Studies of sludge collected from the anaerobic digester of a meat processing company

A thesis
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by

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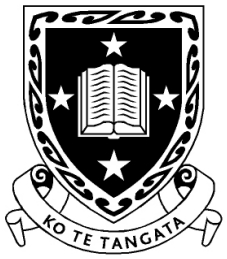

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Abstract

Anaerobic digestion is commonly used as a wastewater treatment step where complex biological substrates are progressively degraded in the absence of oxygen to produce methane and carbon dioxide with hydrogen and volatile acids occurring as intermediate products. These intermediate products are more valuable commodities than methane so there is interest in optimizing their production and recovery from the anaerobic digestion process. In previous work, a sludge sample collected from a local meat processing company was reported to produce significant amounts of hydrogen at ambient temperatures.

In the present study, eleven sludge samples were collected from the same meat processing company and characterized in terms of their solids content, pH, as-collected gas production profiles and gas production profiles and gas production rates, when repetitively batch fed with glucose at their original pH and also at pHs successively lowered to pH 4.5. Similar studies were performed using cellulose as the substrate.

In these studies, no hydrogen was produced by the as-collected sludges, but hydrogen was produced by two of the sludges when batch fed with glucose. Detailed studies of the effect of pH on one of the sludges revealed that hydrogen was produced when the pH was lowered to between 5.2 and 5.4 and batch fed with glucose. No hydrogen was formed when the sludges were batch fed with cellulose under the conditions investigated. Acid conditions severely inhibited gas production rates when both glucose and cellulose were used as substrates. Gas production rates with cellulose substrate were systematically slower than when glucose was used as the substrate.

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Chapter 1 Introduction

1.1 General introduction

Anaerobic digestion is a series of processes in which microorganisms break down biodegradable material in the absence of oxygen. It is widely used to treat wastewater and wastewater sludges and other organic wastes. As part of integrated waste management systems, anaerobic digestion reduces the emissions of landfill gas into the atmosphere. Anaerobic digestion is a renewable energy source because the process produces a methane and carbon dioxide rich biogas suitable for energy production so helping replace fossil fuels. Also, the nutrient-rich solids and fluid left after digestion can be used as fertilizer.

The digestion process begins with bacterial hydrolysis of the input materials in order to break down insoluble organic polymers such as carbohydrates and make them available for other bacteria. Acidogenic bacteria then convert the sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids. Acetogenic bacteria convert these resulting organic acids into acetic acid, along with additional ammonia, hydrogen, and carbon dioxide. Methanogens, finally are able to convert these products to methane and carbon dioxide.

While methane provides a valuable energy product from the digestion process, intermediate products, particularly acetic acid and hydrogen are potentially more valuable. Exploring how the anaerobic digestion can be optimized to produce hydrogen was part of the general aim of the present investigation.

1.2 Background and literature review

This section will describe the historical use of anaerobic digestion in the treatment of biodegradable waste materials and will review recent literature.

1.2.1 The history of anaerobic digestion

Scientific interest in the gasses produced by the natural decomposition of organic matter, was first reported in the seventeenth century by Robert Boyle and Stephen Hale, who noted that flammable gas was released by disturbing the sediment of streams and lakes. In 1808, Sir Humphry Davy determined that methane was present in the gasses produced by cattle manure. The first anaerobic digester was built by a leper colony in Bombay, India in 1859. In 1895 the technology was developed in Exeter, England, where a septic tank was used to generate gas for street lighting. Also in England, in 1904, the first dual purpose tank for both sedimentation and sludge treatment was installed in Hampton. In 1907, in Germany, a patent was issued for the Imhoff tank, an early form of digester. (Wikipedia, 2010)

Through scientific research anaerobic digestion gained academic recognition in the 1930s. This research led to the discovery of anaerobic bacteria, the microorganisms that facilitate the process. Further research was carried out to investigate the conditions under which methanogenic bacteria were able to grow and reproduce. This work was developed during World War II where in both Germany and France there was an increase in the application of anaerobic digestion for the treatment of manure. (Wikipedia, 2010)

1.2.2 The stages of anaerobic digestion

As the process flow diagram for anaerobic digestion is shown in Figure 1-1, upon harvesting or collection, biomass or wastes are chopped and ground for size reduction, and may be subjected to pre-treatment to enhance biodegradation. After primary digestion, suspended solids in the effluent are settled in an anaerobic secondary digester. Some of the active sludge is recycled and the remainder is further processed for use as a fertilizer or animal feed, or subjected to dewatering and/or thermal conversion prior to disposal or combustion. Supernatant may be recycled or processed into a form suitable for disposal. Product gas can be utilized directly or treated to remove carbon dioxide and traces of hydrogen sulphide (Chynoweth and Isaacson 1987).

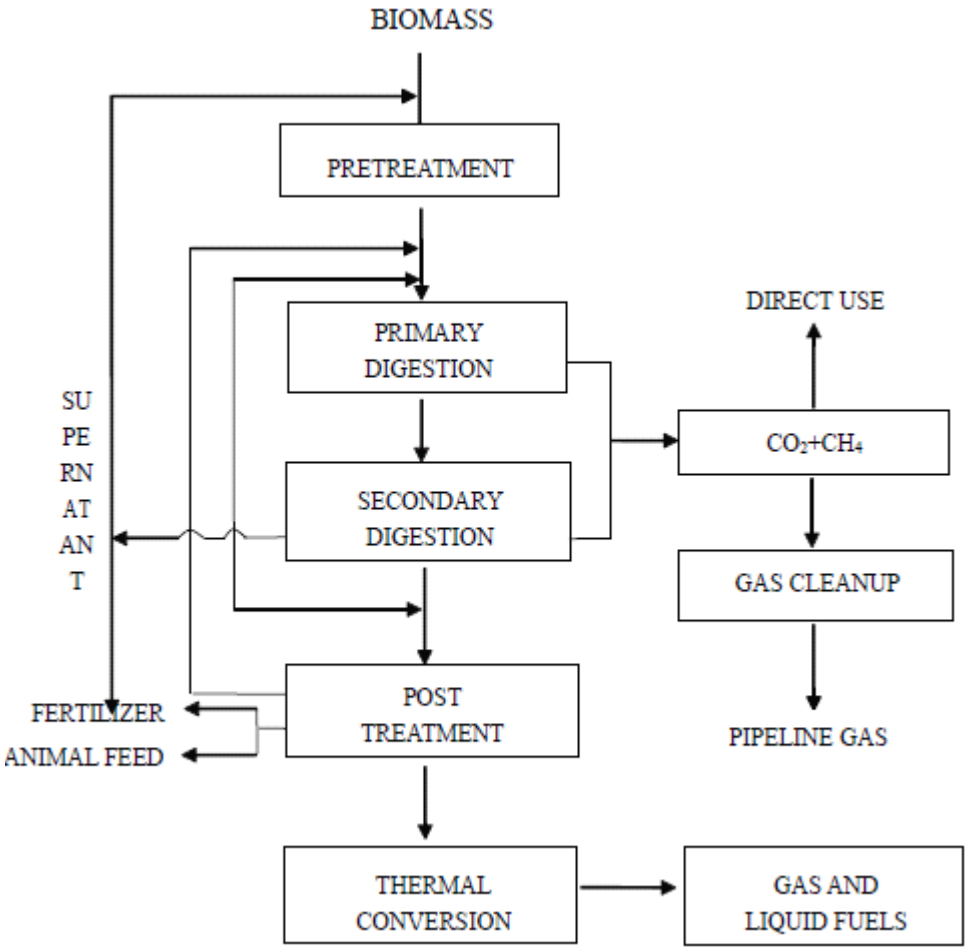


Figure 1-1. Generalized anaerobic digestion process scheme (Li, 2007)

1.2.3 Principle and operation of anaerobic digestion

Anaerobic digestion is a complex process which requires strict anaerobic conditions (oxidation reduction potential (ORP) < -200 mV) to proceed, and depends on the coordinated activity of a complex microbial association to transform organic material into mostly CO2 and methane (CH4) (Qasim, 1999).

Microbial methanogenesis is a natural process occurring in anaerobic environments such as ocean and lake sediments and animal digestive tracts (Chynoweth and Isaacson 1987). Anaerobic digestion processes are widely used in wastewater treatment. The overall anaerobic conversion of biodegradable organic solids to the end products CH_4 and CO_2 was initially

believed to proceed in three stages which occurred simultaneously within the digester. These were: hydrolysis of insoluble biodegradable polymers; the production of acid from smaller soluble organic molecules and CH_4 generation. These stages are generally referred to as hydrolysis, acetogenesis and methanogenesis (Stronach, Rudd et al. 1986).

The hydrolysis step degrades both insoluble organic material and high molecular weight compounds such as lipids, polysaccharides, proteins and nucleic acids, into soluble organic substances (e.g. amino acids and fatty acids) (Appels, Baeyens et al. 2008).

The components formed during hydrolysis are further split during acidogenesis. VFA are produced by acidogenic (or fermentative) bacteria along with ammonia (NH_3), CO_2, H_2S and other by-products (Ghyoot and Verstraete, 1997).

The second stage in anaerobic digestion is acetogenesis, where the higher organic acids and alcohols produced by acidogenesis are further digested by acetogens to produce mainly acetic acid as well as CO_2 and H_2 (Appels, Baeyens et al. 2008). This conversion is controlled to a large extent by the partial pressure of H_2 in the mixture (Wang, Kuninobu et al. 1999).

The final stage of methanogenesis produces methane by two groups of methanogenic bacteria: the first group splits acetate into methane and carbon dioxide and the second group uses hydrogen as electron donor and carbon dioxide as acceptor to produce methane (Appels, Baeyens et al. 2008).

The rate limiting step in the digestion of soluble organic matter from the above scheme was considered to be the production of CH_4 from fatty acid degradation (Stronach, Rudd et al. 1986).

Anaerobic reactors present a unique ecosystem in which diverse groups of bacteria catalyse the conversion of complex organic compounds to methane and carbon dioxide in a highly controlled and coordinated fashion.

Anaerobic degradation of organic matter in a reactor is generally considered to be a two-phase process in which the acidogenic and the methanogenic bacteria must be in a state of dynamic equilibrium, in which the volatile fatty acid (VFA) and other fermentation end-products of hydrolytic/ fermentative bacteria are directly converted to CH_4 and CO_2 by methanogenic species. There were two reactor digesters which have been described by Vavilin, Rytow et al. (1995). In the two reactor digesters, the acidogenic phase is separated from the methanogenesis phase by taking into consideration the difference in growth rates of acidogens and methane-formers. It has been reported in a number of papers that the optimal pH of the acidification process is about 6.0, while the optimal pH of a methanogenic reactor is about 7.0. Recycling of the effluent in a two-phase anaerobic system has been shown to reduce the consumption of alkali required to maintain the pH level in the acidic reactor (Vavilin, Rytow et al, 1995).

The multiphase nature of the process has been revealed by the discovery of hydrogen-producing acetogenic bacteria and by a better appreciation of the limited substratecapabilities of methanogens (Torpy, 1988), and this is displayed in the schematic of figure 1-2 (Li, 2007).

As shown in Figure 1-2, obligate H_2-producing acetogens (OHPA species) oxidize VFA fermentation products, such as propionate, butyrate, etc., to acetate, CO_2 and H_2. OHPA species are also known to be involved in the ß-oxidation of longer-chain fatty acids (stearate, oleate, etc.) (Torpy 1988).

Methane fermentation is a versatile biotechnology capable of converting almost all types of polymeric materials to methane and carbon dioxide under anaerobic conditions. This is achieved as a result of the consecutive biochemical breakdown of polymers to methane and carbon dioxide in an environment in which varieties of microorganisms, including fermentative microbes (acidogens); hydrogen-producing, acetate-forming microbes (acetogens); and methane-producing microbes (methanogens) harmoniously grow and produce reduced end-products.

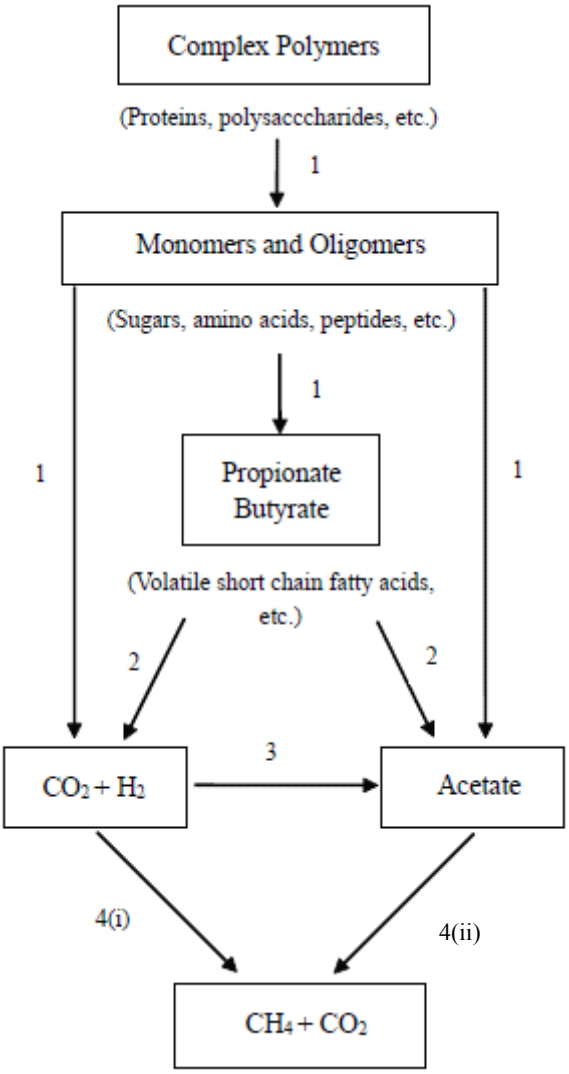


Figure 1-2. Multiphase Nature of Anaerobic Digestion.

1: hydrolytic and non-hydrolitic fermentative bacteria
2: syntrophic acetogens (obligate H_2-producing acetogens)
3: homoacetogens
4.(i): hydrogenotrophic methanogens
(ii): aceticalastic methanogens

1.2.4 The process microbiology of anaerobic digestion

Consortia of microorganisms, mostly bacteria, are involved in the transformation of complex high-molecular-weight organic compounds to methane. Furthermore, there are synergistic interactions between the various

groups of bacteria implicated in anaerobic digestion of wastes. Although some fungi and protozoa can be found in anaerobic digesters, bacteria are undoubtedly the dominant microorganisms. Large numbers of strict and facultative anaerobic bacteria are involved in the hydrolysis and fermentation of organic compounds. There are four categories of bacteria that are involved in the transformation of complex materials into simple molecules such as methane and carbon dioxide. These bacterial groups operate in a synergistic relationship in as much as group 1 has to perform its metabolic action before group 2 can take over, etc.

(1) Group 1-- Hydrolytic bacteria

Consortia of anaerobic bacteria break down complex organic molecules (proteins, cellulose, lignin, and lipids) into soluble monomer molecules such as amino acids, glucose, fatty acids, and glycerol. The monomers are directly available to the next group of bacteria. Hydrolysis of the complex molecules is catalyzed by extracellular enzymes such as cellulases, proteases, and lipases. However, the hydrolytic phase is relatively slow and can be limiting in anaerobic digestion of waste such as raw cellulolytic wastes, which contain lignin.

(2) Group 2-- Fermentative acidogenic bacteria

Acidogenic (i.e., acid-forming) bacteria convert sugars, amino acids, and fatty acids to organic acids (e.g., acetic, propionic, formic, lactic, butyric, or succinic acids), alcohols and ketones (e.g., ethanol, methanol, glycerol, acetone), acetate, carbon dioxide, and hydrogen. Acetate is the main product of carbohydrate fermentation. The products formed vary with the type of bacteria as well as with culture conditions (temperature, pH, redox potential).

VFA are produced by acidogenic (or fermentative) bacteria along with ammonia (NH_3), CO_2, H_2S and other by-products .

(3) Group 3-- Acetogenic bacteria

Acetogenic bacteria convert fatty acids (e.g., propionic acid, butyric acid) and alcohols into acetate, hydrogen, and carbon dioxide, which are used by the methanogens. This group requires low hydrogen tensions for fatty acid conversion; and therefore a close monitoring of hydrogen concentrations is necessary. Under relatively high H_2 partial pressure, acetate formation is reduced and the substrate is converted to propionic acid, butyric acid and ethanol rather than methane.

(4) Group 4-- Methanogens

Anaerobic digestion of organic matter in the environment releases 500-800 million tons [453.6-725.75 metric tons] of methane per year into the atmosphere and this represents 0.5% of the organic matter derived from photosynthesis. The fastidious methanogenic bacteria occur naturally in deep sediments or in the rumen of herbivores. This group is composed of both gram-positive and gram-negative bacteria with a wide variety of shapes. Methanogenic microorganisms grow slowly in wastewater and their generation times range from 2 days at 35°C too as high as 50 days at 10°C. About two thirds of methane is derived from acetate conversion by methanogens. The other third is the result of carbon dioxide reduction by hydrogen.

Molecular hydrogen is formed during different stages of anaerobic digestion. In the hydrolysis stage the bacteria produce fatty acids, carbon dioxide and hydrogen from carbohydrates. During the acetogenesis, bacteria (Syntrophobacter wolinii or Syntrophomonas wolfei) produce acetate, carbon dioxide and hydrogen, or acetate and hydrogen by anaerobic oxidation of propionate and n-butyrate (Rehm, Reed et al. 2000). In this last stage, hydrogen can only be formed when it is consumed by methanogenic bacteria so it does not accumulate (Appels, Baeyens et al. 2008). This can also be achieved by the activity or sulphate reducing bacteria via interspecies electron transfer. The hydrogen concentration can also be decreased in sewage sludge by acetate formation from CO_2 and H_2 (Rehm, Reed et al. 2000).

Acetogenesis of fatty acids or of other reduced metabolites may only function if hydrogen does not accumulate but is consumed by methanogens. In sludge digesters, the hydrogen concentration may be decreased by acetate formation from carbon dioxide and hydrogen.

Several studies determined the effect of hydrogen partial pressure, $p_{H2,}$ on the production of acetic acid, propionic acid and butyric acid (Masse and Droste, 2000). Conversions of propionic acid and butyric acid to acetic acid were found to be thermodynamically possible only when p_{H2} is less than 10^{-4} for n-butyric acid and 10^{-5} atm for propionic acid. They also indicated that when p_{H2} is higher than 10^{-4} atm, the Gibbs free energy change is larger for CO_2 reduction than for the acetate cleavage, resulting in a reduction of CO_2 instead of an acetate cleavage. A decrease in H_2 concentration allows conversion of acetic acid to methane to resume (Rehm, Reed et al. 2000). The methanogenic and sulphate reducing activity of the respective micro-organisms is not sufficient to maintain p_{H2} at the required level (Appels, Baeyens et al. 2008). However, by reversed electron transport electrons may be shifted to a lower ORP suitable for proton reduction (Rehm, Reed et al. 2000).

VFA are the most important intermediates in the anaerobic digestion process, where they are degraded by proton-reducing acetogens in association with hydrogen consuming methanogenic bacteria (Mechichi and Sayadi, 2005). However, the production of VFA can be toxic to micro-organisms, especially to methanogens at a concentration of 6.7- 9.0 mol / m^3 (Batstone, Kelleret al. 2000). These increased concentrations are the result of accumulation due to process imbalances which can be caused by variation in temperature, organic overloading, toxic compounds, etc. (Mechichi and Sayadi, 2005). In such cases, the methanogens are not able to remove the hydrogen and volatile organic acids fast enough. As a result the acids accumulate and the pH decreases to such a low value that the hydrolysis/acetogenesis can be inhibited (Siegert, Banks et al. 2005).

1.2.5 The products of anaerobic digestion

There are three principal products of anaerobic digestion: biogas, digestate and water.

Biogas is the ultimate waste product of the bacteria feeding off the input biodegradable feedstock, and is mostly methane and carbon dioxide, with a small amount hydrogen and trace hydrogen sulfide. As-produced, biogas also contains water vapor, with the fractional water vapor volume a function of biogas temperature (Richards and Cummings, 1991). Most of the biogas is produced during the middle of the digestion, after the bacterial population has grown, and tapers off as the putrescible material is exhausted. The typical composition of biogas is shown in Table 1-1.

Matter	%
CH_4	50-75
CO_2	25-50
N_2	0-10
H_2	0-1
H_2S	0-3
O_2	0-2

Table 1-1. The typical composition of biogas.

Digestate is the solid remnants of the original input material to the digesters that the microbes cannot use. It also consists of the mineralised remains of the dead bacteria from within the digesters. Digestate can come in three forms; fibrous, liquor or a sludge-based combination of the two fractions.

The final output from anaerobic digestion systems is water. This water originates both from the moisture content of the original waste that was treated but also includes water produced during the microbial reactions in the digestion systems. This water may be released from the dewatering of the digestate or may be implicitly separate from the digestate. The wastewater exiting the anaerobic digestion facility will typically have elevated levels of biochemical oxygen demand (BOD) and chemical oxygen demand (COD), these are measures of the reactivity of the effluent and show an ability to pollute. If this effluent was put directly into watercourses

it would negatively affect them by causing eutrophication (Dosta, Gali et al. 2007). As such further treatment of the wastewater is often required.

1.2.6 Influence of the conditional factors in the processes of anaerobic digestion

Several environment factors can affect anaerobic digestion, either by enhancing or inhibiting parameters such as specific growth rate, decay rate, gas production, substrate utilisation, start-up and response to changes input. During the influence factors, temperature, pH, nutrients are described below:

(1) Temperature

Temperature is one of the major influences on all of the above. It has an important effect on the physicochemical properties of the components found in the digestion substrate. It also influences the growth rate and metabolism of micro-organisms and hence the population dynamics in the anaerobic reactor (Appels, Baeyens et al. 2008).

The mesophilic range (25-45 °C) is generally used in anaerobic biological reactor systems as the number of thermophilic anaerobic species is small (Zinder, Anguish et al. 1984). Acetotrophic methanogens are one of the most sensitive groups to increasing temperatures. The degradation of propionate and butyrate is also sensitive to temperatures above 70 °C. The temperature has moreover a significant effect on the partial pressure of H_2 in digesters, hence influencing the kinetics of the syntrophic metabolism. Thermodynamics show that endergonic reactions (under standard conditions), for instance the breakdown of propionate into acetate, CO_2, H_2, would become energetically more favorable at higher temperature, while reactions which are exergonic (e.g. hydrogenotrophic methanogenesis) are less favoured at higher temperatures (Rehm, Reed et al. 2000).

An increasing temperature has several benefits, including an increasing solubility of the organic compounds, enhanced biological and chemical reaction rates, and an increasing death rate of pathogens (thermophilic

conditions) (Boe, 2006).

However, the application of high temperatures (thermophilic) has counteracting effects: there will be an increase of the fraction of free ammonia, which plays an inhibiting role for the microorganisms (Rehm, Reed et al. 2000); but the increasing pKa of the VFA will make the process more susceptible to inhibition (Boe, 2006). Control is thus a very sensitive issue for thermophilic as compared to mesophilic digestion.

Thermophilic bacteria are typically considered to exit and grow within the range 55-80°C; their enzyme systems are physiologically stable at these elevated temperatures, a condition attributable to the presence of heat-stable macromolecules. The optimum temperature of growth of anaerobic microorganisms is 35°C or greater. Because of their slower growth compared with acidogenic bacteria, methanogenic bacteria are very sensitive to small changes in temperature (Stronach, Rudd et al. 1986).

(2) pH

pH is an important parameter in the process of anaerobic digestion. Each group of micro-organisms has a different optimum pH range (Turovskiy, Mathai et al. 2006). The methane bacteria should be held in the pH range of 6.8-7.2 for uninhibited methane formation; for the acid-forming bacteria a more acid pH range is desirable (Stronach, Rudd et al. 1986). Inhibition of the methanogenic step by low pH can lead to an accumulation of volatile fatty acids and the 'souring' of the reactor. The control of pH is fundamental to the maintenance of optimal bacterial growth and/or conversion processes in anaerobic microbial systems (Li, 2007).

The fermentative microorganisms are somewhat less sensitive and can function in a wider range of pH between 4.0 and 8.5 (Hwang, Jang et al. 2004): At low pHs the main products are acetic and butyric acid, while at a pH of 8.0, mainly acetic and propionic acid are produced (Boe, 2006).

The VFAs produced during anaerobic digestion tend to reduce the pH. This

reduction is normally countered by the activity of the methanogenic bacteria, which also produce alkalinity in the form of carbon dioxide, ammonia and bicarbonate (Turovskiy, Mathai et al. 2006). The system pH is controlled by the CO_2 concentration in the gas phase and the HCO_3-alkalinity of the liquid phase. If the CO_2 concentration in the gas phase remains constant, the possible addition of HCO_3-alkalinity can increase the digester pH. A buffering capacity of 70meq $CaCO_3$ / l or a molar ratio of at least 1.4 : 1 of bicarbonate / VFA should be maintained for a stable and well buffered digestion process although it has been shown that especially the stability of the ratio is of prime importance, and not so much its level (Turovskiy, Mathai et al. 2006).

Control of pH within the growth optimum of microorganisms may reduce ammonia toxicity (Bhattacharya and Parkin, 1989). Reducing pH from 7.5 to 7.0 during thermophilic anaerobic digestion of cow manure also increased the methane production by four times (Zeeman, Wiegant et al. 1985). During anaerobic digestion of liquid piggery manure (pH 8), VFAs accumulated to 316 mg/L. Adjustment of pH to 7.4 led to reutilization of VFAs and lowered VFAs concentrations to 20 mg/L. The better performance at pH 7.4 has been attributed to the relief of ammonia-induced inhibition at low pH (Braun, Huber et al. 1981). It should also be noted that both methanogenic and acidogenic microorganisms have their optimal pH. Failing to maintain pH within an appropriate range could cause reactor failure although ammonia is at a safe level (Kroeker, Schulte et al. 1979).

(3) Nutrients

The concentration of main nutrients, such as carbon and nitrogen, can affect the growth of microorganisms and the production of biogas. The optimum ratios of carbon-to-nitrogen for the maximum biogas generation have been suggested to be between 20: 1 and 30: 1 (Rodtong and Anunputtikul, 2005). The presence of trace metals such as molybdenum, selenium (formate dehydrogenase, acetogenic bacteria, methanococcus vanielii etc.), tungsten (formate dehygrogenase, acetogenic bacteria etc.) and nickel (carbon monoxide dehydrogenase, Cl. Pasteurianum etc.) is probably necessary for

the activity of several enzyme systems (Stronach, Rudd et al. 1986).

1.2.7 The advantages and disadvantages of anaerobic digestion (compared with aerobic digestion) in wastewater treatment

(1) The advantages of anaerobic digestion

Anaerobic digestion is particularly suited to wet organic material and is commonly used for effluent and sewage treatment. During the processes of it, there are many advantages which are in the following:

a. Anaerobic digestion uses readily available carbon dioxide as an electron acceptor. It requires no oxygen, the supply of which adds substantially to the cost of wastewater treatment.
b. Anaerobic digestion produces lower amounts of sludge (3-20 times less than aerobic processes).
c. Anaerobic digestion produces methane, which is a useful gas.
d. Energy required for wastewater treatment is reduced.
e. Anaerobic digestion is suitable for high strength industrial wastes.
f. It is possible to apply high loading rates to the digester.
g. Rapid response to substrate addition after long periods without feeding.
h. Process more effectively provides sanitisation / removal of diseases.

(2) The disadvantages of anaerobic digestion (compared with aerobic digestion)

a. Longer star-up time to develop necessary biomass inventory.
b. May require alkalinity and/or specific ion addition, and may require further treatment with an aerobic treatment process to meet discharge requirements.
c. Biological nitrogen and phosphorus removal is not possible.
d. Much more sensitive to the adverse effect of lower temperatures on reaction rates, and may need heating (often by utilisation of process gas) to achieve adequate reaction rates.
e. Increased potential for production of odors and corrosive gases, and hazards may arise from explosion.

(3) Discussion of the advantages of anaerobic treatment processes

Of the advantages cited above, energy consideration, lower biomass yield, fewer nutrients required, and higher volumetric loading are usually considered to be the most important points in the wastewater treatment processes. Therefore, although there are some disadvantages of it, for wastewater with much higher biodegradable COD concentrations and elevated temperatures, anaerobic processes may be more economical.

1.2.8 Feed-stocks for anaerobic digestion

The most important initial issue when considering the application of anaerobic digestion systems is the feedstock to the process.

While the traditional feed-stocks for anaerobic digestion have typically been the degradable solids produced in wastewater treatment, a variety of other agricultural and industrial wastes have been successfully stabilised by anaerobic digestion.

Animal waste includes voided waste from livestock and poultry, wastewater, feedlot runoff, silage juices, bedding, and feed (Zeeman, Wiegant et al. 1985). These wastes are a substantial contributor to non-point source pollution and can affect wetland habitats and contaminate drinking water sources (Krylova, Khabiboulline et al. 1997). Animal waste often has very high total ammonia nitrogen concentrations due to the presence of ammonia as well as protein and urea that readily release ammonia upon anaerobic treatment (Hansen, Angelidaki et al. 1998). Consequently, the principal instability associated with the anaerobic digestion of animal waste is ammonia inhibition (Chen, Cheng et al. 2008). Sudden increases in ammonia concentration in the feedstock are unusual (Hobson, 1991). However, feed slurry that has been stored for some time in the animal house often contains high concentration of ammonia released from decomposition of organic nitrogen. Shock loading of this feed slurry can cause inhibition of anaerobic digesters (Chen, Cheng et al. 2008). In addition to ammonia, swine manure also contains a high sulfate concentration derived from a

protein-rich diet. The inhibition caused by ammonia and by sulfide influences each other (Hansen, Angelidaki et al. 1999). Feed additives (antibiotics chemotherapeutics) for improving food utilization and disinfectants for preventing infectious diseases have been widely used in intensive animal production (Hilpert, Winter et al. 1984). In most cases, these compounds are in very low concentrations (less than 30 ppm) in the waste and are generally not inhibitory (Hobson, 1991). However, some synthetic chemotherapeutics such as Olaquindox may be strongly inhibitory even at 1 mg/L (Hilpert, Winter et al., 1984). This concentration may be reached in practice and special treatments such as pre-dilution may be needed before anaerobic digestion (Poels, Assche et al. 1984).

Crop residues represent another fraction of agricultural waste. Substantial quantities of unused stalks, straws, and bark are produced from a variety of crops, which could be used for energy generation (Kalra and Panwar, 1986). Crop residues typically contain a high lignocellulosic content. Problems such as low gas yield during anaerobic digestion of these materials are usually associated with a high C/N ratio or high lignin content. In addition, the inhibition caused by pesticide and herbicide residues would affect digestion process kinetics (Khalil, Whitmore et al. 1991). Certain plants generate resin extracts which protect them from biological damage. These extracts may be inhibitory to the digestion process (Chynoweth and Isaacson, 1987).

Pretreatments such as acid or base hydrolysis are often employed before anaerobic digestion to increase biogas yield. However, byproducts formed in the pretreatment (fufural, hydroxymethyl fufural, formic acid, and levulinic acid) are potential inhibitors of anaerobic digestion. Microorganisms may eventually adapt and/ or degrade these byproducts, but process kinetics could be affected (Chen, Cheng et al. 2008).

Anaerobes can breakdown material to varying degrees of success from readily in the case of short chain hydrocarbons such as sugars, to over longer periods of time in the case of cellulose and hemicellulose. Anaerobic microorganisms are unable to break down long chain woody molecules such

as lignin. Anaerobic digesters were originally designed for operation using sewage sludge and manures. Sewage and manure are not, however, the material with the most potential for anaerobic digestion as the biodegradable material has already had much of the energy content taken out by the animal that produced it. Therefore, many digesters operate with co-digestion of two or more types of feedstock. For example, in a farm-based digester that uses dairy manure as the primary feedstock the gas production may be significantly increased by adding a second feedstock; e.g. grass and corn (typical on-site feedstock), or various organic byproducts, such as slaugtherhouse waste, fats oils and grease from restaurants, organic household waste, etc.

1.2.9 Recent developments in anaerobic digestion

Hydrogen is a potentially good fuel source for power generation and is currently used in many important industrial applications such as the manufacture of fertilizers. Although hydrogen is typically not used as a fuel source, hydrogen is often thought of as an environmentally superior fuel to hydrocarbons because when hydrogen is burned, it reacts with oxygen to produce environmentally harmless water. Unfortunately, hydrogen does not exist in nature in useable quantities; consequently, it has to be manufactured.

Most hydrogen produced today is manufactured from hydrocarbons such as petroleum or natural gas. The disadvantage of manufacturing hydrogen from these is that they are expensive and non-renewable energy sources. Furthermore, hydrogen manufacturing using petroleum or natural gas consumes substantial amounts of energy and/or requires expensive catalysts, such as platinum based catalysts.

Anaerobic digestion provides a potential alternative to manufacturing hydrogen from petroleum and natural gas. Anaerobic digesters can produce hydrogen from inexpensive and renewable energy sources such as organic wastes (e.g. food processing waste and animal waste). Recent studies have shown that certain strains of bacteria (e.g. bacteria from the genus Clostridium) are particularly effective at producing hydrogen as a

by-product during anaerobic digestion of organic waste material (Hansen and Cheong, 2009).

One problem with digesting organic waste in an anaerobic digester is that organic waste such as manure includes naturally occurring bacteria. Many of these bacteria consume hydrogen. Eventually, an anaerobic digester fed with non-sterile material will create a bacterial culture that is a mixture of competing bacteria, some of which consume hydrogen. Without intervention, hydrogen-consuming bacteria will invariably grow until most or all of the hydrogen being produced is simultaneously consumed.

Several systems have been developed to allow hydrogen to be produced in an anaerobic digester. These systems typically require growing and maintaining pure strains of hydrogen-producing bacteria and sterilizing the material to be digested. These systems are not commercially viable because maintaining a pure strain of bacteria in a digester is difficult and sterilizing the material to be digested is very expensive. (Hansen and Cheong, 2009).

Recently, an improved method has been developed for obtaining quantities of hydrogen-producing bacteria. In this method, a mixed culture of bacteria is heat treated to destroy the hydrogen-consuming bacteria. The hydrogen-producing bacteria survive the heat treatment by creating spores. Thus the treated culture is enriched with hydrogen-producing bacteria as compared to hydrogen-consuming bacteria. The enriched culture is then used to seed an anaerobic digester. (Hansen and Cheong, 2009).

While the production of hydrogen from specially cultured bacteria in reactors that are kept free from competing bacteria is of interest, the inhibition of undesirable bacteria by control of reactor conditions is probably more commercially viable, particularly if the substrate to be used is a biological waste material that would be costly to sterilize.

1.2.10 Previous and current work at the University of Waikato

Li (2007) studied the possibility of extracting the acetic acid intermediate from model systems representing the fluids of an anaerobic digester. She

found that acetic acid could be successfully purged from 3% solutions of acetic acid. (Langdon and Li, 2007). This work has been continued with reactor systems and it has been found that providing the pH is allowed to fall to below pH 5, acetic acid can be purged and recovered as the calcium salt (Langdon, Li et al, 2009).

Early in 2009, a group of undergraduate students reported in a laboratory project report that hydrogen was the major component of the reactor gas produced at ambient temperature from the glucose fed sludge collected from the anaerobic digester of a local meat processing works (Langdon, 2009). This unexpected result provided motivation for the present research.

1.3 Aims of the current study

The general aims of the program of which the current project is part is to determine conditions under which anaerobic digestion can managed to produce higher value products than the methane usually collected. In particular interest is centered on inhibiting methanogenic conversion to methane of the anaerobic digestion intermediates, acetic acid and hydrogen, and extracting the acetic acid and hydrogen as more valuable commodity products.

The specific aims of the present work are:

1. Characterize sludges sampled from the anaerobic digester of a local meat processing plant that had previously been reported to produce hydrogen at ambient temperature. Determine the gas product profiles of the as-collected sludges..

2. Investigate gas production rates and product profiles after successive additions of glucose substrate. The easily digestible glucose substrate was expected to cause a lowering of reactor pH which in turn was expected to affect product composition favoring hydrogen production.

3. Investigate gas production rate and product profile as pH of reactor sludge is systematically lowered using mineral acid (HCl). Literature reports

indicate that methanogenisis is inhibited by low pH.

4. Determine gas production rates and product profiles an when alternative substrate (cellulose) is used in a reactor under similar conditions as used for glucose digestion.

Chapter 2 Materials and methods

2.1 Equipment

1. GC-TCD, PerKinElmer™ instruments, U.S.A
2. Overhead Stirrer, IKA® Labortechnik, RW20 digital, Germany
3. pH meter, Cyberscan 100, Singapore
4. Milli Q Ultrapure Water System, Quantum®, New Zealand
5. Volumetric measuring flask, 100mL, 'E-MIL' BORO, ± 0.10mL in 20°C, England
6. Cylinders, LMS, 250mL, 1000mL ± 5mL In 20°C, Germany
7. Schott Bottles, Schott Duran 500mL, Boeco 1L, 2L, Germany
8. Tube, LEDA-LON, NYLON 12 <1200 SERIES> 4mm DD <E9/598>
9. Oven, Contherm Scientific Ltd
10. Burettes 50ml Pipettes, Jaytec 50×0.1mL, Class B, Ex 20°C Tol: ± 0.1mL
11. Fume Cupboard

2.2 Materials

2.2.1 Laboratory chemicals

1. Glucose, D- Glucose anhydrous, C6H12O6=180.16, Spec. roto (@25 Deg.C) Min +52.5, Max +53.0 Deg. New Zealand
2. Cellulose, Sigmacell® , New Zealand
3. HCl, Hydrochloric Acid 36%, HCl=36.46, Assay Min 36.0, Max 39.0 %w/w, New Zealand
4. Standard gas, Matheson Tri Gas Inc, Micro, MAT 14, Grace Davision Discovery Science, New Zealand
5. Carrier gas, Helium, BOC, New Zealand
6. N_2, BOC, New Zealand
7. H_2, BOC, New Zealand

2.2.2 Anaerobic sludge

Active sludge was obtained from a local meat processing plant which used

anaerobic digestion as a first treatment for its general waste stream made up of processed waste from fellmongery as well as generic meat processing waste. The digester consisted of a clay-lined trench approximately 100 m long, 30 m wide and 6 m deep. It was covered by three large sections of black polythene membrane to collect gas produced and had a thick floating sludge layer that had accumulated over many years of operation.

The sampling points for the 11 samples collected are indicated on figure 2-1.

Figure 2-1. Sampling sites.

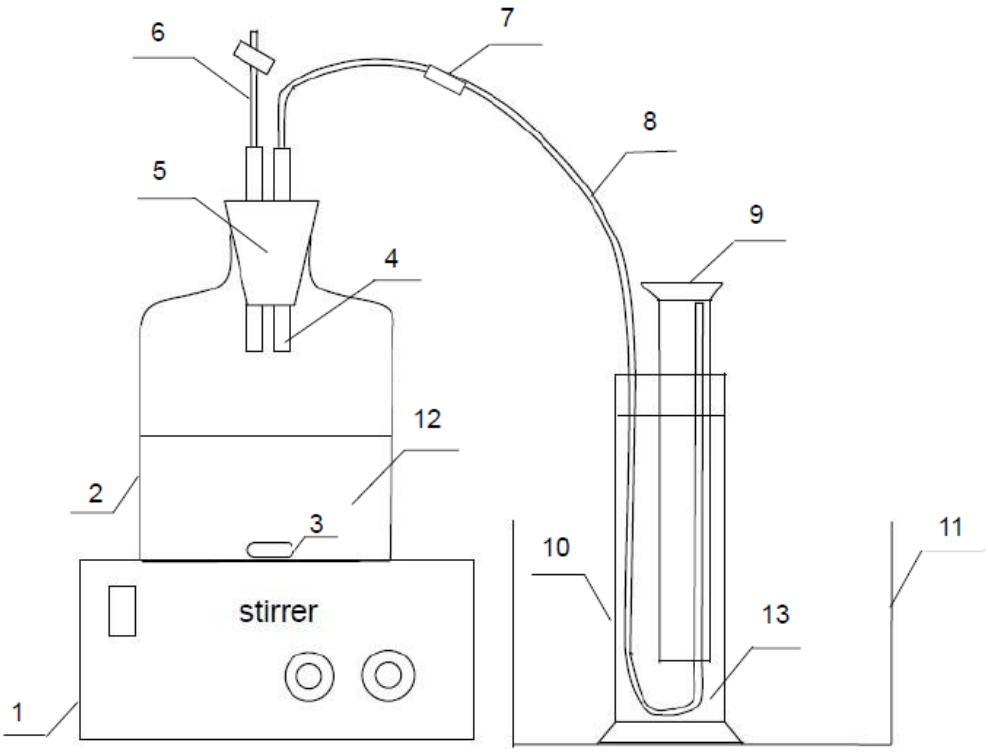
On Figure 2-1, 'S' means 'Sample' and the 11 samples were collected at distances from the outlet as indicated in brackets: S1 (0 m), S2 (4.2 m).

2.3 Methods

2.3.1 Bench scale anaerobic digester

Bench scale anaerobic digesters were used for the digestion studies. They consisted of a Schott bottle (500 mL, 1 L and 2 L) fitted with a two-hole stopper, delivery tubing and valves to control gas flow. The bottle was generally half filled with a sludge substrate and mixed by magnetic stirring. Gas production was measured by water displacement from a measuring cylinder, as illustrated in Figure 2-2. Because our interest was in methane

and hydrogen production, no attempt was made to ensure quantitative collection of the CO_2. Tap water was used in the measuring cylinder without addition of alkali to ensure complete removal of CO_2 or saturation with CO_2 to ensure complete recovery of CO_2.



1 - Stirrer. 2 - Schott bottle. 3 - Stir bar. 4 - Tube for gas getting out of the bottle. 5 - Rubber stopper. 6 - Tube for collecting gas from head space or connecting with a soft rubber tube to make an air lock. 7 - Tap for sucking gas from the measuring cylinder. 8 - Tube for transporting gas. 9 - Cylinder for measuring the gas production volume. 10 - Cylinder for containing water. 11- Tank. 12 - Sludge. 13 - Water.

Figure 2-2. Gas volume measurement system.

In order to ensure anaerobic conditions, it was necessary to purge the sludge head-space with N_2 for at least 5 minutes after addition of substrate to the sludge.

The measuring cylinder used to collect gas was inserted in a larger measuring cylinder and when the volume of water displaced was measured, the collection cylinder was raised until the water levels in each were the

same to ensure that the gas was at atmospheric pressure.

GC was used to analyze head space gas and the gas collected in the measuring cylinder. When gas samples were collected, care was taken to ensure sampling syringes and connecting tubing had been thoroughly purged with the gas to be analysed. In later work, on-line sampling through a 1 mL sampling loop was used.

2.3.2 GC set up

GC theory

The Perkin Elmer GC-TCD instrument was set up for gas analysis. During gas chromatographic separation, the sample is transported via an inert gas called the mobile phase. The mobile phase carries the sample through a coiled tubular column where analytes interact with a material called the stationary phase. For separation to occur, the stationary phase must have an affinity for the analytes in the sample mixture. The mobile phase, in contrast with the stationary phase, is inert and does not interact chemically with the analytes. The only function of the mobile phase is to sweep the analyte mixture through the length of the column.

The stationary phase is chosen so that the components of the sample distribute themselves between the mobile and stationary phase to varying degrees. Those components that are strongly retained by the stationary phase move slowly relative to the flow of the mobile phase. In contrast, components that have a lower affinity for the stationary phase travel through the column at a faster rate. As a consequence of the differences in mobility, sample components separate into discrete bands that can be analyzed qualitatively and quantitatively.

Components of the GC system

The components of the gas chromatographic system used included: (1) a carrier gas supply (argon), (2) a gas sample loop (1.0 mL) for sample introduction, (3) a gas- out tube for gas release, (4) the columns (poropak

and zeolite) and oven, (5) the detector and data collection system.

A thermal conductivity detector (TCD) consists of tiny coiled wires arranged in a Wheatstone bridge configuration. Electric current flows through the filaments making them glow hot, while carrier gas exiting the column flows past the other two filaments. The gas flow carries away excess heat, and the filaments equilibrate. When a sample compound exits the column, the thermal conductivity of the gas flowing around the filaments is changed. Therefore, the filaments get hotter and the balance of the Wheatstone bridge is altered, generating a signal that is amplified and transmitted to the data collection system.

The TCD is used to detect gaseous compounds, such as nitrogen, oxygen, and other non-hydrocarbon compounds. It is a destructive detector that can be used in series only after nondestructive detectors. The TCD has limited target analyte list. Because the TCD detects nitrogen, nitrogen cannot be used as a carrier gas. Details of the GC operation are included as Appendix A.1.

The configuration used in the present work was as follows:

Oven 40 °C, detector temperature 200 °C, injec 110, carrier gas helium 20.0 mL/ min, program time 2.5 min. The helium gas cylinder was set as 700 kPa, and the gas flow program was controlled by valves operated using switches activated by dry air at 500 kPa.
The composition of the reactor gas was determined as following:

1. The GC was calibrated using a 1 mL sampling loop and a certified gas mixture provided by Alltech Associates.

2. The gas to be analysed was passed through the sampling loop and a 1 mL sample was injected onto the column. The percentage of the component gases was calculated using the equation:

$$P_{sample} = \frac{A_{sample}}{A_{s\tan dard}} P_{s\tan dard}$$

Where P_{sample} = percentage composition of sample

$P_{standard}$ = percentage composition of standard

A_{sample} = area of sample

$A_{standard}$ = area of sample

This equation assumes linearity of detector response which is reasonable over the pressure range of less than 1 atmosphere used.

2.3.3 Total solids measurements

A well-mixed 10 mL sample of reactor contents was evaporated in a weighed dish and dried to constant weight in an oven at 104 °C over night, cooled in a desiccators and weighed. The increase in weight over that of the empty dish represents the total solids. The solids content was calculated from:

$$TS = \frac{A - B}{V} \quad ,$$

Where, TS= total solids g L^{-1},

A= weight of dried residue + dish, mg,

B= weight of dish, mg,

V= sample volume, mL.

When use mg as the unit of A and B, use mL as the unit of V, the unit of TS would be g/L.

Chapter 3 Characteristics of the sludges

The collected samples were labeled according to their sampling locations as sample 1 (S1) to sample 11 (S11) (Figure 2-1). However, sample 1 was collected at the exit position and was entirely liquid without any sludge. A large amount of Sample 6 was collected because it came from a site at the middle of the digester and close to where sludge had been sampled previously.

3.1 Characterization of sampled sludges

The method of determining total solids of each sample was shown in section 2.3.3.

Table 3-1. Average total solids of 11 samples.

Sample	Sampling site (m from exit)	Description	TS (g/L)	Standard deviation
S1	0	cloudy liquid, buff	1.43	0.26
S2	4.2	thick sludge, dark brown	51.38	0.19
S3	9.9	thick sludge, dark brown	65.40	0.19
S4	14.0	thick sludge, dark brown	46.73	0.19
S5	19.2	thick sludge, dark brown	66.56	0.17
S6	23.9	thick sludge, dark brown	54.61	0.10
S7	27.6	thick sludge, dark brown	83.74	0.13
S8	32.2	thick sludge, dark brown	52.64	0.03
S9	41.1	thick sludge, dark brown	56.69	0.18
S10	46.3	thick sludge, dark brown	37.41	0.09
S11	59.8	thick sludge, dark brown	44.91	0.17

3.2 Gas product composition formed by as-collected sludges

In a preliminary experiment, measured volumes of as-collected sludges were filled to the mark in 1-L reactor vessels fitted with stoppers and air locks and maintained at ambient temperature of 20 ± 2 ^{o}C. The head space was flushed with nitrogen gas and the reactors were allowed to stand. After periods ranging from 3 to 10 days the head space was connected to the GC system for gas composition determination.

Data for gas composition s determined are summarized in Table 3-2.

Table 3-2. Composition of gasses produced by the as –collected sludges.

	Percentage of H_2 in sample [%]	Percentage of CH_4 in sample [%]	Percentage of N_2 in sample [%]	Percentage of CO_2 in sample [%]
Sample 1	0	0	100	0
sample 2	0.00	2.66	95.58	0.00
sample 3	0.00	2.70	86.74	9.23
sample 4	0.00	7.79	79.62	9.74
sample 5	0.00	0.28	96.66	2.83
sample 6	0.00	2.85	85.46	11.68
sample 7	0.00	4.84	88.87	6.45
sample 8	0.00	7.54	77.32	14.46
sample 9	0.00	44.29	29.20	24.62
sample 10	0.00	6.76	81.19	11.50
sample 11	0.00	42.28	36.68	20.43

None of the as-collected samples produce H_2. The activity in terms of total gas production, as indicated by reduced nitrogen % was highly variable. There was a general trend towards greater activity toward the front end of the reactor and generally methane production correlated with CO_2 production.

The absence of H_2, though not surprising, was disappointing in view of the earlier reported (Langdon, 2009) for sludge collected earlier from the same reactor. However in the earlier investigation, the sludge produced H_2 after being fed with glucose substrate.

3.3 Gas production from the as collected Sample 6

The gas production rate from sample 6 was monitored over a period of 30 day to determine the background gas production profile. Results are summarized in figure 3-1.

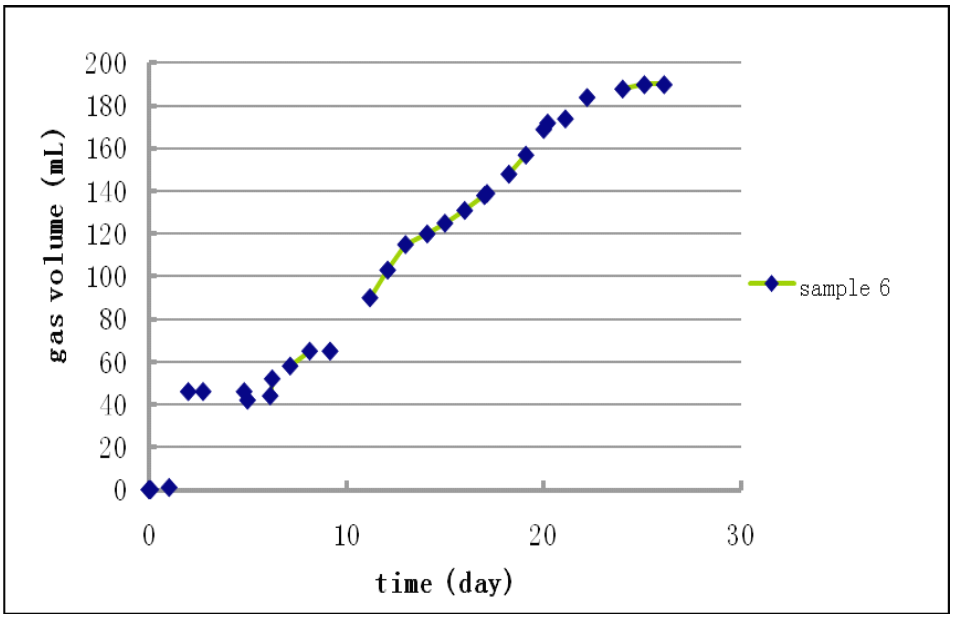


Figure 3-1 Gas production volume of the as collected sample 6 sludge.

After 20 day gas production had virtually ceased indicating that background gas production from the sludge was negligible.

Chapter 4 Studies of the anaerobic digestion of glucose

4.1 Composition of gas produced by aged sludges batch fed with glucose

In order to investigate whether the substrate was a factor in determining the composition of the gas produced, gas production by sludges (previously stored for more than 90 days to ensure minimal background gas production) was analyzed after successive additions of 1 g/L glucose substrate. The reactors (1L Schott bottles).were mounted on magnetic stirrers and fitted with delivery tubes to 250ml measuring cylinders inverted in 500mL measuring cylinders for collecting gas produced by water displacement. As shown in Figure 2-2. The gas produced was sampled as head space (HS) gas and gas collected in the measuring cylinder (MC) when gas production ceased. The experiment was repeated for eight cycles of glucose addition. Representative data for one cycle of glucose addition (cycle 5) are summarized in Table 4-1. The data for the eight cycles are given in Appendix A.2.

Table 4-1. Gas composition of sludge samples during the cycle 5 of glucose addition and sampled when gas production stopped.

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
Sample 2	0.7	0.0	33.4	50.5	57.8	22.1	7.9	27.0
Sample 3	0.0	0.0	0.0	14.8	0.0	78.8	0.0	5.9
Sample 4	0.0	0.0	44.6	51.5	38.2	8.1	14.4	39.9
Sample 5	0.0	0.0	0.0	16.7	0.0	49.1	0.0	32.2
Sample 6	0.2	0.0	6.8	24.3	92.9	58.1	0.0	17.5
Sample 7	0.0	0.0	35.8	52.2	50.6	19.4	13.3	28.1
Sample 8	0.0	0.0	45.0	51.5	49.7	14.9	4.9	33.1
Sample 9	0.0	0.0	37.9	56.9	50.9	10.0	9.6	32.4
Sample 10	0.0	0.0	47.4	56.3	40.6	10.4	12.0	33.2
Sample 11	0.0	0.0	13.6	24.5	82.9	53.9	3.5	21.1

H_2 was detected at low concentrations only in the gas collected from samples 2 and 6 and only for glucose addition cycle 2 (for sample 2) and cycle 5 (for sample 6 and sample 2).

4.2 Gas production rates from successive glucose batch feeding

Gas production curves were then obtained by plotting the volume of water displaced (gas produced) as a function of time during the gas production period. Data are summarized in Figures 4-1 (a) to (h). Original data is presented in Appendix A.3.

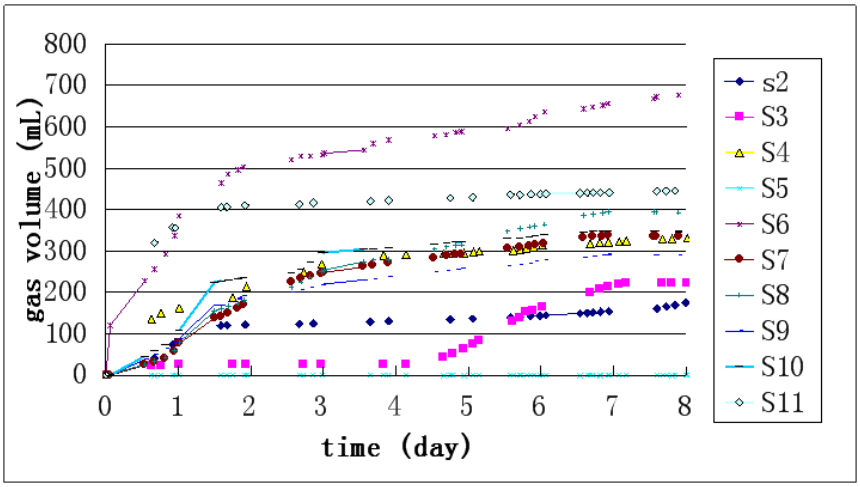


Figure 4-1 (a)

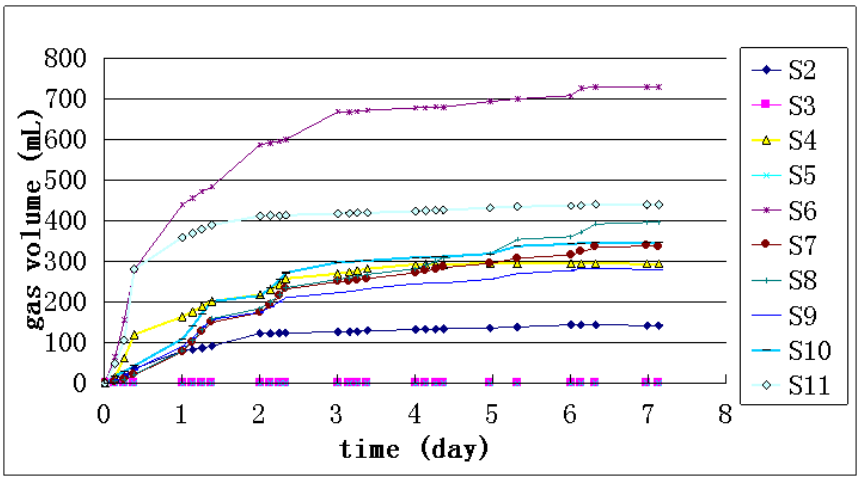


Figure 4-1 (b)

Figure 4-1 (a to b). Gas production profile for digester runs 1 (a) and 2 (b).

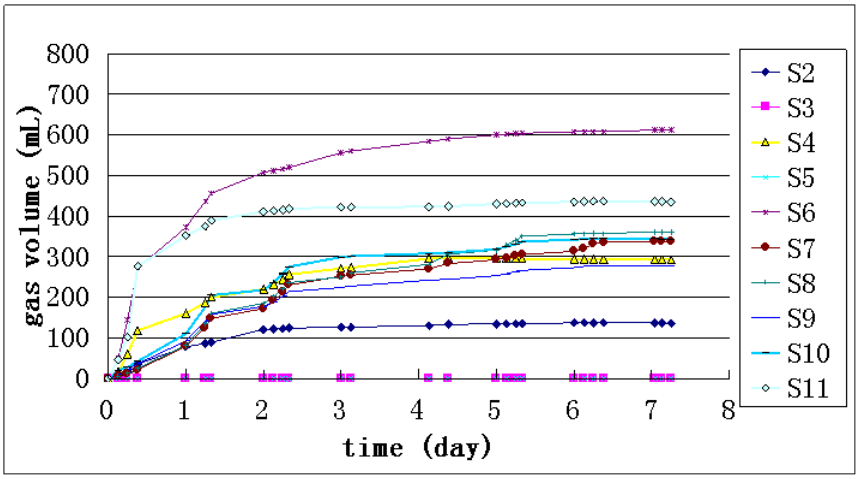


Figure 4-1 (c)

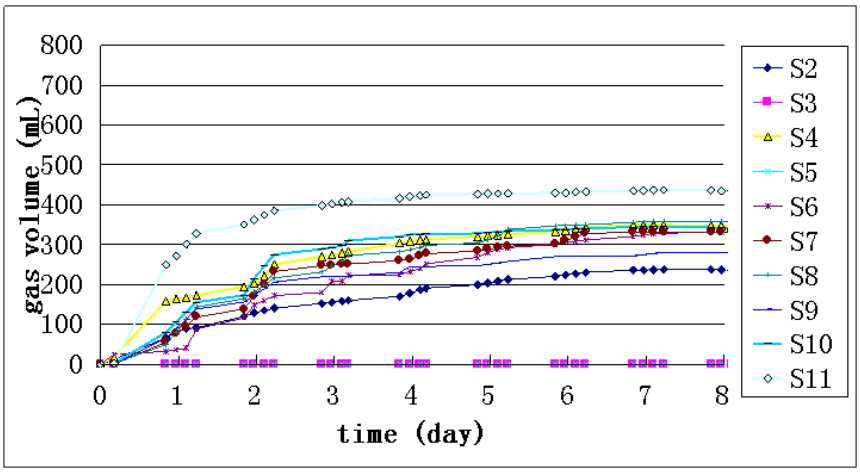


Figure 4-1 (d)

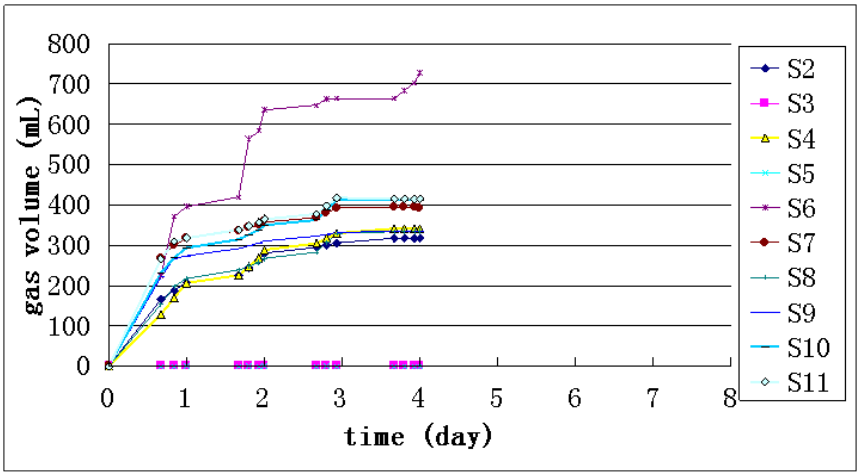


Figure 4-1 (e)

Figure 4-1 (c to e). Gas production profile for digester runs 3 (c), 4 (d) and 5 (e).

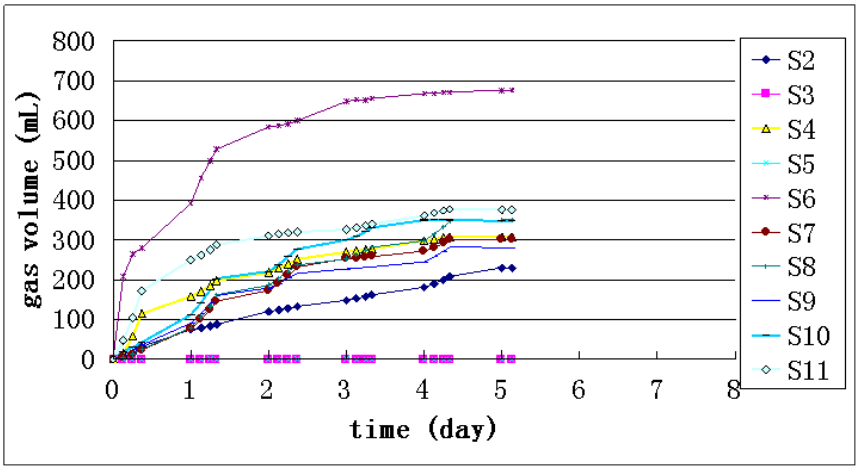


Figure 4-1 (f)

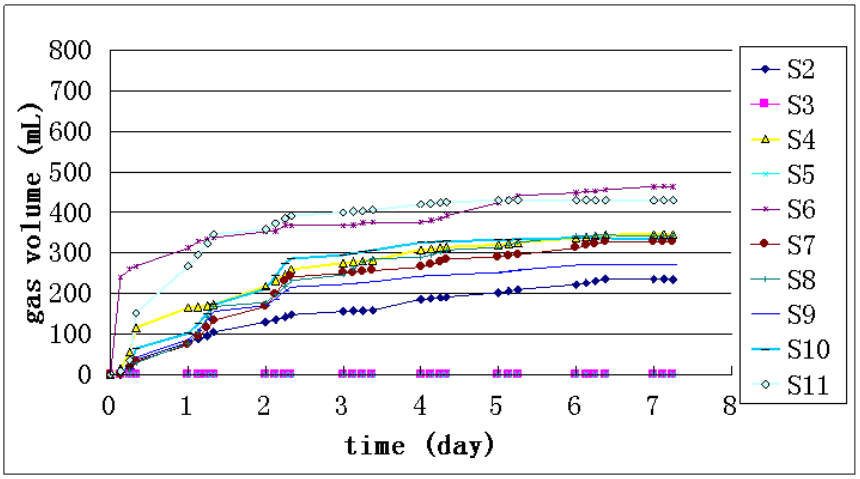


Figure 4-1 (g)

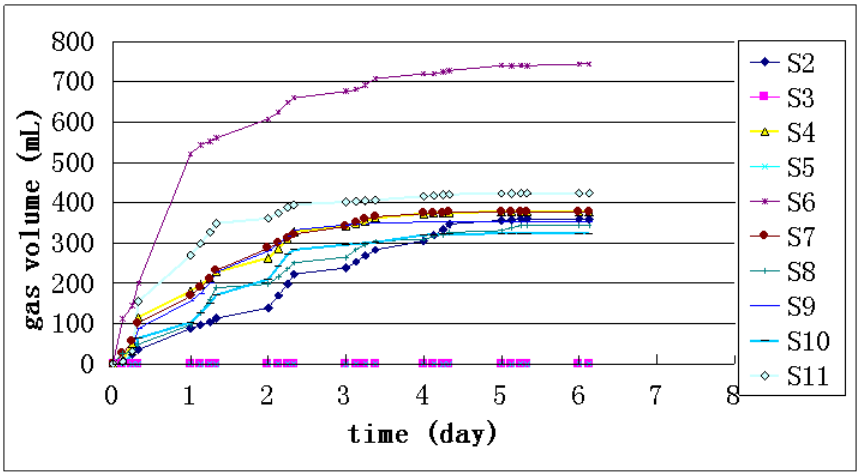


Figure 4-1 (h)

Figure 4-1 (f to h) Gas production profile for digester runs 6 (f), 7 (g) and 8 (h).

The gas production curves indicated that all but sludges S3 and S5 were active and produced some gas. The remaining sludges all produced some gas but at different rates and with different total production volumes. Generally gas production slowed after about 3 days and was essentially complete after 8 days when the runs were terminated.

The sludge S6 was consistently the most active sludge followed in activity by S11. There is no apparent reason why the sludges behaved so differently. The sludge blanket had obviously accumulated over a extended period of time so that variation in age may have been a factor.

4.3 Gas production curves for successive glucose batch feeding of Sample 6

While there was significant variability in the gas production curves for different sludges, the most active sludge, S6 yielded fairly reproducible results after batch feeding. The successive run data of figure 4-1 for S6 are plotted in Figure 4-2 to show this.

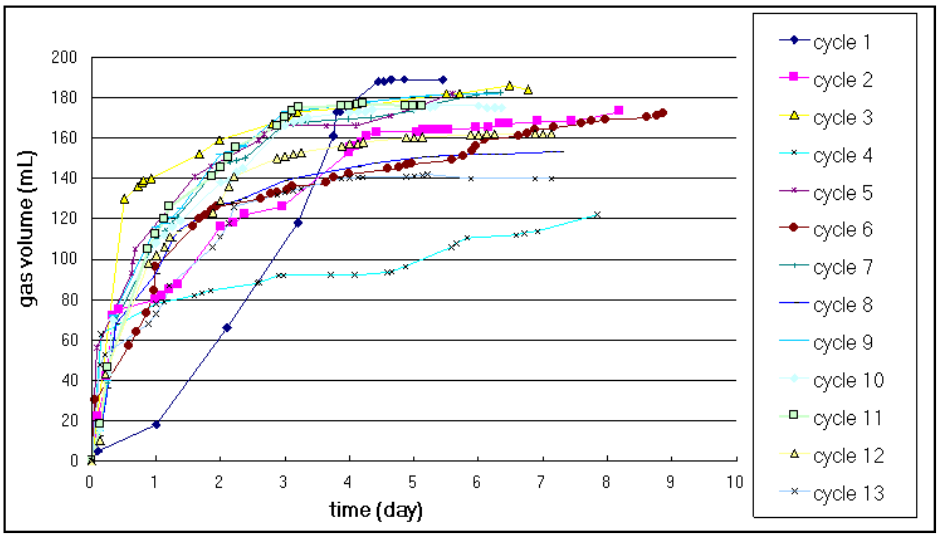


Figure 4-2 Gas production profile of sample 6 for 13 runs.

Figure 4-2 illustrates that gas production for each run usually finished within 8 days, with most production occurring in the first two days,

followed by a gradual decrease from the third day. Cycle 1 and cycle 4 deviated considerably from the average behavior of the other 11 runs. The slow initial gas production rate from run 1 was probably due to the malfunctioning of the stirring over the first day of the experiment.

4.4 The effect of batch feeding with glucose on the sludge pH

After each run for the series of runs described in Sections 3.3 and 4.1, the pH of the sludge was measured. Results are shown in Figure 4-3 and original data are summarized in appendix A.4.

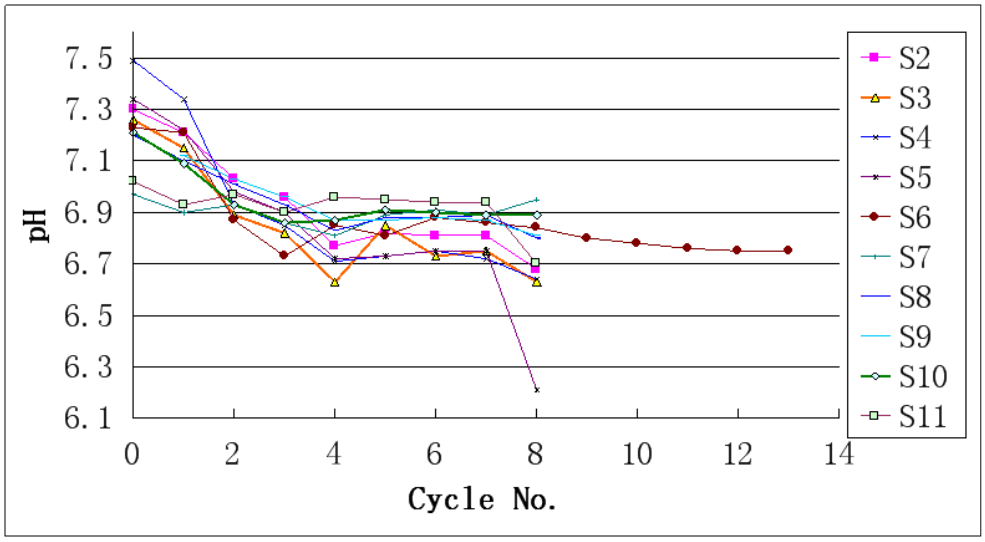


Figure 4-3. pH changes in sludges S2 to S11 during successive batch feeding cycles with 1 g/L glucose.

The decrease in pH with successive batch feeding is consistent with the accumulation of volatile acid due to acidogenic processes occurring more rapidly than methamnogenic processes. This is consistent with the formation of H_2 in at least some of the samples (S2 and S6 see section 3.3 above) after repeated additions of glucose. H_2 formed in the early stages of the digestion is usually consumed with CO_2 by methanogenic bacteria at the later stages of the digestion.

4.5 The effect of pH on the anaerobic digestion of glucose

While repeated additions of glucose substrate led, at least in some cases, to the production of hydrogen and in all cases to the gradual lowering of pH, the strategy did not give a reliable way of modifying the gas production process to favor hydrogen production. According to the literature external pH control is a more reliable way of controlling the digestion to produce hydrogen.

Four 500 mL Schott bottles each containing 250 mL of Sample 6 sludge were set up as shown in Figure 2-2. The pH of the sludges was carefully adjusted using 0.1 mol/L HCl to adjust to provide pHs of pH 4.5, pH 5, pH 5.5, and there was also a ' control ' sample at pH 7.23 without adding any acid.

Repeated additions of glucose at a rate of 1 g/L were used to start the digestion and the gas produced was collected in an inverted measuring cylinder as described in Section 4.1. After each batch addition had ceased producing gas, the pH was measured.

4.5.1 The effect of sludge pH on composition of gas produced from glucose

Data for head space and measuring cylinder gas composition at the end of successive runs are summarized in appendix A.6.

Hydrogen production in the reactor and collection in the measuring cylinder occurred only over the narrow pH range from 5.2 to 5.4 in this experiment. All runs produced hydrogen in this pH range with maximum production of 0.9 % occurring in cycle 3 of glucose addition.

Representative data for gas composition in the measuring cylinder and the head space of the reactor are presented in Figure 4-2. While measuring cylinder composition clearly indicates hydrogen for all runs, there was no measurable hydrogen in the head space.

Table 4-2. Gas composition of sludge samples during the cycle 7 of glucose addition and sampled when gas production stopped.

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.84	0.0	0.0	20.2	23.8	68.8	59.3	10.1	16.2
pH 4.47	0.0	0.0	0.0	0.0	0.0	90.4	0.0	9.2
pH 4.86	0.0	0.0	3.0	5.8	96.2	81.6	0.0	11.9
pH 5.21	0.6	0.0	0.4	28.2	98.4	26.1	0.0	44.7

During the process of anaerobic digestion, the final stage of methanogenesis produces methane by two mechanisms. Acetate is split into methane and CO_2, and CO_2 is reduced to methane by electrons extracted from hydrogen. The later reaction explains why no H_2 was detected in the headspace. The H_2 produced in the reactor was subsequently consumed by methanogenic processes.

4.5.2 The effect of pH on gas production rates and volumes

The experimental system as described in section 4.5 was used to measure gas production curves by plotting the volume of water displaced (gas produced) as a function of time during the gas production period. Nine cycles of glucose additions were monitored in order to determine the long term effects on the sludge of the lowered pH. One sample of unmodified sludge was used as a control.

Data for successive runs are summarized in Figures 1 (a) to (i). Original data is presented in Appendix A.7.

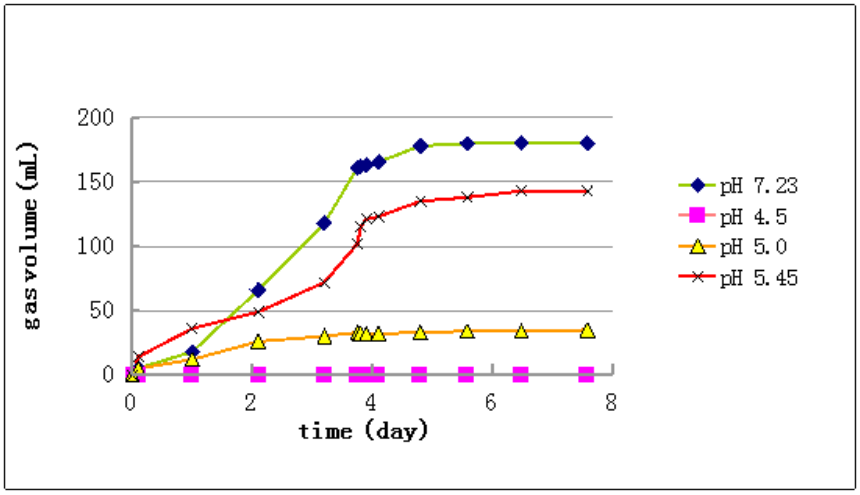


Figure 4-4 (a)

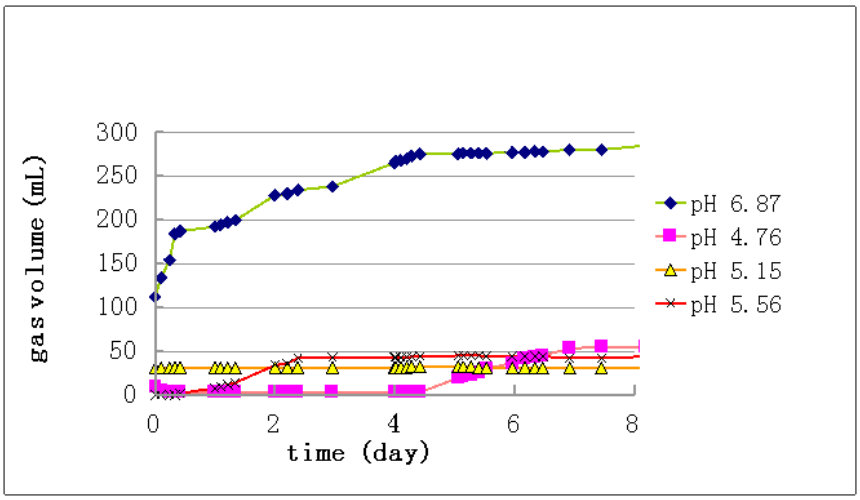


Figure 4-4 (b)

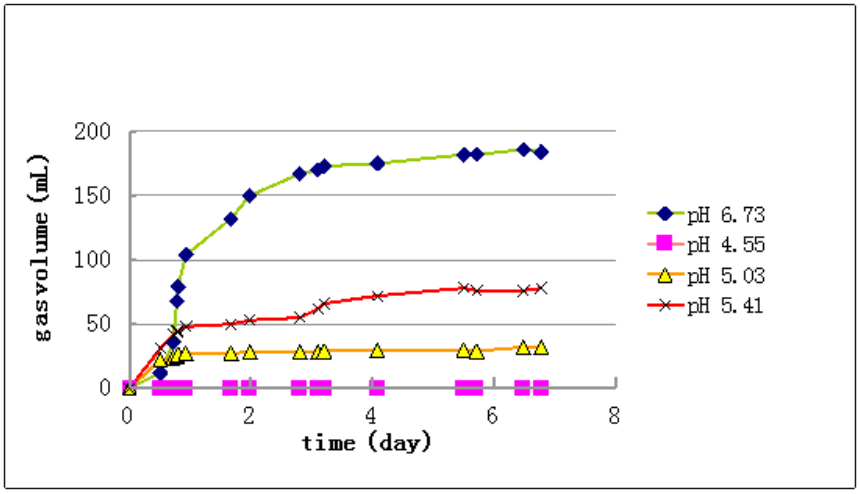


Figure 4-4 (c)

Figure 4-4 (a to c). Gas production profile for digester runs 1 (a), 2 (b), and 3 (c).

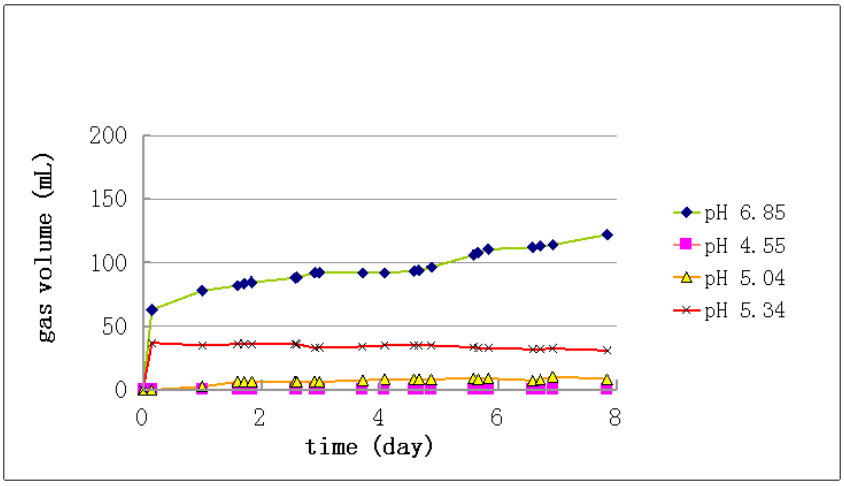


Figure 4-4 (d)

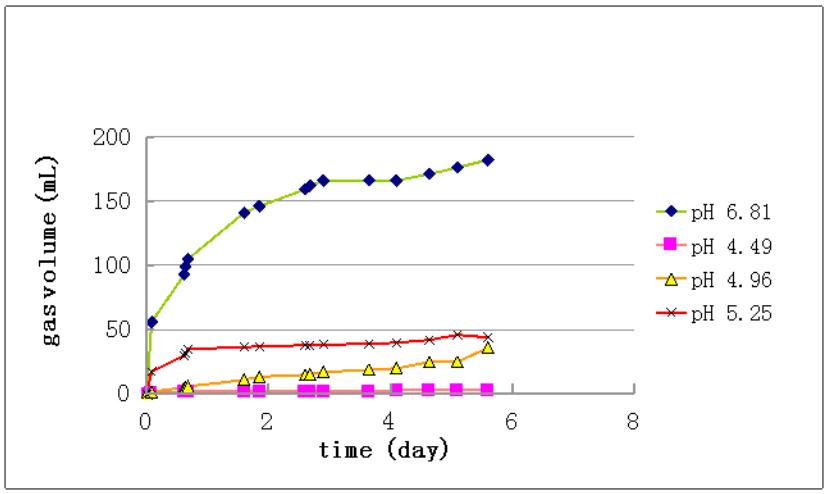


Figure 4-4 (e)

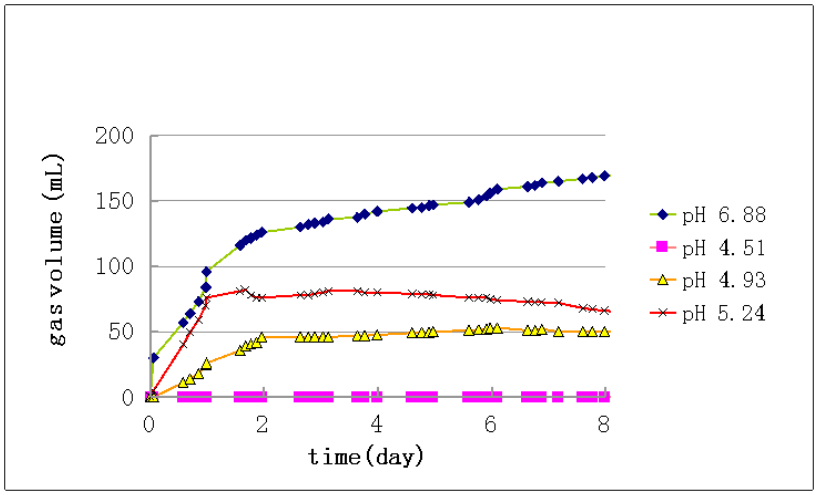


Figure 4-4 (f)

Figure 4-4 (d to f). Gas production profile for digester runs 4 (d), 5 (e) and 6 (f).

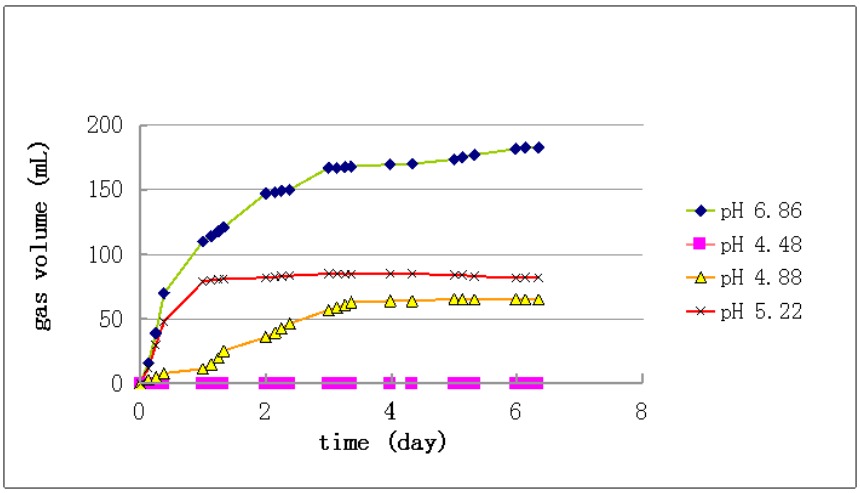


Figure 4-4 (g)

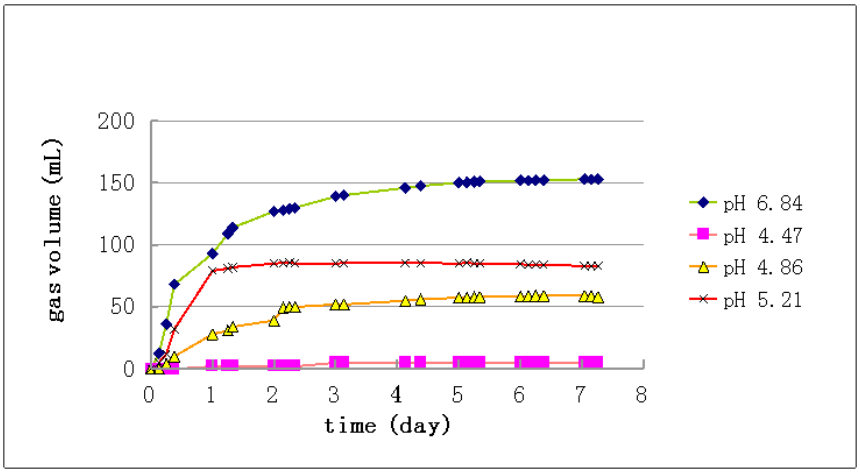


Figure 4-4 (h)

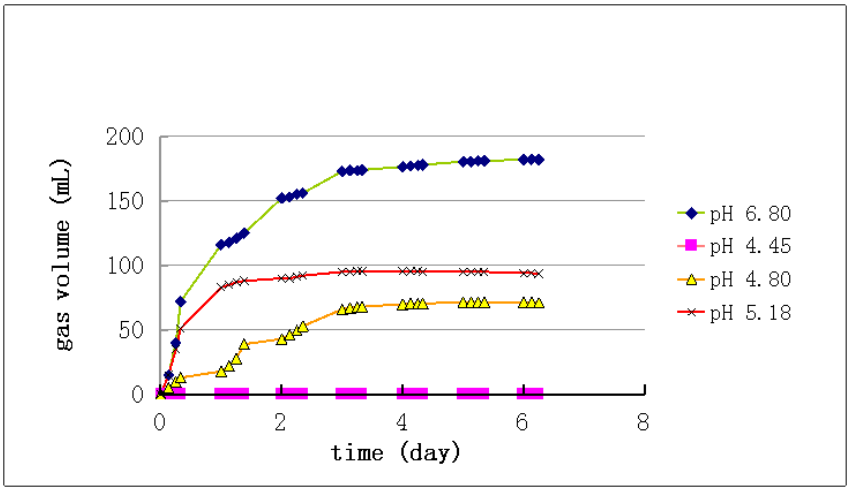


Figure 4-4 (i)

Figure 4-4 (g to i). Gas production profile for digester runs 7 (g), 8 (h) and 9 (i).

Lowering the pH caused a progressive reduction of gas production rates and final gas production volume such that at pH 4.5 very little activity was detected. However at the intermediate pHs of approximately 4.8 and 5.2, gas evolution appeared to decrease after the first few cycles but then recover towards the end of the 9-cycle experiment. Some recover and acclimatization to the lowered pH conditions appeared to have occurred.

4.5.3 The effect of successive glucose additions on adjusted sludge pHs

At end of each run described in Section 4.5.2 the pH of the sludges were measured in order to .determine acid accumulation in the sludge. Data are summarized in Figure 4-5 and original data are included as Appendix A.8.

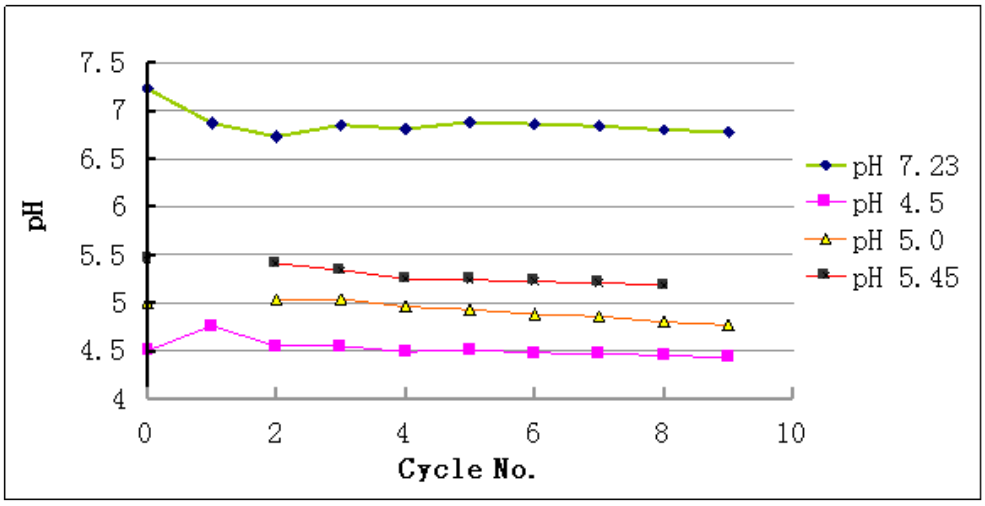


Figure 4-5. pH changes in S6 during successive batch feeding cycles with 1 g/L glucose.

The pH of all sludges fell as a result of the glucose additions. In the case of the control, the pH stabilized at approximately 6.8 after an initial drop of approximately 0.5 pH units. The acidified sludges all showed continuing pH drops during successive glucose additions. These were noticeable for the sludge pHs of 5.0 and 5.45.

Falling pH in the acid sludges is evidence of acid accumulation in these sludges caused by inhibition of methanogenesis.

Chapter 5 Studies of the anaerobic digestion of cellulose

5.1 Introduction

One of the principal objectives of the current study was to investigate the possibility of using low value biomass as feedstock for the production of commodity chemicals. A feedstock that is widely available is woody biomass. Thus experiments were performed using laboratory reagent cellulose powder as a first step towards assessing the digestibility of cellulosic materials.

The experiment procedure was identical to that used for the work with glucose except batch feeding with 1 g/L cellulose instead of glucose was used.

5.2 Gas production curves for successive cellulose batch feeding of Sample 6

In the study of batch feeding sludges with glucose, the most active sludge, S6, yielded fairly reproducible results.. Thus sample 6 was chosen to gain a comparison of gas production using cellulose as the substrate. Gas production data for sludge sample 6 batch fed with cellulose under condition described in Section 4.2 are summarized in figure 5-1.

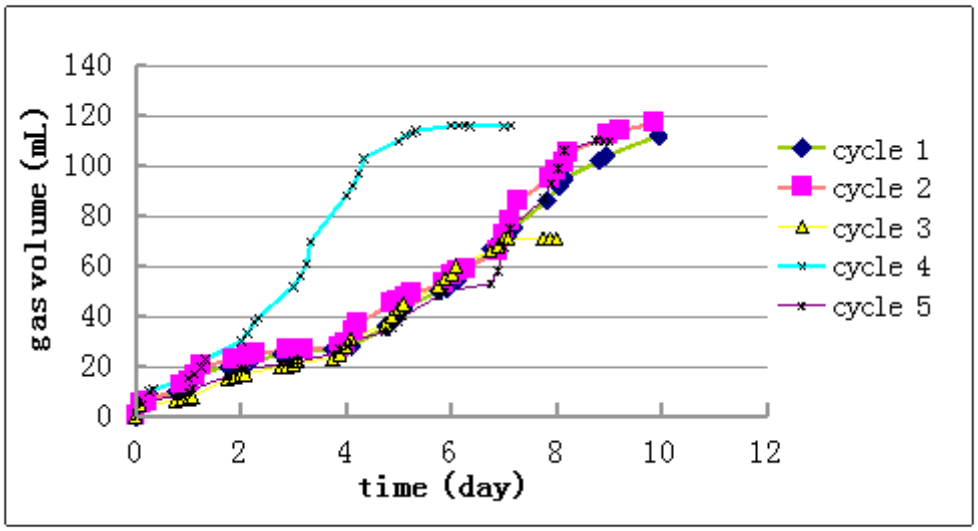


Figure 5-1 Gas production profile of sample 6 for 5 runs.

Figure 5-1 illustrates that gas production for each run usually finished within 10 days, with increasing production during the first 7 days, followed by a gradual decrease from the eighth day. Cycle 4 deviated considerably from the average behavior of the other 4 runs. There was no clear reason of its quick response.

5.3 The effect of sludge pH on composition of gas produced from cellulose.

While repeated additions of cellulose substrate led, no hydrogen production was detected. In order to study the effect of pHs on gas composition from cellulose and compare with that of glucose, further experiment, which was external pH control, was an applicable way of monitoring the digestion to produce hydrogen.

The experiment procedure was identical to that used for the work with glucose except batch feeding with 1 g/L cellulose instead of glucose was used.

Data for head space and measuring cylinder gas composition at the end of the run are summarized in appendix A.9.

Representative data for one cycle of cellulose addition (cycle 3) are summarized in Table 5-1.

Table 5-1. Gas composition of sludge samples during the cycle 3 of cellulose addition and sampled when gas production stopped.

pH of samples	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.97	0.0	0.0	5.5	13.3	93.8	74.4	0.0	11.8
pH 4.43	0.0	0.0	0.0	0.1	0.0	99.5	0.0	0.3
pH 4.94	0.0	0.0	2.7	7.2	96.7	79.4	0.0	13.0
pH 5.48	0.0	0.0	9.1	15.8	90.3	56.0	0.0	27.3

None of sludge sample produced hydrogen during these five cycles of

adding 1 g/L cellulose. There was no CO_2 which was detected by the GC from measuring cylinders, because CO_2 dissolved in the water. All sludges produced methane except the sample around pH 4.5 which was not active.

5.4 The effect of pH on gas production rates and volumes

Gas production curves were obtained by plotting the volume of water displaced (gas produced) as a function of time during the gas production period. One sample of unmodified sludge was used as a control. There were five cycles of cellulose addition, and the experimental system was as same as the description in section 4.1.

Data for successive runs are summarized in Figure 5-2 (a) to (e). Original data is presented in Appendix A.10. Each reactor was set up as the Figure 2-2.

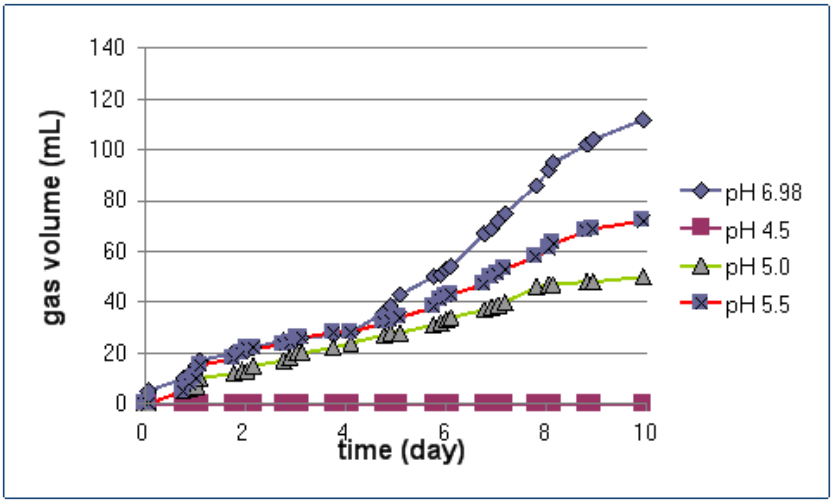


Figure 5-2 (a). Gas production profile for digester runs 1 (a).

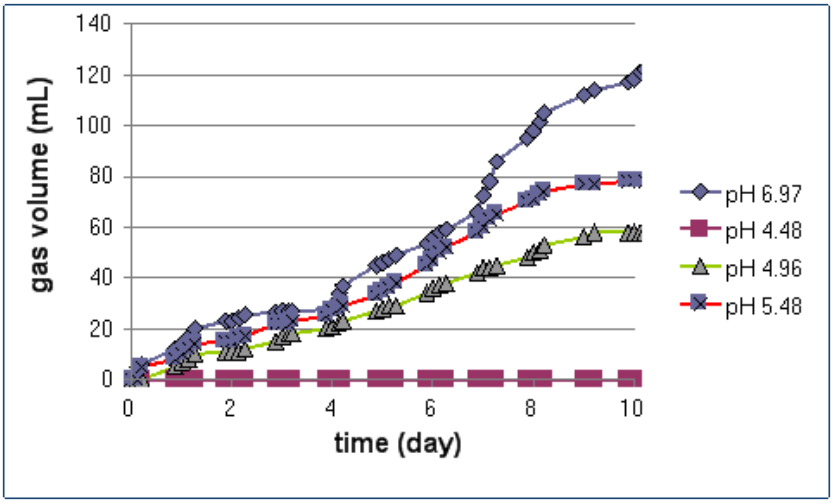


Figure 5-2 (b)

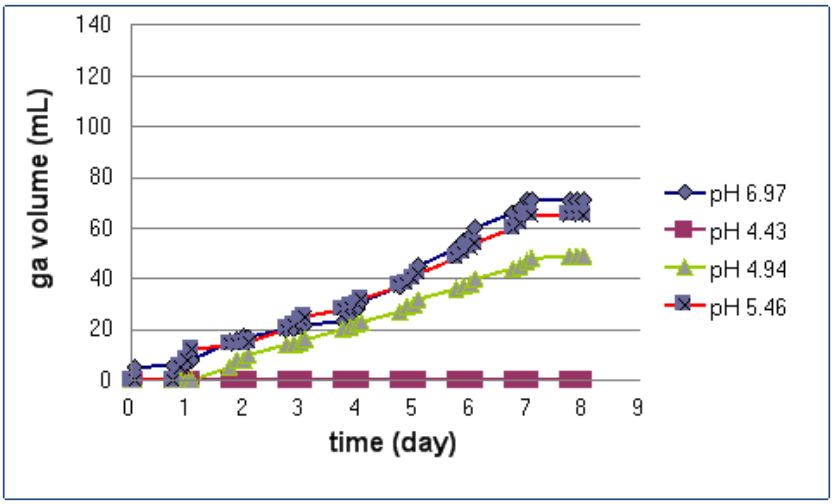


Figure 5-2 (c)

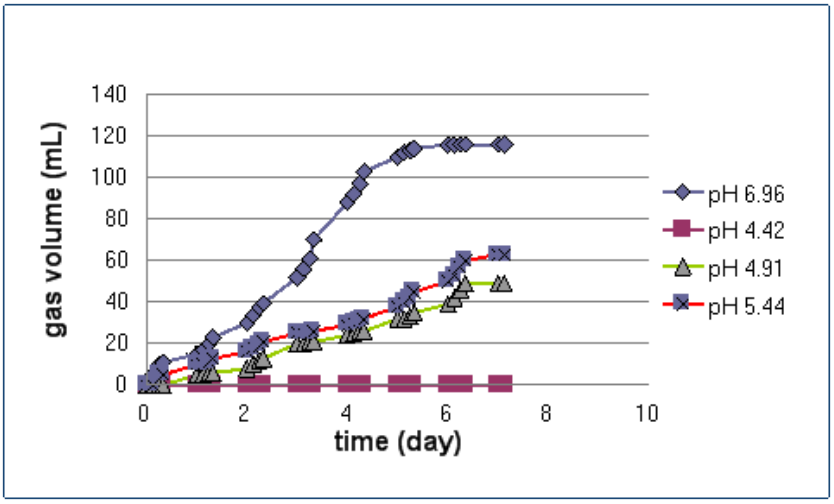


Figure 5-2 (d)

Figure 5-2 (b to d). Gas production profile for digester runs 2 (b), 3 (c), 4 (d).

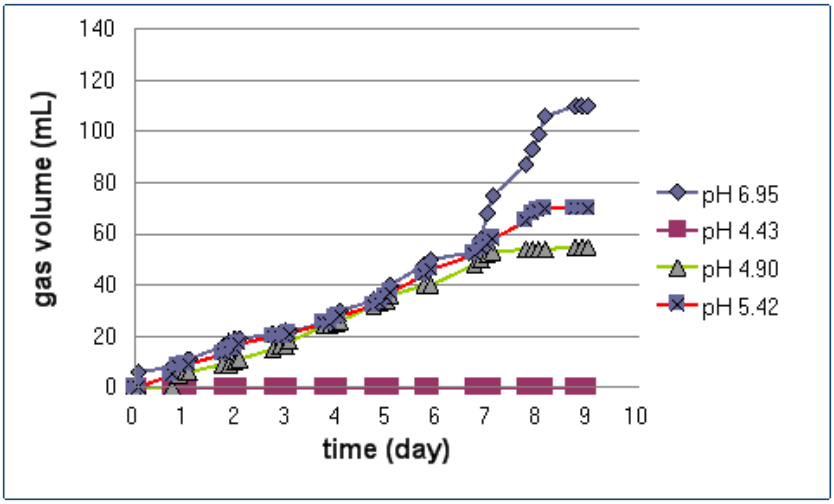


Figure 5-2 (e). production profile for digester runs 5 (e).

It is shown by the above figures that the volume of gas produced was decreased through lowering pH by HCl. Sludge S6 at pH 4.5 was completely inactive towards cellulose which was also the case for glucose, at this pH. After adding 1 g/L cellulose in each sample, response time was longer than that of adding 1 g/L glucose.

5.5 The effect of successive cellulose additions on adjusted sludge pHs

In order to determine acid accumulation in the sludge, the pHs of the sludge samples were measured at the end of each run. The pH changing data are summarized in Figure 5-3, and original data are included as Appendix A.11.

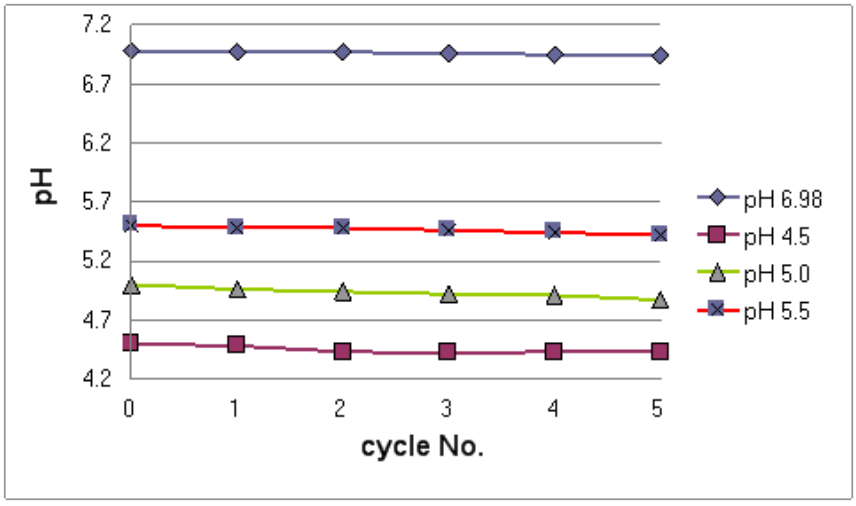


Figure 5-3. pH changes in S6 during successive batch feeding cycles with 1

g/L cellulose.

The sludge samples all showed continuing small pH drops of 0.15 or less during successive glucose additions. This is evidence of acid accumulation and inhibition of methanogenesis.

Chapter 6 Monitoring gas production from GC determination of head space gas composition

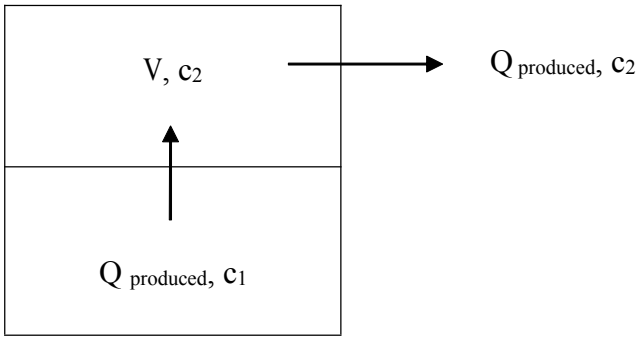
6.1 Introduction

During the experiments described in Chapters 4 and 5 it was often observed that the gas collected in the measuring cylinder was either devoid of carbon or carbon dioxide was present at percentages lower than normally expected. Gas collection was used mainly as a relative indicator of anaerobic activity and no precautions were taken to either absorb all the carbon dioxide by making the water in the measuring cylinder alkaline or avoid dissolution by pre-saturating the water with carbon dioxide.

Carbon dioxide is an important product of the digestion process and so it was considered worthwhile to see if it could be estimated by head space nitrogen dilution without gas collection by displacement of pre-saturated water. It should be possible to achieve this by monitoring reactor head space gas composition during the digestion process.

6.2 Theory

Consider a reactor with headspace of volume, V, flushed with nitrogen. Digestion within the reactor produces gas containing no nitrogen at a volumetric rate, Q, which displaces headspace gas at the same volumetric flow rate. The system is illustrated below.



Mass balance on nitrogen for this system is represented by:

$$V\frac{dc}{dt} = Q_{produced} \cdot c_1 - Q_{produced} \cdot c_2$$

Where c_1 is the concentration of nitrogen in the gas being produced by the digestion, $c_1 = 0$, and c_2 is the concentration of nitrogen in the headspace at a given time during the digestion.

$$\therefore V\frac{dc}{dt} = -Q_{produced} \cdot c_2$$

$$\therefore \frac{dc}{dt} = -\frac{Q_{produced}}{V} \cdot dt$$

But using $c = \frac{P}{Rt,}$ and integrating between t = 0 and t = t gives

$$\int_0^{\varepsilon} \frac{dP}{P} = \frac{1}{V}\int_0^t Q_{produced}dt$$

After time t, the volume of gas produced, V_t, will be

$$V_t = \int_0^t Q_{produced}dt$$

$$\therefore \ln\frac{P_0}{P_t} = \frac{V_t}{V}$$

$$\therefore V_t = V\ln\frac{P_0}{P_t}$$

(Equation 6-1)

Thus the total volume of gas produced after time t can be calculate from the ratio of the nitrogen GC peak areas providing the GC response is linear and the nitrogen dilution is neither too large or too small and there are no changes in gas composition brought about by gas reactions in the reactor.

6.3 Experimental

In order to test the usefulness of equation 6-1, gas production from a sludge batch fed at pH 6.75 was collected.

The reactor, a 500 mL Schott bottle with 250 mL sample sludge S6, was mounted on a magnetic stirrer and fitted with delivery tubes to pass through GC and then to a 250 mL measuring cylinder inverted in a 500 mL measuring cylinder for collecting gas. Both measuring cylinders were filled

with tap water. The sludge was fed with 1 g/L glucose and purged with N_2 for 5 minutes in order to fill the head-space with N_2 before the run started. At t = 0, the head space composition was 100% nitrogen and the gas volume in the measuring cylinder was zero. The gas product profile of the head space and the volume of displaced water were measured regularly throughout the run until gas production stopped after about 2 days. No significant further gas production occurred over the next 8 days. The volumes of gas produced calculated from Equation 6-1 are compared with the gas volumes measured by the measuring cylinder in Figure 6-1 and Appendix A.12. The change in composition of the headspace with run time is shown in Figure 6-2.

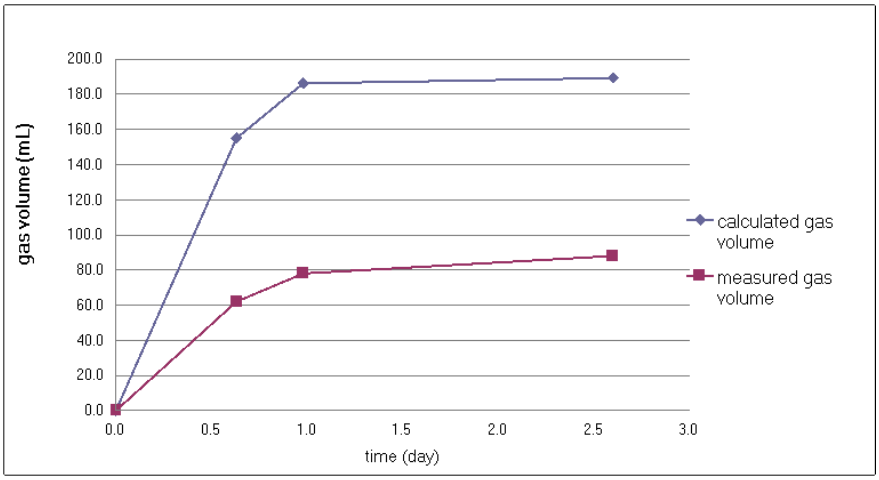


Figure 6-1. Comparison between the calculated gas volume and the measured gas volume.

It is indicated in Figure 6-1 that the measured gas volume was less than the calculated gas volume (approximately 100 mL by the end of the experiment) and the two curves indicated that gas production had essentially ceased after about 1 day. Figure 6-2, shows that the composition of CH_4 and CO_2 increased while that of N_2 decreased, that the ratio of CO_2 to CH_4 stayed essentially constant at approximately 1:1 and that no H_2 was produced.

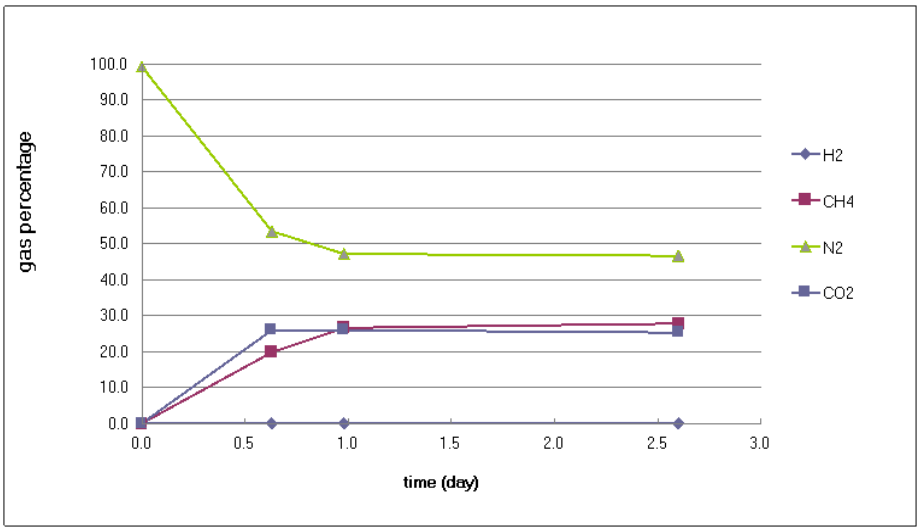


Figure 6-2 . Gas composition at different times during one run.

According to data collected previously under similar conditions, little or no CO_2 was detected in the measuring cylinder, an effect ascribed to dissolution of the CO_2 in the water of the measuring cylinder. In summary, methane and CO_2 were the only gases produced during the run and they were produced in roughly equal volumes. Thus the fact that the volume of gas collected in the measuring cylinder was about half the volume calculated from the dilution of the nitrogen in the head space in consistent with all the CO_2 produced being dissolved in the measuring cylinder water. If this was the case then the volume of CO_2 produced was 100 mL and the volume of methane produced was 90 mL. An error of approximately 10% is indicated.

6.4 Conclusion

This preliminary experiment appears to provide a means of monitoring the production of reactor gases by monitoring head space composition. In principle the method should allow correction for disappearance of reactive gasses such as CO_2 providing relative gas composition remains constant. Further validation of the procedure was beyond the scope of the present project.

Chapter 7 General discussion and conclusions

7.1 General discussion

The objective of this project was to study anaerobic digestion by sludges from an anaerobic digester, which had been reported to produce hydrogen at ambient temperature.

Eleven sludge samples (S1 to S11) were collected from different locations along the plug flow reactor system. The as-collected sludges were characterized in terms of their solids content, pH, and gas production profiles. None of the samples produced hydrogen gas under the condition used in the present study. Slow gas production from the sludges continued for up to 2 months. All of the sludges were stored at room temperature for three months before batch feeding experiments were performed.

When repetitively batch fed with glucose at their original pHs, only the sludges S2 and S6 produced hydrogen and only at low concentrations and only for two cycles of glucose addition. The active sludges all produced some gas but with different total production volumes and at different rates. Generally the gas production slowed after about 3 days and was essentially complete after 8 days when the runs were terminated. The pH decreased with successive batch feeding with 1 g/L glucose, consistent with the accumulation of volatile acid due to acidogenic processes occurring more rapidly than methamnogenic processes.

The pH of sludge S6 was adjusted to pH 4.5, pH 5.0, and pH 5.5 with HCl. The pH of all the systems fell with successive additions of glucose and it was only when the pH of the system that was initially adjusted to pH 5.5 fell to between pH 5.4 and 5.2 that hydrogen was produced. Lowering the pH caused a progressive reduction of gas production rates and final gas production volume such that at pH 4.5 very little activity was detected.

The effectiveness of the sludge in digesting cellulose was investigated in batch fed trials with sludge S6. When repetitively batch fed with 1 g/L

cellulose at the sludge's original pH, gas production for each run usually ceased after the longer period of 10 days. Gas production rate and amount were less than when glucose was used as the substrate.

When the pH of S6 was adjusted to pH 4.5, pH 5.0, pH 5.5 with HCl and batch fed with 1 g/L cellulose for 5 runs, none of sludge samples produced hydrogen. All sludges produced methane except the sample at pH 4.5 which was totally inactive.. Gas production rates with cellulose substrate were systematically slower than when glucose was used as the substrate. The pHs of sludge samples all dropped slightly during successive glucose additions, but the pH decrease was never more than 0.15 pH units. The pH of the system initially at pH 5.5 never fell as low as pH 5.4 the pH at which hydrogen had been detected when cellulose was used as the substrate.

GC analysis of the gas collected in the measuring cylinders indicated highly variable CO_2 compositions ranging from virtually zero to percentages as high or higher than the percentage of CH_4, whereas the percentage of CO_2 in the head space was always significant. Because the water filling the measuring cylinder had not been pre-saturated with CO_2 it was likely that variable amounts of CO_2 was dissolved in this water. While determination of CO_2 not critical to the present studies it would never the less be useful to be able to determine CO_2 digester runs. A method was developed and tested whereby this could be done by following the dilution of the N_2 that was initially the only component in the head space.

7.2 Summary of conclusions

The principal conclusions that can be drawn from the present investigation are:

1. The sludge used in the present studies and collected from the anaerobic digester of a local meat processing company appeared to be different from the sludge studied previously and reported to produce significant amounts of hydrogen at room temperature. None of the 11 samples collected from various positions along the plug

flow reactor for the present work, produced hydrogen as as-collected sludges and only 2 sludges produced hydrogen when batch fed with glucose.

2. Digestion of glucose was rapid at room temperature, generally being essentially complete in about 3 days.

3. Lowering the pH resulted in hydrogen production over the pH range of 5.2 to 5.4 when batch fed with glucose.

4. Digestion was much slower when cellulose was used as the substrate and no hydrogen was produced when the pH was lowered.

5. Preliminary work has shown that it may be possible to follow gas production by on-line monitoring of head space composition.

7.3 Recommendations for further work

It appears that the meat works digester behaves differently at different times of the year, depending upon the seasonal variation of substrate loading. It would be of interest to monitor sludge characteristics over a 12-month period.

The literature provides evidence that anaerobic sludges can be modified to favor hydrogen production by systematic application of stress. More detailed studies of pH stress along with thermal stress, substrate overload stress and oxygen stress would also be of interest.

Gas production by monitoring of head space composition could provide a convenient means of following anaerobic digestion. Further work is required to fully validate this method.

Appendix

A.1 GC-TCD operation

1. Start the system: double click 'TC Nav'
2. Log on. User's name: instrument. Password: generic
3. Click 'Instrument', click 'set up'
   Method: C:\GC\Methods\Lisa\He-H2-CH4-O2-N2-CO2-CO.mth
   Data Path: C:\GC\Data\Nanxin
   Base file name: time pH produce x ml gas
   Click 'OK'
4. When 'Status' shows:

GC--TCD
Ready
ACQ: Ready
I/F: Inst Ready
CMD: None
GC: Ready
A/S: Ready

   Click 'Run', click 'Start Run', click 'GC--TCD', click 'OK'
5. 'Status' shows:

GC--TCD
Active
ACQ: Sampling
I/F: Sampling
CMD: None
GC: Initial
A/S: Ready

6. Click 'View-Real-Time-Plot' to see the real time chart
7. Click 'Reprocess-Results' to see previous charts

A.2 Final head space and measuring cylinder gas compositions for successive cycles of S2 to S11 sludges batch fed with 1g/L glucose

A.2.1 Cycle 1

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
Sample 2	0.0	0.0	51.5	22.9	41.7	64.0	6.8	12.6

Sample 3	0.0	0.0	16.2	8.3	77.2	79.9	5.7	11.43
Sample 4	0.0	0.0	50.0	48.3	47.0	34.7	2.3	16.5
Sample 5	0.0	0.0	0.0	5.7	0.0	76.2	0.0	15.2
Sample 6	0.0	0.0	30.8	41.1	67.0	44.5	1.2	14.3
Sample 7	0.0	0.0	32.4	28.7	61.6	62.1	6.0	8.8
Sample 8	0.0	0.0	72.3	46.8	21.0	20.3	6.6	32.9
Sample 9	0.0	0.0	35.0	25.3	58.4	61.4	6.0	12.8
Sample 10	0.0	0.0	57.4	51.5	35.6	29.0	6.3	18.8
Sample 11	0.0	0.0	19.9	21.7	79.4	62.8	0.0	15.4

A.2.2 Cycle 2

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
Sample 2	0.2	0.0	27.7	24.4	66.5	60.0	4.1	14.4
Sample 3	0.0	0.0	0.0	1.0	0.0	95.2	0.0	3.1
Sample 4	0.0	0.0	54.2	46.2	38.5	27.6	6.8	25.8
Sample 5	0.0	0.0	0.0	6.4	0.0	70.5	0.0	22.8
Sample 6	0.0	0.0	6.7	20.7	92.6	66.4	0.0	12.5
Sample 7	0.0	0.0	50.4	43.1	41.6	27.0	8.0	29.4
Sample 8	0.0	0.0	64.2	50.4	20.8	18.5	11.6	31.0
Sample 9	0.0	0.0	33.8	41.4	59.6	39.5	6.6	18.2
Sample 10	0.0	0.0	67.2	52.6	25.6	17.4	7.0	28.7
Sample 11	0.0	0.0	16.0	17.0	80.5	67.8	3.0	14.5

A.2.3 Cycle 3

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
Sample 2	0.0	0.0	31.2	22.7	65.0	64.7	3.6	12.2
Sample 3	0.0	0.0	0.0	17.4	0.0	73.4	0.0	8.2
Sample 4	0.0	0.0	60.0	51.5	31.3	19.4	7.6	27.8
Sample 5	0.0	0.0	0.0	5.7	0.0	75.7	0.0	17.8
Sample 6	0.0	0.0	18.3	28.4	78.5	59.7	0.8	11.7
Sample 7	0.0	0.0	53.5	46.2	39.4	21.0	6.4	32.0
Sample 8	0.0	0.0	70.7	57.9	18.3	11.0	10.0	30.8
Sample 9	0.0	0.0	35.5	44.8	56.0	35.3	6.9	18.6
Sample 10	0.0	0.0	72.5	56.5	21.4	12.9	6.0	30.2
Sample 11	0.0	0.0	17.2	22.5	77.3	54.8	4.3	21.7

A.2.4 Cycle 4

	% H_2	% CH_4	% N_2	% CO_2

	MC	HS	MC	HS	MC	HS	MC	HS
Sample 2	0.0	0.0	47.4	29.3	49.4	50.8	0.0	17.2
Sample 3	0.0	0.0	0.0	15.5	0.0	72.4	0.0	8.9
Sample 4	0.0	0.0	58.3	53.0	30.2	17.8	7.7	29.1
Sample 5	0.0	0.0	0.0	7.1	0.0	71.9	0.0	21.0
Sample 6	0.0	0.0	21.1	17.7	78.6	61.7	0.0	19.9
Sample 7	0.0	0.0	72.1	55.4	16.3	20.7	10.8	23.2
Sample 8	0.0	0.0	59.0	47.3	34.1	26.2	4.6	25.4
Sample 9	0.0	0.0	34.1	41.4	58.0	38.8	6.4	19.2
Sample 10	0.0	0.0	45.4	49.2	51.9	23.9	0.0	24.3
Sample 11	0.0	0.0	20.0	23.6	76.4	59.0	2.9	15.9

A.2.5 Cycle 5

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
Sample 2	0.7	0.0	33.4	50.5	57.8	22.1	7.9	27.0
Sample 3	0.0	0.0	0.0	14.8	0.0	78.8	0.0	5.9
Sample 4	0.0	0.0	44.6	51.5	38.2	8.1	14.4	39.9
Sample 5	0.0	0.0	0.0	16.7	0.0	49.1	0.0	32.2
Sample 6	0.2	0.0	6.8	24.3	92.9	58.1	0.0	17.5
Sample 7	0.0	0.0	35.8	52.2	50.6	19.4	13.3	28.1
Sample 8	0.0	0.0	45.0	51.5	49.7	14.9	4.9	33.1
Sample 9	0.0	0.0	37.9	56.9	50.9	10.0	9.6	32.4
Sample 10	0.0	0.0	47.4	56.3	40.6	10.4	12.0	33.2
Sample 11	0.0	0.0	13.6	24.5	82.9	53.9	3.5	21.1

A.2.6 Cycle 6

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
Sample 2	0.0	0.0	55.2	30.7	44.7	50.9	0.0	18.2
Sample 3	0.0	0.0	0.0	18.9	0.0	70.2	0.0	10.8
Sample 4	0.0	0.0	67.0	56.5	23.4	12.1	9.5	31.3
Sample 5	0.0	0.0	0.0	7.4	0.0	69.5	0.0	22.3
Sample 6	0.0	0.0	13.8	30.1	86.1	50.0	0.0	19.7
Sample 7	0.0	0.0	78.4	59.3	9.3	15.6	12.2	24.8
Sample 8	0.0	0.0	66.8	51.2	28.4	20.6	4.7	28.1
Sample 9	0.0	0.0	40.2	44.4	51.5	34.2	7.8	20.9
Sample 10	0.0	0.0	46.2	55.9	53.5	16.6	0.0	27.0
Sample 11	0.0	0.0	22.0	24.3	74.1	57.4	3.4	17.8

A.2.7 Cycle 7

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
Sample 2	0.0	0.0	50.2	30.2	49.5	49.2	0.0	19.9
Sample 3	0.0	0.0	0.0	17.8	0.0	71.8	0.0	9.7
Sample 4	0.0	0.0	65.3	61.1	25.4	5.0	9.2	33.6
Sample 5	0.0	0.0	0.0	8.4	0.0	66.1	0.0	24.9
Sample 6	0.0	0.0	17.1	31.1	82.4	48.8	0.0	19.6
Sample 7	0.0	0.0	83.5	61.0	3.4	13.4	13.0	25.4
Sample 8	0.0	0.0	71.0	53.2	23.4	16.9	5.2	29.4
Sample 9	0.0	0.0	39.5	47.5	52.5	29.6	7.7	22.6
Sample 10	0.0	0.0	44.0	61.5	55.9	9.3	0.0	28.4
Sample 11	0.0	0.0	24.7	25.9	70.9	54.4	3.8	19.1

A.2.8 Cycle 8

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
Sample 2	0.0	0.0	34.0	22.9	59.2	64.4	6.4	12.2
Sample 3	0.0	0.0	0.0	19.3	0.0	70.8	0.0	9.2
Sample 4	0.0	0.0	67.0	58.3	23.9	11.1	8.6	30.0
Sample 5	0.0	0.0	0.0	7.1	0.0	69.8	0.0	22.3
Sample 6	0.0	0.0	20.1	36.7	77.4	43.3	1.0	19.1
Sample 7	0.0	0.0	60.9	55.2	30.5	6.7	7.6	37.5
Sample 8	0.0	0.0	71.1	61.3	17.8	5.3	10.4	32.5
Sample 9	0.0	0.0	38.3	50.4	53.3	25.2	7.6	23.6
Sample 10	0.0	0.0	79.7	60.9	12.0	3.2	7.6	35.0
Sample 11	0.0	0.0	18.2	24.3	75.9	50.4	4.9	24.8

A.3 Comparative rates of gas production from sludges S2 to S11 batch fed with glucose

A.3.1 Cycle 1

Time	Gas volume (mL)									
(day)	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	0	0	0	0	0	0	0	0	0	0
0.06					30	0	2	0	0	
0.54					57	24	26	38	46	
0.63		46	136	0						
0.67	40			0	64	30	32	50	58	320
0.76		47	149	0						

0.82					73	40	43	64	75	
0.92	74			0						356
0.95					84	56	58	79	99.5	
0.96	79			0						356
1.01		48	162	0	96	76	79	88	108	
1.5						138	155	170	224.5	
1.59	120			0	116	140.5	161	170	228	406
1.67	121			0						407
1.69					121.5	150	167	177	230	
1.75		49	188	0						
1.82					124	161	173	185	234	
1.9					126	170	181	192	236	
1.92	122			0						410
1.94		49	214	0						
2.56					130	226	212	200	248	
2.67	124			0						413
2.69					132	233	225	207	256	
2.73		50	249	0						
2.82					132	240	237	213	272	
2.86	125			0						416
2.98		50	268	0	133	246	250	218	296	
3.01					134	248	254	220	296.5	
3.56					136	261	274	230	304.5	
3.65	129			0						420
3.69					140	264	278	233	306	
3.83		50	290	0						
3.9	131			0	142	270	280	239	309	422
4.14		51.5	292	0						
4.53					144.5	283	304	250	317	
4.66		82.5	294	0						
4.7					145	287	309.5	252	319.5	
4.75	134			0						428
4.79		103	294.5	0						
4.83					146.5	290	313.5	255.5	321	
4.91					147	292.5	315	258	322	
4.94		126	294.5	0						
5.06	136			0						430
5.07		148	297.5	0						
5.15		165	299	0						
5.54					149	305	348	265	330	
5.58	140			0						436
5.62		256	301.5	0						
5.71	141			0	151	309	354	267	332	436

5.72		276	304	0						
5.8		304	307	0						
5.84					153.5	312	358	271	333.5	
5.86	142.5			0						437
5.9		312	310	0						
5.92					156	315	360	275	336	
5.99	143.5			0						437
6.02		329	314	0						
6.05					159	318.5	364	278	338.5	
6.07	145			0						438
6.54	149			0						440
6.59					161	331	385	286	346	
6.64	150			0						441
6.68		398	318	0						
6.72	151.5			0	162	334	389	288	347.5	441
6.81		412	320	0						
6.82	152.5			0						441
6.85					163	335	392	290	348	
6.93		426	321	0	164	337	395	292	348	
6.94	154			0						441
7.08		438	322	0						
7.18		442	324	0						
7.56					167	336	395	291	348	
7.6	161			0						445
7.68		442	328	0						
7.59					168	336	394	291	348	
7.73	166			0						445
7.81		442	329	0						
7.85	170			0						445.5
7.9					169	335	393	290	347	
8	175			0						446
8.02		442	331	0						
8.1	176			0						450
8.57						334	392	289	346	
8.6	178			0						459
8.64		444	334.5	0						
8.7					170	334	392	288	346	
8.73	178			0						461
8.81		445	337	0						
8.83					171	333.5	391	288	346	
8.91					172	332	390	287.5	345	
8.94	178	445	341	0						464
9.02		445	343	0						

A.3.2 Cycle 2

Time (day)	Gas volume (mL)									
	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	0	0	0	0	0	0	0	0	0	0
0.13	5	0	15	0	16	5	5	10	18	48
0.25	16	0	62	0	39	9	10	20	29	106
0.38	36	0	120	0	70	20	22	34	43	281
1	80	0	164	0	110	78	80	91	110	360
1.13	83	0	176	0	114	102	105	112	140	369
1.25	87	0	189	0	118	127	132	137	172	379.5
1.38	92	0	202	0	121	150	159	157	202	390
2	123	0	218	0	147	174	184	175	216	412
2.13	123	0	231	0	148	193	200	187	234	413
2.25	123.5	0	243	0	149	215	218	201	255	413
2.33	124	0	258	0	150	232	235	211	272	414
3	126	0	270	0	167	250	256	223	298	418
3.15	127	0	274	0	167	252	259	226	299	419
3.25	128	0	278	0	167.5	254	263.5	229	301	420
3.38	130	0	282	0	168	258	268	233	302	420
4	132	0	293	0	169.5	272	282	245	310	423
4.13	133	0	293	0	169.5	276	291	246	310	425
4.26	133.5	0	293	0	170	281	300.5	246	311	426
4.36	134	0	293.5	0	170	285	309	247	312	427
4.96	136	0	295	0	173.5	295	319	256	319	432
5.31	138.5	0	295	0	175	308	354	270	338	435
6	144	0	295	0	177	316	361	278	343	437
6.13	144	0	295	0	181.5	323	372	283	345	438
6.32	144	0	295	0	182.5	335	392	283	347	441
6.98	142	0	294	0	182.5	338	396	280	346	440
7.13	142	0	294	0	182.5	337	396	280	346	440

A.3.3 Cycle 3

Time (day)	Gas volume (mL)									
	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	0	0	0	0	0	0	0	0	0	0
0.13	5	0	16	0	12.5	5	5	10	20	46
0.25	14	0	60	0	36	10	12	22	28	102
0.38	35	0	118	0	68	21	24	36	42	276
1	78	0	160	0	93	79	82	92	110	352
1.25	86	0	187	0	109	125	134	140	176	375
1.33	89	0	200	0	114	148	160	158	205	389

2	121	0	220	0	127	172	185	177	218	411
2.13	122	0	232	0	128	191	202	188	236	413
2.25	123	0	243	0	129	212	220	203	258	415
2.33	124	0	256	0	130	230	236	214	275	418
3	126	0	271	0	139	252	250	224	299	422
3.13	126	0	273	0	140	254	260	227	301	422
4.13	130	0	295	0	146	270	281	242	308	423
4.38	133	0	296	0	147.5	283	306	244	310	424
5	134	0	296	0	150	292	316	253	318	430
5.13	134	0	296	0	150.5	297	328	258	325	431
5.25	134	0	295.5	0	151	302	340	263.5	331	432
5.33	135	0	295	0	151	305	350	266	337	433
6	137	0	294	0	152	313	356	273	342	435
6.13	137	0	293.5	0	152	320	356	276	343	436
6.25	137	0	293	0	152	332	357	277	344	437
6.38	137	0	293	0	152	335	357	277	344	437
7.04	136.5	0	292	0	153	336	361	277	344	436
7.13	136.5	0	292	0	153	336	361	277	343	435
7.25	136	0	292	0	153	336	360	276	343	435

A.3.4 Cycle 4

Time	Gas volume (mL)									
(day)	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	0	0	0	0	0	0	0	0	0	0
0.17	12	0	18	0	6	0	2	0	2	2
0.84	66	0	158	0	8	55	49	65	80	250
0.97	81	0	163	0	9	76	80	90	106	272
1.1	89	0	167	0	10	96	112	113	130	301
1.23	91	0	172	0	22	118	143	138	155	328
1.84	120	0	195	0	29	138	164	156	173	350
1.97	128	0	202	0	37	170	181	172	215	362
2.1	135	0	222	0	40	201	199	189	246	374
2.23	140	0	250	0	43	232	216	206	275	385
2.84	152	0	270	0	45	247	231	219	289	398
2.97	155	0	274	0	52	248	245	220	293	402
3.1	158	0	279	0	52	250	260	220.5	297	406
3.18	160	0	282	0	56	251	273	221	310	408
3.84	170	0	304	0	56	260	282	230	320	416
3.97	178	0	309	0	58	263	287	245	325	421
4.1	187	0	312	0	60	271	293	245	326	423
4.18	191	0	313	0	63	277	298	245	326.5	425
4.84	199	0	320	0	67	284	304	247	328	427

4.97	203	0	322	0	70	288	312	250	330	428
5.1	208	0	324	0	72	292	321	254	332	428
5.23	212	0	326	0	73	295.5	338	257.5	334	429
5.84	220	0	332	0	75	302	347	270	337	430
5.97	224	0	335	0	77	310	348	270	338	430
6.1	227	0	340	0	77	319	349	270	339	432
6.23	230	0	343	0	78	328	350	271	341	433
6.84	236	0	349	0	80	331	356	272	344	435
6.97	236	0	349	0	81	331	357	275	345	436
7.1	237	0	349	0	82	331	358	278	345	436.5
7.23	237	0	349	0	82	332	359	281	345	437
7.84	237	0	348	0	83	332	359	280	343	436.5
7.97	237	0	348	0	83	332	359	280	342	435
8.1	236	0	348	0	83	332	359	280	342	435

A.3.5 Cycle 5

Time (day)	Gas volume (mL)									
	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	0	0	0	0	0	0	0	0	0	0
0.67	166	0	129	0	56	267	154	221.5	232	266
0.84	188	0	170	0	93	300	198	268	270	310
1	208	0	207	0	99	318	218	274	294	318
1.67	226	0	226	0	105	336	239	292	315	339
1.8	245	0	247	0	141	343	248	299	327	348
1.93	264	0	269	0	146	351	258	306	339	357.5
2	281	0	289	0	159	358	268	311	350	365
2.67	295	0	305	0	162	369	283	323	363	378
2.8	301	0	318	0	166	380	307	327	388	398
2.93	306	0	330	0	166	393	330	332	412	418
3.67	318	0	342	0	166	394.5	334	335	413	416
3.8	318	0	342	0	171	394	335	336	413	415
3.93	318	0	341	0	176	394	335	336	412	415
4	318	0	341	0	182	393	335	336	412	415

A.3.6 Cycle 6

Time (day)	Gas volume (mL)									
	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	0	0	0	0	0	0	0	0	0	0
0.13	5	0	15	0	52	5	5	10	20	48
0.25	15	0	58	0	66	12	14	24	30	104

0.37	32	0	115	0	70	23	25	36	43	172
1	75	0	158	0	98	78	80	91	112	250
1.13	79	0	171	0	114	100	106	115	142	262
1.25	83	0	185	0	125	123	132	141	175	276
1.33	88	0	198	0	132	146	162	160	203	288
2	120	0	218	0	146	173	186	179	221	310
2.13	124	0	229	0	147	190	204	190	238	315
2.25	128	0	240	0	148	210	221	205	259	318
2.37	133	0	252	0	150	232	237	216	277	320
3	148	0	270	0	162	253	252	227	300	326
3.13	153	0	273	0	163	254.5	263	229	309	331
3.25	158	0	276	0	163	257	274	231	321	336
3.33	162	0	278	0	164	258	282	232	331	340
4	181	0	298	0	167	272	298	245	350	361
4.13	190	0	302	0	167	280	315	258	351	368
4.25	200	0	306	0	168	291	333	272	351	374
4.33	208	0	308.5	0	168	300	348	282	350	377
5	230	0	308	0	169	301	350	281	348	376
5.13	230	0	308	0	169	301	351	281	348	376

A.3.7 Cycle 7

Time	Gas volume (mL)									
(day)	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	0	0	0	0	0	0	0	0	0	0
0.13	0	0	16	0	60	0	5	0	5	8
0.25	15	0	56	0	65	18	9	14	20	36
0.33	37	0	116	0	67	33	29	43	65	152
1	79	0	165	0	78	73	77	86	102	268
1.13	88	0	167	0	82	93.5	106	110	126	296
1.25	96	0	170	0	83.5	115	140	135	151	324
1.33	105	0	173	0	84.5	134	168	156	172.5	346.5
2	130	0	218	0	88	168	178	170	212	358
2.13	136	0	232	0	88.5	199	197	187	244	373
2.25	142	0	246.5	0	92	232	217	207	275	386
2.33	148	0	260	0	92	241	232	216	286	392
3	156	0	275	0	92	250	246	223	295	400
3.13	157	0	278	0	92	252	262	225	299	402
3.25	158	0	280	0	93.5	254	276	227	303	404
3.38	159	0	282	0	94	256.5	286	229	307	406
4	186	0	308	0	94	265	289	243	326	420
4.13	188	0	310	0	95	272	295	244	327	422
4.25	190	0	313	0	96	279	302	245	328	424

4.33	191.5	0	315	0	98	285	307	245.5	329	425.5
5	202	0	320	0	106	290	314	252	332	430
5.13	206	0	323	0	108	293	320	255	333	430
5.25	210	0	326	0	110.5	295.5	325.5	258	334	430.5
6	222	0	336	0	112	312	342	270	337	430.5
6.13	226	0	339	0	113	318	343	270	337	430.5
6.25	231	0	342	0	113	323	343.5	270.5	337	430.5
6.38	236	0	345	0	114	327	344	270.5	336.5	430
7	236	0	346	0	116	328	343	271	335	430
7.13	236	0	346	0	116	328	343	271	335	430
7.25	235	0	345	0	116	328	343	271	335	430

A.3.8 Cycle 8

Time	Gas volume (mL)									
(day)	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	0	0	0	0	0	0	0	0	0	0
0.13	8	0	10	0	28	25	26	0	0	6
0.25	23	0	50	0	36	56	29	35	23	32
0.33	35	0	116	0	50	102	49	88	64	155
1	88	0	180	0	130	168	97	156	103	269
1.13	97	0	197	0	136	190	126	175	127	299
1.25	104	0	213	0	138	212	161	201	150	327
1.33	113	0	228	0	140	232	189	228	171	349
2	139	0	263	0	152	287	198	280	210	361
2.13	170	0	286	0	156	300	216	298	242	375
2.25	198	0	308	0	162	312	236	316	272	388
2.33	223	0	328	0	165	322	251	332	283	395
3	238	0	343	0	169	341	265	345	296	402
3.13	253	0	349	0	170	350	283.5	346.5	298	404
3.25	269	0	355	0	173	358	297	348	300	405
3.38	283	0	362	0	177	365	305	349	302	407
4	305	0	373	0	180	373	309	352	320	416
4.13	319	0	374	0	180	374	316	352	321	418
4.25	334	0	374	0	181	375	321	353	322	420
4.33	347	0	375	0	182	376	326	353	322.5	421
5	356	0	378	0	185	377	331	354	323.5	423
5.13	358	0	378	0	185	377	338	354	324	423
5.25	360	0	378	0	185	376.5	343	354	324	423.5
5.33	360	0	378.5	0	185	376.5	343	354	324	423.5
6	360	0	377	0	186	376	343	353	323.5	423
6.13	359	0	377	0	186	376	343	353	323.5	423

A.4 pH changes in sludges S2 to S11 during successive batch feeding cycles with 1 g/L glucose

Cycle No.	Run time (day)	pH of each sample									
		S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
0	0	7.30	7.26	7.49	7.34	7.23	6.97	7.20	7.22	7.21	7.02
1	15	7.21	7.15	7.34	7.22	7.21	6.90	7.10	7.12	7.09	6.93
2	7	7.03	6.89	6.93	6.98	6.87	6.93	7.01	7.03	6.93	6.97
3	7	6.96	6.82	6.85	6.90	6.73	6.86	6.93	6.96	6.86	6.90
4	8	6.77	6.63	6.71	6.72	6.85	6.81	6.83	6.87	6.87	6.96
5	4	6.82	6.85	6.73	6.73	6.81	6.89	6.88	6.87	6.91	6.95
6	5	6.81	6.73	6.75	6.75	6.88	6.91	6.88	6.88	6.90	6.94
7	7	6.81	6.75	6.72	6.75	6.86	6.89	6.89	6.87	6.89	6.94
8	6	6.68	6.63	6.64	6.21	6.84	6.95	6.80	6.81	6.89	6.70
9						6.8					
10						6.78					
11						6.76					
12						6.75					
13						6.75					

A.5 Gas production volume of the as collected sample 6 sludge

Time(day)	Gas volume (mL)	Time(day)	Gas volume (mL)
	Sample 6, pH 7.23		Sample 6, pH 7.23
0	0	14.1	120
1.03	1	15	125
2	46	16	131
2.75	46	17	138
4.83	46	17.13	139
5	42	18.24	148
6.15	44	19.1	157
6.25	52	20	169
7.16	58	20.2	172
8.15	65	21.1	174
9.18	65	22.2	184
11.2	90	24	188
12.1	103	25.1	190
13	115	25.3	190

A.6 Variation with pH of product composition formed by S6 (batch fed with 1 g/L glucose)

A.6.1 cycle 1

	% H_2	% CH_4	% N_2	% CO_2
	MC	MC	MC	MC
pH 6.87	0.0	18.2	71.2	9.7
pH 4.76	0.0	16.2	94.9	5.0
pH 5.15	0.0	0.3	99.2	0.0
pH 5.56	0.0	2.8	60.3	36.6

A.6.2 cycle 2

	% H_2	% CH_4	% N_2	% CO_2
	MC	MC	MC	MC
pH 6.73	0.0	5.9	88.5	5.1
pH 4.55	0.0	0.0	99.9	0.0
pH 5.03	0.0	0.2	99.0	0.0
pH 5.41	0.2	0.6	98.1	0.0

A.6.3 cycle 3

	% H_2	% CH_4	% N_2	% CO_2
	MC	MC	MC	MC
pH 6.85	0.0	30.8	44.6	23.4
pH 4.55	0.0	0.9	98.3	0.0
pH 5.04	0.0	0.3	99.5	0.0
pH 5.34	0.9	2.0	96.0	0.0

A.6.4 cycle 4

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.81		0.0		24.2		55.3		19.8
pH 4.49	0.0	0.0	0.0	0.0	0.0	88.8	0.0	10.5
pH 4.96	0.0		0.2		99.2		0.0	
pH 5.25	0.7	0.0	0.3	1.7	97.4	81.8	0.0	16.3

A.6.5 cycle 5

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.88	0.0	0.0	6.7	20.7	92.5	65.3	0.0	12.5
pH 4.51	0.0	0.0	0.0	0.0	0.0	90.0	0.0	9.2

pH 4.93	0.0		0.2		99.6		0.0	
pH 5.24	0.6	0.0	1.5	2.0	97.6	87.4	0.0	9.9

A.6.6 cycle 6

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.86	0.0		18.2		71.9		9.7	
pH 4.48	0.0	0.0	0.0	0.0	0.0	91.4	0.0	8.1
pH 4.88	0.0		0.3		99.1		0.0	
pH 5.22	0.1	0.0	0.3	2.8	98.1	59.2	0.0	36.6

A.6.7 cycle 7

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.84	0.0	0.0	20.2	23.8	68.8	59.3	10.1	16.2
pH 4.47	0.0	0.0	0.0	0.0	0.0	90.4	0.0	9.2
pH 4.86	0.0	0.0	3.0	5.8	96.2	81.6	0.0	11.9
pH 5.21	0.6	0.0	0.4	28.2	98.4	26.1	0.0	44.7

A.6.8 cycle 8

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.80	0.0	0.0	23.0	24.9	66.1	56.0	10.3	18.2
pH 4.45	0.0	0.0	0.0	0.0	0.0	88.8	0.0	11.0
pH 4.80	0.0	0.0	2.8	5.4	96.3	82.8	0.0	11.2
pH 5.18	0.3	0.0	4.4	25.8	95.0	32.6	0.0	40.8

A.6.9 cycle 9

	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.78	0.0	0.0	23.7	26.3	65.8	51.7	10.0	21.1
pH 4.43	0.0	0.0	0.0	0.0	0.0	92.2	0.0	7.4
pH 4.77	0.0	0.0	6.4	5.9	93.0	81.3	0.0	11.8
pH 5.16	0.2	0.0	5.4	26.6	93.5	29.6	0.0	43.5

A.7 Effect of pH on S6 gas production volume, gas production rate and sludge pH during successive batch feeding cycles with 1 g/L glucose

A.7.1 Cycle 1

Time (day)	Gas volume (mL)			
	pH 7.23	pH 4.5	pH 5.0	pH 5.45
0	0	0	0	0
0.1	5	0	5	14
1	18	0	12	36
2.1	66	0	26	49
3.2	118	0	30	72
3.75	161	0	33	102
3.8	162	0	32	115
3.9	163	0	32	121
4.1	165.5	0	32	123
4.8	178	0	33	135
5.58	180	0	34	138
6.48	180	0	34	143
7.58	180	0	35	143
9.3	180	0	35	143

A.7.2 Cycle 2

Time (day)	Gas volume (mL)			
	pH 6.87	pH 4.76	pH 5.15	pH 5.56
0	112	10	31	0
0.1	134	5	31	0
0.24	154	3	31	0
0.33	184	3	31	0
0.42	187	3	31	2
1	192	3	31	8
1.09	194	3	31	9.5
1.21	197	3	31	11.5
1.34	199.5	3	31	14
2	228	3	31.5	34
2.21	230	3	31.5	36
2.38	234	3	31.5	42
2.96	238	3	31.5	43
4	265	3	31.5	43
4.02	267	3	31.5	43

4.1	268	3	31.5	43
4.2	270	3	31.5	43
4.28	273	3	32	43.5
4.42	275	3	32	44
5.06	275	19.5	32	45
5.14	276	21	32	45
5.27	276	22.5	32	45
5.4	276	25	31	45
5.53	276	30	31	44
5.96	277	36.5	31	44
6.17	277	42	31	44
6.34	278	43	31	44
6.47	278	45	31	44
6.92	280	53	31	42
7.45	280	55	31	42
8.2	285	55	31	44
9.04	293	56	31	44

A.7.3 Cycle 3

Time (day)	Gas volume (mL)			
	pH 6.73	pH 4.55	pH 5.03	pH 5.41
0	0	0	0	0
0.51	12	0	22	31
0.72	36	0	24	41.5
0.78	68	0	25	44
0.8	79	0	26.5	44
0.93	104	0	27.5	48
1.67	132	0	27.5	50
1.98	150	0	28	53
2.8	167	0	28	55
3.1	170	0	28.5	62
3.2	173	0	29	66
4.08	175	0	29.5	72
5.5	182	0	30	78
5.71	182	0	29	76
6.48	186	0	32	76
6.77	184	0	32	78

A.7.4 Cycle 4

Time (day)	Gas volume (mL)			
	pH 6.85	pH 4.55	pH 5.04	pH 5.34

0	0	0	0	0
0.15	63	0	0	37
1	78	0	2.5	35
1.6	82	0	6.5	36
1.71	83.5	0	6.5	36
1.84	84.5	0	6.5	36
2.57	88	0	6.5	36
2.6	88.5	0	6.5	36
2.9	92	0	6.5	33
2.98	92	0	6.5	33.5
3.71	92	0	7.5	34
4.08	92	0	8.5	35
4.58	93.5	0	8.5	35
4.66	94	0	8.5	35
4.87	96.5	0	8.5	35
5.58	106	0	9	33.5
5.66	108	0	8.5	33
5.83	110.5	0	9	33
6.58	112	0	7.5	32
6.71	113	0	8	32
6.92	114	0	10.5	32.5
7.84	122	0	8.5	31

A.7.5 Cycle 5

Time (day)	Gas volume (mL)			
	pH 6.81	pH 4.49	pH 4.96	pH 5.25
0	0	0	0	0
0.08	56	1	2	17.5
0.62	93	2	5	30
0.64	99	2	5.5	32
0.68	105	2	6	35
1.6	141	2	11	36.5
1.85	146	2	13.5	37
2.6	159	2	15	38
2.68	162	2	15.5	38
2.9	166	2	17	38.5
3.65	166	2	19	39
4.1	166	3	20	40
4.64	171	3	25	42
5.1	176	3	25	46
5.6	182	3	36	44

A.7.6 Cycle 6

Time (day)	Gas volume (mL)			
	pH 6.88	pH 4.51	pH 4.93	pH 5.24
0	0	0	0	0
0.06	30	0	0	5
0.58	57	0	11	40
0.7	64	0	14	49.5
0.85	73	0	18	59
0.98	84	0	24.5	70
0.99	96	0	26	76
1.58	116	0	35.5	81
1.68	120	0	39	82
1.77	121.5	0	40.5	78
1.87	124	0	42	76
1.96	126	0	46	76
2.64	130	0	46	78
2.78	132	0	46	78
2.89	133	0	46	79
3.04	134	0	46	80
3.13	136	0	46	81
3.64	137.5	0	47	81
3.77	140	0	47	80
3.99	142	0	47.5	80
4.6	144.5	0	49	79
4.77	145	0	49.5	78.5
4.9	146.5	0	49.5	78.5
4.97	147	0	50	78
5.6	149	0	51	76
5.77	151	0	51.5	76
5.91	153.5	0	52	76
5.97	156	0	52.5	75
6.1	159	0	53	74
6.63	161	0	51	73
6.76	162	0	51	72.5
6.88	164	0	52	72.5
7.17	165	0	50	72
7.6	167	0	50	68
7.77	168	0	50	67
7.98	169	0	50	66
8.63	170	0	50	64
8.77	171	0	49.5	64
8.88	172	0	49.5	63

A.7.7 Cycle 7

Time (day)	Gas volume (mL)			
	pH 6.86	pH 4.48	pH 4.88	pH 5.22
0	0	0	0	0
0.13	16	0	3	12
0.25	39	0	5	30
0.38	70	0	8	48
1	110	0	11.5	79
1.13	114	0	15	80
1.25	118	0	20	80.5
1.33	121	0	25.5	81
2	147	0	36	82
2.15	148	0	39.5	82.5
2.25	149	0	43	83
2.38	150	0	46.5	83.5
3	167	0	57	85
3.13	167	0	59	85
3.26	167.5	0	61	84.5
3.36	168	0	63	85
3.98	169.5	0	64	85
4.33	170	0	64	85
5	173.5	0	65.5	84
5.13	175	0	65	84
5.32	177	0	65	83
5.98	181.5	0	65.5	82
6.13	182.5	0	65	82
6.34	182.5	0	65	82

A.7.8 Cycle 8

Time (day)	Gas volume (mL)			
	pH 6.84	pH 4.47	pH 4.86	pH 5.21
0	0	0	0	0
0.13	12.5	0	0	5
0.25	36	0	4	12
0.38	68	0	10	32
1	93	2	28	79
1.25	109	2	31	81
1.33	114	2	34	82
2	127	2	39	85
2.15	128	2	49.5	85.5

2.25	129	2	50	86
2.34	130	2	50	85
3	139	5	52	85
3.13	140	5	52	85.5
4.13	146	5	55	85.5
4.38	147.5	5	56	85.5
5	150	5	57.5	85
5.13	150.5	5	57.5	85.5
5.25	151	5	58	85
5.34	151	5	58	85
6	152	5	58.5	84.5
6.13	152	5	58.5	84
6.25	152	5	59	84
6.38	152	5	59	84
7.04	153	5	59	83
7.15	153	5	59	83
7.26	153	5	58	83

A.7.9 Cycle 9

Time (day)	Gas volume (mL)			
	pH 6.80	pH 4.45	pH 4.80	pH 5.18
0	0	0	0	0
0.13	15	0	5	15
0.25	40	0	10	35
0.33	72	0	13	51
1	116	0	18	83
1.13	118	0	22	85
1.25	121	0	28	87
1.38	125	0	39	88
2	152	0	43	90
2.13	153	0	46	90
2.25	155	0	49.5	91
2.35	156	0	53	92
3	173	0	66	95
3.13	173.5	0	67	95
3.25	173.5	0	67.5	95.5
3.33	174	0	68	95.5
4	176.5	0	70	95.5
4.13	177	0	70.5	95.5
4.25	177.5	0	70.5	95.5
4.33	178	0	70.5	95
5	180.5	0	71.5	95

5.13	180.5	0	71.5	95
5.25	181	0	71.5	95
5.35	181	0	71.5	95
6	182	0	71.5	94
6.13	182	0	71.5	94
6.25	182	0	71	93.5

A.8 pH changes in S6 during successive batch feeding cycles with 1 g/L glucose

Cycle No.	Run time (day)	pH 7.23	pH 4.5	pH 5.0	pH 5.45
1	10	6.87	4.76	5.15	5.56
2	11	6.73	4.55	5.03	5.41
3	7	6.85	4.55	5.04	5.34
4	8	6.81	4.49	4.96	5.25
5	6	6.88	4.51	4.93	5.24
6	10	6.86	4.48	4.88	5.22
7	7	6.84	4.47	4.86	5.21
8	8	6.80	4.45	4.80	5.18
9	7	6.78	4.43	4.77	5.16

A.9 Variation with pH of product composition formed by S6 (batch fed with 1 g/L cellulose)

A.9.1 Cycle 1

pH of samples	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.98	0.0	0.0	6.4	13.5	93.1	74.4	0.0	11.5
pH 4.5	0.0	0.0	0.0	0.0	0.0	99.8	0.0	0.1
pH 5.0	0.0	0.0	2.3	7.6	97.2	78.9	0.0	13.2
pH 5.5	0.0	0.0	10.3	16.9	89.4	55.0	0.0	27.3

A.9.2 Cycle 2

pH of samples	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.97	0.0	0.0	6.6	13.1	92.7	72.8	0.0	13.7
pH 4.48	0.0	0.0	0.0	0.0	0.0	99.4	0.0	0.2
pH 4.96	0.0	0.0	3.1	8.5	96.3	78.7	0.0	12.3
pH 5.48	0.0	0.0	10.8	17.6	88.8	52.5	0.0	29.4

A.9.3 Cycle 3

pH of samples	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.97	0.0	0.0	5.5	13.3	93.8	74.4	0.0	11.8
pH 4.43	0.0	0.0	0.0	0.1	0.0	99.5	0.0	0.3
pH 4.94	0.0	0.0	2.7	7.2	96.7	79.4	0.0	13.0
pH 5.48	0.0	0.0	9.1	15.8	90.3	56.0	0.0	27.3

A.9.4 Cycle 4

pH of samples	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.96	0.0	0.0	5.8	15.2	93.5	67.4	0.0	16.9
pH 4.42	0.0	0.0	0.0	0.1	0.0	99.4	0.0	0.4
pH 4.92	0.0	0.0	3.7	8.0	95.8	77.0	0.0	14.4
pH 5.46	0.0	0.0	10.2	15.0	89.1	56.3	0.0	27.9

A.9.5 Cycle 5

pH of samples	% H_2		% CH_4		% N_2		% CO_2	
	MC	HS	MC	HS	MC	HS	MC	HS
pH 6.95	0.0	0.0	4.9	13.1	94.6	72.5	0.0	13.8
pH 4.43	0.0	0.0	0.0	0.1	0.0	98.9	0.0	0.6
pH 4.91	0.0	0.0	4.1	8.1	95.3	77.3	0.0	14.0
pH 5.44	0.0	0.0	10.1	16.7	89.1	49.5	0.0	33.4

A.10 Effect of pH on S6 gas production volume, gas production rate and sludge pH during successive batch feeding cycles with 1 g/L cellulose

A.10.1 Cycle 1

Time(day)	Gas volume (mL)			
	pH 6.98	pH 4.5	pH 5.0	pH 5.5
0	0	0	0	0
0.08	5	0	0	0
0.77	10	0	5	5
0.9	12	0	6	8
1.03	15	0	7	10
1.11	17	0	10	15

1.77	20	0	12	18
1.94	21	0	13	20
2.04	21	0	13	22
2.17	22	0	15	22
2.77	25	0	17	23
2.9	25.5	0	18	24
3.03	25.5	0	20	26
3.13	26	0	20	26
3.76	27	0	22	28
4.11	28	0	24	28
4.77	36	0	27	32
4.9	38.5	0	28	33
5.09	43	0	28	34
5.76	50	0	31	38
5.91	51	0	32	41
6.01	53	0	33	42
6.1	54	0	34	43
6.77	67	0	37	47
6.92	69	0	38	50
7.05	72	0	39	51
7.18	75	0	40	53
7.81	86	0	46	58
8.06	92	0	47	61
8.14	95	0	47	63
8.81	102	0	48	68
8.94	104	0	48	69
9.94	112	0	50	72

A.10.2 Cycle 2

Tme (day)	Gas volume (mL)			
	pH 6.97	pH 4.48	pH 4.96	pH 5.48
0	0	0	0	0
0.13	5	0	0	0
0.21	6	0	0	5
0.88	12	0	5	8
1.01	14	0	7	10
1.14	16.5	0	8	12
1.27	20	0	10	14
1.88	23	0	11	15
2.01	23	0	11	15
2.14	24	0	11	16
2.27	25.5	0	12	17

2.88	26.5	0	15	22
3.01	27	0	17	22
3.14	27	0	18	22
3.21	27	0	18	23
3.88	27.5	0	20	25
4	29	0	21	27
4.13	34	0	22	28
4.21	37	0	23	29
4.88	45	0	27	33
5	46	0	28	35
5.13	47.5	0	29	36
5.27	49	0	29	38
5.88	53.5	0	34	45
6	56	0	36	47
6.13	58	0	37	50
6.27	59	0	38	52
6.88	66	0	42	58
7	72.5	0	44	60
7.13	78	0	44	63
7.27	86	0	45	65
7.88	95	0	48	70
8	98	0	50	71
8.13	101.5	0	51	73
8.21	105	0	53	74
9	112	0	56	77
9.21	114	0	58	77
9.88	117	0	58	78

A.10.3 Cycle 3

Time (day)	Gas volume (mL)			
	pH 6.97	pH 4.43	pH 4.94	pH 5.46
0	0	0	0	0
0.08	5	0	0	0
0.75	6	0	0	0
0.88	7	0	0	5
1	7	0	0	8
1.08	8	0	0	12
1.75	15	0	5	14
1.88	16	0	8	14
2	17	0	8	14
2.08	17	0	10	15
2.75	20	0	14	20

2.88	20	0	14	22
3	21	0	15	24
3.08	22	0	16	25
3.75	23	0	20	28
3.88	25	0	21	29
4	28	0	22	30
4.08	31	0	23	32
4.75	37	0	27	37
4.88	40	0	29	38
5	43	0	30	40
5.08	45	0	32	42
5.75	52	0	36	48
5.88	55	0	37	50
6.01	57	0	38	52
6.08	60	0	40	54
6.75	66	0	44	60
6.88	68	0	45	62
7	71	0	47	65
7.08	71	0	48	65
7.75	71	0	49	65
7.88	71	0	49	65
8	71	0	49	65

A.10.4 Cycle 4

Time (day)	Gas volume (mL)			
	pH 6.96	pH 4.42	pH 4.91	pH 5.44
0	0	0	0	0
0.13	5	0	0	0
0.25	10	0	0	5
0.33	11	0	0	5
1	15	0	5	10
1.13	17	0	5	10
1.25	20	0	6	11
1.33	23	0	6	13
2	30	0	8	16
2.13	33.5	0	10	18
2.25	37.5	0	12	19
2.33	39.5	0	13	21
3	52	0	20	25
3.13	56	0	20	25
3.25	61	0	21	25
3.33	70	0	21	26

4	88	0	24	29
4.13	92	0	25	30
4.25	97	0	26	31
4.33	103	0	26	32
5	110	0	32	38
5.13	112	0	32	40
5.25	113	0	33	42
5.33	114	0	35	45
6	116	0	39	50
6.13	116	0	42	53
6.25	116	0	46	57
6.35	116	0	49	60
7	116	0	49	63
7.13	116	0	49	63

A.10.5 Cycle 5

Time (day)	Gas volume (mL)			
	pH 6.95	pH 4.43	pH 4.90	pH 5.42
0	0	0	0	0
0.08	6	0	0	0
0.75	8	0	0	5
0.88	8	0	5	8
1	10	0	6	9
1.08	11	0	6	9
1.75	16	0	9	13
1.88	18	0	9	14
2	19	0	10	15
2.08	19	0	11	17
2.75	21	0	15	20
2.88	21	0	16	20
3	22	0	16	20
3.08	22	0	18	21
3.75	25	0	24	25
3.88	27	0	24	25
4	28	0	25	27
4.08	30	0	26	28
4.75	34	0	32	32
4.88	35.5	0	33	33
5	38	0	34	35
5.08	40	0	36	37
5.75	48	0	40	44
5.88	50	0	40	46

6.75	53	0	48	52
6.88	58	0	50	54
7	68	0	52	56
7.11	75	0	53	58
7.77	87	0	54	65
7.9	93	0	54	68
8.02	99	0	54	69
8.15	106	0	54	70
8.75	110	0	55	70
8.88	110	0	55	70
9	110	0	55	70

A.11 pH changes in S6 during successive batch feeding cycles with 1 g/L cellulose

Cycle No.	Run time (day)	pH of each sample			
Original		6.98	4.5	5.0	5.5
1	13	6.97	4.48	4.96	5.48
2	12	6.97	4.43	4.94	5.48
3	8	6.96	4.42	4.92	5.46
4	8	6.95	4.43	4.91	5.44
5	9	6.94	4.43	4.87	5.42

A.12 Gas composition and comparison between the calculated gas volume and the measured gas volume at different times during one run

Time (day)	percentage of gas at different times (%)				calculated gas volume (mL)	measured gas volume (mL)
	H_2	CH_4	N_2	CO_2		
0.0	0.0	0.0	99.4	0.0	0.0	0.0
0.6	0.0	19.8	53.5	26.0	155.0	62.0
1.0	0.0	26.6	47.2	26.0	186.2	78.0
2.6	0.0	27.8	46.7	25.3	189.2	88.0
2.9	0.0	29.0	44.6	26.2	200.2	92.0

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