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ENHANCEMENT OF HYDROGEN AND METHANE PRODUCTION FROM CO-DIGESTION OF FOOD WASTE AND CHICKEN MANURE

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ENHANCEMENT OF HYDROGEN AND METHANE PRODUCTION FROM CO-DIGESTION OF FOOD WASTE AND CHICKEN MANURE

By

MOHD FAIZ BIN MAT SAAD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

March 2017

To my parents,
Mat Saad bin Awang &
Hasnah binti Mohd Noor

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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March 2017

Chair : Nor'Aini Abd Rahman, PhD
Faculty : Biotechnology and Biomolecular Sciences

The demand for clean energy from renewable resources stimulates biohydrogen and biomethane production from agro-food waste as an alternative fuel to replace fossil fuel. A combined production of biohydrogen and biomethane has attracted growing attention of researchers and industries worldwide due to their potential as fuel substitute. Biosynthesis of biohydrogen and biomethane from food waste and chicken manure fermentation initiates clean technologies for energy generation thus provide the solution for waste treatment. Despite that, biogas production of hydrogen and methane have limiting factors that relate to soluble metabolites and active microorganisms. This inhibition effects can be overcome by optimizing several factors for biohydrogen and biomethane production. The objectives of this study were to determine the best ratio of food waste and chicken manure for biogas production in batch fermentation and to evaluate the effect of different inoculums and heat treatment upon selected inoculum on biohydrogen and biomethane production besides the microbial diversity in the fermentation using Next Generation Sequencing (NGS) of 16S ribosomal RNA were also carried out. The batch fermentation was conducted using 150 mL serum bottles incubated in anaerobic condition. Food waste with composition ratios of 3:1:1 of carbohydrates, protein and fiber were used as substrate added with chicken manure freshly collected from poultry farm. Biohydrogen and biomethane production were tested for the effects of different substrate ratio, different inoculums and heat treatment on selected inoculums. Temperature and initial pH were kept constant at 35°C and initial pH 7. Biohydrogen and biomethane from food waste and chicken manure was performed at different ratio (40:60, 50:50, 60:40 and 70:30 (v/v)) inoculated with aeration tank sludge (ATS), return activated sludge (RAS) and palm oil mill effluent (POME) sludge. Heat treatment was carried out at 80°C for 20 minutes to eliminate the nonsporing bacteria. Biogas was collected daily throughout 10 days fermentation and the composition of hydrogen and methane in the biogas was

analyzed by gas chromatography. The highest biogas yield obtained was 111.72 NmL/g TSS for the experiment conducted at 50:50 (v/v) substrate ratio added with RAS as inoculum without heat treatment. The highest percentages of hydrogen and methane produced were 53.35% and 52.85%, respectively. Microbial assessment was performed by using Next Generation Sequencing (NGS) of 16S ribosomal RNA technique. *Clostridium* sp. was related to biohydrogen production methanotroph such as *Cyclobacteriaceae, Saprospiraceae* and *Chloroflexi* that were inhibited after the heat treatment. Heat treatment of inoculums is not suitable for the production of both biohydrogen and biomethane since it inhibits the methanogens. Thus, controlling operating conditions were important for hydrogen-producing bacteria as well as methanogens for biohydrogen and biomethane production.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENAMBAHBAIKAN PENGHASILAN HIDROGEN DAN METANA MELALUI CAMPURAN BERSAMA SISA MAKANAN DAN NAJIS AYAM

Oleh

MOHD FAIZ BIN MAT SAAD

Mac 2017

Pengerusi : Nor'Aini Abd Rahman, PhD
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Permintaan terhadap tenaga tenaga bersih daripada sumber boleh diperbaharui telah menggalakkan penghasilan gas biohidrogen dan biometana sebagai alternatif kepada bahan bakar daripada bahan api fosil. Kombinasi pemprosesan biohidrogen dan biometana telah semakin menarik minat para penyelidik dan industri seluruh dunia disebabkan oleh potensinya sebagai pengganti bahan bakar. Biohidrogen dan biometana dari penapaian sisa makanan dan najis ayam merupakan teknologi bersih untuk penjanaan tenaga seterusnya adalah penyelesaian untuk rawatan sisa. Namun, penghasilan hidrogen dan metana mempunyai faktor kekangan yang berkait dengan metabolit dan mikroorganisma aktif. Kesan kekangan ini boleh ditangani dengan mengoptimumkan beberapa parameter untuk penghasilan biohidrogen dan biometana. Objektif kajian ini dijalankan adalah untuk menentukan nisbah terbaik antara sisa makanan dan najis ayam untuk penghasilan biogas, untuk mengetahui kesan penggunaan inokulum berbeza dan rawatan haba ke atas inokulum terpilih untuk penghasilan biohidrogen dan biometana dan untuk mengetahui kepelbagaian mikrob di dalam fermentasi menggunakan Next Generation Sequencing (NGS) untuk 16S DNA ribosom untuk mengenalpasti dan membandingkan bakteria yang wujud di dalam sampel yang tidak diberi rawatan dengan rawatan haba. Penapaian kelompok telah dijalankan menggunakan botol serum 150 mL yang dieram dalam keadaan anaerobik. Sisa makanan yang diambil dari kafeteria dengan nisbah komposisi 3:1:1 dari karbohidrat, protein dan serat digunakan sebagai substrat dicampur dengan najis ayam yang diambil segar dari ladang ayam. Penghasilan biohidrogen dan biometana diuji untuk kesan menggunakan substrat berbeza, inokulum berbeza dan kesan rawatan haba terhadap inokulum yang dipilih. Suhu dan pH di kekalkan pada 35°C dan pH permulaan 7. Sisa makanan dan najis ayam digabungkan pada nisbah berbeza (40:60, 50:50, 60:40 and 70:30 (v/v)) dan pada tiga inokulum berbeza (enapcemar tangki pengudaraan (ATS), enapcemar diaktifkan kembali (RAS) dan

enapcemar efluen kilang kelapa sawit (POME)) digunakan sebagai inokulum. Rawatan haba dijalankan kepada inokulum yang dipilih pada 80°C selama 20 minit bagi menyingkirkan bakteria yang tidak diingini. Biogas dikumpulkan setiap hari selama 10 hari dan komposisi hidrogen dan metana di dalam biogas dianalisa menggunakan kromatografi gas. Penghasilan biogas tertinggi ialah 111.72 NmL/g TSS untuk eksperimen yang dijalankan pada nisbah ratio 50:50 (v/v) yang dicampur bersama RAS sebagai inokulum tanpa rawatan haba. Peratus tertinggi penghasilan biohidrogen dan biometana adalah 53.35% dan 52.85%, setiap satu. Mikroorganisma dinilai menggunakan Next Generation Sequencing (NGS) dari teknik 16S ribosomal RNA. *Clostridium* sp. berkait dengan penghasilan biohidrogen dan metanogen seperti *Cyclobacteriaceae, Saprospiraceae* dan *Chloroflexi* bertanggungjawab ke atas penghasilan metana. Rawatan haba didapati tidak sesuai bagi penghasilan serentak biohidrogen dan biometana kerana ianya menghalang metanogen. Justeru, kawalan keadaan semasa penghasilan biohidrogen dan biometana adalah sangat penting untuk membantu pertumbuhan bakteria penghasil hidrogen dan juga metanogen untuk penghasilan biohidrogen dan biometana.

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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 Master of Science. The members of the Supervisory Committee were as follows:

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This is to confirm that:

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
ATS	Aeration tank sludge
Biohydrogen	Biological hydrogen
Biomethane	Biological methane
Biohythane	Biological hydrogen and methane
BLAST	Basic local alignment search tool
BOD	Biological oxygen demand
$C_6H_{12}O_6$	Glucose
CH_4	Methane
CO_2	Carbon dioxide
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
FISH	Fluorescent *in situ* hybridization
F/M	Food to microorganism
GC	Gas Chromatography
H_2	Hydrogen
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
HNO_3	Nitric acid
HPLC	High performance liquid chromatography
MSW	Municipal solid waste
NmL	Normalize volume in mL
NADH	Nicotinamide adenine dinucleotide

NaOH	Sodium hydroxide
NCBI	National Center for Biotechnology Information
O_2	Oxygen
PCR	Polymerase chain reaction
POME	Palm oil mill effluent
rRNA	Ribosomal ribonucleic acid
SD	Standard deviation
TS	Total solid
TSS	Total suspended solid
TVS	Total volatile solid
VSS	Volatile suspended solid

LIST OF NOMENCLATURE

$C_{H, i}$	Fraction of hydrogen gas in the headspace of the bottle measured using gas chromatograph in the current (%/100)
$C_{H, i-1}$	Fraction of hydrogen gas in the headspace of the bottle measured using gas chromatograph in the previous time interval (%/100)
e	2.718281828
H	Cumulative biohydrogen produced (mL)
P	Biogas production potential (mL)
Rm	Rate of biogas production (mL/h)
$V_{G,I}$	Total biogas volume at current (mL)
$V_{G,I-1}$	Total biogas volume at previous time interval (mL)
V_{H}	The total volume of gas in the headspace of reactor (mL)
$V_{H, i}$	Cumulative biohydrogen production at current (mL)
$V_{H, i-1}$	Cumulative biohydrogen production at previous time intervals (mL)
$V_{M, i}$	Cumulative biomethane production at current (mL)
$V_{M, i-1}$	Cumulative biomethane production at previous time intervals (mL)
λ	Lag phase (h)
t	Fermentation tome (h)

CHAPTER 1

INTRODUCTION

1.1 Introduction

Majority of the world's energy demand today rely on fossil fuels, which are being depleted. Besides, the energy production from fossil fuels that have impacts on the environment such as greenhouse emissions, global warming, climate change and ozone layer depletion are causing an urgent need for renewable energy (Azbar & Levin, 2012). Hydrogen and methane are two major gaseous energy carriers that can address all the above concern as a viable alternate energy source.

Hydrogen is labelled as the cleanest fuel because when it burnt, it released energy and the only product left is water. Utilization of hydrogen can be described as a growing trend of the future society due to its zero-carbon emission and has two times energy yield than any hydrocarbon fuel (Cai et al., 2004). The development of society based on hydrogen is however has a drawback due to its cost-intensive in both processes and operations. By utilizing the waste materials such as food waste, treatment costs will be reduced due to the abundance and low material cost.

Methane in the other hand is used as a vehicle fuel in natural gas form. As for the chemical industry purpose, methane is usually being used as favorite feedstock for the production of methanol, acetic acid, acetic anhydride and hydrogen. Comparing methane with other fossil fuels, it is claimed as more environmentally friendly than other fossil fuels such as gasoline, petrol and diesel, it is however limited by its narrow range of flammability, high ignition temperature and slow burning speed. These weaknesses in methane are interestingly complements perfectly by the hydrogen because it increases the H/C ratio. By adding hydrogen, the narrow range of flammability of methane can be improved, in that way improving the fuel efficiency. The flame speed of methane will be greatly increased and the high ignition of methane can be reduced by the addition of hydrogen (Falco & Basile, 2015).

Various attempts have been carried out in order to use cheap and renewable sources for a fuel substitute. Substrate such as food waste (Kim et al., 2008; Pan et al., 2008) palm oil mill effluent (Ismail et al., 2010), wastewater (Yang et al., 2007), dairy manure (Amon et al., 2007) and chicken manure (Abouelenien et al., 2010) have been studied to produce biohydrogen and biomethane. The usage of carbon and nitrogen sources readily available in food waste and chicken manure for biohydrogen and biomethane production will generate less economic burden for the future by turning food waste and chicken manure into energy source while overcome the pollution created by these waste.

One way of producing biohydrogen and biomethane is through anaerobic degradation of complex substrate. Anaerobic digestion is a series of biological process during which microorganisms break down organic matter in the absence of oxygen environment. Anaerobic digestion could be used trimmings and solid waste, with varying degrees of degradation. Through a process called methanogenesis, anaerobic digestion produce methane biogas that can be used as a renewable energy source. Production of biogas from anaerobic digestion of biomass is a technology that can produce renewable energy and also minimize the environmental risks related with manure and food waste. Co-digestion of multiple biosolid wastes, which optimize the nutrients and bacterial diversity can use those wastes of digestive process is an appealing approach for exceeding the order of biotransformation. Many effective co-fermentation processes using different substrates have shown great increase in biogas production, contrast with single digestion of the substrates.

Fermentative biogas production is a very complex process (Wang & Wan, 2009). Thus, serious consideration should be taken to control the surrounding conditions such as temperature, pH, substrate concentration and nutrient supply to achieve high biogas yield (Wang & Wan, 2009). Controlling the conditions of the fermentation is important since it can affect the rates of biogas production, product formation (organic acids, solvent, biohydrogen and biomethane), bacterial activies and growth rates, also may change metabolic pathways of hydrogenase enzyme and methanogenic bacteria (Pan et al., 2008). Fermentative biogas production from mixed culture was simple and less tendency of contamination when compared to single culture. The effectiveness of biogas production from mixed culture as inoculums have been verified by several researchers from anaerobic sludge (Pan et al., 2008; Yusoff et al., 2009) compost (Akutsu et al., 2009; Lee et al., 2008), sewage sludge (Kim et al., 2008) and cattle manure sludge (Cheong & Hansen, 2007). Anaerobic fermentation using mixed culture was suitable for biohydrogen and biomethane production as the substrate was utilized by microorganism with fast rate, simple to operate without requirement of light and oxygen supply (Chong et al., 2009; Valdez-Vazquez & Poggi-Varaldo, 2009).

More than one substrate are simultaneously consumed in the same reactor in co-digestion. If the combination of substrates are selected correctly, co-digestion will give benefits to the process due to more preferable buffering capacity, more suitable carbon – nitrogen ratio ,more diverse nutrient content or dilution of inhibiting compounds which will resulting a higher biogas yields and biologically a more balance process (Chen et al., 2008; Karthikeyan & Visvanathan, 2013). In co-digestion, adjustment of pH and moisture in the reactor can also be done (Esposito et al., 2012). Total nutrients and water can be balanced by combining different substrates, e.g. wet nutrient-poor substrates combined with nutrient-rich dry matter. For efficient co-digestion, substrate characteristics must be known (Karthikeyan & Visvanathan, 2013), e.g. substrates that contain high amount of nitrogen (e.g. chicken manure) and other inorganic nutrients should be digested with low-nitrogen and high-carbon content substrates (e.g. food waste) to minimize the inhibition of ammonium nitrogen. This kind of substrate mixture is proposed to inhibit total pH drops (Esposito et al., 2012). Combination of manures and highly biodegradable food such as food waste will form a good mixture for co-digestion (Karthikeyan & Visvanathan, 2013). Esposito et al. (2012) proved a quicker and more stable digestion process when highly biodegradable substrates and ammonia-rich substrate were co-digested. When pig manure was co-digested with kitchen waste, Kuglarz et al., (2011) observed an increment in methane production rates up to 60%.

1.2 Problem Statement

There are many ways that food waste can affect our environment. Food waste is food that initially is for eating being thrown away, generally at consumer or retail level. This is a huge problem in industrialized country, where a cheaper option which is throwing away is often favorable than reusing. In reality, food waste is usually avoidable**.** Increased generation of food waste is both a national and global problems, with an estimation of 70% from the municipal solid waste is originated from the food waste (Wang et al., 2005). Methane gas, a gas 25 times stronger than carbon dioxide at trapping heat in the atmosphere is produced when wasted food is thrown away and break down together with other organic materials (Stabnikova et al., 2006). This will lead to the releases of greenhouse gases in the environment.

Accumulations of greenhouse gases in the atmosphere are contributing to climate change and global warming worldwide. Food waste can also give a big impact on dumping ground, or landfills, and how they give impact to the surrounding environment. Degradation of food waste releases nutrients, which can drift out of landfill waste and spread out to the surrounding environment. Accumulation of nutrients can be a trouble because they can pollute groundwater and waterways. This will lead to the pollution of the water source (Ding & Wang, 2008).

A Malaysia's food production lifecycle, or supply chain can also contribute on greenhouse and affect the environment. For instance, to produce, harvest, transport, process, package, distribute and market all food products, soils, water, natural resources and energy are used. The energy and resources invested by the supply chain to deliver food to our house is lost with the food leftover (Sakai et al., 2008). One of the factor that contribute total greenhouse gas emission in Malaysia is the food supply chain. Starting from direct emissions of agricultural machinery and those attributed to energy including transportation, production of food, processing and distribution to retailers. By changing the wasteful habits for a greener approach to buy, prepare and manage the food, significant environmental and greenhouse benefits can be achieved when all people play their parts.

While there are numerous reports and studies that drawn up to solve the food waste generation, avoidance and managements are sparse and difficult to conduct and verify. This is due to a range of factors related to the context and process of food waste generation (Sakai et al., 2008). At present, food waste is considered as common waste which is worthless and is therefore not segregate in a proper way. In addition, where kitchen waste and other organic waste are differentiated from other general wastes, they are often considered together, making it difficult to calculate the proportion of this waste that is food only. On the other hand, separating food waste physically is not practical besides health and safe risk. Due to these factors, it is difficult to manage the food waste generation and to structure the programs for food management practices. One of the wise ideas of managing these wastes is to utilize the organic waste and convert it into value-added and sustainable product such as bioenergy.

Due to the increasing demand and supply of chicken product, Malaysia is facing a serious issue in managing chicken manure. A large amount of chicken manure generated gives environmental problem such as spreading of pathogens, odorous compound and emission of greenhouse gases. In Sinar Harian Kelantan edition dated 26 December 2011, one of the villagers of Kampung Cherang, Chabang Empat, Tumpat Kelantan said that the chicken farm bring the unpleasant odours to the village for the past eight year. It gave negative effect to human health and the quality of life for people living around chicken farm areas. These are some of the examples of the serious environmental problems that need to overcome quickly by the proper treatment of chicken manure. The presence of nitrogen in chicken manure will pollute ground water and increase the level of nitrate in drinking water.

There are many factors limiting biogas production including pH, temperature, headspace volume and nature of substrate (Yusof et al., 2014). Separate biohydrogen and biomethane production has been well researched and documented in the literature. Although some researchers found that carbohydrate rich waste feedstock are appropriate substrate for biogas (hydrogen) production, there are new studies that are being explored with mixed substrate to investigate co-fermentation. Co-digestion also can deal with the limitation of source for substrate and inoculums. Biogas yield and production rate of biogas were vary even for a specific substrate relying on the inoculums.

1.3 Objectives of Study

To date, information for biohydrogen and biomethane production has been studied from different kind of substrate. In this study, food waste and chicken manure were used as substrates for biohydrogen and biomethane production. However, biohydrogen is inhibited by hydrogen consuming bacteria, as well as biomethane production inhibited by several limiting factors. Thus, optimizing parameters one factor at a time are critical to eliminate the inhibition effects to enhance high yield of biogas production. The objectives of this study are :

1. To determine the best ratio of food waste and chicken manure for biogas production in batch fermentation.

2. To determine the effect of different inoculums and heat treatment upon selected inoculum on biohydrogen and biomethane production from co-digestion of food waste and chicken manure in one step fermentation.

3. To determine the microbial diversity in the fermentation using Next Generation Sequencing (NGS) of 16S ribosomal RNA to identify and compare the bacteria present in the samples with untreated and heat treated inoculum.

1.4 Scope of Study

The proposed research of investigating the enhancement of biohydrogen and biomethane production from food waste and chicken manure was carried out in three stages; evaluation of mixing ratio of food waste and chicken manure, determine the effect of different source of inoculums, and assessment on heat treatment for inoculums for co-digestion of food waste and chicken manure. The details scope for each objective was stated below:

1.4.1 Evaluation of Mixing Ratio of Food Waste and Chicken Manure

The evaluation focused on the comparison of different mixing ratio of food waste and chicken manure besides determining the characteristics of the substrates that were used along the experiments. The mixing ratio of 40:60, 50:50, 60:40 and 70:30 (v/v) of food waste and chicken manure were studied. The statistical analyses were performed using MATLAB software and Microsoft Excel 2010.

1.4.2 Determine the Effect of Different Inoculums

Three types of different inoculums were tested to get the most suitable inoculums to be co-digested with the substrates. Aeration tank sludge (ATS), return activated sludge (RAS) and palm oil mill effluent (POME) sludge were used in this study. The best mixing ratio were used to study the effect of different inoculums. The deciding factors that were used in choosing the best inoculums are based on high yield of biohydrogen and biomethane production. The statistical analyses were performed using Statistica 13.0 Software, MATLAB software and Microsoft Excel 2010.

1.4.3 Evaluate the Effect of Heat Treatment on Inoculums

The production of biohydrogen and biomethane were optimized by studying the effect of heat treatment on the selected inoculums. The inoculums were heat treated at 80°C for 20 minutes. MATLAB software and Microsoft Excel 2010 were used to perform the statistical analyses. The influencing factors of producing the high yield of biohydrogen and biomethane were also discussed.

1.4.4 Assessment of Microbial Diversity

Microbial characterization study was carried out to determine the phyla of the available microorganisms for the samples of heat treated and non-treated inoculums. The selected samples were sent to First BASE Laboratories Sdn. Bhd. for 16S ribosomal RNA (rRNA) analysis. To perform the data analysis, SILVA119, BlasyN and MEGAN5 software were used. The taxonomy and phylogeny of the samples were also discussed.

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BIODATA OF STUDENT

Mohd Faiz bin Mat Saad was born on 29th of December 1990 in Military Hospital Terendak Camp, Malacca and is the youngest of a family of 5 siblings. He started his education in Malaysia by attending Sekolah Rendah Kebangsaan Kem Terendak (1) for the primary school requirement from 1997 to 2002. He then attended Sekolah Menengah Kebangsaan Ghafar Baba, Masjid Tanah for the lower secondary school from 2003 to 2005 before continued his upper secondary school at Sekolah Menengah Sains Muar, Johor starting from 2006 to 2007. He then continues his matriculation at Pusat Asasi Sains Universiti Malaya, Kuala Lumpur until 2008. After completing the section of his study life, he then decided to further his studies in Universiti Kuala Lumpur for a Bachelor's Degree in Biosystem Engineering Technology that took four years to complete and was awarded the degree in 2013.

During his Bachelor's Degree period, he was selected as the President of Biosystem Engineering Technology and involved in many student activities. In his final semester, he was awarded with a Dean List Award and decided to continue his study. He then pursued a Master's Degree in Environmental Technology is September 2013. He also has presented posters and attended several workshops and seminars in Malaysia.

PUBLICATION

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The demand for clean energy from renewable resources stimulates biohydrogen and biomethane production from agro-food waste as an alternative fuel to replace fossil fuel. A combined production of biohydrogen and biomethane has attracted growing attention of researchers and industries worldwide due to their potential as fuel substitute. Biosynthesis of biohydrogen and biomethane from food waste and chicken manure fermentation initiates clean technologies for energy generation thus provide the solution for waste treatment. Despite that, biogas production of hydrogen and methane have limiting factors that relate to soluble metabolites and active microorganisms. This inhibition effects can be overcome by optimizing several factors for biohydrogen and biomethane production. The objectives of this study were to determine the best ratio of food waste and chicken manure for biogas production in batch fermentation and to evaluate the effect of different inoculums and heat treatment upon selected inoculum on biohydrogen and biomethane production. The batch fermentation was conducted using 150 mL serum bottles incubated in anaerobic condition. Food waste with composition ratios of 3:1:1 of carbohydrates, protein and fiber were used as substrate added with chicken manure freshly collected from poultry farm. Biohydrogen and biomethane production were tested for the effects of different substrate ratio, different inoculums and heat treatment on selected inoculums. Biogas was collected daily throughout 10 days fermentation and the composition of hydrogen and methane in the biogas was analyzed by gas chromatography. Microbial assessment was performed by using Next Generation Sequencing (NGS) of 16S ribosomal RNA technique. *Clostridium* sp. was related to biohydrogen production methanotroph such as *Cyclobacteriaceae, Saprospiraceae* and *Chloroflexi* that were inhibited after the heat treatment. Heat treatment of inoculums is not suitable for the production of both biohydrogen and biomethane since it inhibits the methanogens. Thus, controlling operating conditions were important for hydrogen-producing bacteria as well as methanogens for biohydrogen and biomethane production.