Journal of Environmental Science and Health, Part A

Evaluation Of Pressurised Carbon Dioxide Pre-Treatment Aimed At Improving The Sanitisation And Anaerobic Digestibility Of Co-Settled Sewage Sludge

--Manuscript Draft--

Full Title:	Evaluation Of Pressurised Carbon Dioxide Pre-Treatment Aimed At Improving The Sanitisation And Anaerobic Digestibility Of Co-Settled Sewage Sludge
Manuscript Number:	
Article Type:	Original Research
Keywords:	Biogas; sewage sludge; pre-treatment; faecal indicator organism; pathogen
Manuscript Classifications:	Biofuels/Biomass; Effluents; Environmental microbiology; Pollutants; Sludge and sewage waste treatment; Water/wastewater
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Order of Authors:	Maryam Mushtaq, PhD
	Charles James Banks, PhD
	Sonia Heaven, PhD

View Letter

August 14, 2018

RE: "Evaluation Of Pressurised Carbon Dioxide Pre-Treatment Aimed At Improving The Sanitisation And Anaerobic Digestibility Of Co-Settled Sewage Sludge"
Manuscript ID JESHA-2018-0020

Dear Sir,

Please see attached a revised copy of the above mentioned manuscript. The author has tried level best to do all the recommended corrections and provide additional information where recommended. However, if there is still something that needs to be corrected please don’t hesitate to get in touch.

Looking forward to listen from you.

Sincerely yours,

Maryam Mushtaq

Title:

Evaluation Of Pressurised Carbon Dioxide Pre-Treatment Aimed At Improving The Sanitisation And Anaerobic Digestibility Of Co-Settled Sewage Sludge

Authors' names and affiliations:

Maryam Mushtaq[1], Charles J. Banks, Sonia Heaven

Faculty of Engineering and the Environment, University of Southampton, Southampton SO17 1BJ, UK.

Abstract

This work reports on the use of pressurised CO_2 pre-treatment to improve methane yield and pathogen indicator organism die-off in co-settled sewage sludge. Four semi-continuous mesophilic anaerobic digesters were fed on co-settled sewage sludge to establish a baseline for performance and stability. One pair of digesters was then fed with co-settled sewage sludge pre-treated by P CO_2 at 2800 kPa for 23 hours. The trial continued for 70 days during which specific biogas and methane production, volatile solids destruction and loss of viability of *Escherichia coli* was monitored in test and control digesters. The pre-treatment had no positive influence on any of these parameters which was further confirmed using batch biochemical methane potential tests and direct measurement of die-off of *E. coli* and *Salmonella enterica* in samples of different sizes treated in pressure vessels of different sizes and in matrices of nutrient medium and co-settled sewage sludge. Pressurised CO_2 pre-

[1] Corresponding author Maryam Mushtaq, now at School of Environmental Sciences, University of Guelph Ridgetown Campus, Ridgetown, Canada N0P 2C0. email mushtaq@uoguelph.ca

treatment was effective at killing faecal indicator bacteria in nutrient medium but ineffectual in sewage sludge, strongly suggesting that the nature of suspending matrix was a principle determining factor. Paper concludes that pressurised CO_2 pre-treatment is not a satisfactory approach to improve either biogas production or pathogen destruction in anaerobic digestion.

Keywords

Biogas, sewage sludge, pre-treatment, faecal indicator organism, pathogen, cell disruption

Introduction

Sewage Sludge (SS) is an inevitable product of waste water treatment which is rich in organic matter and pathogens, has obnoxious odour [1] and is amenable to biological decomposition. [2] Anaerobic Digestion (AD) has a strong potential to stabilise, sanitise and minimize the putrescible nature of SS. [2] The outputs of AD are biogas (a mixture of carbon dioxide and methane) and an innocuous organic residue called digestate which can be applied to agricultural land to recycle valuable organic matter and nutrients. [3] Various pre-treatment techniques have been applied to municipal SS prior to anaerobic digestion (AD), including thermal, chemical, thermo-chemical, sonication, biological, enzymatic and mechanical methods. [4,5] These are generally aimed at making the organic matter more easily accessible to anaerobic micro-organisms, and thereby enhancing the digestibility and biogas production as well as decreasing the quantity of sludge for final disposal. Additional benefits of some of these pre-treatments may potentially also include improved sludge dewatering, reduction in foaming and enhanced pathogen kill. [6] Pre-treatment, however, adds greater complexity to the process chain, has implications for both capital and operating expenditure and to be judged successful is usually required to show a net economic benefit. [7] A novel process reported by Spooner et al.,[8] which uses pressurised biogas (35-45% CO_2) to pre-treat secondary sludge

biosolids before anaerobic digestion was reported to enhance biogas production by around 30-40% . The aim of the current work was to validate or repudiate this claim, with the additional aim of assessing whether this type of pre-treatment could also contribute towards enhanced pathogen removal.

The detrimental effect of pressurised carbon dioxide (P CO_2) on bacterial growth was first reported by Valley and Rettger [9] , but it is only over the last two decades that P CO_2 has become established as a technique for non-thermal sterilisation [10], its application to date, however, has been largely confined to the food industry. The principle of P CO_2 treatment is that CO_2 dissolves in the aqueous phase of a liquid to form carbonic acid (H_2CO_3) which diffuses into any microbial cells present. This causes a drop in intracellular pH [11] which affects the enzymatic activities [12] and triggers a series of complex interrelated processes leading to the loss of cell viability. [10] The effect is augmented when pressure is released as CO_2 dissolved in the cytoplasm expands causing the microbial cells to rupture and release their contents. [13] The potential benefit in anaerobic digestion is that this cell rupturing process will make the feed biosolids more readily available to anaerobic bacteria, thereby helping to overcome the rate-limiting hydrolysis step of AD. [8] The process could therefore boost biogas production with the additional benefit of potentially enhancing digestate sanitisation through inactivation of pathogenic bacteria. [8] The present evaluation used the P CO_2 pre-treatment on co-settled sewage sludge, a mixture of settled solids from wastewater and the excess biosolids from activated sludge treatment, which was then fed to a mesophilic anaerobic digester. Any potential for improvement in process performance was monitored with reference to biogas production and removal of *Escherichia coli* as a pathogen indicator organism.

Materials and Methods

Digesters

Four 5-L capacity digesters with a 4-L working volume were used in the study. These were constructed from PVC tube with gas-tight top and bottom plates. The top plate was fitted with a gas outlet, a feed port sealed with a rubber bung, and a draught-tube liquid seal through which a stainless-steel asymmetric bar stirrer was inserted with a 40 revolutions per minute (RPM) motor mounted directly on the top plate. Temperature was maintained at 37 ± 0.5 degree Celsius (^{o}C) by water circulated through an external heating coil. The digesters were connected to tipping-bucket gas counters with continuous data logging. Calibration of gas counters was checked weekly by collecting the gas in a Tedlar bag (SKC Ltd, Blandford Forum, UK): the volume was then measured accurately in a weight-type water displacement gasometer. [14] All gas volumes reported are corrected to standard temperature and pressure of 0 ^{o}C, 101.325 kilo pascal (kPa) as described by Walker *et al.* [14] Semi-continuous operation was achieved by daily removal of digestate through an outlet port in the base plate before adding feed via the feed port in top plate (Figure 1).

Inoculum and Feedstock

The digesters were filled to working volume with inoculum obtained from a mesophilic anaerobic digester at Millbrook Wastewater Treatment Plant (WWTP), Southampton, UK. Co-settled Sewage Sludge (CSS) from the same site was used as feedstock throughout the experimental period.

Feedstock Pre-Treatment

P CO_2 pre-treatment was carried out by adding 0.6 L of the CSS feedstock to a 2.2-L capacity stainless steel pressure vessel (Prosep Filter Systems Ltd. West Yorkshire, UK, model No.

530110XN10B10V). The vessel was fitted with a pressure gauge to measure vessel pressure and one port was fitted with a valve through which it could be pressurised with CO_2 and through which the pressure could subsequently be released (Figure 2a). The vessel was pressurised to 2800 kPa by connection to a cylinder of CO_2 (BOC Ltd, UK) and gas transfer at the liquid interface promoted throughout the pressurisation period of 23 hours by orbital shaking of the vessel and its contents at 100 rpm (Barloward Scientific Ltd, Staffordshire, UK). These treatment conditions were selected based on the best inactivation rate of *E. coli* obtained in the initial studies. [15] At the end of the pressurisation period, the pressure was rapidly released through the outlet valve via a tube into an expansion chamber designed to minimise respirable aerosol release to the atmosphere. During the testing period treated CSS was prepared daily alongside an unpressurised control using the same source material.

In follow-up testing CO_2 pressurisation was also carried out in the pressurisation unit of a bomb calorimeter (Cal2K Eco, South Africa) with approximately 250 millilitre (mL) capacity (Figure 2b).

Digester Operation

Before the start of the trial all 4 digesters were initially operated for 173 days on untreated CSS to allow performance to stabilise.[16] The current study then used a data period equivalent to 6 hydraulic retention time (HRT) (90 days) to provide baseline values for comparison of the operational stability and other digestion parameters for all 4 digesters running under identical conditions on the same feed. Two digesters were then switched to feeding with the pressure-treated sludge for a period of 71 days (4.7 HRT), while the other two continued to receive untreated material. Operational conditions throughout were designed to maintain an organic loading rate (OLR) of 2.5 g volatile solids (VS) L^{-1} day^{-1} at a

Hydraulic Retention Time (HRT) of 15 days with the VS of the feed material adjusted to 3.75% by water addition. The digesters were monitored for pH, alkalinity, ammonia, biogas, biogas composition, volatile solids (VS) destruction and concentration of *E. coli* in the digestate. Gas composition was measured on samples collected in a gas sampling bag over a 24-hour period.

Biochemical Methane Potential (BMP) Testing

This was carried out in 0.5-L static glass reactors in a temperature-controlled water bath at 35 ^{o}C with biogas collected in water displacement gasometers filled with a barrier solution of acidified saline (75% saturated NaCl, pH 2), to minimise CH_4 dissolution. Methane content of the biogas was measured by gas chromatography and all gas volumes were corrected to STP 0 °C and 101.325 kPa.[14] The results were expressed in terms of net cumulative specific methane yield per gram of substrate added. Substrates tested were treated and untreated samples of CSS and thickened waste activated sludge (WAS) mixed at inoculum to substrate ratios of 5:1 and 3:1 on a VS basis respectively. Inoculum-only blank controls were run in parallel.

BMP results were further analysed using a pseudo-parallel first-order model (Equation 4.1) to identify readily degradable and more slowly degradable components. [17]

Methane production is therefore governed by two rate constants *k1* and *k2*:

$$Y = Y_{max} (1 - Pe^{-k1t} - (1-P)e^{-k2t})$$

Where

Y is the cumulative methane yield at time *t*

Y_{max} is the ultimate methane yield

k1 is the first order rate constant for the proportion of readily degradable material

k2 is the first order rate constant for the proportion of less readily degradable material

P is the proportion of readily degradable material

Analytical Methods

Total (TS) and volatile solids (VS) were measured according to Standard Methods 2540 G. [17] pH was determined using a Jenway 3010 meter (Bibby Scientific Ltd, UK) with a combination glass electrode calibrated in buffers at pH 4, 7 and 9.2 (Fisher Scientific, UK). Alkalinity was measured by titration with 0.25N H_2SO_4 to endpoints of pH 5.7 and 4.3, allowing calculation of total (TA), partial (PA) and intermediate alkalinity (IA). [18] Total ammonia nitrogen (TAN) concentration was determined using a Kjeltech steam distillation unit according to the manufacturer's instructions (Foss Ltd, Warrington, UK). Biogas composition (CH_4 and CO_2) was determined using a Varian star 3400 CX Gas Chromatograph with a porapak column operated at 50 ^oC and a thermal conductivity detector. The GC was calibrated with a standard gas mix of 65% (v/v) CH_4 and 35% (v/v) CO_2 (BOC, UK).

Concentration of *E. coli* was determined by the Most Probable Number (MPN mL^{-1}) technique as described in method 2 of 'Methods for the isolation and enumeration of *Escherichia coli* including verocytotoxigenic *Escherichia coli*. [20] All microbiological reagents were obtained from Oxoid Ltd (Basingstoke, UK) and all sterile media and equipment requirements met by sterilisation at 121°C for 15 min.

Results and Discussion

Digestion Performance and Stability

The inoculum used for CSTR reactors collected from Millbrook WWTP in Southampton had 3.5 % TS of wet weight (%WW) out of which, 2.3 % were VS (%WW). Nearly 66% of TS in the inoculum were VS. The properties of feed stock (CSS) varied slightly over the course of trial with the TS ranging from 4.0 to 6.6 % of WW, and an average VS of 4.1 %WW. The variability in TS can be seen in Figure 3a, but the proportion of volatile matter remained very similar at around 78 %TS. To ensure a constant loading and HRT in the digesters the CSS was fed at a VS content of 3.75 %WW except towards the end of the trial where the VS of the sampled material dropped to around 3.4 %WW. During this period the HRT was kept constant at 15 days and the OLR was allowed to vary.

The pH of all the digesters remained in the range 7.2 to 7.3 [21], with no apparent difference due to the CO_2 pressure pre-treatment. The digestate TAN showed a very gradual increase in concentration over the trial period (Figure 3b) but remained within the range 1.1 - 1.35 g L^{-1} which is typical of that found in a sewage sludge digester, and well below the concentration that may become inhibitory to methanogens. [21] There was no difference in TAN concentration between the control and the test digesters (Figure 3c), such as might have been expected if the pressure treatment was leading to extensive cell disruption and improved hydrolysis. If this had occurred then more nitrogen would have been released from cell protein and reduced to ammonia as the carbon components were degraded, although this might then have been taken up by additional microbial biomass in response to the increase in effective OLR. Total, partial and intermediate alkalinity were measured: the ratio of IA/PA is shown in Figure 3d and did not exceed a value of 0.6. The digesters can therefore be regarded

as operating stably without any accumulation of volatile fatty acids [22], and again with no apparent difference between test and control digesters.

Average specific biogas production was 0.499 L g^{-1} VS ^{added} (Standard deviation 0.023). In the pre-trial period from day 0-90 this showed small variations that tended to follow the same pattern in each of the 4 digesters (Figure 4). This type of natural variation can be expected when running an experiment of this type and may be caused by small differences in daily feed due to slight inhomogeneity of the feedstock material. A similar pattern can be seen after the start of the CO_2 pressurisation trial and throughout its duration, but in this case, there is also a slight downward trend in all digesters. The reason for this is unknown but is evidently related to the properties of the sludge itself, which appeared to be more dilute during the summer period as indicated by the lower %TS and VS obtained in the later part of the trial. It may be that in warmer weather some of the more putrescible components in the sludge were degraded at the treatment works in sedimentation and/or storage tanks before sampling took place. There was no evidence, however, of any benefit in terms of increased biogas yield as a result of the CO_2 pressurisation pre-treatment. This can be explained by the treatment of CSS with P CO_2 at sub-critical conditions (<31.1 ^{o}C and 7380 kPa) at which CO_2 has low penetration power as compared to supercritical conditions (>31.1 ^{o}C and 7300 kPa); further the presence of solids and less water content of CSS might also have played a role in hindering the action of this pretreatment. [10]

As expected the specific methane production graph (Figure 4) showed a similar trend to that for specific biogas production: the average specific methane yield in the last 30 days of the trial was 0.313 and 0.307 L CH_4 g^{-1} VS ^{added} for the control and test digesters respectively. VS destruction was calculated both by simple difference in percentage solids entering and

leaving the digester, and also by a mass balance taking into account loss of mass in the biogas. The result in both cases was very similar, with values for the two test digesters showing 47.7 and 49.1% destruction and the two controls 48.5 and 48.8% destruction. Performance was therefore typical of a single stage high-rate mesophilic digestion process for sewage sludge. [23]

Enumeration of E. coli.

The concentration of *E. coli* in the co-settled sludge on the day of collection ranged from 1.30 x 10^5 to 2.80 x 10^6 MPN mL^{-1} (Figure 5) and decreased on storage of the material under refrigerated conditions. This die-off at 4 ^oC under the storage conditions used was initially assessed on single sample with an initial concentration of around 1.3 x 10^5 MPN g^{-1} wet weight which declined to 1.6 x 10^4 MPN g^{-1} wet weight by day 14. Although not excessive, this natural loss of *E. coli* on storage needs to be taken into account to quantify die off as a result of digestion; as the natural die-off could not be determined for each batch of daily feed, however, the results are presented as a comparison between the test and control digesters over the test period. This data is also compared to that in the pre-trial condition where the digesters were running under identical conditions.

The concentration of *E. coli* in the digesters over the study period ranged from 10^2 to 10^4 MPN mL^{-1}, which was on average about two log reductions below the concentration in feedstock (10^5 to 10^6 MPN mL^{-1}). This is typical of the performance of a mesophilic digester fed on co-settled sewage sludge. [20] There was no apparent difference in *E. coli* destruction before and after the start of the CO_2 pressurisation treatment. The general pattern of removal in all the digesters was similar, suggesting that the differences on a sample-to-sample basis

probably reflected the *E. coli* load entering the digester, which can also be seen to vary in the feed sludge.

Although, the pre-treatment conditions applied in this trial were much more stringent than those described by Spooner *et al.*, [8], where a pressure of 600 kPa with an exposure time of up to 60 minutes was recommended for waste activated sludge pre-treatment. The results obtained in this study thus did not validate the findings of Spooner *et al*., therefore it was decided to carry out some additional batch tests including a biochemical methane potential (BMP) assay and using *Salmonella* as an additional means of quantifying pathogen die-off. Since the natural concentration of *Salmonella* in CSS was low, a pure culture of *Salmonella enterica* (ATCC 14028) obtained from National Collection of Industrial Marine and Food Bacteria (NCIMB) Aberdeen, Scotland UK was used to spike the CSS to a cell density of 10^6 CFU mL^{-1} in all experiments.

Batch Die-Off

A sample of CSS (600 g) was pressure treated for 23 hours at 2800 kPa with initial and final concentrations of *E. coli* and *Salmonella* determined using the MPN technique. The results showed only 0.2 and 0.3 log reductions in numbers, respectively, confirming that the P CO_2 treatment was almost ineffectual in a CSS medium. This was surprising as previous studies [15] using a pure culture of *E. coli* grown in nutrient broth and treated with CO_2 at the same pressure and exposure time had shown an 8-log reduction in numbers, albeit with a smaller sample size and in the smaller pressure vessel.[15] It was therefore considered possible that the change in relative surface area for gas transfer had influenced the effectiveness of the treatment, and two further experiments were conducted to investigate this using *E. coli* and *Salmonella*. In the first, 100 g aliquots of broth culture containing the test organisms and of

CSS spiked with *S. enterica* were treated in the two pressure vessels. The results (Table 1) showed there was little difference between log reductions in the small and large pressure vessels for both *E. coli* (0.3 and 0.4) and *Salmonella* (1.4 and 1.3), respectively. In the second experiment the sample size was varied and 600 g and 3 g aliquots of broth culture and CSS were treated in large and small vessels. For CSS, treatment of the smaller sample size of 3 g in the small vessel showed a 3.0 log reduction in *E. coli*, compared to only 0.2 log reduction for the 600 g sample in the large vessel; this difference was less marked for *Salmonella* with log reductions of 2.6 and 1.6 (Table 1). Treating equal amounts (100 g) of broth culture of *E. coli* in the small and large vessels resulted in an 8-log reduction, equivalent to complete inactivation. There was, however, a fall in log reduction to 5.4 in the large vessel when the amount treated was increased to 600 g. Together these results show that the vessel size by itself is unimportant; the sample size influences die off; but the major factor appears to be the nature of matrix. Lower water content (as compared to the nutrient broth) and presence of suspended solids and fat/grease matrix were considered as major factors in protecting the test microorganisms and reduced inactivation in CSS in comparison to the nutrient broth. [10]

BMP assay results

The BMP test ran for 45 days, until cumulative net specific methane production reached a plateau. Average specific methane production from the untreated and treated CSS and WAS samples is shown in Figure 6. There was a small amount of variability between test samples of each substrate: this was slightly greater for the WAS, probably reflecting the very small quantity of VS^{added} in the test due to the low solids content of this material even after settlement thickening. As expected, the BMP of the CSS (0.304 and 0.316 L CH_4 g^{-1} VS^{added} for untreated and treated CSS, respectively) was higher than for the WAS (0.251 and 0.259 L CH_4 g^{-1} VS added for untreated and treated WAS, respectively). Although there appeared to

be minor differences between the average specific methane yields for treated and untreated samples, the difference of around 4% in each case was too small in relation to the natural variation seen in the test to be confirmed as significant, as indicated by the overlapping error bars in Figure 6.

Coefficients from kinetic modelling of average methane production values for replicate samples are shown in Table 2. For both untreated and treated CSS, 90% of methane was released in the first 10 days confirming that a semi-continuous AD system operating at a short retention time of about 15 days might be expected to yield a high proportion of the methane potential. Methane release from the WAS took slightly longer and appeared to reach a maximum value after 15 days indicating that a slightly longer retention time would be preferable in a semi continuous system and that pre-treatment to accelerate degradation is likely to show benefits when using this substrate. As can be seen, however, there was no significant difference between the kinetic coefficients for the treated and untreated samples in either substrate. This gave support to the view that, under the conditions used, no significant improvement in the specific methane yield of either substrate is likely to occur in semi-continuous digestion.

Conclusions

Digester operation over the 160-day experimental period showed performance and stability parameters typical of a sewage sludge digester treating co-settled sludge. The test digesters fed with CO_2 pressurised sludge however showed no enhancement in either biogas production or *E. coli* destruction under the test conditions used. This result was slightly unexpected, as P CO_2 treatment is recognised as an effective means of sterilizing consumer food products,[10] and earlier work by the authors had also shown it to be effective at reducing

the numbers of both *E. coli* and *Salmonella* as pure cultures in nutrient medium.[15] Batch experiments showed that both sample size and the nature of substrate affected the die-off of faecal test organisms, and it seemed as if co-settled sewage sludge in some way protected the organisms from this type of treatment. Batch biochemical methane potential tests also showed the pre-treatment to be ineffectual at increasing methane yield. The results are contrary to those previously found in research by other authors. [8] These results can be explained by the fact that majority of the studies involving the inactivation of pathogens in food products have used P CO_2 in supercritical range as compared to the current study where the treatment conditions are in sub-critical range. In supercritical range, CO_2 has a higher solvating power and higher temperature enhances the diffusivity of CO_2 in suspending medium hence resulting in higher inactivation of microorganisms. Moisture content of suspending medium also plays key role in microbial inactivation. [10] Although, encouraging results were obtained by the authors for inactivation of both test microorganisms in nutrient broth, [15] the results were not replicable in the case of CSS. It can be linked to the fact that the suspended solids present in CSS acted as a shield and prevented the action of P CO_2 on microorganisms. Moreover, lower water content and fats/oils present in the CSS may have prevented the same treatment conditions (temperature/ pressure) to provide the same results as with the nutrient broth. There is a possibility that stringent treatment conditions (temperature/ pressure) might improve the performance of this technique in terms of both pathogens kill-off and biogas yield. However, at higher pressure and temperature the process will become energy intensive and expensive; making it in suitable for use in a wastewater treatment plant. It can therefore be concluded that P CO_2 pretreatment under tested conditions is unlikely to be useful for application in wastewater treatment industry.

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List of figure captions

Figure 1. Image and cut-away diagram showing construction of the 5-L digester vessels, method of mixing and temperature control.

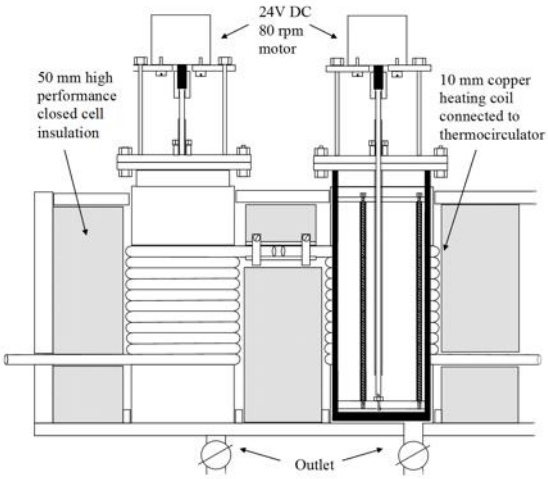
Figure 2. P CO_2 treatment vessels used: (a) 2.2-L Prosep filter, (b) small vessel with pressure regulated filling system.

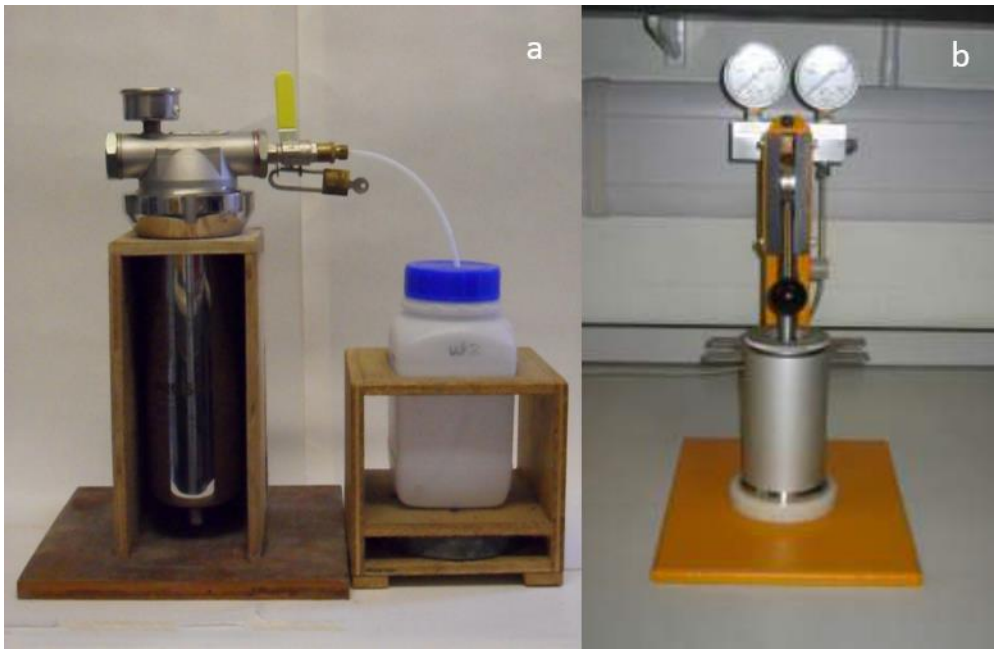
Figure 3. Results during the experimental period for: (a) Feedstock TS and VS, (b) digestate TAN concentration, (c) total alkalinity and (d) IA/PA ratio in test and control digesters**.** Vertical dotted lines indicate start of feeding with CO_2-pressurised digestate.

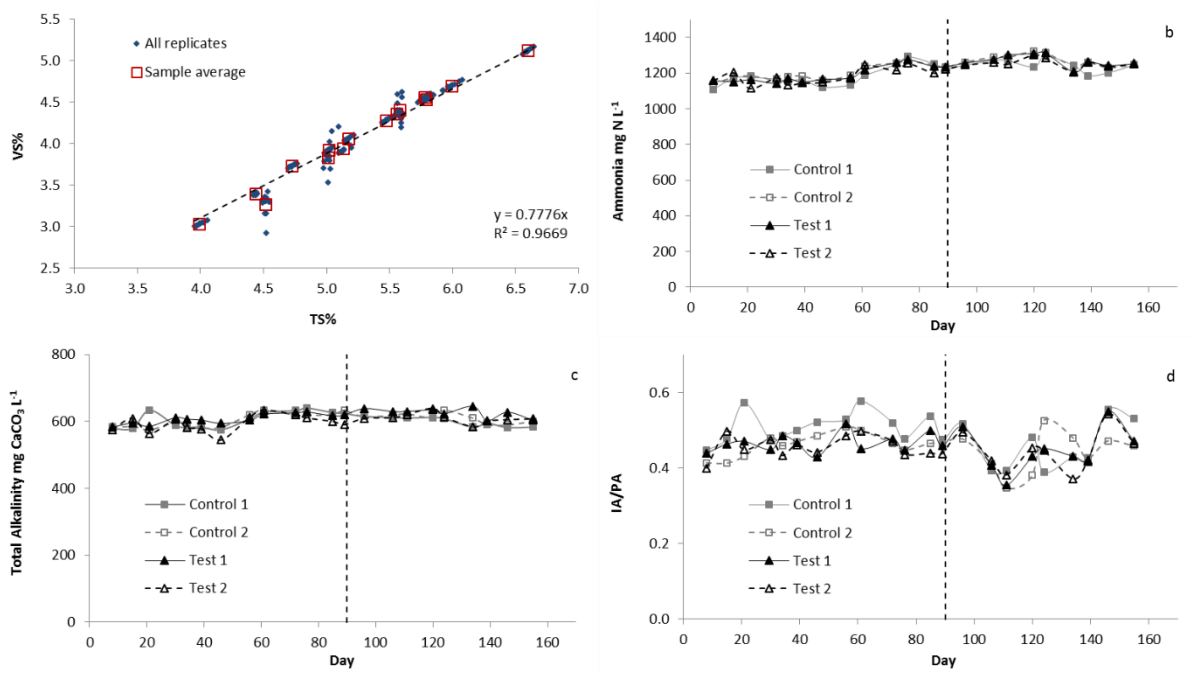
Figure 4. Gas production in test and control digesters during the experimental period: (a) specific biogas production, (b) specific methane production. Vertical line indicates start of feeding with CO_2-pressurised digestate.

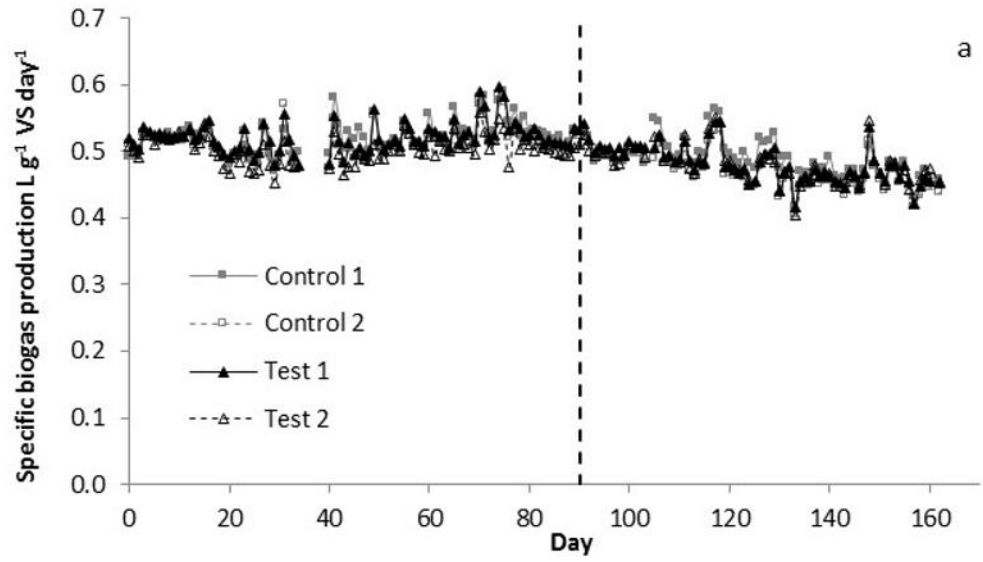
Figure 5. *E. coli* numbers in fresh feedstock before storage and in control and test digesters. Vertical line indicates start of feeding with CO_2-pressurised digestate.

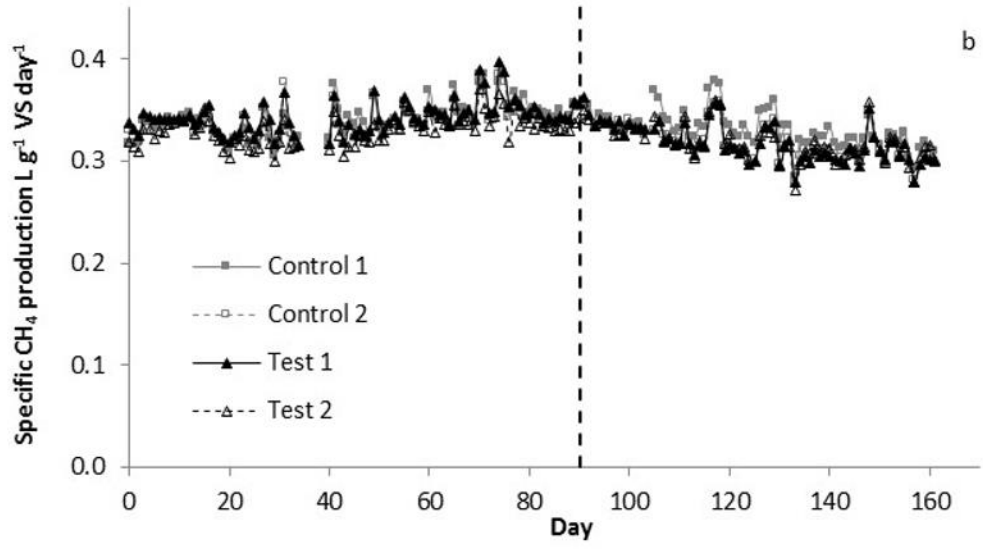
Figure 6. Net cumulative specific methane production in BMP tests for treated and untreated CSS and WAS. Error bars show range of values at selected intervals.

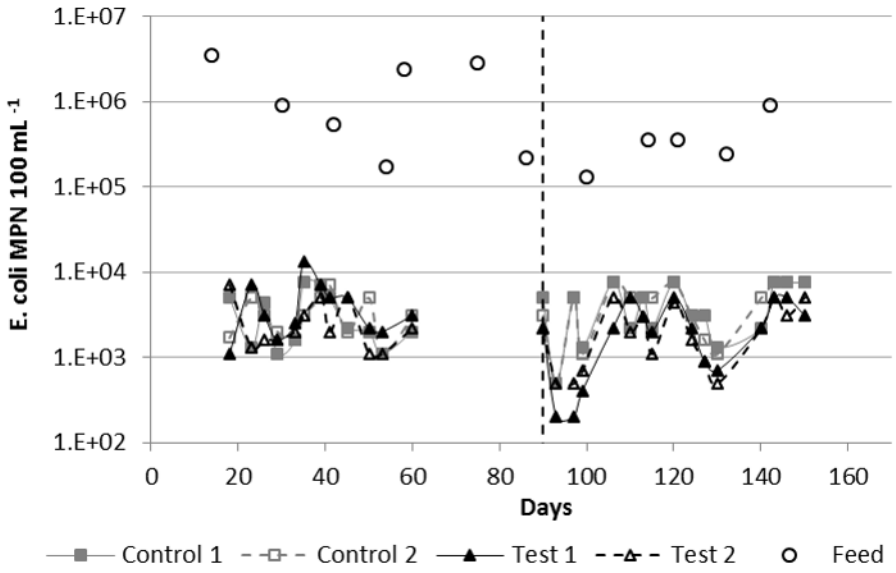

24V DC
80 rpm
motor
50 mm high
performance
closed cell
insulation
10 mm copper
heating coil
connected to
thermocirculator
Outlet

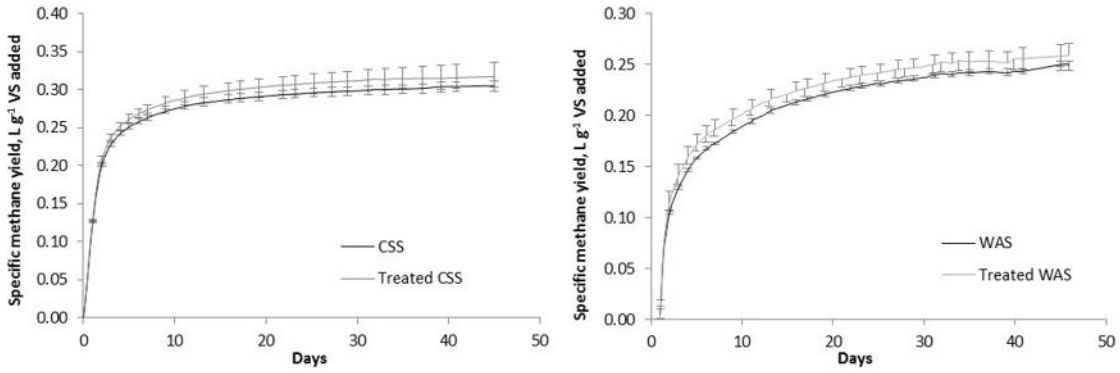

a
b

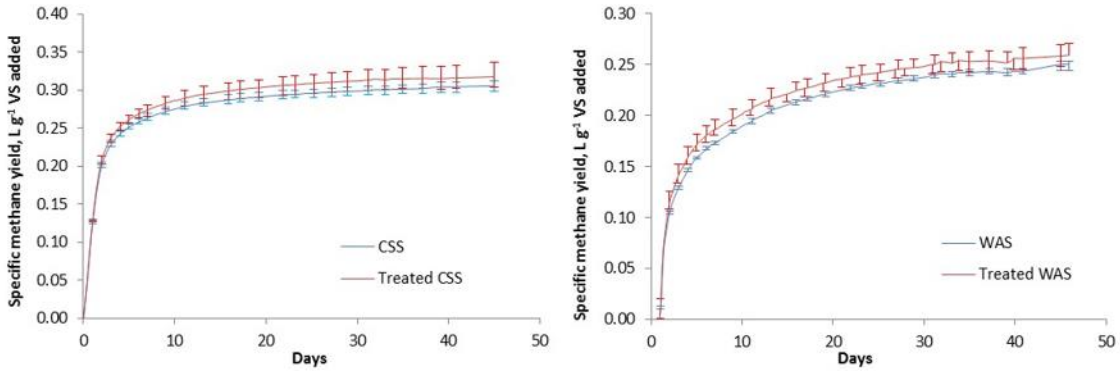

All replicates
Sample average
y = 0.7776x
R² = 0.9669
VS%
TS%
Ammonia mg N L-1
Control 1
Control 2
Test 1
Test 2
Day
b
Total Alkalinity mg CaCO3 L-1
c
IA/PA
d


Specific biogas production L g-1 VS day-1
a
Control 1
Control 2
Test 1
Test 2
Day


Specific CH4 production L g-1 VS day-1
b
Control 1
Control 2
Test 1
Test 2
Day


E. coli MPN 100 mL -1
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
0
20
40
60
80
100
120
140
160
Days
Control 1
Control 2
Test 1
Test 2
Feed


Specific methane yield, L g-1 VS added
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
40
50
Days
CSS
Treated CSS
Specific methane yield, L g-1 VS added
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
40
50
Days
WAS
Treated WAS


Specific methane yield, L g-1 VS added
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
40
50
Days
CSS
Treated CSS
Specific methane yield, L g-1 VS added
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
40
50
Days
WAS
Treated WAS

Tables

Table 1 Log reduction in indicator organisms from P CO_2 treatment in different conditions and matrices

Substrate	Conditions	Small vessel		Large vessel	
		E. coli	*Salmonella*	*E. coli*	*Salmonella*
CSS	Same amount [a]	0.3	1.4	0.4	1.3
	Different amounts [b]	3.0	2.6	0.2	1.6
Culture broth	Same amount [a]	7.9	-	7.9	-
	Different amounts [b]	7.5	-	5.4	-

[a] 100 g in both pressure vessels

[b] 3 g and 600 g in large and small pressure vessels, respectively

Table 2 Kinetic coefficients obtained for average of replicated samples of each substrate

Parameter	Untreated CSS	Treated CSS	Untreated WAS	Treated WAS
Ym	0.304	0.316	0.251	0.259
P	0.77	0.77	0.45	0.48
k1	0.81	0.79	1.72	1.69
k2	0.09	0.09	0.10	0.11
R^2 [a]	0.9992	0.9991	0.9994	0.9991

[a] R^2 = correlation coefficient for modelled and experimental values

Table

Substrate	Conditions	Small vessel		Large vessel	
		E. coli	*Salmonella*	*E. coli*	*Salmonella*
CSS	Same amount [a]	0.3	1.4	0.4	1.3
	Different amounts [b]	3.0	2.6	0.2	1.6
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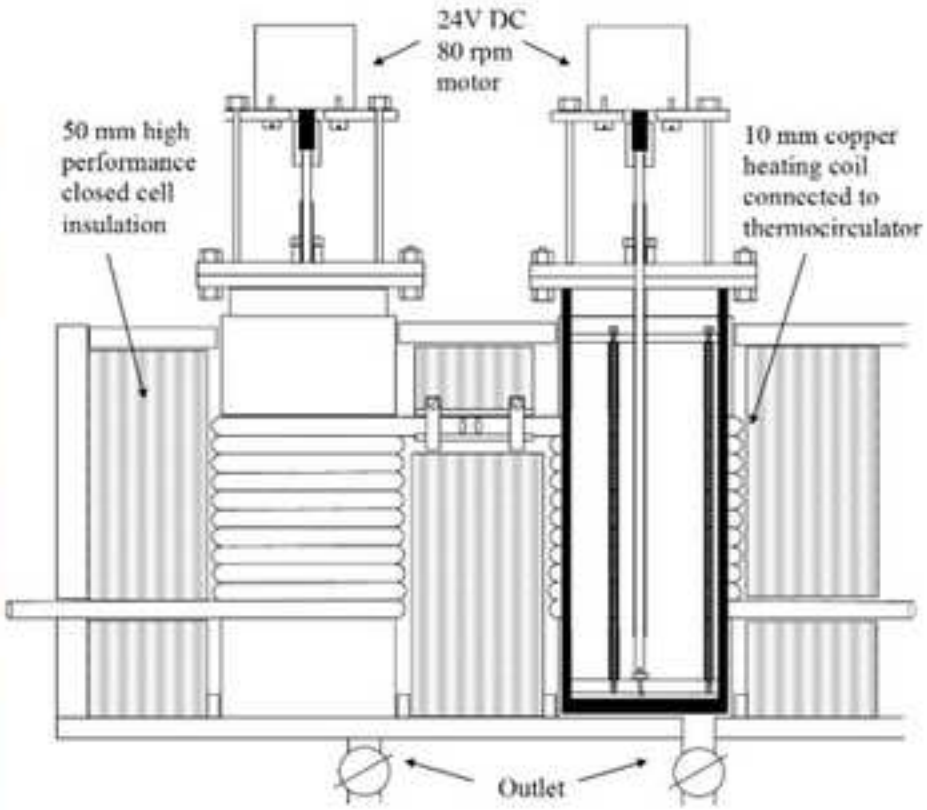
Figure


24V DC
80 rpm
motor
50 mm high
performance
closed cell
insulation
10 mm copper
heating coil
connected to
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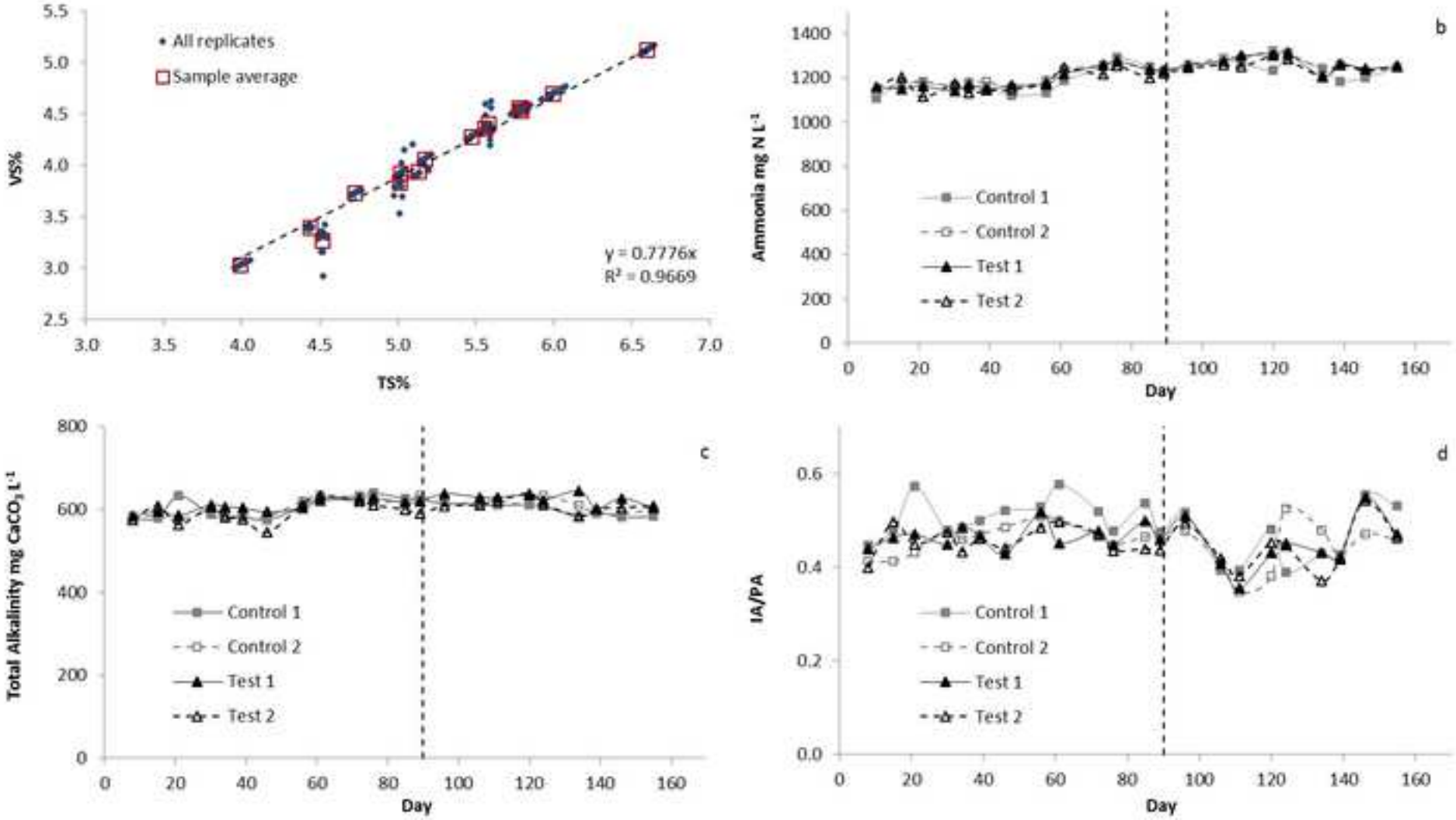
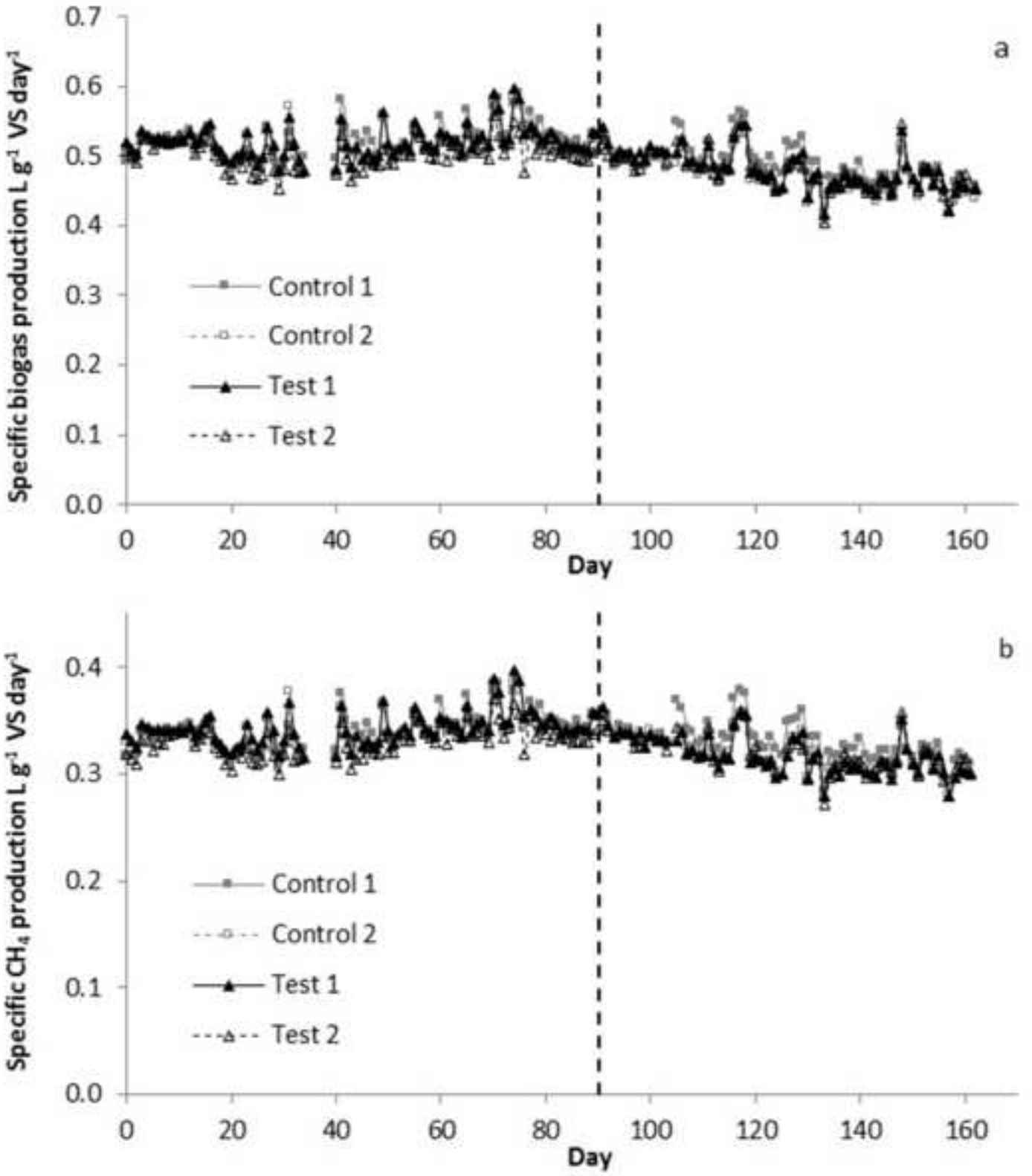
Outlet

Figure

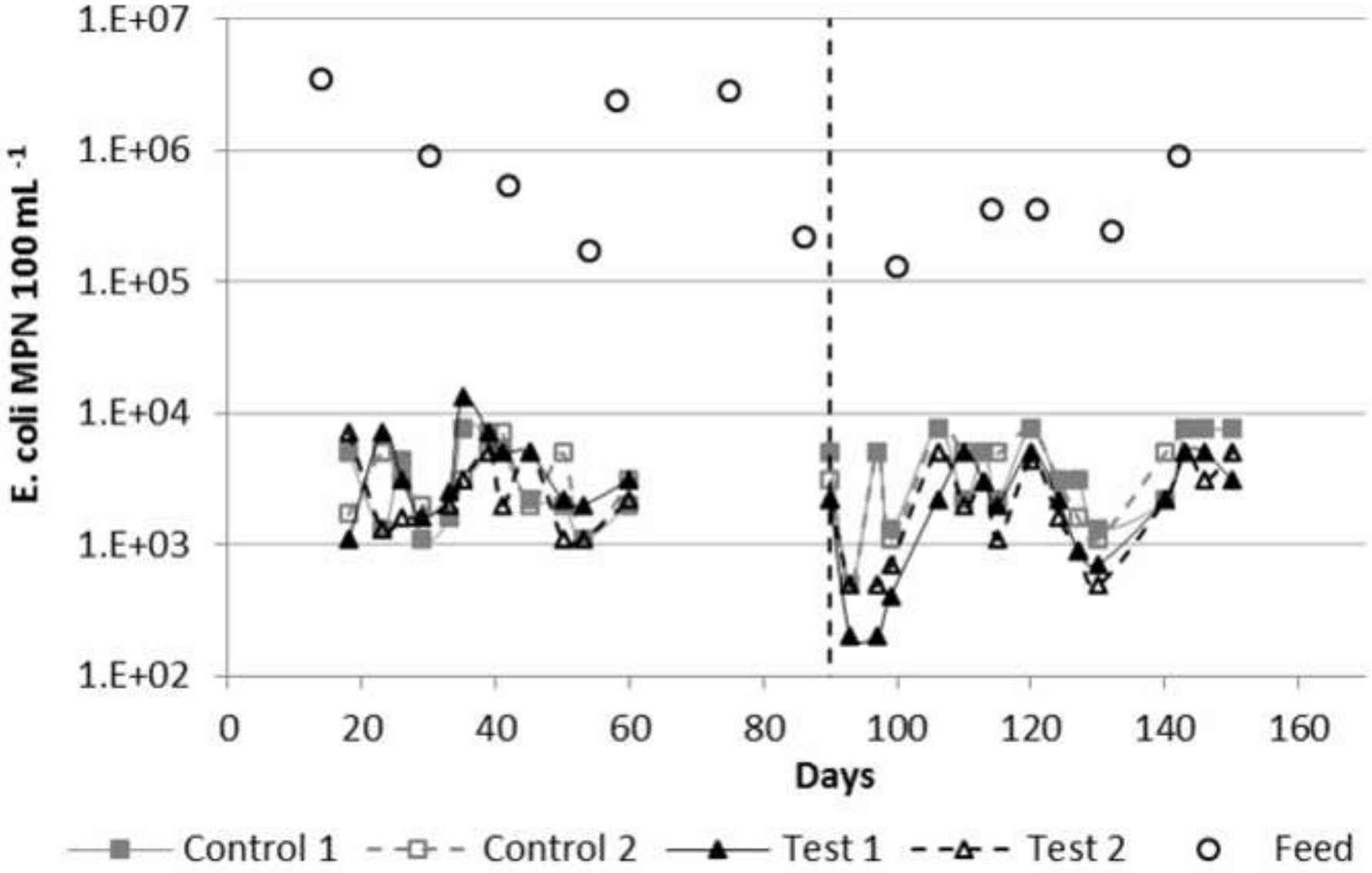
Figure


All replicates
Sample average
y = 0.7776x
R² = 0.9669
VS%
TS%
b
Ammonia mg N L⁻¹
Day
Control 1
Control 2
Test 1
Test 2
c
Total Alkalinity mg CaCO₃ L⁻¹
d
IA/PA

Figure


a
Specific biogas production L g-1 VS day-1
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Control 1
Control 2
Test 1
Test 2
0
20
40
60
80
100
120
140
160
Day
b
Specific CH4 production L g-1 VS day-1
0.4
0.3
0.2
0.1
0.0
Control 1
Control 2
Test 1
Test 2
0
20
40
60
80
100
120
140
160
Day

Figure


1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
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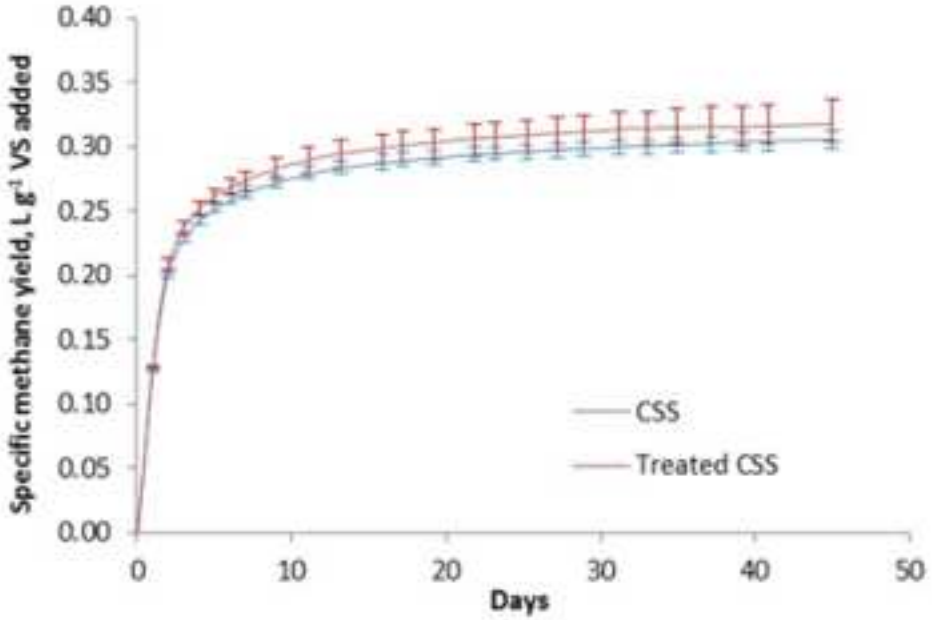
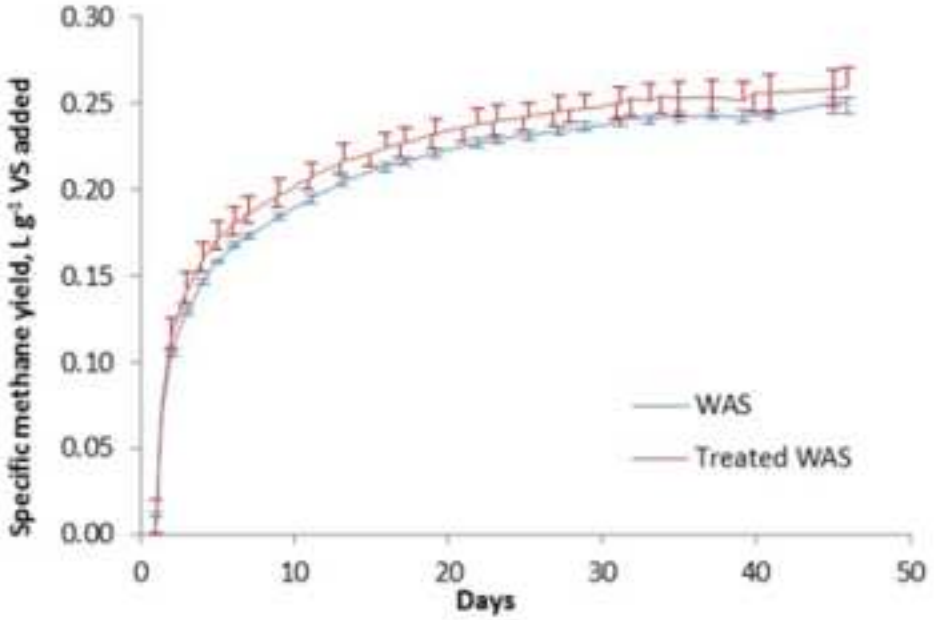
E. coli MPN 100 mL -1
0
20
40
60
80
100
120
140
160
Days
Control 1
Control 2
Test 1
Test 2
Feed

Figure


Specific methane yield, L g-1 VS added
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
40
50
Days
CSS
Treated CSS


Specific methane yield, L g-1 VS added
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
10
20
30
40
50
Days
WAS
Treated WAS