EVALUATION OF PROCESS PERFORMANCE AND MICROBIAL PROFILE OF HOUSEHOLD FOOD WASTE ANAEROBIC DIGESTION

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By

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December 2018

Chair : Assoc. Prof. Latifah Abd Manaf, PhD
Faculty : Environmental Studies

Interest in household food waste treatment has increased in recent years due to the growing rate of its generation. In spite of the renewed attention on anaerobic digestion of household food waste, process stability is always becoming a concern among the practitioners. Moreover, anaerobic digestion performance at steady-state and the comprehensive characterizations of microbial community from wet to dry technologies were not compared in parallel. The main focus of this research work was to evaluate the process performance and microbial profile on household food waste anaerobic digestion. Biochemical methane potential (BMP) assays was conducted to access the suitability and feasibility of the substrate with different total solid (TS) content in 1 L reactor. The reactor were labelled as BMP.5 (5% TS), BMP.10 (10% TS) and BMP.15 (15% TS). Batch and semi-continuous experiments were conducted to evaluate and compare their process performance with TS content ranged 5 – 15%. Batch reactor (200 L) were labelled as B.5, B.10 and B.15 and semi-continuous reactor (10 L) were labelled as C.5, C.10 and C.15 for TS 5%, 10% and 15%, respectively. Co-digestion (1 L) was carried out to improve batch system with higher total solid contents (20 – 25% TS). All reactors configuration utilized in this study is employed in triplicate. Finally, microbial study utilizing Illumina next generation sequencing on steady-state of each anaerobic reactor was conducted. BMP.15 obtained the highest methane (CH_4) yield (409 mL CH_4/g VS) and mass reduction (75.4 – 78.3%). Better performance on volatile solid reduction (85.6%) and soluble chemical oxygen demand removal (77%) was seen in C.15. Higher methane production (425 mL CH_4/g VS) was obtained from B.15. Approximately 3-folds increase in TS contents from 5 – 15%, the average of volatile solid (VS) reduction increased 2-folds (from 33% to 63%) for batch reactor while semi-continuous reactor reached 80 – 86% of reduction. The average methane yield increased 4-folds (240.4 – 425.0 mL CH_4/g VS) in batch reactor and 1.8-folds (269.5 – 347.8 mL CH_4/g VS) in semi-continuous reactor ($\rho < 0.05$). In co-digestion experiments higher TS content from 20% to 25% were evaluated. The results revealed that by increasing TS concentration, the methane production improved 1.5-fold from mono-digestion. Highest methane production (475 mL CH_4/g VS) was obtained in co-digestion reactor with 25% TS. Co-digestion had synergistic effect on methane production with the highest level of synergy (α = 1.61 – 2.14). Illumina MiSeq data showed significant shift of bacterial communities and that the phyla included *Bacteroidetes*, *Firmicutes*, *Synergistetes* and *Chloroflexi*. The relative abundance of phylum *Bacteroidetes* increased while *Chloroflexi* decreased at increasing TS content from 5% to 25%. *Methanosarcina* were abundant and dominant during the steady-state of anaerobic digestion at higher solid content in the reactor. *Methanomicrobiales* were mostly dominant in reactor working with lower solid content. In summary, a higher cumulative methane yield and better performances in terms of solid and mass organic reduction were achieved at higher TS content of AD. These findings also revealed the influenced of TS contents ($\rho < 0.01$) on the behavior of the microbial community involved in anaerobic digestion of household food waste.

Keywords: High Solid Anaerobic Digestion; Household Food Waste; Illumina Sequencing; Low Solid Anaerobic Digestion; Next Generation Sequencing.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENILAIAN PRESTASI PROSES DAN PROFIL MIKROB KE ATAS PENCERNAAN ANAEROBIK SISA MAKANAN RUMAH

Oleh

NURULJANNAH KHAIRUDDIN

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Keprihatinan merawat sisa makanan isi rumah telah meningkat sejak kebelakangan ini disebabkan oleh pembuangan sisa tersebut semakin bertambah. Walaupun terdapat pertambahan aplikasi pencernaan anaerobik terhadap sisa makanan, memastikan kestabilan teknologi ini masih lagi menjadi isu di kalangan pengamalnya. Selain itu, prestasi pencernaan anaerobik pada keadaan stabil dan pencirian terhadap komuniti mikrob dari teknologi basah ke kering tidak dibandingkan secara selari. Objektif utama kajian ini adalah untuk menilai prestasi proses dan profil mikrob pada pencernaan anaerobik sisa makanan isi rumah. Ujian “biochemical methane potential” (BMP) dilakukan untuk mengkaji kesesuaian substrat dengan mengunakan kandungan pepejal yang berbeza (TS) di dalam reaktor 1 L. Reaktor dilabel sebagai BMP.5 (5% TS), BMP.10 (10% TS) dan BMP.15 (15% TS). Eksperimen “batch” dan “semi-continuous” telah dijalankan untuk menilai dan membandingkan prestasi proses dengan kandungan pepejal TS 5 – 15%. Reaktor “batch” (200 L) dilabel sebagai B.5, B.10 dan B.15 dan reaktor “semi-continuous” (10 L) dilabelkan sebagai C.5, C.10 dan C.15 untuk TS 5%, 10% dan 15%. Eksperimen “co-digestion” (1 L) dijalankan untuk memperbaiki sistem “batch” dengan kandungan pepejal yang lebih tinggi (20 – 25% TS). Konfigurasi reaktor untuk setiap eksperimen diulang-kaji sebanyak tiga kali. Akhir sekali, kajian mikrob menggunakan Illumina penjujukan gen pada keadaan stabil bagi setiap reaktor anaerobik telah dijalankan. Dari kajian ini, didapati bahawa, BMP.15 memperoleh hasil metana tertinggi (CH_4) (409 mL CH_4/gVS) dan pengurangan jisim pepejal (75.4 – 78.3%). Prestasi yang lebih baik terhadap pengurangan jisim pepejal (85.6%) dan penyingkiran “soluble chemical oxygen demand” (77%) dapat dilihat berlaku pada reaktor C.15. Pengeluaran metana yang lebih tinggi (425 mL CH_4/gVS) diperoleh dari B.15. Kira-kira 3 kali ganda peningkatan kandungan TS dari 5 hingga 15%, purata pengurangan pepejal pepejal meningkat 2 kali ganda (dari 33% hingga 63%) untuk reaktor “batch” manakala reaktor “semi-continuous” mencapai 80 – 86% pengurangan. Hasil metana purata meningkat 4 kali ganda (240.4 – 425 mL CH_4/gVS) dalam reaktor “batch” dan 1.8 kali ganda (269.5 –

347.8 mL CH_4/gVS) dalam reaktor “semi-continuous” (ρ <0.05). Prestasi pencernaan dengan kandungan TS yang lebih tinggi 20 – 25% dinilai. Hasilnya menunjukkan bahawa dengan meningkatkan kepekatan TS dari 20% hingga 25%, pengeluaran metana bertambah 1.5 kali ganda daripada “mono-digestion”. Pengeluaran metana paling tinggi (475 mL CH_4/gVS) diperolehi dalam reaktor pencernaan bersama dengan 25% TS. Tambahan pula, “co-digestion” mempunyai kesan sinergistik terhadap pengeluaran metana (α=1.61– 2.14). Data Illumina MiSeq menunjukkan peralihan ketara komuniti bakteria termasuk *Bacteroidetes*, *Firmicutes*, *Synergistetes* dan *Chloroflexi*. Kelimpahan relatif *Bacteroidetes* meningkat sementara *Chloroflexi* menurun pada peningkatan kandungan TS dari 5% hingga 25%. *Methanosarcina* dominan didapati dalam keadaan stabil semasa pencernaan anaerobik pada kandungan pepejal yang lebih tinggi. *Methanomicrobiales* pula dominan didapati dalam reaktor dengan kandungan pepejal yang lebih rendah. Secara ringkasnya, hasil metana kumulatif yang lebih tinggi dan prestasi proses yang lebih baik dari segi pengurangan organik dan jisim pepejal dicapai di dalam reactor yang mempunyai kandungan TS yang lebih tinggi. Penemuan ini juga mendedahkan pengaruh kandungan TS (ρ <0.01) kepada tingkah laku komuniti mikrob yang terlibat dalam pencernaan anaerobik sisa makanan isi rumah.

Kata kunci: Pencernaan Anaerobik Pepejal Tinggi; Sisa Makanan Rumah; Illumina Sequencing; Pencernaan Anaerobik Pepejal yang Rendah; Illumina Penjujukan Gen.

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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 Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
ADF	Acid Detergent Fibre
ADL	Acid Detergent Lignin
ANOVA	Analysis of Variance
APHA	American Public Health Association
BD	Biodegradability
BMP	Biochemical Methane Potential
Bp	Base pair
C:N	Carbon to Nitrogen Ratio
CH_4	Methane
CoAD	Co Anaerobic Digestion
CS	Cattle Slurry
CSTR	Continuous Stirred Tank Reactor
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
EMY	Experimental Methane Yield
FAN	Free Ammonia Nitrogen
FAO	Food Association Organization
FISH	Fluorescence in-situ hybridization
FW	Food Waste
GHG	Greenhouse Gas
GPR	Gas Production Rate
HRT	Hydraulic Retention Time
HS-AD	High Solid Anaerobic Digestion
LFG	Landfill Gases
LS-AD	Low Solid Anaerobic Digestion
MHLG	Ministry of Housing and Local Government
MP	Methane Production
N.D	Not Determined
NDF	Neutral Detergent Fibre
NGS	Next Generation Sequencing
OCB	Organic Content Balance
OFMSW	Organic Fraction of Municipal Solid Waste
OLR	Organic Loading Rate
OUT	Operational Taxonomic Unit
PCR	Polymerase Chain Reaction
RF	Response Factor
SCOD	Soluble Chemical Oxygen Demand
SE	Standard Error
SMP	Specific Methane Production
SRT	Solid Retention Time
TA	Total Alkalinity
TAN	Total Ammonia Nitrogen
TMY	Theoretical Methane Yield
TOC	Total Organic Carbon
TRFLP	Terminal Restriction Fragment Length polymorphism
TS	Total Solid
UASB	Up-flow Anaerobic Sludge Blanket

VFA	Volatile Fatty Acids
VS	Volatile Solid
VS_r	Volatile Solid Reduction

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nowadays, the accumulation of food waste (FW) has eventually become a global issues (Capson-Tojo et al., 2016). In Malaysia, FW is the largest component of municipal solid waste (MSW) stream, approximately 44.5% and is still increasing (Ministry of Urban Wellbeing Housing and Local Government, 2016). According to Jabatan Pengurusan Sisa Pepejal Negara (JPSPN, 2013), FW generated was 1.38 kg/capita/day per person in Malaysia. The total of daily FW production from household level is about 9, 685 metric tonnes and reduced to 8, 492 metric tonnes as the FW moves along the transportation routes from household to the disposal site (JPSPN, 2013).

This situation occurred due to the fact that FW contained high moisture content approximately 55% enhances the rapid degradation of the organic materials encouraged the production of leachate. Nayathinka et al. (2018), defined leachate as any contaminated liquid effluent that percolated through a solid such as FW and leached out of some of the organic constituents. Hence, indiscriminate decomposition of household FW contribute to larger contamination of land, water, air and public nuisance when leachate penetrates to the environment.

Anaerobic digestion (AD) is the most attractive and cost-effective technology for treating sorted organic fraction of MSW (OFMSW), especially FW (Braguglia et al., 2016). Various AD processes have been widely developed in many countries as FW treatment (Cho et al., 2013; Arlunbatar et al., 2014; Fisgativa et al., 2016). So far, two main types of AD technologies have been developed according organic loads concentration in terms of total solids (TS) content of the feedstock; low solid-AD (LS-AD) also known as conventional wet (≤10% TS) and high solid-AD (HS-AD) and modern dry (≥10% TS) technology (Angelonidi and Smith, 2015). Higher TS content in anaerobic reactor promote higher organic removal which determines the treatment capacity and efficiency (Karthikeyan and Visvanathan, 2013).

On the other hand, according to Mata-Alvarez et al. (2014), anaerobic co-digestion is the simultaneous digestion of two or more substrates, a preferable option to improve reactor stability and AD performance. Banks et al. (2011) recommended on-farm co-digestion of dairy waste and source sorted FW as the most effective method of making FW economically viable. Moreover. In addition to the high methane production per unit volume of reactor, co-digestion can offers many other advantages such as dilution of inhibitory substances, nutrient balance, and accomplishment of the required moisture contents in the reactor feed, reducing the emission of greenhouse gases to the atmosphere, synergetic effects of microorganisms and increasing the load of biodegradable organic matter (El-Mashad and Zhang, 2010; Zhang et al., 2012).

Anaerobic Digestion (AD) is a multi-stage biochemical process including hydrolysis, acidogenesis, acetogenesis and methanogenesis. The microbial communities are different at each of the stage and play different role of function during the degradation process. According to Wang et al. (2017) there are 50 kinds of bacteria in hydrolysis and acidogenesis such as *Clostridium*, *Bacteroidetes* and *Bacillus*. Whereas, methanogen has been found 65 kinds of species belonging to 3 orders, 7 families and 19 genus considered as archaea group. *Methanobacterium*, *Methanococcus*, *Methanomicrobium* and *Methanosarcina* are the main archaea responsible for methane production in AD (Imachi et al., 2007; Nielsen et al., 2007). Illumina sequencing, as a next generation sequencing has gained increasing attention as a novel tool for studying microbial diversity. Currently, this technology has been widely and successfully used to characterize the microbial communities and structures in various environmental samples.

1.2 Problem Statement

There are four elements of problem statement addressing the gaps in this study as depicted in Figure 1.1. Firstly, in spite of growing interest and attention on anaerobic digestion (AD) of household food waste (FW), process instability is always becoming a concern among the AD practitioners. AD of household FW is a complicated treatment process due to its homogeneity and the characteristics of household FW leads the anaerobic reactors inefficiency. Secondly, process instability is serious limitation to AD system caused from several inhibitor factors. According to Chuimenti et al. (2018), in order to maintain process stability, the anaerobic reactor is always operated with low organic loading rate (OLR) from 1 – 4 g VS/L/d with lower solid contents (TS <5%) or increase the hydraulic retention time (HRT). However, these resulting sloppy the process performance in terms of biogas production and material recovery**.** Moreover, treating FW from household level with low solid concentration is inefficient and uneconomical.

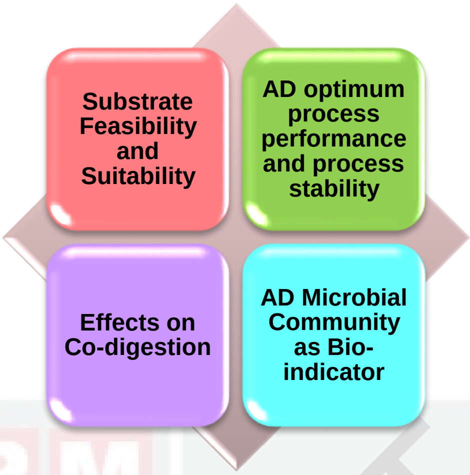

Figure 1.1 : Problem Statements of the Study

Thirdly, anaerobic co-digestion exhibits better process efficiency than the mono-digestion by offering complementary benefits such as better yield, nutrient availability, lower feed volume, substrate variability, toxicity dilution and synergism. However there are more challenges in improving the feasibility and suitability of this technology. A better understanding on co-digestion of FW with other organic waste such as cattle slurry will pave the way for a more efficient and sustainable mass and energy recovery.

Finally, understanding anaerobic microbiome is necessary to identify microbial-based indicators. Although microbial indicators cannot be detected as quickly as process parameters due to limitations of monitoring techniques, it is recognized that fluctuations in process parameters and AD performance are the results of microbial succession due to inhibitors and environmental stress (Lebuhn et al., 2015). Thus, changes and shifts in microbial community as reactor alarming indicators will occur earlier than changes in process parameters. However, the microbial diversity and the shifts of microbial during the process are still remains uncertain. Furthermore, the available literature is simply on about AD performance comparisons (Banks et al., 2011; Scano et al., 2014; Choong et al., 2016; Kopsahelis et al., 2018). Moreover, the AD performance at steady-state and the comprehensive characterizations of microbial community in AD of household FW from high to low solids (wet and dry technologies) were not compared in parallel.

1.3 Research Objectives

The general objective of this study is to investigate the process performance and microbial activity on anaerobic digestion (AD) of household food waste (FW). The specific objectives of this study are as follow:

1. To analyse the fractional, characteristics and feasibility of household FW as sole substrate in anaerobic digestion.
2. To examine the relationship between increasing organic loads concentrations (TS content) for maximum operation in different anaerobic digestion feeding modes.
3. To investigate the effects and synergism of improved anaerobic conversion by co-digestion of household FW with other organic waste (cattle slurry).
4. To elucidate the microbial structure and function during the steady-state of anaerobic digestion reactors.

1.4 Scope of Study

This research work comprises with four research scopes as described in Figure 1.2. Firstly, involves the characterisations work on compositions and fractionations analysis. Begun with the collection of food waste (FW) from household within one month operation, the samples was then sorted and characterized based on its major constituents (Agamuthu et al., 2012). The characterisation work evaluates household FW characteristics (cooked rice, fruit and vegetable residues, animal fats and others), biofibre contents such as soluble, cellulose, hemicellulose and lignin, material composition such as total solid (TS), volatile solid (VS) and elemental analysis (carbon, hydrogen, oxygen, nitrogen and sulphur). These assessments is to evaluate the feasibility and suitability household FW as substrate. Next, 1.0 L biochemical methane potential (BMP) assays were conducted to investigate the potential of household FW in anaerobic digestion (AD) in term of methane production potential and solid mass and organic reduction aspects such as Soluble-COD (SCOD) and VS removal.

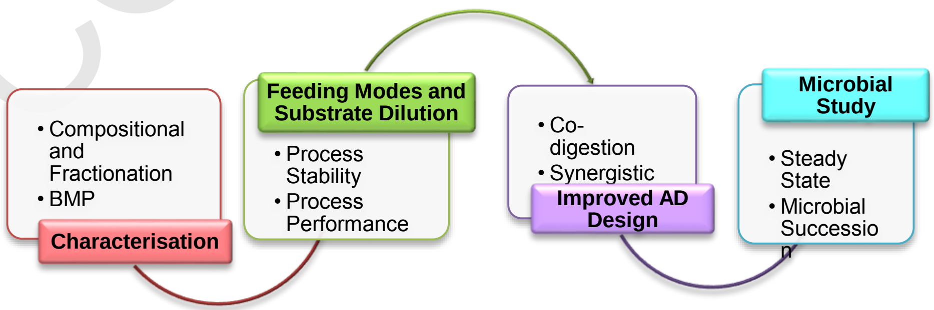

Figure 1.2 : Scope of Study

The second scope is to access the impact of different TS contents on low solid anaerobic digestion (LS-AD) and high solid anaerobic digestion (HS-AD) in different anaerobic reactor feeding modes. LS-AD operates at ≤5% TS; while HS-AD works at ≥10% TS. Considering 200.0 L batch and 10.0 L semi-continuous reactor operations, the effect of the feeding modes and dilution on AD of household FW was evaluated to study the process stability and steady-state conditions at various TS contents (5%, 10% and 15%). All the operational parameters (pH, VFA patterns, SCOD and VS removal) and the performance (methane production and organic load reduction) of the AD were compared using statistical means and interpretations.

The third attempts is improving and enhance the performance of HS-AD process through 1.0 L batch co-digestion technology. Moreover, the study also pays attention on synergistic effect of the co-digestion considering the energy recovery and mass balance evaluations. Cattle slurry (CS) was utilized as co-substrate with household FW combining feedstock which can increase organic loads concentrations and improve performance relative refer to mono-digestion by diluting the inhibitory compound such as volatile fatty acids (VFA) and providing macronutrient and micronutrient to the system. The principal objective of this study is to access the key biodegradability in term of organic reduction and methane production from the impacts on higher TS concentrations (20 – 25% TS) in co-digestion at thermophilic conditions.

Finally, the last scope of study is on the microbiology of AD treating household FW during the steady-state of the system. With the respect to different anaerobic experimental design, this study unravels the microbial succession during the steady-state of AD using modern Illumina Next Generation Sequencing (NGS) Technology.

1.5 Significance of Study

Knowing the fact that, household food waste (FW) characterisation is suitable for potential feedstock in anaerobic digestion (AD), many researches on AD of FW has getting interest globally. Despite that, in Malaysia AD of FW is still at the stage of development compared to treating other organic waste that has been establish in Malaysia such as AD of palm oil or animal manure. However, these experiences cannot be directly applied to AD of household FW. Especially, due to the complex composition of FW on its rheological properties is different from other organic waste such as palm oil waste and animal manure. Food waste from household level has commonly lower pH (pH = 4 – 6) while palm oil and animal manure have higher pH (pH = 7 – 8) which is suitable for maintaining the optimum AD (pH = 7.2). In order to maintain the stable operation, the anaerobic reactor treating FW especially from household usually operated at low organic loading concentrations (Tampio et al., 2014). Hence in order to increase the efficiency on AD of household FW, this study provide an insight of AD working on different organic loads concentration in terms of total solids (TS) content.

Despite that, AD of household FW often encounters some drawbacks such as low initial pH. Addition of chemicals such as calcium carbonate ($CaCO_3$) or sodium hydroxide (NaOH) is unsuitable as the chemical compound can damage the rheological properties of household FW although the pH in the system are at optimum range (7 – 7.2). Consequently, denatured the microbial communities finally, halt the AD process. To overcome the deficiencies of mono-digestion, co-digestion the simultaneous AD of household FW with other organic wastes was developed to improve the operational stability in terms of buffering capacity. This study employed cattle slurry as co-substrate with household FW combining substrate can increase organic loads concentrations and improve performance relative refer to mono-digestion by diluting the inhibitory compound such as volatile fatty acids (VFA) and providing macronutrient and micronutrient to the system which beneficial to produce better soil amendment or fertiliser from digestate.

Since the process is a series of biochemical reactions conducted by various types of microorganism, it is believed that the performance of AD of FW is directly related to the functional group of microbes. This study unravels microbial communities and structures during the steady-state on different spectrums of household food waste anaerobic digestion in different organic loads concentrations. It is expected that the findings can facilitate the development of more efficient full-scale HS-AD system to achieve a high-rate of organic material reduction and methane production as renewable energy resource. As in the national key economic areas, the oil, gas and energy sector would gain benefits from this research because by-product anaerobic digestion technology could be used to supply energy to small community; hence comply with a the national strategic plan. Moreover, the development of the treatment plant in vicinity of the community would potentially increase public participation thus will significantly reduce the potential of environmental deteriorations.

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