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The Use of Biochar in High-Solids Anaerobic Digestion

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Abstract

The use of anaerobic digesters for the production of energy in the form of methane-containing biogas can greatly benefit resource-constrained communities. Biogas can be used as an alternative fuel to wood, coal and crop waste which have traditionally been used for cooking. The household air pollution caused by cooking with solid fuels is a major cause of premature deaths in resource-constrained communities. Further use of anaerobic digesters could be achieved by targeting highly abundant, yet underutilised feedstocks, such as chicken litter.

Manures mixed with bedding material, such as chicken litter, are suitable for processing in high-solids anaerobic digesters. High-solids anaerobic digesters have a lower water requirement which makes them useful for processing dry feedstocks and suitable for areas where water is scarce. The lower water use results in a higher volumetric efficiency (methane yield per digester volume) compared with conventional low-solids anaerobic digesters. However, high-solids anaerobic digesters can have a lower methane yield per mass organic material added and slower rates of methane production. Using biochar (pyrolysed biomass), as an additive, may be a simple and robust method to improve performance of high-solids anaerobic digesters.

The majority of previous work on biochar use in anaerobic digesters has focussed on the application to low-solids anaerobic digesters. There are, however, many differences between the two process configurations. High-solids anaerobic digesters have a higher concentration of inhibitors, lower diffusion rates of intermediate products in the bulk sludge and are predominately batch operated. As a result, the effect of biochar on low and high-solids anaerobic digesters is expected to vary. To develop a deeper understanding on the effect of biochar use in high-solids anaerobic digesters a series of experimental investigations was conducted and the findings are presented in this body of work.

In high-solids anaerobic digesters ($\gtrsim$ 20% total solids) processing chicken litter, it was determined that the addition of biochar produced from wood-pellets results in a 35–42% reduction in the lag time before methane production starts. By comparison, the lag time is reduced by only 17% in digesters with biochar operating in low-solids conditions (5% total solids). Compared with digesters without biochar, the peak daily methane occurs earlier in digesters operating at 10% and 20% solids. This effect does not occur at 5% total solids. The shorter lag time also allows for a shorter retention time and a greater throughput of feedstock over multiple batches.

In high-solids anaerobic digesters, the use of wood-pellet biochar prevents a reduction in the peak methane yield and minimises an increase to the lag time which occurs when using a higher feedstock-to-inoculant ratio. The use of wood-pellet biochar in high-solids anaerobic digesters also resulted in methane yield increases of up to 32%. The increased methane yield corresponded with a reduction in the concentration of propionate, a key intermediate substrate, at the end of the 90-day digestion period by up to 95%.

The parent material and dosage of biochar also varies its effect on high-solids anaerobic

digesters. The addition of biochar produced from wood-pellets was superior to adding biochar produced from wheat straw or sheep manure. Biochar produced from wheat straw and sheep manure were detrimental to digester performance. The addition of these biochars increased the lag time compared with digesters without biochar. The benefits of wood-pellet biochar addition were related to its graphitic, as opposed to amorphous, carbon structure and low-ash content.

Wood-pellet biochar dosages of 1 $g_{TS\text{-}char}/g_{TS\text{-}feed}$ caused the greatest reduction in lag times and largest increases to the methane yield. These high dosages result in a high consumption rate of biochar. However, biochar can be recovered and re-used. The addition of re-used wood-pellet biochar can also improve performance to a greater degree compared with the addition of pristine wood-pellet biochar. Re-used wood-pellet biochar increased methane yields by up to 69% compared with digesters without biochar. Increased methane yields occurred due to enhanced degradation of volatile fatty acids. However, the beneficial effects of re-using biochar are variable.

The reduced lag times and increased methane yields are likely due to interactions between the biochar and the microorganisms. It is possible that the addition of biochar increases the rate of methane production through the facilitation of direct interspecies electron transfer (DIET). The *Methanosaetaceae* family which participates in DIET was the dominant methanogen on the biochar. Reduced interspecies distance because of microbial attachment onto biochar and biofilm formation, as observed using scanning electron microscopy, may also cause the improvements to performance.

Declaration by author

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Accepted publications included in this thesis

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2. Indren, M., Birzer, C.H., Kidd, S.P., Hall, T., Medwell, P.R., 2020. Effects of biochar parent material and microbial pre-loading in biochar-amended high-solids anaerobic digestion. Bioresource Technology 298, 122457. https://doi.org/10.1016/j.biortech.2019.122457

Submitted manuscripts included in this thesis

3. Indren, M., Birzer, C.H., Kidd, S.P., Medwell, P.R., 2020, High-solids anaerobic digestion with varying dosages of wood-pellet biochar and biochar re-use, Submitted to the Journal of Environmental Management

4. Indren, M., Birzer, C.H., Kidd, S.P., Medwell, P.R., 2020, The use of biochar in high-solids anaerobic digestion of chicken litter and the effect of feedstock-to-inoculant ratio, Submitted to the Journal of Environmental Chemical Engineering

Other publications during candidature

Conference papers

1. Indren, M., Cheruvu, N., Birzer, C., Medwell, P., 2017. Biochar production and characterisation — A field study, in: 2017 IEEE Global Humanitarian Technology Conference IEEE, pp. 1–5. https://doi.org/10.1109/GHTC.2017.8239333

2. Indren, M., Birzer, C., Medwell, P., Kidd, S., 2018. Biochar Addition in High-Solids Anaerobic Digestion of Poultry Litter, in: 2018 IEEE Global Humanitarian Technology Conference (GHTC), pp. 1–8. https://doi.org/10.1109/GHTC.2018.8601893

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Contents

Chapter 1

Introduction

1.1 Research Motivation

There are approximately 2.7 billion people on Earth who rely on solid fuels such as coal, wood, crop waste or manures for cooking (Bonjour et al., 2013; Edwards et al., 2017). The household air pollution caused by cooking with solid fuels is estimated to contribute to 4 million premature deaths per annum (Forouzanfar et al., 2016). Household air pollution is one of the major risk factors to a healthy life (Forouzanfar et al., 2016). People in resource-constrained communities within sub-Saharan Africa, South Asia, East Asia and Andean Latin America are the most likely to use solids fuels and therefore are most affected by household air pollution (Bonjour et al., 2013).

Using clean-cooking fuels such as biogas is an alternative to solid fuels. Clean-cooking fuels offer the potential to improve public health in resource-constrained communities. Biogas is a combustible gas consisting of methane (60–70%), carbon dioxide (30–40%) and trace amounts of hydrogen sulphide, hydrogen, nitrogen and water vapour (Appels et al., 2011). It has a low heating value of 13–25 MJ/m^3 (Li et al., 2017). Biogas can also be used in gas engines for electricity generation or purified and used as a transport fuel (Scarlat et al., 2018).

As well as being an alternative to solid fuels, biogas may also be an alternative to liquefied petroleum gas (LPG). LPG is a clean-cooking fuel which is gaining popularity in developing countries (Rosenthal et al., 2018). However, the production of LPG is not possible or economically feasible in many countries. For example, Nepal is reliant on India for its LPG supply. A two-month blockade at the India-Nepal border in 2015 caused widespread shortages of LPG as well as other essential supplies (Poudyal et al., 2019). By contrast, biogas can be produced in any country using widely abundant feedstocks, such as manure.

The production of biogas occurs via a biological process called anaerobic digestion. During this process a diverse group of microorganisms break down organic materials in an oxygen-free environment. A wide variety of organic materials such as manure, sewage sludge, crop residues or food wastes are all suitable for the process.

Chicken litter, a waste product from chicken-meat (broiler) production is a highly abundant organic material. Chicken-meat production, which is often conducted in 40–50 day growing cycles, produces approximately 0.7–2 tonnes of chicken litter (dry weight)/1000 chickens per cycle (Bolan et al., 2010). There is a growing global demand for chicken meat. Chicken meat is predicted soon to become the most commonly consumed meat consumed worldwide (Bennett et al., 2018). This increased consumption is mostly because of increasing consumption in developing countries (Boland et al., 2013). This abundance of waste material and the need for clean-cooking fuels makes biogas production from chicken litter an attractive option.

Compared with sewage sludge, cow manure or swine manure, chicken litter is less frequently used in anaerobic digesters (Fuchs et al., 2018; Scarlat et al., 2018). Common uses of chicken litter are as a fertiliser or for energy production by direct burning (Abouelenien et al., 2009). These solutions have their own drawbacks. Burning of chicken litter can lead to air pollution. The application of chicken litter as a fertiliser solely near the farm, a common practice in developing countries (Williams, 2013) can cause excessive concentrations of nutrients in surface water and ground-water (DeLaune et al., 2006). Using chicken litter in anaerobic digesters provides a unique opportunity for energy production and improved waste management. However, modifications to conventional anaerobic digester designs and process modifications are required to ensure it is a viable feedstock for biogas production.

A digester design suitable for the processing of chicken litter is a high-solids anaerobic digester. These digesters operate with a total solids (TS) content in the bulk sludge $\gtrsim 20\%$. By comparison, low-solids anaerobic digesters use total solids contents of $\lesssim 10\%$ (Abbassi-Guendouz et al., 2012). A significant advantage of high-solids anaerobic digesters is their lower water requirement. This advantage is most prominent when processing dry feedstocks. Chicken litter has a total solids content typically in the range of 30–80% TS. In addition, the lower water volume significantly reduces the digester tank volume. As a result, high-solids anaerobic digesters have a higher volumetric efficiency (volume methane/volume digester) compared with low-solids anaerobic digesters (Li et al., 2013). Finally, the typical digester design for high-solids anaerobic digesters are leach bed digesters which are not affected by large fragments of biomass which can become clogged in low-solids stirred tank digesters (Chanakya et al., 1997).

There are certain draw-backs of high-solid anaerobic digesters which limit their widespread use. Due to the lower water volume, a higher concentration of substances which can inhibit the activity of methane-generating microorganisms, such as ammonia, is expected. Furthermore, water is essential for the enzyme-facilitated conversion of solid material into soluble products which microorganisms consume, a process termed hydrolysis. Water also facilitates the diffusion of these soluble products within the bulk sludge of a digester (Batstone and Jensen, 2011). As a result, high-solids anaerobic digestion of manure produces less methane per gram of organic material than low-solids anaerobic digesters processing the same feedstock (Tait et al., 2009; Li et al., 2013). There is a need for methods to improve performance of high-solids anaerobic digesters. These methods must be simple and robust for digesters operated in rural locations or

resource-constrained communities.

1.2 Biochar in High-Solids Anaerobic Digesters

A robust method for improving methane yields in high-solids anaerobic digesters is the inclusion of biochar in the bulk sludge (Sun et al., 2019; Paritosh and Vivekanand, 2019). Biochar is a solid residue produced from pyrolysis of biomass (heating in a low-oxygen environment). Biochar has traditionally been used as a soil additive. A promising feature of this method is that it can be retrofitted to the millions of anaerobic digesters already constructed worldwide.

Biochar addition reduces the lag time before methane production starts and increases the cumulative and peak daily methane yields (Lü et al., 2016; Wang et al., 2017; Pan et al., 2019). The mechanisms which facilitate these improvements are not fully understood. However, a growing body of research suggests these changes occur due to relationships between the biochar and microorganisms which attach to its surface (Lü et al., 2018). The majority of this research has been conducted in low-solids anaerobic digesters. By contrast, the effects of biochar addition in high-solids anaerobic digesters are not well understood. Due to lower mass transfer rates (Bollon et al., 2013) a lower degree of biochar to microorganism contact is expected. Furthermore, greater ammonia-stress is expected which could negate positive effects of biochar addition. There is a need to better understand the effects of biochar addition in high-solids anaerobic digesters.

One of the most attractive features of using biochar as an additive is the ability to use a variety of parent materials and robust technologies for its production. For example, biochar can be produced from wood, manure or crop residues (Enders et al., 2012) in earth pits, rotary kilns, furnaces and gasifiers (Schimmelpfennig and Glaser, 2012). However, the variety of parent materials and technologies used for biochar production has made a determination of key properties that result in improved digester performance difficult. As a result, a deeper understanding of the effect of parent material used for biochar production and the variations to anaerobic digester performance is required.

The addition of biochar can be useful in batch-operated high-solids anaerobic digesters. These are a common type of digester used in rural areas (Riggio et al., 2016). Batch digesters need an inoculant in the bulk sludge to achieve a short lag time before methane production starts (Yap et al., 2016; Cui et al., 2011; Li et al., 2016). An example of an inoculant is the digestate from a previous batch. However, the high ammonia concentration in the bulk sludge from digesters processing nitrogen-rich feedstocks such as chicken litter is undesirable. An inoculant from another farm can be sourced but the transport costs can be high for remote farms. As biochar can be produced on-site and also shortens methane production lag times it could be an alternative to high volumes of inoculant. Understanding the relationship between inoculant volume and biochar use will result in reduced inoculant consumption rates in batch digesters processing nitrogen-rich feedstocks.

The biochar dosage used in an anaerobic digester is an important aspect of this process modification. High dosages will result in higher costs for the operator. Higher costs arise from additional time taken to produce biochar and the cost for the parent materials. However, low biochar dosages will prevent microorganisms to contact the biochar. Therefore, limitations on the improvements to anaerobic digester performance can occur. Previous work has shown improvements to anaerobic digesters performance occurs with an increasing biochar dosage (Wang et al., 2018a; Fagbohungbe et al., 2016; Sun et al., 2019; Paritosh and Vivekanand, 2019). Using higher biochar dosages compared with previous work in high-solids anaerobic digesters (Sun et al., 2019; Paritosh and Vivekanand, 2019) is expected to result in further improvements to performance.

The costs associated with high dosages of biochar can be reduced through biochar recovery and re-use. The re-use of biochar could also improve the rate of methane production. The process of microbial attachment onto biochar can take several days (Kuroda et al., 1988) and the microbial population on biochar can change over time (Lü et al., 2016). A pre-loaded microbial community on the biochar may result in greater improvements to performance compared with the addition of pristine biochar. However, there is little understanding of the effects of biochar pre-loaded with microorganisms on anaerobic digester performance.

This research project determines the effects of biochar addition on the performance of high-solids anaerobic digesters. The variability of the effects of biochar addition with varying process conditions and biochar properties are investigated. The knowledge developed will contribute to a greater level of reliability of anaerobic digesters and a wider level of adoption of this technology.

Chapter 2

Literature Review

2.1 Anaerobic Digestion of Chicken Litter

An abundant feedstock for anaerobic digesters is manure from chicken farms. Wastes from chicken farms are highly attractive for biogas production as there are approximately 20 billion chickens in the world (Bennett et al., 2018) and the distribution of chickens closely follows the distribution of the human population as show in Figure 2.1. There are two different manure-based wastes from chicken farms. Waste from egg-laying hens, termed chicken manure and waste from chicken-meat farms (broiler chickens) termed chicken litter. Chicken manure does not contain bedding material (Fuchs et al., 2018). Chicken litter contains both manure and bedding material such as wood shavings, peanut hulls or rice hulls. Compared to the numerous studies on chicken manure, there are relatively fewer focussed on biogas production from chicken litter (Singh et al., 2010b; Fuchs et al., 2018).

The suitability of an organic material for biogas production is defined by its volume of methane produced per gram of volatile solids (VS) in the material. The volatile solids content is a measure of the organic matter available for biological degradation. Under identical operating conditions, chicken manure (no bedding material) has a higher methane production potential (259 ml/g-VS) than dairy manure (204 ml/g-VS), goat manure (159 ml/g-VS) and horse manure (155 ml/g-VS) (Kafle and Chen, 2016). The methane production potential is only surpassed by swine manure (323 ml/g-VS) (Kafle and Chen, 2016). The methane production from chicken litter has been reported in the range of 74–195 ml/g-VS (Webb and Hawkes, 1985; Costa et al., 2012; Gangagni Rao et al., 2013; Zahan et al., 2018; Aguilar-Moreno et al., 2020). The methane production from chicken litter is lower than chicken manure because of the lignin content of the bedding material. The lignin content is included in the volatile solids content, however, lignin does not significantly degrade in anaerobic digesters (Liew et al., 2012). Despite the lower methane production potential of chicken litter, it has still a large potential for use as a feedstock in anaerobic digesters. This arises from its widespread geographical availability and increasing global chicken-meat consumption (Bennett et al., 2018).

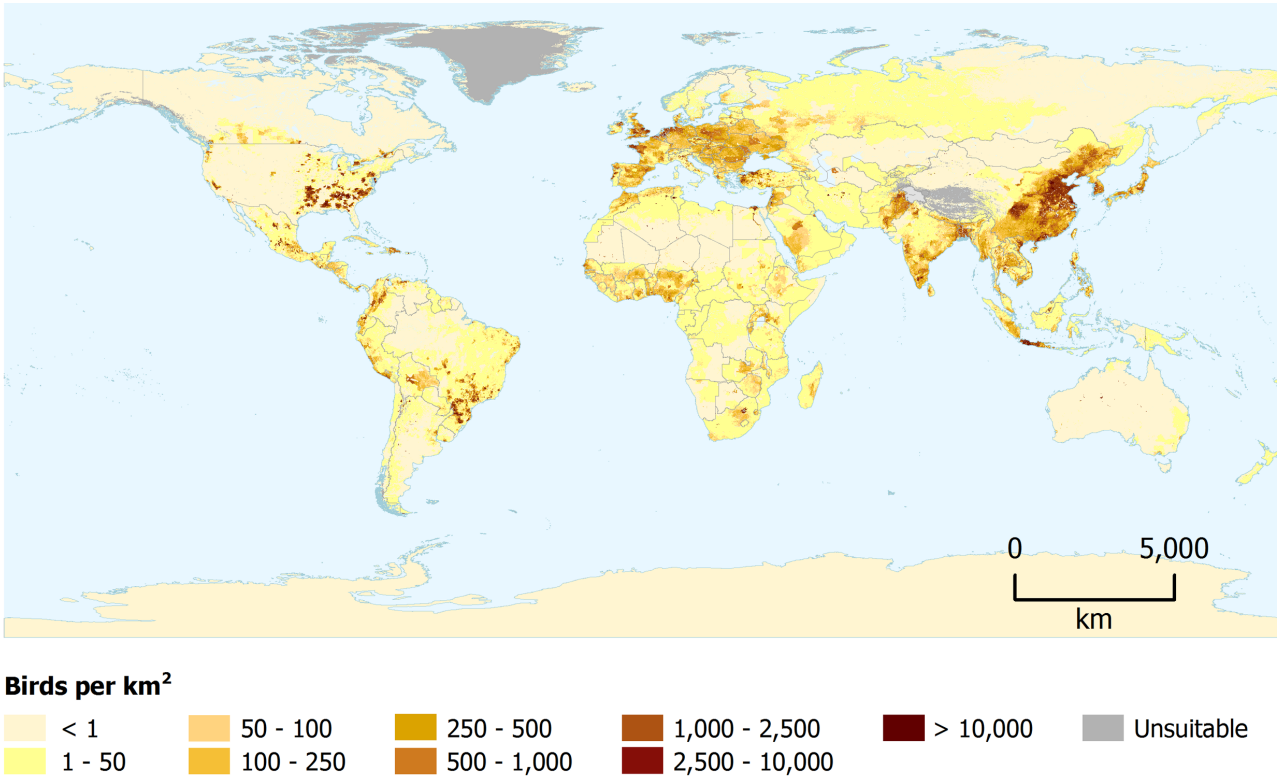

Figure 2.1: Global distribution of chickens (Robinson et al., 2014)

2.2 Biological Principles of Anaerobic Digestion

Methane production from organic materials occurs through a biological process termed anaerobic digestion. Anaerobic digestion occurs naturally in areas where oxygen is limited such as water-logged soils, landfills and intestinal tracts (Ward et al., 2008). Engineered systems designed to harness this process are called anaerobic digesters. Anaerobic digesters rely on different groups of microorganisms, working in cooperation, to allow for the degradation of complex feedstocks into methane. There are four main steps of the process, as shown in Figure 2.2:

1. Hydrolysis is the conversion of carbohydrates (such as cellulose and hemicellulose), proteins and lipids into soluble organic molecules. This reaction is facilitated by the excretion of enzymes from acid-generating bacteria. Hydrolysis produces sugars, amino acids and long-chain fatty acids.

2. Acidogenesis (acid-generation)/fermentation is the conversion of hydrolysis products into alcohols and volatile fatty acids (VFAs), such as propionate and butyrate.

3. Acetogenesis (acetate-generation) is the degradation of the alcohols and volatile fatty acids into acetate. Acetate can also be produced from hydrogen and carbon dioxide, termed homoacetogenesis (HA). The reverse process, acetate oxidation (AO) can also occur.

4. The final step, methanogenesis (methane-generation) occurs via two main pathways. Aceticlastic (acetate-cleaving) methanogenesis or acetate oxidation coupled with hydrogenotrophic (hydrogen-consuming) methanogenesis.

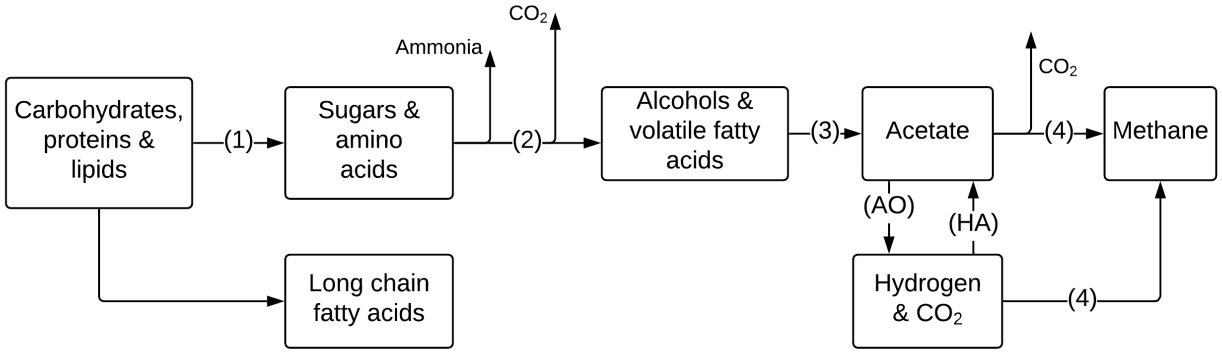

Figure 2.2: Schematic diagram of the main steps in anaerobic digestion; (1) hydrolysis, (2) acidogenesis/fermentation, (3) acetogenesis, (4) methanogenesis. Acetate oxidation (AO) and homoacetogenesis (HA) are also shown. Adapted from Batstone and Jensen (2011)

The conversion of acetate (CH_3COOH) into methane (shown in Equation 2.1) is facilitated by only a limited number of methane-generating microorganisms (methanogens). However, these are an important group of methanogens. Two thirds of biologically-generated methane is derived from acetate (Liu et al., 2008). Aceticlastic methanogens belong to the order *Methanosarcinales*. Only members of the genus *Methanosaeta* within the *Methanosaetaceae* family and the genus *Methanosarcina* within the *Methanosarcinaceae* family can conduct aceticlastic methanogenesis (Batstone and Jensen, 2011). *Methanosaeta* has a slower growth rate yet can grow at lower acetate concentrations than *Methanosarcina* (De Vrieze et al., 2012). The *Methanosarcinaceae* family is mixotrophic, meaning it can use both methane production pathways.

$$CH_3COOH \longrightarrow CH_4 + CO_2 \qquad \Delta G^{\circ\prime} = -33\,\mathrm{kJ/mol} \tag{2.1}$$

The strictly hydrogenotrophic methanogens belong to the orders *Methanomicrobiales* and *Methanobacteriales*. Hydrogenotrophic methane generation relies on syntrophy (a beneficial partnership) between acetate oxidising bacteria and the hydrogen consuming methanogens (Liu and Whitman, 2008). Acetate oxidation is a thermodynamically unfavourable reaction (Schink and Stams, 2006) as shown by the positive Gibbs free energy change ($\Delta G^{\circ\prime}$) in Equation 2.2. If the oxidation products are immediately used by methanogens, as shown in Equation 2.3, (Liu and Whitman, 2008) the overall reaction (the sum of Equation 2.2 and Equation 2.3) becomes thermodynamically favourable.

$$CH_3COOH + 2\,H_2O \longrightarrow 2\,CO_2 + 4\,H_2 \qquad \Delta G^{\circ\prime} = +94.9\,\mathrm{kJ/mol} \tag{2.2}$$

$$4\,H_2 + 2\,CO_2 \longrightarrow CH_4 + 2\,H_2O \qquad \Delta G^{\circ\prime} = -135.0\,\text{kJ/mol} \tag{2.3}$$

The efficiency of this syntrophic partnership between acetate oxidising bacteria and hydrogenotrophic methanogens is dependant on the rapid transfer and consumption of hydrogen in the bulk sludge of an anaerobic digester. The transfer of intermediate products between microorganisms in the bulk sludge occurs by diffusion. The rate of diffusion is calculated using Fick's law, as described in Equation 2.4 (de Bok et al., 2004).

$$J = D \times a \times \frac{C_p - C_c}{d} \tag{2.4}$$

In Equation 2.4, J is the diffusion rate (mol/sec), D is the diffusion coefficient of the bulk sludge (m^3/sec), a is the surface area of the microorganisms (m^2), $C_p - C_c$ is the concentration of the intermediate product at the producing and consuming microorganism (mol/m^3), respectively, and d is the distance between two microorganisms (m). As highlighted by Equation 2.4, the rate of hydrogen transfer can be increased by reducing the distance between partnering microorganisms. This can occur through the granulation of the bulk sludge, adding extra methanogens or constructing aggregates of microorganisms (Stams and Plugge, 2009). This could include microorganisms attached to a surface.

2.3 High-Solids Anaerobic Digestion

2.3.1 Process Description

High-solids anaerobic digestion, also termed solid-phase or dry digestion, is a process configuration which uses a total solids (TS) content $\gtrsim$ 20% in the bulk sludge. This is in contrast to conventional anaerobic digesters which use semi-solids, 10–20% TS, and low-solids conditions $\lesssim$10% TS. High-solids anaerobic digesters are the most suitable design when processing feedstocks with an "as received" total solids content $\gtrsim$15%, as shown in Figure 2.3. High-solids anaerobic digesters are frequently used for biogas production from manures with bedding material. These manures include swine litter (Tait et al., 2009; Yap et al., 2016), horse litter (Cui et al., 2011), cow litter (Riggio et al., 2017) and poultry litter (Bayrakdar et al., 2018).

A significant benefit of using high-solids conditions is the lower volume of water required. As a result, high-solids anaerobic digesters are smaller in volume (Li et al., 2013) and have a lower capital cost (Li et al., 2018b). Lower water requirements are also useful in areas where water is scarce. A benefit of a smaller volume digester is a superior volumetric efficiency. The volumetric efficiency is defined as the unit volume methane produced per unit volume of the bulk sludge. Anaerobic digesters operating at 20% total solids can have a volumetric efficiency 50–300% higher than a digester operating at 5% total solids (Li et al., 2013, 2018b).

High-solids anaerobic digesters are an appropriate design for processing chicken litter. The suitability of processing chicken litter in high-solids anaerobic digesters arises from its total solids

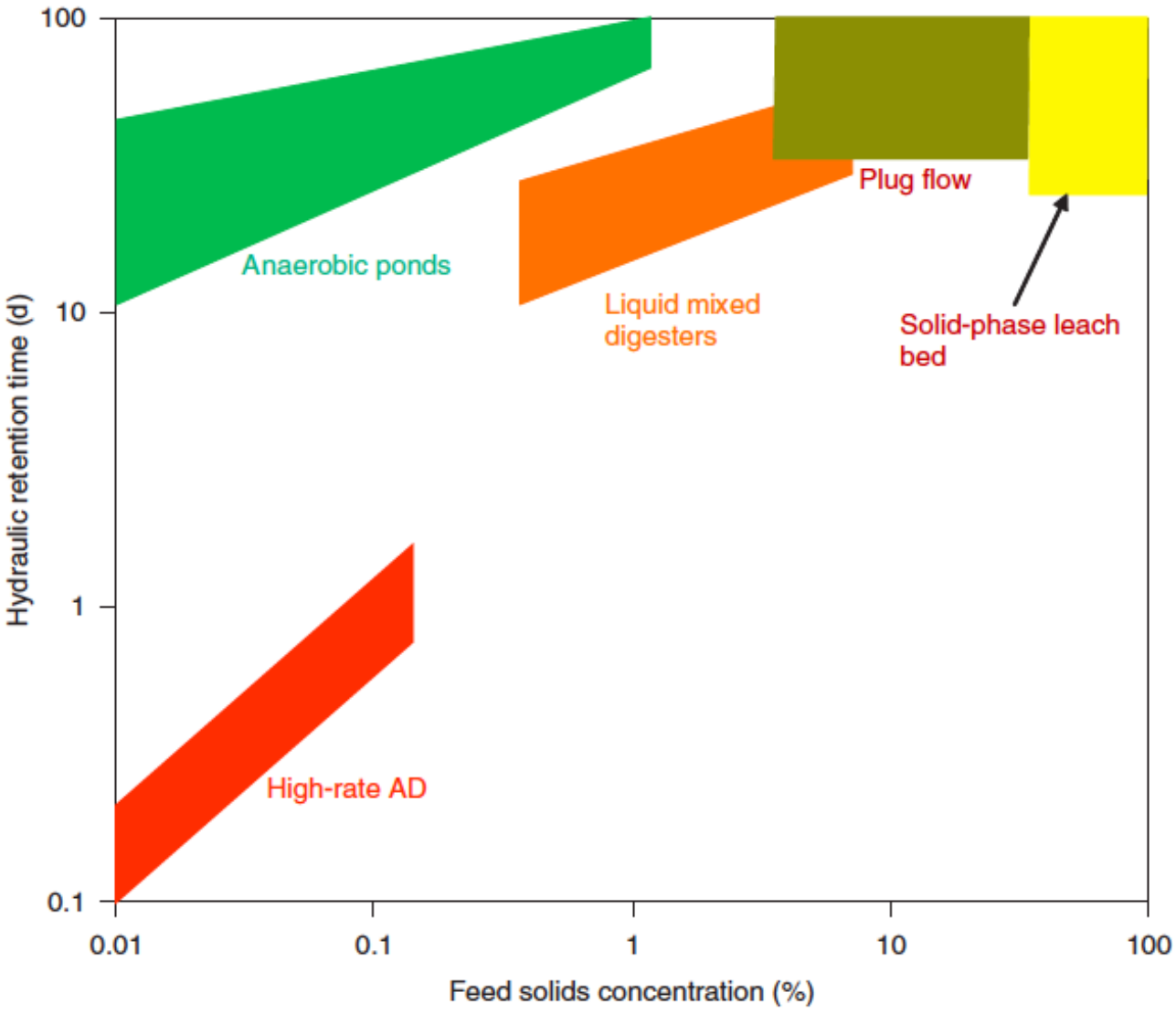

Figure 2.3: Anaerobic digestion technology selection sorted by retention time (vertical axis) and total solids content (horizontal axis) (Batstone and Jensen, 2011)

content of >60%, as shown in Table 2.1, and the presence of bedding material. Bedding material is unsuitable for inclusion in conventional low-solids anaerobic digesters, which are generally mixed tank digesters as shown in Figure 2.3, due to the high likelihood of clogging in narrow inlet pipes and the inability to remove material that does not degrade anaerobically (Chanakya et al., 1997).

Batch-operated high-solids anaerobic digesters require an appropriate feedstock-to-inoculant (F/I) ratio for optimum performance. The inoculant, which can be the digestate from another digester or a previous batch provides the community of microorganisms required for anaerobic digestion to occur. Its chemical composition can also buffer against drops in pH common during startup of a digester (Li et al., 2016). High F/I ratios increase the lag time before methane production starts (Bujoczek et al., 2000; Tait et al., 2009; Motte et al., 2013; Xu et al., 2016). For example, in high-solids anaerobic digestion of spent-bedding from a horse stable, the lag times were 1, 4 and > 30 days at volatile solids-based F/I ratios of 2, 4 and 6, respectively (Cui et al., 2011). In high-solids anaerobic digestion of chicken manure using a TS-based F/I ratio of 9

Table 2.1: Properties of chicken litter from analysis of farms in the USA and China

Parameter	Survey of 106 farms in Alabama, USA (Stephenson et al., 1990)		Survey of 111 farms in China, various locations (Shen et al., 2015)		Combined data from nine studies in the USA (Edwards and Daniel, 1992)	
	mean	SD	mean	SD	mean	range*
Total solids, TS (%)	80	6.0	36	9.0	76	71–88
Volatile solids (% of TS)	-		62	11	-	
Ash (% of TS)	25	9.0	28	14	-	
C (% of TS)	-		34	8.8	38	27–41
H (% of TS)	-		5.1	1.9	-	
N (% of TS)	-		3.7	1.3	4.1	1.7–6.7
S(% of TS)	0.5	0.1	0.9	0.6	-	
P (% of TS)	-	0.5	1.1	5.5	1.4	0.8–2.5
C/N	-		9.2		9.3	

* Standard deviation (SD) not reported

no methane production occurred even after 120 days (Bujoczek et al., 2000). There is still a need to determine a suitable F/I ratio for high-solids anaerobic digestion of chicken litter. In addition, methods allowing for high F/I ratios will benefit the user by enabling a greater percentage of the digester volume to be used for the feedstock.

2.3.2 Process Indicators and Key Parameters

Process indicators and key parameters that affect anaerobic digester performance are the concentration of inhibitors, pH, VFA concentration, total alkalinity and the temperature of the bulk sludge. Ammonia is a significant inhibitor at high concentrations. Ammonia is produced through anaerobic degradation of nitrogen-containing compounds (Kayhanian, 1999). In chicken litter, nitrogen is found in undigested proteins and in urea (Bujoczek et al., 2000). High ammonia concentrations inhibit the activity of methanogens and hence limits the widespread application of chicken litter in anaerobic digesters (Rajagopal et al., 2013; Fuchs et al., 2018).

The total ammonia-nitrogen (TAN) content in anaerobic digesters comprises of free ammonia-nitrogen (FAN) and the ammonium ion ($NH_4{}^+$). FAN is gas in solution while the ammonium ion ionically binds to water (Kayhanian, 1999). The proportion of FAN and ammonium is determined by a pH and temperature-dependent relationship, as described by Equation 2.5 (Hansen et al., 1998).

$$[FAN] = [TAN] \times \left(1 + \frac{10^{-pH}}{10^{-(0.09018+\frac{2729.92}{T})}}\right)^{-1} \tag{2.5}$$

In Equation 2.5, $[FAN]$ is the concentration of free ammonia-nitrogen, $[TAN]$ is the concentration of total ammonia-nitrogen and T is the temperature in kelvin. Equation 2.5 demonstrates a high pH and high temperature promote the formation of FAN. Figure 2.4 shows the relationship between pH, TAN, $NH_4{}^+$ and FAN on the degree of inhibition of aceticlastic

methanogens. A pH in the bulk sludge of 6–7 and 6–7 g-TAN/l completely inhibits aceticlastic methanogens and the major inhibitor is the ammonium ion. However, at a pH of 7–8.5 just 3 g-TAN/l is required and FAN is the major inhibitor (Astals et al., 2018).

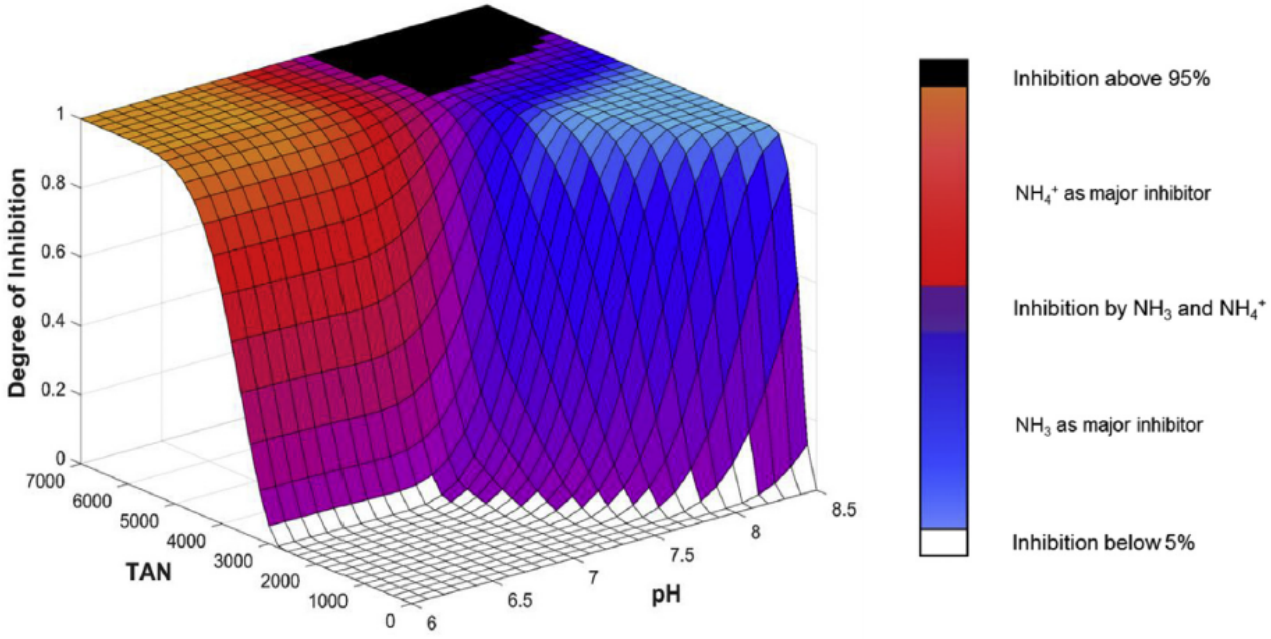

Figure 2.4: Predicted ammonia-nitrogen inhibition profile of acetate-consuming methanogens with varying total ammonia-nitrogen and pH (Astals et al., 2018)

In addition to ammonia there are other inhibitors to the anaerobic digestion process. For example, chicken litter contains sulphur (Table 2.1). Sulphate-reducing bacteria compete with hydrogenotrophic methanogens for hydrogen (Holmes and Smith, 2016). In addition, high concentrations of the soluble salts of calcium, magnesium, sodium and potassium or metals such as copper, zinc, chromium, cadmium, nickel and lead can also inhibit activity of the methanogens (Mccarty and Mckinney, 1961; Chen et al., 2008).

The pH in the bulk sludge of anaerobic digester is predominately determined by the concentration of weak acids (e. g. VFAs and long chain fatty acids) and weak bases (e. g. free ammonia-nitrogen). A drop in the pH of the bulk sludge can occur when the activity of the methanogens are inhibited (Chen et al., 2008). High-solids anaerobic digesters generally have a high total alkalinity due to a high FAN concentration (Batstone and Jensen, 2011) and hence are well buffered against pH drops. The high total alkalinity, reported as grams of calcium carbonate per litre (g-$CaCO_{3eq}$/l) will cause the bulk sludge to maintain a high pH. Therefore, FAN will be the major inhibitor to the methanogens when manure such as chicken litter is used as the feedstock for anaerobic digesters.

VFAs, a key intermediate in the process, are acids with three to six carbon atoms. The main VFAs in anaerobic digesters are acetate, propionate and butyrate (Wang et al., 1999; Felchner-Zwirello et al., 2013). VFAs accumulate during digester startup or when the process is inhibited (Gallert and Winter, 2008). High VFA concentrations in the bulk sludge signify a loss of potential methane production (Chen et al., 2008). Methanogens do not directly consume the

three to six carbon atom VFAs (Schink, 1997). The degradation of these acids into substrates that methanogens can consume, acetate and hydrogen, leads to increases in the methane yield from anaerobic digesters (de Bok et al., 2004; Barua et al., 2018). However, the degradation of these acids are endergonic under standard conditions. Equation 2.6 and Equation 2.7 show a positive $\Delta G^{\circ\prime}$ for the degradation of propionate and butyrate (Schink, 1997).

$$CH_3CH_2COO + 2\,H_2O \longrightarrow CH_3COO + CO_2 + 3\,H_2 \qquad \Delta G^{\circ\prime} = +76\,kJ/mol \tag{2.6}$$

$$CH_3CH_2CH_2COO^- + 2\,H_2O \longrightarrow 2\,CH_3COO^- + 2\,H^+ + 2\,H_2 \qquad \Delta G^{\circ\prime} = +48.3\,kJ/mol \tag{2.7}$$

The degradation of propionate and butyrate can only proceed if the products are immediately scavenged by methanogens. A syntrophic (partnering) relationship is needed between multiple groups of microorganisms for their degradation (Schink and Stams, 2012). The overall reactions of propionate and butyrate degradation are thermodynamically favourable as shown by the negative $\Delta G^{\circ\prime}$ in Equation 2.8 and Equation 2.9. Reducing the distance between partnering microorganisms will increase the diffusion rate of intermediate products, as discussed in Section 2.2 and increase the likelihood of these reactions occurring.

$$4\,CH_3CH_2COO^- + 4\,H^+ + 2\,H_2O \longrightarrow 7\,CH_4 + 5\,CO_2 \qquad \Delta G^{\circ\prime} = -249\,kJ/4\text{ mol propionate} \tag{2.8}$$

$$2\,CH_3CH_2CH_2COO^- + 2\,H^+ + 2\,H_2O \longrightarrow 5\,CH_4 + 3\,CO_2 \qquad \Delta G^{\circ\prime} = -177\,kJ/2\text{ mol butyrate} \tag{2.9}$$

The temperature of the bulk sludge has a variety of effects on the process. In general, methane production rates increase due to increased rates of microbial activity (Batstone and Jensen, 2011; Jang et al., 2017). The three ideal ranges for anaerobic digestion are psychrophilic 10–30°C, mesophilic 30–40°C and thermophilic 40–70°C. Pyschrophilic digesters are un-heated. This is suitable for locations which have air temperatures ranging from 18–30°C such as sub-Saharan Africa (Rupf et al., 2015). The use of un-heated anaerobic digesters in Nepal and northern China, where air temperatures are 10–15°C is less effective (Rupf et al., 2015). Mesophilic and thermophilic digesters are heated and typical laboratory test temperatures are 37°C and 55°C, respectively (Holliger et al., 2016).

2.3.3 Rate-Limiting Steps

Despite the benefits and suitability of using chicken litter in high-solids anaerobic digesters, introduced in Section 2.3.1, there are some limitations to the process. These include lower

hydrolysis rates (step 1 in Figure 2.2) and a higher concentration of free ammonia-nitrogen which inhibits methanogenesis (step 4 in Figure 2.2).

The rate of hydrolysis is affected by the diffusion rate of products in the bulk sludge (Equation 2.4). The step involves excretion of a hydrolytic enzyme, diffusion of the enzyme to the complex organic matter and a reaction to form a soluble substrate. The soluble substrate then diffuses through the bulk sludge. Due to the multiple diffusion steps involved, the rate of hydrolysis is affected by the diffusion coefficient of the bulk sludge. In a digester operating with a total solids content of 8% the diffusion coefficient of the bulk sludge (D in Equation 2.4) is approximately 50 times lower compared with water. By comparison, using a total solids content of 25% (high-solids anaerobic digestion) the diffusion coefficient is 185 times lower than water (Bollon et al., 2013). The variation in the diffusion coefficient in the bulk sludge has a non-linear effect on the peak daily methane yield. Increasing the total solids content from 10% to 30% can decrease the peak daily methane yield by around 60% (Abbassi-Guendouz et al., 2012).

Methanogenesis can also be a rate limiting step. For digesters processing chicken litter, ammonia and VFA concentrations are expected to be key selectors for the dominant methanogens. Within the aceticlastic methanogens, the *Methanosataceae* family has a lower resistance to ammonia stress than the *Methanosarcinaceae* family. Furthermore, hydrogen-consuming methanogens also have a higher resistance to ammonia-stress than *Methanosaetaceae* (Angelidaki and Ahring, 1993; Schnürer and Nordberg, 2008). However, *Methanosaetaceae* dominates over *Methanosarcinaceae* when there is low concentrations of acetate in the the bulk sludge (Demirel and Scherer, 2008). The maintenance of a diverse microbial population is needed to ensure stable methane production with varying operating conditions.

Due to both lower rates of hydrolysis and methanogenesis, an anaerobic digester processing chicken manure operating for 30 days at 5% total solids has a 45% higher cumulative methane than a digester operating at 10% total solids, while a digester operating at 20% total solids has no significant methane production (Li et al., 2013). As a result, an inhibited anaerobic digester operating at 20% total solids performs worse on both a methane yield and volumetric efficiency basis than a low-solids anaerobic digester (Li et al., 2013). There remains a need to improve the efficiency of methane production from high-solids anaerobic digestion operating under ammonia stress. This will improve the viability of using chicken litter and a wide range of manures with bedding material for biogas production.

2.4 Biochar in Anaerobic Digesters

There are several solutions to improve performance of anaerobic digesters processing feedstocks where a high concentration of ammonia is expected. A common approach is to dilute the feedstock with water to decrease the concentration of ammonia. However, the benefits of high-solids anaerobic digestion are lost if the bulk sludge is diluted below 20% total solids. Mixing

the manure with a low-nitrogen feedstock such as wheat straw, termed co-digestion, is a possible solution for high-solids anaerobic digesters (Li et al., 2013). Transport distances and seasonal availability of the second feedstock are key determinants for the viability of co-digestion (Li et al., 2017). Therefore, there are geographic limitations to the widespread use of co-digestion. A variety of processes for ammonia removal are possible (Abouelenien et al., 2010; Desloover et al., 2015) but are unlikely to be suitable for resource-constrained communities. Robust and low-cost solutions for enhanced biogas production from anaerobic digesters are needed.

One appropriate option to improve the performance of anaerobic digesters is the use of additives. A variety of additives have been investigated for anaerobic digesters. These include biochar (Pan et al., 2019), activated carbon (Poirier et al., 2017), magnetite (Cruz Viggi et al., 2014) and zeolites (Milán et al., 2001). The use of biochar as an additive is particularly attractive for resource-constrained communities. Biochar is the solid residue from pyrolysis. Pyrolysis is the heating of biomass in a low-oxygen environment. Biochar can be produced using a range of agricultural wastes. These include wood, crop residues and manures (Singh et al., 2010a; Enders et al., 2012). It is produced using robust technology such as top-lit up-draft gasifiers (Kirch et al., 2020), cookstoves (Birzer et al., 2014), charcoal stacks (Schimmelpfennig and Glaser, 2012), rotary kilns (Schimmelpfennig and Glaser, 2012) and earth-pits (Cornelissen et al., 2016). The use of biochar does not require redesigning anaerobic reactors and may make systems more resilient to process disturbances (Lovley, 2017).

Biochar has traditionally been used to improve soil fertility (Singh et al., 2010a; Pandit et al., 2018) and adsorb contaminants from water (Mohan et al., 2014). By comparison to the parent material used biochar production, biochar generally has a higher resistance to biological degradation, a higher proportion of carbon in an aromatic chemical structure (Baldock and Smernik, 2002), and a higher specific surface area due to its porous nature (Schimmelpfennig and Glaser, 2012). These properties allow biochar to participate in redox reactions by accepting and donating electrons (Klüpfel et al., 2014; Prévoteau et al., 2016), absorb organic and inorganic contaminants (Mohan et al., 2014), and increase the water holding capacity (Gray et al., 2014) and acid-buffering capacity of the material it is mixed with (Fryda and Visser, 2015).

In anaerobic digesters, biochar addition has eliminated the lag time (Cruz Viggi et al., 2017) or shortened the lag time before methane production starts by 27–69% (Wang et al., 2018a; Pan et al., 2019). Biochar addition has also increased the maximum daily methane production rate by 5–40% (Lü et al., 2016; Wang et al., 2018a) and the cumulative methane yield by 27–69% (Jang et al., 2017; Pan et al., 2019). These effects have been observed in a wide variety of process configurations. As a result, biochar is a promising low-cost solution that could be used in the millions of existing anaerobic digesters in resource-constrained communities.

2.4.1 Potential Mechanisms for Enhanced Performance

There are a variety of mechanisms which may explain the improved performance of anaerobic digesters due to the addition of biochar. The addition of biochar results in both microbial and chemical changes in the bulk sludge. As a result, identification of the most suitable biochar for the desired process configuration is not yet possible (Cruz Viggi et al., 2017).

Mechanisms that involve changes to the microbial community are likely to be the most important for improved performance of high-solids anaerobic digestion of chicken litter. Several studies have shown preferential attachment of *Methanosaeta* (De Vrieze et al., 2012; Lü et al., 2016; Zhao et al., 2016) or *Methanosarcina* (Dang et al., 2016; Lei et al., 2019) onto biochar. The attachment of methanogens and bacteria in close proximity while on the biochar would be expected to increase the diffusion rate of intermediate products between partnering microorganisms (Wang et al., 2016a; Martins et al., 2018), as described in Section 2.2. The proximity of partnering microorganisms could explain the greater degradation rates of VFAs such as propionate and butyrate in digesters with biochar (Zhao et al., 2016; Cruz Viggi et al., 2017).

The attachment of microorganisms onto the biochar can also lead to biofilm formation (Luo et al., 2015; Cooney et al., 2016). A biofilm is a community of microorganisms embedded in an extracellular polymeric substance (Flemming et al., 2016). Microorganisms within a biofilm have a greater resistance to environmental stresses (Flemming et al., 2016). For example, biofilm formation allows for greater methane production rates in salt-stressed anaerobic digesters (Gagliano et al., 2017). The formation of biofilms could allow for increased methane production rates from the ammonia sensitive *Methanosaetaceae* family.

Conductive materials such as biochar, activated carbon, carbon cloth and magnetite can also stimulate direct interspecies electron transfer (DIET) in anaerobic digesters (Chen et al., 2015; Zhao et al., 2016; Lei et al., 2019). The methanogens known to participate in DIET are *Methanosaeta* (Rotaru et al., 2014) and *Methanosarcina* (Chen et al., 2015). DIET involves the transfer of an electron to an attached methanogen from the degradation of intermediate products such as ethanol or propionate (Rotaru et al., 2014; Cruz Viggi et al., 2014), by an electron-donating bacteria such as *Geobacter*. It is suggested *Methanosaeta* grows faster when it receives some of its energy from DIET compared with just the metabolism of acetate alone (Holmes et al., 2017; Wang et al., 2018b).

DIET has only been directly confirmed to occur in co-culture studies using pure strains of microorganisms (Summers et al., 2010; Rotaru et al., 2014; Chen et al., 2015). It is difficult to make conclusions about mechanisms in systems where multiple pathways for electron transfer, such as interspecies hydrogen transfer, also operate simultaneously (Wang et al., 2016b; Martins et al., 2018). However, identification of conditions where DIET may occur by identifying key methanogens in more complex can provide additional evidence to investigations conducted in pure culture systems. This will allow for a greater understanding of the key mechanisms which result in improved anaerobic digester performance in larger-scale anaerobic digesters.

2.4.2 The Effects With Varying Total Solids Content

The majority of studies have investigated the use of biochar in low-solids anaerobic digesters (5–10% TS) (Mumme et al., 2014; Zhao et al., 2015; Luo et al., 2015; Lü et al., 2016; Zhao et al., 2016; Poirier et al., 2017; Cruz Viggi et al., 2017; Ma et al., 2019). The studies of biochar use in high-solids anaerobic digesters have not been under ammonia-stressed conditions (Sun et al., 2019; Paritosh and Vivekanand, 2019). Recent research suggests the benefits of biochar addition only occur when the anaerobic digestion process is under stress (Shao et al., 2019). Therefore, the benefits of biochar use in anaerobic digesters processing a nitrogen-rich waste, such as chicken litter, may be expected to be greater with an increasing total solids concentration. This is due to an increased degree of ammonia stress on the process. However, a higher total solids content in an ammonia-stressed digester may prevent attachment of microorganisms and hence the benefits of biochar addition from occurring. This is due to both the high ammonia concentration inhibiting the activity of methanogens and a reduced diffusion coefficient of the bulk sludge. There is a need to understand how the effects of biochar use varies with the total solids content of a digester processing a nitrogen-rich waste such as chicken litter.

2.4.3 Effects With Varying Feedstock-to-Inoculant Ratios

The use of biochar may allow for a reduced volume of inoculant in a high-solids anaerobic digester. In low-solids anaerobic digesters not operating under ammonia stress, the addition of biochar reduces lag times either consistently (Wang et al., 2018a) or to a greater degree at higher F/I ratios (more feedstock and less inoculant) (Cai et al., 2016; Li et al., 2018a). In thermophilic high-solids anaerobic digestion the use of biochar increased the methane yield at F/I ratios of 4 and 6 compared with the controls (Meng et al., 2020). At mesophilic temperatures there will be a reduced level of microbial activity and in high-solids conditions, increasing the F/I ratio will simultaneously reduce the initial methanogen population and increase the ammonia stress. These differences are expected to reduce the likelihood of methanogens attaching to biochar. As a result, there is a need to understand the relationship between biochar addition, methane production performance and F/I ratios in ammonia-stressed high-solids anaerobic digestion operating at mesophilic temperatures.

2.4.4 The Effects of Biochar Parent Material

A wide variety of parent materials for biochar production have been used. Biochar produced from wood (Fagbohungbe et al., 2016; Cruz Viggi et al., 2017), rice husk (Fagbohungbe et al., 2016), rice straw (Yuan et al., 2018), wheat bran (Cruz Viggi et al., 2017), wheat straw (Shen and Zhu, 2016), cow manure (Jang et al., 2017; Yuan et al., 2018), chicken manure (Pan et al., 2019) and worm manure (Wang et al., 2017) have all improved anaerobic digester performance. By contrast, biochar produced from walnut shells (Linville et al., 2017), cardboard (Li et al., 2019), cow manure (Sun et al., 2019) and corn stover amended with iron (Zhang et al., 2019a)

have shown detrimental effects on performance. Detrimental performance has been reported to be caused by excessive concentrations of light metal ions such as sodium, potassium, magnesium, calcium and aluminium (Linville et al., 2017). Another explanation for poor performance has been the adsorption of enzymes needed for hydrolysis out of the bulk sludge (Li et al., 2019). Due to their lower water content, high-solids anaerobic digesters are more likely to be affected by biochar induced inhibitors and hydrolysis adsorption compared with low-solids anaerobic digesters. As a result, there is a need to explore the effects of biochar produced from different parent materials in high-solids anaerobic digesters.

2.4.5 Effects With Varying Dosages

A sufficient biochar dosage is required to observe the maximum possible improvements to anaerobic digester performance. The biochar dosage used in low-solids anaerobic digesters has ranged from 2–15 g-biochar/l-bulk sludge (Lü et al., 2016; Fagbohungbe et al., 2016; Zhao et al., 2015; Wang et al., 2018a). On a dry weight-based dosage, this is approximately 0.4–6.25 g-biochar/g-feedstock. In high-solids anaerobic digesters, dosages of 2–30 g-biochar/l, have increased methane yields and shortened lag times (Sun et al., 2019; Paritosh and Vivekanand, 2019). In these studies, the dry weight-based dosage was not reported. In studies in low-solids anaerobic digestion where the effect of biochar dosage has been investigated, a greater percentage reduction in lag time has been observed with increasing biochar dosage (Lü et al., 2016; Fagbohungbe et al., 2016; Wang et al., 2018a). It is possible that higher biochar dosages, than previously used in high-solids anaerobic digesters can improve performance further.

2.4.6 The Recovery and Re-use of Biochar

The recovery of biochar and re-use in a subsequent batch could reduce the costs (both time and money) associated with biochar production. It would also allow for higher biochar dosages. An additional benefit of re-using the biochar is that microorganisms and biofilms are already attached to the biochar at the beginning of the digestion period. For example, the addition of re-used magnetite improves methane production rate by 70% compared with adding pristine magnetite (Baek et al., 2017). The benefits were attributed to the attachment of *Methanosaeta* and the facilitation of DIET. The re-use of biochar is a promising option. Biochar does not significantly degrade under anaerobic conditions (Mumme et al., 2014) and its porous structure can facilitate microbial attachment. However, it is unclear how the re-use of biochar will affect methane production performance compared with pristine biochar.

2.5 Research Gaps

The following research gaps have been identified and are addressed in this thesis.

1. The majority of investigations of biochar addition to anaerobic digesters have used total solids contents of 5–10%, as highlighted in subsection 2.4.2. These findings may not be applicable to high-solids anaerobic digesters (20% TS) due to differences in mass transfer rates and the concentration of inhibitory products. Chapter 3 addresses the following aim and objective:

    - Aim: identify the effects of biochar addition as a function of the total solids content in ammonia-stressed anaerobic digesters.
    - Objective: determine if the effects of biochar addition on methane production performance are consistent using total solids contents of 5%, 10% and 20%. Determine changes to chemical conditions and the population of methanogens with varying total solids content.

2. In batch anaerobic digestion, using a higher F/I ratio allows for a greater proportion of the digester volume to be allocated to the feedstock. Subsection 2.3.1 described how the use of higher F/I ratios decreases anaerobic digester performance by increasing lag times and reducing peak daily methane production rates. As biochar has been shown to reduce lag times there is a significant potential to increase viable F/I ratios through the addition of biochar. The effects of biochar addition with varying F/I ratios has been investigated in low-solids anaerobic digesters and thermophilic high-solids anaerobic digesters (subsection 2.4.3), however, there is still uncertainty if these results are applicable to mesophilic high-solids anaerobic digesters. Chapter 4 addresses the following aim and objective:

    - Aim: identify the influence of biochar addition as a function of the F/I ratio in a mesophilic high-solids anaerobic digester.
    - Objective: determine the upper limits of F/I ratio which are viable as a result of biochar addition. Also, determine if low F/I ratios remove the need to add biochar to high-solids anaerobic digesters. Determine changes to chemical conditions and the population of methanogens with varying feedstock-to-inoculant ratios.

3. Biochar produced from a wide variety of parent materials have been shown to improve anaerobic digester performance. However, some studies suggest the inclusion of biochar that contains soluble light metal ions may inhibit the process (subsection 2.4.4). High-solids anaerobic digesters have a higher susceptibility to inhibition due to their lower water content compared with low-solids anaerobic digesters (subsection 2.3.2). The comparatively fewer studies reporting negative effects of biochar addition may be due to the focus of low-solids anaerobic digesters. Chapter 5 addresses the following aim and objective:

- Aim: identify the effects of adding biochar produced from parent materials, which are likely to be available in rural areas, on methane production performance from high-solids anaerobic digesters.
- Objective: determine physical and chemical differences between the types of biochar that lead to variations on high-solids anaerobic digester performance

4. The biochar dosage is a key parameter for improved anaerobic digesters performance to be observed. In low-solids anaerobic digesters, increases to performance are observed with increasing biochar dosages. Higher biochar dosages than used in studies in high-solids anaerobic digesters highlighted in subsection 2.4.5 are possible which may lead to further improvements to performance. Chapter 6 addresses the following aim and objective:

   - Aim: identify the changes to lag time and methane yields as a function of biochar dosage in a high-solids anaerobic digester.
   - Objective: determine the effect of biochar dosages greater than 30 g-biochar/l on high-solids anaerobic digestion. Compare the benefits to performance the changes to methane yield basis and the annual use of biochar with varying dosages.

5. The re-use of biochar has potential to improve methane production performance and lower the costs associated with high biochar dosages. The re-use of an alternative additive, magnetite, has shown greater benefits to anaerobic digesters compared with pristine magnetite (subsection 2.4.6). It is not clear if re-using biochar can improve methane production performance. Chapters 5 and 6 both address the following aim and objective 1 while Chapter 6 also addresses objective 2:

   - Aim: identify the effect of re-using biochar on high-solids anaerobic digester performance.
   - Objective 1: determine the effect on methane production from the addition of pristine and re-used biochar.
   - Objective 2: determine the effect of varying dosages of re-used biochar on anaerobic digester performance.

2.5.1 Summary

Table 2.2 shows an outline of the work and the key parameters investigated. The table shows the results from each chapter will inform the parameters used for subsequent studies. An initial assumption of feedstock to inoculant ratios, biochar dosage and type were based on literature reviewed in this section.

Table 2.2: Summary of research plan

Research gap	Total solids (%)	Feedstock to inoculant ratio	Biochar parent material	Biochar dosage ($g_{TS\text{-}char}/g_{TS\text{-}feed}$)
1: Chapter 3	5, 10, 20[§]	2[*]	Wood pellet[*]	1[*]
2: Chapter 4	20[†]	0.5, 1, 2[§]	Wood pellet[*]	1[*]
3: Chapter 5	20[†]	2[†]	Wood pellet, sheep manure and wheat straw[§]	1[*]
4: Chapter 6	20[†]	2[†]	Wood pellet[†]	0.25, 0.5 and 1[§]
5: Chapter 5 and 6	20[†]	2[†]	Re-used wood pellet[§]	0.25, 0.5 and 1[§]

[§] Parameter under investigation.
[*] Selected based on literature review of comparable studies.
[†] Selected based on experimental results.

Chapter 3

Effect of Total Solids Content on Anaerobic Digestion of Poultry Litter with Biochar

The following publication has been incorporated as Chapter 3:

Indren, M., Birzer, C.H., Kidd, S.P., Medwell, P.R., 2020. Effect of total solids content on anaerobic digestion of poultry litter with biochar. Journal of Environmental Management 255, 109744. https://doi.org/10.1016/j.jenvman.2019.109744

Principal author	Mathu Indren
Contribution to the paper	Performed literature review. Identified research gaps. Developed experimental protocols. Conceived and designed experimental assay for completing the study aims and objectives. Sourced organic materials for methane production assay. Conducted experiments. Analysed and interpreted data. Produced figures and tables. Wrote and edited manuscript. Produced responses to reviewer comments. Corresponded with journal regarding the reviewer comments.
Contribution	70%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature and date	

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

1. the candidate's stated contribution to the publication is accurate (as detailed above);
2. permission is granted for the candidate in include the publication in the thesis; and
3. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Co-author	Cristian Birzer
Contribution to the paper	Conception and design of the project. Analysis and interpretation of research data. Critical revision of article and reviewer comments.
Signature and date	

Co-author	Stephen Kidd
Contribution to the paper	Conception and design of the project. Analysis and interpretation of the research data.
Signature and date	

Co-author	Paul Medwell
Contribution to the paper	Conception and design of the project. Analysis and interpretation of research data. Critical revision of article and reviewer comments.
Signature and date	

Chapter 4

The Use of Biochar in High-Solids Anaerobic Digestion of Chicken Litter and the Effect of Feedstock-to-Inoculant Ratio

The following manuscript has been incorporated as Chapter 4.

Principal author	Mathu Indren
Contribution to the paper	Performed literature review. Identified research gaps. Developed experimental protocols. Conceived and designed experimental assay for completing the study aims and objectives. Sourced organic materials for methane production assay. Conducted experiments. Analysed and interpreted data. Produced figures and tables. Wrote and edited manuscript.
Contribution	70%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature and date	

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

1. the candidate's stated contribution to the publication is accurate (as detailed above);
2. permission is granted for the candidate in include the publication in the thesis; and
3. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Co-author	Cristian Birzer
Contribution to the paper	Conception and design of the project. Analysis and interpretation of research data. Critical revision of article.
Signature and date	

Co-author	Stephen Kidd
Contribution to the paper	Conception and design of the project. Acquiring data. Analysis and interpretation of the research data.
Signature and date	

Co-author	Paul Medwell
Contribution to the paper	Conception and design of the project. Analysis and interpretation of research data. Critical revision of article.
Signature and date	

The use of biochar in high-solids anaerobic digestion of chicken litter and the effect of feedstock-to-inoculant ratio

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Abstract

High-solids anaerobic digestion is a method to produce energy from manure even if mixed with bedding material. The feedstock (F) to inoculant (I) ratio is a key parameter in high-solids anaerobic digesters. The F/I ratio determines the lag time before methane production starts and the required retention time. This study shows the addition of wood-pellet biochar can increase the viable F/I ratio in high-solids anaerobic digesters. Using biochar reduced the lag time by 41%, 48% and 42% at F/I ratios 0.5, 1 and 2, respectively. The addition of biochar also prevented a 33% reduction in the peak daily methane yield when the F/I ratio was increased from 0.5 to 2. Using a lower F/I ratio results in a more diverse population of methane-generating microorganisms in the bulk sludge. The F/I ratio does not affect the diversity of the methane-generating microorganisms on the biochar.

Keywords: F/I ratio, biochar, high-solids anaerobic digestion, chicken litter, biogas, anaerobic digestion

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1. Introduction

Anaerobic digestion is the biological decomposition of organic matter, such as animal manure, in the absence of an oxygen source. Two beneficial by-products of anaerobic digestion are methane-containing biogas and digestate. Biogas can be used for cooking, electricity generation or purified and used as a transport fuel. Digestate is the solid or liquid residue of an anaerobic digesters and may be used as a fertiliser. The production of both energy and fertiliser make anaerobic digesters a unique and valuable technology.

Chicken litter, a waste product from chicken-meat production, is an abundant form of organic waste worldwide. The global consumption of chicken meat is growing faster than any other meat type [1, 2]. This presents a waste management issue but an opportunity for energy generation. Chicken litter is currently underutilised in anaerobic digesters. This is due to a large proportion of nitrogen-containing compounds in the waste, such as undigested proteins and urea [3, 4], which decompose to form ammonia. Methane-generating microorganisms are sensitive to high ammonia concentrations [5].

High-solids anaerobic digesters are an anaerobic digester type which operate using a total solids (TS) content >20%. These digesters are suitable for processing relatively dry feedstocks such as chicken litter (TS $\geq$35%). High-solids anaerobic digesters in rural locations are generally operated as batch digesters [6, 7]. In batch anaerobic digestion an inoculant is needed to minimise the lag time before methane production begins [8–10]. The lag time determines the retention time needed per batch. Using a large amount of inoculant, a low feedstock-to-inoculant (F/I) ratio, can increase costs for the operator. If the inoculant is effluent from sourced from another location, using low F/I ratios will increase transport costs for the operator. The inoculant may also be the bulk sludge from a previous batch. However, the bulk sludge from digesters processing chicken litter would contain inhibitory concentrations of ammonia [11]. Methods to minimise the amount of inoculant (maximising the F/I

ratio) will improve the viability of biogas production from chicken litter.

Using biochar, a porous, carbon-rich product from pyrolysis of biomass, is a promising method to improve high-solids anaerobic digester performance. The addition of biochar reduces lag times by 20–60% in low-solids digesters [12, 13]. A benefit of using biochar over greater volumes of inoculant arise from the ability to produce biochar on-site using wood-based wastes. Wood-based wastes are a common bedding material in chicken farms. Biochar can also be produced using robust equipment such as gasifiers (including cook-stoves) [14–17] or earth-pits.

Improved performance of anaerobic digesters with biochar is suggested to be caused by; (1) reduced distance between partnering microorganisms while attached to biochar and (2) electrical-based interactions between methanogens and other bacteria [18, 19]. These mechanisms require contact between microorganisms and the biochar and are expected to vary with changes to the F/I ratio. Increasing the F/I ratio will lower the initial microbial population and hence the likelihood microorganisms will be in proximity of a biochar particle. In addition, with higher F/I ratios, ammonia stress will increase because of increased proportions of chicken litter in the digesters. Therefore the reduced activity of microorganisms due to ammonia may also prevent attachment of microorganisms onto biochar.

In low-solids anaerobic digesters not operating under ammonia stress, the addition of biochar reduces lag times either consistently [13] or to a greater degree at higher F/I ratios (more feedstock and less inoculant) [12, 20]. In thermophilic high-solids anaerobic digestion the addition of biochar into digesters operating at F/I of 4 and 6 increased methane yields compared with controls [21]. However, it is difficult to extrapolate data obtained from thermophilic conditions to mesophilic conditions due to lower microbial activity and changes to free ammonia-nitrogen concentrations. As a result, investigations into the relationship between high-solids anaerobic digester performance, F/I ratio and biochar addition at mesophilic temperatures are required.

This study aims to determine the influence of varying F/I ratio and biochar addition on the performance of a mesophilic high-solids anaerobic digester. The objectives are to; (1) determine changes in methane yield and production rate due to both F/I ratio and biochar addition and (2) evaluate the impact of biochar addition with varying F/I ratios in terms of chemical conditions in the bulk sludge and the microbial population in the bulk sludge and attached to the biochar.

2. Methods

2.1. Methane production assay

The methane production assays was performed using 250 ml glass bottles. The assays were performed in triplicate to account for heterogeneity of the feedstock and biological variations. The biogas produced was measured by the displacement of a saturated sodium chloride solution [22]. The biogas volume was corrected to dry gas at 0°C [23].

The chicken litter, was sourced from a litter distributor (Infield, South Australia) from a meat-chicken farm. The chicken litter was a mixture of faeces and pine wood-shavings. A 10 kg sample of chicken litter was collected at the end of the growing-cycle. A representative 1 kg sample of the chicken litter was prepared by mixing the 10 kg sample and taking 10 $\times$ 100 g sub-samples as described by John and Combs [24].

The inoculant was the de-watered effluent from an anaerobic digester located in a wastewater treatment facility (SA Water, South Australia). The facility processes a combination of municipal wastewater and wastewater from a slaughterhouse. To reduce its methane production potential, yet maintain an active microbial population the inoculant was maintained at 37°C for three days.

The biochar was produced using wood-pellets in an auto-thermal top-lit up-draft gasifier (TLUD) [25]. Each batch processed in the TLUD contained 2.1 kg of wood-pellets. 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 had a pH of 10.3 (in water), a total solids content of 97% and the following dry weight-based contents of ash (3%), carbon (88%), nitrogen (0.2%), and hydrogen (2%).

The feedstock and inoculant were loaded into the digesters at VS-based F/I ratios of 0.5, 1 and 2. A constant working wet weight of the inoculant, feedstock and water of 100 g was used for both digesters with biochar and control digesters. The total solids content of the digesters was adjusted to 20% using Milli-Q water, but did not include the total solids content of the biochar. The biochar was added on a 1/1 total solids-based ratio of biochar to chicken litter.

The head-space in each bottle was flushed with high-purity nitrogen gas to produce anaerobic conditions. The digesters were maintained at 37°C in a temperature-controlled room. Mixing occurred by inverting the bottles for 10 seconds, once per day and five times per week.

2.2. Biogas analysis

The composition of CH_4, CO_2 and H_2 in the biogas was analysed by extracting the headspace of the digesters three times per week into 10 ml gas-tight syringes. The biogas samples were passed through a gas chromatograph equipped with a thermal conductivity detector (Agilent, 490 MicroGC). A 10 metre, 5Å molecular sieve was used for analysis of CH_4 and H_2. The column was set at 80°C and used argon at 200 kPa as the carrier gas. The CO_2 analysis was conducted on a PoraPLOT U, 10 metre column at 80°C. The column used helium at 150 kPa as the carrier gas. The injector temperature of the gas chromatograph was 110°C.

2.3. Physical and chemical analyses

The total solids content of the chicken litter, inoculant and biochar were determined by drying the materials at 105°C [26]. Volatile solids (VS) analysis was conducted by ashing at 550°C [26] in a thermogravimetric analyser (Mettler Toledo, TGA-DSC2). To account for the heterogenous nature of the chicken litter and inoculant, analyses of TS and VS for these materials were conducted in sextuplet. Elemental analysis (carbon, hydrogen and nitrogen) was performed on the dried materials using a Perkin Elmer, 2400 Series II elemental analyser.

Aqueous samples of the chicken litter, inoculant and bulk sludge were prepared by diluting 5 g of the material in 20 ml of Milli-Q water. The sample was mixed for 20 minutes, centrifuged at 2000G for 10 minutes and separated into a liquid and solid fraction. If present, biochar was not removed from the bulk sludge. The pH of the aqueuos samples was determined using a pH probe (Mettler Toledo, InLab Expert Pro®). The pH was recorded immediately after the aqueous samples were prepared. Total alkalinity was determined by titration of the supernatant against 0.1 N HCl to an end-point pH of 4.4 [27]. The concentration of volatile fatty acids was determined by titration of the supernatant against 0.1 N HCl between points 5 and 4.4 [28]. Total ammonia-nitrogen was determined colorimetrically according to the salicylate method and a spectrophotometer [29]. The free ammonia-nitrogen (FAN) concentration was calculated according to equation 1 [30].

$$FAN = TAN \times \left(1 + \frac{10^{-pH}}{10^{-(0.09018+\frac{2729.92}{T})}}\right)^{-1} \quad (1)$$

In Equation 1, the term T is the temperature in kelvin.

2.4. Microbial population analysis

The microbial population of the chicken litter, inoculant, bulk sludge and biochar on completion of the 90-day assay was analysed. The DNA was extracted from these samples using a PowerSoil DNA isolation kit (Quiagen, Germany). 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 Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, USA).

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 [31]. 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. Further details on the qPCR methodology have been reported previously [32].

2.5. Scanning electron microscopy

A Philips XL30 scanning electron microscope (SEM) was used to examine the morphology of the biochar and to analyse changes due to its addition in an anaerobic digester. At the end of the methane production assay, the biochar was residual sludge was removed by washing the biochar with phosphate buffered saline (PBS). Additional details on the sample preparation methodology are described in previous work [33].

2.6. Data Analysis

To assess both the level of repeatability of the assay and to determine chemical changes over time, one set of 18 digesters was operated for 30 days and a second set of 18 digesters was operated for 90 days. Digesters from the 90-day assay were not opened after 30 days mark as earlier trials indicated opening the digesters affected the total methane yield measurements. One digester in the 30-day assay (F/I = 1 control) and 90-day assay (F/I = 2 with biochar) was lost due to inadequate sealing of the digester. The data from these digesters were excluded from the analyses.

The modified Gompertz equation (Equation 2), was used to estimate the potential methane yield, the maximum daily methane production rate and methane production lag time.

$$M(t) = A \times exp\left\{-exp\left[\frac{R_{max} \times e}{A}(\lambda - t) + 1\right]\right\} \quad (2)$$

In Equation 2, $M(t)$ is the methane production at time t (day), A is the potential methane production (ml/g-VS), e is exp(1) $\approx$ 2.71828; R_{max} is the maximum daily methane production rate (ml/g-VS/day) and λ is the lag time (days). These parameters were calculated using the Grofit package [34] of R-project software (version 3.5.0)

Statistical analysis included one-way analysis of variance (ANOVA) with a significance of 0.05 and the Tukey post-hoc test for comparison of mean values between each scenario.

3. Results and Discussion

3.1. Feedstock and inoculant characteristics

Table 1 shows the properties of the chicken litter and the inoculant. There is a significantly higher total ammonia-nitrogen concentration (5.5 g-TAN/kg) in the chicken litter than the inoculant 0.8 g-TAN/kg. As a result, the inoculant will dilute the ammonia concentration in the chicken litter. There will be a greater level of dilution with increasing F/I ratios. The dominant methane-generating microorganism in the inoculant is the *Methanosaetaceae* family. There are no methane-generating microorganisms in the chicken litter, highlighting the need for an inoculant.

3.2. Effect of F/I ratio and biochar addition on methane yields

Figure 1 shows the daily methane yield from digesters using volatile solids-based feedstock-to-inoculant (F/I) ratios of 0.5, 1 and 2. Data are shown for digesters with and without biochar (control). The dotted line shows the values from the 30-day assay and the solid lines

Table 1: Characteristics of the chicken litter and de-watered anaerobic digester sludge (inoculant)

	Chicken litter	Inoculant
Total solids, TS (wt%)	60 ± 2	17 ± 1
Volatile solids, VS (wt%)	48 ± 2	10 ± 1
VS (% of TS)	80 ± 3	59 ± 1
Carbon (% of TS)	34.6 ± 0.5	25.6 ± 0.3
Hydrogen (% of TS)	5.4 ± 0.1	5.4 ± 0.02
Nitrogen (% of TS)	4.7 ± 0.2	5.4 ± 0.1
Oxygen (% of TS)*	35	23
Ash (% of TS)	20 ± 3	41 ± 1
C/N	7.4 ± 1.4	4.8 ± 1.4
pH	8.9 ± 0.01	8.4 ± 0.01
Total alkalinity (g-$CaCO_{3eq}$/kg)	26.0 ± 2	2.3 ± 0.08
Volatile fatty acids (g/kg)	3.0 ± 0.7	2.3 ± 0.4
Total ammonia-nitrogen (g-TAN/kg)	5.5 ± 0.3	0.8 ± 0.2
Methanosaetaceae (ng/µl/g)	ND	4.8 ± 0.4
Methanosarcinaceae (ng/µl/g)	ND	ND
Methanobacteriales (ng/µl/g)	ND	ND
Methanomicrobiales (ng/µl/g)	ND	0.05 ± 0.07
Total microorganisms (ng/µl/g)	130 ± 49	444 ± 86

* Calculated: O% =100 - ash% - (C%+H%+N%)

ND = not detected

show the values from the 90-day assay. The markers show the daily methane yield from each biological replicate and the line shows the mean value. After 90 days, the daily methane production rate is ≤ 0.1 ml/g-VS/day in all digesters and no further methane production is expected.

Figure 1 shows the daily methane production rate in the control digesters is affected by increasing the F/I ratio. In the control digesters, using an F/I ratio of 0.5 produces the largest peak daily methane yield (2.1 ml/g-VS/day). This peak methane yield occurs at day 36. At an F/I ratio of 1 and 2, the maximum daily yield decreases to 1.3 and 1.4 ml/g-VS/day, respectively. At an F/I ratio of 0.5 there is also a well-defined peak in the daily methane yield. By comparison at F/I ratios of 1 and 2, there is no sharp drop in the daily methane yield after the peak daily yield occurs. In these digesters, the daily methane yield is roughly constant between days 30 to 50.

The addition of biochar can prevent the reduction in daily methane yield and the flattening of the daily methane production curve with an increasing F/I ratio. The maximum daily methane yields were 2.1, 2.3 ml/g-VS/day at F/I ratios of 0.5, 1 and 2.4 ml/g-VS/day at an F/I ratio of 2. In addition, there is a sharp drop in the daily methane yield after the peak daily methane production rate at all three F/I ratios. The elongated methane production time in digesters without biochar at higher F/I ratios will be detrimental to operational performance. These digesters will require a longer retention time to maximise their cumulative methane yield. As a result, this will limit the number of batches that can be started per year. Using biochar allows for higher F/I ratios and therefore greater throughput of chicken litter.

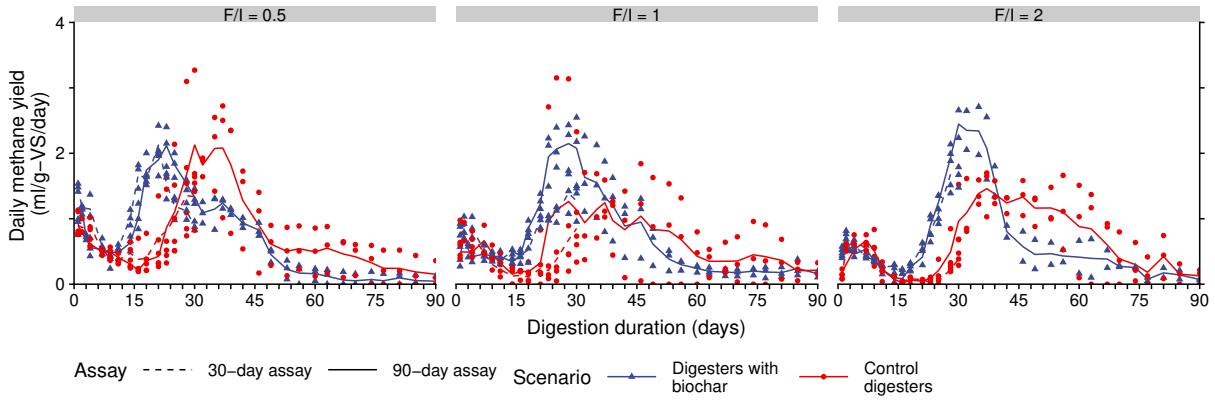

Figure 1: Daily methane yield, normalised based on the volatile solids (VS) content of the inoculant and chicken litter, from digesters with biochar and control digesters (without biochar) using a VS-based feedstock-to-inoculant (F/I) ratio of 0.5, 1 and 2. The lines show the mean and markers show the range from the replicates.

Figure 2 shows the cumulative methane yield from digesters without biochar and the controls at the three F/I ratios. The markers show the range of values from the replicate digesters and the line shows the mean value. The dotted line shows the mean yield from the 30-day assay and the solid line shows the mean yield from the 90-day assay. The cumulative methane yield does not change significantly with varying F/I ratio or due to biochar addition. The methane yield in the control digesters ranges from 56–63 ml/g-VS. By comparison, in digesters with biochar, the cumulative methane yield ranges from 57–62 ml/g-VS.

The changes to the methane production rate with varying F/I ratio and the addition of biochar observed in Figure 1 and Figure 2 are quantified using the Gompertz equation. Table 2 shows the fit of the measured cumulative methane yield from the 90-day assay to the Gompertz equation (described in equation 2). Data are shown for the lag time, the peak daily methane production rate and the potential methane yield.

Table 2 shows that in increased F/I ratio results in a longer lag time before methane production starts. In the control digesters, the lag time increased from 15.8 days at an F/I

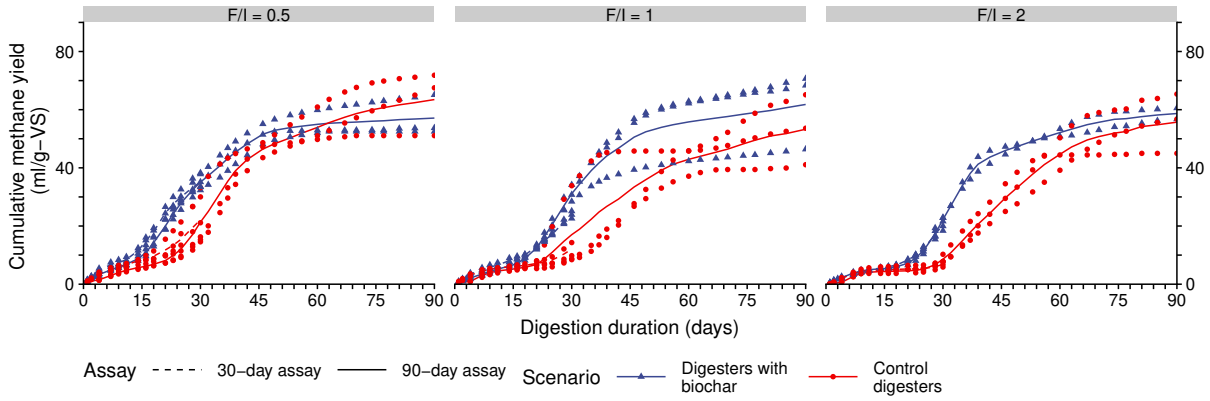

Figure 2: The 30 and 90-day cumulative methane yield, normalised based on initial volatile solids (VS) content of chicken litter and inoculant, from digesters with biochar and the control digesters (without biochar) using a VS-based feedstock-to-inoculant (F/I) ratios of 0.5, 1 and 2. The lines show the mean and markers show the range from replicates.

ratio of 0.5 by 56% to 24.7 days at an F/I ratio of 2. At an F/I ratio of 1, one of the replicates without biochar exhibited a shorter lag time and higher maximum daily methane yield than the other two replicates. The other two biological replicates from the 90-day assay and the three replicates from the 30-day assay at an F/I ratio of 1 do not show a shortened lag time or higher maximum daily yield.

The results at an F/I ratio of 1 in the control digesters need to be interpreted with caution. Variation in methane yield from digesters under environmental stress has been suggested to occur due to adaptation of the microbial community to the feedstock [35]. In addition, the heterogeneous distribution of chicken litter and inoculant will result in different areas of VFAs and ammonia within the bulk sludge. Due to this variation, the median value was used as an estimate of the lag time for this particular scenario.

The addition of biochar reduces the lag time at all three F/I ratios. At an F/I ratio of 0.5, 1 and 2, the lag time in digesters with biochar was reduced by 41%, 48% and 42%, respectively, compared with the control digesters. Remarkably, digesters using biochar and

Table 2: Summary of the kinetic parameters according to the Gompertz model for digesters with varying feedstock-to-inoculant ratios.

F/I	Scenario	Peak daily production rate (ml/g-VS/day)	Lag time (days)	Potential methane yield (ml/g-VS)
0.5	Biochar	1.7 ± 0.1	9.3 ± 0.8	56.6 ± 0.7
0.5	Control	1.6 ± 0.1	15.8 ± 1.2	63.2 ± 1.7
1	Biochar	1.8 ± 0.2	12.8 ± 1.4	60.3 ± 1.6
1	Control	1.0 ± 0.1	23.6* ± 2.5	55.5 ± 3.8
2	Biochar	1.8 ± 0.1	14.3 ± 1.0	60.2 ± 1.1
2	Control	1.4 ± 0.1	24.7 ± 1.1	55.6 ± 1.3

* Median value, all other data are represented as mean ± standard deviation.

an F/I ratio of 2 have a shorter lag time than the control digesters using an F/I ratio of 0.5.

ane yield in the control digesters decreased as the F/I ratio increased. The maximum daily yield was 2.1 mL CH_4/g-VS/day at an F/I ratio of 0.5 and decreased by 29% to 1.5 mL CH_4/g-VS/day at an F/I ratio of 2. In digesters with biochar, the maximum daily methane production rate, did not decrease with increasing F/I ratio. The maximum daily methane yields were 2.2, 2.3 and 2.5 mL CH_4/g-VS/day at F/I ratios of 0.5, 1 and 2, respectively. Figure 1 also shows the addition of biochar causes the the maximum daily methane yield to occur earlier. Compared with the controls, the maximum daily methane yield in digesters with biochar occurred 7 days earlier at both F/I ratios of 0.5 and 2.

3.3. Effect of F/I ratio and biochar addition on chemical conditions in the bulk sludge

Table 3 shows the pH, total ammonia-nitrogen (TAN), free ammonia-nitrogen (FAN), volatile fatty acids (VFA), total alkalinity (TA) and the VFA/TA ratio on completion of the 30-day and 90-day assays. Table 3 shows all the digesters are operating at a high pH (8.2–8.9). At this pH range, FAN, as opposed to the ammonium ion, is expected the main inhibitory component of total ammonia-nitrogen [36]. The concentration of FAN did not vary with the F/I ratio or due to biochar addition. However it did vary with time.

After 30 days, in all digesters, the FAN concentration is similar regardless of biochar addition or F/I ratio (0.6–0.9 g-FAN/kg). This concentration of FAN is expected to lower the growth rate of methanogens. The growth rate of aceticlastic (acetate-consuming) and hydrogenotrophic (hydrogen-consuming) methanogens is expected to be 20% and 40% of non-inhibited growth rates, respectively [37]. This slower growth rate combined with a smaller population of methane-generating microorganisms could explain the longer lag times with increasing F/I ratios.

After 90 days, the FAN concentration has increased substantially. In all digesters, the FAN concentration is within a range of 1.2–1.9 g-FAN/kg after 90 days. Increases to FAN

Table 3: Chemical conditions on completion of both the 30-day and 90-day assays for digesters with varying feedstock-to-inoculant ratios.

Assay (days)	F/I	Scenario	pH	Total ammonia-nitrogen (g/kg)	Free ammonia-nitrogen (g/kg)	Volatile fatty acids (g/kg)	Total alkalinity (g/kg)
30	0.5	Biochar	8.5 ± 0.2	2.7 ± 0.1	0.8 ± 0.3	2.5 ± 0.5	8.9 ± 0.2
	0.5	Control	8.4 ± 0.1	2.9 ± 0.3	0.7 ± 0.2	6.2 ± 1.8	9.4 ± 0.2
	1	Biochar	8.5 ± 0.1	3 ± 0.3	0.9 ± 0.1	6.8 ± 0.6	11 ± 0.3
	1	Control	8.3 ± 0.1	2.9 ± 0.3	0.6 ± 0.3	12.9 ± 1.0	11.4 ± 1
	2	Biochar	8.2 ± 0.4	2.9 ± 0.4	0.5 ± 0.3	5.1 ± 2.9	11.8 ± 0.7
	2	Control	8.3 ± 0.1	3.5 ± 0.2	0.7 ± 0.1	12.9 ± 0.4	13.1 ± 0.4
90	0.5	Biochar	8.8 ± 0.1	3.1 ± 0.2	1.4 ± 0.2	0.9 ± 0.7	9.1 ± 0.4
	0.5	Control	8.8 ± 0.2	2.9 ± 0.4	1.2 ± 0.1	1.3 ± 0.2	9.9 ± 0.9
	1	Biochar	8.7 ± 0.1	4 ± 0.5	1.5 ± 0.4	1.7 ± 1.4	11.8 ± 0.1
	1	Control	8.9 ± 0	3.5 ± 0.5	1.8 ± 0.3	2.4 ± 0.4	12.4 ± 0.3
	2	Biochar	8.8 ± 0.1	4.4 ± 0	1.9 ± 0.2	1.5 ± 0.4	13.6 ± 0.6
	2	Control	8.7 ± 0	4.6 ± 0.4	1.8 ± 0.2	3.3 ± 0.6	15 ± 0.7

concentration are likely caused by further anaerobic degradation of nitrogen. At these concentrations complete inhibition of both aceticlastic and hydrogenotrophic methanogens is expected.

The concentration of VFAs can be an indicator of the degree of degradation of the feedstock and the activity of methanogens. Table 3 shows the VFA concentration changes due to F/I ratio and biochar addition. After 30 days, the VFA concentration in the control digesters using an F/I ratio of 0.5 is 6.2 g/kg. At an F/I ratio of 1 and 2 the VFA concentration is 108% higher. A lower VFA concentration/greater VFA degradation after 30 days is expected in digesters with a lower F/I ratio due to their greater cumulative methane yield at this time stage of the digestion period. Similarly, in the digesters with biochar, after 30 days, there is a 90–150% lower VFA concentration compared with the controls digesters at the same F/I ratio. These results are consistent with the greater cumulative methane production in digesters with biochar within the first 30 days.

After 90 days the VFA concentration in all control digesters is lower than the 30-day concentration. In the controls, after 90 days the F/I ratio ranges from 1.2–1.8 g/kg. The addition of biochar causes a reduction in the VFA concentration. After 90 days at an F/I ratio of 0.5 there is a 45% reduction in VFA concentration in digesters with biochar compared with the controls. At an F/I ratio of 2 there is a 122% reduction.

The lower VFA concentrations in the bulk sludge due may be expected to correlate with greater cumulative methane yields. However, cumulative yields were not changed due to changes to the F/I ratio or because of biochar addition.

The reduction in VFA concentrations yet unchanged methane yield may be due to the decoupling of the VFA degradation step with hydrogenotrophic methanogenesis. The reduction in VFA concentrations may occur through the degradation of VFAs such as acetate, propionate and butyrate into carbon dioxide, hydrogen and formate [38]. The bacteria which facilitate this reaction have a high resistance to ammonia stress [39]. Hydrogenotrophic

methanogens can then consume the oxidation products for the production of methane [40]. This would be expected to increase methane yields. However, the syntrophic relationship between VFA degrading microorganisms and hydrogenotrophic methanogens can be disrupted in ammonia-stressed digesters [41]. As a result, VFA degradation can occur without causing an increase to methane yields.

The total alkalinity of the bulk sludge is an indicator of the sensitivity of the system to pH changes due to VFA accumulation. All digesters show a high total alkalinity (8.9–15 g/kg). The total alkalinity increases in the control digesters with increasing F/I ratios. This is expected as the concentration of total ammonia-nitrogen, which is a weak base, also increases with F/I ratios. At the same F/I ratio, there is no significant difference in the total alkalinity between digesters with biochar and the controls.

The high total alkalinity suggests variation in lag times with F/I ratios are likely driven by varying concentrations of total ammonia-nitrogen or changes to the microbial population. This is in contrast to findings in low-solids digesters. The use of biochar in low-solids digesters increased the total alkalinity of the bulk sludge and prevented long lag times caused by drops in pH at the start of the digestion period [13, 42].

3.4. Effect of biochar and F/I ratio on methanogens

Analysis of the population of methane-generating microorganisms (methanogens) was conducted to understand the relationship between methane yields and the microbial population in the bulk sludge. The targeted methanogens were the acetate-consuming *Methanosaetaceae* family, the mixotrophic (acetate or hydrogen-consuming) *Methanosarcinaceae* family and the hydrogen consuming orders *Methanobacteriales* and *Methanomicrobiales*. The population of the methanogens in the bulk sludge in digesters using biochar and in the controls at F/I ratios of 0.5 and 2 are shown in Figure 3. Also, the total microbial population (both bacteria and archaea) is shown.

Figure 3 shows the *Methanosaetaceae* family was dominant in the bulk sludge. The concentration of this family ranges from 2.4–4.2 ng/µl/g-bulk sludge. The concentration is not significantly affected by the F/I ratio or biochar addition. The high concentration of *Methanosaetaceae* in the bulk sludge is likely due to its dominance in the inoculant (Table 1).

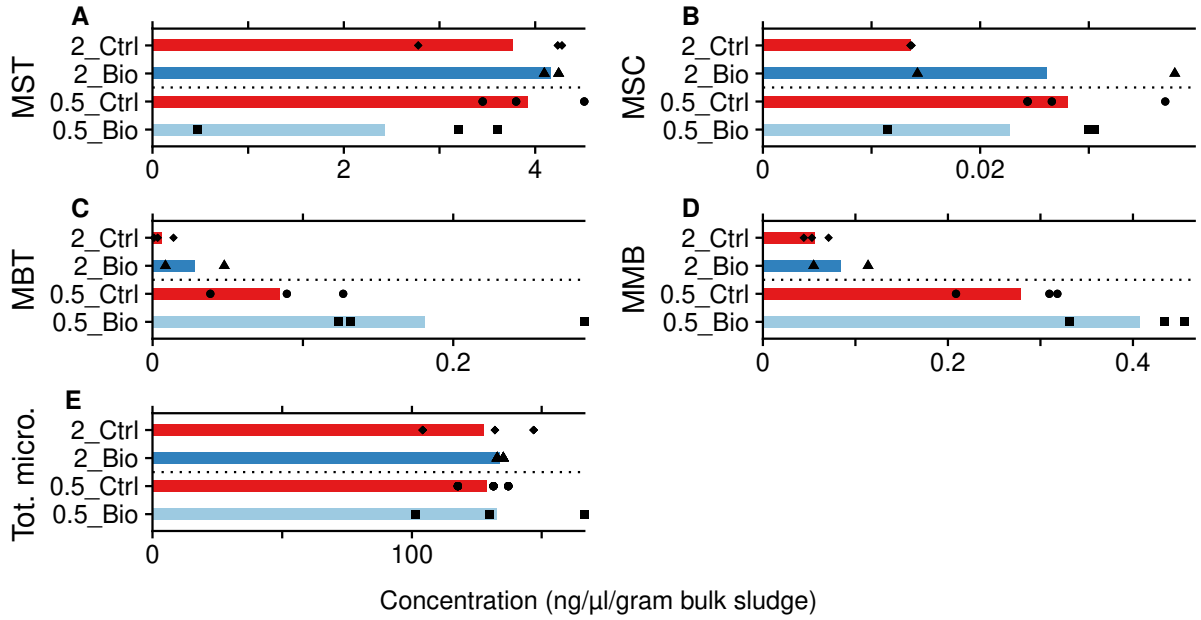

Figure 3: The concentration of DNA from (A) Methanosaetaceae (MST), (B) Methanosarcinaceae (MSC), (C) Methanobacteriales (MBT), (D) Methanomicrobiales (MMB) and (E) total microorganisms (Tot. micro.) in the bulk sludge. Samples were taken 90 days at F/I ratios of 0.5 and 2 in digesters with biochar (Bio) and in the controls (Ctrl).

There are also only small variations in the *Methanosarcinaceae* and the total microorganism concentration ($\approx$ 125 ng/µl/g-bulk sludge) with varying F/I ratios. The *Methanosarcinacea* family had an approximately 100 times lower concentration ($\approx$ 0.02 ng/µl/g-bulk sludge than *Methanosaetaceae*. The figure also shows there is variation in the concentration of the methanogens between the biological replicate digesters. This is expected given the observed variability in methane yields.

The concentration of the hydrogen-consuming methanogens in the bulk sludge is affected by the F/I ratio. At an F/I ratio of 0.5 the *Methanobacteriales* concentration in the bulk sludge of the control digesters (0.08 ng/µl/g-bulk sludge) is 8 times higher than the concentration at an F/I ratio of 2 (0.01 ng/µl/g-bulk sludge). The *Methanomicrobiales* population in the control digesters is approximately 5 times higher at an F/I ratio of 0.5 (0.28 ng/µl/g-bulk sludge) compared with an F/I ratio of 2 (0.06 ng/µl/g-bulk sludge). A trend of greater concentration of hydrogen-consuming methanogens at lower F/I ratios was also observed in digesters with biochar. There were no significant changes in concentration of methanogens between digesters with biochar and controls at the same F/I ratio.

At an F/I ratio of 0.5, the increase in the concentration of hydrogenotrophic methanogens may cause improvements to anaerobic digester performance. As hydrogenotrophic methanogens have a greater tolerance to ammonia stress than aceticlastic methanogens [43] the increase in concentration of these methanogens may explain the shorter lag times with lower F/I ratios.

Microorganisms in the bulk sludge also attach to the biochar. Scanning electron microscopy (SEM) was conducted for visual inspection of microbial attachment onto biochar. Figure 4 shows the porous structure of the biochar. The pores range from 5–20 µm in diameter. These pores are expected to be large enough to allow for microbial colonisation within the internal volume of biochar.

Figure 5 shows multiple types of microorganisms within proximity of each other. Shorter distances between microorganisms can increase the rate of reactions between partnering microorganisms [44]. Figure 6 shows one portion of biochar featuring microorganisms within a suspected biofilm. Within a biofilm microorganisms are in proximity but also less susceptible to environmental stress [45]. Biofilm formation or attachment within the internal volume of biochar could lead to an increased microbial activity despite inhibitory conditions in the bulk sludge.

Figure 7 shows the concentration of the targeted methanogens and total microorganisms

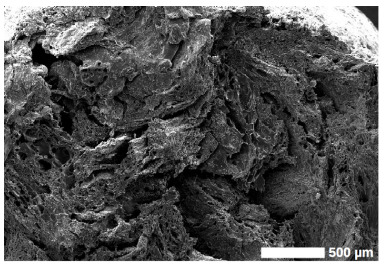

Figure 4: Cross section of wood-pellet biochar

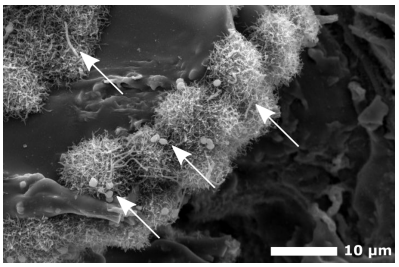

Figure 5: A multi-species community of microorganisms attached to the biochar surface. Arrows highlight different types of microorganisms

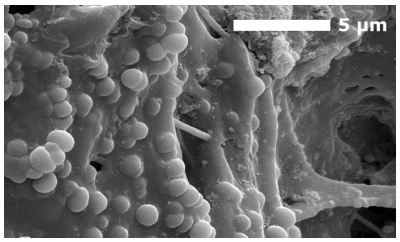

Figure 6: Microorganisms attached to the biochar possibly within a biofilm.

associated with biochar at the end of the 90-day assay at F/I ratios of 0.5 and 2. The figure shows *Methanosaetaceae* is the dominant methanogen attached to the biochar at both F/I ratios (1.9–2.8 ng/µl/g-bulk sludge). The concentration of *Methanosaetaceae* on the biochar is within the same order of magnitude as the concentration in the bulk sludge.

In contrast to the attachment of *Methanosaetaceae* there is no significant attachment of the other methanogens. The *Methanosarcinaceae* concentration was less than 0.004 ng/µl/g-biochar. Both the hydrogenotrophic methanogens had concentrations on the biochar 1000 times lower than the concentration in the bulk sludge. This study confirms the affinity of *Methanosaetaceae* to biochar shown in previous work [19].

The concentration of total microorganisms attached to the biochar varied with the F/I ratio. At an F/I ratio of 0.5, the concentration is 25 ng/µl/g-bulk sludge which is around 25% of the concentration in the bulk sludge. At and F/I ratio of 2 the total microorganisms attached to the biochar increases to 75 ng/µl/g-bulk sludge. This concentration is approximately 50% of the concentration in the bulk sludge. This could suggest increasing ammonia stress, induced by higher F/I ratios leads to greater microbial attachment, possibly through

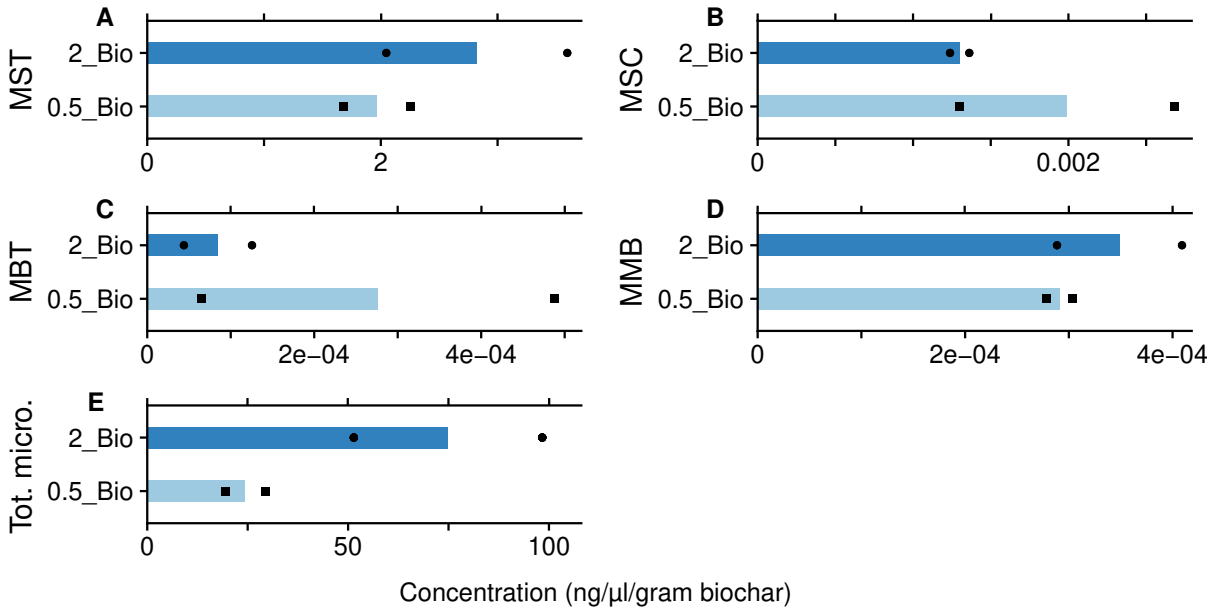

Figure 7: The concentration of DNA from (A) Methanosaetaceae (MST), (B) Methanosarcinaceae (MSC), (C) Methanobacteriales (MBT), (D) Methanomicrobiales (MMB) and (E) total microorganisms (Tot. micro.) associated with the biochar. Samples were taken 90 days at F/I ratios of 0.5 and 2 in digesters with biochar (bio). Two biological replicates were used in this assay.

the formation of biofilms. This result must be interpreted with caution because of the small sample size and variation between the biological replicates.

The selective promotion of *Methanosaetaceae* on the biochar may allow for an increased rate of methane production through the direct inter-species electron transfer (DIET) mechanism. Biochar can facilitate DIET between *Methanosaeta* species and electron-donating bacteria, also attached to the biochar, such as *Geobacter* [46, 47]. As a result, through the DIET mechanism, *Methanosaeta* does not rely solely on acetate for its metabolism. The addition of biochar may reduce the negative effects of a slow diffusion rate of acetate in the bulk sludge of high-solids anaerobic digesters. The facilitation of this mechanism may be one explanation for the reduced lag times and greater peak daily methane yield in digesters with

biochar compared with the control digesters.

4. Conclusions

In high-solids anaerobic digesters, the addition of biochar reduces the lag time before methane production commences. In addition, the use of biochar prevents a drop in the peak daily methane yield with an increasing F/I ratio. The percentage reduction in lag time due to biochar addition is not significantly changed by varying F/I ratio. Lowering the F/I ratio resulted in a more diverse methanogen population in the bulk sludge. However, lowering the F/I ratio does not reduce the degree of attachment of methanogens onto the biochar. There is selective growth of *Methanosaetaceae* on biochar at all three F/I ratios investigated. The use of biochar is a viable alternative to lower F/I ratios to ensure rapid startup of high-solids anaerobic digesters.

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Appendix A. Calibration of Real-time PCR

The key parameters from the standard curves produced using the methodology as described in Section 2.4 are shown in Table A.1.

Table A.1: Standard curves for each primer set

Parameter	Methano-bacteriales	Methano-microbiales	Methano-sarcinaceae	Methano-saetaceae
Linear range (ng/µL)	21.7–0.00217	11.3–0.00113	15–0.0015	2.6–0.00026
Slope (mean)	-3.692	-3.393	-3.5773	-3.3953
Slope r^2	0.99752	0.9949	0.99747	0.99867
Intercept	17.92	17.98	15.73	19.61
PCR efficiency	87%	97%	90%	97%

Appendix B. Digester loading

The mass of chicken litter, inoculant, biochar and water added to each digester is shown in Table B.2

Table B.2: Experimental design for biochar addition to digesters with varying feedstock-to-inoculant ratio

	chicken litter			Inoculant			Water	Biochar		With biochar	Control
F/I ratio	Wet weight (g)	TS (g)	VS (g)	Wet weight (g)	TS (g)	VS (g)	Wet weight (g)	Wet weight (g)	TS (g)	TS%	TS%
0.5	9.1	5.4	4.3	84.9	14.6	8.6	6.0	5.6	5.4	24%	20%
1.0	14.3	8.5	6.8	66.8	11.5	6.8	19.0	8.8	8.5	26%	20%
2.0	20.0	12.0	9.5	46.8	8.0	4.8	33.2	12.4	12.0	28%	20%

Chapter 5

Effects of Biochar Parent Material and Microbial Pre-Loading in Biochar Amended High-Solids Anaerobic Digestion

The following publication has been incorporated as Chapter 5: Indren, M., Birzer, C.H., Kidd, S.P., Hall, T., Medwell, P.R., 2020. Effects of biochar parent material and microbial preloading in biochar-amended high-solids anaerobic digestion. Bioresource Technology 298, 122457. https://doi.org/10.1016/j.biortech.2019.122457

Principal author	Mathu Indren
Contribution to the paper	Performed literature review. Identified research gaps. Developed experimental protocols. Conceived and designed experimental assay for completing the study aims and objectives. Conducted experiments. Analysed and interpreted data. Produced figures and tables. Wrote and edited manuscript. Produced responses to reviewer comments. Corresponded with journal regarding the reviewer comments.
Contribution	70%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature and date	

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

1. the candidate's stated contribution to the publication is accurate (as detailed above);
2. permission is granted for the candidate in include the publication in the thesis; and
3. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Co-author	Cristian Birzer
Contribution to the paper	Conception and design of the project. Analysis and interpretation of research data. Critical revision of article and reviewer comments.
Signature and date	

Co-author	Stephen Kidd
Contribution to the paper	Conception and design of the project. Analysis and interpretation of the research data. Acquiring microbial population data as it required significant judgement and input.
Signature and date	

Co-author	Tony Hall
Contribution to the paper	Acquiring VFA data as it required significant intellectual judgement and input. Critically revising manuscript to contribute to its interpretation.
Signature and date	

Co-author	Paul Medwell
Contribution to the paper	Conception and design of the project. Analysis and interpretation of research data. Critically revising article and reviewer comments.
Signature and date	

Chapter 6

Effect of Wood Biochar Dosage and Re-use on High-Solids Anaerobic Digestion of Chicken Litter

The following publication has been incorporated as Chapter 6: Indren, M., Birzer, C.H., Kidd, S.P., Hall, T., Medwell, P.R., 2021. Effect of wood biochar dosage and re-use on high-solids anaerobic digestion of chicken litter. Biomass and Bioenergy 144, 105872. https://doi.org/10.1016/j.biombioe.2020.105872

Principal author	Mathu Indren
Contribution to the paper	Performed literature review. Identified research gaps. Developed experimental protocols. Conceived and designed experimental assay for completing the study aims and objectives. Sourced organic materials for methane production assay. Conducted experiments. Analysed and interpreted data. Produced figures and tables. Wrote and edited manuscript.
Contribution	70%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature and date	

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

1. the candidate's stated contribution to the publication is accurate (as detailed above);
2. permission is granted for the candidate in include the publication in the thesis; and
3. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Co-author	Cristian Birzer
Contribution to the paper	Conception and design of the project. Analysis and interpretation of research data. Critical revision of article.
Signature and date	

Co-author	Stephen Kidd
Contribution to the paper	Conception and design of the project. Analysis and interpretation of the research data.
Signature and date	

Co-author	Tony Hall
Contribution to the paper	Acquiring data. Contribution of knowledge.
Signature and date	

Co-author	Paul Medwell
Contribution to the paper	Conception and design of the project. Analysis and interpretation of research data. Critical revision of article.
Signature and date	

Chapter 7

Discussion

This chapter summarises the investigations presented in Chapters 3–6 which were aimed to address the five research gaps identified in Chapter 2. In addition, a discussion on the application of the use of biochar in anaerobic digesters for resource constrained communities is provided. Finally, an analysis on the repeatability of high-solids anaerobic digestion assays is shown.

7.1 Summary of Results

7.1.1 The Use of Biochar with Varying Total Solids Content

The majority of previous research focused on the use of biochar in anaerobic digesters operating at a total solids content of 5–10%. To address this research gap an investigation, presented in Chapter 3, was conducted to identify the effects of wood-pellet biochar on methane production from poultry litter as a function of the digester total solids (TS) content (5%, 10% and 20% TS). Previous work showed increasing the total solids content from 10–30% decreased the peak daily methane yield by around 60% (Abbassi-Guendouz et al., 2012). Lower rates of methane production also occurred with increasing total solids content due to lower diffusion rates of soluble intermediate products throughout the digester (Xu et al., 2014; Bollon et al., 2013).

The results in Chapter 3 show that the addition of biochar had greater performance improvements in using high-solids anaerobic digesters (20% TS) compared with low-solids anaerobic digesters. This was due to greater increases in the daily methane yield and greater percentage reductions in lag times. As a result of these improvements, subsequent studies (Chapters 4,5 and 6) used high-solids conditions for investigations on the effects of feedstock/inoculant (F/I) ratio, biochar types and dosages.

7.1.2 The Use of Biochar with Varying Feedstock-to-Inoculant Ratios

Studies which have focussed on the addition of biochar in low-solids anaerobic digesters showed either consistent of greater percentage reduction in lag times when using higher F/I ratios (more

feedstock and less inoculant) (Wang et al., 2018a; Cai et al., 2016; Li et al., 2018a). The second research gap identified in this project was the unknown effects of biochar addition with varying F/I ratios in high-solids conditions.

The results of Chapter 4 show that the addition of biochar at prevented a drop in peak daily methane yield which occurs when increasing the F/I ratio (F/I = 0.5, 1 or 2). There is also a consistent effect on the percentage reduction in lag time across the three F/I ratios investigated. The results of Chapter 4 suggests suggest biochar has greater benefits in high-solids anaerobic digesters operating with higher F/I ratios. The practical benefit is a greater volume of feedstock (chicken litter) that can be added to each batch. As a result of the greater performance improvements at higher F/I ratio, the subsequent investigations (Chapters 5 and 6) used F/I ratios of 2.

7.1.3 Effect of Biochar Parent Material

A wide variety of parents materials have been used for biochar production and added into anaerobic digesters. The parent materials include wood (Fagbohungbe et al., 2016; Cruz Viggi et al., 2017), agricultural wastes such as rice husk (Fagbohungbe et al., 2016), wheat bran (Cruz Viggi et al., 2017), wheat straw (Shen and Zhu, 2016) or manures (Jang et al., 2017; Pan et al., 2019; Wang et al., 2017) have all shown to improve digester performance. There were also only a few studies report detrimental effects of biochar addition to anaerobic digesters (Linville et al., 2017; Li et al., 2019; Sun et al., 2019; Zhang et al., 2019a) Excessive concentrations of light metal ions (such as sodium, potassium, magnesium, calcium and aluminium) within the biochar was suggested in one study where poor performance was observed (Linville et al., 2017). To date, only a limited number of studies have focused on the effect of the biochar parent material on the performance of high-solids anaerobic digesters. This was the third research gap identified.

The results of Chapter 5 showed that only biochar produced from wood pellet improved high-solids anaerobic digester performance compared with the controls. The results of the physical and chemical analysis of the biochar shown in Chapter 5 suggest the high ash content of both wheat straw and sheep manure biochar contribute to increasing the lag time compared with the controls. In addition, the low bulk density of wheat straw biochar substantially decreased the volumetric efficiency of the biochar. The high volumetric efficiency of high-solids anaerobic digesters is a key advantage over low-solids digesters which should be maintained when adding biochar.

The benefits of using wood-pellet biochar compared with sheep manure of wheat straw biochar in high-solids conditions at an F/I ratio of 2 imply that no further benefit would be gained by adding sheep manure or wheat straw biochar at lower F/I ratios of lower TS contents. In addition, the subsequent study (Chapter 6) also used wood-pellet biochar.

7.1.4 Effect of Biochar Dosage

An aim of chapter 6 was to identify the impact of dosage levels of both biochar produced from wood-pellets on the performance of high-solids anaerobic digesters processing chicken litter. Studies of biochar addition in high-solids anaerobic digesters have used dosages of 2–30 g/l (Paritosh and Vivekanand, 2019; Zhang et al., 2019b). These dosages used in high-solids conditions are low on a mass-basis due to the substantially lower working volumes. The fourth research gap identified was that very little is known about the effect of biochar dosages greater than 30 g/l in high-solids anaerobic digesters. An objective of Chapter 6 was to determine the effects of wood-pellet biochar dosage (0.25, 0.5 and 1 $g_{TS\text{-}char}/g_{TS\text{-}feed}$ on methane yield from a high-solids anaerobic digester.

The results of chapter 6 show wood-pellet biochar dosage of at least 1 $g_{TS\text{-}char}/g_{TS\text{-}feed}$ or 59 $g_{TS\text{-}char}$/l, was required to increase the 90-day methane yield compared with the control digesters. This dosage also results in the largest percentage reduction in lag time. All four investigations presented in this thesis use a wood-pellet biochar dosage of 1 $g_{TS\text{-}char}/g_{TS\text{-}feed}$. It is possible lower dosage would be sufficient in systems under a lower degree of stress due to lower TS contents or lower F/I ratios. The effects on biochar dosage under these conditions would need to be experimentally determined and could be the subject of future work.

7.1.5 Effect of Biochar Re-use

An aim of chapter 5 and 6 and the fifth research gap shown in Chapter 2 was to determine the impact of re-using biochar on anaerobic digester performance. The re-use of another additive, magnetite improves methane production rate by 70% compared with adding pristine magnetite (Baek et al., 2017), however the effect of re-using biochar was unknown.

The results in Chapters 5 and 6 show the effects of re-using biochar can be variable. The results in Chapter 5 show substantial improvements in methane yield compared with the addition of pristine biochar while the results of Chapter 6 show no benefit. Future work is required to identify the key conditions which will lead to improvements in methane yield due to the re-use of biochar. The population of propionate oxidising bacteria on the re-used biochar should be quantified. Furthermore, a comparison of the effects of adding re-used biochar compared with an equivalent proportion of digestate could provide a deeper understanding on the effects of re-used biochar. In addition, the economic viability of biochar re-use should be investigation. Options to recover biochar floatation and skimming which may not be feasible in all situations. Also, a determination of the value of the biochar/digestate mixture compared with standard digestate (without biochar) is required.

7.1.6 Optimum Process Parameters

Overall, the results in Chapters 3–6 suggest that using biochar in a high-solids digesters leads to greater performance improvements (20% TS). A high F/I ratio and biochar dosage also

results in the largest percentage improvements to performance. Wood is the preferred parent material for biochar production and a high bulk density of the biochar is beneficial. The re-use of biochar is possible, however, further cost-benefit analyses are required before this practice is implemented.

7.2 Application in Resource-Constrained Communities

A motivation for this project was to improve the performance of anaerobic digesters using a method which is appropriate for resource-constrained communities.

The use of anaerobic digesters as a replacement of solid cooking fuels has a variety of benefits. For example, the use of biogas in India has resulted in lower levels of household air pollution, reduced firewood use and time savings for household cooks (Lewis et al., 2017). Other benefits include an increased level of energy security through lower volumes of fuel imports and an improved method for waste management (Rupf et al., 2015; Scarlat et al., 2018). These benefits have led to the construction of millions of domestic-scale anaerobic digesters globally. There is an estimated 30–40 million in China, 4.5 million in India, 250 000 in Nepal and 30 000 in sub-Saharan Africa (Scarlat et al., 2018). However, anaerobic digesters have a reputation for being difficult to operate over the long term (Bond and Templeton, 2011).

Chapters 3–6 showed that the addition of wood-pellet biochar produced in a top-lift up-draft gasifier (TLUD) results in performance improvements anaerobic digesters under a wide variety of operating conditions. As a TLUD is a low-emissions cook-stove used for cooking in resource constrained communities it is plausible that this is a viable additive to anaerobic digesters. The implementation of this process modification would require determining areas where there is a high availability of feedstocks for both biogas and biochar production, determining the cost of the types of biochar production technologies available in geographic regions and a cost of high-solids anaerobic digesters compared with low-solids anaerobic digesters. These costs would be evaluated against the economic, social and environmental benefits that wider use of biochar in resource-constrained communities would facilitate.

This work has also shown that through the use of biochar chicken litter is also a viable feedstock for biogas production. There are high densities of chickens in China, South Asia and Africa, as depicted in Chapter 2. . Many of these areas with a high population density of chickens are the same areas where household air pollution from cooking with solid fuels is a significant health concern.

The use of chicken litter as feedstock requires further use of high-solids anaerobic digesters in resource constrained communities. Previous work has shown that batch operated high-solids anaerobic digesters are feasible for processing manures with bedding materials in rural and resource constrained communities (Svensson et al., 2006; Yap et al., 2016; Riggio et al., 2017). Semi-continuous and plug-flow high-solids anaerobic digesters exist but have large energy requirements (Batstone and Jensen, 2011; Fagbohungbe et al., 2015). A robust small-scale

high-solids anaerobic digester processing plant biomass can produce 1.2–1.4 m^3-biogas/day/m^3-digester (Svensson et al., 2007). Low-solids anaerobic digesters commonly used by households for biogas production in resource-constrained communities are 2–8 m^3 (Bond and Templeton, 2011; Ferrer et al., 2011). Therefore, a high-solids anaerobic digester of a similar size, which is operating efficiently could potentially produce the 1.5–2 m^3 of biogas per day required to cook two meals for a family of five (Bond and Templeton, 2011).

7.3 Variability of High-Solids Anaerobic Digestion Assays

The bench-top methane production assays described in Chapters 3–6 have been used in numerous studies to determine the performance of anaerobic digesters using a wide variety of process conditions (Angelidaki et al., 2009; Raposo et al., 2011; Kafle and Chen, 2016). Due to the biological nature of the process, variations in the methane yields and production rates from repeated assays are expected. Previous work, using low-solids anaerobic digesters has established significant variations in the methane production rate from repeated assays (Raposo et al., 2011). However, there is limited information on the repeatability of the cumulative methane yields and production rates in high-solids anaerobic digestion assays.

This section show the repeatability of methane production from anaerobic digesters operating at 20% total solids (TS) through a compilation of data from Chapters 3–6. Data are shown for high-solids anaerobic digesters processing chicken litter, using de-watered sludge as an inoculant at a volatile solids (VS)-based feedstock-to-inoculant ratio of 2. In addition, an assessment of the repeatability of adding wood-pellet biochar into these digesters at a dosage of 1 $g_{TS\text{-}char}/g_{TS\text{-}feed}$ is shown.

The cumulative methane yield from the repeated anaerobic digestion assays presented in Chapters 3–6 for digesters without biochar (control) and digesters with wood-pellet biochar are shown in Figure 7.1 (A) and Figure 7.1 (B), respectively. The daily methane yield from these repeated assays are shown in Figure 7.2 (A) and Figure 7.2 (B). The markers in the figures represent the value from each biological replicate and the dashed lines show the mean value from the biological replicates presented in each chapter. The mean cumulative and daily methane yields from the four studies, represented by the solid line, were calculated using the R Software package and the *geom_smooth* function with a span of 0.2 and the locally-estimated scatterplot smoothing (loess) method.

Figure 7.1 (A) shows the mean cumulative methane yield after 90 days for the control digesters is 52 ± 7 ml/g-VS (mean ± standard deviation, n = 11). The addition of biochar results in a small increase in the cumulative methane yield. The mean methane yield after 90 days from digesters with biochar is 65 ± 10 ml/g-VS (n = 11). The relative standard deviation is 13% and 15% for the controls and digesters with biochar, respectively. This is comparable to the 10% relative standard deviation in the cumulative methane yield from an assessment of the repeatability of low-solids anaerobic digesters reported by Raposo et al. (2011).

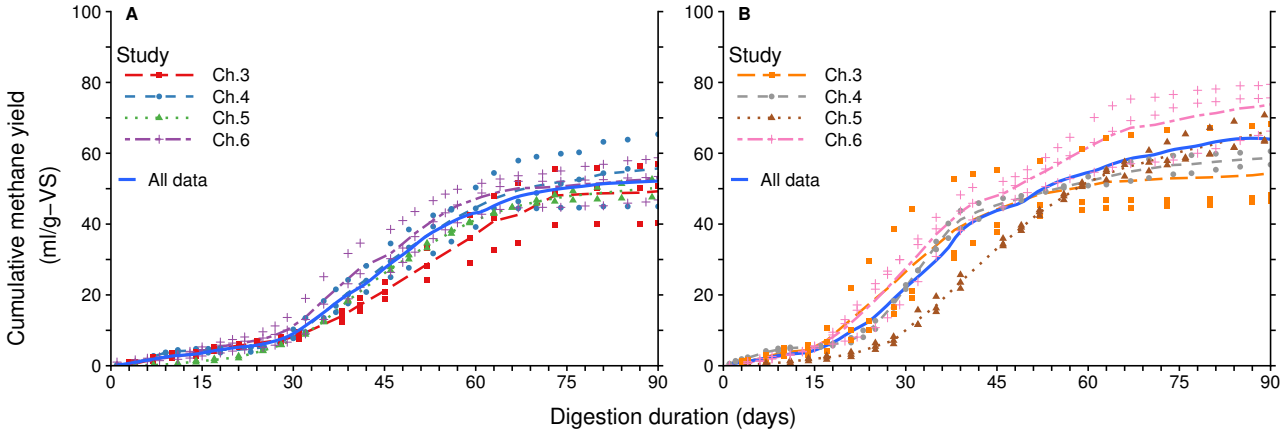

Figure 7.1: The cumulative methane yield from experiments in Chapters (Ch.) 3, 4, 5 and 6. The methane yield is normalised based on the initial volatile solids (VS) content of poultry litter and inoculant. Digesters are operated at 20% total solids (TS) and use a feedstock/inoculant ratio of 2. Data are shown for (A) digesters without biochar and (B) digesters with a biochar dosage of 1 $g_{TS\text{-}char}$/1 $g_{TS\text{-}feed}$. The solid line shows the mean from all four studies. The dashed lines show the mean from each individual study, markers show the range of values from all replicates.

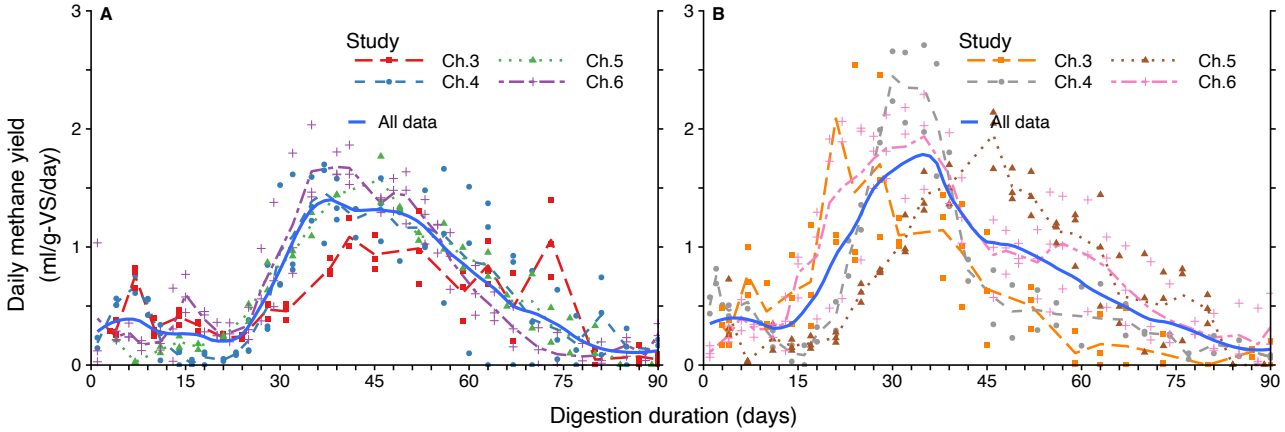

Figure 7.2: The daily methane yield from experiments in Chapters (Ch.) 3, 4, 5 and 6, normalised based on the initial volatile solids (VS) content of poultry litter and inoculant. Digesters are operated at 20% total solids (TS) and use a feedstock/inoculant ratio of 2. Data are shown for (A) the digesters without biochar and (B) digesters with a biochar dosage of 1 $g_{TS\text{-}char}$/1 $g_{TS\text{-}feed}$. The solid blue line shows the mean from all four studies. The dashed lines show the mean from each individual study, markers show the range of values from all replicates.

Figure 7.2 shows the mean lag time before methane production commences is approximately 22 days in the control digesters. The lag time is reduced by approximately 35% to 14 days in digesters with biochar. There is variability in the lag times between each study. In the control digesters, the lag time calculated in each study ranged from 20–25 days. In digesters with biochar, the calculated lag time was 13–14 days in the results from Chapters 3, 4 and 6 but was 25 days in the results from Chapter 5. As a result, there are also variations in the day at which the peak daily yield occurs. By contrast, there was a only a small variation in the value of the peak daily yield. In the controls, the mean peak daily methane yield is 1.5 ± 0.3 ml/g-VS/day and occurs between days 37–46 in all four studies. In digesters with biochar, the peak daily yield is 2.1 ± 0.2 ml/g-VS/day. The peak daily yield occurs between days 21–35 in the results from Chapters 3, 4 and 6 but at day 46 in the results from Chapter 5.

7.3.1 Possible Sources of Variability

The variability in the methane yields and the lag time may be caused by the changes to the activity or diversity of the microbial community in the inoculant. In digesters with biochar, the level of contact between biochar and the microorganisms may also cause variability.

To maintain a consistent level of microbial activity in the inoculant, a fresh batch of anaerobic digester effluent was collected prior to the commencement of each assay. Despite this effort to maintain consistency, changes to the microbial population in full-scale anaerobic digesters occur over time (De Vrieze et al., 2016). As the inoculant was collected at different times over two years, changes to the microbial population is expected. However, there was no change to the dominant methane-generating microorganism (methanogen) in the inoculant. It is possible that the diversity of the bacterial population or the activity of the microorganisms may have changed. For example, the activity of propionate oxidising bacteria decreases due to storage of anaerobic digester effluent for six weeks (Gallert and Winter, 2008). The variations to the activity of propionate oxidising bacteria may be significant. The propionate concentrations after 90 days can vary substantially between biological replicate digesters as shown in Chapter 5 and Chapter 6.

Maintaining a consistent level of microbial activity of the inoculant may reduce variability between assays conducted at different times. One option is to keep storage times of inoculant to a minimum. In the results presented across Chapters 3–6 the storage time was less than three weeks for all studies. However, the storage time varied due to limited access to the wastewater treatment plant. A storage time of less than one week would be expected to reduce variability due to changes to the activity of key microorganisms and is recommended for future work.

The same batch of chicken litter, stored at 4°C, was used in all four studies. The same batch was used because the composition of chicken litter collected from the same farm varied between each growing cycle. For example, chicken litter collected at a later date had a higher proportion of litter to faeces and smaller size wood shavings. Due to this variation, this second batch of chicken litter was discarded. As a result of the long-term storage, the total solids content of the chicken litter decreased from 60% TS in the earliest study (Chapter 4) to 42% TS in the latest study (Chapter 6). However, there were only minor changes to key parameters. The total ammonia-nitrogen (TAN) content increased by 4% and the VS/TS ratio decreased from 80% to 75%. This suggested the chicken litter did not degrade significantly over the storage period. As the inoculant was the main source of microorganisms an active microbial population in the chicken litter was not required.

To evenly distribute the biochar throughout the anaerobic digester, the biochar was thoroughly mixed with the chicken litter and inoculant at the beginning of the assay. However, due to the viscosity of the bulk sludge, the biochar particles did not continue to move through the bulk sludge when the digesters were mixed by inversion once per day. This may have led to a variable level of contact between microorganisms and the biochar in the early stages of the digestion period. This could explain the substantially longer lag time for the set of three

digesters in Chapter 5.

To account for the heterogeneity of biochar distribution in the bulk sludge, smaller biochar particles than the 10–20 mm in length and 4–6 mm in diameter pellets could be used. Also, an increased number of replicates may be used. Six replicates, as opposed to three used in these studies, are possible but will limit the number of scenarios that can be investigated in one experimental campaign. Using less than three replicates would not be recommended for high-solids anaerobic digesters.

7.3.2 Recommendations

The heterogenous nature of the bulk sludge, activity or diversity of the microbial community in the inoculant and distribution of biochar all can contribute to variability. To reduce variability the inoculant storage time should be minimised. In addition, an increased number of replicates (> 3) should be considered. In digesters with biochar, smaller particle sizes may cause a more even distribution of biochar in the bulk sludge and reduced variability. This may be the subject of future work.

Chapter 8

Conclusions

Improved performance of high-solids anaerobic digesters processing chicken litter, a widely available yet underutilised feedstock, can result in a greater availability of biogas worldwide. To achieve this aim, biochar, a by-product from a top-lit updraft gasifier (TLUD) was investigated as an additive to anaerobic digesters. The use of a by-product from a TLUD in an anaerobic digester is a low-cost and appropriate method for improved anaerobic digester performance.

This project developed an understanding on the use of wood-pellet biochar in ammonia-stressed anaerobic digesters with varying total solids (TS) content. In high-solids anaerobic digesters, an understanding on the effect of wood-pellet biochar as a method to increase the feedstock to inoculant (F/I) ratios was developed. Also, an understanding of the effects of wood-pellet biochar dosages and the recovery and re-use of biochar was revealed. In addition, the effects of biochar produced from manure and crop wastes on high-solids anaerobic digesters was gained.

8.1 Effect of Biochar Addition with Varying Process Conditions

The majority of previous research has focused on the use of biochar in anaerobic digesters operating at a total solids content of $\leq$10%. In Chapter 3, an investigation on the effect of wood-pellet biochar in digesters operating at 5%, 10% and 20% total solids was conducted. The study found biochar reduces the lag time before methane production to a greater degree in digesters with a higher total solids content. In addition, in digesters operating at 10% and 20% total solids, the use of biochar causes an increase in the peak daily methane yield compared with the controls. The peak daily methane yield also occurs earlier in digesters at 10% and 20% total solids compared with the controls. By contrast, no changes to peak daily methane yield occurs in digesters operating at 5% total solids due to the addition of biochar.

The assessment of the population of methane-generating microorganisms in Chapter 3 shows

the increases to the total solids content of a digester does not lower attachment of microorganisms onto the biochar. A lower degree of attachment was expected due to higher concentrations of ammonia and lower mass transfer rates in high-solids conditions. The greater ammonia concentration in the bulk sludge with increasing total solids content may lead to preferential attachment of the ammonia-sensitive methanogens, *Methanosaetaceae*, on the biochar. The methanogens may exist in a biofilm. Microorganisms attached to the surface of the biochar within a biofilm were observed using scanning electron microscopy.

In high-solids anaerobic digesters (20% TS) a suitable F/I ratio is needed to ensure the rapid startup of methane generation. In Chapter 4, the addition of wood-pellet biochar in digesters using three volatile solids (VS)-based F/I ratios (F/I = 0.5, 1 and 2) was investigated. The addition of biochar results in a consistent percentage reduction in lag time compared with the controls across the three F/I ratios. Using biochar also prevents a reduction in peak methane yield that occurs when increasing the F/I ratio in digesters without biochar. As a result, the use of biochar allows for higher F/I ratios to be viable in high-solids anaerobic digesters. For practical applications, this knowledge will allow for an increased throughput of chicken litter in each batch and a lower inoculant consumption rate.

The results in Chapter 4 shows the use of a higher F/I ratio does not lower the degree of the dominant methanogen, *Methanosaetaceae* on the biochar. A lower degree of attachment was expected due to the lower initial microbial concentration and higher ammonia stress. However, using low F/I ratios results in a greater diversity of methanogens in the bulk sludge. This is likely caused by a greater initial population of methanogens and a lower initial ammonia content. The results also show the alkalinity of biochar is not a key factor leading to improved performance in ammonia-stressed high-solids anaerobic digesters. The total alkalinity of the bulk sludge is determined by the ammonia concentration in the bulk sludge. The selective attachment of *Methanosaetaceae* to the biochar with varying F/I ratios and total solids content suggests improved anaerobic digester performance occurs through interactions between *Methanosaetaceae* and the biochar.

8.2 Effect of Varying Biochar Characteristics

The results shown in Chapters 3–6 show the addition of biochar produced from wood-pellets in a TLUD gasifier consistently improves high-solids anaerobic digester performance. In Chapter 5 the effects of wood-pellet biochar on high-solids anaerobic digesters was compared with the addition of biochar produced from wheat straw and sheep manure. The graphitic carbon structure of wood-pellet biochar and its low ash content result in performance improvements to anaerobic digesters. The graphitic structure may allow for *Methanosaetaceae*, the dominant methanogen attached to the biochar after 90 days in the digesters, to use the direct interspecies transfer (DIET) mechanism for greater degradation of propionate as observed in Chapter 5 and Chapter 6.

The low ash content of wood-pellet biochar eliminates the introduction of inhibitory elements

such as sodium, sulphur and potassium into the bulk sludge. These elements were present in higher concentrations in biochar produced from wheat straw and sheep manure. The addition of wheat straw biochar and sheep manure biochar to high-solids anaerobic digesters caused longer lag times compared with digesters without biochar. In addition, the high bulk density of wood-pellet biochar allows for a smaller percentage increase in the digester working volume compared with the addition of wheat straw biochar, which has a low bulk density.

Chapter 6 shows the biochar dosage is a key parameter which determines its effectiveness in high-solids anaerobic digesters. The effect of wood-pellet biochar addition at 0.25, 0.5 and 1 $g_{TS\text{-}char}/g_{TS\text{-}feed}$, which is equivalent to 18, 34 and 59 $g_{TS\text{-}char}$/l, respectively, was investigated. Previous research of biochar addition to high-solids anaerobic digesters have used biochar dosages of up to 30 g/l. A wood-pellet biochar dosage of 1 $g_{TS\text{-}char}/g_{TS\text{-}feed}$ was required for increases to the 90-day methane yield compared with the controls to be observed. The percentage reduction in lag time was also greatest with the largest biochar dosage.

Higher dosages of wood-pellet biochar increase the working volume of the digester. As a result, a decreased volumetric efficiency (m^3-methane/m^3-digester) compared with digesters without biochar occurs if the digesters are operated until no further methane production occurs. However, there is a crossover time point before which digesters with biochar have a superior volumetric efficiency to the control digesters. Using a shorter retention time allows for more batches to be processed per year and a greater throughput of chicken litter.

Chapter 5 and Chapter 6 show the effect of re-used wood-pellet biochar compared with pristine wood-pellet biochar on high-solids anaerobic digester performance. Chapter 5 shows wood-pellet biochar recovered from a high-solids anaerobic digester (20% TS) after 90 days can significantly increase methane yields from high-solids anaerobic digesters compared with digesters amended with pristine biochar. The beneficial effects of re-used of biochar are likely caused by microorganisms in proximity and within biofilms on the biochar resulting in syntrophic degradation of propionate an isovalerate.

The re-use of biochar is a promising method to reduce biochar consumption, however, its beneficial effects to the cumulative methane yield can be variable. The beneficial effects also vary depending on the duration that the biochar has spent in an anaerobic digester. As opposed the beneficial effects of re-using biochar taken from a high-solids digesters after 90 days the addition of biochar re-used after 30 days in a low-solids anaerobic digester did not result in performance, as shown in Chapter 5. Furthermore, in contrast to the improvements to anaerobic digester performance due to the addition of re-used biochar from a high-solids digester shown in Chapter 5, the results in Chapter 6 did not show any benefit to the methane yield, relative to the addition of pristine biochar. However, the reduction in the isobutyrate concentration (Chapter 6) suggested re-used biochar can still enhance syntrophic degradation of volatile fatty acids even when methane yields are not substantially increased.

8.3 Future Work

The work described in this thesis adds important insights into the performance of high-solids anaerobic digesters with changes to process conditions and biochar characteristics. There is still further work required before this promising process variation can be applied with assurances of performance improvements.

A greater understanding of the mechanisms resulting in increased biochar performance is required. The preferential attachment of *Methanosaetaceae* on the biochar and the increased volatile fatty acid degradation allows for the possibility that *Methanosaetaceae* attached to wood-pellet biochar is using the DIET mechanism. However, additional information is needed to confirm this hypothesis. Firstly, a determination of the population of the electron-donating bacteria on the biochar is needed. In addition, a determination of the methanogenic pathway, either aceticlastic or hydrogenotrophic, used by the attached methanogens is needed. Furthermore, the significance of biofilm formation which could affect both the electron-donating capacity and the interspecies distance of microorganisms is required. These investigations could determine if DIET or increased rates of hydrogen/formate transfer is the key mechanism allowing for improved performance.

A deeper understanding of the key properties of wood-pellet biochar that leads to improved digester performance is also required. Investigating the effect of the pyrolysis temperature and hence the graphitic structure of the biochar in an auto-thermal TLUD gasifier could provide useful insights. In a TLUD, pyrolysis temperature can be varied by altering the primary air input. A TLUD is suitable for biochar production in resource-constrained communities, however, variations to pyrolysis temperatures may be more controllable in biochar producing reactors that are not auto-thermal. One such reactor is a tube furnace. A comparison of the properties of biochar from tube furnaces to biochar from a TLUD gasifier and the effects on anaerobic digester performance will also be useful.

The variable effect of re-used wood pellet biochar on the methane yield and propionate degradation, shown in Chapter 5 and Chapter 6, indicates further development of this method is required. It is possible the variable effects are due to ammonia-stress or the large biochar particle size. The use of smaller particles of re-used biochar and pre-loading the biochar in a digester using lower ammonia concentrations should be investigated.

To develop an understanding of the mechanisms allowing for increased methane yields with re-used biochar, the population of propionate oxidising bacteria on the re-used biochar should be quantified. Furthermore, a comparison of the effects of adding re-used biochar compared with an equivalent proportion of digestate could provide a deeper understanding on the effects of re-used biochar.

The abundance of crop wastes and manures warrant further investigations of these materials as parent materials for biochar production. Continuous use of wood-based biochar, if not re-used, could lead to deforestation. Inhibition of methane generation may be avoided by using lower

dosages of wheat straw and sheep manure biochar. Alternatively, biochar produced from wheat straw or manure may be useful to inhibit methane generation from ruminants or rice paddies soils. Furthermore, inhibition of methane production resulting in high concentrations of VFAs may provide an alternative revenue stream for users. For example, Chapter 5 shows hexanoate, which is a valuable chemical compound (Liu et al., 2017), is produced in the inhibited digesters due to the addition of sheep manure biochar.

Applied research of biochar in high-solids anaerobic digesters should focus on using feedstocks producing greater methane yields. The methane yields from high-solids anaerobic digesters amended with pristine biochar ($\approx$ 65 ml/g-VS) or re-used biochar (up to 84 ml/g-VS), compared with yields from low-solids anaerobic digesters ($\approx$ 100 ml/g-VS), suggests the use of biochar alone does not make chicken litter a viable feedstock. Improvements to the methane yield per gram of volatile solids can be gained by sourcing chicken litter from a farm using a bedding material with a lower lignin content than wood-shavings. This would improve the anaerobic degradability and hence the methane yield. Alternatively, chicken litter may be mixed (co-digested) with a low-nitrogen feedstock such as wheat straw. The co-digestion of chicken litter coupled with the addition of biochar may make chicken litter a viable feedstock for biogas production from high-solids digesters in resource-constrained communities.

The improvements to performance suggest the use of biochar in larger systems that incorporate a scalable digester design is warranted. A leach-bed anaerobic digester is a scalable digester design that could incorporate biochar. In leach-bed digesters, biochar can be added into the leachate tank or in the bulk sludge. Using biochar in the leachate storage tank is an appropriate method of retaining the biochar over multiple batches. This may lead to greater volatile fatty acid degradation and methane yields as observed in Chapter 5. As an alternative, biochar can be added into the bulk sludge of a leach-bed digester. The biochar could be completely mixed with the bulk sludge only partially mixed. Partial mixing could involve a bed of biochar at the bottom of the digester which remains in the reactor over multiple batches.

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Appendix A

Biochar Production and Characterisation — A Field Study

2017 IEEE Global Humanitarian Technology Conference (GHTC), San Jose, CA, USA, 19–22 Oct 2017

Appendix B

The Effect of Biochar Addition on Biogas Production from Poultry Litter

2018 IEEE Global Humanitarian Technology Conference (GHTC), San Jose, CA, USA, 18–21 Oct 2018