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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.

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 yield, 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 g_{vs}/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_2 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 gas-chromatography 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_2 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_2 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 yields. 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_{added}) yielding mL CH_4/g_{vs}.

Table 1
Methane yields (CH_4) in terms of mL CH_4/g_{vs} of B-AD on the basis of substrate particle size (in mm) and initial substrate concentration (VDI, multiples of the standard) or in g_{vs}/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_4	SE	VDI	g_{vs}/L	size	CH_4	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					

Solubles	Cellulose	Hemicellulose	Lignin	C/N	TS	VS
28% ± 2.3	32% ± 1.9	23% ± 1.4	14% ± 1.7	52.34 ± 1.1	92.73% ± 1.2	92.57% ± 1.1

2.3.2. Continuous wet anaerobic digestion experiments (CW-AD)

CW-AD tests were carried out in 5 L continuously CSTRs in a triplicate arrangement [23]. The fermentors were filled with 5 L of the inoculation sludge and incubated without fresh substrate addition until residual biogas production ceased completely. Substrate feeding was then carried out daily (particle size: <10 mm), biogas production was monitored continuously, methane content was measured daily and fermentation parameters were evaluated weekly.

2.3.3. Solid-state anaerobic digestion (SS-AD)

SS-AD experiments (Figs. 1 and 3) were carried out in packed-bed type solid-state fermentors of a unique design; the reactors consisted of a solid-phase reservoir tray with a working volume of 3 L and a 5 L percolate reservoir tank. Biogas yields were monitored constantly; methane content was measured daily. Manual feeding, subsequent mixing of the sludge and the feed with a steel spatula was carried out weekly; and percolation of the solid-phase biomass and flushing of the headspace with N_2 was carried out twice a week (particle size: <10 mm). A schematic for the SS-AD system is shown in Supplementary Fig. 1.

In CW-AD and SS-AD the differences in feeding rates were taken into account in the calculation of gas productivities, the methane values were divided as a function of the given substrate VS_{added} yielding mL CH_4/g_{vs}/feeding phase.

2.4. Bio-methane concentration

Bio-methane concentrations in the headspace were measured on a daily basis with an Agilent 7880 Gas-chromatograph (GC), on a HP Molesieve column, with a length of 30 m and an inner diameter of 0.53 mm, equipped with a Thermal Conductivity Detector (TCD). The carrier gas was Ar, oven (column) temperature was 37 °C, flow velocity was 1.2 mL/min and detector temperature was 160 °C. 250 μL Hamilton syringes were used to inject 100 μl of gas sample.

2.5. Fermentation parameters

Total organic acid, total acid capacity (VOA/TIC) values were measured using a Pronova FOS/TAC 2000 device; pH was measured using a Radelkis OP-211/2 device. OLR (Organic loading rate) values are provided in the Results section. HRT (Hydraulic retention time) values were not calculated because for solid substrates that have no precise volume they are not relevant.

2.6. Statistical analysis

Analysis of variance (ANOVA) test were carried out using the R programming language [24].

3. Results and discussion

3.1. Batch fermentation of corn stover: effect of substrate concentration

To assess the biogas production from <10 mm to <2 mm corn stover monosubstrate B-AD reactors were assembled with several differing substrate concentrations, i.e. 8.33, 10.00, 11.67, 15.00 and

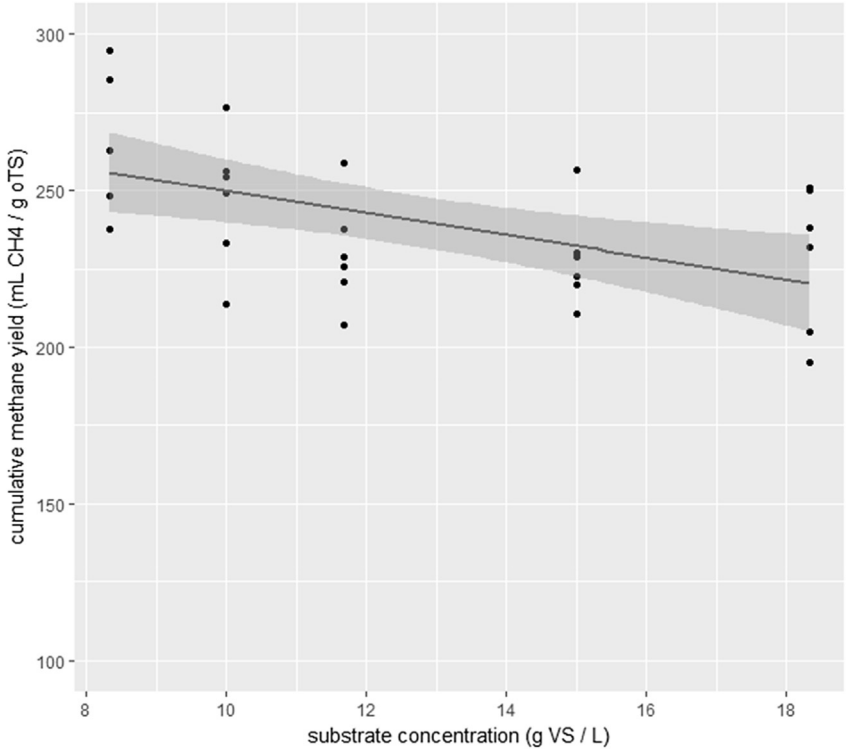

Fig. 1. Total methane yields regarding the B-AD experiments as a function of substrate concentration. Grey line: correlation of total methane yields and substrate concentration, grey area: 95% confidence interval region of the regression line.

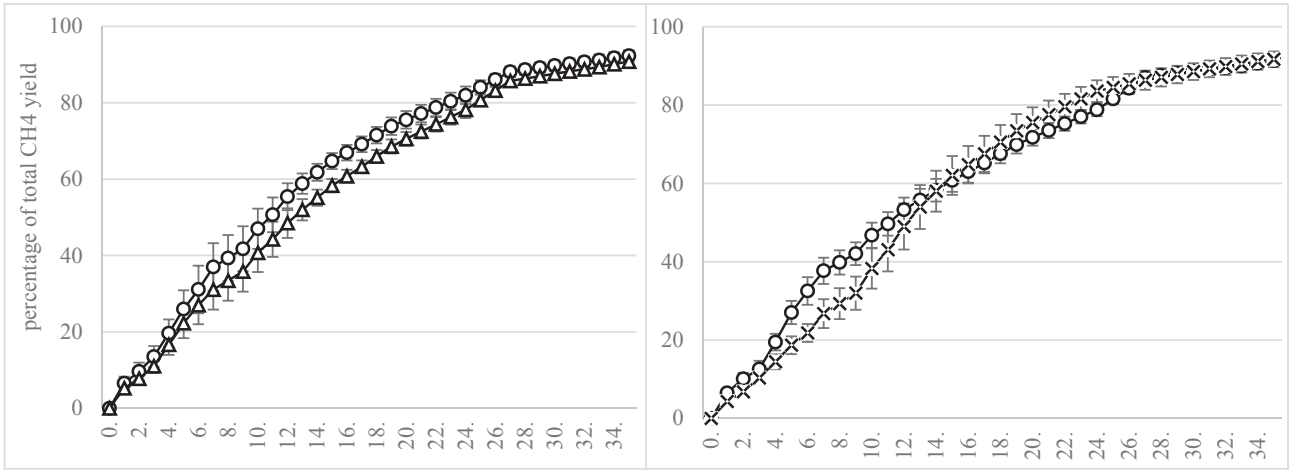

Fig. 2. A. and **B.** Mean proportional total methane generation of the fermenters on the basis of particle size (A) at particles size <2 mm (circles) and <10 mm (triangles); and substrate concentration (B) at 8.33 g_{vs}/L (circles) and 18.33 g_{vs}/L (X-es).

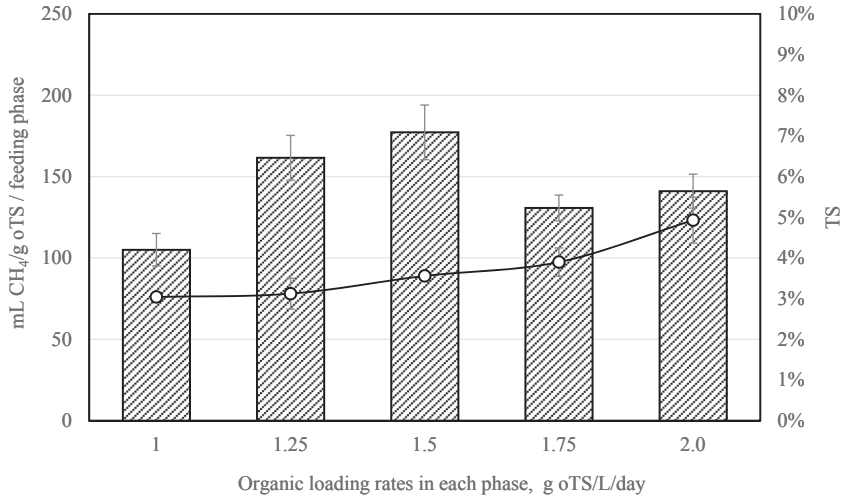

Fig. 3. Specific CH_4 yields (bars) regarding the feeding phases and respective TS values (circles) of the fermentors (percentage) in CW-AD.

18.00 g_{vs}/L, which were equivalent to VDI concentration ratios of substrate vs. inoculum of 1.0, 1.2, 1.4, 1.8 and 2.2. By the end of the 72-day period, the daily methane evolution was less than 0.5% of the total methane yields. 90% of the total methane yield was achieved by day 35, whereas 95% was produced by day 43. Fig. 1 shows the relationship between substrate concentrations and specific methane yields.

Linear regression model and Pearson's correlation coefficient (ρ) was calculated. A moderate negative correlation of −0.517 was found (Fig. 1.) with a *p*-value of 0.0033, corroborating the negative effect of elevated substrate concentrations on specific methane yields.

The corresponding methane yield data are collected in Table 1.

The analysis using ANOVA indicated statistically not significant differences between the <2 mm and <10 mm sized samples (*p*-value = 0.082) regarding the total methane yields of all substrate concentrations. Pairwise comparisons for each substrate concentration regarding the total methane yields for 2 and 10 mm substrate sizes yielded also non-significant differences (*p*-values for concentrations of 8.33, 10.0, 11.67, 15.00 and 18.33 are: 0.25, 0.17, 0.15, 0.27 and 0.07 respectively). However, the average rate of biomethane generation was higher in the <2 mm than in the <10 mm samples. During the first 3 weeks the mean difference was 5.06%, which decreased to 3.68% by day 35 (Fig. 2A). Alteration in the substrate concentration did cause a significant difference in terms of total methane yields (*p*-value = 0.0066). Similar differences to those seen in the case of particle size were observed in the rate of methane generation: during the first 3 weeks the mean difference between VDI 1x (8.33 g_{vs}/L) and VDI 2.2x (10.0 g_{vs}/L) was 4.96%, which decreased to 2.94% by day 35 (Fig. 2B.). The time required for the fermentations to produce 90% of the total methane yield were shorter with an average of 4 days in the <2 mm particle size CS samples than in the ones containing <10 mm sized CS.

These findings are in line with those reported by Böjti et al. (this issue). Similar results were obtained using wheat and/or rice straw or other lignocellulosic biomass. As early as in 1988 Sharma and coworkers [25] found that particle size of 0.1–0.4 mm was optimal for several fibrous biomass AD substrates. The employed milling technology influences the resulting biogas yields [26]. A gradual improvement of biogas kinetics in the particle size range 0.76 and 0.20 mm was found in the case of wheat straw, but the overall methane yield did not change accordingly [27]. Differences in the behavior of wheat and rice straw was noted in mesophilic B-AD tests although generally the 0.75 mm average sized particles gave

the best results [28]. This is very close to the <2 mm size range found optimal in our studies. Similar conclusions were drawn by Scherer and colleagues recently [29]. The main conclusion to be drawn from these and our studies is that decomposition kinetics rather than methane yield is affected by particles size and the various lignocellulosic biomass sources have somewhat different optimal particle size ranges for most efficient decomposition albeit within a narrow size breadth. It is noteworthy that the lignin content of cereal straws was significantly higher (23.5%, [29]) than that of the corn stover (14%, see above) but apparently this did not have as pronounced effect on biodegradability of the lignocellulosic biomass as the particle size.

The results from our B-AD experiments yielded an average methane production of 269.2 mL CH_4/g_{vs} (SD: 23.1) for an initial substrate concentration of VDI 1x (8.33 g_{vs}/L), i.e. as prescribed by the VDI protocol, and substrate to inoculum ratio (S/I) of 0.5 (average of particle sizes <2 and < 10 mm). This is lower than the 360 mL/g_{vs} (SD: 0.003) reported for similar batch fermentation carried out for 70 days [30]. Nevertheless, a very similar result to our findings has been reported recently [29], i.e. 271 mL CH_4/g_{vs} for wheat straw. The biological methane potential tests carried out on CS determined 260.7 mL CH_4/g_{vs} (SD: 8.9) with a S/I = 0.5 in accordance with VDI standards [31]. This is close to the 234 mL CH_4/g_{vs} measured on 1.9 mm wheat straw particles [32] indicating that subcellular structures of lignocellulosic biomass sources do not influence extensively the biogas yield if similar operational parameters are employed. Using S/I = 3, however, caused all fermentations to fail when CS was used as mono-substrate. In our experiments S/I = 1.25 did not cause process failure although the inhibitory effect of elevated substrate concentration was clearly apparent. In this experimental arrangement floating layer formation-associated problems were not encountered.

3.2. Continuous AD experiment under wet conditions in CSTR (CW-AD)

The inoculum source affects the anaerobic digestion process greatly [33]. Therefore the remaining digestate from the batch fermentation experiments, which was adapted to CS, was used to amend the fresh inoculum sludge in the CW-AD tests. The inoculum was incubated to remove residual biogas before it was mixed with the digestate from B-AD. Since both particle sizes in B-AD yielded about the same amount of methane, yet the energy demand for milling is evidently higher in the case of <2 mm particle size and the <10 mm particle size is more appropriate selection from an industrial point of view, this was chosen as substrate in the following experiments. Feeding phases denote periods of the fermentation, during these periods the fermentors were fed with the respective OLR. The duration of the feeding phases were 9, 21, 15, 10 and 24 days.

In order to adapt the system to an increasing substrate concentration, the initial organic loading rate (OLR) of 1 g_{vs}/L/day (recommended by the VDI protocol 4630) was gradually elevated. The specific methane yields for each feeding regime are presented in Fig. 3. The initial 105 mL CH_4/g_{vs}/phase increased to 162 when the OLR was elevated to 1.25. 1.5 g_{vs}/L/day was the optimal OLR, yielding the highest specific methane production, i.e. 177 mL CH_4/g_{vs}. Further increase of OLR resulted in lower specific methane yields (data not shown).

Fig. 4 presents the pH and the VOA/TIC values of the fermentations. The drop in the methane yields in the feeding period of 1.75 g oTS/L/day was accompanied by a slight change in pH and imbalanced fluctuation of VOA/TIC. When the substrate loading rate was elevated to 2 g oTS/L/day, the pH dropped and the VOA/TIC raised, indicating that the digesters started to accumulate VFAs and deter the metabolism of the methanogenic archaea. The low methane yield in this period was most likely due to the increased VOA levels brought about by the elevated TS, which exceeded the buffering capacity of the system. Nevertheless, these operational parameters remained within the tolerable range and the system still produced a fairly good amount of methane (Figs. 4 and 5.). Since our aim was to find the highest loading rate and TS value that did not cause the fermentors to collapse yet, the loading rate was not increased above 2 g oTS/L/day. After 3 weeks the feeding was stopped completely, and after some resting period (of 5 days) the sludge was transferred into the solid state fermentors.

Available data on the continuous fermentation of CS is limited in the relevant literature. Batch studies have been used more frequently to evaluate the biodegradability and methane production potential of organic substrates. Li and colleagues elevated the OLR in their experiments to 4 g_{vs}/L/day [31], although they used a previously proven appropriate ratio [34] of corn stover and chicken manure and found stable methane yields of 223 ± 7 mL/gc_{vs}. Compared to that, our results are lower but the mono-digestion of corn stover is more challenging for the microbes resulting from the high C/N ratio and TS content.

3.3. Solid-state AD experiments (SS-AD)

To adapt the inoculum to dry conditions, the sludge from the previous CSTR experiment was transferred to two identical and parallel operated solid-state anaerobic digesters (SS-AD), was supplemented with additional CS (particle size: <10 mm) to maintain about 25% of total solid content or to around 15% VS (organic loading = 20 g_{vs}/L). Feedstock:effluent ratio (F: E) is considered an important parameter of SS-AD assembly [35], thus in order to reactors be 'healthy', this was set to 1. The fermentation process was monitored in the form of VFA composition and methane production measurements (Fig. 5.). On the basis of feeding occasions the total fermentation process was divided into 5 phases, which were termed the following: Transfer, Adaptation, Stabilization, Inhibition and Starvation. After the initial high organic loading, the subsequent feeding rates (phases 2–4) were lowered to 12 gvs/L.

The Transfer phase showed very low methane production, probably as the consequence of a high VOA concentration due to the shock feeding during the transfer of the sludge and sudden increase of biomass loading to reach SS-AD condition. After the initial shock and a lower amount of organic loading, the reactors recovered and the methanogens started to convert the VFAs into a reasonable methane yield in the Adaptation period. The highest methane (98.5 mL/g_{vs}) production was observed during phase 3. In the next period (Inhibition) propionate accumulated and methane evolution started to decrease. The cause of this is not evident, as the conditions were not altered; perhaps inhibitory lignin mounted up or the system became nitrogen or trace element limited. Nevertheless, methanogenesis continued during this phase (80.7 mL/g_{vs}) suggesting that long-term biogas production from CS mono-substrate could be achieved in this SS-AD design. In the last phase of the SS-AD experiment (Starvation) the reactors were not fed any longer to see how much residual methane was produced. During this phase nearly all of the remaining VFAs were utilized by the microorganisms, only some acetate was left by the end of day 63. Methane production was low and eventually stopped.

Very high VOA levels and especially high acetate concentrations are indicators of process imbalance [36]. The propionate to acetate ratio is also generally regarded as an important parameter of methanogenesis, thus the accumulation of these acids were tested for correlations against methane yields. The values from the Starvation phase, when the fermentation stopped, were left out from

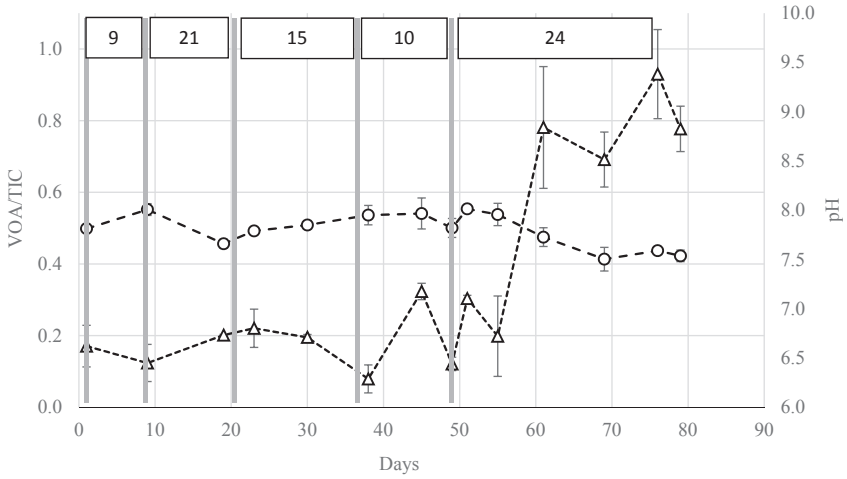

Fig. 4. VOA/TIC (triangles) and pH values (circles) during CW-AD. Vertical grey lines indicate the start of the phases with organic loading rates of 1, 1.25, 1.5, 1.75 and 2 g oTS/L/day, respectively (see Fig. 3). Numbers in boxes above indicate the number of days of each respective phase.

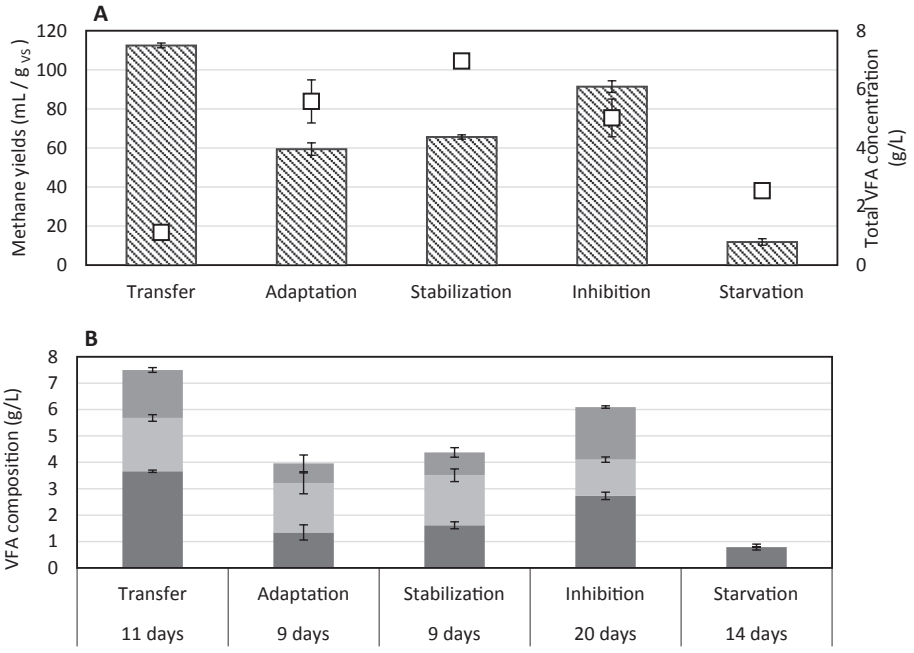

Fig. 5. A.) Phase-specific methane yields (rectangles) and total VFA concentrations (VOA, bars) and **B.)** phase-specific VFA composition (acetate: dark; propionate: light grey; isobutyrate: dark grey bars) during SS-AD. Numbers below the diagram indicate the number of days of each respective phase.

the calculation.

For acetate (Fig. 6. A.) an R^2 value of 0.721 and a *p*-value of 0.008 was found, corroborating a significant linear negative correlation and that indeed high acetate levels indicated disturbed methanogenesis. As established by Hill et al., in 1987 [37], acetate levels in wet-operating biogas fermenters above 0.8 g/L signaled impending digester failure. With a TS content of approximately 3 times as high in SS-AD, the alarming acetate levels above 2.5 g/kg$_{vs}$ observed in these experiments are in accordance with the earlier CW-AD results.

In the case of propionate to acetate ratio a quadratic polynomial equation fitted the data best: an R^2 value of 0.357 and *p*-value of 0.112 were found (Fig. 6. B.). Although the correlation found is neither strong nor significant, it suggests that extreme propionate concentration may be associated with inhibition of the methanogenesis. Inhibition occurred at propionate to acetate ratios higher than 1.35, which also seem to be in line with Hill et al. [37]. For VOA levels (Fig. 6. C.) an R^2 value of 0.740 and a *p*-value of 0.011 was found, indicating a significant linear negative correlation with methane generation, perhaps as a consequence of a drop in pH levels, in the samples with VOA levels of 6 g/kg$_{vs}$ or higher (reactors shortly after the transfer). In their recent work Franke-Whittle et al. [38] found that if the reactors had a good buffering capacity, then VFA accumulation alone is neither causing the fermentors to fail nor the composition of the methanogenic population to alter significantly. In accordance with that finding, our results suggests that only a radical elevation in these process parameters were detrimental to the fermentation, i.e. above 1.4 propionate to acetate ratio and 2.8 or 6 g/kg$_{vs}$ for acetate and VOA concentration, respectively.

SS-AD of CS showed promising results, after the transfer from CW-AD to SS-AD the microbial community recovered well, but

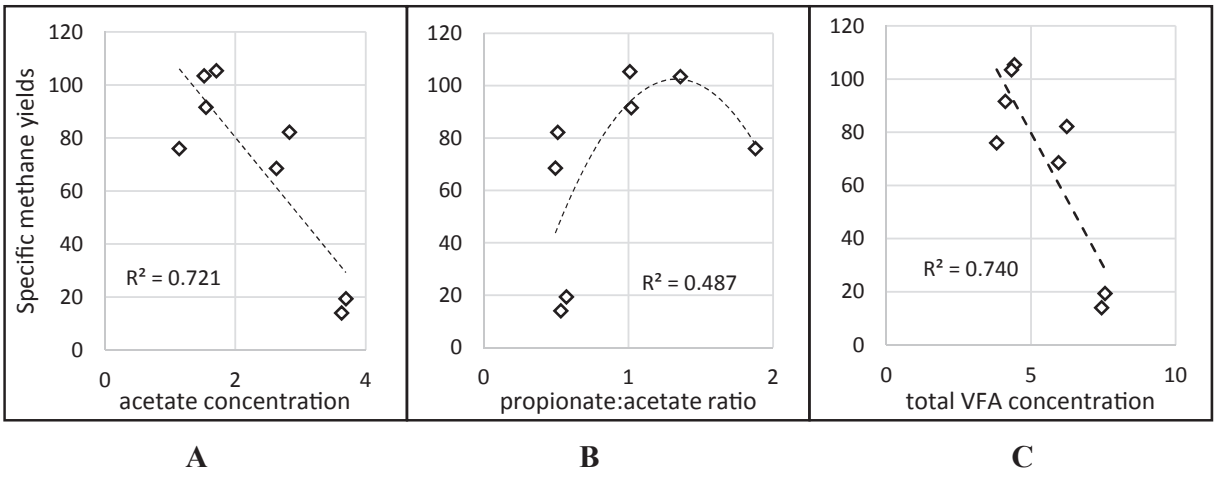

Fig. 6. Correlation of acetate concentration (g/kg$_{vs}$) A.), propionate:acetate ratio B.) and VOA (g/kg$_{vs}$) and C.) to methane yields (mL CH_4/g$_{vs}$). R^2 values for the regression lines are indicated.

methane yields were less, compared to wet fermentation conditions (177 vs. 105 mL CH_4/g$_{vs}$). This difference may be the consequence of high organic loading in the "transfer" period. Brown and colleagues [18] compared batch SS-AD to CW-AD of different lignocellulosic feedstocks. They found only a slight difference in methane yields between SS-AD and CW-AD of corn stover (132 vs. 124 mL CH_4/g$_{vs}$), however volumetric biogas productivity (Vp, mL CH_4/g$_{vs}$/L fermentor) of SS-AD was 7-fold greater than that with CW-AD. In our experiments a slightly higher Vp of 28.62 (SE = 5.58) was observed in the CW-AD, relative to 25.11 (SE = 4.26) in the case of SS-AD. We employed a F:E ratio of 1, instead of the recommended 3 [14], which may be the reason for the low Vp.

Microbial community changes drive the different productivities of the fermentations, thus monitoring the changes in the microbial communities may give insights into how to operate the fermentors more efficiently. A comparison of the microbial community in CW-AD against SS-AD were presented in a recent work [39] and the community profiles were shown to be very similar at least at higher taxonomic ranks, although process parameters, substrates and technology differed between the wet and dry biogas fermentations analyzed in the study. The results illustrated that core community taxa perform key functions in biomass decomposition and methane synthesis. Regarding methanogenesis, it was found that *Methanoculleus bourgensis* MS2T dominated the dry fermentation process, suggesting the adaptation of members belonging to this species to specific fermentation process conditions. The microbial changes during the adaptation process that took place in our work will be evaluated and reported separately via metagenomic assessment in order to deepen our understanding of the underlying microbiological ecology.

4. Conclusions

The batch AD experiments showed that CS could be a suitable substrate in batch fermentors to an initial concentration of at least 18.00 g$_{vs}$/L without pretreatment. The differences between total methane yields of <2 and < 10 mm particle sizes were small, but the fraction composed of smaller particle size decomposed faster.

Mono-fermentation of CS could be carried out via CSTR to an OLR of at least 2 g$_{vs}$/L/day. The sludge was adapted to dry conditions, and subsequently semi-continuous fermentation using the SS-AD technology could be carried out. In order to deeply understand the underlying changes in the microbial ecology, further experiments with the focus of metagenomic evaluation of the process are in progress.

As far as we know AD of CS (or other lignocellulosic substrates for that matter) in a laboratory-scale continuously fed CSTR, followed by a semi-continuously fed SS-AD system in a sequential adaptation manner, has not been studied before. This combination could be a useful strategy in industrial scale biogas fermentation to handle the accumulating undigested biomass and regain the biogas potential from lignocellulosic materials.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.anaerobe.2017.05.015.

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