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PII: S0960-8524(17)31442-6
DOI: http://dx.doi.org/10.1016/j.biortech.2017.08.128
Reference: BITE 18737

To appear in: *Bioresource Technology*

Received Date: 5 July 2017
Revised Date: 18 August 2017
Accepted Date: 20 August 2017

Please cite this article as: Rennuit, C., Triolo, J.M., Eriksen, S., Jimenez, J., Carrèlre, H., Hafner, S.D., Comparison of pre- and inter-stage aerobic treatment of wastewater sludge: effects on biogas production and COD removal, *Bioresource Technology* (2017), doi: http://dx.doi.org/10.1016/j.biortech.2017.08.128

Comparison of pre- and inter-stage aerobic treatment of wastewater sludge: effects on biogas production and COD removal

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Abstract

The aim of this study was to investigate thermophilic (55°C) aerobic digestion (TAD) as pre- and inter-stage treatment of sludge anaerobic digestion and to analyse the change in organic matter accessibility and complexity. Pre-treatment decreased methane yield (up to -70%), due to oxidation losses whereas inter-stage treatment slightly improved overall methane yield (+2.6%) and total COD removal (+5%) compared to control. Anaerobic degradability and COD removal in the second anaerobic stage significantly increased, by 13 to 40%. Organic matter fractionation showed that TAD led to an increase in sludge organic matter accessibility in all cases. Organic matter complexity, measured by fluorimetry, increased after TAD pre-treatment whereas it remained constant after inter-stage treatment. TAD was shown to

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Preprint submitted to Bioressource Technology *August 23, 2017*

be more efficient if applied to a more recalcitrant substrate and should thus be used as inter-stage treatment to avoid decreasing methane production.

Keywords: recalcitrance, accessibility, complexity, organic matter loss, methane

1. Introduction

Anaerobic digestion is a proven technology for energy recovery and sludge stabilisation (Pèrez-Elvira et al., 2006). Most substrates–but especially lignocellulosic and bacterial cell biomass–are only partially degraded during anaerobic digestion, and various treatments to increase anaerobic conversion of recalcitrant organic matter have been developed (Carrère et al., 2010; Monlau et al., 2013). Chemical and physical treatments led to increase conversion efficiency but are often energy-intensive and expensive, and chemical treatments can harm downstream biological processes (Pèrez-Elvira et al., 2006). To avoid those drawbacks, biological treatments can be used.

Combined aerobic-anaerobic biological treatments more completely degrade sludge and other organic wastes than either does alone. Despite some comparison, exactly how organic matter utilisation differs between aerobic and anaerobic communities is not clear (Burton, 1992; Kumar et al., 2006; Dumas et al., 2010; Tomei et al., 2011; Monlau et al., 2013; Braguglia et al., 2014; Cheng et al., 2015).

Among aerobic treatments, thermophilic aerobic digestion (TAD) has been combined with anaerobic digestion to increase biogas production and organic matter destruction of municipal wastewater sludge. From literature, effect of TAD pre-treatment on COD and VS reduction are unanimous but

effects on biogas production are inconsistent (Jang et al., 2014; Dumas et al., 2010; Hasegawa et al., 2000; Pagilla et al., 2000; Ward et al., 1998). One study reported an increase in biogas production from swine manure (Pagilla et al., 2000) and another from wastewater sludge (Jang et al., 2014) but in the latter case it is not clear whether TAD really increased overall methane production as COD mass balance and methane production were not consistent. In other studies aerobic pre-treatment did not affect or even decreased biogas production despite an increase in substrate destruction and anaerobic degradability (Ward et al., 1998; Hasegawa et al., 2000). Co-treatment, where some of the digestate recirculated to the digester is treated in a TAD reactor (65°C), led to similar results (Dumas et al., 2010). In general, TAD as a pre-treatment for biogas production has not been popular because it oxidises organic matter, leaving much less substrate available for anaerobic conversion (Le, 2006).

Substrates of biological origin contain a mix of materials with a wide range in degradability (Rittmann and McCarty, 2001), and it is the most degradable of these that is oxidised to the greatest extent during aerobic biological treatment. The place of the biological treatment in a production chain influences the success of the process. We hypothesised that inter-stage TAD can increase anaerobic conversion and reduce oxidation loss by ensuring that the most degradable substrate is converted to methane prior to aerobic treatment. This study compared the use of TAD as a pre- and inter-stage treatment in terms of biogas production and organic matter removal during anaerobic digestion of municipal wastewater sludge. Changes in organic matter accessibility and complexity for both configurations were also investi-

gated. Furthermore, we proposed a simple framework for understanding and evaluating aerobic biological treatments.

2. Materials and Methods

Four experiments were carried out: two with pre-treatments (P1 and P2), and two with inter-stage treatments (I1 and I2) (Fig. 1). They provided data to evaluate anaerobic degradability and methane production from TAD effluent, to characterise the effects of the TAD treatment and to measure the overall effect of the treatment chain on methane production and COD removal.

2.1. Substrates

Original substrates were raw municipal wastewater sludges and digestates from a wastewater treatment plant producing biogas (VCS, Ejby Mølle, Denmark; treating capacity 385 000 person equivalents) (Table 1). The digesters at the plant are fed a mixture of primary (60%), dewatered secondary sludge (40%) and highly degradable organic waste (depending on availability). Secondary sludge dewatered by centrifugation (including polymer addition) was the substrate in P1, P2, and I2. Secondary sludge alone was used because it is generally to be more recalcitrant to biogas conversion than primary sludge. For I1, original substrate was the full-scale original feed in order to better assess the effect of treatment under the plant conditions.

2.2. Batch thermophilic aerobic digestion

The TAD reactor was 3 L, aerated with compressed air, heated to 55°C by a heating plate and stirred by three flat blade impellers. TAD feed was de-

watered secondary sludge for pre-treatment experiments and digested sludge for inter-stage treatment experiments.

TAD inoculum was collected from a semi-continuous TAD reactor that had been running for at least two weeks, and was fed secondary dewatered sludge every 4 to 5 days. Inoculum was taken before feeding to ensure that its COD was low. Reacting mass was about 1.5 kg to provide sufficient headspace in case of foaming. Inoculum-to-substrate ratio was 1:4 based on wet mass. Mixing rate was > 1150 rev·min^{-1} to break up foam. Aeration rate was 0.25 L·kg^{-1}·min^{-1} (L air per kg reacting mass) at the start of TAD and remained constant for P1 and I2. It was not adjusted after each sampling for P2 and I1 and aeration rate was 0.36 and 0.4 L·L^{-1}·min^{-1} at the end of P2 and I1 respectively. Duration of P1 was 5 days with sampling every 24 h. The other experiments lasted 24 h with 3-4 intermediate samplings (data not shown). Initial samples taken after mixing of TAD inoculum and substrate but before aeration served as controls to evaluate the effect of TAD treatment. For P2, I1 and I2, heat-only samples (55°C, no aeration) were included to assess the heat effect. Following TAD or heat treatment, all samples were subjected to anaerobic digestion.

2.3. Anaerobic digestion

First stage anaerobic digestion for I1 took place at the full-scale digester of the wastewater treatment plant (37°C, average HRT of 28.6 days in 2015).

For I2, first stage anaerobic digestion was conducted at 37°C for 25.5 days in (20 L) stirred reactor in batch mode. Anaerobic inoculum was digestate from the same wastewater treatment plant in all cases. Inoculum-to-substrate ratio was 1:1 based on wet mass (COD ratio ca. 0.5:1).

Post-TAD anaerobic digestion was carried in batch in the laboratory for 20 days at 37°C. Reacting mass (50-100 g) was put into 0.1-0.5 L glass serum bottles, sealed with butyl septa and screw caps, and flushed with N_2. Target substrate mass was 10 to 16 g of effluent from the TAD reactor. Inoculum-to-substrate ratio was relatively high (2.5:1 based on COD) to avoid any inoculum limitation. Quality of inoculum was checked according to VDI (2006). Contribution of the anaerobic inoculum to the methane volume was measured in inoculum-only bottles and subtracted. All conditions were run in triplicate.

Biogas volume was measured every five days or more frequently using syringes. Measurements were checked using a gravimetric approach (Hafner et al., 2015). Gas samples were collected at each volume measurement in 10 mL vacuum vials and analysed for methane and carbon dioxide using a gas chromatograph equipped with a thermal conductivity detector (Agilent 7890A, column: J&W 113-4332GS – GASPRO, oven temperature 250°C).

2.4. Sample handling and analysis

COD was measured in triplicate using Hach COD vials (Hach Company, Loveland, CO, USA) based on sample mass. If necessary, samples were stored at 4°C before analysis for a maximum of 2 days.

Evaluation of accessibility and complexity of the organic matter before and after TAD treatment was done on frozen samples from P1 and I2 following Jimenez et al. (2014, 2015). Bioaccessibility was quantified based on COD solublisation after extractions with successively stronger chemicals (Jimenez et al., 2015). This approach is based on the assumption that bioaccessibility follows chemical accessibility, as it has been shown for wastewater sludge by

Jimenez et al. (2014). Fractionation resulted in six fractions as defined in Fig. 2. The more COD is found in the top fractions (DOM, SPOM, REOM), the more the substrate is considered to be accessible (Fig. 2). Fractionation was done in duplicate. Successive extractions were done on 1.5-3 g pellets using around 10-30 mL of extractant (8 mL for 1 g of pellet).

Complexity was quantified based on 3D fluorescence spectroscopy (Perkin Elmer LS55) for one replicate per extracted fraction. Excitation wavelengths ranged from 200 to 600 nm with an increment of 10 nm. Based on coordinates of excitation-emission wavelengths, resulting spectra were divided in seven zones corresponding to biochemical family-like fluorescence. The simplest molecules (e.g. amino acids) are located in the zones 1 to 3 and the more complex molecules are located in the zones 4 to 7. Finally, the proportion of total fluorescence in each zone was calculated by integrating the fluorescence intensity and zone area (Jimenez et al., 2015).

Complexity characterisation is a qualitative tool as only aromatic molecules can be quantified by fluorimetry.

To characterise the changes, complexity was related to the abundance of each fraction obtained in the accessibility analysis.

2.5. Data treatment

Data processing and statistical analysis was done in R (R Core Team, 2017). Cumulative methane production was calculated using the biogas package (v. 1.6) (Hafner and Rennuit, 2015) and statistical analysis using the stats package (R Core Team, 2017). Effect of treatment was evaluated using a two-factor (factors were experiment and a binary factor for TAD treatment) analysis of variance (ANOVA) at $\alpha = 0.05$.

Anaerobic degradability was calculated by dividing the methane production by the theoretical methane production expected from 1 g of COD (350 $mL \cdot g^{-1}$) (Rittmann and McCarty, 2001). To evaluate anaerobic degradability of TAD effluent, methane production was normalised by the COD of the TAD effluent. To calculate anaerobic degradability of initial sludge methane production at the different stages was normalised by the COD of the initial sludge (TAD inoculum contribution subtracted). Characterisation of the TAD treatment was done by analysing changes in accessibility and complexity, as well as by COD mass balance. COD mass balance was conducted by normalising the residual COD, oxidised COD and COD converted into methane by the COD of the TAD influent (containing the TAD inoculum). COD conversion to methane (g COD per g substrate wet mass) during the anaerobic stage was calculated by dividing cumulative methane production after 20 days (normalised by substrate wet mass) by 350 $mL \cdot g^{-1}$ (mL CH_4 per g COD) (Rittmann and McCarty, 2001). Overall performance of the treatment chain was calculated by normalising the methane production and COD removal by the COD of the initial sludge and TAD inoculum contribution was subtracted. Normalisation of methane production were made using the COD concentration of the substrate.

2.6. Oxidation losses and anaerobic degradability

We assumed that aerobic biological treatment consists of two processes with opposing effects on methane production: 1) increase in substrate anaerobic degradability and 2) loss of substrate through oxidation by the microorganisms carrying out the treatment, and based on the oxidation losses, we quantified the minimal increase in anaerobic degradability required to in-

crease methane production. This implies that a successful treatment would increase methane production (overall methane production) only when increased anaerobic degradability (after treatment) is higher than the loss due to substrate consumption and oxidation. Hence, the mass of COD converted into methane from the treatment process must be greater than the mass of COD converted into methane into the control process, as shown in Eq. 1.

$$d_2 > d_1/(1-l) \tag{1}$$

where d_2 and d_1 are the fractional conversion of COD to methane after the treatment and for the control control and l is the fraction of initial substrate COD lost to oxidation during treatment (in $g{\cdot}g^{-1}$ (g COD per g total COD)).

If the potential fractions anaerobically converted to methane before and after the treatment (d_1 and d_2) are known, maximum loss of substrate by oxidation could be found by solving Eq. (1) for l.

3. Results and discussion

3.1. Anaerobic degradability and methane production after TAD treatment

Contrary to TAD treatment of raw sludge (P1 and P2), anaerobic degradability and methane production increased with the treatment of digested sludge (I1 and I2) (Fig. 4). Anaerobic degradability of TAD effluent was reduced by more than half after 1 day of pre-treatment (from 0.43 to 0.19 in P1 and 0.67 to 0.19 in P2).

It increased by 13 to 40% with inter-stage treatment (from 0.19 to to 0.22 after 1 d for I1, and from 0.14 to 0.20 after 1 d for I2, maximal increase for I1 was after 4.6 h, where anaerobic degradability increased from 0.19 to

0.24). In I1, a maximum increase in methane production from stage 2 of around 25% was obtained at 4.6 h in TAD and around 40% more methane was obtained after 24 h in TAD in I2 (Fig. 4) Heat control reactors yielded in average increases of 10% and 10-20% of second stage methane production for P2 and I1 respectively while a decrease of around 9% was observed in P2. A COD loss of around 5% was observed after the heat control in P2 (from 64.3 ± 0.34 to 61.3 ± 0.65 g·kg^{-1}). No changes in COD after the heat treatment in I1 and I2 were observed(data not shown). The increase observed after inter-stage treatment was close to the +50% increase by micro-aeration found by Hasegawa et al. (2000) after pre-treatment of secondary sludge and considering the low anaerobic degradability of the digested sludge compared to the one of raw sludge (0.19 vs 0.64 in I1, and 0.14 vs 0.76 in I2) this percentage of increase was quite large.

Treating digested sludge, as done with inter-stage treatment, reduced oxidation losses and resulted in a larger increase in anaerobic degradability of remaining substrate than did pre-treatment of raw sludge. For a successful pre-treatment, calculations with eq. 1 show that with the pre-treatment as it was done on raw sludge it was impossible to increase methane production (from the calculations anaerobic degradability d_2 should increase by 85% and more than 100% (P1 and P2)). This theory was confirmed by the experimental results. For treatment of digested sludge, increase in anaerobic degradability should be at least 18 to 33% (Eq. 1), which could be achieved in I1 and I2.

3.2. Characteristics of TAD influent and effluent

3.2.1. COD mass balance and accessibility

For all experiments, COD removal in TAD increased monotonically with treatment time (intermediates times not shown) but treatments effects on the raw and digested sludge were different.

Treatment of digested sludge by TAD resulted in much less COD removal in TAD than treatment of raw sludge (COD was reduced by 17 ± 1% and 6.8 ± 0.8% after 24 h for I1 and I2 respectively vs. 31 ± 3 and 32 ± 2% for P1 and P2, Fig. 3).

The relative increase in DOM (compared to total COD after TAD) was around 10% for both treatments (Fig. 5) showing that accessibility was improved after TAD.

Those results are in accordance with Ward et al. (1998) and Hasegawa et al. (2000) who found an increase in solubilisation after TAD.

However, differences were observed in the the pool size of the DOM fraction: it was not affected by pre-treatment and increased with interstage (+0.8% vs +76% increase in pool size) (Fig. 5). All other fractions decreased with pre-treatment (from -24 to -83% for PEOM and REOM). During inter-stage treatment PEOM and NEOM fractions remained stable while REOM and SEOM significantly decreased (-50 and -43%) and SPOM was reduced by 18%.

Accessibility after inter-stage TAD was most likely improved by the mean of solubilisation of hydrolysis products while the increase after pre-treatment is most probably explained by the large reduction in total COD observed after pre-treatment.

The additional DOM fraction observed after the inter-stage treatment may have been derived from solubilisation of the SPOM and REOM compartment as they were reduced by inter-stage treatment (SPOM and REOM together decreased by 2.4 g·kg^{-1} while DOM increased by 2.5 g·kg^{-1} (g COD per kg wet mass)). The real mechanism might be a more complex transfer between compartments more than a direct solubilisation from less accessible layers to DOM. As the effect measured in heat controls was much less than half of the observed effect on solubilisation in TAD (the heat control increased the DOM size by +24%), it can be concluded that solubilisation was due to microbial activity under aeration and was enhanced by thermophilic temperature.

Can solubilisation alone explain the changes in methane production observed? From a COD mass balance, DOM could not be the only source of CH_4, since the CH_4 produced was greater than the sample DOM both before and after TAD treatment (7.9 vs 5 and 6.9 vs 5.8 g·kg^{-1} g COD per kg wet, for P1 and I2 after treatment). Complexity changes in other less accessible compartments due to aerobic treatment could have also influenced methane production (as for instance, it occurred for the REOM after inter-stage TAD (Fig. 6)).

Moreover, not all the DOM produced was converted into CH_4. The increase in DOM fraction was greater than the COD converted into methane: compared to the reference 2 g·kg^{-1} was converted to methane while 2.5 g·kg^{-1} (g COD per kg wet mass) was solubilised in the DOM during the inter-stage treatment. This was also true for the heat control. Hence, in accordance with (Kim et al., 2013), it cannot be assumed that there was a simple re-

lationship between increased COD fraction and higher methane production found in this study.

3.2.2. Complexity

Total fluorescence percentage of complex zones (4 to 7) was higher after both treatments (except for REOM after inter-stage) and compared to control the proportion of complex zones was greater after pre-treatment than after inter-stage (Fig. 6). These trends were also observed for the most represented organic fractions (DOM, SEOM and PEOM). When relating changes in complexity to the abundance of each fraction it was found that complexity clearly increased after pre-treatment while it remained in the same range after inter-stage.

In general the complexity of digestate was greater for all fractions as compared to secondary sludge except for the PEOM fraction.

The most accessible organic matter fractions (DOM, SPOM, REOM) constituted a smaller part of the overall COD in digested sludge as compared to secondary raw sludge ($16 \pm 0.5\%$ versus $12 \pm 0.9\%$). Digestate was less accessible and more complex than the raw secondary sludge, as found by Aemig et al. (2016). This is also supported by the much lower anaerobic degradability measured in digestate.

As a large COD reduction and an increase in DOM complexity was observed after pre-treatment, it seems that most of the COD solubilised was directly oxidized during pre-treatment and that hydrolysis products were less oxidised during inter-stage treatment (less COD was removed and DOM complexity remained similar). Kinetics of hydrolysis and uptake of soluble products for oxidation may play a role.

During TAD, soluble products from recalcitrant substrates would be more slowly oxidised than the ones from less recalcitrant substrates. Slower oxidation would give the opportunity to use the hydrolysis products for anaerobic digestion where a longer retention time would facilitate their degradation and conversion into methane. Results suggest that the more recalcitrant (less accessible and more complex) the substrate, the less its hydrolysis products are oxidised during TAD, leaving more soluble organic matter for conversion to methane.

Hence the positive effect of TAD might be related to a more efficient hydrolysis in aerobic conditions (compared to anaerobic) and a slower uptake rate of hydrolysis products for oxidation in TAD. Further, it seems that TAD as a pre-treatment for anaerobic digestion is effective only if applied to a complex-like substrate with low anaerobic degradability. This difference in recalcitrance of the substrate could explain the difference in relative effects of TAD observed here and in previous studies. The few results in literature showing a positive effect on methane production (Jang et al., 2014; Pagilla et al., 2000) may be due to a 'sufficient' recalcitrance of the initial substrate used (mix wastewater sludge and swine manure).

3.3. Process performances

3.3.1. Overall methane production and COD removal

Inter-stage treatment slightly increased overall methane production. An increase of 1.8 and 2.6% of total methane production was found for I1 and I2 (from 20.9 to 22.3 and 21.8 to 22.4 $LCH_4 \cdot kg^{-1}$) (Table 2). Inter-stage treatment increased total COD removal by 5 to 8% compared to the control (from 69.1 to 72.3 and 74.8% for I1 with 4.6 and 24 h in TAD, and from 81.6 to

86.3% for I2 with 24 h in TAD) (Table 2). Total COD removal was >70% in both inter-stage experiments. Stage 1 accounted for >90% of total methane production and 64 to 76% of total COD removal (Table 2). Relative to other non-biological treatments, the increase found in this inter-stage study is small. With physical or chemical inter-stage treatments, increases of overall yields from 14 to 33% have been found (acidic and alkaline co-treatment (referred to as post-treatment by the authors) studied by Takashima and Tanaka (2014) and Li et al. (2013) or thermal inter-stage with CO_2 stripping proposed by Nielsen et al. (2011)). Inter-stage treatment of swine manure with TAD of 1 day SRT increased overall methane production by 25% (Pagilla et al., 2000). The small extent of the increase in total methane production observed with inter-stage treatment can be partly explained by the high production of methane during the first stage (>90% of methane production and >70% of COD removal) but also by the low anaerobic degradability of the substrate entering the second stage of anaerobic digestion. The estimate of methane production from the first stage for I1 was probably overestimated, since highly degradable organic wastes were included as digester feed at this plant, increasing first-stage methane production.

Anaerobic degradability of substrates in their first digestion ranged from 0.44 to 0.76 but it was only 0.19 and 0.14 in stage 2 for the control reactors in I1 and I2. A shorter first stage digestion might have led to different results because degradability of digested sludge could have been higher.

TAD pre-treatment decreased total methane production from 55 to 70% in P1 and P2 (from 4.7 to 2.1 and 13.9 to 4.1 $LCH_4 \cdot kg^{-1}$ (Table 2). Intermediate samples collected in P2 showed that COD reduction in TAD increased

and anaerobic conversion decreased monotonically with TAD retention time (data not shown). In P1, 31 ± 3% (± standard error) of the initial COD was converted into methane in the control condition and more COD was degraded during the pre-treatment (37 ± 3%) than during anaerobic digestion (Table 2). A similar trend was found in P2: pre-treatment resulted in total COD reduction of 41 ± 11% after 24 h (Table 2). Compared to control (anaerobic digestion only), total COD removal was improved by 20% in P1 (from 31 to 37%) but decreased by 20% in P2 (from 52 to 40%)(Table 2). Contrary to inter-stage TAD, total COD removal was not systematically increased with TAD pre-treatment time (intermediates times not shown). While globally more COD was removed after 24 h in TAD than for the control in P1, best removal was achieved for the control sample for P2, meaning that the anaerobic digestion following the TAD treatment in P2 was less efficient to remove COD than was anaerobic digestion of raw sludge. This difference might be linked to the composition of the sludge which was different even though it came from the same waste water plant. COD from raw sludge in P2 was 76.3 compared to 44 $g \cdot kg^{-1}$, it contained also more DM and VS than the sludge from P1 (Table 1). The TAD might have converted some of the more readily accessible and simple organic matter from the high COD sludge into less accessible and more complex organic matter, hindering the subsequent anaerobic digestion.

Reported effect of non-biological pre-treatment on overall methane production from sludge ranges from 11% for low temperature treatment (50°C, 48 h) to 88% for acidic pre-treatment or ultra-sonication (Tyagi and Lo, 2011) (increases in anaerobic degradability and in total methane production were

assumed to be identical as no COD losses generally occur during chemical or physical treatment).

It ranges from none to 40% for biological pre-treatments (Carrère et al., 2010; Jang et al., 2014) but the later case the real effect on overall methane production is not clear as the increase in methane production needs to account for VS and COD losses during the pre-treatment.

In P1 and I1, best COD removal was achieved for the longest time in TAD and did not correspond to the optimal treatment time for methane production (no treatment for P1 and 4.6 h for I1). Thus it is difficult to propose general treatment conditions that could apply for sludge in general and treatment optimisation would need to be adapted to the sludge and in some cases a compromise between COD removal and CH_4 production.

3.3.2. Viability of TAD pre- and inter-stage treatments

Improved destruction of COD by the use of TAD treatment could reduce sludge production and associated disposal costs. Compared to non-biological treatments, TAD has the advantage of avoiding any input or disposal of chemicals and is effective for sludge hygienisation and does not require external heat at full scale (Ward et al., 1998; Layden et al., 2007). Any potential increase in methane and COD destruction must be compared to the cost of aeration and of a more complex system to evaluate full-scale feasibility. Effectiveness of TAD treatment could almost certainly be improved through selection of optimal operating conditions (including reducing the retention time of the first-stage anaerobic digestion and optimising retention time and aeration in TAD). An advantage of inter-stage configuration over pre-treatment is that treated material has a much lower dry matter concentration than with

pre-treatment, possibly reducing aeration costs and equipment wear and tear. An inter-stage configuration for sludge thermal treatment was recommended by Nielsen et al. (2011) and Ortega-Martinez et al. (2016) using batch assays and full scale data.

However, inter-stage treatment requires the investment in an additional reactor which might not pay off. In this way, pre-treatment might be more profitable. If the choice of pre-treatment is made, it should be applied to a sufficiently complex substrate to benefit methane production. Another possibility to minimise the reactors requirements is the use of co-treatment where TAD effluent is recirculated back to the initial digester as proposed by Dumas et al. (2010). However, this configuration did not increase methane production. In co-treatment, the anaerobic digester cannot be run in batch and ensure that all the material is degraded to a sufficient extent, which seems to be one of the important parameter for the success of the treatment. Moreover, the use of only one anaerobic reactor might hinder the possibility for the micro-organism community to adapt to the quality of the substrate treated.

In order to increase methane production by exploiting the complementarity of anaerobic and aerobic biodegradation, it is necessary to minimise the loss of organic matter to oxidation while increasing anaerobic degradability. This work was based on thermophilic aerobic digestion of wastewater sludge but this approach may be effective for other substrates and biological treatments. Understanding how and why hydrolysis and subsequent uptake and metabolism of hydrolysis products differs between aerobic and anaerobic conditions, and degradable and recalcitrant material, will be essential for

optimising aerobic treatment for biogas production.

4. Conclusions

TAD used as inter-stage treatment successfully reduced oxidation losses and did not decrease total methane production. Overall increase in methane production for TAD inter-stage treatment was low (1.8 to 2.6%) but optimisation of treatment conditions could improve it. TAD proved to be a useful pre-treatment for complex substrates as it could increase anaerobic degradability of digested sludge (>40%). Adding a short aerobic stage to anaerobic digestion can substantially increase COD removal (up to 2-fold change in COD removal for treatment of digested sludge). More work is needed to understand how TAD increases anaerobic degradability of poorly accessible and complex substrates.

E-supplementary data for this work with details on derivation for eq. 1, results for CH_4, COD for all times, calculated oxidation losses and accessibility and complexity of initial substrates can be found in e-version of this paper online.

5. Acknowledgements

Funding was provided by the Danish Council for Strategic Research under the work program "BioCap - Bioenergy production from residual biomass through a novel integrated carbon chain pathway" (grant number 3047-00006B). Some work was also founded by the Foundation Idella. We are very grateful to Torben Hansson (KBM, SDU, Denmark) for providing the reactor used in the TAD experiments and for his helpful suggestions. Last

but not least, we would like to thank our Korean BioCap partners for fruitful discussions and support in technology transfer. Special thanks go to Hyun Min Jang and Prof. Jong Moon Park in POSTECH for their help and introduction to the TAD technology.

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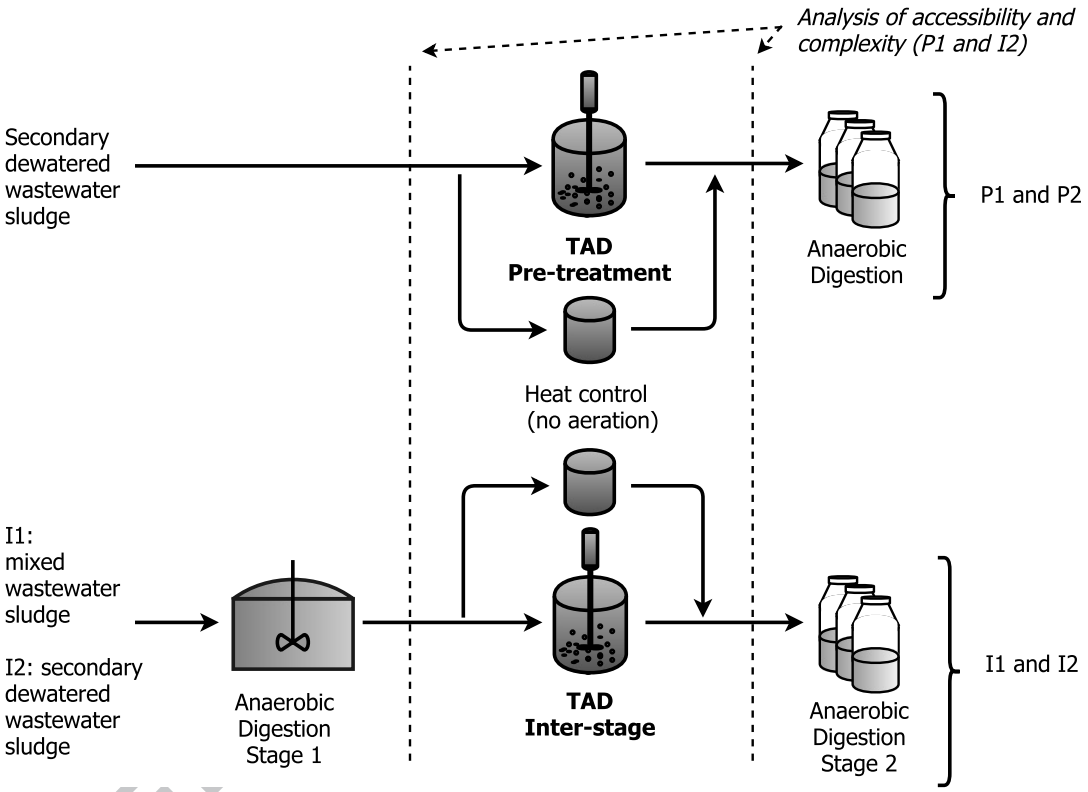

Figure 1: Treatment sequence used in P1, P2, I1 and I2. The first stage of anaerobic digestion was done either in a full scale biogas plant or in a laboratory continuously-stirred reactor. Post-TAD anaerobic digestion was done in batch reactors in the laboratory. Heat contro was for P2, I2 and I2.

Table 1: Characteristics of the municipal wastewater sludge used as substrate.

Experiment	Pre-treatment		Inter-stage treatment			
	P1	P2	I1		I2	
Substrate	Secondary sludge	Secondary sludge	Mixed sludge (original)[1]	Digested mixed sludge	Secondary sludge (original)	Digested secondary sludge
COD ($g \cdot kg^{-1}$)	44.0 (0.97)	76.3 (0.91)	86.3 [2]	31.2 (3.24)	76.3 (0.91)	36.5 (1.98)
DM ($g \cdot kg^{-1}$)	39.9 (0.20)	66.0 (0.02)	-	27.2 (1.11)	69.1 (4.42)	40.3 (0.01)
VS ($g \cdot kg^{-1}$)	28.6 (0.06)	48.0 (0.09)	-	15.0 (0.55)	50.3 (3.25)	23.0 (0.09)

Figures presented in parenthesis correspond to the standard deviation ($n = 3$ for COD, $n = 2$ for DM and VS).

[1] Feed to first stage anaerobic digestion as described in section 2.1.

[2] COD estimated from COD mass balance as the sum of the COD in digestate and the COD converted into methane during stage 1. Calculation was based on CH_4 production from full scale (19.3 $L \cdot kg^{-1}$ (L CH_4 per kg wet mass)), measurement of COD in digestate and conversion of COD in stage 1 as in Rittmann and McCarty (2001) (1 g COD yields 350 mL CH_4).

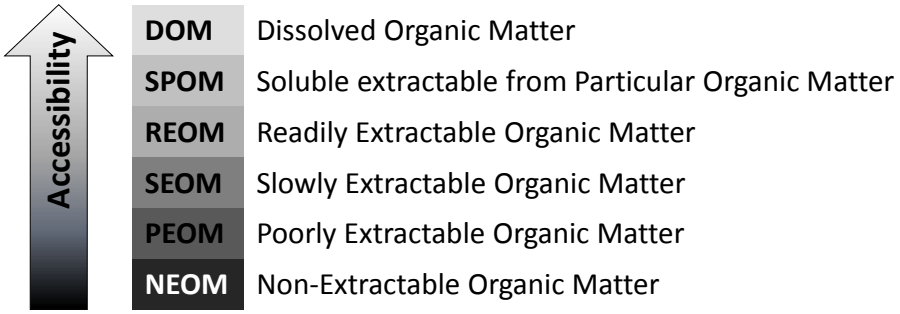

Figure 2: Definition and accessibility of the different organic matter fractions obtained by the organic matter fractionation.

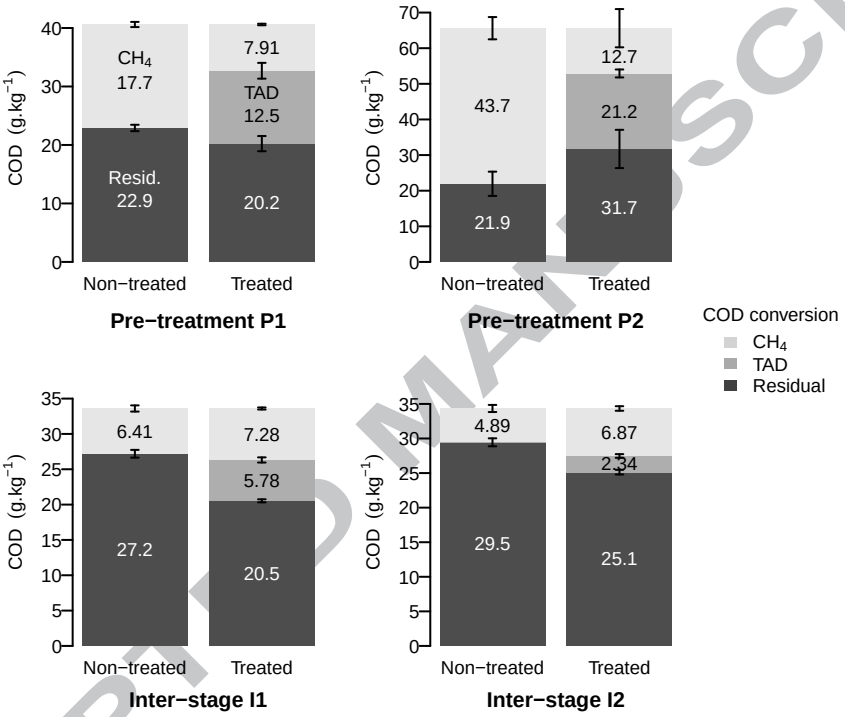

Figure 3: Conversion of wastewater sludge COD during pre- and inter-stage thermophilic aerobic digestion (TAD) and subsequent anaerobic digestion in four experiments. COD is expressed per mass of initial wet sludge. TAD treatment time was 24 h and methane production was evaluated after 20 days.

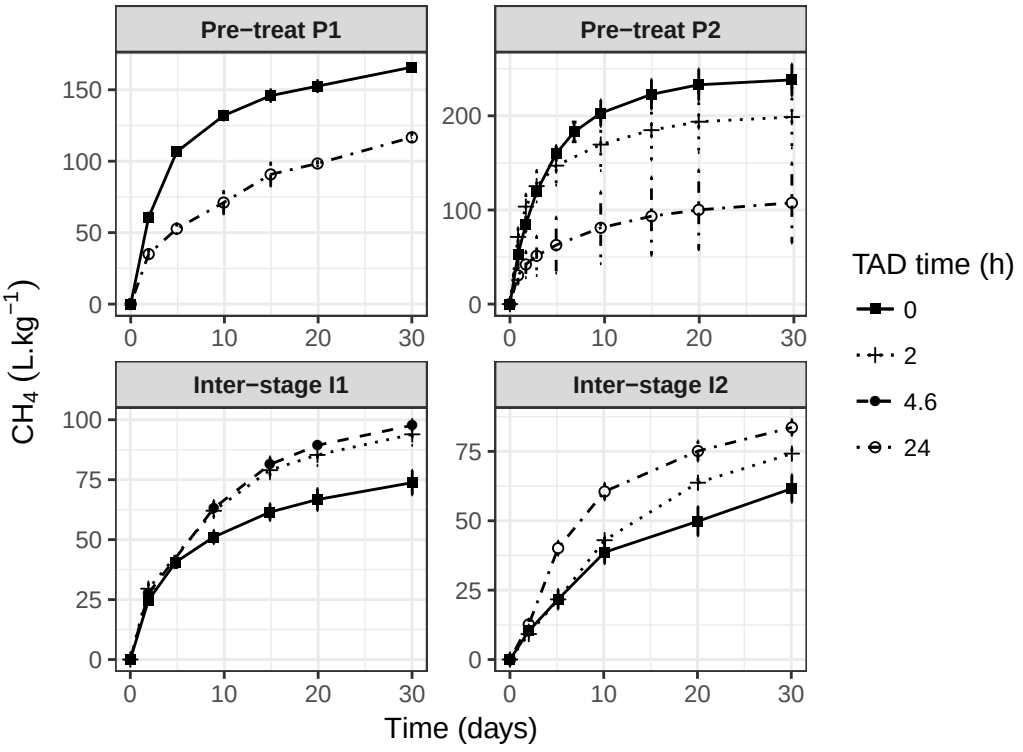

Figure 4: Cumulative methane production normalised per mass of COD in TAD effluent. Times in TAD showing the largest effect compared to the control are presented as well as an intermediate one.

Table 2: Effect of the different treatment steps on COD removal and methane production

Experiment		TAD time	COD mass balance				COD removal			
			COD initial [1]	CH_4 stage 1 [2]	COD lost TAD	CH_4 stage 2	Stage 1	TAD	Stage2	Total
		(h)	($g \cdot kg^{-1}$)	($L \cdot kg^{-1}$)	($g \cdot kg^{-1}$)	($L \cdot kg^{-1}$)	%	%	%	%
Pre-treatment	P1	0	44 (0.10)	-	-	4.7 (0.52)	-	-	30.7 (3.38)	30.7 (0.00)
		24	44 (0.10)	-	10.2 (1.58)	2.1 (0.39)	-	23.2 (3.58)	13.7 (2.50)	37 (4.37)
	P2	0	76.3 (0.91)	-	-	13.9 (1.57)	-	-	52.2 (5.91)	52.2 (0.00)
		24	76.3 (0.91)	-	19.7 (1.90)	4.1 (2.97)	-	25.8 (2.51)	15.2 (11.13)	41 (11.41)
Inter-stage treatment	I1	0	86.3 (1.00)	19.3 (1.00)	-	1.6 (0.25)	63.9 (3.39)	-	5.2 (0.84)	69.1 (0.00)
		4.6	86.3 (1.00)	19.3 (1.00)	1.6 (0.58)	2 (0.24)	63.9 (3.39)	1.9 (0.67)	6.5 (0.80)	72.3 (3.57)
		24	86.3 (1.00)	19.3 (1.00)	4.3 (0.66)	1.8 (0.24)	63.9 (3.39)	5 (0.76)	5.9 (0.80)	74.8 (3.59)
	I2	0	76.3 (0.91)	20.4 (1.00)	-	1.4 (0.27)	76.2 (3.85)	-	5.3 (1.02)	81.6 (0.00)
		24	76.3 (0.91)	20.4 (1.00)	2 (0.40)	2 (0.21)	76.2 (3.85)	2.6 (0.52)	7.5 (0.77)	86.3 (3.99)

[1] COD of original substrate (before any treatment) in g COD per kg wet mass. See Table 1 for more details on initial substrates.

[2] in L CH_4 per kg wet mass. Can be divided by 0.35 to get the COD value.

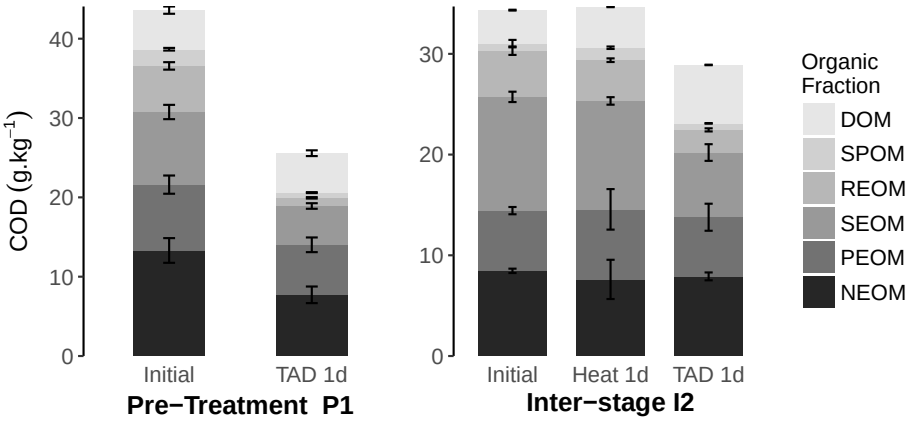

Figure 5: COD of the different organic fractions, based on the fractionation of organic described in Section 2.4 and Figure 2.

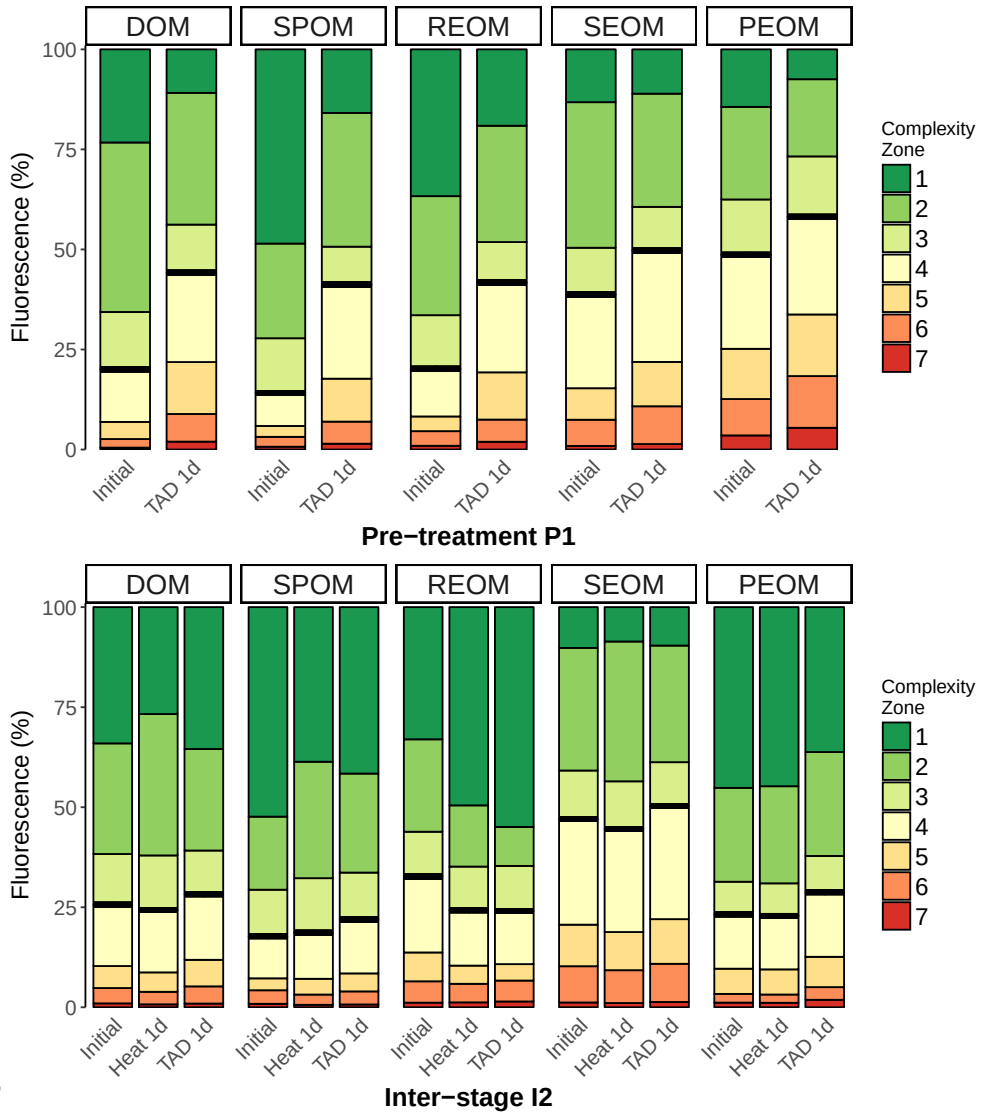

Figure 6: Complexity as a percentage of total fluorescence of each organic fraction obtained by the fractionation of organic matter (Section 2.4 and Figs. 2 and 5). Complexity increases from zone 1 to 7. The bold between zones 3 and 4 indicates the boundary between complex and simple organic matter.