Effect of total solids content on anaerobic digestion of poultry litter with biochar

Mathu Indren[a,c,*], Cristian H. Birzer[a,c], Stephen P. Kidd[b,c], Paul R. Medwell[a,c]

[a]*School of Mechanical Engineering,*
[b]*School of Biological Sciences,*
[c]*Humanitarian and Development Solutions Initiative,*
The University of Adelaide, South Australia, 5005, Australia

Abstract

Methane production via anaerobic digestion of poultry litter provides a pathway for energy production from an abundant waste product. Recent studies have shown the use of biochar (pyrolysed biomass) can decrease methane production lag times and increase peak daily yields from ammonia-stressed low-solids anaerobic digesters. Due to the variety of feedstocks and digester configurations used, research to date has not yet determined the effect of biochar addition as a function of the digester total solids content. This study shows the addition of biochar reduces the lag time by a greater percentage in the digesters with a higher total solids content. There was a 17%, 27% and 41% reduction lag time due to biochar addition at total solids contents of 5%, 10% and 20%, respectively. The peak daily methane yield increased by 136% at 10% total solids. There was no significant increase in the peak yield at 5% total solids, while there was a 46% increase at 20% total solids. Real-time PCR analysis confirms the *Methanosaetaceae* family, which is a key methanogen due to its ability to facilitate direct interspecies electron transfer while attached to biochar, preferentially attaches to biochar. Furthermore, this research shows the attachment of the *Methanosaetaceae* family, does not decrease with increasing total solids content. A potential negative effect of biochar addition, a reduced volumetric efficiency, can be negated by using a shorter retention time. This new understanding will help to improve predictions of the impact of biochar

*Corresponding author
Email address: mathu.indren@adelaide.edu.au (Mathu Indren)

addition for new digester designs operating in semi-solids and high-solids conditions.

Keywords: Anaerobic digestion, Biochar, Poultry litter, Total solids content, Biogas

1. Introduction

Anaerobic digestion is the biological degradation of organic matter by a diverse group of microorganisms in the absence of oxygen. During anaerobic digestion, organic material is converted to biogas, which is approximately 65% methane and 35% carbon dioxide. Biogas is a combustible gas which can be used for energy generation. Various types of organic matter can be used as a feedstock for anaerobic digesters. Poultry litter, a waste product from poultry meat production, is a highly abundant form of organic matter. Poultry production is the fastest-growing meat source worldwide, with the majority of increased consumption coming from developing countries (Delgado, 2003). However, poultry litter is underutilised as a feedstock for anaerobic digesters.

Anaerobic digestion is a multi-step process consisting of hydrolysis, acidogenesis (acid generation), acetogenesis (acetate generation) and methanogenesis (methane generation). Under specific conditions, each step can be rate-limiting. In digesters processing poultry litter, the rate-limiting steps can be hydrolysis and/or methanogenesis (Batstone and Jensen, 2011). Hydrolysis is the conversion of complex particulate materials, which cannot be used by anaerobic microorganisms, into soluble substrates. It can be a rate-limiting step due to the high proportion of particulates and solids in poultry litter. Methanogenesis can be rate-limiting as the activity of methane-generating microorganisms (methanogens) decreases with increasing ammonia concentrations (Chen et al., 2008; Rajagopal et al., 2013; Yenigün and Demirel, 2013).

The total solids (TS) content (a measure of the water content) is a key parameter in anaerobic digestion of poultry litter which affects hydrolysis and methanogenesis rates. Increasing the total solids content from 10–30% can cause a decrease in the peak daily methane yield by around 60% due to lower hydrolysis rates (Abbassi-Guendouz et al., 2012). Lower rates of methane production can also occur with increasing total solids content due to lower diffusion rates of soluble interme-

diate products throughout the digester (Bollon et al., 2013; Xu et al., 2014). Also, in ammonia-stressed digesters increasing the total solids content from 5% to 20% results in a 7-fold decrease in total methane yield (Li et al., 2013). Despite these disadvantages, the volumetric efficiency, defined as the unit volume methane produced per unit volume of the bulk sludge, increases with increasing total solids content, when methanogenesis is not inhibited. As a result of lower capital costs, high-solids digesters have greater economic viability than low solids digesters when processing a mixture of dairy manure, corn stover and tomato residues (Li et al., 2018), a mixture that is not expected to cause ammonia inhibition. Methods to improve methane performance of ammonia-stressed semi and high-solids digesters will improve the viability of using poultry litter, a highly abundant waste product, for methane production.

One method to improve the performance of anaerobic digesters is the addition of biochar. Biochar is a solid residue from pyrolysis of biomass, which has traditionally been used as a soil-additive (Lehmann and Joseph, 2009).In ammonia-stressed low-solids digesters, biochar has been shown to decrease the lag time before methane production starts and increase the peak daily methane yield (Lü et al., 2016; Pan et al., 2019; Wang et al., 2017). The suggested mechanisms for enhanced methane production include: (i) improved resistance to acid-stress due to the acid-buffering capacity of biochar (Wang et al., 2017); (ii) improved resistance to ammonia stress via attachment of microorganisms resulting in the formation of biofilms (Mumme et al., 2014; Sossa et al., 2004) ; and (iii) an improved rate of methanogenesis via direct interspecies electron transfer (DIET) between bacteria and methanogens both attached to the biochar surface (Rotaru et al., 2014).

In anaerobic digestion of poultry litter, the influence of these mechanisms is expected to vary with the total solids content. The effect of total solids content in digesters with biochar is not well understood. Ammonia-stress on the methanogenesis step will increase due to a lower amount of water dilution. Furthermore, the attachment of microorganisms onto the biochar requires sufficient contact between microorganisms and biochar. The level of contact is expected to decrease with

increasing total solids content due to lower rates of mass transfer within the bulk sludge. These variations in conditions within the bulk sludge indicate a need to understand the effect of biochar addition as a function of the total solids content.

This study aims to to identify the effects of wood-pellet biochar on methane production from poultry litter as a function of the digester total solids content. The specific objectives are to determine changes in methane production in terms of yield, production rate and volumetric efficiency. In addition, changes in the chemical conditions and population of methanogens are analysed at each total solid regime.

2. Methodology

2.1. Anaerobic digestion assay

The anaerobic digesters were 500 ml glass bottles. The volume of biogas was measured by displacement of saturated sodium chloride solution (Walker et al., 2009). The volume of biogas was corrected to dry gas at 0°C (Richards et al., 1991). There were triplicate digesters for each testing scenario. The control digesters did not include biochar. Each digester was flushed with high-purity nitrogen gas to generate anaerobic conditions. The digesters were placed in a 37°C temperature-controlled room. Mixing of the digesters was conducted for 10 seconds, once per day, five days per week. At 20% total solids, the digesters were mixed by inversion while the digesters at 10% and 5% were mixed by swirling. The total solids content of the digesters was set at 20%, 10% and 5% using Milli-Q water. The calculation of total solids did not include the total solids content of the biochar. The weight of each material added is shown in the supplementary data (Table A1).

2.2. Characterisation of materials

The feedstock, poultry litter with wood-shavings the bedding material was sourced from a farm in South Australia. The source of methane-generating microorganisms (inoculant) was centrifuged anaerobic digester effluent from a wastewater treatment facility (SA Water, South Australia). The

volatile solids-based feedstock to inoculant (F:I) ratio was 2. This ratio was chosen to maximise the amount of poultry litter in the digester. Prior to the methane production assay, the inoculant was maintained at 37°C for three days to reduce its residual methane production potential, while maintaining an active microbial population.

The biochar was produced using commercially available wood-pellets in a top-lit up-draft gasifier (TLUD) (Kirch et al., 2018). The composition of the wood-pellets were a mix of timber waste from multiple timber mills around Australia. The TLUD contained 2.1 kg of wood-pellets per batch. The inner diameter of the TLUD was 98 mm. The peak temperature inside the TLUD was approximately 800°C, with an average residence time of 2.5 hours. The biochar was 10–20 mm in length and 4–6 mm in diameter. The biochar was added at an equivalent dry mass to the poultry litter and its dosage was constant across all three total solids regimes.

2.3. Biogas analysis

Samples of biogas were collected periodically using 10 ml gas-tight syringes. The composition of CH_4, CO_2 and H_2 in the gas was determined by a gas chromatograph with a thermal conductivity detector (Agilent, 490 MicroGC). The composition of CH_4 and H_2 was determined on a 5Åmolecular sieve 10 metre column, at 80°C using argon at 200 kPa as the carrier gas. The concentration of CO_2 was determined using a PoraPLOT U, 10 metre column at 80°C using helium at 150 kPa as the carrier gas. The injector temperature was set to 110°C.The gas chromatograph was calibrated using standard gases of known concentrations (CAC Gas, New South Wales, Australia).

2.4. Physical and chemical analyses

The total solids content was determined by drying samples at 105°C (Clesceri et al., 1999b). The volatile solids content was determined by ashing the materials at 550°C (Clesceri et al., 1999b) in a thermogravimetric analyser (Mettler Toledo, TGA-DSC2). The Elemental analysis (carbon, hydrogen and nitrogen) was performed in triplicate using a Perkin Elmer 2400 Series II elemental

analyser. The oxygen fraction was calculated as the difference of the CHN component and ash fraction. The sulphur content was assumed to be negligible. Prior to elemental analysis, the materials were oven-dried at 60°C.

Liquid samples of the inoculant, poultry litter and bulk sludge for pH, total alkalinity and total ammonia-nitrogen analysis were made by diluting 5 g of the bulk sludge in 20 ml of Milli-Q water, homogenising for 20 minutes and centrifuging at 2000G for 10 minutes. The pH of the supernatant was analysed by a pH probe (Mettler Toledo, InLab Expert Pro®) without stirring and recorded immediately. A two-point calibration of the pH probe was conducted before analysis. Total alkalinity was analysed by titrating the supernatant against 0.1 N H_2SO_4 to an end-point pH of 4.4 (Clesceri et al., 1999a). Total ammonia-nitrogen was analysed using the colorimetric salicylate method (Forster, 1995). The free ammonia-nitrogen concentration was calculated according to the relationship given by Hansen et al. (1998).

The total volatile fatty acid concentration of the inoculant, poultry litter and bulk sludge was determined by titrating the supernatant against 0.1 N H_2SO_4 between points 5 and 4.4 (Sun et al., 2017). Liquid samples for volatile fatty acids (VFA) measurements were prepared as described for pH analysis in digesters using 10% and 20% total solids only. At 5% total solids the VFA concentration was low and dilution with water resulted in a concentration outside the valid range. The total alkalinity of the biochar was determined by titration against 0.5 M NaOH (Singh et al., 2017). The volatile fatty acid and ammonia content was not determined for biochar as it was expected the concentrations would be significantly lower than concentrations in the inoculant and poultry litter.

2.5. Microbial population analysis

The population of methane-generating microorganisms in the inoculant, digester bulk sludge and on the biochar was analysed. In digesters using 5% and 10% total solids, 5 ml of the bulk sludge was centrifuged at 2000G for 10 minutes to produce a solid biomass pellet within the centrifuge

tube. It was not necessary to centrifuge the bulk sludge samples at 20% total solids to produce a biomass pellet. The DNA was extracted from the solid biomass samples and the biochar using a PowerSoil DNA isolation kit (Quiagen, Germany). The biochar samples were crushed using a mortar and pestle prior to DNA extraction. The quality of extracted DNA was checked using a 0.5% agarose gel stained with gel red. The quantity of DNA extracted was determined using a spectrophotometer (NanoDrop Technologies, Wilmington, USA).

Quantitative polymerase chain reaction (qPCR) was conducted using an iCycler (Bio-Rad Laboratories, Hercules, CA) to determine the abundance of *Methanobacteriales*, *Methanomicrobiales*, *Methanosaetaceae* and *Methanosarcinaceae* using previously developed primer sets (Yu et al., 2005). These families and orders account for the majority of methanogens commonly found in anaerobic digesters (De Vrieze et al., 2016; Li et al., 2014).

Quantitative PCR (qPCR) was conducted using an iCycler (Bio-Rad Laboratories, Hercules, CA) to determine the abundance of *Methanobacteriales*, *Methanomicrobiales*, *Methanosaetaceae* and *Methanosarcinaceae* using previously developed primer sets (Yu et al., 2005). The qPCR procedure was a two-step amplification that used initial denaturation at 95°C for three minutes, followed by 39 cycles of denaturing at 95°C for 10 seconds and simultaneous annealing and elongation at 55°C for 30 seconds. The final step included generating a melt curve by cycling at 65-95°C at 0.5°C per minute to check for primer dimer formation and product specificity. Each qPCR reaction was 20 µL in volume and used 3 µL of target DNA, 0.5 µL of forward and reverse primer each, 10 µL of SSO Advanced SYBR Green Supermix (Bio-Rad Laboratories, Hercule, CA) and 6 µL of nuclease-free water. Standard curves of target DNA were constructed using three technical replicates of 10-fold dilutions of standard DNA supplied by Deutsche Sammlung von Mikroorganismen und Zellkuturen GmbH (DSMZ, Braunschweig, Germany).

2.6. Scanning electron microscopy

A scanning electron microscope (XL30, Philips) was used to investigate the attachment of microorganisms on the biochar at each total solids regime. The biochar was first washed with phosphate-buffered saline (PBS) to remove loosely attached sludge and then placing the samples in a fixative containing 4% paraformaldehyde and 1.25% glutaraldehyde, in PBS. The samples were then dehydrated, firstly using ethanol/water mixtures containing 70%, 90% and 100% ethanol and then using hexamethlydisilazane (HDMS)/ethanol mixtures of 50% and 100% HDMS.

2.7. Analysis of kinetic parameters

Equation 1 is the modified Gompertz equation. The equation was used to model the potential methane yield, maximum daily methane production rate and methane production lag time. It has been used in other studies to quantify the changes in process performance due to biochar addition (Fagbohungbe et al., 2016; Lü et al., 2016; Pan et al., 2019). The parameters were calculated using the Grofit package (Kahm et al., 2010) in R (version 3.5.0).

$$M(t) = A \times exp\left\{-exp\left[\frac{R_{max} \times e}{A}(\lambda - t) + 1\right]\right\} \quad (1)$$

$M(t)$ is the total methane yield at time t (day), A is the potential methane yield (ml/g-VS), e is exp(1)≈2.71828; R_{max} is the maximum daily methane production rate (ml/g-VS/day) and λ is the lag time (days).

2.8. Analysis of results

The statistical analysis was used as a complementary tool to the experimental data to discuss changes due to biochar addition. Statistical analysis was conducted using R (version 3.5.0) and included one-way analysis of variance (ANOVA) with a significance value of 0.05. The Tukey post hoc test, with a significance value of 0.05, was used for a comparison of mean values between each scenario.

3. Results and discussion

3.1. The effect of total solids and biochar addition on total methane methane production

Figure 1 shows a time series of the total methane yield over 90 days, for digesters with biochar and the control digesters (without biochar) using total solids contents of 5%, 10% and 20%. The mean total yield is represented by the line and the range is represented by markers. Figure 1 indicates that the total methane yield after 90 days is not strongly affected by the presence of biochar. The total methane yield after 90 days is highest at 5% total solids, in both digesters with biochar and controls. Increasing the total solids from 5% to 10% decreased the mean total methane yield from 107 ml CH_4/g-VS by 30% in digesters with biochar and 28% in the controls. Increasing the total solids content from 5% to 20% decreased the total methane yield by 53% in digesters with biochar and 50% in the controls. The difference in the total methane yield after 90 days between digesters with biochar and the controls is less than 5% at the same total solid regime. This indicates the ammonia inhibition and the lower anaerobic degradability of nitrogen-rich substrates cannot be improved through the use of biochar.

Despite the unchanged total methane yield, Figure 1 shows digesters with biochar have a shorter lag time before methane production commences at all three total solids regimes. The lag time may be estimated by fitting the total methane production curve to the Gompertz model (equation 1). The model-predicted lag time, peak daily methane yield and potential methane yields are shown in Table 1. Table 1 also shows the percentage reduction in lag time due to biochar addition increases with increasing total solids content. At 5%, 10% and 20% total solids, the addition of biochar reduced the lag time by 17%, 27% and 41%. Possible causes of the long lag time at 5% TS is discussed in section 3.3.

Poultry litter varies in composition, in particular, the initial ammonia concentration and presence and type of bedding material. This makes comparisons between the methane yields achieved in other studies difficult. The methane yields at 5% and 10% total solids are 41–45% lower than

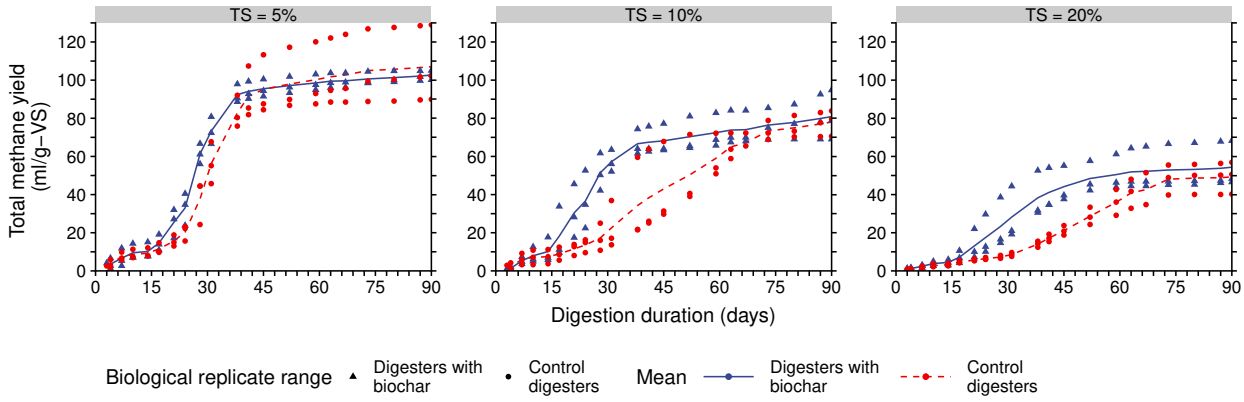

Figure 1: Normalised total methane yield over 90 days. Normalised yield is based on the initial volatile solids (VS) content of both the poultry litter and inoculant. Data are presented for control digesters and digesters with biochar at 5%, 10% and 20% total solids (TS). The lines show the mean and markers show the range of values from three biological replicates.

Table 1: Summary of the Gompertz model parameters for digesters with biochar and controls and with varying total solids contents

Scenario	Lag time (days)		Peak daily methane yield (ml/g-VS/day)		Potential methane yield (ml/g-VS)	
	mean	SD	mean	SD	mean	SD
TS = 5%, biochar	15.4	0.7	4.9	0.3	101.3	1.3
TS = 5%, control	18.6	1.4	4.5	0.6	106.3	2.8
TS = 10%, biochar	9.9	1.4	2.8	0.3	76.5	1.9
TS = 10%, control	13.5	2.5	1.4	0.1	89.0	7.5
TS = 20%, biochar	12.8	2.3	1.6	0.2	55.1	2.4
TS = 20%, control	21.6	1.8	1.0	0.1	58.5	4.0

yields previously reported for poultry litter (Li et al., 2013). This may be explained by the lack of bedding material used by Li et al. (2013). Also, the heterogeneous nature of the material and the high level of ammonia stress may have contributed to the variation in methane yields between the replicate digesters. Li et al. (2013) reported standard deviations of the total methane yield of 24 ml and 6 ml CH_4/g-VS at TS contents of 5% and 10%, respectively. By comparison, the standard deviations from replicate digesters in this study are 16 ml and 6 ml CH_4/g-VS at 5% and 10% total solids, respectively. At 20% total solids, Abouelenien et al. (2016) observed a 76% higher methane yield than this study, however, poultry litter without bedding material was used and the variation between replicates was not shown.

3.2. The effect of total solids and biochar addition on the daily methane production rate

Figure 2 shows the daily methane yield over 90 days, for digesters with biochar and the control digesters (without biochar) using total solids contents of 5%, 10% and 20%. The mean daily yield is represented by the line and the range of values are represented by the markers. The figure shows the daily methane yield varies over time. There is a small peak in the daily methane yield within the initial seven days which is followed by a second peak that occurs over 10-20 days later. After the occurrence of the peak daily yield, there is a rapid drop-off for digesters at 5% and 10% TS. After 90 days, the methane production is insubstantial for all scenarios.

Methane production within the initial seven days is likely caused by the presence of VFAs, in both the feedstock and the inoculant, as shown in Table 2. The VFAs are readily degraded by methane-generating microorganisms. It would be expected the initial VFA concentration will vary significantly between types of litter and storage time before its use in an anaerobic digester. This initial methane production from VFAs is not representative of the anaerobic degradability and will vary depending on the storage time of the poultry litter. Therefore, the peak daily methane yield referred to throughout this paper is the methane production that occurred after the first seven days.

The effect of biochar addition on the peak daily yield varies with the total solids content of the

Table 2: Characteristics of the poultry litter with wood-shavings bedding, de-watered anaerobic digester sludge (inoculant) and wood-pellet biochar.

Parameter	Poultry litter		Inoculant		Biochar	
	mean	SD	mean	SD	mean	SD
Total solids, TS (wt%)	47	2	17	1	97	1
Volatile solids, VS (wt%)	37	2	10	1	96	1
VS (% of TS)	80	3	59	1	98	0.1
Carbon (% of TS)	34.6	0.5	25.6	0.3	88.0	1.1
Hydrogen (% of TS)	5.42	0.1	5.43	0.02	1.9	0.1
Nitrogen (% of TS)	4.67	0.2	5.39	0.06	0.22	0.01
Oxygen (% of TS)*	35	23	7			
Ash (% of TS)	20	3	41	1	3	0.1
C/N	7.4	1.4	4.8	1.4	404.3	18.5
pH	8.94	0.01	8.40	0.01	10.3	0.4
Total alkalinity (g-$CaCO_{3eq}$/kg)**	26.0	2	2.29	0.08	8.6	1.2
Volatile fatty acids (g/kg)	2.96	0.7	2.29	0.4	ND	
Total ammonia-nitrogen (g-TAN/kg)	5.5	0.3	0.83	0.2	ND	

* Determined by subtraction: O = 100-(C+H+N+ash)

** Acid titration used for poultry litter and inoculant, acidification and back-titration against a base used for biochar.

ND = not determined, see Section 2.4 for details.

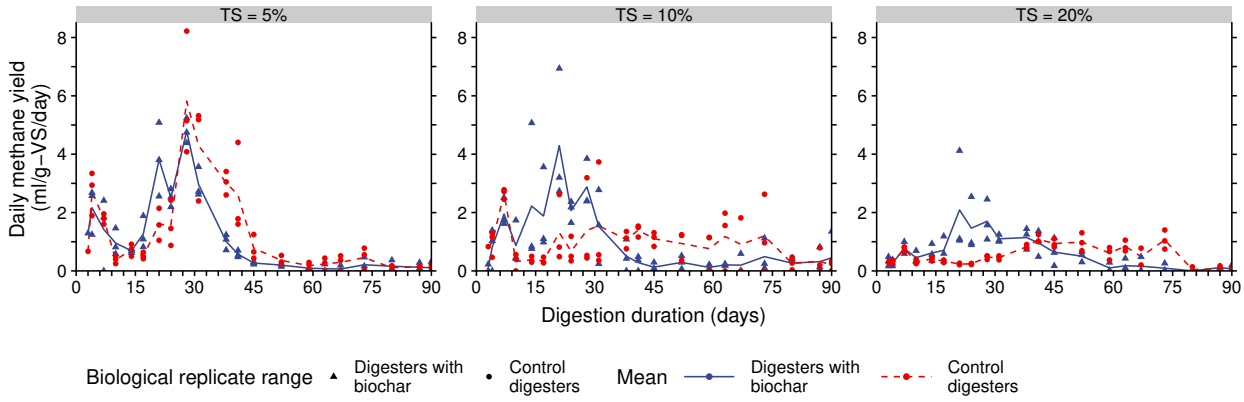

Figure 2: The normalised daily methane yield over 90 days. The normalised yield is based on the initial volatile solids (VS) content of both the poultry litter and inoculant. Data are shown for control digesters and digesters with biochar at 5, 10 and 20% total solids (TS). The lines show the mean and the markers show range from three biological replicates.

digester. At 5% total solids, the peak daily yield in digesters with biochar was not significantly different ($p>0.05$) to the peak yield in the controls. In addition, the peak yields occurred at roughly the same day. This is in contrast to 23–47% increases in the peak yield in low-solids ammonia-stressed digesters processing wastewater sludge where wood-based biochar was also used (Lü et al., 2016). It is not clear why the peak daily methane yield at 5% total solids was not increased in this study.

At 10% total solids, digesters with biochar have a 136% higher ($p<0.05$) peak daily yield than the controls. The peak daily yield occurs around day 21 in digesters with biochar. In the controls, the daily methane yield curve is flatter and there is no pronounced peak in daily yield as shown in digesters with biochar.

At 20% total solids the addition of biochar had a less pronounced effect on the daily yield compared with digesters operating at 10% total solids. The peak daily methane yield was 46% higher in digesters in biochar than in the controls, however, this increase was not statistically significant ($p>0.05$). The inability to achieve statistical significance could be caused by the smaller percentage

Table 3: Chemical conditions of the bulk sludge after 90 days in digesters with varying total solids contents and with the addition of biochar.

Scenario	pH		Total ammonia-nitrogen (g-TAN/kg)		Free ammonia-nitrogen (g-FAN/kg)		Volatile fatty acids (g/kg)		Total alkalinity (g-$CaCO_{3eq}$/kg)		VFA/TA	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
TS = 5%, biochar	8.0	0.0	2.4	0.6	0.3	0.07	0.4	0.2	6.3	0.2	0.06	0.04
TS = 5%, control	8.0	0.1	2.4	0.5	0.3	0.11	1.0	1.1	6.9	0.0	0.14	0.16
TS = 10%, biochar	8.3	0.0	4.4	0.2	0.8	0.07	1.1	0.2	12.2	0.5	0.09	0.01
TS =10%, control	8.3	0.1	4.2	0.9	0.7	0.12	1.4	0.0	13.8	0.6	0.10	0.01
TS = 20%, biochar	8.7	0.1	4.5	0.4	1.8	0.13	2.1	0.8	14.1	2.0	0.14	0.04
TS = 20%, control	8.5	0.1	6.5	0.5	1.9	0.13	3.8	0.2	17.0	0.5	0.22	0.01

changes and biological variations between the replicate digesters. The figure also shows the peak yield occurs earlier. In digesters with biochar at 20% total solids, the peak methane yields occurred between days 21–38, while in the controls the peak yields occurred between days 42 and 75.

3.3. The effect of total solids content and biochar addition on acid-stress

To compare the difference in the acid-buffering capacity of the bulk sludge due to biochar addition at each total solids regime, analysis of the pH, total alkalinity and volatile fatty acids (VFA) was conducted. The concentration of these chemical parameters, as well as the concentration of total ammonia-nitrogen, are shown in Table 3.

Table 3 shows the substantially lower total alkalinity at 5% TS (6.3–6.9 g-$CaCO_{3eq}$/kg) compared with 12.2–13.8 g-$CaCO_{3eq}$/kg at 10% and 14.1–17.0 at 20% TS correlates with a higher total ammonia-nitrogen concentration. This is expected as ammonia is a weak base. In addition, the

total alkalinity is unaffected by wood-pellet biochar addition. The lower total alkalinity means the digesters at 5% total solids are more susceptible to acid-stress caused by VFA production. This could have resulted in a pH drop below the ideal range of 6.5–8.5 (Sung and Liu, 2003) in the early stages of digestion. This may explain the longer methane production lag times at 5% total solids (15.4–18.6 days) compared with lag times in digesters at 10% (9.9–13.5 days) total solids. To support this possibility, digesters at 5% total solids have a lower final pH (8.0 ± 0.1) compared with digesters at 10% total solids (pH of 8.3–8.7). Measurements of pH at intermediate time points were not collected as preliminary experiments showed opening the digesters for sample collection affected the measured methane yield.

As wood-pellet biochar has a low total alkalinity (Table 2), it is likely the reduction in lag time due to wood-pellet biochar addition is not due to acid buffering capacity at any total solids regime. The slower rate of degradation and higher ammonia concentration at 10% and 20% total solids reduces the degree of acid-stress. This suggests biochar with a low total alkalinity, such as wood-based biochar is suitable for use in digesters at 10% and 20% total solids. However, at 5% total solids, the lag time caused by acid-stress could be reduced using biochar with a higher total alkalinity such as biochar produced from vermicompost (Wang et al., 2017).

3.4. The effect of total solids content and biochar addition on ammonia-stress

The degree of ammonia inhibition at each total solids regime was analysed by measurements of both total ammonia nitrogen (TAN) and free ammonia-nitrogen (FAN) concentrations at the end of the 90-day digestion period. The data are shown in Table 3. The lowest TAN concentration was recorded at 5% total solids, 2.4 g TAN/kg in both digesters with biochar and controls. This occurs due to a lower amount of water dilution. At 20% total solids, the TAN concentration was 170% higher ($p<0.05$) in controls but only 90% higher in digesters with biochar.

Compared with the TAN concentration, there was a larger increase in the FAN concentration with increasing total solids. At the same total solids regime, there was no statistically significant

difference ($p>0.05$) in the FAN concentration between digesters with biochar and controls. The lowest FAN concentration of 0.3 g-FAN/kg was recorded at 5% total solids in both digesters with biochar and controls. At 10% total solids the concentration increased by 229% ($p<0.05$) in digesters with biochar and by 141% in the controls. At 20% total solids the concentration was increased by 621% ($p<0.05$) in digesters with biochar and by 531% ($p<0.05$) in the controls. The higher FAN concentration occurs due to a higher total ammonia-nitrogen as well as a higher pH. A high pH shifts the equilibrium between the ammonium ion and free ammonia-nitrogen, towards the formation of free ammonia-nitrogen (Hansen et al., 1998). The inhibition caused by FAN can explain the 27–37% lower total methane yield at 10% total solids as well as the 47–57% lower yield at 20% total solids compared with digesters operating at 5% total solids.

3.5. The effect of total solids content on biochar-microorganism interactions

3.5.1. Microorganisms in the bulk sludge

To further understand the effect of total solids and biochar addition on the methane production process, analysis of the population of methane-generating microorganisms (methanogens) was conducted. Methane production in anaerobic digesters occurs via two main pathways: (i) the cleaving of acetate into methane and carbon dioxide (acetoclastic methanogenesis); and (ii) consumption of hydrogen and the reduction of carbon dioxide into methane (hydrogenotrophic methanogenesis) (Holmes and Smith, 2016). Each pathway is facilitated by a different group of methanogens.

Figure 3 shows the relative abundance of the targeted methanogens in the bulk sludge after 90 days. Data are presented for digesters with biochar and controls, at each total solids regime, and for each of the targeted methanogens. The targeted methanogens were the strictly acetate-consuming *Methanosaetaceae* family, the acetate or hydrogen-consuming *Methanosarcinaceae* family, and the strictly hydrogen-consuming orders *Methanobacteriales* and *Methanomicrobiales*. The population of these methanogens is presented as a relative abundance of the DNA detected from the targeted family/order as a percentage of all the DNA extracted from the bulk sludge. Data from each bio-

logical replicate represent one sample taken from each of the replicate digesters.

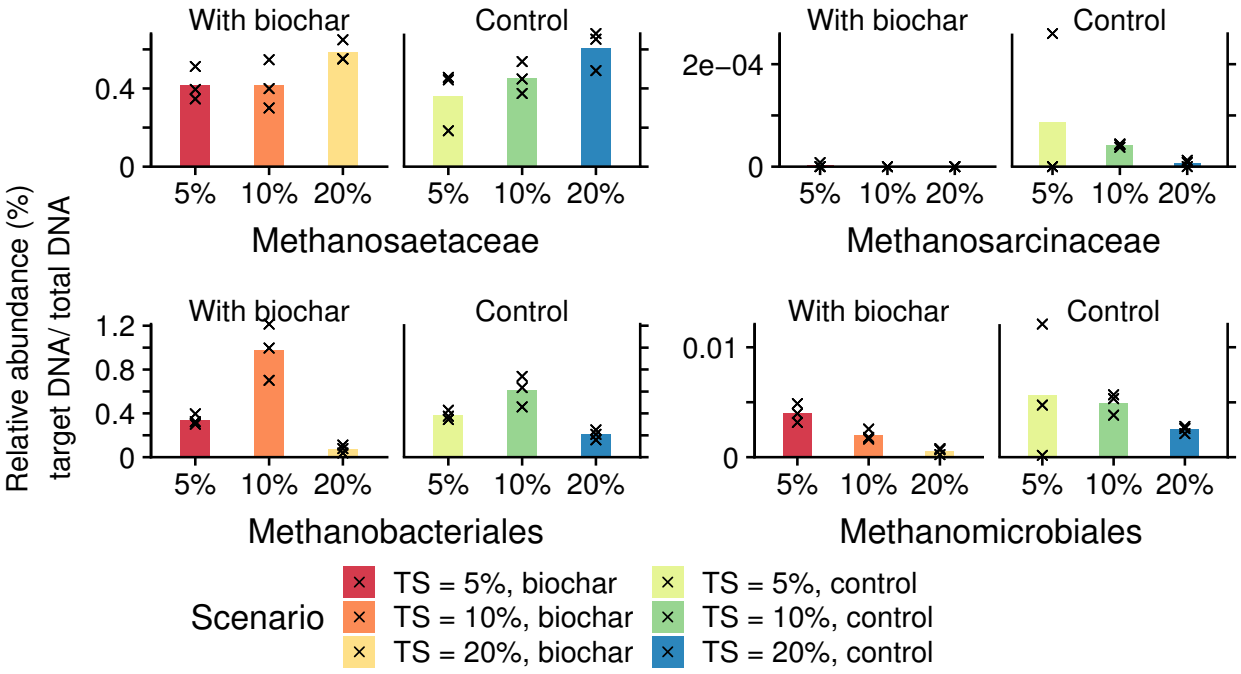

Figure 3: The relative abundance of target methane-generating microorganisms against total DNA extracted from the bulk sludge after 90 days. Bars show the mean value and markers show the variation between biological replicates.

Figure 3 shows only the populations of *Methanosaetaceae* and *Methanobacteriales* are significant in the bulk sludge. These methanogens have a relative abundance greater than 0.01% in the bulk sludge at all total solids regimes. The population of methanogens in the bulk sludge changes with the total solids content, yet is unaffected by biochar addition. These findings are similar to the changes in chemical conditions in the bulk sludge.

At 5% total solids, the relative abundance of *Methanosaetaceae* and *Methanobacteriales* in the bulk sludge was approximately equal ($\approx$ 0.4% of total DNA). At 10% total solids, the relative abundance of *Methanosaetaceae* (0.40–0.45% of total DNA) is lower than the relative abundance of *Methanobacteriales* (0.7-0.9% of total DNA). At 20% total solids, the relative abundance of

Methanobacteriales decreases to 0.1-0.2% of total DNA. At this total solids regime, *Methanosaetaceae* was the dominant methanogen (≈ 0.6% of total DNA).

The presence of *Methanosaetaceae* in the bulk sludge is likely due to its dominance in the digesters at the start of the digestion period. It accounts for all the methanogens in the inoculant (wastewater treatment plant sludge, Figure A2). The higher proportion of *Methanobacteriales* at 5% and 10% total solids may be due to a larger degradation rate of complex organics allowing for the diversification of the microbial population. A low population of hydrogenotrophic methanogens in ammonia-stressed digesters operating at 15% total solids has been observed (Dai et al., 2016). This may be due to competition for hydrogen with sulphate-reducing bacteria (Holmes and Smith, 2016) or the low level of gas/liquid mass transfer with an increasing total solids content (Abbassi-Guendouz et al., 2012).

The increase in hydrogen-consuming methanogens with lower total solids may be due to their higher resistance to ammonia stress. They are generally more dominant when the free ammonia-nitrogen content rises above 0.13–0.33 g-FAN/L (Schnürer and Nordberg, 2008). This threshold was achieved at all total solids regimes in this study. Therefore their growth rate is not limited by the free ammonia-nitrogen concentration in the digesters. It is not clear why *Methanobacteriales* grew in favour of the methanogens from the other hydrogen-consuming order, *Methanomicrobiales*, however, other studies have shown similar results (Lü et al., 2016; Yang et al., 2017). The presence of ammonia-tolerant methanogens explains the increase in total and peak daily methane yield at 5% and 10% total solids.

3.5.2. Microorganisms associated with the biochar

The methanogens associated with the biochar as a function of digester total solids content were analysed. Figure 4 shows the relative abundance of the targeted methanogens attached to the biochar at the end of the 90-day digestion period. The data are presented as percentage of the total DNA from all microorganisms extracted from the biochar. The biological replicates represent

one biochar pellet taken from each of the replicate digesters.

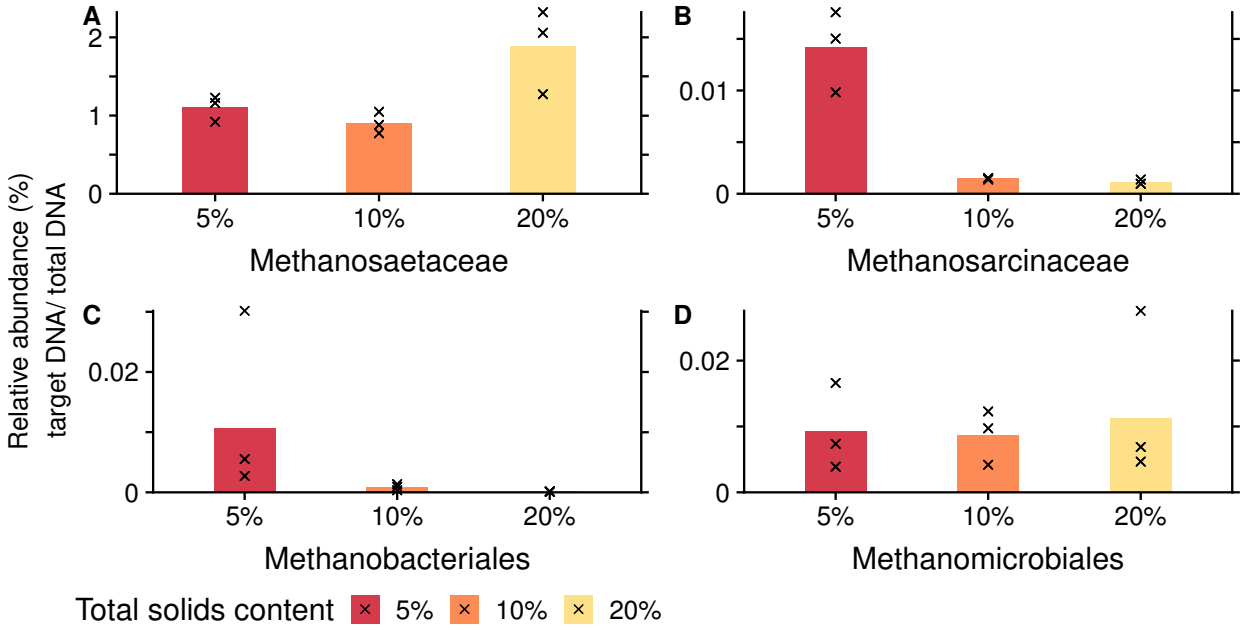

Figure 4: The relative abundance of target methane-generating microorganisms against total DNA from all microorganisms associated with the biochar. Biochar samples were collected after 90 days in digesters. Bars show the mean value and markers show the variation between the biological replicates.

Figure 4 shows the *Methanosaetaceae* family is the dominant methanogen associated with the biochar (1–1.8% of total DNA). All other methanogens consist of less than 0.05% of total DNA. This indicates *Methanosaetaceae* is preferentially attached to the biochar surface. If the degree of attachment was only related to the relative abundance of methanogens in the bulk sludge, attachment of *Methanomicrobiales* onto the biochar would be expected at 5% and 10% total solids.

There is no statistically significant change ($p>0.05$) in the proportion of *Methanosaetaceae* associated with the biochar with digesters at 10% or 20% total solids compared with digesters at 5% total solids. The lower rate of mass transfer with an increased total solids content was expected to decrease the amount of biochar/methanogen contact and the level of attachment. A possible explanation for not seeing this effect may be the higher ammonia concentration leading to the

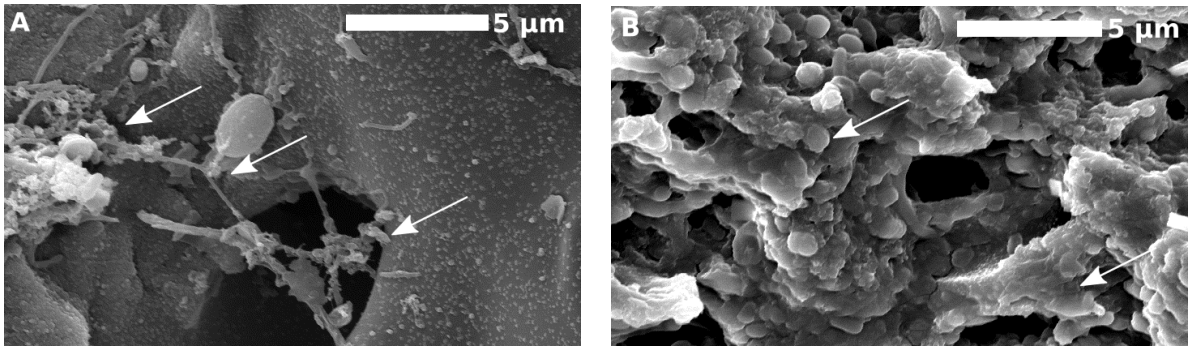

Figure 5: Wood-pellet biochar taken from a digester at 5% (A) and 20% (B) total solids after 90 days. The arrows highlight microorganisms in a possible biofilm.

attachment of *Methanosaetaceae* within a biofilm. **??**(A) and (B) show possible biofilm formation on biochar taken from digesters at both 5% and 20% total solids, respectively. Microorganisms are known to form biofilms when under environmental stress (Petrova and Sauer, 2012), such as high ammonia concentrations. It has been suggested that *Methanosaetaceae* has a lower tolerance to ammonia compared to hydrogenotrophic methanogens such as *Methanobacteriales* (Schnürer and Nordberg, 2008). Future research could involve investigations into the population of methanogens as well as bacteria that exist within these biofilms.

Another possibility is the presence of biochar increases the overall porosity of the bulk sludge. The porosity of bulk sludge in digesters with total solids content greater than 15% is known to decrease during the digestion period, which results in liquid movement through preferential channels (André et al., 2015). This would be expected to decrease the level of biochar/microorganism contact. As biochar does not decompose under anaerobic conditions, its physical presence in the bulk sludge may allow for greater flow of water through bulk sludge. The increased porosity of the bulk sludge may be more important in high-solids leach-bed digesters where a liquid is recirculated through the digester to improve mass transfer rates.

An additional benefit of the preferential attachment of *Methanosaetaceae* onto the biochar sur-

face is the possibility of direct interspecies electron transfer (DIET) between methanogens and other bacteria. In co-culture studies, biochar has been shown to facilitate methane production between *Methanosaeta* species and electron-donating bacteria, also attached to the biochar, such as *Geobacter* species (Rotaru et al., 2014; Zhao et al., 2016). As a result, through interaction with other microorganisms, via the DIET mechanism, *Methanosaeta* does not rely solely on acetate for its metabolism. This could lead to the shorter lag times in digesters with biochar. This mechanism may be of greater importance with an increasing total solids content as the diffusion rate of acetate will decrease with an increasing total solids content. This may explain the greater reduction in lag time due to biochar addition with an increasing total solids content.

3.6. Practical implications at each total solids regime

The use of an additive that does not degrade under anaerobic conditions will decrease the digester volumetric efficiency. This has the possibility of negating the benefits of using a digester with a higher total solids content. Figure 6 shows the volumetric efficiency of the digesters at the three total solids regimes. Due to a lower amount of water, the volumetric efficiency increases with increasing total solids content. This figure can be compared with Figure 1, in which the methane yield is normalised per gram volatile solids and the yield decreases with increasing total solids content.

The digesters with biochar have a 10–11% lower volumetric efficiency after 90 days than the controls at the same total solids regime. Digesters with biochar approach their maximum volumetric efficiency earlier. This is due to their shorter lag times at all total solids regimes and earlier peak yields at 10% and 20% total solids. Digesters with biochar have a larger volumetric efficiency than the controls before 41 days at 5% total solids, 63 days at 10% total solids and 67 days at 20% total solids. At these time points, digesters with biochar have achieved 89%, 83% and 89% of the Gompertz-model potential methane yield (Figure A3). By comparison, at these time points, controls digesters have achieved 86%, 73% and 74% of the Gompertz-model potential methane yield.

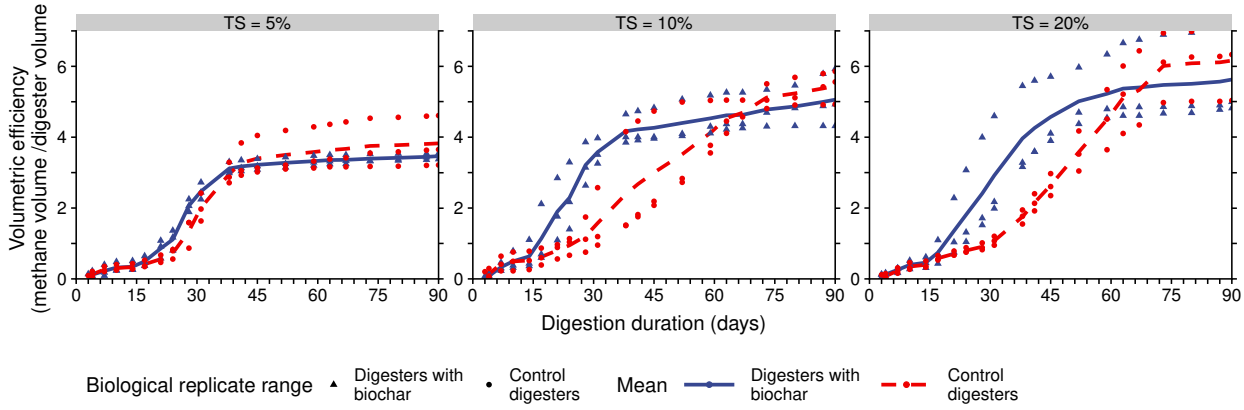

Figure 6: Effect of biochar addition on the volumetric efficiency of a digester. The volumetric efficiency is the volumetric methane yield per unit digester working volume. Data are given for digesters with total solids (TS) content of 5%, 10% and 20%. Markers show the range of values from the three biological replicates for each scenario and the lines represent the mean value.

Thus, a superior volumetric efficiency can be achieved in digesters with biochar by using a shorter retention time.

4. Conclusions

The analysis of methane production as a function of total solids content (TS = 5%, 10% and 20%) has extended knowledge of biochar-enhanced anaerobic digestion. To date, the majority of studies using biochar in anaerobic digesters have focused on low-solids conditions. This study shows the percentage reduction in lag time due to biochar addition increases with an increasing total solids content. In addition to the shorter lag time, at 10% and 20% total solids, there are increases to the peak daily methane yield and the peak daily yield occurs earlier. These findings could lead to increased viability of operating digesters at higher total solids content despite lower total methane yields. Also, this study has shown there is a cross-over time point before which digesters with biochar have greater volumetric efficiency than the control digesters.

Biological-based interactions, such as the formation of biofilms or electrical-based interactions

between *Methanosaetaceae* with the biochar, likely leads to the reduced lag time at all total solids regimes. Increasing the total solids content does not reduce concentrations of *Methanosaetaceae* attached to the biochar surface.

5. Supplementary Material

Supplementary material for this work can be found in online version of the paper.

6. Acknowledgements

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Appendix A. Supplementary Material

Appendix A.1. Digester loading

Table A1: The mass of material added to the digesters in each scenario.

TS (without biochar)	Feedstock			Inoculant			Water	Biochar		TS (with biochar)
	Wet weight (g)	TS (g)	VS (g)	Wet weight (g)	TS (g)	VS (g)	Wet weight (g)	Wet weight (g)	TS (g)	
20%	26.8	12.6	10.0	49.2	8.4	5.0	29.1	13.0	12.6	28%
10%	26.8	12.6	10.0	49.2	8.4	5.0	134.2	13.0	12.6	15%
5%	26.8	12.6	10.0	49.2	8.4	5.0	344.5	13.0	12.6	8%

Appendix A.2. Methane production from biological replicates

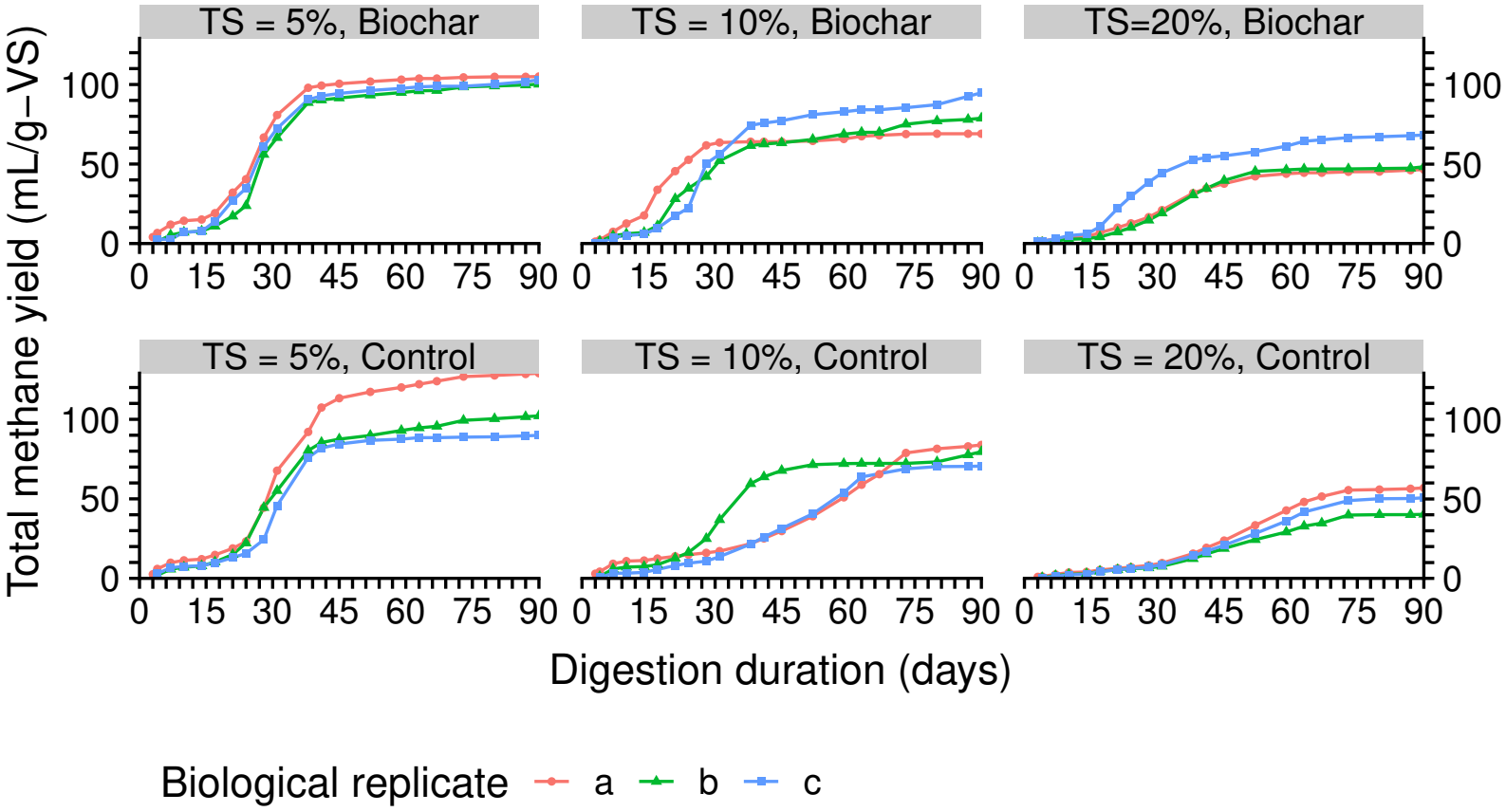

Figure A1: The total methane yield over 90 days, normalised based on initial the volatile solids (VS) content of poultry litter and inoculant, from digesters with varying total solids content, with biochar and the control digesters (without biochar). The markers show the methane yield from each biological replicate digester

Appendix A.3. Microbial population of inoculant

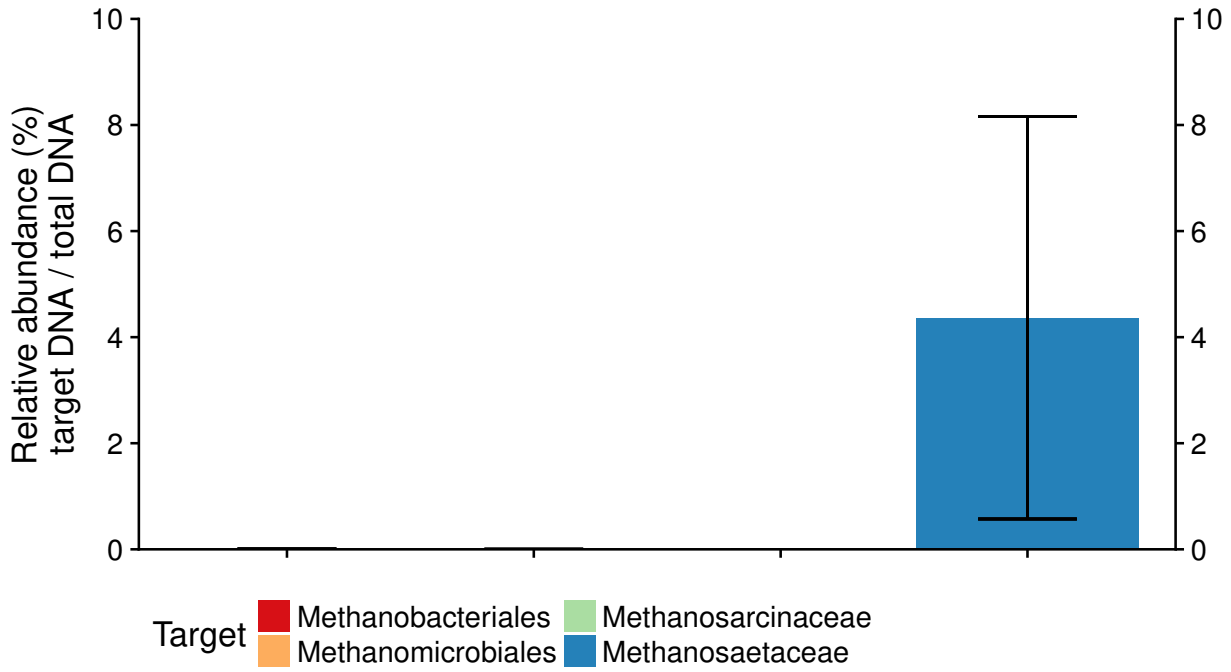

Figure A2: The relative abundance of methane-generating microorganisms (methanogens) against total DNA in the inoculant (de-watered wastewater treatment plant sludge). Error bars show the standard deviation from the biological replicates.

Appendix A.4. Digestion time

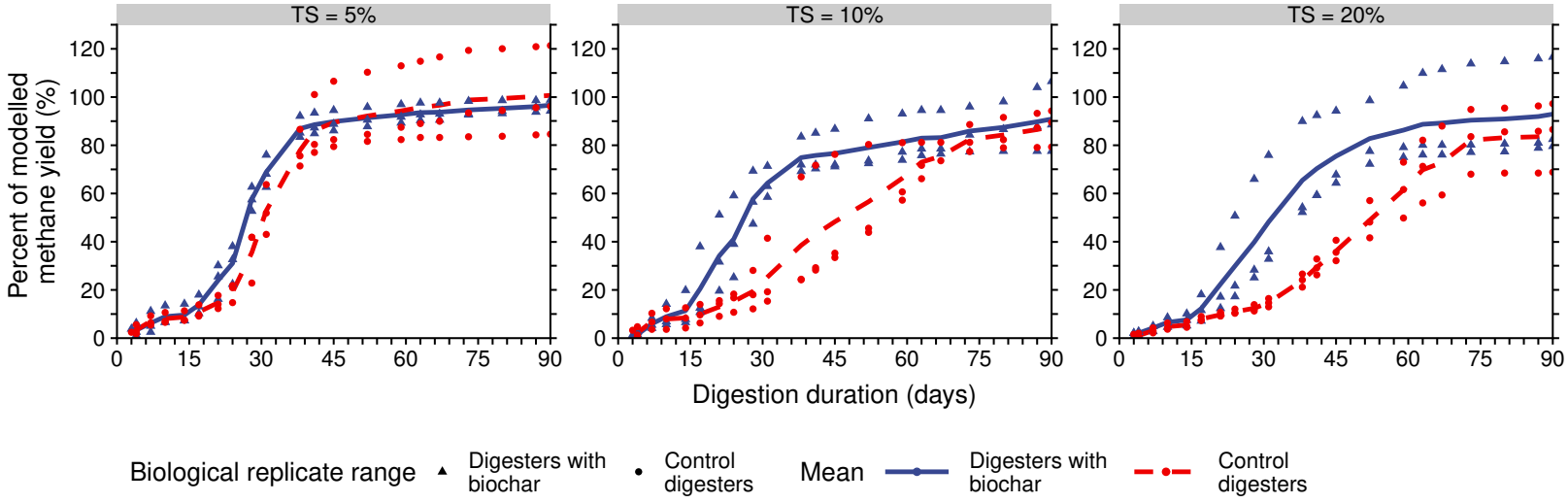

Figure A3: The time needed for digesters with biochar and controls (without biochar) using total solids contents of 5%, 10% and 20% to produce a percentage of the maximum Gompertz model methane yield. The markers represent the range of three biological replicates. The lines show the mean value.