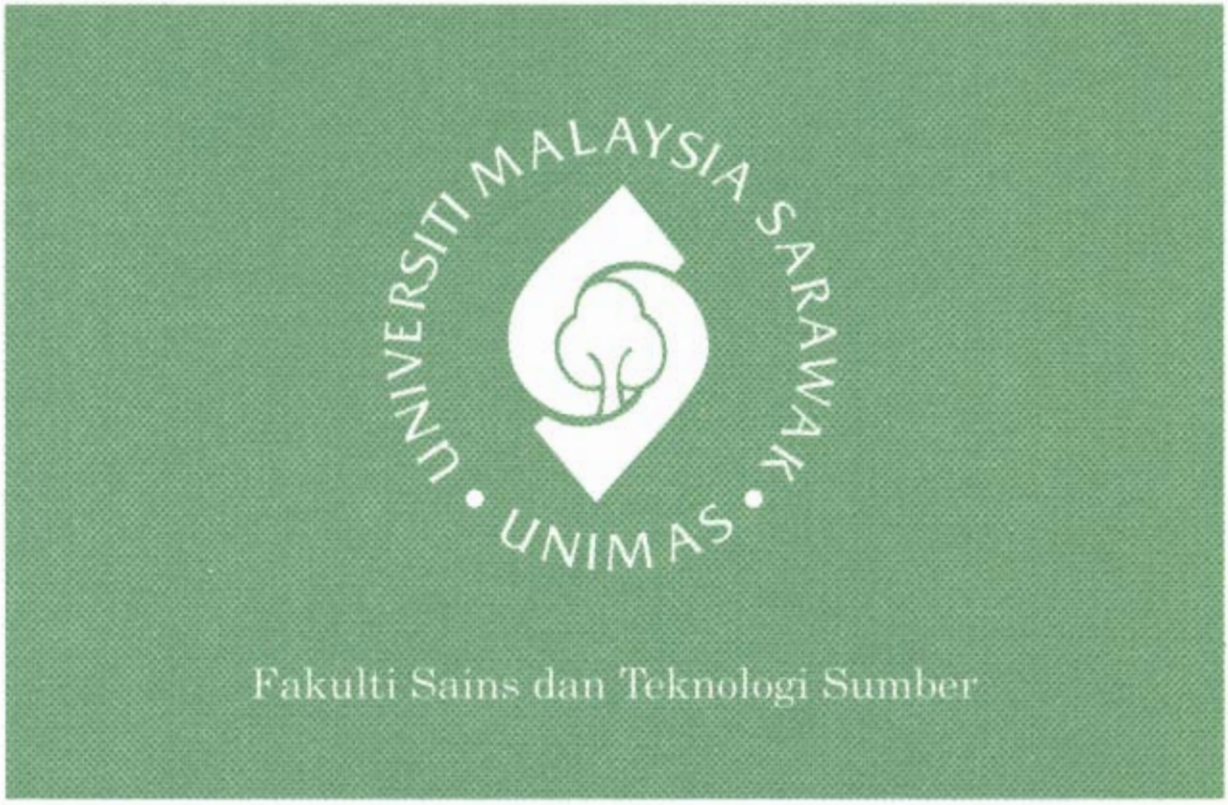

PRODUCTION OF BIOGAS FROM KITCHEN WASTE: A PRELIMINARY STUDY

Thong Sharon

Bachelor of Science with Honours
(Resource Biotechnology)
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Thong Sharon

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This project is submitted in partial fulfillment of the requirement for the degree of
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Supervisor: Prof. Madya Dr Kasing Apun

Co-supervisor: Dr Lesley Maurice Bilung

Resource Biotechnology
Department of Molecular Biology

Faculty of Resource Science and Technology
Malaysia Sarawak University
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List of Abbreviations

AD	Anaerobic digestion
bp	Base pair
CH_4	Methane
CO_2	Carbon dioxide
DNA	Deoxyribonucleic acid
H_2	Hydrogen
Mcr	Methyl-coenzyme reductase
O_2	Oxygen
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
16S rRNA	Small ribosomal ribonucleic acid

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Thong Sharon

Biotechnology Resource Programme
Faculty of Science and Resource Technology
Universiti Malaysia Sarawak

ABSTRACT

There is a worldwide increasing interest in producing alternative energy from organic waste such as sewage sludge, garden waste, or animal dung. In this report, kitchen waste which consisted of vegetable waste was investigated for its potential in biogas production. The experiment was carried out using a self-assembled lab-scale 2-L anaerobic digester. The digester was designed for batch feeding to avoid disturbance of anaerobic digestion during the experiment. A total of 600g of kitchen waste was added to 130g cow dung and allowed for anaerobic digestion for 21 days in room temperature. The total volume of biogas generated after 21 days was 65.5cm^3. Analysis of the microbes in the cow dung revealed that *Staphylococci* as the main organism whereas *Enterococcus* was isolated from pre-treated kitchen waste.

Keywords: Anaerobic digester, biogas, kitchen wastes

ABSTRAK

Pencarian tenaga altenatif daripada bahan buangan organik seperti mendapan kumbahan, bahan buangan kebun dan tinja haiwan semakin meningkat di seluruh dunia. Dalam laporan ini, sisa dapur yang mengandungi sayur-sayuran sahaja digunakan untuk menyelidik potensinya untuk menghasilkan biogas dan kualiti biogas yang dihasilkan. Eksperimen ini dijalankan dengan menggunakan pencerna anaerobik 2L skala makmal. Rekaan pencerna ini disesuaikan untuk tujuan suapan kelompok supaya proses pencernaan tidak akan diganggu sepanjang eksperimen. Sejumlah 600g sisa dapur dan 130g tinja lembu digunakan untuk proses pencernaan anaerobik selama 21 hari dalam suhu bilik. Jumlah isipadu biogas yang dihasilkan selepas 21 hari ialah 65.5cm^3. Analisis mikroorganisma dalam tinja lembu telah menunjukkan kehadiran Staphylococci sebagai organisma utama dan Enterococcus telah diasingkan dari sisa dapur yang telah melalui pra-rawatan.

Kata Kunci: Pencerna anaerobik, biogas, sisa dapur

1.0 Introduction

There is a worldwide increased interest in looking for biogas as an alternative source of energy from organic waste, such as garden waste, kitchen waste, and sewage sludge. Biogas is produced by microbes involved in degrading of organic matter in anaerobic condition. However, little is known regarding the microbiology involved in such treatment method.

Biogas constitutes of about 50 – 70% methane, 30 - 40% CO_2, and the rest is made up of traces of elements of hydrogen, nitrogen, and H_2S (Ilori *et al.*, 2007). There are many experiments carried out worldwide on production of biogas from different organic waste especially in sewage sludge (Elango *et al.,* 2006), municipal organic waste (Hansen *et al.,* 2006), cattle dung (Meher *et al.,* 1993), banana and plantain peels (Ilori *et al.,* 2007).

In this report, kitchen waste is chosen because it is the main waste generated in our country and little research has carried out by using kitchen waste. In Malaysia, 0.5 tonnes of food waste from restaurant, food courts, market, households are produced each year (Lew and Mohamed, 2003). Food wastes are normally disposed off to landfill site. Instead of disposing these large quantities of these wastes, they can be utilized to produce energy source.

Treatment of kitchen waste in anaerobic digester is found to be effective because of its low requirement in nutrients, and methane produced as byproduct, can be combusted to produce electricity (Rittmann and McCarty, 2001). Methane in biogas is produced by

methanogen bacteria. Methanogen community is sensitive to temperature, pH, salinity and tend to be more stable in moderate environments. Methanogens are obligate anaerobes, thus their isolation and cultivation is somewhat elusive due to technical difficulties encounter in handling them under completely oxygen-free conditions.

The objectives of this study are:

i) To design a lab-scale anaerobic digester that can be applied to produce biogas from kitchen waste.

ii) To isolate and characterize microbial populations from kitchen waste by culturing, microscopial and molecular biology methods.

iii) To investigate feasibility and potential to produce biogas from vegetable waste.

2.0 Literature Review

2.1 Methanogen

Phylogenetically, methanogens are *Archaebacteria*, a group of microbes that are distinguished from true bacteria by a number of characteristics, including the possession of membrane lipids composed of isoprenoids, ether-linked to glycerol or other carbohydrates and distinctive ribosomal RNA sequences (Ferry *et al.*, 1993). The catabolic pathways of methanogen can be divided into three groups: i) CO_2-reducing pathways use a series of four two-electron reduction to convert CO_2 or bicarbonate to methane. ii) The methylotrophic pathways catabolize compounds that contain methyl groups, such as methanol, which transfer to a methyl carrier and reduced to methane. iii) Aceticlastic pathway splits acetate, oxidizes the carboxyl group to CO_2 and reduces the methyl group to methane. Methanogens are responsible in CH_4 production in wide variety of anaerobic habitats such as anaerobic bioreactor, marine and freshwater sediments, bogs, geothermal habitats and gastrointestinal tracts. They interact with other anaerobes that break down complex compound such as carbohydrate. Methanogens are divided into five orders: *Methanobacteriales*, *Methanococales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanopyrales*. Hierarchy of their taxonomy is based essentially on 16s rRNA sequencing. The aceticlastic methanogens, *Methanosarcina and Methanosaeta (Methanothrix)*, are relatively slow growing genera with 24 hours doubling times. Hence they will have to compete with more rapidly-growing hydrogenotrophs (hydrogen oxidizing methanogens) with one to 4 hours doubling times (Malina *et al*, 1992).

Moreover, the methanogenic bacteria are affected by the accumulation of hydrogen, and maintenance of low hydrogen levels is important.

2.2 Anaerobic Digestion

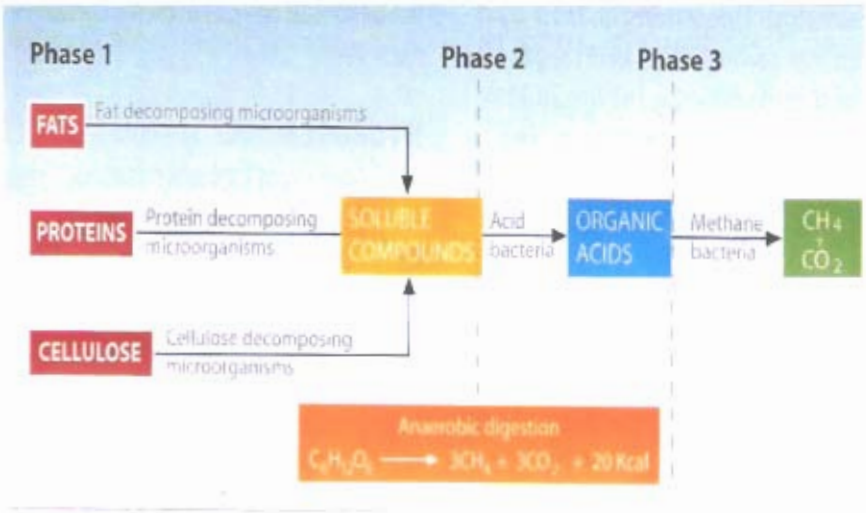

Figure 1: Three phases in the microbiology of biogas production Adapted from Biofuel: The Fifth Utility symbiosis on Octerber, 2006.

Anaerobic digestion is a natural process which converts organic waste into biogas; it takes place spontaneously in natural surroundings such as digesters, landfills, and bogs. It is a complex reaction which involves interaction between microbial populations. The chemistry of the anaerobic digestion producing biogas involve i) hydrolysis, acidogenesis, acetogenesis and methanogenesis as shown in Figure 1. It is usually performed at mesophilic (30-35°C) and sometimes at thermophilic (50-60°C). Methanogen and acetogen act in a symbiotical way, hydrogen producing acetogen create an anaerobic conditions and substrate such has acetate

for methanogen. Methanogens utilize intermediates of the acetogens, to reduce toxic conditions for the acetogens.

Anaerobic digestion plants are used to produce electricity and heating for local villages. Biogas plants have been built in Sweden to produce vehicle fuel for town buses. Anaerobic digestion is also widespread in other parts of the world. India and Thailand have several thousand small scale plants. Several substrates for anaerobic digestion have been study, these include banana and plantain peels (Ilori *et al.*, 2007), municipal organic waste (Hansen *et al.*, 2006), domestic sewage (Elango *et al.*, 2006) and cotton waste (Isci and Demirer, 2007).

2.1.1 Hydrolysis and Acidogenesis

The organic matter is hydrolyzed by enzymes such as cellulose, amylase, protease and lipase produced by microorganisms. Hydrolytic bacteria break down complex carbohydrate, proteins and, fats into soluble compound. Lipids are hydrolyzed by lipases into fatty acids. Protein converted to amino acids by protease, amino acids produced are then hydrolyzed to fatty acids such as acetate, propionate, and butyrate, and to ammonia. Polysaccharides such as cellulose, starch, and pectin are hydrolyzed by celluloses, amylases, and pectinases respectively. The extent of hydrolysis depends largely on the chemical nature of the organic matter being degraded. Essentially all of the oligosaccharides from carbohydrate polymers during hydrolysis are fermented to methane, and so the extent of polymer hydrolysis determines the yield of methane (Smith *et al.*, 1988). Acidogenic bacteria which are commonly found in digester include species of *Butyrivibrio, Propionibacterium, Clostridrium,*

Bacteroides, Ruminococcus, Acetivibrio, Bifidobacterium, Eubacterium, Peptostreptococcus, Peptococcus, Selenomonas, Lactobacillus, Streptococcus and members of *enterobacteriaceae* (Iannotti *et al.*, 1982).

2.1.2 Acetogenesis

Although some acetate and H_2 are directly produced by acidogenic fermentation of sugar and amino acids, both products can also be derived from acetogenic bacteria. Monomers are then metabolized by acetogen with the production of hydrogen, carbon dioxide, and volatile fatty acids. The acid-producing acetogenic bacteria create an anaerobic condition which is essential for the methanogens.

2.1.3 Methanogenesis

Methanogenesis is a natural process occurring under strictly anaerobic environment such as animal digestive tract. The methanogen, methane-producing bacteria play a key role in terminal step of anaerobic digestion. They break down fatty acids into simpler molecules such as water, carbon dioxide and methane, removing the smell and producing biogas (Fulford, 1988).

2.3 Biogas

Biogas is mixture of colourless, flammable gas obtained by anaerobic digestion of organic materials like garden waste, kitchen waste or chicken dung. Production of biogas involves interactions of a few microorganism populations. Anaerobic biogas will have about 67% methane, 30% CO_2, and the rest is made up of traces of elements of hydrogen, nitrogen

and H_2S (Hori *et al.*, 2007). Biogas is cleaner and more convenient to use than traditional fuels, such as firewood, dried dung and kerosene, it gives a hot, clean flame that does not dirty pots or irritate the eyes (Fulford, 1988). Methane is the main component of biogas, an important renewable energy source, particularly in undeveloped area where they still use firewood for heating and cooking (Bagi *et al.*, 2007). In Tarzania, biogas produced from animal dung and human waste has been used in producing electricity for cooking, heating water and other heating process (Robi, 2008). Similarly in Cuba, a plant that operates in the a motel in Villa Clara, 300kms from Havana, produces nearly 300 cubic meters of gas a day, enough to run the motel's kitchen and barbecue (Grogg, 2007). Anaerobic waste treatment plant in Belgium had produced enough biogas to generate energy to meet its own energy demand, providing electricity for 2000 homes. Furthermore, there are biogas powered trains in Sweden and biogas powered bus in France that operated by using biogas.

Extensive research has been done to intensify production of biogas (Bagi *et al.*, 2007). In addition, production of biogas has been investigated using different sources, such as banana and plantain peels (Ilori *et al.*, 2007), municipal organic waste (Hansen *et al.*, 2006), domestic sewage (Elango *et al.*, 2006) and cotton waste (Isci and Demirer, 2007). In Malaysia, reported research on biogas, included hydrogen content in biogas using cow by Vijayaraghavan *et al.* (2006) and operation of anaerobic digester for palm oil mill effluent treatment (Yacob *et al.*, 2005). However, based on available literature, there has not been any systematic study in Malaysia on the production of biogas from vegetable waste.

2.4 Kitchen Waste

Food and kitchen waste are biodegradable waste which can be solid and semi solid. Kitchen waste is derived from food materials such as vegetable, meat scraps, excess or spoiled prepared food, and other discards from domestic or commercial kitchen. It is mainly composed of carbohydrate, lipids, cellulose and protein. In Malaysia, 0.5 tonnes of food waste from restaurant, food courts, market, households are produced each year (Lew and Mohamed, 2003). According to Rittmann and McCarty (2001), treatment of kitchen waste in anaerobic digester is effective because low requirement in nutrients, and methane produced can be combusted to produce electricity. BBC News (2008) reported that England government is considering building biowaste plants in towns and cities across England which turn food waste into energy.

2.5 Molecular Markers

With the recent development of molecular marker, it is now possible to have better understanding on functions of microbial communities in natural ecosystem and precise description of them, especially for microorganisms that are difficult to cultivate such as methanogens. They are obligate anaerobes and some require long incubation period.

2.5.1 Methyl-coenzyme M reductase gene

A molecular marker widely used for studies of methanogen communities is the gene coding for MCR, enzyme unique to methanogenic microbes. The enzyme catalyzes the reduction of methyl-coenzyme M to methane and free coenzyme M. MCR is composed of

three different subunits, α, β, and γ which are encoded by *mcrA, mcrB* and *mcrG* gene (Jerry, 1992). *Mcr* is a good molecular marker because its detection shows the presence of methanogen. The α-subunit of the *mcr* gene has been suggested as a useful genetic marker. The *McrI* gene is unique to methanogens and found in all methanogen species (Springer, 1995).

2.5.2 Small subunit 16S rRNA

Among the three existing ribosomal RNA, 5S, 16S/18S, and 23S/28S, the 16S rRNA is the most widely used marker. It has become an important tool for analyzing natural microbial populations because it has highly conserved primer binding sites and hypervariable regions which can provide specific-specific signature sequences useful for bacterial identification to species level. Primers for the study of methanogens have been developed to amplify various region of the 16S rRNA. Some primers are specific to MCR α-subunit and some target Archeae generally. The 16S rRNA and methyl-coenzyme reductase α subunit (*mcrA*) genes show the same phylogenetic relationships among methanogens, hence *mcrA* can be used for phylogenetic analysis in place of the 16S rRNA gene. *McrA* is specific to methanogen, so non-specific amplification of non-methanogens can be avoided.

3.0 Materials and Methods

3.1 Digester Design

A 2-L laboratory scale anaerobic digesters was initially constructed from a cylindrical closed plastic bottle with a feeding inlet at the mouth of the bottle and a gas outlet near to the neck of the bottle as shown in Figure 2. The amount of gas produced was measured by the downward displacement of water technique using a pipette. The digester was operated at room temperature (30 ± 2 °C).

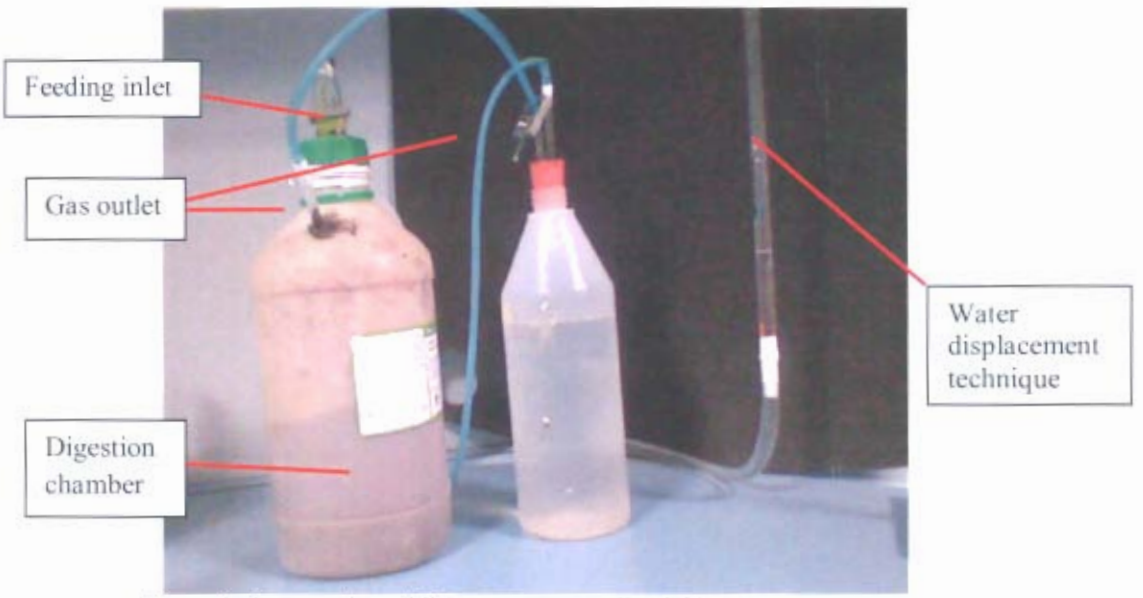

Figure 2: Construction of digester 1 with feeding inlet

However, this digester did not work well for biogas production since oxygen entered the digester during feeding of kitchen waste. After several unsuccessful attempts, a new digester was constructed with a slightly different design. The new digester constructed did not

have feeding inlet to minimize presence of oxygen in the digester and draining pipe is placed near the bottom of the digester for collection of sludge sample.

Figure 3: Construction of digester 2 without feeding inlet

3.2 Pretreatment and preparation of feedstock

For digester 1, mixed kitchen waste was used and consisted mainly of rice, vegetables, and meats. The kitchen waste used in this digester did not undergo pre-treatment. A total of 300g of untreated kitchen waste was added to 100ml of distilled water and 100g of chicken dung and mixed in the digester. The digester was allowed to undergo stabilization for two weeks. After stabilization week, 300g of untreated kitchen waste was added into the digester through feeding inlet every two days for two weeks. This experiment was stopped when there was no biogas production throughout the experiment.

As for the digester 2, vegetable waste consisted mainly of cucumber skins, carrots, and cabbages was used. The vegetable waste was pretreated for a week before it was added into the digester. About 1.4kg of vegetable waste was briefly homogenized using a blender, 300ml of distilled water was added and allowed to undergo initial fermentation without agitation in a 2L Schott bottle for a week at room temperature. After pretreatment, about 600g of pretreated vegetable waste was added to 130g cow dung and 600ml distilled water and mixed in the digester. It was allowed to run for 25 days and the volume of gas produced was recorded.

3.3 Isolation and assessment of microbial populations

For digester 2, microbial species in pre-treated kitchen waste and cow dung were identified before they were placed into the digester. The isolate was cultured on anaerobe basal medium and incubated in an anaerobic jar at room temperature for two to four days. Individual colonies were purified and identified morphologically by light microscope after Gram staining.

4.0 Results

4.1 Isolation and assessment of microbial populations

From the isolation of microbes, two bacteria species were isolated from cow dung and pre-treated kitchen waste. The organisms isolated from cow dung were gram positive cocci and showed pale pink and opaque colonies when cultivated on MacConkey agar. This indicated growth of *Staphylococci* on MacConkey agar. *Enterococcus* species isolated from pre-treated kitchen waste showed red colonies on MacConkey agar, result of gram negative cocci was obtained by further identification through Gram staining.

4.2 Biogas production

From the experiment conducted in the laboratory, a set of results that contain the daily gas production was obtained. The amount of biogas produced daily from kitchen waste was documented in Table 1 and was illustrated in Figure 4. Total biogas production from kitchen waste was documented in Table 2 and was illustrated in Figure 5. As shown in the graph, there was no lag period but highest biogas production of 27.6cm^3 was shown upon the commencement of the experiment. However, the trend of the graph showed a sudden drop in biogas production on the third day of anaerobic digestion followed by a rise on the fifth day. On the third, 13th, 15th, 18th, 19th, 20th, 21th day, there was reduction in biogas production in the digester. The digester had produced a total of 65.5cm^3 of biogas after 21 days of experiment.

Retention time (day)	biogas production (cm^3)
1	27.6
2	21.6
3	-2.9
4	8.3
5	16.6
6	0.4
7	0
8	0
9	4.7
10	2.9
11	0.3
12	0.3
13	-6.3
14	0
15	0.8
16	-1.2
17	0
18	-1.3
19	-2.1
20	-2.5
21	-1.6

Table 1: Daily biogas production. Negative shown on the table indicated that biogas in the digester was being used instead of being produced. (Method to measure biogas produced on daily basis - the reading of biogas produced each day was obtained by deducting total biogas produced on previous day.)

Retention time (day)	Biogas production (cm^3)
1	27.6
2	49.2
3	46.3
4	54.6
5	71.2
6	71.6
7	71.6
8	71.6
9	76.3
10	79.2
11	79.5
12	79.8
13	73.5
14	73.5
15	74.3
16	73.1
17	73.1
18	71.8
19	69.7
20	67.2
21	65.6

Table 2: Total biogas production. Amount of biogas produced daily was added accumulatively.

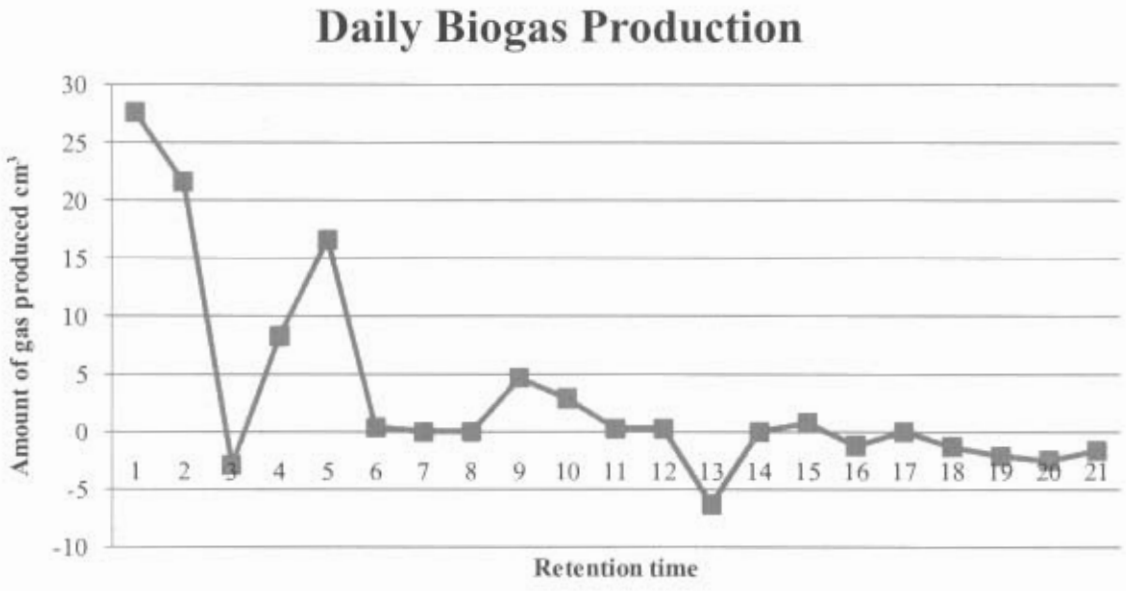

Figure 4:Amount of biogas produced from vegetable waste on a daily basis

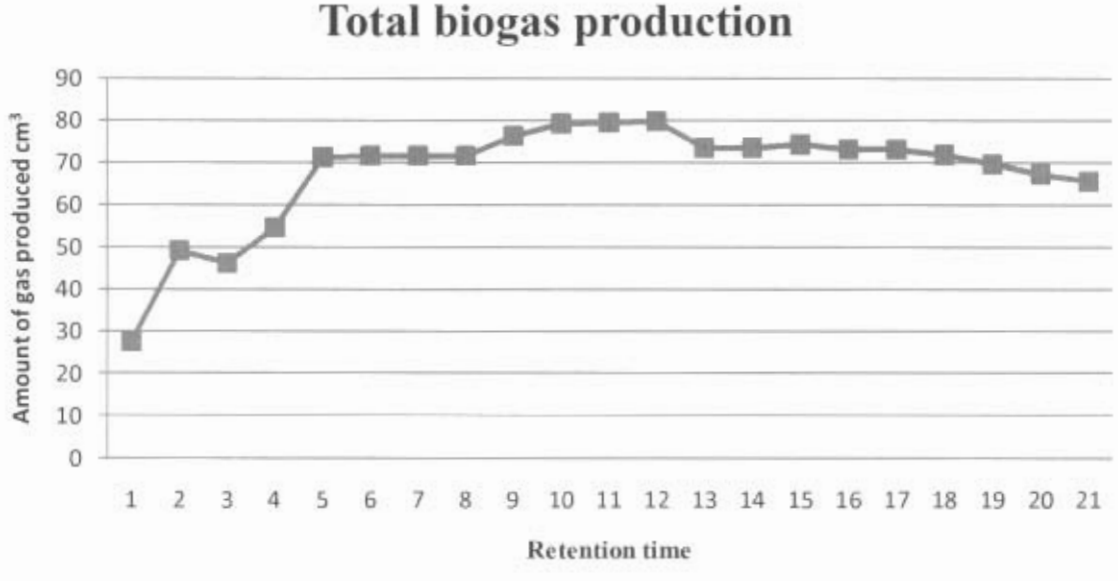

Figure 5: Total biogas production produced from vegetable waste

5.0 Discussion

In this study, two anaerobic digesters were constructed as the first digester failed to produce biogas. The failure to produce biogas can be caused by presence of oxygen in anaerobic digester. Methanogenic decomposition is a process occurring only under strict anaerobic conditions (Chynoweth, 1987). During periodic feeding of kitchen waste into the digester, oxygen may have entered the digester through the feeding inlet which consequently failed methanogenic decomposition. Hence, another batch-type digester without feeding inlet was constructed to address this problem where feed was added once into the digester and fermentation was allowed to proceed until gas production ceased. The digester was fortified with cow dung which provides all the right bacteria to start fermentation process.

In order to achieve higher production of biogas, the organic materials must be highly degradable. Kitchen waste used in this study consisted only of vegetable, which are cellulosic. Cellulosic materials have been described as fibrous microfibrils wrapped in a continuous barrier of middle lamella consisting of lignin and hemicelluloses. In the native state, the accessibility to cellulose is presumably limited to only the openings at both ends of a long and thin structure (Tsao, 1987). Size reduction is viewed to increase the number of exposed ends by cutting the long structure into many short ones. Furthermore, cellulosics are generally resistant to hydrolysis by enzymes or acids because of cellulose structure and lignin barrier (Tsao, 1987). Longer time is needed to break down cellulose to produce fermentable sugars. Hence, pre-treatment of kitchen waste was carried out. Size reduction by homogenizing the kitchen waste had increased the number of exposed ends which increase hydrolysis of cellulose by enzyme. Fermentation of homogenized kitchen waste for one week allowed

hydrolysis of cellulose to produce fermentable sugars which later can be used by microbes in digester to produce ethanol, organic acids, and other products.

Hydrolytic reaction is the primary step in anaerobic digestion and one that control the rate of the process (Mah, n.d.). Hence, appropriate microbial populations presence at this step is very important. *Enterococcus* spp. and *Staphylococci* have been isolated from this experiment. Both of these bacteria have found to be involved in hydrolytic reaction.

Enterococcus spp. isolated from kitchen waste were lactic acid bacteria. They have a fermentative metabolism in which they convert carbohydrates to lactic acid (Gilmore and Clewell, 2002). This showed that *Enterococcus* spp. might have involved in fermentation of pre-treated kitchen waste. *Staphylococci* isolated from cow dung found to be involved in first stage of anaerobic digestion. According to Fischetti (2000), glucose catabolism of staphylococcal species predominantly proceeds through the Embden-Meyerhof Parnas (EMP). The major end product of anaerobic glucose metabolism is lactate which will be the substrate needed by transitional bacteria.

Biogas produced from this experiment was low in quantity. Research carried out by Ilori *et. al.* (2007) had produced a total of 13,356 cm^3 of biogas from 2.5kg mixture of banana and plantain peels, 8,800cm^3 and 2,409cm^3 from 2.5kg banana and 2.5kg plantain peel respectively. From the results obtained, as shown in Figure 4, the graph pattern shown was not as expected which is supposed to be a bell-shaped pattern. Based on available literature, there was usually lag period shown in daily biogas production graph where it is the period required for bacteria to build up to a population large enough to ferment the kitchen waste (Ilori *et. al.*, 2007). However, there were no lag period observed in this study, this might due to the fact that