The Effect of pH Control on Ammonium Release in Anaerobic Digestion

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Abstract

Anaerobic digestion is a process whereby microorganisms break down waste material into simpler compounds, while simultaneously producing biogas that is generally high in methane and carbon dioxide content (60 –70 % and 30 –40 %, respectively) and a nutrient-rich by-product called the digestate. The energy potential of this process cannot be overstated; it is estimated that a feed of one ton of biowaste generally translates to an electricity yield of 250 kWh. However, the digestate is a typically overlooked by-product in anaerobic digestion studies due to a fixation on the energy-dense biogas product. The digestate can be in either liquid or solid form, this study focused on the liquid digestate. The liquid digestate is generally high in valuable nutrients like nitrogen, potassium, and phosphorus which are essential for plant growth. This indicates that the liquid digestate can be an effective fertiliser. Currently, farmers habitually apply digestate on farmlands because of its high macronutrient concentration. However, an underexplored avenue is the use of liquid digestate as an organic fertiliser for soilless agriculture. Although soilless agriculture provides a multitude of advantages over conventional agriculture, such as providing better nutrient distribution while also saving on both land and water, it is still dependant on harmful mineral fertilisers. Given the major growth occurring in the soilless agriculture sector and the need for more sustainable fertilisation strategies in food production processes, liquid digestate has been promoted to a more prominent stream in the circular production of human nutrition. In this regard, it is important to understand the rate and extent of fertiliser production in anaerobic digestion process.

In this study, the pH of the anaerobic digestion process was controlled at three different set points (6, 7, and 8) for three different substrates (banana peels, cow dung, and red lentils) in order to determine the ammonium release characteristics at each set point. This was achieved by using two different set-ups; One system employed a daily pH adjustment (Daily dosing system or DDS) while the other system (Continuous Dosing Set-up or CDS) employed pH corrections every minute. The results indicated that a pH of 7 is the optimal set point for both ammonium release as well as the gas production rate. This pH value provided average percentage differences of 20 % and 22 % in terms of ammonium release and gas production when compared to the runs that were performed without pH control. In terms of a comparative analysis between precise pH control, that was performed every minute and pH control that was performed once a day, there were differences present

in the gas production profiles, with the CDS providing enhanced rates compared to the DDS. The CDS provided an average percentage increase of 50 % compared to the DDS in terms of gas production. However, there was a negligible difference in the ammonium release rate.

Keywords: Anaerobic digestion; pH; liquid digestate; nitrogen; ammonium

Publication

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"*If you win without sacrifice, you enjoy it, but it's more satisfying when you have struggled.*"
- Andrés Iniesta

Contents

List of Figures

List of Tables

1 Introduction

Anaerobic digestion is a process whereby microorganisms break down waste material into simpler compounds, while simultaneously producing biogas that is typically high in methane and carbon dioxide content (60–70 % methane and 30–40 % carbon dioxide) with trace amounts of hydrogen sulphide along with some water vapour (Mir, Hussain & Verma, 2016). Methane, in particular, is a highly desirable product because it is an energy dense fuel that can be easily stored (McDonald, 1990). The energy potential of this process cannot be overstated; the Environmental and Energy Study Institute estimates that a feed of one ton of biowaste generally translates to an electricity yield of 250 kWh (Velivela *et al*, 2020). This process has been exploited successfully by countries throughout the European Union in recent years. Countries such as Germany, Spain, and The Netherlands have anaerobic digestion plants that convert organic waste (primarily food waste, green waste, and agricultural waste) into biogas. As recently as 2020, the European Biogas Association reported that there were approximately 20 000 biogas and biomethane units in operation (a striking increase from roughly 10 000 units in 2010), this amounted to 191 TWh of total energy production, which, in turn, accounted for around 4.6 % of the European Union's gas consumption. However, this sector is expected to increase fivefold by 2050, with estimates suggesting that the energy production from biogas plants could be as high as 1000 TWh, which would account for around 30–40 % of the EU's total gas consumption (Akhiar, Zamri, *et al*, 2020; Association *et al*, 2020).

However, a typically overlooked by-product of the anaerobic digestion process is the digestate. The digestate is the material that remains after the anaerobic digestion process, it is generally high in valuable nutrients such as nitrogen, potassium and phosphorus, all of which are considered essential for plant growth (Ajala *et al*, 2022; McGrath, Spargo & Penn, 2014). This indicates that digestate has the potential to be an extremely effective fertiliser. The digestate is comprised of two phases, namely, solid and liquid. Solid digestate is habitually used by farmers as livestock bedding or composted with minimal processing (Tamošiūnas, Khiari & Jeguirim, 2022). Liquid digestate, on the other hand, has seen a vast increase in usage by farmers as a fertiliser that can be applied on farmlands because of its high macronutrient concentration. The nitrogen that is available in the liquid digestate is generally in the form of either ammonium or ammonia depending on the pH of the solution (Makádi, Tomócsik & Orosz, 2012). Anaerobic digestion may produce between 1.5–6.5 g/L of nitrogen in the liquid digestate, with around 60–80 % of that nitrogen typically being ammonium (Makádi *et al*, 2012; Akhiar, Battimelli, *et al*, 2017; Loria *et al*, 2007), however, the ammonium content in the digestate generally depends on the type of feedstock that is used, with protein-rich feedstock customarily providing higher ammonium content in the digestate. This indicates that liquid digestate

could be suitable as a fertiliser for soilless agriculture (Makádi *et al*, 2012).

Soilless agriculture is a possible solution to the myriad of problems that are currently plaguing the agricultural sector. Not only does this approach save on both land and water; but it also has the added advantages of having better control over the nutrients and water that are delivered to the plants, making it easier to grow healthy plants consistently (K El-Kazzaz & A El-Kazzaz, 2017; Sengupta & Banerjee, 2012). However, similarly to conventional farming, soilless agriculture remains largely dependent upon harmful mineral fertilisers, although admittedly in smaller quantities (Tsukagoshi & Shinohara, 2020). There has been surprisingly limited research done on the use of liquid digestate in soilless agriculture; this is, in large part, due to the fact that organic fertilisers tend to be poisonous to plants (Mupambwa *et al*, 2019). However, there has been a recent surge in the number of researchers who are interested in liquid digestate as fertiliser, with varying degrees of success. Some researchers argue that the use of digestate in hydroponics leads to poor plant growth, this poor growth is typically attributed to low concentrations of plant-available macronutrients such as phosphorus and sulphur in the liquid digestate, as well as ammonia phytotoxicity (Mupambwa *et al*, 2019; Neal & Wilkie, 2014; Asp *et al*, 2022), whereas other researchers argue that the use of digestate in hydroponic systems has a beneficial effect on plant growth (Bergstrand, Asp & Hultberg, 2020; Ronga *et al*, 2019; Stoknes *et al*, 2016; Pelayo Lind *et al*, 2021). However, it should be noted that an auxiliary step was utilised in most of the cases that reported beneficial plant growth, this step typically involved converting the ammonium from the digestate to nitrates before the fertiliser was introduced to the hydroponic unit. This is because plants can absorb nitrogen as either ammonium or nitrate, however, the total uptake of nitrogen usually consists as combination of the two. Although plants may be able to utilise ammonium for growth, they typically prefer a higher concentration of nitrates than ammonium in standard nutrient solutions (Tabatabaei, Fatemi & Fallahi, 2006).

In this study the emphasis is placed on the production of liquid digestate in anaerobic digestion. Conventionally, the liquid digestate was only seen as a minor by-product from the anaerobic digestion process and little emphasis was placed on the mineralisation rates within the digester. Given the major growth occurring in the soilless agriculture sector and the need for more sustainable fertilisation strategies in these food production processes, liquid digestate has been promoted to a more prominent stream in the circular production of human nutrition. In this regard it is important to understand the rate and extent of fertiliser production in anaerobic digestion process. This will aid in the design of novel digester processes, where liquid digestate is selectively removed in order to counter ammonia inhibition while simultaneously optimising fertiliser production. Accordingly, this study scrutinises the ammonia production rates in batch digestation under different pH control strategies in order to gain more insight on the time-dependant mineralisation

characteristics of the process.

2 Literature

Anaerobic digestion is a natural, chronological process in which organic materials are broken down into simpler compounds in the absence of oxygen. The reactor in which this process occurs is called an anaerobic digester (Cioabla *et al*, 2012). There are several factors that affect the overall performance of an anaerobic digester; namely, the pre-treatment of the biomass, biomass concentration, moisture content in the feedstock, the carbon to nitrogen (C/N) ratio, the inoculum, temperature, pH, and particle size (Lohani & Havukainen, 2018).

2.1 Process chemistry

Before discussing the different conditions necessary for successful anaerobic digestion, it is important to understand the processes that make up anaerobic digestion. This process is widely considered as having four sequential steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Chen & Neibling, 2014). However, some scholars syncopate the acidogenesis and acetogenesis steps. This is because it can be difficult to show the discordance between these steps due to both steps typically producing H_2 and CH_3COO^- (Zhang *et al*, 2017; Anukam *et al*, 2019).

The first step, hydrolysis, is typified by a decomposition of complex organic polymers (proteins, fats, and polysaccharides) into simpler, soluble polymers like peptides, saccharides, and fatty acids. The breakdown of these complex polymers is facilitated by hydrolytic microorganisms like amylase, cellulase, protease, and lipase (Mir *et al*, 2016). Parawira (2004) suggests that proteins are reduced to amino acids and small peptides, carbohydrate sources are converted into a variety of monosaccharides including glucose, xylose and galactose, and lipids are decomposed into long—chain fatty acids. This is a relatively slow step and is often cited as the rate limiting step in anaerobic digestion because the breakdown of proteins and fats by hydrolytic microorganisms can take several days (Mustafa, Calay & Román, 2016).

The second step, acidogenesis, is the step in which acidogenic bacteria further degrade and convert the soluble compounds that were produced during the hydrolysis step to simpler molecules like hydrogen, carbon dioxide, ammonia, and short-chain organic acids such as butyric and propanoic acid (Mustafa *et al*, 2016). These acids are commonly referred to as volatile fatty acids (VFAs). Given that ammonia is one of the focal points of this study, understanding how it might be produced is of great importance. Sangavai & Chellapandi (2019) postulate that the production of ammonia in anaerobic digestion

is primarily due to the digestion of amino acids to organic acids using the Stickland reaction in which one amino acid is used to reduce another, releasing ammonia in the process. This ammonia may then take up a hydrogen ion from the solution to become an ammonium ion, thereby acting as a base and increasing pH. However, it should be noted that the pre-eminent products from this step are the VFAs that typically lower the pH.

In the acetogenesis step, the products from acidogenesis are further broken down to acetic acid, organic acids, and acetate. This stage is also referred to as the dehydrogenation stage because hydrogen gas is usually formed as a waste product (Mir *et al*, 2016; Mustafa *et al*, 2016). This step can also be used as a test for the efficiency of biogas production in the process, this is because it is estimated that up to 70 % of the total methane formed is from the reduction of CH_3COO^- (Anukam *et al*, 2019).

In methanogenesis, the final step, archea known as methanogens convert the acetate, and other products that were produced in the acetogenesis stage to methane, carbon dioxide, and water (Mustafa *et al*, 2016). This process may follow two different pathways, using either acetate or hydrogen and carbon dioxide as the primary reactants. However, the aceticlastic pathway (acetate as the primary reactant) is typically favoured (Anukam *et al*, 2019). In this final step, methane-rich biogas is formed. The composition of the biogas varies depending on the feedstock but the general consensus is that it is approximately 60 % methane and 40 % carbon dioxide with trace amounts of hydrogen sulphide and hydrogen (Abdel-Hadi, 2008; Mathew *et al*, 2015; Fardmanesh, Pourdarbani & Najafi, 2020). These impurities hamper efforts to commercialise biogas because they contribute towards reducing the energy density of the gas. There have been numerous experiments performed with the aim of purifying the biogas from anaerobic digestion; a popular method is incorporating steel wool, water and silica gel. The steel wool reacts with hydrogen sulphide, the water is used to absorb the carbon dioxide, and the silica gel is used to reduce the presence of water vapour in the biogas. This increases the methane concentration from approximately 68 % to 90 % (Nallamothu, Teferra & Rao, 2013). Figure 1 gives an illustration of the steps discussed in this section.

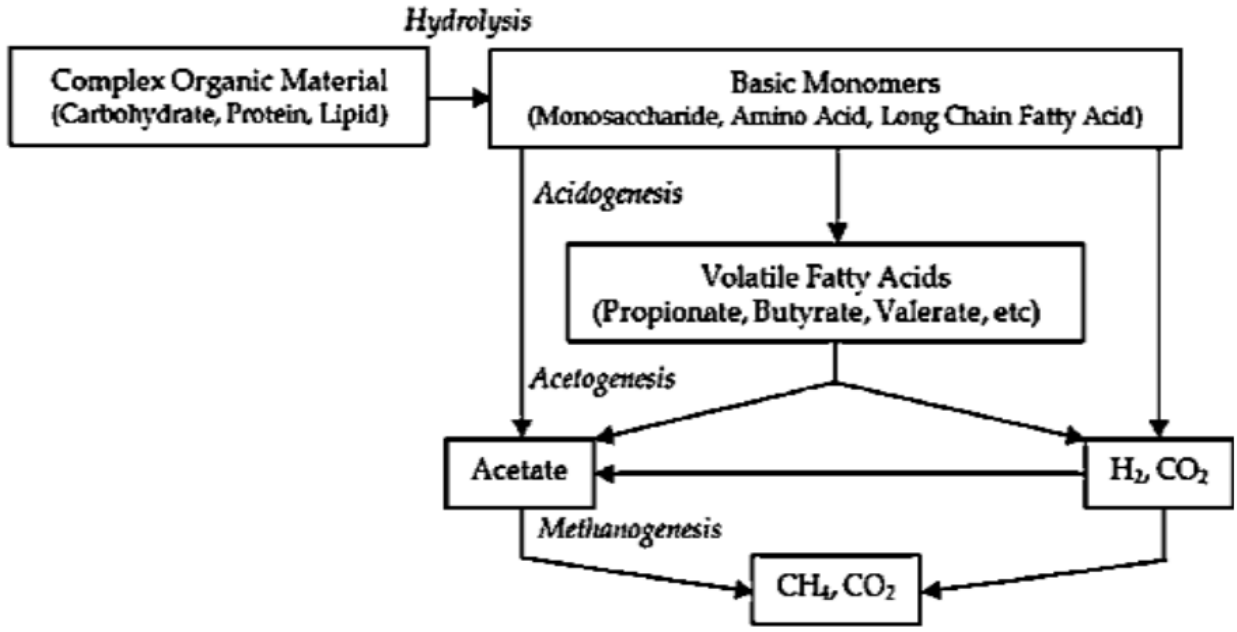


Figure 1: Steps of the anaerobic digestion process (Suleiman, Kevin & Ajayi, 2018).

Although the reaction steps in anaerobic digestion are viewed as sequential steps, it is important to realise that simultaneous occurrence of these steps happen in both batch and continuous anaerobic digesters. For batch systems certain time ranges may be associated with the 4 reaction steps although significant overlap occurs between the steps.

2.2 Pre-treatment

There are various, well-documented pre-treatment techniques that are often employed to ameliorate the efficiency of the anaerobic digestion process. These techniques are predominantly mechanical, chemical, thermal, and biological methods that typically enhance one of the four steps discussed in Section 2.1 (Ariunbaatar *et al*, 2014).

Mechanical pre-treatments fracture solid particles of the substrate. This, in turn, increases the surface area of the substrate, allowing for improved interaction between the substrate and anaerobic bacteria (Carrère *et al*, 2010). The adverse effects of a large particle radius are discussed in greater detail in Section 2.8. There are numerous mechanical pre-treatments that have been studied, such as sonication, liquid shear, maceration, and high-pressure homogenisation (Ariunbaatar *et al*, 2014).

Maceration, sonication, and high-pressure homogenisers are widely regarded as the simplest forms of mechanical pre-treatment. Sonication is characterised by a vibrating probe that mechanically disrupts the cell structure and floc matrix (Elliott & Mahmood, 2007).

A study performed by Carrère *et al* (2010) found that sonication resulted in an enhancement in the production of biogas by 24 – 140 % in batch systems, and a 10 – 45 % enhancement in continuous systems. However, it should be noted that not all studies concur with these findings, Sandino *et al* (2005) found the effect of sonication to be negligible in terms of biogas production. A high-pressure homogeniser (HPH) is characterised by increasing the pressure of the substrates in the reactor by several hundred bars, and then homogenising the substrates under strong depressurisation. The cavitation induces internal energy, which disrupts the cell membranes (Mata-Alvarez, Macé & Llabres, 2000). Engelhart *et al* (2000) found that employing this method resulted in a 30 % biogas enhancement, which could reduce the working volume of digesters by up to 23 %. Maceraction involves soaking the substrate in a liquid to reduce the particle size. Angelidaki & Ahring (2000) found that this pre-treatment method increased the biogas production by up to 20 %. The main advantages of mechanical pre-treatments are that they are inexpensive, reduce odour generation, are easy to implement, and provide better dewaterability of the final anaerobic residue (Pérez-Elvira, Diez & Fdz-Polanco, 2006; Toreci, Kennedy & Droste, 2009).

Thermal pre-treatment is another popular method that has been researched thoroughly. This technique often leads to pathogen removal, improving the dewatering performance of the final anaerobic residue, and it reduces the viscosity of the digestate, which often leads to less complicated digestate handling (Carlsson, Lagerkvist & Morgan-Sagastume, 2012; X Liu *et al*, 2012). Thermal pre-treatment, like mechanical pre-treatment, aims to disintegrate the cell membranes, which results in better solubility of organic compounds (Ferrer *et al*, 2008). There are two common practices that are followed in terms of thermal pre-treatment; thermal pre-treatment at low temperatures (temperatures lower than 110 °C), and thermal pre-treatment at high temperatures (temperatures larger than 110 °C) (Ariunbaatar *et al*, 2014).

Studies performed by Barjenbruch & Kopplow (2003) and Prorot *et al* (2011) both suggest that pre-treatment at temperatures below 100 °C does not truly degrade complex molecules, they simply aid in the deflocculation of macromolecules. However, this is not to say that low temperature pre-treatment is nugatory, Skiadas *et al* (2005) found that pre-treatment at temperatures as low as 70 °C have a definitive effect on pathogen removal; whereas Appels *et al* (2010) found that biogas production increased by as much as 20 times after applying low temperature techniques for an hour (90 °C).

High temperature pre-treatment has also been an area of great interest to numerous scholars. These studies are usually characterised by temperatures well above 110 °C. X Liu *et al* (2012) found that the thermal pre-treatment of food waste at a temperature of 175 °C led to an 11.7 % decrease in biomethane production. This is largely due to the

melanoidins that form from reducing sugars, proteins, and amino acids. Ma *et al* (2011) found more success by using a temperature of 120 °C, this increased the biomethane production by 24 %.

Chemical pre-treatment is used to break down organic compounds through the use of strong acids, bases or oxidants (Ariunbaatar *et al*, 2014). Since the anaerobic digestion process naturally tends towards a decrease in pH, alkali pre-treatment methods are generally preferred in industrial applications (H Li *et al*, 2012). With alkali pre-treatment, the early reactions involved include solvation and saponication, which swell the solids, this increases the surface area available to anaerobic microbes. However, it should be noted that biomass might consume some of the alkali, which means that higher alkali reagents may be required for the desired enhancement (Carlsson *et al*, 2012; Hendriks & Zeeman, 2009; Modenbach & Nokes, 2012; Torres & Lloréns, 2008). Although alkali pre-treatment is the most commonly applied chemical pre-treatment process, acid pre-treatment methods are useful for certain feedstocks. This method is more advantageous for lignocellulosic substrates because it decomposes the lignin (which is viewed as being a typically complex biomass structure), whilst simultaneously enhancing hydrolytic microbes since they tend to thrive in acidic conditions (Mussoline *et al*, 2013). However, one must exercise caution when using this method, strong acids may produce by-products that inhibit the anaerobic digestion process such as furfural and hydroxymethylfurfural (HMF) (Modenbach & Nokes, 2012). There are other disadvantages associated with this method that cannot be overlooked, such as the high costs of acids and the loss of fermentable sugars due the the break down of complex material (Taherzadeh & Karimi, 2008; D Kumar & Murthy, 2011).

Ozonation is a chemical pre-treatment method in which no chemical residue remains, meaning that this method does not increase the salt concentration in the digester. Moreover, this method provides additional advantages such as disinfecting pathogens; and it acts as a strong oxidant that decomposes into radicals, which then react with organic substrates by either direct or indirect reactions depending on the structure of the reactant, this leads to the compounds being more biodegradable and more accessible to the anaerobic microbes (Kameswari, Kalyanaraman & Thanasekaran, 2011; Carballa *et al*, 2007; Weemaes *et al*, 2000; Kianmehr, Parker & Seto, 2010).

Biological pre-treatment methods may include both aerobic or anaerobic methods, and generally includes the addition of enzymes such as peptidase, carbohydrolase, and lipase to the digester in order to facilitate the process (Ariunbaatar *et al*, 2014). Güelfo *et al* (2011) found that composting prior to anaerobic digestion resulted in much higher specific microbial growth rate than in untreated feedstocks. This was likely due to greater volatile fatty acid formation due to enhanced hydrolytic and acidogenic bacteria activity (Lim &

JY Wang, 2013). However, this ultimately leads to a volatile solids loss of 19.5 % and a decrease in methane production (Brummeler & Koster, 1990; Mshandete *et al*, 2005).

2.3 Effect of C/N ratio

The carbon to nitrogen ratio, commonly referred to as the C/N ratio, is often considered as the most important aspect for successful anaerobic digestion. The nitrogen promotes microbial growth, while the carbon is the energy source for the microbes (Mir *et al*, 2016). It is, therefore, unsurprising that scholars like Mir *et al* (2016) and Igoni *et al* (2008) suggest that the rate of carbon consumption is up to 35 times higher than that of nitrogen consumption. However, the most important aspect in terms of C/N ratios is that the carbon and nitrogen should be consumed in such a way that a nitrogen shortage does not occur, and excess nitrogen does not build up in the reactor. With this in mind, the optimal C/N ratio is often cited as 30:1 (Mir *et al*, 2016; Igoni *et al*, 2008). If the ratio is too small, this would lead to an excess in nitrogen, which would lead to the production and build up of nitrogen in the reactor. This build up could lead to an increase in pH that kills off the microbes in the bacteria.

The two principal forms of inorganic ammonia nitrogen in anaerobic digestion are ammonium nitrogen ($NH_4^+ - N$) and free ammonia nitrogen ($NH_3 - N$), the form depends on the pH and temperature of the solution (Yenigün & Demirel, 2013). At 25 °C, ammonia production is generally favoured at pH values larger than 9.25, whereas ammonium is preferred at pH values lower than 9.25 (Hillel & Hatfield, 2005). The build up of nitrogen in the system could cause a phenomenon called ammonia inhibition. Although both forms of inorganic ammonia nitrogen can inhibit the process, the free ammonia nitrogen (FAN) in particular is considered the more powerful inhibitor to methanogens above threshold concentrations (Jiang *et al*, 2019). This phenomenon is more likely to occur in complex substrates such as manure or with the organic fraction of municipal solid waste (OFMSW) (Yenigün & Demirel, 2013).

The threshold concentration for total ammonia nitrogen (TAN), *i.e.*, free ammonia nitrogen plus ammonium nitrogen, varies greatly in literature. Values typically range from 1500 – 7000 mg/L (Rajagopal, Massé & Singh, 2013). However, this is usually because most studies directly relate inhibition with TAN concentration instead of FAN concentrations, which is regarded as the main inhibitor of methanogenic activity. When one considers FAN concentrations only then the range is much more stringent, with values ranging from 700 – 2000 mg/L (Rajagopal *et al*, 2013). The main factors that affect FAN concentrations are the temperature and pH. A study performed by Kayhanian (1999) demonstrated that there is a sixfold increase in the FAN concentration at thermophilic

temperatures (50 – 60 °C) compared to mesophilic temperatures (30 –40 °C) at the same pH. A similar study performed by Fernandes *et al* (2012) showed that at a temperature of 35 °C and a pH of 7, the FAN concentration accounted for less that 1 % of the TAN, but at the same temperature and a pH of 8, the FAN concentration accounted for 10 % of the TAN.

Considering that one of the main aims of this study is to produce ammonium, it is important to understand how the C/N ratio of the feedstock may affect the final ammonium concentrations in the anaerobic digestion process. Table 1 summarises experiments that were performed by X Wang *et al* (2012) in which the total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) were tested for feedstocks with different C/N ratios.

Table 1: A summary of the total ammonia nitrogen (TAN), free ammonia nitrogen (FAN), and ammonium nitrogen ($NH_4^+ - N$) at different C/N ratios (X Wang *et al*, 2012).

C/N ratio	TAN (mg/L)	FAN (mg/L)	$NH_4^+ - N$ (mg/L)
15	2614	223	2391
20	1800	70	1730
25	712	9.1	702.9
30	604	7.5	596.5
35	444	2.2	441.8

With Table 1 in mind, it must be stressed that this study aims to provide a nitrogen-rich fertiliser for soilless agriculture. Taking this into account, one must compare current nutrient solutions in order to ascertain the feasibility of this study. One of the most popular artificial nutrient solutions is called Hoagland solution. The nitrogen content in Hoagland solution is 210 mg/L (Hoagland, Arnon, *et al*, 1950), which is well below the reported values in Table 1 meaning that the product from this study will likely have to be diluted in order to be used as an effective fertiliser.

Another factor to consider with the C/N ratio is how the biomass concentration affects anaerobic digestion performance. The biomass refers to the organic material that is fed into the reactor, this biomass typically acts as the substrate required by the microbes in the reactor. If the reactor is fed with a large biomass concentration that has a high carbon to nitrogen ratio, it may prematurely terminate the digestion process due to the pH being too low; whereas, high concentration biomass with a low carbon to nitrogen ratio may release large amounts of ammonia into the system, this could increase the pH and prematurely terminate the process as well. With this in mind, an optimal biomass

concentration is crucial for the performance of the anaerobic digester. Unfortunately, there is a lot of conjecture about this particular topic. However, most scholars seem to agree on a solids concentration of 5 to 10 g/L with the biomass suspended in water when dealing with liquid anaerobic digestion (Dohaei *et al*, 2020). Solid-state anaerobic digestion, on the other hand, typically occurs at solid concentrations that are higher than 15 % (Y Li, Park & Zhu, 2011). This is because this type of digestion is usually used to treat substrates that generally have a high solid content, such as lignocellulosic biomass and municipal solid waste (Zhou & Wen, 2019).

2.4 Inoculum

Two main inocula attract the most attention in literature: manure and anaerobic digester effluent. These are obvious choices because anaerobic digestion occurs naturally in the ruminant stomachs of cattle, the anaerobic digestion in theses livestock tend to be extremely efficient at producing methane. Up to 14.5 % of global greenhouse emissions are estimated to be due to livestock (PJ Gerber *et al*, 2013). Whereas, anaerobic digester effluent would already have all of the microbes required for anaerobic digestion.

There are various properties of animal manure that makes it extremely suitable as inocula for anaerobic digestion: It supplies nutrients for bacterial growth, acts as a pH buffer, contains a lot of bacteria that facilitate anaerobic digestion, and it may help in dissolving substrates with lower water content in co-digestion (Rojas *et al*, 2010). If the inoculum is not utilised correctly, it may become ineffective. Therefore, it is crucial to establish the optimum ratio of substrate to inoculum that will yield the most efficient digestion. Studies done by Latinwo & Agarry (2015) and Verma, Singh & Rai (2007) both suggest that a ratio of 1:1 on a concentration basis provides the best methane yield. Whereas O'Sullivan *et al* (2010) suggest using 1 mL of digestate from a previously successful digester for every 100 mL in a new digester.

2.5 Effect of temperature

Temperature affects the efficiency of anaerobic digestion to a great extent. There are two types of temperature-dependant organisms that can be used for anaerobic digestion, mesophilic and thermophilic. Thermophilic organisms typically thrive at temperatures in the range of 50 – 60 °C, whereas mesophilic organisms prefer relatively lower temperatures, in the range of 30 –40 °C (Ahring, 1994; Lohani & Havukainen, 2018).

If one only takes reaction kinetics into account, then a comparison between mesophilic and thermophilic organisms would be futile. This is because, normally, reaction rates increase exponentially with an increase in temperature, which would make the thermophilic system faster (Schoolfield, Sharpe & Magnuson, 1981). However, one cannot just consider reaction kinetics when discussing anaerobic digestion. Thermophilic systems pose more challenges and disadvantages compared to their mesophilic counterparts. Principally, thermophilic bacteria are significantly more sensitive to temperature fluctuations than mesophilic bacteria, this makes thermophilic systems less stable and more prone to failure unless a proper temperature control system is employed (Kiely, 2007). Additionally, thermophilic systems require additional energy input compared to mesophilic systems. These problems, coupled with the fact that thermophilic systems often produce low quality digestate in terms of nutrients, mean that mesophilic systems are preferred over thermophilic systems in industry (Igoni *et al*, 2008).

2.6 Effect of pH

The effect of pH on anaerobic digestion cannot be overstated. The reactions that were discussed in Section 2.1 are all dependant on different enzymes; these enzymes operate optimally in different pH ranges. Enzymes can be viewed as proteins that act as biological catalysts; and as such, physical factors such as temperature, concentration, and pH may affect enzyme activity. Extreme changes in the pH may change the shape of the enzyme; which, in turn, alter the active sites of the enzymes. This is referred to as denaturing. Different enzymes operate in different ranges of pH and will be denatured if the environment strays too far from the appropriate range (Lubert, John & Jeremy, 2015). This is an important consideration in anaerobic digester design for two reasons; the different processes described in Section 2.1 have different pH ranges and if the pH of the reactor drifts too far from an optimal point, one or more of the reactions will be inhibited and thus slow the whole process down.

The first two steps that were discussed in Section 2.1, hydrolysis and acidogenesis, have a lower optimal pH than the final two steps, acetogenesis and methanogenesis (Verma *et al*, 2007; Zhang *et al*, 2017). There is still some conjecture on what the actual pH values should be but the general consensus among scholars is that the optimal pH for hydrolysis and acidogenesis is in the range of 5.2 – 6.5, and the optimal pH range for acetogenesis and methanogenesis is 6.5 – 8.2 (Vermaak & Dobson, 2016; Zhang *et al*, 2017).

This poses a significant challenge when designing an anaerobic digester. Each of the four steps are crucial in the anaerobic digestion process but they have different optimal pH ranges. Nevertheless, researchers have found many different approaches that may be

able to deal with this conundrum. However, an anaerobic digester that is left to its own devices naturally varies in pH in any case. This phenomenon will be discussed first before considering ways in which the adverse effects that pH variance causes in the anaerobic digestion process can be mitigated.

To abate complexity, a batch anaerobic digester will be considered for this section. The first two steps of anaerobic digestion (hydrolysis and acidogenesis) produce volatile fatty acids (VFAs). This usually acts to decrease the pH of the digester shortly after a run begins (Igoni *et al*, 2008). If this decrease in pH is too drastic, it may lead to the termination of the digestion process (Anukam *et al*, 2019). However, in a successful run, the pH often stabilises due to the digestion of the volatile fatty acids by acetogens and methanogens, which triggers an increase in the pH. Whether the pH decrease is terminal or not is largely dependent on the type of substrate that is fed into the reactor. Rojas *et al* (2010), for example, found that materials like cow dung tend to act as a pH buffer that prevents significant changes in the pH of the process. Another example is a study that was performed by Mathew *et al* (2015), they compared the anaerobic digestion profiles of water hyacinth and the aquatic fern, *salvinia*, in the same reactor. It was found that the water hyacinth produced far less VFAs compared to the *salvinia*. It was postulated that the reason for this discrepancy was because the VFAs in the hyacinth experiment were converted to methane at the same rate as the VFAs were produced; whereas the production of VFAs outpaced the conversion of VFAs to methane in the *salvinia* experiment.

However, the first two steps are not the only ones in which the pH may drastically affect the performance of the digester; the final two steps may pose a similar quandary. The volatile solids (VS) may become depleted in these steps, which would lead to the termination of the process; or the pH may increase excessively and destroy the acetogens and methanogens, leading to process termination. As mentioned in Section 2.1, acetogenesis and methanogenesis produce CO_2, water, and methane. This increases the pH of the process, however, the reaction of carbon dioxide with hydroxide produces bicarbonate ions which act as a buffer in the digester (Tao *et al*, 2020).

Additionally, the pH of the digester is interlinked with the C/N ratio of the biomass. As discussed in Section 2.3, a low C/N ratio can lead to ammonia inhibition. This leads to an increase in the pH to the point where the pH is harmful to the microbes that facilitate anaerobic digestion. There are three main avenues that have been explored in great detail with regards to pH; the first is controlling the pH at a constant pH, the second is allowing the pH to vary naturally, the third is segregating the process according to the different processes, which will be discussed in greater detail Section 2.7.

The general consensus among scholars is that a pH of 7 is the optimum pH for methane production (Igoni *et al*, 2008). However, there is some leeway for the control of this pH. Cioabla *et al* (2012) state that the optimum pH range for anaerobic digestion is 6.8 – 7.2; while other sources like Mustafa *et al* (2016) state that the process may still operate at acceptable efficiencies within a pH range of 5.5 – 8.2. However, pushing the process towards the extremities of this range tends to reduce the speed of the process.

The most popular route that is followed is allowing the process' pH to vary naturally. A study by Mathew *et al* (2015) followed this approach and it resulted in a better methane yield than a similar study that was performed by O'Sullivan *et al* (2010) in which the pH was controlled. It is evident that the natural process resembles the optimums in pH for each of the four processes present in anaerobic digestion. This is seen in the pH profiles throughout the process; the pH at the beginning of the process is lower, which favours the hydrolysis and acidogenesis steps, and the pH increases over time as the VFAs and organic acids that are produced from the first two steps are consumed and ammonia is produced. This approach has many advantages; chief among those advantages is that it requires no chemical inputs. However, as discussed in Section 2.3, some feedstock may favour earlier process steps which could inhibit methanogenesis. One such example is food waste, which tends to rapidly produce VFAs, this could terminate the process early. High pH values may have adverse effects on the process as well, lower C/N ratios in feedstock tend to create an uncontrollable increase in the pH of the system, similarly terminating the process. In academic studies, pH control is recommended.

2.7 Reactor configuration

A solution to achieving optimum pH levels for the different reaction steps is the segregation of process units according to the four different processes of anaerobic digestion. Physically separating the acidogens and methanogens may result in higher methane production. This could be done by configuring the digester as a two-phase anaerobic digester in which the first stage could be optimised for hydrolysis and acidogenesis, resulting in more degradation of substrates (Hartmann & Ahring, 2005; Parawira, Murto, *et al*, 2005). This would involve operating one part of the reactor at a lower pH, which would promote hydrolysis and acidogenesis, and then transferring the products from this section to another section in the reactor that operates at a slightly higher pH, where acetogenesis and methanogenesis are promoted. A study done by Zhang *et al* (2017) performed a study in which this scheme was applied, they reported much higher methane yields using this process compared to the conventional process of having all of the processes occurring at once.

A two-stage anaerobic digester is widely regarded as being superior to the traditional, single-stage digester. This type of digester consists of a hydrolytic-acidogenic stage, followed by a methanogenic stage. This system provides increased pH control, an increase in the possible loading rate, a higher potential for removing pathogens, an increase in the specific activity for methanogens, and an increase in the volatile solids reduction (Blonskaja, Menert & Vilu, 2003; Bouallagui *et al*, 2005).

The pH can be monitored in one of two simple and straightforward ways; it can be measured using a pH probe that is attached inside the digester; or small amounts of the digestate can be withdrawn from the reactor at regular intervals and then the pH can be measured. The only caveat with these methods of pH monitoring is that the digestate has to be well mixed in order to draw conclusions on the pH of the digester. However, controlling the pH requires more care. It is essential that the control measures that are utilised do not interfere or inhibit the digestion process. Three substances are often preferred when controlling the pH of an anaerobic digester; these are: sodium bicarbonate, lime, and calcium carbonate (Mathew *et al*, 2015; Mir *et al*, 2016). None of these substances are known to inhibit the anaerobic digestion process. Moreover, they act as buffers that assist in the protecting the reactor from large fluctuations in pH. However, one must exercise caution when using lime or calcium carbonate because it may cause precipitation (Mir *et al*, 2016; Igoni *et al*, 2008).

A more recent breakthrough is that of the temperature phased anaerobic digester. This system follows a similar principle to that of the two-stage anaerobic digester; the first stage is a digester at thermophilic temperatures (45 – 75 °C), and the second is at mesophilic temperatures (30 – 40 °C). This system provides higher methane yields compared to single stage digesters as well as a digestate that is both nutrient-rich and pathogen free (Sung & Santha, 2003; Riau, De la Rubia & Pérez, 2010). A study done by Lee *et al* (2009) showed that a temperature phased anaerobic digester with the first stage operating at 70 °C and the second at 55 °C performed better than single stage thermophilic digesters in terms of biogas production. Two approaches to temperature control stand out from literature: the use of a water bath and use of a water jacket. Water baths are typically preferred for smaller digester units. An example of this is given in Figure 2.

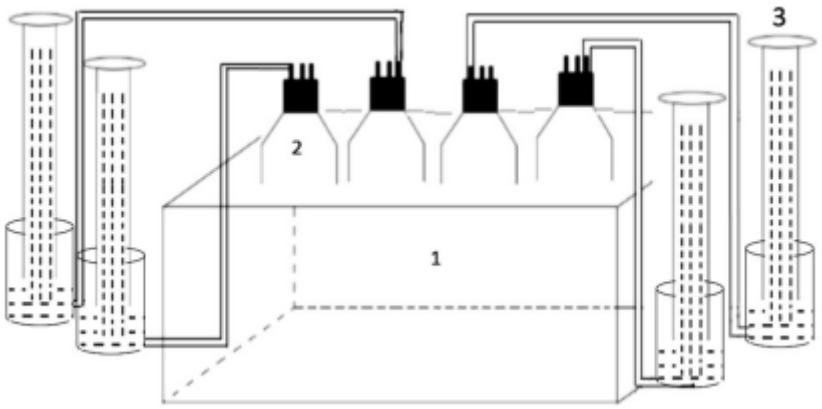


Figure 2: Experimental setup of laboratory scale anaerobic digester: 1 Temperature-controlled shaking water bath, 2 Anaerobic digester, 3 Graduated cylinder (Mathew *et al*, 2015).

However, the design seen in Figure 2 is generally only considered for lab-scale digesters, this is because water baths are infeasible on a large scale due to the amount of energy that would be required to heat the water uniformly and the volume of water required. Industrial systems typically employ water jackets. These are typically more complex in their design and construction, however, they provide better temperature control because forced convection increases the convection coefficient (Cengel & Ghajar, 2007). An example of the use of a water jacket for temperature control in anaerobic digestion is shown in Figure 3.

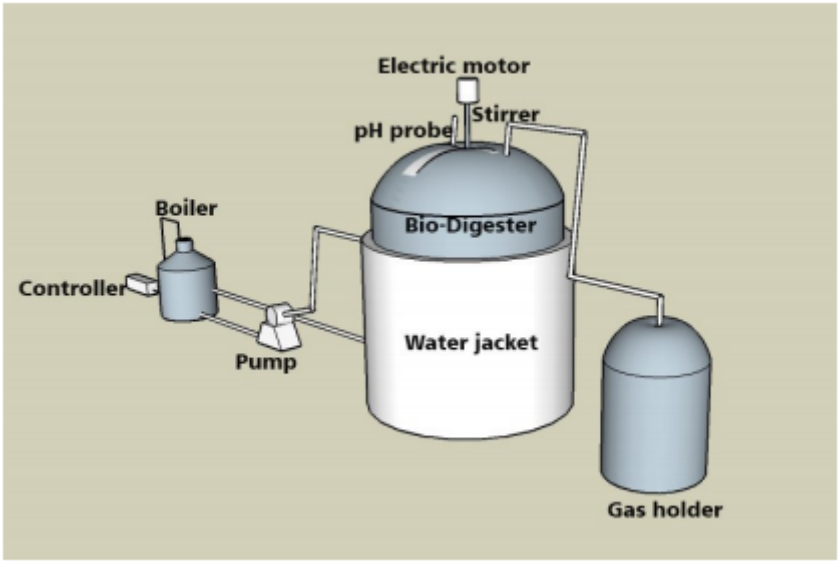


Figure 3: An example of an anaerobic digester with a water jacket for temperature control (Omotoso Agbede *et al*, 2020).

2.8 Particle size

As mentioned in Section 2.2, pre-treatment is essential because it reduces the size of the particles, which increases the available substrate surface area to anaerobic bacteria, improving interaction between the substrate and the anaerobic bacteria (Carrère *et al*, 2010). Verma *et al* (2007) suggest a particle size of 5 mm as the optimum size for digestion, with no real improvement in the methane yield below this particle size.

Larger particles typically result in lower chemical oxygen demand degradation and a lower methane production yield (Esposito *et al*, 2011). They also tend to reduce the maximum substrate utilisation rate of the anaerobic microbes (I Kim, Kim & Hyun, 2000). These factors all work towards increasing methane yield and digestate quality.

2.9 Digestate

The digestate is the material that remains in the digester after the anaerobic digestion process. The digestate is comprised of two phases, namely, solid and liquid. Although the solid digestate is extremely useful for practical applications such as livestock bedding and may be composted with minimal processing, the liquid digestate is of particular interest in this study because of its high macronutrient concentration (Tamošiūnas *et al*, 2022). The digestate typically contains a large amount of mineral nitrogen, which is easily accessible for plants. Furthermore, it contains other macronutrients like potassium and phosphorus, which are essential for plant growth. A significant element in the digestate characteristics is the feedstock. Protein-rich feedstocks conventionally provide higher ammonium content in the digestate (Makádi *et al*, 2012). Table 2 summarises the macronutrients that are typically present in the digestate. It should be noted that the studies performed by Asp *et al* (2022) and Risberg *et al* (2017) reported concentrations in kg/ton, these were converted to units of g/L using a standard density of 1.03 kg/L as reported by M Gerber & Schneider (2015).

Table 2: Liquid digestate macronutrient characteristics.

Feed	Total N (g/L)	$NH_4^+ - N$ (g/L)	P (g/L)	K (g/L)	Source
Swine manure	2.93	2.23	0.93	1.37	(Loria *et al*, 2007)
Food waste	-	5.20	0.63	1.30	(Ren *et al*, 2020)
Household waste, food waste, manure, and slaughter waste	5.45	3.81	0.26	1.54	(Asp *et al*, 2022)
Distiller's waste and cereals	6.59	4.22	-	-	(Risberg *et al*, 2017)
Slaughterhouse waste and food waste	7.82	5.15	-	-	(Risberg *et al*, 2017)
Distiller's waste	5.76	3.19	-	-	(Risberg *et al*, 2017)
Pig slurry and cabbage	4.42	3.19	-	-	(Risberg *et al*, 2017)
Manure and organic waste	4.63	3.81	-	-	(Risberg *et al*, 2017)
Cow manure	3.50	2.26	-	-	(Risberg *et al*, 2017)
Cow, pig, and chicken manure	4.94	3.70	-	-	(Risberg *et al*, 2017)
Cow manure and slaughterhouse waste	5.56	3.40	-	-	(Risberg *et al*, 2017)
Cow manure	4.22	2.88	-	-	(Risberg *et al*, 2017)
Food waste and household waste	5.25	3.50	-	-	(Risberg *et al*, 2017)
Organic waste, silage and grease	5.66	3.60	-	-	(Risberg *et al*, 2017)

Another important aspect to consider is the total volatile solids that are present in the substrate. Volatile solids are substances that can easily transform from their solid forms to their vapour forms without going through a liquid phase. Table 3 shows the volatile solids concentrations in each of the feeds.

Table 3: Total volatile solids, as a percentage of the dry mass, in each feed used for the experiments conducted.

Substrate	Total volatile solids (% DM)	Source
Banana Peels	86.29 ± 0.16	(MT Khan *et al*, 2016)
Cow dung	87.3 ± 1.8	(Dustan, 2002)
Lentils	88	(Thomsen, 2014)

3 Experimental

Two different set-ups were used for the experiments performed over the course of this study. The first was a batch set-up constructed for manual dosing once a day, the second set-up included continuous dosing with the aim of controlling the pH on a minute-to-minute basis; these set-ups were named the daily dosing setup (DDS) and continuous dosing setup (CDS) respectively.

3.1 Materials

The cow dung was collected from the University of Pretoria Experimental Farm located on the Hillcrest campus. The cow dung was sourced from dairy cows. The pH was controlled by using a 1 M solution of NaOH and a 1 M solution of HCl. Imbo red lentils (500 g) as well as Cavendish bananas were procured from a local supermarket. Deionised water was also acquired from the University of Pretoria laboratories. The feed material for the CDS was the same as those used in the DDS experiments. However, the pH was controlled by using a 0.25 M solution of NaOH and a 0.25 M solution of HCl. The environmental impacts of the chemicals that were used were considered, and it was observed that the chemicals that were used were added in sufficiently small dosages to assume negligible effects due to accumulation. It should also be noted the main aim of this dissertation was to determine the effect of altering pH on the anaerobic digestion process, the fear with adjusting the organic load was that it would add extra variables to the experiment that would not be accounted for.

3.2 Analysis

A DLAB Single-Channel Adjustable Pipette was used to extract samples from the Schott bottles. An Agilent Technologies Cary 60 UV-vis spectrophotometer was used to analyse the samples for ammonium. A Bluelab® pH Probe connected to LabVIEW (Laboratory Virtual Instrument Engineering Workbench), was used for pH measurements. A Radwag PS 8000/X digital lab scale was used to measure the mass of the chemicals and initial masses of the feedstocks required in the experiments.

The initial mass of each feed was measured by a Radwag PS 8000/X digital lab scale. The pH was measured using a Haoshi H101 pH Electrode. The temperature inside the reactors was measured by a Maxim DS18B20 temperature sensor. The pH was controlled by using a Precision Peristaltic Pump and Intelligent Stepper Controller. The pump,

stepper controller, pH and temperature sensors were all coupled to an Arduino MEGA 2560.

Each digestate sample was analysed using a Merck Spectroquant Ammonium Test and then measuring the absorbance of the mixture of the sample and the test on an Ultraviolet–visible spectrophotometer set at a wavelength of 690 nm. The absorbance could then be related to ammonium concentration through a previously calibrated ammonium absorbance-concentration curve. The samples were taken by opening the lid of the digester and drawing the sample with the pipette and then closing the lid after extracting the sample.

3.3 Apparatus

An Orbital Shaker-Incubator ES-20/60 was used as the main vessel for the DDS experiments. The incubator had enough space for six 250 mL Schott bottles. An 8 mm hole was drilled into each bottle's lid in order to allow for gas capturing with a tube. The gas was captured with six 500 mL graduated cylinders that were inverted and submerged in water; the gas production rate could then be correlated with the water displaced in the cylinders over the course of the experiment. The final set-up and a schematic of this apparatus are shown in Figures 4 and 5 respectively.

Figure 4: The Daily Dosing Set-up (DDS).

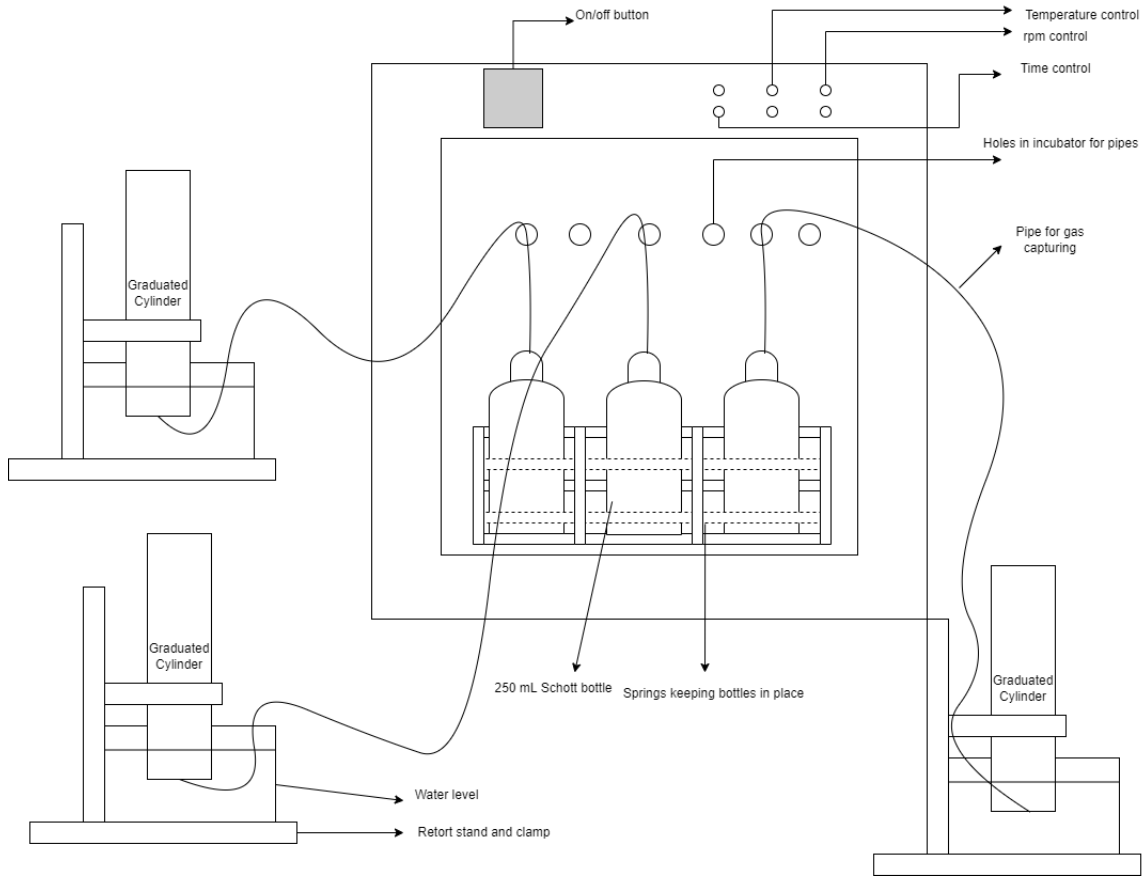


Figure 5: A schematic of the Daily Dosing Set-up (DDS).

Two identical reactors were constructed for the Continuous Dosing Set-up (CDS) experiments. Each reactor required an acrylic tube with an outside diameter of 110 mm, an inside diameter of 104 mm and a height of 140 mm, and a square plexiglass base (200x200x10 mm). A Daihan Scientific Digital Hotplate Stirrer MSH-20D was used to control the temperature and mix the digester contents with a stirrer bar. The reactors were constructed by attaching the clear acrylic tubes to the square base using magma bond (C1). The lid for each of the reactors was a PVC end cap with an inside diameter of 110 mm, PTFE tape was placed in the space between the lid and the tube, providing an airtight seal. Both the reactors would be operated on stirrer plates, each mixed by a Daihan Scientific Digital Hotplate Stirrer MSH-20D with a stirrer bar. The lid had various holes drilled through it allowing for sampling, charging, temperature control, a gas outlet, and pH control. The gas was captured in the same fashion as the DDS experiments. The peristaltic pumps were used to regulate the pH inside the reactors. The pH electrodes and the pumps were coupled to an Arduino MEGA 2560 in order to employ an on/off control scheme to achieve the desired set-point. The final set-up and a schematic of this apparatus are shown in Figures 6 and 7 respectively.

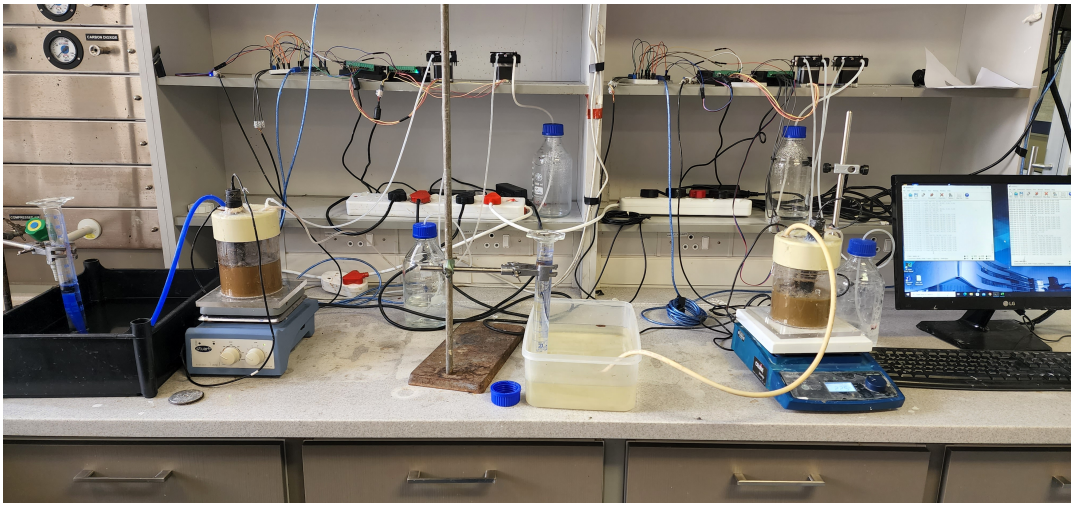


Figure 6: The Continuous Dosing Set-up (CDS).

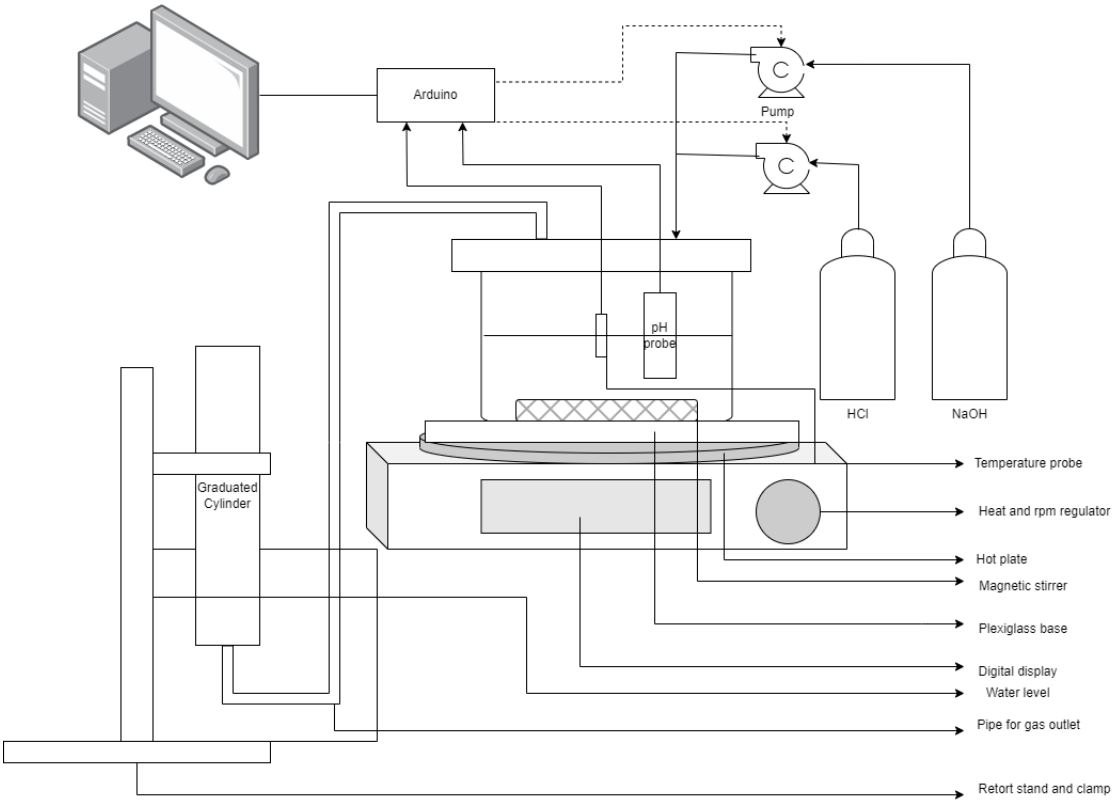


Figure 7: A schematic of the Continuous Dosing Set-up (CDS).

3.4 Experimental procedure

For the DDS experiments, firstly, three banana peels each with a mass of 100 g were dried in an oven for 24 hours at 70 °C to determine the dry mass to wet mass ratio of the banana peels, the average moisture content of the banana peels was found to be 85 % on

a mass basis, which correlates well with literature (MR Khan & Perveen, 2010; Abano, Sam-Amoah, *et al*, 2011). The full results from these tests are given in Table 4. Six 250 mL Schott bottles were used for each experiment.

Three separate feeds were then prepared for each experiment. Each feed was prepared such that the total dry solids in each bottle would be 5 % of the total mass. The lower concentration value was chosen because higher solid concentrations would require separation in order to use the analytical tools that were available. The first feed was prepared with 52.5 g of cow manure and 105 mL of deionized water. The second feed was prepared with 26.25 g of cow dung, 26.25 g of banana peels, and 105 mL of water. The third feed was prepared with 26.25 g of cow dung, 3.94 g of dry red lentils and 130 mL of water. The value for the moisture content of cow manure that was used for the experiments was 85 %, this is generally the value that is found in literature (Xin *et al*, 2018; Taylor, 1917).

The starting masses of 7.88 g (dry basis) of each type of feedstock were then blended and placed in the 250 mL Schott bottles and placed into a shaker incubator at a specified rpm of 150 and a temperature of 35 °C for the duration of each experiment. Each feed had a duplicate bottle for each pH condition. The pH in each bottle was measured and adjusted daily with a standard solution of 1 M NaOH or 1 M HCl as necessary depending on the experimental requirements. A wide range of incubation times for batch anaerobic digestion have been reported in literature. Some sources state that 7 days is enough for complete degradation of organic substrates, however, others state that 30 days is the optimal period for complete degradation (Kivaisi & Eliapenda, 1995; Raposo *et al*, 2008; Owen *et al*, 1979). 21 days was chosen as the end for each experiment. There were two reasons for this decision. Firstly, two preliminary experiments were performed for much longer durations (40 days). These experiments showed that ammonium released seized after the first 15 days of each experiment. Secondly, time constraints related to the dissertation necessitated choosing a reasonable timeframe for each experiment, therefore, 21 days was chosen as the ending of the batch AD experiment. The ammonium concentration in each bottle was measured on days 0, 1, 5, 9, 13, 17, and 21 by extracting a 2 mL sample from each bottle.

Table 4: Wet masses, dry masses, and moisture content of each banana peel sample tested.

	Wet mass (g)	Dry mass (g)	% Moisture
Sample 1	103	16.3	84.2
Sample 2	100	14.4	85.6
Sample 3	98	15.7	83.9
Average	100	15.5	84.6

For the CDS experiments, only two of the feeds were considered, the cow dung only and cow dung and banana peel feed. Each feed was prepared such that the total dry solids in each flask would be 5 % of the total mass, however, due to the reactors being slightly larger in volume, the initial masses of each feed had to change. This change in initial masses was so that the probes could be submerged in the solution without interfering with the stirrer bar. The first feed was prepared with 175 g of cow dung and 350 mL of deionized water. The second feed was prepared with 87.5 g of cow dung, 87.5 g of banana peels, and 350 mL of deionized water.

The feedstock was placed in each reactor at a specified rpm of 150 and a temperature of 35 °C for the duration of each experiment. The pH and dosing data was captured for every minute of each experiment, whereas the ammonium concentration in each bottle was measured on days 0, 1, 5, 9, 13, 17, and 21 by extracting a 2 mL sample from each reactor.

Once the experiment started, the Arduino would be activated. The Arduino received signals from the pH meter and the temperature probe that were captured in a text file, these signals were then used to control both the pH and temperature in the reactors. The Arduino was linked to two peristaltic pumps; with each pump connected to a 0.25 M NaOH and 0.25 M HCl solution respectively. These pumps were actuated depending on the pH set point required for the experiment. Simple on/off control was employed for the pH control, with a signal being received every minute during the experiments, meaning that the pumps could be actuated every minute to control the pH. In the event of an experiment that did not require pH control, the pumps were deactivated, however, the pH and temperature data was still captured to a text file.

4 Results and Discussion

Two types of experiments were performed over the course of this study. The first was performed with pH correction once a day, the set-up used to perform these experiments was referred to as the Daily Dosing Set-up (DDS). A second, comparative experiment was designed to determine the effect of continuous dosing as opposed to dosing once a day; this experiment recorded pH data every minute with the aim of controlling the pH of the solution on a minute-to-minute basis, the set-up used to perform these experiments was named the Continuous Dosing Set-up (CDS). The experiments that were performed over the course of this study are summarised in Table 5.

Table 5: A summary of all the experiments performed. Two ticks indicate that the experiment was performed for both the CDS and DDS, one tick indicates that the experiment was only performed for the DDS.

Feed	pH 6	pH 7	pH 8	No pH control
Cow dung only	✓✓	✓✓	✓✓	✓✓
Banana peels and cow dung	✓✓	✓✓	✓✓	✓✓
Red lentils and cow dung	✓	✓	✓	✓

4.1 The DDS

In the first experiment, there was no acid or base dosing. This was done to determine the digestion characteristics of each feedstock to better understand the influence each feedstock had on the pH. The pH characteristics of each feed is shown in Figure 8.

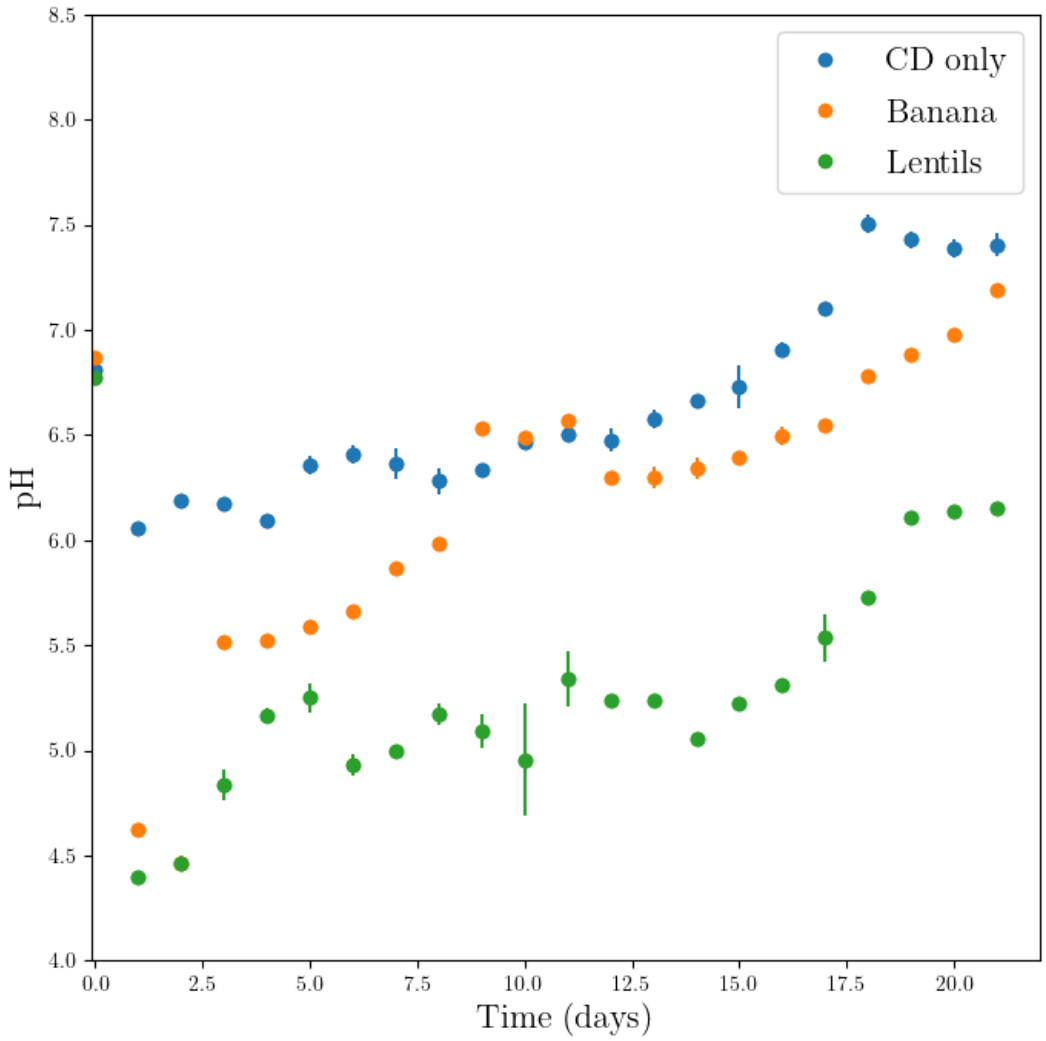


Figure 8: pH characteristics of each feedstock with no pH adjustments for the DDS.

Figure 8 depicts a sharp initial decrease in the pH on the first day, then a steep incline in the pH on the second day, and then a steady increase in the pH until a plateau is reached in all the different feedstocks at around day 18. There is a noticeable difference in the pH of the lentil feedstock compared to the cow dung only and banana feedstocks. This could be attributed to the fact that the feed is protein-rich and as such it was expected to produce far more ammonium than the other two feedstocks (Makádi *et al*, 2012; Kryvoruchko *et al*, 2009). The production of ammonium typically correlates with the first two steps of the anaerobic digestion process (hydrolysis and acidogenesis) and these first two steps generally occur at lower pH values than the rest of the process (JK Kim *et al*, 2006; Parawira, Read, *et al*, 2008; Adekunle, Okolie, *et al*, 2015), this explains why the pH of the lentil feedstock was notably lower than that of the other two feedstocks.

The lentil substrate was also the only substrate that was cooked before being placed in the reactors. This may be considered as a thermal pre-treatment step in the process.

Thermal pre-treatment is a universally accepted method of augmenting the anaerobic digestion process because it accelerates the degradation of the substrate, which provides an easily digestible fraction of the substrate (Saragih *et al*, 2019; Ariunbaatar *et al*, 2014; VK Nguyen *et al*, 2021; Pilli *et al*, 2020). Another major difference between the three substrates is the fact that lentils contain much less lignocellulosic material compared to the other two substrates. Lignocellulosic bio-mass, especially the lignin content, has been reported as having an inhibitory effect on the anaerobic digestion process due to the complexity of the biomass structure (ATW & Zeeman, 2009; Sawatdeenarunat *et al*, 2015; Paul & Dutta, 2018; MU Khan *et al*, 2022). Lentils typically contain 1.2 to 1.8 % of lignin, whereas cow dung and banana peels range from 8 to 14 % and 8 to 15 % respectively (Srivastava & Vasishtha, 2013; P Kumar *et al*, 2009; Wen, Liao & Chen, 2004; Liao *et al*, 2006; Bakar *et al*, 2021; Kabenge *et al*, 2018).

Commonly, anaerobic digester plants are operated with continuous feeding. This usually results in slightly more stable pH patterns. Generally, the initial pH patterns are quite similar, however, the difference in pH characteristics is much larger towards the end of the digestion process, with the continuously fed reactor typically providing a more stable pH pattern (Sihlangu *et al*, 2021). Figure 9 shows the acid/base dosing of each feed at the different pH set points.

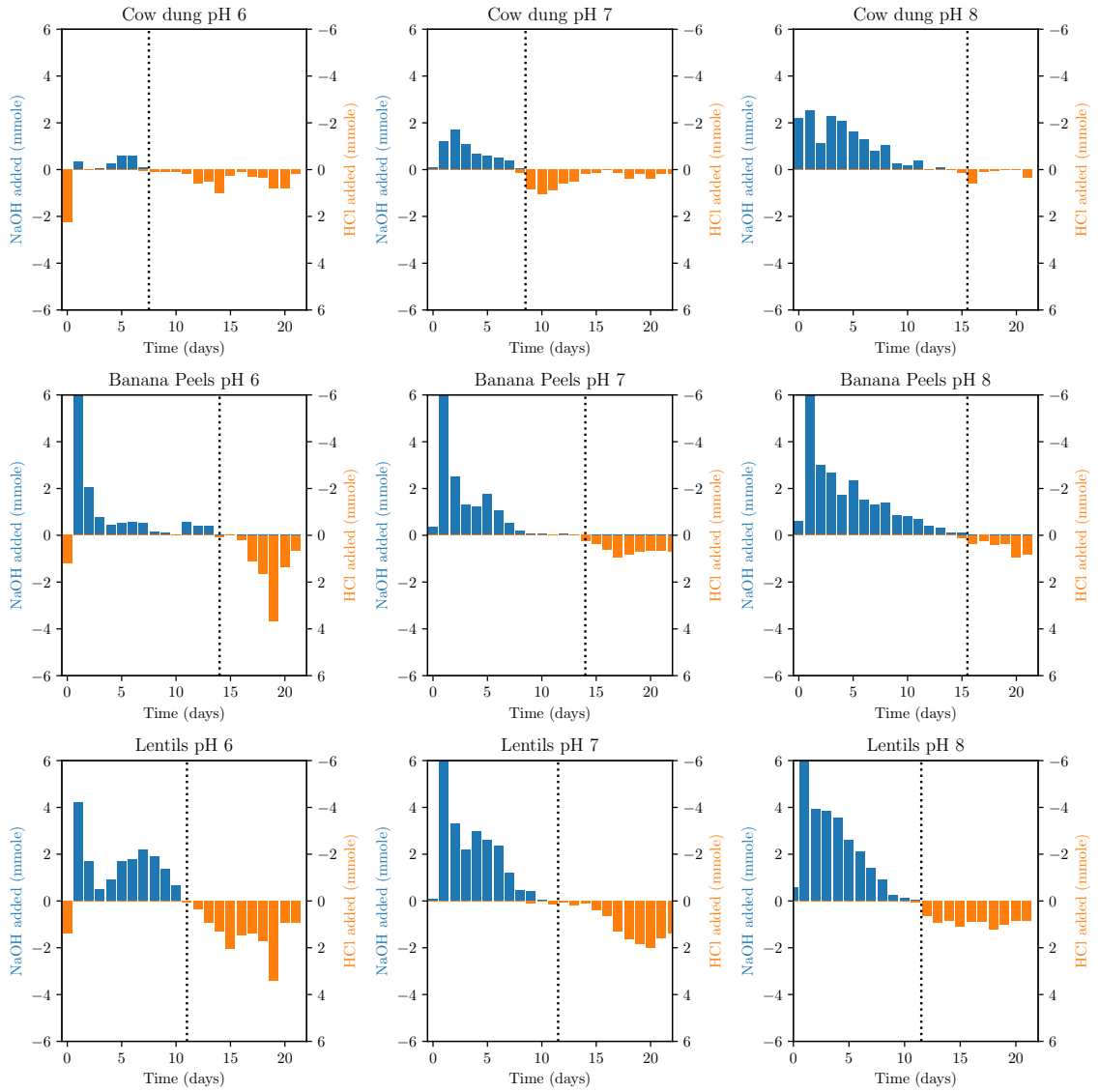


Figure 9: A comparison of the acid/base dosing of each feed at the different pH set points for the DDS. The blue represents the amount of sodium hydroxide added, the orange represents the amount of hydrochloric acid added. The dotted line represents the switchover point for each experiment (*i.e.*, when HCl had to be dosed instead of NaOH)

From Figure 9, the runs at a higher pH set point require more sodium hydroxide to reach the set point, however, the time taken to reach the switch point (when hydrochloric acid must be added instead of sodium hydroxide to maintain the set point) does not vary significantly with each feed. It is noted from Figure 9 that at a pH of 8 the cow dung and banana peel feeds take much longer to reach their switchover point. This could be attributed to the fact that the anaerobic digestion process is sub-optimal at such a relatively high pH. Typically, the process of anaerobic digestion prefers pH values between 6.8–7.2 (Cioabla *et al*, 2012; Moosbrugger *et al*, 1993; Adekunle, Okolie, *et al*, 2015). This inferiority in digestion at the higher pH is further validated by the insignificant gas

production shown in Figure 12. These two substrates had almost identical switch points for the experiments performed at pH values of 6 and 7. However, the cow dung substrate had a faster switch point compared to the banana peel substrate at these pH values. Figure 9 also illustrates how the lentil substrate showed little variation in the switch point characteristics (when hydrochloric acid must be added instead of sodium hydroxide to maintain the set point) compared to other two substrates. The environmental impacts of the chemicals that were used were considered, and it was observed that the chemicals that were used were added in small dosages. It should further be noted that the main aim of this dissertation was to determine the effect of altering pH on the anaerobic digestion process, the fear with adjusting the organic load to control the pH was that it would add extra variables to the experiment that would not be accounted for. Figure 10 shows the ammonium concentrations of each feed at different pH set points.

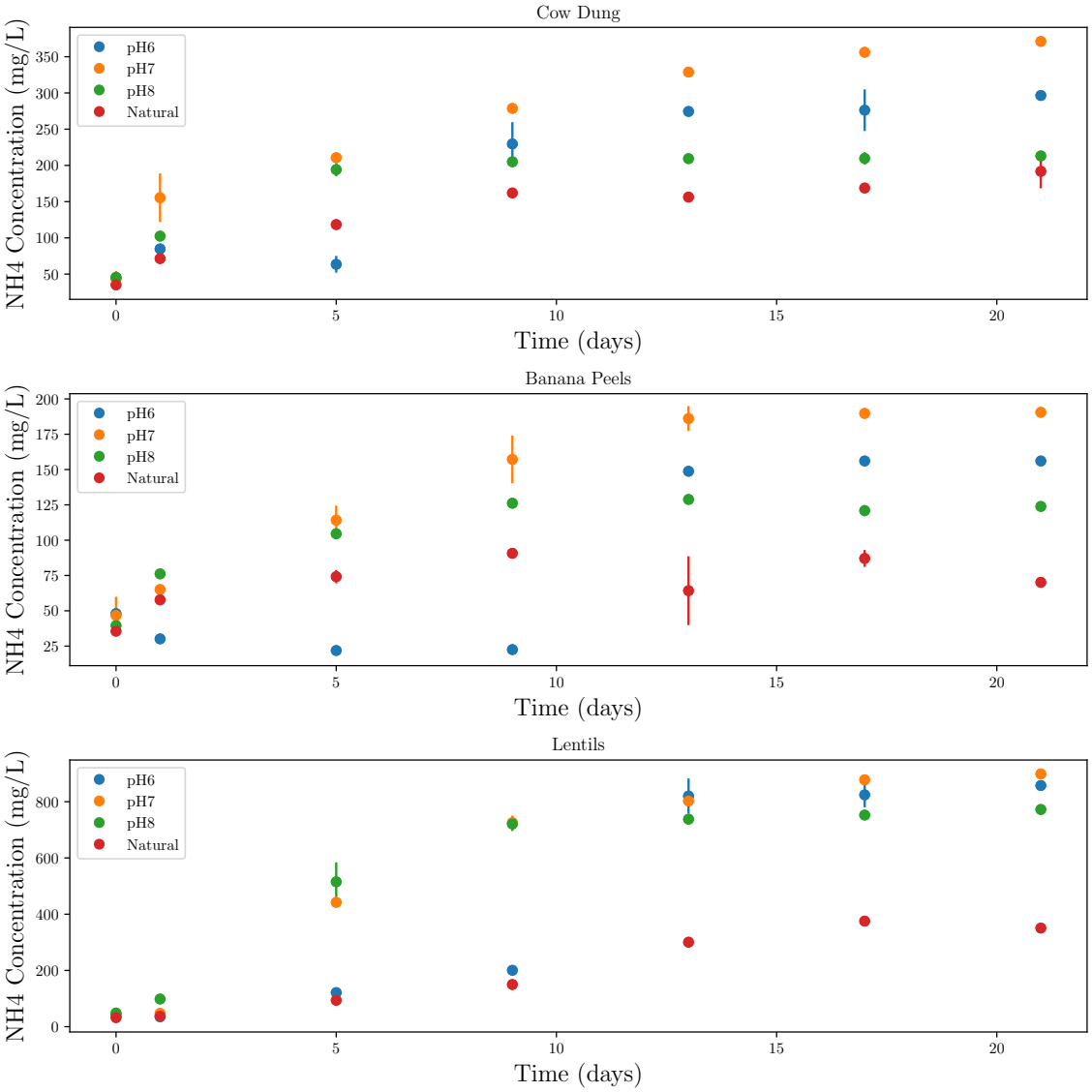


Figure 10: The ammonium concentrations of all of the feeds for the experiments performed in the shaker incubator at different pH values for the DDS. Each concentration value is an average of the two repeats that were performed for each feed and pH value.

Figure 10 demonstrates that a pH of 7 is generally preferable for all the feeds in terms of ammonium released, although it could be argued that the differences in concentrations for the lentils are negligible. This figure also shows how the ammonium that is released in the lentil feedstock is much higher than the other two feeds; this could be attributed to the fact that the lentil feedstock contains more protein compared to the other two feedstocks and as such it was expected that it would release the largest amount of ammonium (as previously proposed) (Makádi *et al*, 2012). Although the pH 7 run was optimal for ammonium release in all the feeds, the significant advantage it had over the other set points in the cow dung and banana peel substrates is less pronounced in the lentil substrate, where much more nitrogen was released. This is likely an indication that the

readily digestible protein fraction of a protein-rich feed is insensitive to pH compared to the more complex lignocellulosic part of the feed.

More significant insight can be drawn from Figure 10; firstly, the pH control aided in extracting ammonium from the feedstock into the liquid. The optimal was a pH of 7, outperforming the runs without pH control by an average percentage difference of 20 %. The higher pH provided the lowest amount of ammonium released, the ammonium concentrations for the experiments performed at a pH of 6 were only marginally better than those of the pH 8 experiments; there was an average percentage difference of 5.6 % between the two pH values. An explanation for these results can be obtained from anaerobic digestion theory; anaerobic digestion has four process (hydrolysis, acidogenesis, acetogenesis and methanogenesis) (Wukovits & Schnitzhofer, 2009; Miao *et al*, 2018), the first steps are typically at a lower pH and the final step is generally at a higher pH value, and these steps are sequential at the start of the process. It is widely accepted that the optimal pH is around 6.8–7.2 for optimal digestion (Cioabla *et al*, 2012; Moosbrugger *et al*, 1993; Adekunle, Okolie, *et al*, 2015), this explains why the ammonium concentrations for the experiments performed at pH values of 7 and 8 are similar at the beginning and then the pH 8 starts to plateau. In contrast the lower pH run stagnates the production of ammonium at the beginning of the run as the lower pH stunts the process. This can also be seen in Figure 12, the gas production is much slower at the beginning of the process for the experiments at a pH of 6 compared to the other runs (except for the run without pH control). Figure 10 also shows that the runs without pH control performed poorly compared to the pH-controlled runs, there was an average percentage difference of 20.3 % between the runs performed at a pH value of 7 and the runs without pH control. If one considers Figure 12, the gas production from the runs without pH control was much lower than the controlled runs, this fact coupled with Figure 8, shows how the run without pH control had not reached the optimal pH range by the end of the run. It is plausible that this could be the reason for the decrease in ammonium and gas production. The uncontrolled experiments consistently produced the least amount of ammonium for each substrate, indicating that pH control is important for ammonium release in anaerobic digestion. The final ammonium concentrations for each feed and pH condition is shown in Table 6.

Table 6: A summary of all the ammonium concentrations for each feed and pH condition. All results are reported in units of mg/L.

Feed	pH 6	pH 7	pH 8	No pH control
Cow dung only	297	371	213	192
Banana peels and cow dung	156	191	123	70.2
Red lentils and cow dung	858	899	772	351

Since there seems to be a correlation between the pH control and ammonium concentration, a composite figure of the ammonium and cumulative sodium hydroxide added for each pH set point was made. This is seen in Figure 11.

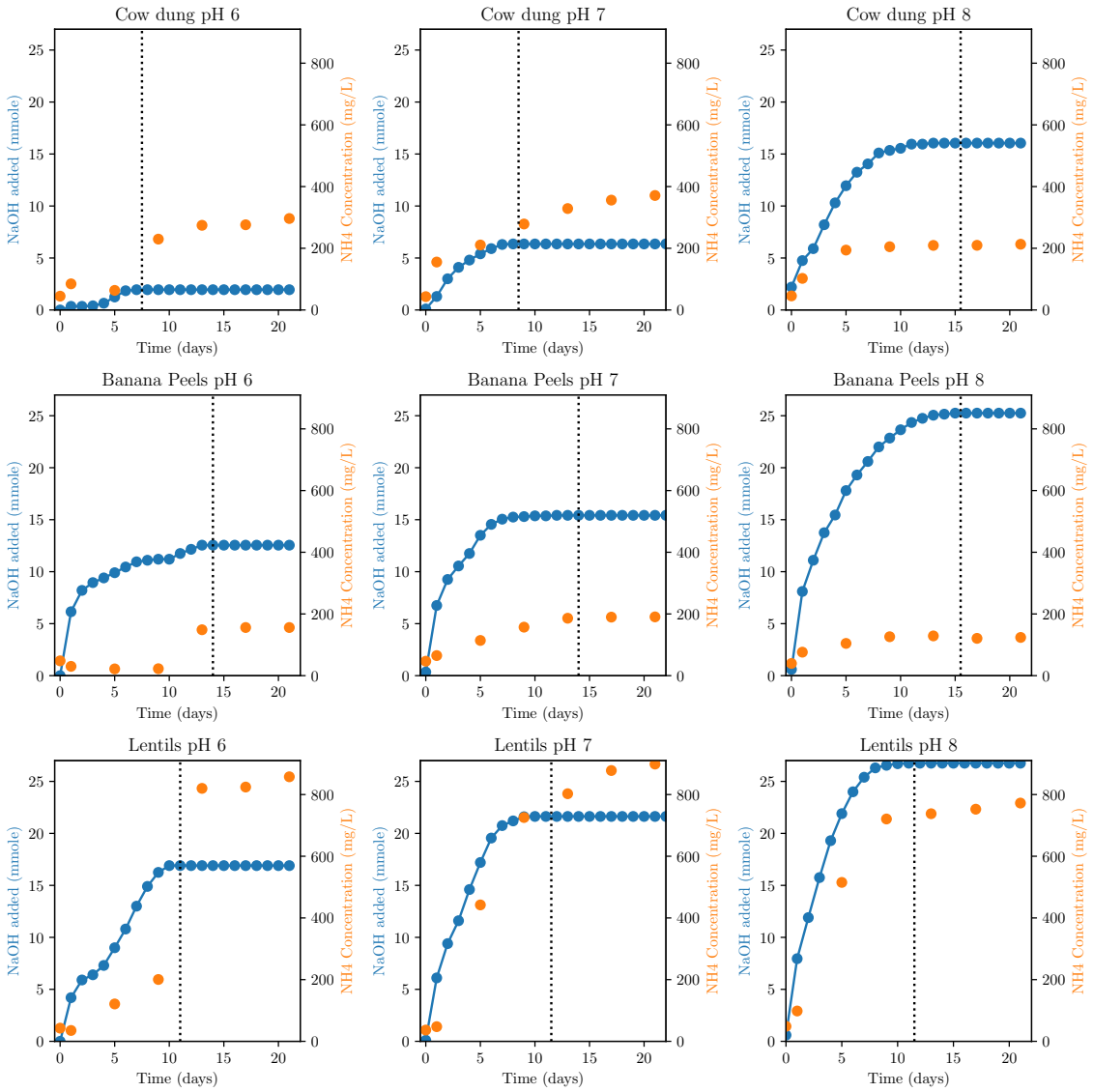


Figure 11: A composite figure of the cumulative sodium hydroxide dosing and the ammonium concentration on each y-axis respectively for the DDS. The orange markers represent the ammonium concentrations, and the blue markers represent the cumulative sodium hydroxide dosing. The NaOH added is in mmole.

Figure 11 shows a clear increase in the sodium hydroxide required as the pH set point increases for each pH-controlled run. This figure also shows that the difference in the dosing amount of sodium hydroxide between the banana peel and lentil substrate is relatively low despite the vast differences in the ammonium concentrations, this indicates that the additional amino acid breakdown that is required for the lentil substrate does not have in influence on acidifying the mixture. Figure 11 further displays a sharp increase in ammonium concentrations after the switch point for the experiments performed at a pH of 6. This indicates that the ammonium release is initially inhibited at a lower pH. The pH has an inhibitory effect on the ammonium concentrations before the switch over point (i.e., in the initial phase of the process). This is clear when one examines Figure 11, the

ammonium concentrations before the switch over point in the runs that were conducted at a pH of 6 produce much less ammonium in the initial phase of the runs. There is a distinct lag phase that is associated with the lower pH value. However, once the switch over point is reached (changeover from alkali to acid dosages), there is a distinct increase in ammonium production, similar to the results that were observed with the other two pH values. It should further be noted that the switchover point signifies the moment at which there was a change from alkali to acid dosage (the moment at which acid dosing began), it does not show the extent to which the alkali dosing had decreased prior to the switch point. In the cases that the sharp increase in ammonium production does not correlate well with the switchover, the alkali dosage had dropped significantly before the switch point. This can be seen in Figure 9, especially with the runs performed at a pH of 7. There is a clear and significant decrease in the amount of sodium hydroxide that was dosed before the switch point (some days did not even require dosing). This shows that the runs had reached the point at which they did not require alkali dosing to maintain the pH set point, however, the pH had not increased enough to necessitate acid dosing.

It is of paramount importance to ascertain whether the ions that are introduced to the reactor via the control strategy affect the ammonium concentration and gas production. Studies have shown that certain metal ions can have inhibitory effects on the anaerobic digestion process, especially Mg^{2+}, Ca^{2+}, and Na^{+} (Hou, Ji & Zang, 2018). However, the amounts at which these ions affect the anaerobic digestion process to a notable extent are well above the amounts that were used in this experiment. Na^{+} ions were introduced into this system as part of the pH control strategy, a known process inhibitor in anaerobic digestion, it impacts methanogens by either increasing the osmotic pressure or a complete dehydration of microorganism, however, only Na^{+} ion concentrations of 5–10 g/L inhibit methanogenic activity and biogas production, which was well above the concentrations that were used in these experiments (Xiao *et al*, 2022; Xiaolong *et al*, 2006; Rinzema, Lier & Lettinga, 1988; Y Liu & Boone, 1991). On the other hand, some scholars state that the addition of Na^{+} ions actually augment the anaerobic digestion process, but the positive effects of Na^{+} ions were only observed at concentrations that ranged from 1–2 g/L (Xiaolong *et al*, 2006; Hou *et al*, 2018). The Na^{+} ion concentrations that were used in this experiment never surpassed a value of 1 g/L for any experiment. The highest concentration of Na^{+} ions that were added to the system at any point during the experiment was 0.6 g/L, as seen in Figure 11.

Another ion that could skew the data is the Cl^{-} ion. Studies have shown that it may adversely affect the methanogenic performance, however, the concentration values that any deterioration in methanogenic performance occurs range from 15–20 g/L (Zhao *et al*, 2018). Once again, these values were well above the ranges that were used in these experiments, the Cl^{-} ion concentrations that were used in these experiments ranged from

0.04– 0.53 g/L. Figure 12 shows the gas production of each feedstock at different pH set points.

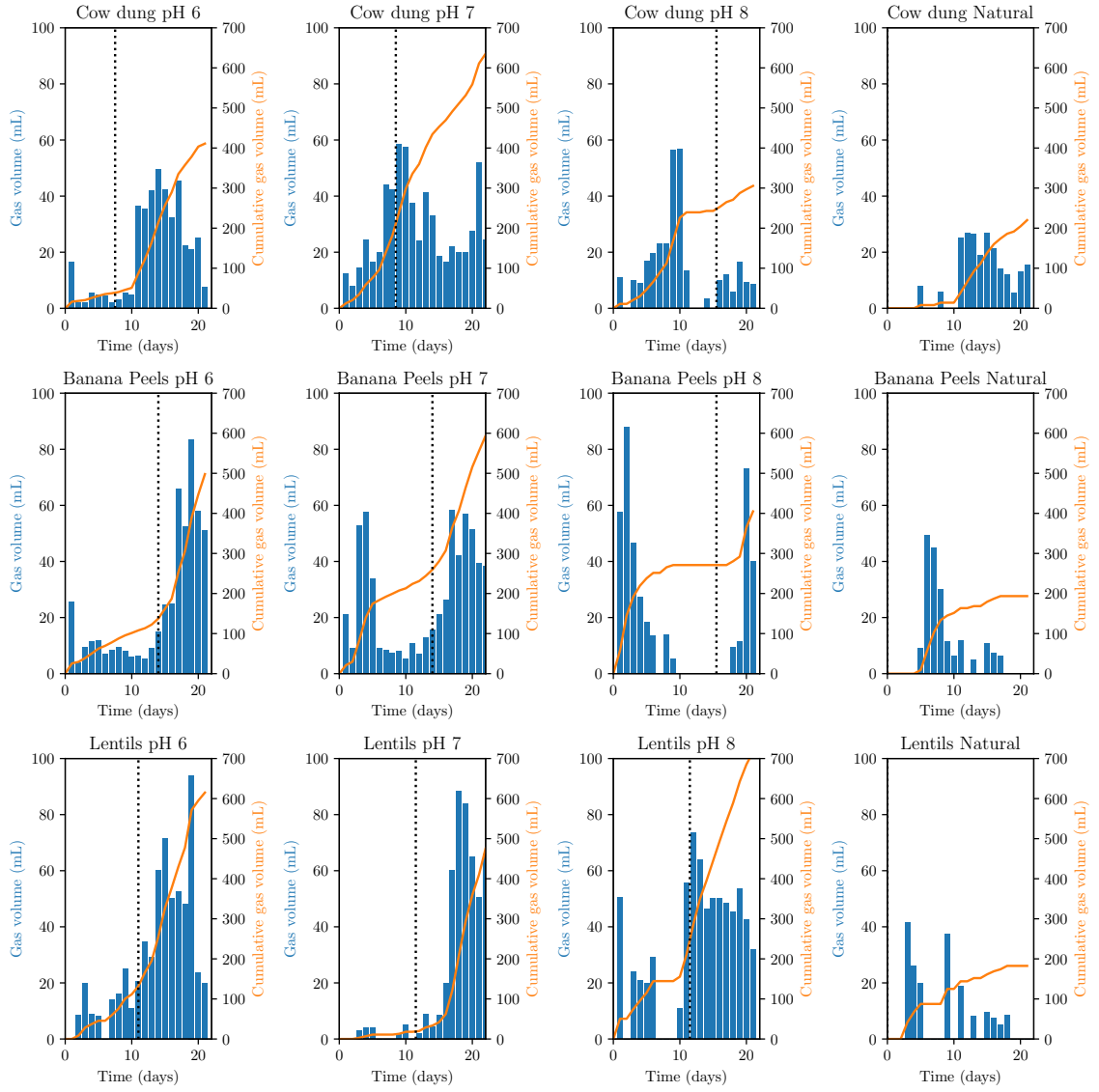


Figure 12: Gas volumes produced of each feed at different pH set points for the DDS. The orange line represents the cumulative water displaced over time, whereas the blue represents daily water displacement.

When one examines the results seen in Figure 12, it is evident that the gas production at a pH of 6 is inhibited at the beginning of the experiment. This makes it apparent that the lower pH has an inherent inhibitory effect on the anaerobic digestion process as whole (*i.e.*, in terms of gas production and ammonium production, the inhibition is not just localised to ammonium production). Figure 12 also shows how the uncontrolled pH experiments were comprehensively outperformed in terms of gas production by the experiments that had pH control. The uncontrolled pH experiments typically had a relatively large lag phase at the beginning of the experiments in which no gas was

produced, whereas the pH-controlled experiments typically started producing gas much earlier. The pH-controlled experiments also consistently produced more gas than the experiments without pH control.

Figure 12 shows that the gas production is left skewed at a lower pH value (*i.e.*, the mean gas produced is less than the median), indicating that perhaps the lower pH hinders the anaerobic digestion process slightly more than the other pH set points; whereas the higher pH value produces gas more sporadically compared to the other two set points. A pH of 7 seems to be the optimal for most of the feeds (outperforming the run without pH control of 6 by an average percentage difference of 7.5 %). The run without pH control seems to corroborate these findings, at the beginning of each run without pH control the pH was still relatively low resulting in limited gas production, when the pH started approaching neutral values the gas production picked up indicating that the lower pH stunts the gas production of the process. The runs without pH control were outperformed by the runs performed at a pH of 7 by an average percentage difference of 22 %. These findings generally correlate well with results that were determined by other studies. Jayaraj, Deepanraj & Sivasubramanian (2014) found that a pH of 7 was optimal for biogas production for food waste compared to pH values of 5, 6, 8, and 9. However, in their study, a pH value of 8 performed marginally better than a pH value of 6. Keramati & Beiki (2017) performed a similar study, but they only controlled the pH at values of 7, 8, and 9. They also found that the optimal pH value for biogas production was 7.

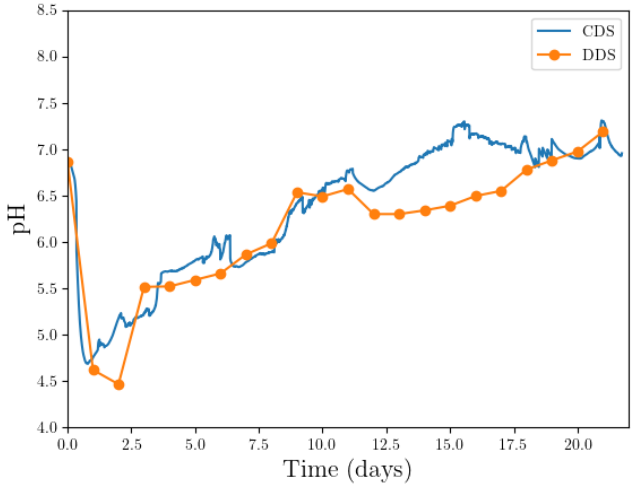
Figure 12 also shows clear evidence of an accelerated gas production after the switch point is reached. There is an inflection in the cumulative gas production that almost perfectly correlates to the switch point in all the pH-controlled experiments. This inflection is not present in the runs without pH control which gives further credence to the fact that the acceleration in gas production is correlated to the switch point. The sudden onset of accelerated gas production in the neat samples can be correlated with the sudden increase seen in the pH around the 11^{th} day of the experiment, as seen in Figure 8. The lag phase that was observed in the neat samples occurred when the pH of the system was still relatively low, the gas production accelerated as soon as the pH started to steadily increase, this clearly indicates a regime change in the experiments. The total gas produced for each feed and pH condition is shown in Table 7.

Table 7: A summary of the total gas produced for each feed and pH condition. All results are reported in units of mL.

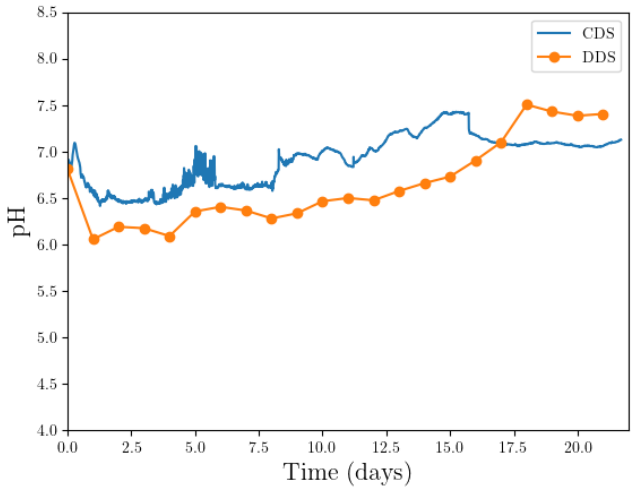
Feed	pH 6	pH 7	pH 8	No pH control
Cow dung only	411	611	306	220
Banana peels and cow dung	498	556	405	194
Red lentils and cow dung	615	410	717	183

4.2 CDS *versus* DDS

These experiments were slightly more complex in nature compared to the experiments that were performed in the shaker flasks. They had online measurements for the pH and temperature, as well as online pH control. The primary aim for these experiments was to compare the results obtained from controlling the pH once a day to the results obtained from controlling the pH on a minute-by-minute basis. With this in mind, it was of paramount importance to make sure that external factors did not interfere with the prescribed independent variables; therefore, a comparison of the pH between the runs without pH control for the shaker incubators and the online set-ups was made. This comparison can be seen in Figure 13.



(a) Comparison of the banana peel substrate.



(b) Comparison of the cow dung substrate.

Figure 13: Comparative plots of the CDS *versus* the DDS runs. a) depicts the comparison between the two banana peel runs and b) depicts the comparison between the cow dung only runs.

The results from Figure 13 are encouraging. This is because the CDS and the DDS experiments have very similar pH profiles without any pH control. This shows that there is very little difference in terms of the set-ups if one runs the set-ups without external control, signifying that any future differences will be due to the differences in the control strategies implemented. Figure 14 shows the comparison of the cumulative dosing of sodium hydroxide in a system that was controlled by continuous dosing through online measurements *versus* the shaker flasks that were controlled by measuring the pH every day and dosing accordingly.

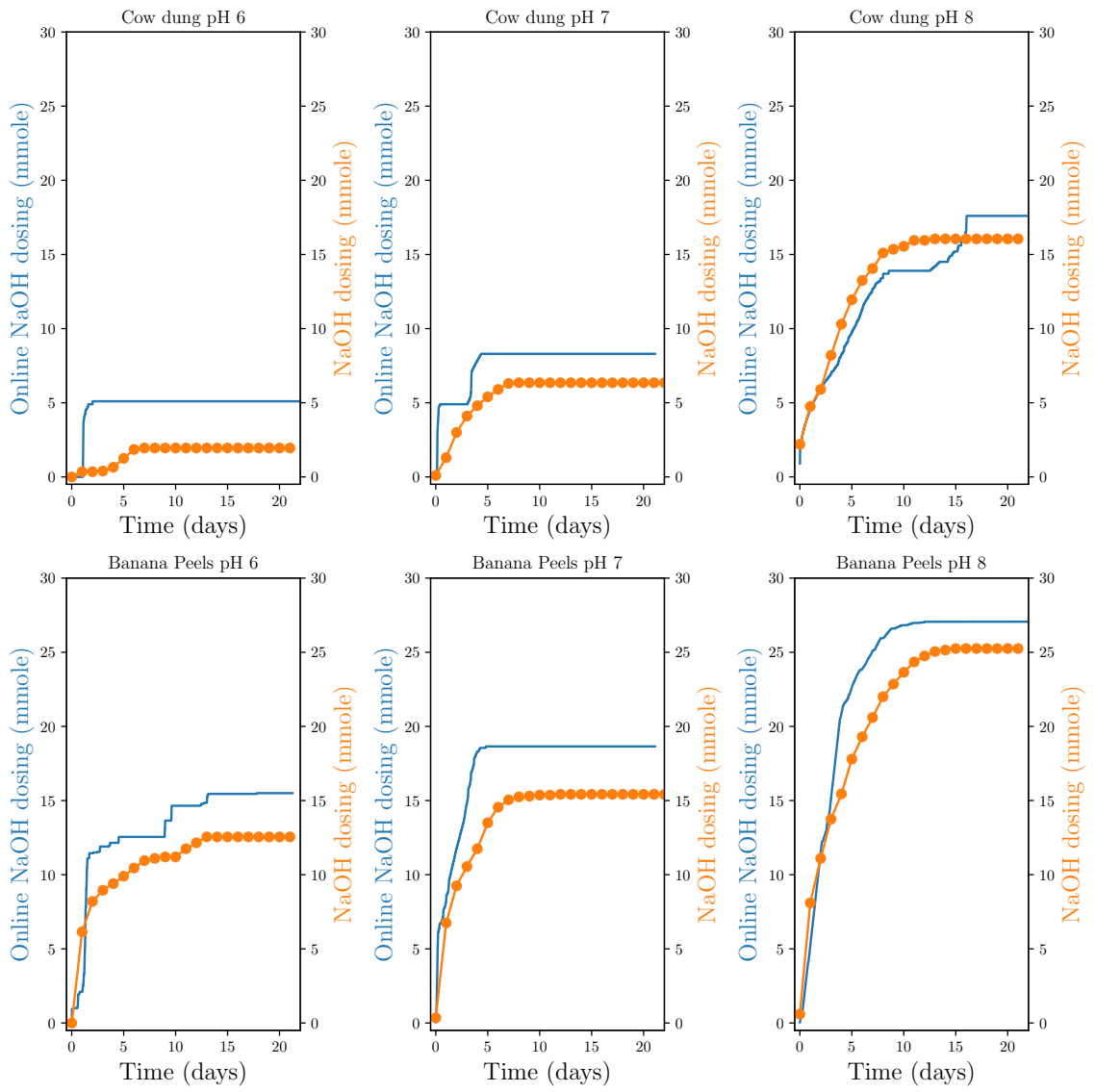


Figure 14: A comparison of the cumulative dosing of NaOH for the CDS *versus* the DDS at different pH set points. The orange represents the DDS whereas the blue represents the CDS.

Figure 14 illustrates that the CDS typically provided peaks of sodium hydroxide much earlier than the DDS, it is also clear that the CDS generally has a higher peak than that of the DDS. This could be because the CDS has a much better pH control strategy than the DDS. Since the pH is measured every minute in the CDS it has more stringent pH control, it is plausible that the solids conversion was better for the CDS. This would explain why there is generally more sodium hydroxide dosed in the CDS experiments compared to the DDS experiments. Unfortunately, the solids content was not measured as it had the potential to shed more light on the differences. Figure 15 shows the comparison between the ammonium concentrations of the CDS *versus* DDS experiments.

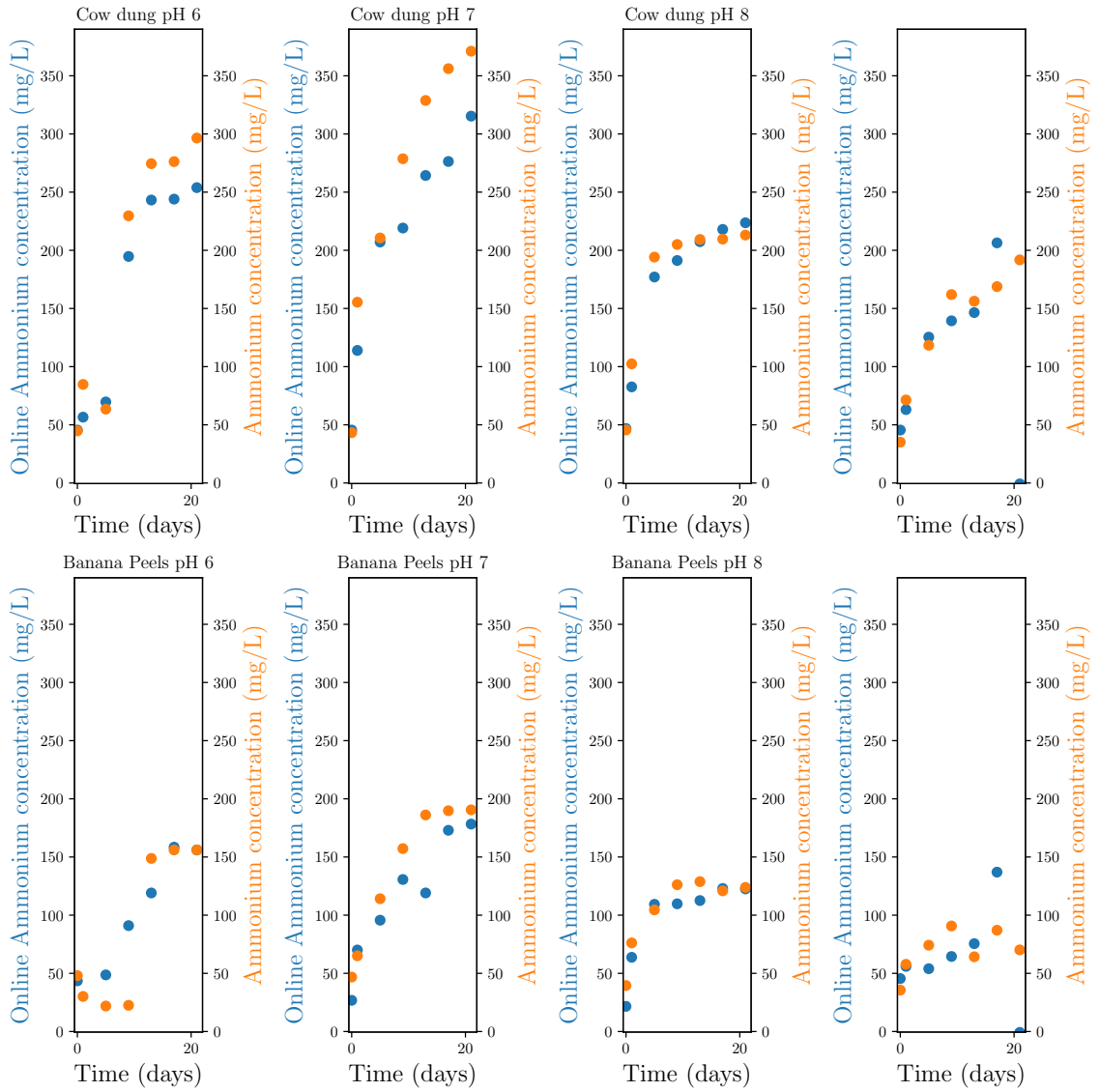


Figure 15: A comparison of the ammonium concentrations for the CDS *versus* the DDS at different pH set points. The orange represents the DDS whereas the blue represents the CDS.

Figure 15 shows that the difference in ammonium production between the two systems is insignificant. However, the increase in ammonium concentrations in the CDS runs is slightly more consistent than that seen in the DDS runs. This is seen especially in the experiments that were performed at a pH of 6; the ammonium increased at a steady rate for the continuous dosing, whereas the DDS provided a much steeper increase in ammonium concentrations over a smaller period. Figure 16 shows the gas production of the CDS *versus* the DDS runs.

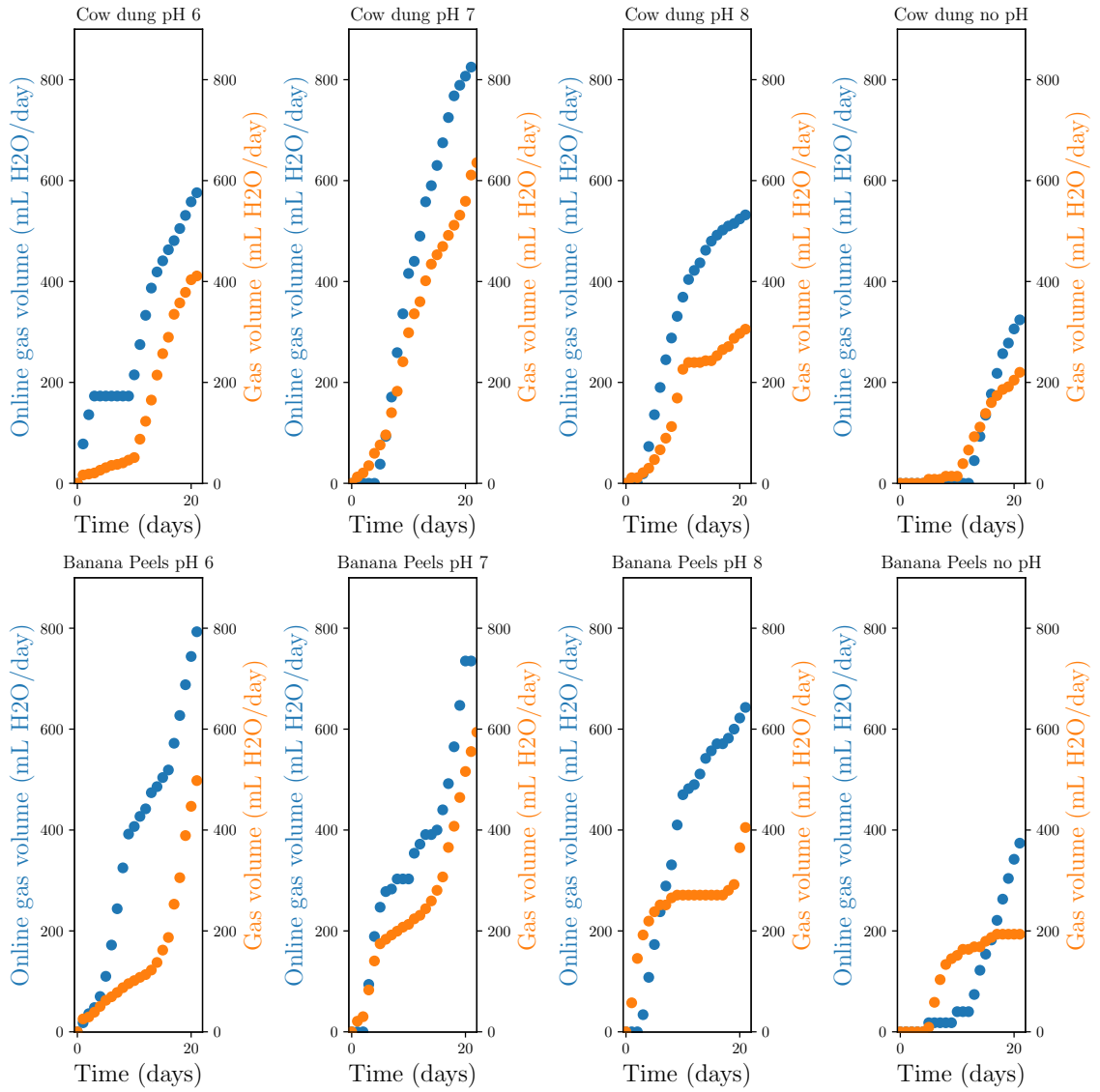


Figure 16: A comparison of the cumulative amount of water displaced by the gas created for the CDS *versus* the DDS at different pH set points. The orange represents the DDS whereas the blue represents the CDS.

Figure 16 shows the most notable differences between the two different set-ups, there seems to be more periods in which the gas production stagnates for the DDS runs, however, for the CDS runs the gas production seems to be more consistent. The gas production typically increases steadily for the CDS runs whereas there seems to be more frequent periods in which gas production stagnates for the DDS runs. This could be attributed to the fact that the methanogens (bacteria responsible for methane production) are typically sensitive to pH fluctuations (Certification, 1992), These pH fluctuations are inherently more drastic in the DDS because pH corrections were only performed once a day, whereas pH corrections were performed every minute in the CDS. The delay in pH corrections in the DDS resulted in more drastic pH fluctuations compared to the CDS

which most likely disrupts the methanogenic bacterial activity. This leads to an average percentage increase of 50 % in gas production from the DDS to CDS. Figure 17 shows the pH values of both set-ups. For the DDS experiments, each data point represents the pH before any dosing corrections were made on a particular day. Figure 17 clearly illustrates how the delay in pH corrections in the DDS resulted in more drastic pH fluctuations; whereas the CDS pH values stayed relatively close to the set point.

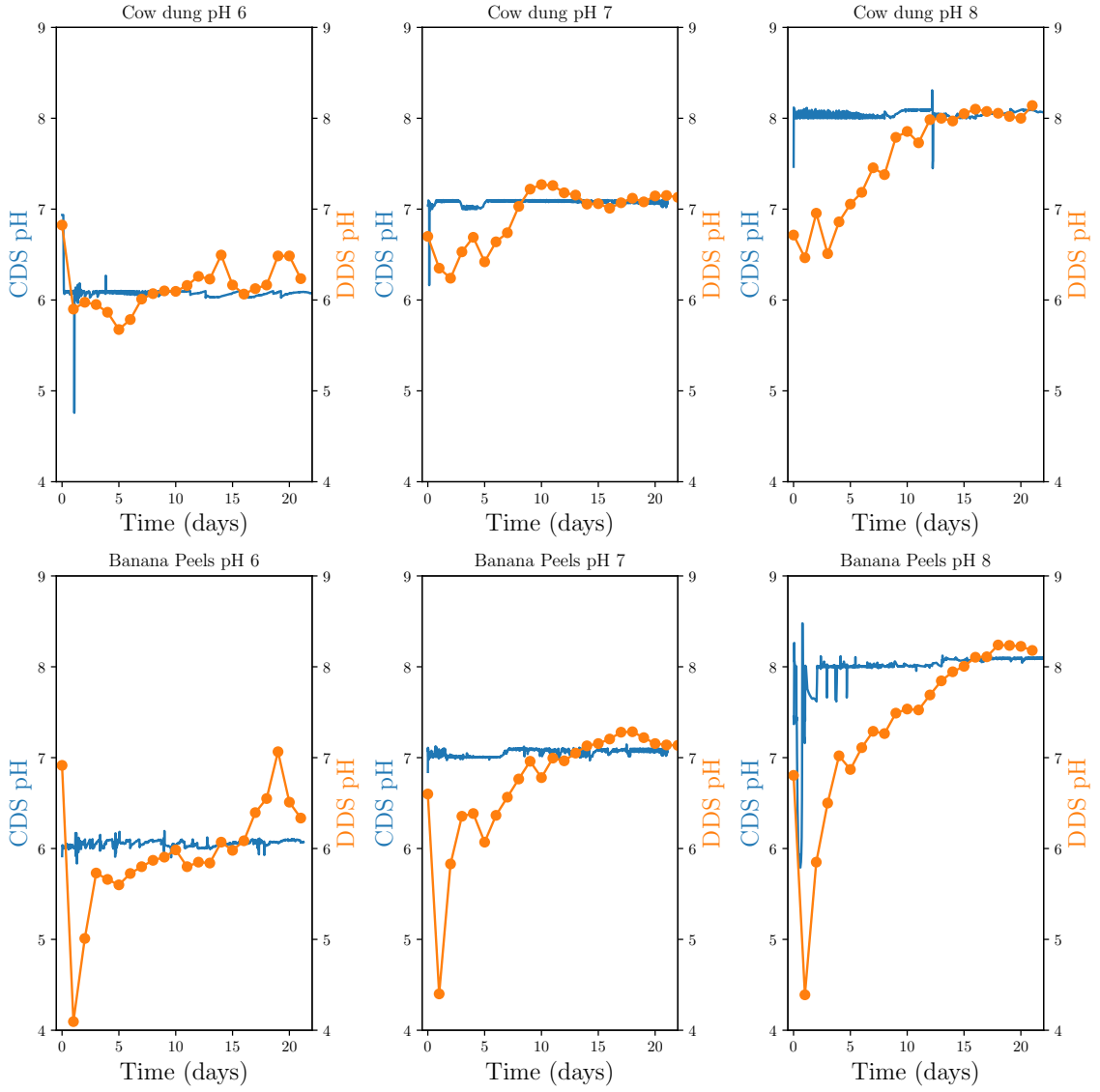


Figure 17: A comparison of the pHs for the CDS *versus* DDS at different pH set points. The orange represents the DDS whereas the blue represents the CDS. FOr the DDS experiments, each data point represents the pH each day before any dosing corrections were made.

5 Conclusions and Recommendations

It is evident that pH control has a profound effect on the ammonium release rate as well as the gas production rate. A pH of 7 is clearly the optimal set point for both ammonium release as well as the gas production rate. This pH value provided average percentage differences of 20 % and 22 % in terms of ammonium release and gas production when compared to the runs that were performed without pH control. The results also show that the substrate that contained a larger amount of easily accessible protein produced significantly more ammonium compared to the more lignocellulosic substrates that were tested. In addition, it was noted that the enhanced ammonium concentrations from the protein-rich substrate did not significantly affect the amount of base required for neutralisation.

The substrate had a strong influence on the pH switch point from base to acid dosing. The actual pH set point had a significant effect on the switch point on the protein-lean substrates. However, the differences in pH values are largely insignificant for the protein-rich substrate indicating that the additional amino acid breakdown that is required for the lentil substrate does not have in influence on acidifying the mixture.

There appeared to be an inherent inhibitory effect on both gas and ammonium production associated with a low pH at the beginning of the anaerobic digestion process. This inhibitory effect was not observed at higher pH values. The switch point was observed to be crucial in terms of gas production. There was a clear acceleration in the gas production observed after the dosing switch point.

In terms of the comparative analysis between the CDS and the DDS, there were differences present in the gas production profiles, with the CDS providing enhanced rates compared to the DDS. The CDS provided an average percentage increase of 50 % compared to the DDS in terms of gas production. There was a negligible difference in the ammonium release rate between the different set-ups, which indicates that precise pH control has a more pronounced effect on the methanogenesis phase of anaerobic digestion compared to the hydrolysis, acidogenesis, and acetogenesis steps.

A recommendation for future experiments is to control the pH at different set points (*i.e.* controlling the pH at a lower value at the beginning of the experiment and then increasing the set point in the second half of the experiment) as this could ameliorate the different steps in the anaerobic digestion process. Another recommendation would be to determine the C/N ratios of each feedstock (this was not possible in this study due to a limited amount of analytic equipment). An additional recommendation would be to determine a solid conversion for each run.

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