced biogas was measured on a daily basis via gaschromatography to evaluate methane yields.

#### 2.3.1. Batch anaerobic digestion (B-AD)

B-AD experiments were carried out in 0.5 L glass reactors in triplicates. Substrate concentration, the amount of inoculum and diluting water were calculated according to VDI 4630 protocol [22]. The fermentation volume was 120 mL, leaving a headspace of 380 mL. The reactors were flushed with N<sub>2</sub> to ensure anaerobic conditions and were sealed with butyl rubber stoppers and aluminum caps. Gas sampling for methane concentration measurement and flushing the headspace with N<sub>2</sub> to remove the residual biogas were carried out on a daily basis. To assess the total methane yield of the substrate, the experiments were run for 72 days. A negative control sludge, containing no added substrate, was used to evaluate the residual methane potential of the inoculum, which was subtracted from the test fermentations' methane vields. The fermenters were not stirred, but were shaken manually each day before the chromatography measurement. The methane values were divided with amount of the given substrate (VS<sub>added</sub>) yielding mL CH<sub>4</sub>/g<sub>vs</sub>.

Table 1

Methane yields (CH<sub>4</sub>) in terms of mL CH<sub>4</sub>/g<sub>vs</sub> of B-AD on the basis of substrate particle size (in mm) and initial substrate concentration (VDI, multiples of the standard) or in g<sub>vs</sub>/L. The *mean* columns indicate the mean methane yields of the given size or concentration category. SE = standard error.

VDI	$g_{vs}/L$	size	CH <sub>4</sub>	SE	VDI	$g_{vs}/L$	size	CH4	SE
1.0	8.33	2	281.2	16.4	mean	mean	2	248.5	24.91
1.0	8.33	10	257.1	25.2	mean	mean	10	232.6	23.28
1.2	10.00	2	260.0	14.7	1.0	8.33	mean	269.2	23.12
1.2	10.00	10	234.5	21.3	1.2	10.00	mean	247.3	21.54
1.4	11.67	2	218.9	11.0	1.4	11.67	mean	229.8	17.50
1.4	11.67	10	240.7	16.9	1.8	15.00	mean	228.2	15.67
1.8	15.00	2	236.2	18.1	2.2	18.33	mean	228.5	23.43
1.8	15.00	10	220.2	9.7					
2.2	18.33	2	246.3	7.2					
2.2	18.33	10	210.7	19.1					

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