ANAERODIC DIGESTION OF FOOD WASTE WITH CHICKEN MANURE FOR HYDROGEN AND METHANE PRODUCTION

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By

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ANAEROBIC DIGESTION OF FOOD WASTE WITH CHICKEN MANURE FOR HYDROGEN AND METHANE PRODUCTION

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Chairman: Nor' Aini Abdul Rahman, PhD
Faculty: Biotechnology and Biomolecular Sciences

Recently, environmental concerns associated with animal manure and food waste management generated from the feedlot farming and restaurants in Malaysia needs to be carefully addressed. Biogas production from anaerobic digestion of chicken manure and food waste is regarded as an alternative, due to the simultaneous benefits of environmental pollution control and meeting the national energy demands. Although anaerobic digestion is a common process for treatment of chicken manure and food waste. However, mono-digestion of food waste often leads to digester instability due to the rapid conversion of the easily digestible food waste to volatile fatty acids (VFAs) resulting in a drastic pH drop if no sufficient buffering capacity is present. Therefore the approaches include co-digestion with chicken manure could be beneficial to enhance balance of nutrients, and synergistic effect of microorganisms. The inhibitions effects of biogas production can be overcome by optimizing the physical parameters during hydrogen and methane gas production. The objectives of this study were to establish the optimum operating parameters for hydrogen and methane production from co-digestion of food waste with dry and fresh chicken manure mixed at different ratio. The batch fermentation was conducted using 150 mL serum bottles incubated in anaerobic condition at mesophilic temperature. Food waste was taken from cafeterias with composition ratios 2:1:1 carbohydrate, protein and fiber were prepared. Chicken manure collected from the chicken farm was diluted with water at ratio 1:1. Hydrogen and methane production were performed at different ratio of chicken manure and food waste (0:100, 10:90, 20:80, 30:70, 40:60, 50:50 and 100:0) at initial pH 7. The selected ratio from serum bottle was tested in 500 mL anaerobic digesters with working volume 400 mL at temperature at 35°C. The comparison between dry chicken manure and fresh chicken manure with food waste shows that the highest biogas (972 mL) produced was mixture of fresh chicken manure with food waste in batch system at ratio of 30:70. The highest hydrogen and methane yields obtained were 133.3 and 26.6 mL/gVS, respectively. The community structures of microorganism and their metabolic capability play important roles in hydrogen and methane fermentation processes. The 16S metagenomics analysis was conducted. Tagged 16S rRNA gene pyrosequencing analysis for selected ratio 30:70 of CM:FW showed that the seed culture comprised largely from phyla Proteobacteria, Bacteroidetes, and Firmicutes. During mesophilic hydrogen fermentation, phylum of Firmicutes (40%) was dominant at day 1, while phylum of Firmicutes (15%) was dominated at day 13. Clostridium sp. was the main species detected in the acidogenic phase, while Methanosaeta consilii and Methanosaeta hungatei were detected during methanogenic phase. In this study, the anaerobic digestion was scaled up using 5 L stirred tank bioreactor and was used to improve the biogas production from co-digestion of chicken manure with food waste at ratio 30:70 in batch operation. The experiment was carried out at different temperatures (30, 35, 45 and 55°C). The initial pH was set up at 5.0 and 7.0 for hydrogen and methane gas production, respectively. Gompertz and logistic model were used to evaluate kinetics of hydrogen and methane gas production. The result showed that the reactor with operating temperature of 45oC achieved maximum cumulative hydrogen and methane gas production. The maximum hydrogen and methane yield were 112.4 and 130.87 mL/gVS, respectively. After that, the optimum temperature of 45oC was selected for semi continuous modes using 5L bioreactor. The effect of co-digestion chicken manure with food waste on hydrogen and methane production was investigated at different organic loading rate (OLR) and hydraulic retention time (HRT). The digestion was conducted at varied HRT of 10 and 15 d, and OLR of 0.8 and 1.24 (g VS)/L.d for methane production while for hydrogen production at HRT (18 h, 12 h and 4 h) at OLR (17.7, 34.8 and 50.4) gVS/L.d. The pH was controlled for hydrogen and methane gas production at 5.0-5.5 and 6.0-7.0, respectively. The results indicated that stable hydrogen and methane gas production were achieved from co-digestion of chicken manure and food waste. The highest hydrogen and methane yields were 127.4 and 388.4 mL/g VS at OLR 34.8 and 0.8 g VS/L.d, respectively.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENGURAIAN ANAEROBIK SISA MAKANAN DENGAN NAJIS AYAM UNTUK PENGHASILAN HIDROGEN DAN METANA

Oleh

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Baru-baru ini, perhatian terhadap alam sekitar berkaitan dengan pengurusan tinja binatang dari tempat perladangan dan kedai makan perlu diberikan penelitian. Penghasilan biogas daripada penguraian anaerobik daripada najis ayam dan sisa makanan merupakan alternatif sesuai dengan manfaat-manfaat seperti pengawalan alam sekitar dan juga keperluan tenaga negara. Walaupun penguraian anaerobik merupakan proses yang biasa digunakan untuk rawatan najis ayam dan sisa makanan. Penguraian sisa makanan secara mono menghasilkan ketidakstabilan penguraian disebabkan pertukaran cepat penguraian sisa makanan kepada volatile fatty acids (VFAs) yang menyebabkan pH menurun jika tidak cukup kapasiti bufer yang ada. Maka, pendekatan termasuk campuran dengan najis ayam boleh menjadi kebaikan kepada peningkatan kestabilan nutrien dan kesan sinergi mikroorganima. Kesan pengurangan penghasilan biogas ini boleh ditangani dengan mengoptimakan parameter fizikal semasa proses penghasilan hidrogen dan metana. Tujuan kajian ini dijalankan adalah untuk menentukan parameter operasi optima untuk penghasilan hidrogen dan methana daripada gabungan najis ayam yang kering dan yang segar bersama dengan sisa makanan pada nisbah yang berbeza. Fermentasi kelompok telah dijalankan menggunakan 150 mL botol serum yang direndam dalam keadaan anaerobik. Sisa makanan yang diambil daripada kafeteria dengan nisbah komposisi 3:1:1 (karbohidrat, protein dan serat) disediakan. Najis ayam dikumpul dari ladang ayam dan disediakan pada nisbah 1:1 air dengan najis ayam.

Hidrogen dan metana dijalankan pada nisbah najis ayam dan sisa makanan yang berbeza (0:100, 10:90, 20:80, 30:70, 40:60, 50:50 and 100:0) pada permulaan pH 7. Nisbah yang terpilih daripada botol serum kemudian diuji di dalam 500 mL botol pecerna anaerobik yang dijalankan di dalam makmal pada jumlah kerja 400 mL pada Suhu 35oC. Keputusan menunjukkan gabungan najis ayam segar dengan sisa makanan dapat menghasilkan biogas yang paling tinggi (972 mL) di dalam system ini pada nisbah 30:70. Penghasilan hidrogen dan metana tertinggi adalah pada 133.3 dan 26.6 mL/gVS, masing-masing Struktur komuniti mikroorganisma dan keupayaan metabolik memainkan peranan penting dalam proses fermentasi hidrogen dan metana. Analisis metanogenomik telah dijalankan. Tanda 16S rRNA gen jujukan analisis bagi nisbah yang terpilih iaitu 30:70 (najis ayam: sisa makanan) menunjukkan kelompok benih terdiri daripada bakteria dari Proteobakteria phylum, Bacteroidetes, dan Firmicutes. Semasa fermentasi hidrogen pada keadaan mesopilik, phylum Firmicutes (40%) adalah dominan pada hari pertama, manakala phylum of Firmicutes (15%) didominasi pada hari 13. Clostridium sp. adalah spesies utama yang dikesan dalam fasa asidogenik, manakala Methanosaeta consilii dan Methanosaeta hungatei dikesan semasa fasa metanogenik. Dalam kajian ini, penguraian secara anaerobik ditingkatkan skala kepada reaktor 5 L dan digunakan untuk memperbaiki penghasilan biogas daripada gabungan najis ayam dan sisa makanan pada nisbah 30:70 dalam operasi kelompok. Kajian ini dijalankan pada suhu yang berbeza (30, 35, 45 and 55°C). pH permulaan pada 5.0 and 7.0 untuk hidrogen dan metana gas. Model Gompertz dan Logistic digunakan dalam kajian ini untuk mengkaji kinetik ke atas penghasilan gas hidrogen dan metana. Keputusannya menunjukkan reaktor pada suhu 45oC mencapai penghasilan gas hidrogen dan metana paling tinggi. Pencapaian hidrogen dan metana paling tinggi adalah 112.4 dan 130.87 mL/gVS, masing-masing. Kemudian, suhu yang optimum pada 45oC dipilih untuk dijalankan dalam reaktor tangki 5 L. Kesan najis ayam bersama sisa makanan ke atas penghasilan hidrogen dan metana dikaji pada kadar pemuatan organik (OLR) dan masa pengekalan hidraulik (HRT). Masa pengekalan hidraulik yang berbeza pada 10 dan 15 hari dengan kadar pemuatan organik (OLR) 0.8 dan 1.24 (g VS)/L.d untuk penghasilan metana dan bagi penghasilan hidrogen, masa pengekalan hidraulik (HRT) (18, 12 dan 4 jam) pada kadar pemuatan organik (OLR) adalah (17.7, 34.8 dan 50.4) gVS/L.d. pH dikawal semasa penghasilan hidrogen dan metana pada 5.0-5.5 dan 6.0-7.0, masing-masing. Keputusan menunjukkan penghasilan hidrogen dan metana stabil dicapai daripada penguraian campuran najis ayam dengan sisa makanan. Penghasilan hidrogen dan metana paling tinggi pada 127.4 dan 388.4 mL/g VS pada OLR 34.8 dan 0.8 g VS/L.d, masing-masing.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

CM	Chicken manure
FW	Food waste
H_2	Hydrogen
CH_4	Methane
GC	Gas chromatography
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
MSW	Municipal solid waste
NaOH	Sodium hydroxide
TS	Total solid
TSS	Total suspended solid
TVS	Total volatile solid
VSS	Volatile suspended solid
VFA	Volatile fatty acid
C:N	Carbon:Nitrogen
HRT	Hydraulic retention time
OLR	Organic loading rate
rRNA	Ribosomal ribonucleic acid
CO_2	Carbon dioxide
KW	Kitchen waste
TAN	Total ammonium nitrogen
ATP	Adenosine triphosphate

AD	Anaerobic digestion
KOH	potassium hydroxide
BLAST	Basic local alignment search tool
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reduction
NH_3-N	Ammonia nitrogen
CSTR	Continuous stir tank reactor
KFC	Kentucky Fried Chicken
ATP	Adenosine triphosphate
WAS	Waste activated sludge
AcoD	Anaerobic co-digestion
Mt	Million tons

CHAPTER 1

INTRODUCTION

1.1 Background

The global growth in energy demand has induced for alternative energy sources. A more efficient deployment of renewable energy sources will facilitate a reduction in greenhouse gas emissions and air pollution (Poeschl et al., 2010). Nowadays, air pollution and global warming are the major concerns arisen in the natural environment of human being. This issue could be attributed to the enormous evolution of greenhouse gases (GHG) (CO_2), methane (CH_4) and nitrous oxide (N_2O) which have been obtained from combustion of fossil fuels concurrently with increasing world population (Hosseini et al., 2013). Therefore, renewable energies offer an environmental friendly alternative to fossil fuels and for a lesser contribution to climate change. Sustainable and clean renewable energy such as biofuel can be used to eliminate or reduce methane and carbon dioxide emissions, by replacing the fossils fuel which emits greenhouse gases (GHG) that produced global warming.

Biogas is an environmentally advantageous energy source which is mostly comprised methane (60%) and carbon dioxide (35-40%). Biogas is the gas evolved from a process known as anaerobic digestion (AD). AD is known as the degradation of organic compounds to simple substances by microorganisms which live as syntrophy under the lack of oxygen with releasing biogas (Christy et al., 2014). AD has been found as a biological process for the transformation of waste materials to energy sources through the treatment of various organic waste such as municipal solid waste, food waste, industrial waste, sewage sludge, animal manure and agricultural residues (Yong et al., 2015).

Food waste is an easily biodegradable organic matter with high moisture, carbohydrate, lipid, and protein contents represents a source of bioenergy. AD of food waste is facing many technical challenges. One important technical challenge is that when lacking concise process control and optimization, harmful intermediate compounds can be easily produced, reducing system stability, or causing low methane yield or foaming (Grimberg et al., 2015). One common type of system instability is caused by the rapid conversion of the easily digestible food waste to volatile fatty acids (VFAs) at an early stage of the digestion process, resulting in a drastic pH drop if no sufficient buffering capacity is present (Banks et al.,2011; Zhang et al.,2012). Therefore, the approaches include co-digestion with chicken manure or using waste activated sludge to adjust C:N, adding trace elements to accelerate the growth of methanogens and methane formation. However, mono-digestion of food waste often leads to digester instability and even failure at higher organic loading rates (OLR, above 2.5 g VS/L/d), especially under thermophilic conditions, due to the accumulation of VFAs and ammonia inhibition (Zhang et al., 2013). Co-digestion of food waste with manure, sewage sludge, and lignocellulosic biomass could be beneficial

due to dilution of toxic chemicals, enhanced balance of nutrients, and synergistic effect of microorganisms (Zhang et al., 2014).
Animal manure is considered to be an excellent co-substrate because of its high buffer capacity and a wide variety of nutrients (Mata-Alvarez et al., 2014).

Nowadays, the demand for chicken meat and chicken eggs had increased in Malaysia. The upward trend of chicken meat consumption is seen in Malaysia from 36 to 39 kg of per capita consumption from 2000 to the year 2011 and approximately, in the year 2010 (Jayaraman et al., 2013). The amount of the manure, for instance, has been estimated 0.08-0.1 kg/day for chicken (Avcioğlu et al., 2012). Therefore, the uncontrolled rapid development of chicken farm in Malaysia has subsequently produced untreated chicken manure which has potential to create environmental problems. Since the organic matter in the chicken manure is biodegradable, anaerobic digestion of these wastes can be considered as an alternative method to minimize the amount of waste and recover energy by the production of methane.

The productions of biogas have a pathway for biogas production. During fermentation, first pathway generated of acidogenesis and acetogenesis in anaerobic digestion process (AD), but rapidly consumed by methanogenesis bacteria in the single phase digestion (Chananchida el., 2013). Separation of acidogenesis and methanogenesis in the two phase AD system can recover both hydrogen and methane (Hallenbeck et al., 2012; Kapdan et al., 2006). Several studies, however, demonstrated that the two phase AD achieved higher overall degradation efficiency and is more advantageous than the single phase system for the treatment of the waste feedstocks containing a large fraction of recalcitrant organic matters such as food waste (Pan, 2008). The two-stages fermentation process with hydrogen and methane production in respective reactor can increase the energy recovery efficiency (Lee and Chung, 2010). It was considerable potential to enhance the economic feasibility of waste treatment.

Biogas can be produced through anaerobic degradation complex substrate. Thus, controlled environmental conditions such as pH, temperature, substrate concentration and nutrient supply should be taken into consideration to achieve high biogas production such as methane and hydrogen. The controlled conditions can affect rates of biogas production, product formation (organic acids), growth rates, bacterial activities and also may change methabolic pathway. However, biogas fermentation is still considered to be an unstable and unmanageable technology. Advances in molecular biology have enhanced our understanding of the microbial ecology relevant to methane fermentation. Understanding the microbial mechanism of biogas fermentation will contribute to the development of improved processes. In theory, the large volume of data available on the subject should inform the analysis of how various factors, such as carbon source, temperature, retention time, loading rate and reactor type affect the microbial community.

However, it is difficult to reach clear conclusions. One important reason for this difficulty is the complexity and flexibility of the microbial community during methane fermentation. Correlating various factors with the microbial community during methane fermentation will become much easier, if variables are studied systematically, fixing some of the key factors while varying others. Since microorganisms within a community are specialized, they are each dependent on others to provide them with substrates and or metabolize their products to favor their metabolic activity. In order to thrive, each bacterial or methanogen species also requires a specific combination of physical and chemical conditions, such as pH, temperature, and salinity in addition to substrate availability. Thus, while microbial communities from different environments can perform anaerobic digestion through the same general steps of hydrolysis, acidogenesis, acetogenesis and methanogenesis, the particular microbial species that populate them tend to vary between environments depending on physical and chemical conditions. Since biomethanation is performed by microbial communities, additional improvements in manure digester performance could be accomplished by selecting or manipulating particular groups of microorganisms that populate them. However, communities from anaerobic manure digester remain largely uncharacterized, so a deeper understanding of population structure, as well as metabolic properties and interactions, are necessary in order to successfully improve performance through microbiological manipulation (André-Denis et al., 2013).

1.2 Problem statements

According to Food and Agricultural Organization (FAO, 2012), one third of food produced globally for human consumption is lost along the food supply chain. In many countries food waste are currently landfilled or incinerated together with other combustible municipal wastes for possible recovery of energy. However, these two approaches are facing more and more economic and environmental stresses. Amounts of food waste are generated every day worldwide (Zong et al., 2009). However recycling methods, such as composting and feed stuffing, are not proper methods of treating food waste due to its high salinity. The rapid development of useless materials will face serious problem to the local authorities due to the cost transportation and disposal municipal solid waste (MSW). The overloaded capacity in the landfill brings up land shortage due to the strategic area and land price for landfill purpose. Rapid urbanization and population increment become more challenging as the number of MSW generated rises (Azni, 2009). The food waste is, for the most part, disposed of in landfill. In light of rapidly rising costs associated with energy supply and waste disposal and increasing public concerns with environmental quality degradation, conversion of food wastes to energy is becoming a more economically viable practice. However recycling methods, such as composting and feed stuffing, are not proper methods of treating food waste due to its high salinity.

As is known, food waste (FW) is a classic organic waste for H_2 and CH_4 production (Lee et al., 2010). But sole FW digestion is easy to make VFA and NH_3-N inhibition in the fermentation system due to lower pH (4-5) and C:N ratio (10-15) (Wang et al., 2006). However using food waste for hydrogen production may resulted in a problem where the inoculums would be outgrown by the normal flora in food waste. Therefore, the proper inoculums concentration needs to be optimized. An increase in substrate concentration could increase hydrogen production to a certain level. However, an excessive substrate concentration can cause a build-up of volatile fatty acids (VFAs) in the system, leading to a decline of pH in the reactor and could inhibit the growth of hydrogen producers (Fan et al., 2006). Therefore, the optimum substrate concentration as well as an addition of the buffer at suitable concentration to counteract a decrease in pH would remove this limitation.

Animal manure contains a high concentration of nitrogen (N) and phosphorus (P), which causes nutrient imbalance and pollution in environment. Furthermore, the livestock manure contains the residues of some harmful substances such as growth hormone, antibiotics and heavy metals. On the other hand, microorganisms in the animal manure could contaminate the environment, which in turn causes the outbreak of the human diseases. In this regard, it has been found that the disposal of the livestock manure has a polluting impact on the environment which contaminates air, soil and water sources. Hence, the treatment of animal manure and slurries by AD process has the beneficial out comes of producing quality fertilizer, reduction of odors and microbial pathogens with the sustainable production of energy source as biogas.

The factors that affect the biogas production such as carbon source, temperature, retention time, loading rate and reactor type affect the microbial community. However, it is difficult to reach clear conclusions. One important reason for this difficulty is the complexity and flexibility of the microbial community during methane fermentation. Correlating various factors with the microbial community during methane fermentation will become much easier, if variables are studied systematically, fixing some of the key factors while varying others.

The key biohydrogen-producing microbes may originate from substrates, microbial seed or both. The use of complex microbial seed cultures as starting inocula is advantageous for biohydrogen production from complex organic substrates. These advantages include higher operating stability and tolerance to indigenous microbes present in the feedstock, as well as capability for producing a wide range of hydrolytic enzymes (Argun and Kargi, 2009). Food waste is a common substrate for biogas production. Typically, it comprised of starch and fiber polysaccharides i.e. cellulose and hemicellulose, proteins and lipids as major constituents and vitamins and ash as minor constituents (Kapdan and Kargi, 2006).

According to the previous study of renewable energy sources, mostly focussing on co-digestion of food waste with dairy manure (Agyeman and Tan, 2014), food waste with cow manure (Zhang et al., 2013b), and food waste with food vegetable waste (Sun et al., 2014). Most of the study from the previously of chicken manure with different kind of substrates by investigating the effect of co-digestion of chicken manure (CM) and organic fraction of municipal solid waste (OFMSW) on biogas production have done in an anaerobic digester which is focused on organic loading rate and co-digestion of the substrates. So far, the study on food waste with chicken manure not study yet by researcher. Since the organic matter in the chicken manure is biodegradable, anaerobic digestion of these wastes can be considered as an alternative method to minimize the amount of waste and recover energy by the production of methane and the characteristics of food waste such as high concentration of carbohydrate and high digestibility has been proved to be used to mix with chicken manure in fermentation due to food waste can be supplied to mixed culture, cheap and renewable (Pan et al., 2008). Therefore, the study of chicken manure with food waste was used according to the following objectives.

1.3 Objectives

The hydrogen and methane production has been studied from the co-digestion at different ratio of food waste with chicken manure. The objectives of this study were:

To evaluate hydrogen and methane production from the co-digestion of food waste with chicken manure at different ratio in a batch system

To assess microbial community in both hydrogenesis and methanogenesis stage in food waste and chicken manure by using 16S Metagenomics Analysis
To evaluate the performance of the hydrogen and methane fermentation phase using a kinetic model for anaerobic digestion at different temperature in 5 L of reactor

To determine the effect of hydraulic retention time (HRT) and organic loading rate (OLR) in semi continuous system reactor using 5 L of reactor for the hydrogen and methane production

1.4 Research scope

The scope of the study is given below:

1. To investigate the effect co-digestion process of dry CM and Fresh CM with food waste at mixing ratios of 0:100; 10:90; 20:80; 30:70; 40:60, 50:50 and 100:0 on the biogas production potential in batch mode operation.

2. The best performance of biogas production between dry CM and fresh CM with food waste was chosen to perform in the reactor at 500 mL with control the pH.

3. The highest hydrogen and methane gas production was selected to study microbial community in both hydrogenesis and methanogenesis in food waste with chicken manure by using 16S Metagenomics Analysis.

4. The best performance of hydrogen and methane yield was performed in the biggest scale of reactor at 5 L to study kinetic model for anaerobic digestion at different temperature in the batch system.

5. The effect of the different temperature that have a potential to produce the highest methane and hydrogen gas was selected to study the effect of hydraulic retention time (HRT) and organic loading rate (OLR) in semi continuous system reactor (CSTR).

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