BIOGAS PRODUCTION
FROM POULTRY MANURE WASTEWATER
USING SOIL MIXED CULTURE

NINA FARHANA BINTI MOHD JAMALUDIN

MASTER OF ENGINEERING (BIOPROCESS)

UNIVERSITI MALAYSIA PAHANG

BIOGAS PRODUCTION FROM POULTRY MANURE WASTEWATER USING SOIL MIXED CULTURE

NINA FARHANA BINTI MOHD JAMALUDIN

Thesis submitted in fulfillment of the requirements
for the award of the degree of
Master of Engineering (Bioprocess)

Faculty of Chemical Engineering and Natural Resources
UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2016

UNIVERSITI MALAYSIA PAHANG

DECLARATION OF THE THESIS AND COPYRIGHT

Author's full name : Nina Farhana Binti Mohd Jamaludin

Date of birth : 13th September 1988

Title : Biogas Production from Poultry Manure Wastewater using Soil Mixed Culture

Academic Session : SEM II 2016

I declare that this thesis is classified as:

☐ **CONFIDENTIAL** (contains confidential information under the Official Secret Act 1972)

☐ **RESTRICTED** (contains restricted information as specified by the organization where research was done)

☑ **OPEN ACCESS** I agree that my thesis to be published as online open access (Full text)

I acknowledge that Universiti Malaysia Pahang reserve the right as follows:

1. The Thesis is the Property of Universiti Malaysia Pahang.
2. The Library of Universiti Malaysia Pahang has the right to make copies for the purpose of research only.
3. The Library has the right to make copies of the thesis for academic exchange.

Certified By:

________________________ (Student's Signature)

________________________ (Signature of Supervisor)

880913-
New IC/ Passport Number
Date:

PM.IR.DR. NORAZWINA ZAINOL
Name of the Supervisor
Date:

SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis, and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Master of Engineering (Bioprocess).

Signature :

Name of supervisor : PM.IR.DR.NORAZWINA ZAINOL

Position : SENIOR LECTURER

Date :

STUDENT'S DECLARATION

I hereby that the work in this thesis is my own except for the quotations and summaries which have been duly knowledge. The thesis has not been accepted for any degree and is not currently submitted for the award of any other degree.

Signature :
Name : NINA FARHANA BINTI MOHD JAMALUDIN
ID. Number : MKB11004
Date :

DEDICATION

This work is dedicated to:

My late father,
who I miss every single day,
my mother and parent in law,
who always supporting me from behind,
my siblings,
who always making my day felt brighter,
my supervisor,
who I adore so much,
and my friends,
who has helped me in many ways and,
my Syafiq, Sofea and Alif,
who I love more than any words would say,
I will make up some times for us to be together
Insha'Allah

Thank you for everything.

ACKNOWLEDGEMENTS

In the name of Allah, the most gracious and merciful;

Praise and gratitude to Allah SWT, for His blessing that made this thesis possible.

I would like to give my special appreciation to my supervisor, PM.Ir.Dr. Norazwina Zainol for her constant guidance and support. Her constructive comments and suggestions throughout my study have been crucial in the process of completing this thesis.

I would also like to express my gratitude to Universiti Malaysia Pahang for giving me the opportunity to complete this research. Without both the financial aids and grants, this thesis may yet still incomplete. Not forgetting al the personnel and individual involved during this research including lectures, colleagues, laboratory instructors of Chemical Engineering and Natural Resources Faculty and Environmental Laboratory of Civil Engineering and Earth Resources Faculty. Thank you for the knowledge you shared.

Last but not least, I would like to thank my family, especially my husband (Syafiq) and beloved children (Sofea and Alif) for their unending support throughout this study. Thank you for the love, encouragement and prayers since the start of this study. I would not go this far without them. Thank you.

ABSTRACT

The resource limitation of fossil fuels and the problems arising from their combustion has led to widespread research on renewable energy resources. Currently, biogas has a great potential as renewable energy in Malaysia due to abundantly available resources. According to Malaysia Department of Veterinary Services, poultry industry in Malaysia was growing annually which contributed in the rising amount of poultry manure wastewater (PMW). Biogas resources from wastewater have potential energy of 7800 TJ/y. Thus, utilizing the increasing amount of PMW, into biogas production was suggested in this study. The design of experiment (DOE) of this study utilized Response Surface Method (RSM) by Design Expert Software. Firstly, biological treatment using soil water was conducted because according to few researchers, more than 1000 mg/L of ammoniacal nitrogen (AN) present in PMW will cause inhibition. The initial AN concentration of PMW used in this study was up to 1490 mg/L. The best suggested condition for PMW treatment from the software was agitation (0 rpm), reaction time (5 hours), type of soil water (SSW), soil to water ratio (1:6) and PMW: soil water (1:4). Utilization of this conditions resulted in 81.90% of AN removal. Next, factorial analysis was conducted to analyze factors affecting biogas production. From the result, agitation gave the highest contribution to biogas production by 24.09%. This showed that agitation was the most affecting factor in this study. Agitation ensures efficient transfer of organic material for the active microbial biomass, to release gas bubbles trapped in the medium and to prevent sedimentation of denser particulate matter. The best suggested condition for factorial analysis of PMW by the software was agitation (120 rpm), reaction time (3 days), substrate to inoculum ratio (4:1), process system (batch), and type of substrate (treated PMW). After factorial analysis, process optimization was conducted. The suggested optimum conditions by the software were validated at agitation (120 rpm) and reaction time (3.3 days). Under this condition, 0.00397 L/g COD of biogas yield was obtained with 30% of methane content. This counts for 5.82% error from predicted value. The result from this study showed that utilization of PMW was a suitable method in biogas production. Along with the process, biological treatment was proved to be applicable as treatment method for AN removal to avoid inhibition.

ABSTRAK

Had sumber bahan api fosil dan masalah yang timbul dari pembakarannya membawa kepada penyelidikan meluas kepada sumber tenaga boleh diperbaharui. Kini, biogas mempunyai potensi besar sebagai tenaga boleh diperbaharui di Malaysia kerana sumbernya banyak didapati. Menurut Jabatan Perkhidmatan Veterinar Malaysia, industri ternakan di Malaysia semakin meningkat setiap tahun yang menyumbang kepada peningkatan jumlah air sisa ayam (PMW). Sumber biogas daripada air sisa mempunyai potensi tenaga 7800 TJ/y. Maka, penggunaan PMW yang semakin meningkat untuk pengeluaran biogas telah dicadangkan dalam kajian ini. Reka bentuk eksperimen (DOE) kajian ini menggunakan kaedah permukaan bermuka (RSM) oleh perisian Design Expert. Sebagai permulaan, rawatan biologi menggunakan air tanah telah dijalankan kerana menurut beberapa penyelidik, lebih 1000 mg/L nitrogen ammonia (AN) dalam PMW akan menyebabkan perencatan. Kepekatan awal AN PMW yang digunakan dalam kajian ini adalah sehingga 1490 mg/L. Rawatan PMW yang dicadangkan dari perisian itu adalah pergolakan (0 rpm), masa tindak balas (5 jam), jenis air tanah (SSW), nisbah tanah kepada air (1:6) dan nisbah PMW kepada tanah air (1:4). Seterusnya, analisis faktorial telah dijalankan untuk menganalisis faktor yang mempengaruhi pengeluaran biogas. Dari keputusan, pergolakan penyumbang tertinggi bagi pengeluaran biogas sebanyak 24.09%. Ini menunjukkan bahawa pergolakan adalah faktor yang paling mempengaruhi dalam kajian ini. Pergolakan memastikan pemindahan cekap bahan organik untuk mikrob aktif biojisim, melepaskan gelembung gas terperangkap dan mencegah pemendapan bahan lebih padat. Keadaan dicadangkan untuk analisis faktorial PMW oleh perisian adalah pergolakan (120 rpm), masa tindak balas (3 hari), nisbah substrat kepada inokulum (4:1), sistem proses (kelompok), dan jenis substrat (PMW terawat). Selepas analisis faktorial, proses pengoptimuman telah dijalankan. Keadaan optimum yang dicadangkan oleh perisian telah disahkan pada pergolakan (120 rpm) dan masa tindak balas (3.3 hari). Dalam keadaan ini, 0.00397 L/g COD hasil biogas telah diperolehi dengan 30% kandungan metana. Ralatnya adalah 5.82% daripada nilai yang diramalkan. Hasil daripada kajian ini menunjukkan bahawa penggunaan PMW adalah kaedah yang sesuai dalam pengeluaran biogas. Bersama-sama dengan proses ini, rawatan biologi telah terbukti boleh diguna pakai sebagai kaedah rawatan untuk penyingkiran AN untuk mengelakkan perencatan.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

α	Distance from the centre point of the design
β_0	Intercept
β_1	Linear coefficients
β_2	Linear coefficients
β_{11}	Quadratic coefficients
β_{22}	Quadratic coefficients
β_{12}	Logarithmic coefficient
ºC	Degree celsius
%	Percent
±	Plus minus

LIST OF ABBREVIATIONS

AN	Ammoniacal nitrogen
ANN	Artificial Neural Network
ANOVA	Analysis of variance
BOD	Biological oxygen demand
C	Carbon
CCD	Central composite design
CEC	Cations exchange capacity
CHP	Combine heat and power
CO_2	Carbon dioxide
CH_4	Methane
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
d	day
DI	Distilled water
DF	Degree of freedom
DOE	Design of experiment
F/M	Food to microorganism
F-value	Fishers test value
h	hour
HRT	hydraulic retention time
g	gram
GA	Genetic Algorithm
m	meter
mm	milimeter
Ml	mililiter
m^3	meter cubic
m.t	metric tonnes
mg	milligram
kg	kilogram
kWh	kilowatt per hour
kmol	kilo mol

L	Liter
N	Nitrogen
MC	Moisture content
OFAT	One-factor-at-a-time
P	Phosphorus
PMW	Poultry manure wastewater
POME	Palm oil mill effluent
PFS	Poultry farm soil
PFW	Poultry farm soil water
PSL	Poultry soil
p-value	Probability value
R^2	Coefficient of determination
R1	Response
Rpm	Revolution per minute
RSM	Response surface method
SMC	Soil mixed culture
SS	Sandy soil
SSPS	Statistical Package for the Social Sciences SPSS
SSW	Sandy soil water
SW	Soil water
TFD	Two-level factorial design
TJ	Terajoule
TS	Total solid
UMP	Universiti Malaysia Pahang
USA	United States of America
VS	Volatile solid
y	year

CHAPTER 1

INTRODUCTION

1.1 Research overview

The energy need in Malaysia is met by both renewable and non renewable energy sources. This due the fact that Malaysia was rich with relatively cheap and plentiful supply of conventional fossil fuels such as oil (approximately 3 billion barrels), natural gas (1.61 trillion cubic meters) and coal (776 milion tons) as well as non renewable energy sources like hydro power, solar power and biomass (Joanta, 1996). However, past and current economic growth in the country is fueled mostly by fossil fuels. The maximum electricity energy demand projections are 40,515 MW for the year 2020 (Yusoff, 2006). Figure 1.1 shows the primary energy demand in Malaysia that indicates a rapid increase for year 2030. Malaysia has also committed to reduce its carbon intensity by 40% of its 2005 value, which is going to be a grow energy intensity of natural gas resources in Malaysia (Badariah, 2010). One of the solutions would be utilization of abundantly available renewable energy resources in Malaysia such as animal waste into biogas production.

The utilization of biogas production from animal waste in Malaysia was considered in the early eighties. However, it was felt not attractive at that time, mainly because there were no large scale livestock industries and farm animals were normally scattered so that the collection of wastes was difficult. Biogas production was however practicable in pig farms and palm oil mils (Shariffadeen, 1980). Indeed, SIRIM (The Standard Industrial Research Institute of Malaysia) carried out a 60 kVA pilot program of biogas production in a poultry farm. The project however was later abandoned due to the difficulty of handing of the scattered waste, not the technically of the biogas production system (Othman et al., 1996). Thus, this study presents in our attempt to reintroduce the biogas production from poultry manure wastewater (PMW).

According to Malaysia Department of Veterinary Services (DVS, 2014) poultry industry in Malaysia was growing at rate of 3.03% to 6.77% from 2004 to 2013. Along with the increasing production of poultry, the amount of PMW is also rising. Thus, utilizing and optimizing the increasing amount of PMW, into biogas production was a solution suggested in this study in order to meet the increasing energy demand in Malaysia while recovering abundantly available renewable energy resources in Malaysia. It is proposed to incorporate the latest development in the technology achieved to date, so that the problems faced before in optimizing biogas production from poultry manure wastewater could be overcome.

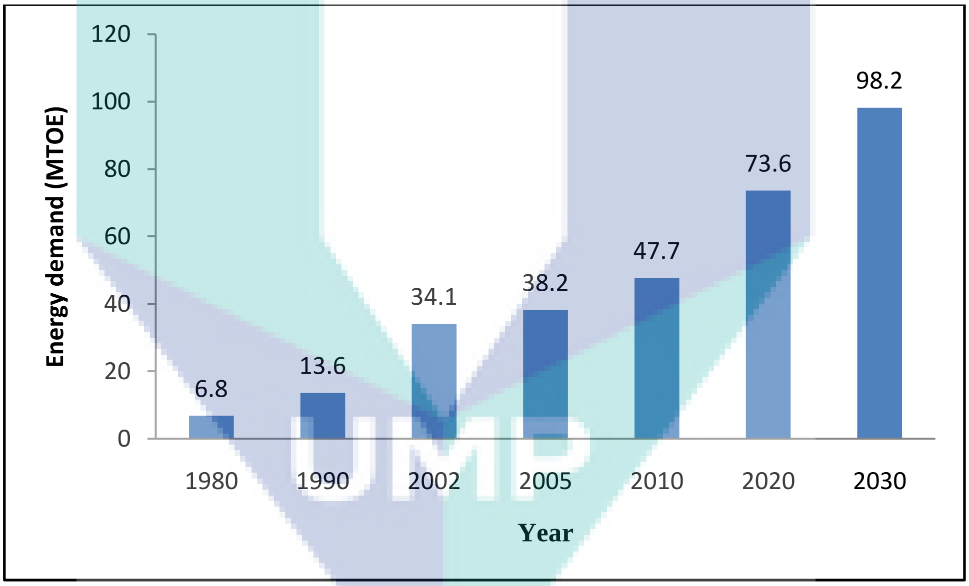


Figure 1:1: Energy demand in Malaysia, MTOE (Million tons of oil equivalents) (APEC, 2006).

1.2 Problem statement

There are several research issues highlighted in this study. In general, poultry manure wastewater (PMW) can be transformed into biogas through anaerobic digestion process. The first issue was regarding the microorganism used in the anaerobic digestion process. Some researchers used algae like *Chlamydomonas reinhardtii*, *Scenedesmus obliquus*, (Mussnug et al., 2010), *S.obiquus* and *Phaeodactylum* (Zamalloa et al., 2012). However, the use of algae involves high processing cost for dewatering, nutrient supplementation, and oil extraction which makes the production economically unfavorable (Singh and Gu, 2010). On the other hand, it is proven that pure culture from soil and wastewater gave biogas yield of 0.401-0.487 L/g VS added with 52-54.9% of methane content (Prajapati et al., 2013). However, usage of pure culture is not favorable due to high maintenance (Kleerebezem and Loosdrecht, 2007). Thus, soil mixed culture was suggested in this study to improve anaerobic digestion. It would results in the lysis or disintegration of cells and release of intracellular matter that becomes more accessible to anaerobic microorganisms, thus optimizing the biogas production (Demirer and Othman, 2008; Vindis et al., 2009).

Second issue was the method used in optimizing the biogas production. There are many methods used in designing optimizing technique in biogas production (Walid et al., 2007; Mahanty et al., 2014; Kana et al., 2012). Conventionally, one factor at-a-time (OFAT) method has been commonly used (Czitrom, 1999). This method ignored the effect of interactions between factor since other factor is maintained constant (Walid et al., 2007). Other than that, Kana et al., (2012) used Artificial Neural Network (ANN) coupling with Genetic Algorith (GA). This technique is complicated and based on the principle of survival of the fittest. Thus, Response Surface Method (RSM) was utilized in this research as a statistical approach which varies all factors at once. Plus, it allowed determination whether interactions between the factors occurred and also to obtain quantitative cause-effects relationships (Santos et al., 2010)

In addition, there were few researchers highlighted that high concentration of ammoniacal nitrogen (AN) present in the poultry manure wastewater will cause inhibition to biogas production (Abouelenien et al., 2009; Magbanua et al., 2001; Bujoczek et al., 2000; Callaghan et al., 2002). Thus, it is difficult to optimize the biogas production. However, according to Miles (2008), soil can acts as filter, exchanger, and absorber, in wastewater. It can treat and degrade organic materials because organic matter and bacteria are food for soil microorganisms. Thus, biological treatment using soil water was selected to overcome the AN inhibition in PMW so that biogas production could be optimized.

1.3 Objectives

The objectives of this research were:

a. To characterize the soils and poultry manure wastewater (PMW)
b. To determine the best suggested condition for treatment of PMW
c. To analyze the factors affecting biogas production from PMW
d. To optimize the process of biogas production from PMW

1.4 Scope of study

The scopes of this research were based on the research objectives:

i. To characterize the chemical and physical properties of soils used in treatment and biogas production process of PMW
ii. To characterize the chemical properties of PMW before and after treatment
iii. To conduct biological treatment of PMW using soil water
iv. To utilize the Response Surface Method (RSM) in treatment, factorial analysis and process optimization of PMW
v. To analyze the following factors for treatment of PMW by using two-level factorial design (TFD): agitation, reaction time, type of soil water (SW), soil to water ratio and PMW to SW ratio

vi. To analyze the following factors which affect biogas production by using two-level factorial design (TFD): agitation, reaction time, substrate to inoculum ratio, process system and type of substrate

v. To optimize biogas production process using central composite design (CCD)

vii. To utilize Design Expert Software in RSM analysis for suggested optimum condition

viii. To evaluate the biogas production in term of biogas yield and methane content

ix. To validate the suggested optimum condition for biogas production

1.5 Organization of the thesis

The important information of this research was presented in five chapters of this thesis. In the first chapter, introduction to this research was presented. It also includes the problem statement, objectives and also scopes of the research.

In the second chapter, literature review of this research was presented. Thoroughly information was explained which includes the process detailed with substrates, inoculum and selected factors affecting the biogas production. The explanation of RSM utilization in two-level factorial design (TCD) and central composite design (CCD) were also included. Chapter summary was included as to summarize the literature review of this research.

Chapter 3 focused on materials and methods of this research. This chapter includes the material and methods used such as the collection techniques and the preparation of the samples. This chapter emphasized on main the experimental techniques on biogas production which includes the PMW treatment, factorial analysis and process optimization of biogas production. Analysis of the whole research was also included in this chapter.

Chapter 4 discussed the acquired results and findings. It was divided into five subchapters; characterization, treatment of PMW, biogas production from PMW, application of treatment to biogas production and comparison to other researchers. Basically, in this chapter, each result for this research was presented and then discussed on why such results were obtained. The results were also compared to other researchers to supports the findings.

In the last chapter, conclusions and recommendations were presented. Briefly, Chapter 5 was segregated into five subchapter; conclusion on characterization, treatment, factorial analysis, process optimization of PMW and also recommendations. The conclusion of the research based on the objectives whether achieved or not. Recommendations for future work were also included.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Past and current economic growth globally is fueled mostly by fossil fuels. However, there are concerns in the increased use of fossil fuels. The concern are with fossil fuel combustion which has release toxic compounds and oxides of nitrogen and sulfur into the atmosphere and becoming air pollutants (Chynoweth et al., 2001). But still, in Malaysia, the energy need is relatively high and was expected to increase at a rate of 9.5% per year as the economy is projected to grow at a high rate. The primary energy demand is expected to increase close to 100 MTOE (million ton of oil equivalent) (APEC, 2006). Thus, there is an ongoing quest to develop sustainable, affordable, and environmentally sound energy from renewable and are an environmentally clean energy source, and have potential to significantly reduce consumption of fossil fuels (Abbasi et al., 2012). Considering the benefits of environmental pollution control and meeting national energy needs, biogas production technology has been chosen as attractive option in recent years (Harikishan and Sung, 2003). More, abundantly available renewable energy resources in Malaysia could be utilized into biogas production. Biogas has no geographical limitations and the technology of producing can be relatively simple (Taleghani and Kia, 2005).

2.2 Biogas production

Biogas production could be achieved by anaerobic digestion. Anaerobic digestion is a technology that can extract biogas from biological degradation of organic matters. In the anaerobic digestion process of degrading the organic matters into biogas, there is a sequence of reactions involved; hydrolysis, acidogenesis (including acetogenesis) and methanogenesis (Poh and Chong, 2009). Hydrolysis is where organic compound are hydrolyzed into smaller units, such as sugars, amino acids, alcohols, and long-chain fatty acids. Next, is the step of acidogenesis. In this step, acidogenic bacteria will break down these sugar, fatty acids and amino acids into organic acids which mainly consist of acetic acid (from acetogenesis) together with hydrogen and carbon dioxide. Hydrogen and carbon dioxide will be utilized by hydrogenotropic methanogens while acetic acid and carbon dioxide will be utilized by acetoclastic methanogens to give biogas as a final product. The anaerobic digestion process was summarized in Figure 2.1.

The valuable component of biogas is methane (CH_4) from the balance being carbon dioxide (CO_2) and small percentage of other gases (Poh and Chong, 2009). The proportion of methane depends on the feedstock and the efficiency of the process, with the range for methane content being 40% to 70%. (Peavy et al., 1985). Biogas is saturated and can be use in a boiler to produce hot water or steam. The most common use is where the biogas fuels an internal combustion gas engine in a Combined Heat and Power (CHP) unit to produce electricity and heat (Davies, 2006). Besides biogas, anaerobic digestion also produces a solid and the liquid residue called digestate which can be used as a soil fertilizer. The quality of digestate obtained will vary according to the feedstock used (Themelis and Verma, 2004).

In particular, biogas is different from the other energies on two parts. Firstly, it is a high methane fuel and is an ideal fuel because it is comparatively clean. Secondly, biogas is important in controlling and collecting organic waste material and producing fertilizer and water for use in agriculture. Biogas resources from wastewater and livestock manure, have potential energy of 7800 and 13,800 TJ/y, respectively (Prasertsan and Sajjakulnukit, 2006). Composition, energy content, density and molar mass of biogas are listed in Table 2.1.

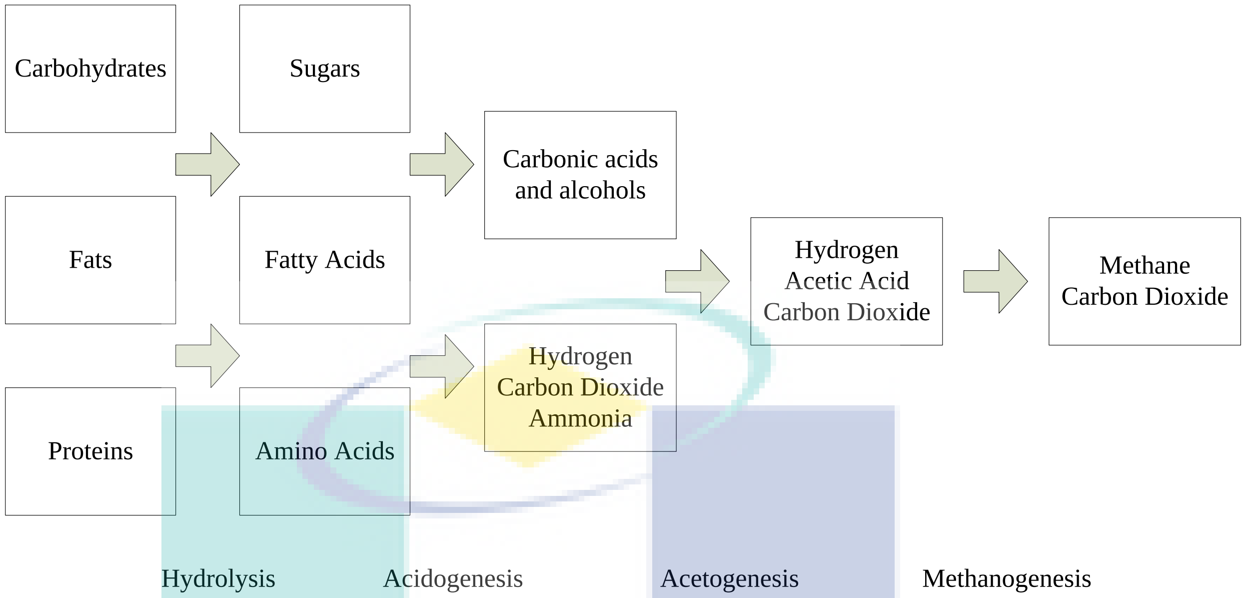


Figure 2.1: Anaerobic digestion process (Peavy et al., 1985)

Table 2.1: General features of biogas (Deublein and Steinhauser, 2008)

Composition	55-70 % methane (CH_4) 30-45 % carbon dioxide (CO_2) Traces of other gases
Energy content	6.0-6.5 kWh/m^3
Fuel equivalent	0.60-0.65 L oil/m^3 biogas
Explosion limit	6-12 % biogas in air
Ignition temperature	650-750°C
Critical pressure	75-89 bar
Critical temperature	-82.5°C
Normal density	1.2 kg/m^3
Smell	Bad eggs
Molar mass	16.043 kg/kmol

2.2.1 Substrates for biogas production

Overall performance of anaerobic digestion is strongly dependent on the substrates used, the biogas yield, the energy input, the source for the digestion process, direct emissions from the process and the use of digestate (Borjesson and Berglund, 2005). Substrates used in anaerobic digestion have different biogas yields due to their energy content. Biogas yields from different type of substrates could be obtained from Table 2.2.

An option of substrates for anaerobic digestion process is fruit and vegetable wastes. Fruit and vegetables wastes tend to have low total solid and volatile solid, and are easily degraded in anaerobic digestion. However, the rapid hydrolysis of these substrates may lead to acidification of a digester and this would result in inhibition in methanogenesis. Thus, these substrates require either co-digestion with other substrates or addition of alkaline buffer to ensure stable performance (Hills and Roberts, 1982; Knol et al., 1978). So, more cost needed for anaerobic digestion of fruit and vegetables wastes.

Other option of substrates for the process is municipal solid waste (MSW). MSW perhaps are the most variable feedstock as the biogas yield depends not only on the sorting method, but also on the location from which the material was sourced and the time of year of collection. Different locations have lifestyle and cultural differences. However, if MSW not separated at source, a considerable amount of pre-processing is required to remove plastics, metals, glass and any other objects that will not be suitable for anaerobic digestion.

Another option of substrate for the process is manure wastewater. Manure wastewater is considered as a waste product from animal breeding. If manure wastewater is used as biogas substrates, there are higher emissions compared to conventional manure wastewater digestion. Using manure wastewater for biogas production will reduces the volume of greenhouse gases normally released during storage (Husted 1994). . Biogas yield from manure wastewater varies widely between livestock types (Table 2.2).

Table 2.2: Biogas yield from different type of substrates (Ward et al., 2008)

Substrates	Biogas yield (m^3/kg VS)
Manure wastewater	
Pig	0.356
Dairy cattle	0.148
Beef cattle	0.328
Poultry	0.480
Fruit and vegetable waste	
Banana peel (Robusta variety)	0.277
Mango (Neelum variety)	0.373
Rotten tomato (mean of variety)	0.298
Potato peel	0.267
Cauliflower leaves	0.190
Cauliflower stem	0.331
Municipal solid waste	
Boiled rice	0.294
Cabbage	0.277
Mixed food waste	0.472
Mechanically sorted (fresh)	0.222
Mechanically sorted (dried)	0.215
Hand sorted	0.205
Yard waste (blend)	0.143
Paper (office)	0.369
Paper (printed newspaper)	0.100
MSW and corn silage	0.110
MSW and cattle manure	0.030
MSW and digested sludge	0.290

Based on Table 2.2, poultry manure wastewater is among a suitable option of substrate for anaerobic digestion process due to it high biogas yield. Anaerobic digestion is suitable for poultry breeding farm because large amount of wastewater is produced due to the use of litter material and these farms use too much energy for heating purposes (Demirci and Demirer, 2004). Therefore, anaerobic digestion is a valuable disposal alternative for poultry manure wastewater producing biogas. It is an advantageous because of its positive energy balance and would result in smaller quantities of sludge compares to aerobic treatment. For these reason, the method is increasingly used (Beccari et al., 1996). Until now, many investigations have been conducted on applicability of anaerobic processing of poultry manure wastewater. Adderley et al., (1976) carried out a study on optimal condition of anaerobic digestion of poultry manure wastewater. Part of their finding was rates of biogas yield in anaerobic digestion process could be improved by considering the sensitiveness of microorganism used to the selected factors.

On the other hand, Atuanya and Aigbirior (2002) examined the feasibility of applying the UASB reactor for treatment of poultry wastewater. They carried out studies for 95 days in a 3.5 L continuous flow UASB at 26-34°C to assess the treatability of poultry wastewaters. They reported that the maximum COD removal was found to be 78% for an OLR of 2.9 kg COD/ (m^3 day) at 13.2 hour HRT. They also concluded that the average biogas recovery was obtained to be 0.26 m^3 CH_4/kg COD with an average methane content of 57% at 30°C.

There was a study conducted by Abouelenien et al., (2009), regarding to the production of biogas from poultry manure. The study was studied in mesophillic conditions at 37°C under laboratory conditions using repeated batch culture system. Biogas was successfully produced after an acclimation period about 254 day. A total volume of 31 ml g^{-1} VS of biogas was produced, despite the presence of high level of ammonia of 8-14 g-N kg^{-1}. This demonstrates that spontaneous acclimation of the methanogenic consortia to high levels of ammonia could occur and result in production of biogas even under high percentage of total solid (25%) and a high level of ammonia, but in long period of time. This finding was supported by Magbanua et al., (2001). They were also able to obtained biogas from poultry manure wastewater after long period of time. Only after 99 day of digestion using 17.4% TS and 14.6%VS, they were able to collect very low amount of biogas, which did not exceed 0.0009 L/g VS. These gave different outcomes compared to a study by Bujoczek et al., (2000), that reported nearly no production of biogas from digested poultry manure wastewater with different total solid percentage, even after 120 day at 35°C. These conditions could be explained by Callaghan et al., (2002). They concluded that high concentration of free ammonia present in poultry manure, causing ammoniacal nitrogen (AN) inhibition to the process. Several mechanical, thermal, chemical, or biological treatment methods have been considered to improve anaerobic digestion process. In the light of this concern, biological treatment was chosen in this study as to overcome the AN inhibition in biogas production from poultry manure wastewater.

2.2.2 Treatment of poultry manure wastewater

Treatment by biological removal of ammoniacal nitrogen (AN) from PMW was an approach to reduce AN inhibition. Soil water was used for the purpose. Soils can reduce ultimate sludge quantity, destroys most of pathogens present in the sludge, and eliminates unpleasant smell problems (Zabranska et al., 2000). Effectiveness of the treatment was studied using five selected factors. The factors were agitation, reaction time, type of soil water (SW), soil to water ratio (soil: water) and PMW to SW ratio (PMW: SW). The ranges for all five factors are based on certain literature review (Table 2.3) and also suitability to the selection of method.

The first selected factor was agitation. Agitation would require significant attention to the treatment efficiency. In this study, the AN removal runs at aerobic conditions and according to Yetilmezsoy and Sapci-Zengin (2009), air flows play an important role in AN volatization. As air introduced, it begins to agitate the solution, creating a removal pathway for dissolved free ammonia to volatize and leave the solution. In a study conducted by Abouelenien et al., (2010), on AN removal by biogas recycle, the agitation speed used was ranges from 10 to 35 rpm, using 500 ml flask. Based on Hygnstrom et al., (2011), agitation is the ability of the soil to treat wastes as in the amount of accessible soil particle surface area. It is indeed necessary factor to study as to ensure the efficiency of the treatment.

Other selected factor was reaction time. In a treatment study conducted by Abouelenien et al., (2010), different reaction time was implemented, ranges 4-10 days. They were able to improved anaerobic digestion by AN removal. In a study by Shiota et al., (2002), the best reaction time for AN removal was 2.8 days. They conducted a strategy in wastewater treatment process for significant reduction of excess sludge production. In a combined thermophilic aerobic process and conventional anaerobic digestion study by Dumas et al., (2010), only one day were taken for good treatment process and ensured high biogas yield in return. Since it has proven to affect the treatment process, this is a factor that is worth to look into.

Another selected factor that would greatly affect the treatment efficiency was PMW to SW ratio (PMW: SW). In a study conducted by Abouelenien et al., (2010), the ratio used was 1:1 on treated poultry manure wastewater to raw poultry manure. The study reported that 80% of total nitrogen in chicken manure was converted to ammonia and 82% of the produced ammonia was removed. Total production of ammonia was higher in the case of mixed substrate including raw poultry manure that contained higher content of nitrogen. This factor is important to study as it has advantages of decreasing the volume of the waste, which in turn reduced the cost of transportation, the space for storage, the reactor size and the use of fuel.

Soil contains a complex biological community of microorganism, including bacteria, protozoa, and fungi, among others. Some of these organisms feed on the organic matter in wastewater (Hygnstrom et al., 2011). Soil did contain high amount and variety species of bacteria such as *Enterobacter Soli* (Jamaludin and Zainol, 2011). Different types of soil gave different treatment efficiency. In this study, two types of soil were selected in order to determine which gave more effect on treatment.

One factor worth studying was soil to water ratio (soil: water). Based on Hygnstrom et al, (2011), soil contains complex microorganism and some of these microorganism feed on the organic matter in the poultry manure wastewater. It is crucial to know the soil: water because microorganisms involved in the treatment require water for their metabolic activities. So, it helps in chemical and biological activities of soil

(Forster-Carneiro, 2008). This factor study was carried out to investigate the effects of different soil to water ratio in which gave better treatment.

Other than treatment, inoculum for biogas production was also important. This due to the fact that inoculum provide bacteria responsible for the anaerobic digestion process (Chamy and Ramos, 2011).

2.3 Inoculum for biogas production

Poultry manure wastewater was acclimatized with soil mixed culture in anaerobic condition. This acclimatization produced inoculum. Inoculum refers to the source of microorganism used for biogas production. Biogas production is facilitated by facultative and anaerobic bacteria (Kelleher et al., 2002).

The soil mixed culture was developed using selected soil. The soil selected was collected besides the poultry barn where the raw poultry manure was taken. This due to reason similar materials is often used to acclimatize a new digester, reducing the start-up time (Ward et al., 2008). The soil mixed culture was acclimatized with PMW because in a study by Chamy and Ramos (2011), it is better to develop inoculum that already adapted to the waste. Biogas production performance is given by the substrate concentration and the inoculum origin. Therefore it is better to carry out the anaerobic digestion with the organic matter that already adapted to the waste.

Soil mixed culture was developed using soil. Soil is defined as the unconsolidated mineral or organic matter on the surface of the earth that has been subjected to and shows effects of the genetic and environmental factors of climate (including water and temperature effect), and macro-microorganisms, conditioned by relief acting on parent material over a period of time (Soil Science Society of America, 2008). Soil can remove multiple contaminants, use minimum energy and chemicals, and promote water reuse and nutrient recovery. This make the used of soil mixed culture as one of the most suitable option while reducing cost compare to use chemical and additives and to ensure highest productivity in returns.

2.4 Important factors affecting biogas production

Biogas production is influenced by many chemical and physical parameters. Several studies were carried out on biogas production from poultry manure to understand the process dynamic and reactors configurations so that the technology could be applied easily at the poultry farm with less cost (Gangagni et al., 2008; Salminen and Rintala, 2002). The determination of anaerobic digestion of wastewater can be based by various factors, among which the agitation, and substrate to inoculums ratio (substrate: inoculum) (Chamy and Ramos, 2011). Effects of five factors were selected in this study. The five factors were agitation, reaction time, substrate: inoculums, process system, and type of substrate. The ranges for all five factors are based on certain literature review and also suitability to the process system.

The first selected factor was agitation. Agitation has a large effect on biogas production process. In a review by Ward et al., (2008), agitation is applied to basic reactor for biogas production. This is to ensure efficient transfer of organic material for the active microbial biomass, to release gas bubbles trapped in the medium and to prevent sedimentation of denser particulate material. Based on Kaparaju et al., (2008), the structure of microbial substrate will be disrupted by vigorous continuous mixing. On contrary, it has been shown that low speed agitation allowed a digester to better absorb the disturbance of shock loading than did high speed agitation (Gomez et al., 2006). Reducing the agitation could improved performance and could also stabilize a continuously-mixed unstable digester (Stroot et al., 2001). Inadequate agitation on the other hand, will results in foam production due to overloaded (Water Environment Federation, 1995). Therefore, this factor was considered as main factor to be studied.

Other selected factor was reaction time. This factor is important to be studied. It is because the major drawbacks of anaerobic digestion are its long reaction time and low degradation efficiency for organic matter (Appels et al., 2011). Anaerobic digestion of poultry waste is preferably to operate at shorter reaction time so as to meet the requirement of economics and environmental benefit. This is because, under short reaction time, the decomposition of organic matter can be achieve efficiently without accumulation of excessive residual and intermediate products like volatile fatty acids

(Ndon and Dague, 1997). Reaction time depends on other factors, such as type of feed stock used (Sakar et al., 2009). Based on Sakar et al. (2009), the reaction time of biogas production from poultry manure was studied between 13.2 h to 91 days. This factor is a suitable factor in the determination of optimum condition of biogas production.

Another selected factor was process system. A variety of process systems are being used for biogas production from poultry manure wastewater. Production of biogas could be in continuous process. However, its use is limited near to the site of the biogas plant (Kapdi et al., 2005). Atuanya and Aigbirior (2002) applying the upflow anaerobic sludge blanket (UASB) reactor for biogas production of poultry wastewater. They concluded that the average biogas production obtained to be 0.26 L/g COD with an average methane content of 57%. Callaghan et al., (2002) was able to established biogas production from cattle slurry, fruit and vegetable wastes and chicken manure by continuous system. They concluded that methane yield was improved from 0.23 to 0.45 L/g VS added. So, it has proven that type of process system would affect the biogas production process and this factor is worth to study for.

A proper substrate: inoculum will allow adequately evaluating the biogas production potential and thus optimizing it. The selection of substrate: inoculum is crucial as for the assessment of anaerobic biodegradability of solid wastes (Lopes et al., 2004). High substrate: inoculum gave higher biogas production than when the ratio is lower (Angelidaki et al., 2006 and Fernandez et al., 2001). Based on Forster-Carneiro et al., (2008), the best performance for the digester was substrate: inoculums of 3:10. It increased reaction time between 20 and 60 days and biogas yield of 0.49 L/g VS. In case of the anaerobic biodegradability of solid waste, the use of highly active anaerobic inoculum will reduce significantly the experimental time. Plus, it could reduce the amount of inoculum required in full scale batch digesters, consequently the corresponding digester volume (Obaja et al., 2003).

In anaerobic digestion, failure to maintain the balance in microorganism involved is the primary cause of reactor instability (Demirel and Yenigun, 2002). Inhibitory substances are often found to be the leading cause of anaerobic reactor upset and failure since they are present in substantial concentrations in wastewater. So, the type of substrate is important to determine the performance of biogas production. According to Liu and Sung, (2002), AN concentrations below 200 mg/L are beneficial to anaerobic process. However, AN inhibition can start at ammonia content up to 1000 mg/L (Liu et al, 2012). In this study, two type of substrate (PMW and treated PMW) was selected to study the effect of this AN inhibition to biogas production process.

2.5 Utilization of response surface method (RSM)

Based on a study by Dornburg et al., (2006), many optimization techniques were developed for optimization. Previously, one factor at-a-time (OFAT) method has been commonly used in optimizing biogas production (Czitrom, 1999). However, this method is time consuming and ignored the effect of interactions between parameter since other factors are maintained constant. Plus, it is practically impossible for the search to accomplish an appropriate optimum in a finite number of experiments (Walid et al., 2007). Some also used simplex-centroid mixture design. However, this method is more suitable for multi-response optimization especially in the optimization of anaerobic co-digestion process (Mahanty et al., 2014). Kana et al., (2012) utilized artificial neural network (ANN) coupling with genetic algorithm (GA). However, this technique is complicated because it mimics the process of mutation and selection fundamental to evolutionary processes and is based on the principle of survival of the fittest.

So, response surface method (RSM) was utilized in this study as a statistical approach which varies all factors at once could give an estimation of the combined effect of variables selected and their significance. RSM is a widely used modeling technique functioned to develop, improve and optimize the response variable in the statistical design of experiments (Bas and Boyaci, 2007). There are several types of design in RSM such as two-level factorial, plackett-burman, d-optimal and central composite (Santos et al., 2010).

Eppinger et al., (2014) utilized plackett-burman design in parameter optimization. This design is specialized design for 2 to 31 factors where factor is varied over 2 levels. However, this design can be use if to assume the absence of two-factor interactions. It is only useful for ruggedness testing where users hope to find little or no effect on the response due to any of the factors. Table 2.3 provides a scale for strength of correlation for evaluating the coefficient of determination (R^2) (Zady, 2000). Mohd Salleh et al., (2011) carried out the optimization process by comparing central composite and two-level factorial. The coefficient of determination (R^2) obtained was 0.998 and 0.96 for central composite and two-level factorial, respectively. This implies that central composite has the higher accuracy compared to others. From the statistical analysis using analysis of variance (ANOVA), the program will suggest the best fitted model and provide a response graph for the measured response.

Table 2.3: Strength of correlation

Size of R^2	Interpretation
0.90 to 1.00	Very high correlation
0.70 to 0.89	High correlation
0.50 to 0.69	Moderate correlation
0.30 to 0.49	Low correlation
0.00 to 0.29	Little if any correlation

Using RSM, a three dimensional surface graph for the responses will be modeled out where the optimization point can be easily obtained (Bas and Boyaci, 2007). From the example of three-dimensional response surface plot (Figure 2.2), the optimal response can be visualized its respective value on independent variables (Bradley, 2007). The proper analysis of RSM will shows the local maximum, local minimum and ridge lines on the topography of response surface and identifies the optimal response region for the design (Olayiwola et al., 2011; Montgomery, 2001).

Overall, RSM is used in optimizing the conditions of tested variables in maximizing the response of an experiment. The optimization in RSM has demonstrated the use of a central composite factorial design by determining the optimum conditions leading to the high yield of biogas production. Thus, smaller and less time consuming experimental designs could generally suffice for the optimization of many processes (Adinarayana and Ellaiah, 2002).

Many reports revealed by using RSM, the response is maximized. Beside, the period of research also decreased. In other ways, RSM helps in saving time and money. This is because less chemical or resources used in less experimental run. This study utilized RSM by using Design Expert Version 7.1.6 software (Stat-Ease Inc., Minneapolis, USA). The software applies important statistical and mathematical methods to find the best model to describe the response data.

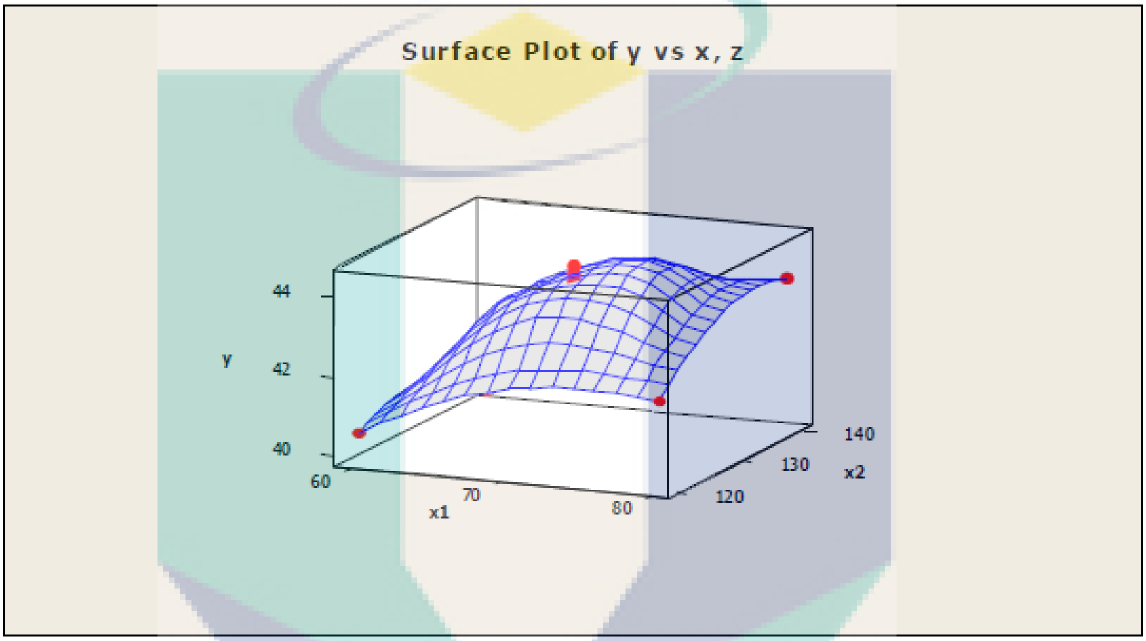


Figure 2.2: Response surface plot

2.5.1 Two-level factorial design (TFD) for factorial analysis

Two-level factorial design (TFD) was used in factorial analysis. It was based on the statistics fundamental principle, randomization, replication and duplication (Santos et al., 2010). Overall, it simplifies the factorial analysis by studying the interactions among the factors over a range of values in a statistical manner. Previously, TFD was successfully employed for the biogas production from palm oil mill effluent (POME) (Zinatizadeh et al., 2010), municipal solid wastes (Markidis et al., 2013) and fruit wastes (Wikandari et al., 2013).

Referring to Zinatizadeh et al., (2010), anaerobic digestion of POME was modeled and analyzed. Experiment were conducted based on a general factorial design and analyzed using response surface method (RSM). As various responses were investigated in this study and different degree of polynomial models was used for data fitting.

In a study by Markidis et al., (2013), two-level four factors factorial design was used. It was used to designed experiment for decomposition of municipal solid wastes stored in wrapped bales. The factorial analysis revealed that there were two main factors that significantly affected the biogas production.

Wikandari et al., (2013) conducted a study on improving biogas production from fruit wastes. The inhibitory effect of fruit flavors on anaerobic digestion was investigated. Complete factorial design was used for experimental design. For statistical analysis, analysis of variance (ANOVA) with significance level of 0.05 was performed using statistical package for the social sciences (SPSS).

Conclusively, TFD was used for the designing model and provides statistical analysis in a multi-factors experiment. In other word, factorial design allowed determination whether interactions between the factors occurred and also to obtain quantitative cause-effects relationships (Santos et al., 2010). It was applied as a statistical method to screen the selected factors on how they affect the production and observe their interaction with one another.

2.5.2 Central composite design (CCD) for process optimization

According to Bezerra et al., (2008), experimental design namely central composite design (CCD) is the most utilized design of optimization. CCD was utilized in the development of analytical procedures compared to the others as their low efficiency of the latter especially for a numbers of variables (Bezerra et al., 2008). Previously, CCD was successfully employed for the biogas production from livestock wastes (Molinuevo-Salces et al., 2010), palm oil mill effluent (Zinatizadeh et al., 2007) and winery wastewater (Riano et al., 2011).

Molinuevo-Salces et al., (2010) employed CCD in designing experiments and determine individual and interactive effects over biogas production and removal of volatile solids. They are using swine and poultry manure as substrate of anaerobic digestion. Statistical analysis allowed them to set initial conditions and parameter to achieve best outputs for real-scale plant operation and/or co-digestion mixtures design.

CCD was successfully applied by Zinatizadeh et al., (2007) to determine the optimum operational conditions for the anaerobic digestion of pre-treated palm oil mil effluent (POME). They concluded that CCD was an effective tool to compare the results obtained from the anaerobic treatment of the two different types of pre-treated POME with different characteristics.

Based on Riano et al., (2011), CCD was applied in designing batch experiments from anaerobic co-digestion of swine manure with winery wastewater. The response of biogas yield was then evaluated by using second order polynomial function. The coefficient of determination (R^2) was calculated to achieve the proportion of data variability that is explained by the model, thus the quality of fit to the model.

In CCD, all factors are studied in 5-levels which are –α, -1, 0, +1, +α. In this study, there were two independent variables, which are agitation speed and reaction time, thus there were a total of 13 experiments employed including five centre points to evaluate the curvature and effects of interaction between independent variables. The factorial design for this experiment is $(\pm 1, \pm 1)$, centre point is (0, 0) and star point is $(\pm\,\alpha, 0)$ (Gunst, 1996).

A quadratic regression model was used to express the biogas yield as a function of two independent variables as in Eq. 1:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_{11}^2 + \beta_{22} x_{22}^2 + \beta_{12} x_1 x_2 \qquad \text{(Eq. 2.1)}$$

where y represents the measure response of the biogas yield (L/g COD), x_1 andx_2 were the coded independent variables which were agitation speed (rpm) and reaction time (days) respectively; β_0 is the intercept; β_1 and β_2 are the linear coefficients; β_{11} and β_{22} are the quadratic coefficients; and β_{12} is the logarithmic coefficient (Chauhan et al., 2013). All the coefficients of the response surface equation can be determined by using CCD in RSM.

The relationships between predicted and experimental results were illustrated and analyzed by CCD. Using CCD, the goodness of fit was determined by coefficient of determination, R^2 while the statistical significance of the regression model was checked by the Fisher statistical test (F-test) in analysis of variance (ANOVA) (Chauhan et al., 2013). Effects with a confidence level higher than 95 % (p-value less than 0.05) were preferable to represent the reliability of a result (Azami et al., 2011).

2.6 Chapter summary

Biogas can be produced by anaerobic digestion process. In order to give high yield of biogas, AN inhibition to the process needed to be conquered first. Biological removal of AN from PMW by using soil water was selected in this study as treatment. This due to the facts that biological method is the most suitable option while reducing cost compare to use chemical and additives and to ensure highest productivity in returns. The determination of biogas production efficiency from wastewater can be based by various factors (Chamy and Ramos, 2011). Five factors were selected in this study. The five factors were agitation, reaction time, process system, substrate: inoculum and type of substrate. The ranges for all five factors were based on literature review and suitability to the process system. The design of factorial analysis utilized two-level factorial design (TFD) while design of process optimization utilized central composite design (CDD). Both designs utilized response surface method (RSM) in Design Expert Software.

CHAPTER 3

MATERIALS AND METHODS

3.1 Process flow of materials and methods

The process flow chart of the research materials and methods was shown in Figure 3.1. The methodology was divided into four main contents as to achieve four objectives. The four main contents were characterization, treatment, factorial analysis and process optimization of poultry manure wastewater (PMW). The procedure was started with collection and preparation of samples. Next, samples were characterized to achieve the first objective. Treatment was then applied to avoid the ammoniacal nitrogen (AN) inhibition to achieve second objective. After that, inoculum was prepared by using soil mixed culture.

The most important parts of the research were factorial analysis and process optimization to achieve objective three and four. Both factorial analysis and process optimization utilized Response Surface Method (RSM). The experimental design for factorial analysis was developed by using two-level factorial design in RSM. The experimental design for process optimization was developed by using central composite design (CCD) in RSM. After process optimization, validation run was performed in order to validate the optimum condition resulted from the analysis. All statistical analysis was performed by utilizing Design Expert software.

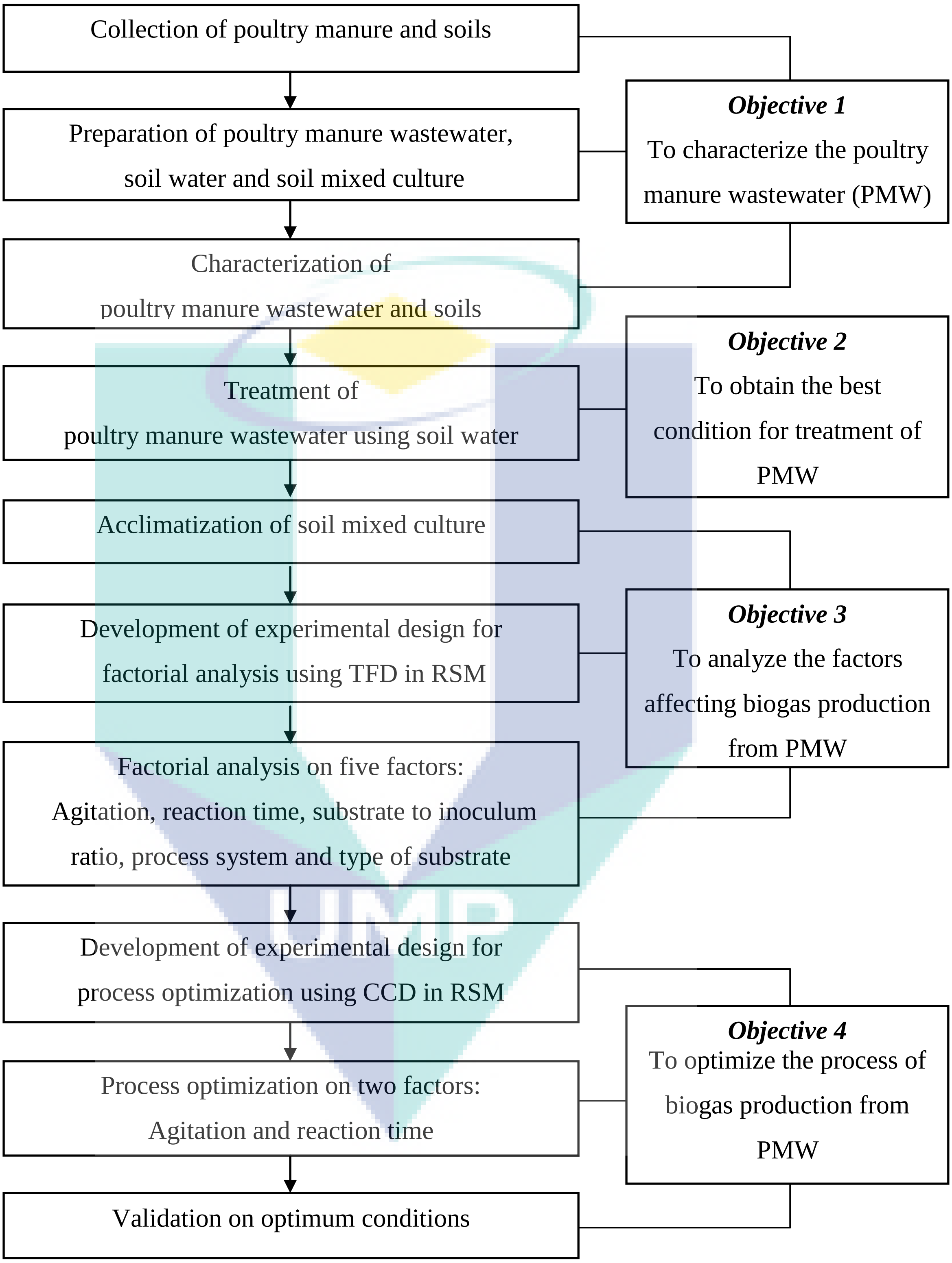


Figure 3.1: Process flow chart

3.2 Poultry manure wastewater

3.2.1 Collection of poultry manure

Poultry manure was collected from a moderate size poultry farm located at Kuantan, Pahang (Malaysia). The site where the poultry manure was taken was named Site A (Appendix B1.1). Poultry manure was collected in bulk plastic container and then stored at 4°C to minimize substrate decomposition and odor.

3.2.2 Preparation of poultry manure wastewater

Initially, large particle was removed from poultry manure. Then, it was diluted with distilled water to produce PMW. According to Gonzalez-Fernandez et al., (2008), substrate will not produce significant biogas quantities if not diluted. The feed ratio was constant throughout the experiment at 1:1 ratio (v/v), and was mixed thoroughly for 5-10 minutes. The PMW was then characterized and kept at 4°c until further used in order to avoid early digestion.

3.3 Treatment of poultry manure wastewater

3.3.1 Collection of soils

In treatment of PMW, there were two types of soils collected. The collected soils were sandy soil (SS) and poultry farm soil (PFS). The SS was collected at one specific site of University Malaysia Pahang (UMP), Malaysia namely Site B (Appendix B1.2). The PFS was collected near poultry farm area namely Site C (Appendix B1.3). Soils were taken in bulk plastic containers and then were stored at 4°C, prior to use.

3.3.2 Characterization of soils

The SS (from section 3.3.1) was characterized for chemical and physical properties. The tests methods for soil characterization were summarized in Table 3.1.

Table 3.1: Summary of test method for soils characterization

No.	Parameter	Unit	Test method (Appendix B3)
1	Nitrogen (N)	%	Determination of Total Nitrogen by Micro Kjedahl Method
2	Moisture Content (MC)	%	Moisture Content and Loss of Ignition
3	Carbon (C)	%	Determination of Carbon
4	Conductivity	-	Determination of Conductivity in Soil
5	Available Phosphorus	ppm	Available Phosphorus in Soil
6	Cation Exchange Capacity (CEC)	cmol/kg	Determination of Exchange Cations by ICP & CEC by Distillation Method
7	Coarse sand	%	Determination of Soil Texture
8	Fine sand	%	Determination of Soil Texture
9	Silt	%	Determination of Soil Texture
10	Clay	%	Determination of Soil Texture

3.3.3 Preparation of soil water

There were two types of soil water (SW), namely sandy soil water (SSW) and poultry farm soil water (PFW). SW was prepared by mixing each soil (from section 3.3.1) with distilled water thoroughly for 5-10 minutes.

3.3.4 Preliminary study

Preliminary study was conducted to determine the ranges of factors for the biological treatment of PMW.

Two shake flasks with working volume of 250 ml filled with poultry manure, each was then added up with distilled water (control), and another one with SSW. The conditions for this study were fixed at mixing ratio (v/v) of 1:4 (PMW to distilled water or SSW), reaction time of 2 hours and no agitation. The conditions were based on study by Sung and Liu (2003) and Liu et al., (2012). The response of preliminary study was percentage of AN removal.

3.3.5 Experimental design

Five factors were selected for study were agitation, reaction time, type of soil water (SW), soil to water ratio (soil: water) and PMW to soil water ratio (PMW: SW). Based on the preliminary results, the ranges of factors for treatment were suggested (Table 3.2). The design of experiment (DOE) was generated by using two-level full factorial (TFD) in RSM. A total of 32 runs were made with five factors TFD (2^5).

Table 3.2: Ranges of factors for PMW treatment

Factor	Symbol	Type	Low coded (-1)	High coded (+1)
Agitation (rpm)	A	Categorical	0	200
Reaction time (hour)	B	Numerical	2	5
Type of soil water	C	Categorical	SSW	PFW
Soil: water	D	Categorical	1:6	1:1
PMW: SW	E	Categorical	1:4	2:3

3.3.6 Experimental set up for treatment

Experiments were performed at Environmental Laboratory of Faculty of Chemical and Natural Resources Engineering, UMP. The treatment was conducted by using 250 ml shake flask in aerobic condition. The flasks were filled with PMW first, and then reaction time started as soon as SW were added. Each flask was then run based on conditions in Table 3.3 (actual factor) and Table 3.4 (coded value).

3.3.7 Analysis of treatment

The response of treatment was the percentage of AN removal. The percentage of AN removal was calculated by using Eq. 3.1. The value of initial and final AN were tested by using HACH Spectrophotometer DR5000 following Method 8155 (Appendix B4.5). Response were analyzed using analysis of variance (ANOVA) combined with the F-test to evaluate significant factors at the level of 5% ($p<0.05$).

$$AN\ removal\ (\%) = \left(\frac{Initial\ \ AN\ concentration\ \ -Final\ \ AN\ concentration}{Initial\ \ AN\ concentration}\right) \times 100\,\% \quad \text{(Eq. 3.1)}$$

Table 3.3: Experimental table for PMW treatment (actual factor)

Run	Agitation (rpm)	Reaction time (h)	Type of soil water	Soil: water	PMW: SW
1	200	2.00	PFW	1:1	1:4
2	200	2.00	PFW	1:1	2:3
3	0	5.00	PFW	1:1	1:4
4	200	5.00	PFW	1:6	1:4
5	0	2.00	SSW	1:6	1:4
6	0	2.00	SSW	1:1	1:4
7	200	2.00	SSW	1:1	1:4
8	200	5.00	SSW	1:6	2:3
9	200	2.00	PFW	1:6	2:3
10	200	5.00	SSW	1:1	2:3
11	0	5.00	PFW	1:6	2:3
12	200	2.00	PFW	1:6	1:4
13	200	5.00	PFW	1:1	1:4
14	0	5.00	PFW	1:1	2:3
15	200	2.00	SSW	1:1	2:3
16	0	2.00	PFW	1:1	1:4
17	200	2.00	SSW	1:6	2:3
18	200	2.00	SSW	1:6	1:4
19	0	2.00	PFW	1:6	1:4
20	0	5.00	SSW	1:1	1:4
21	0	2.00	SSW	1:1	2:3
22	0	2.00	SSW	1:6	2:3
23	200	5.00	PFW	1:1	2:3
24	0	5.00	SSW	1:6	2:3
25	200	5.00	SSW	1:6	1:4
26	0	5.00	SSW	1:1	2:3
27	200	5.00	PFW	1:6	2:3

28	0	2.00	PFW	1:1	2:3
29	0	5.00	SSW	1:6	1:4
30	200	5.00	SSW	1:1	1:4
31	0	2.00	PFW	1:6	2:3
32	0	5.00	PFW	1:6	1:4

*PMW = poultry manure wastewater, *SW = soil water, *SSW= Sandy soil water *PFW= Poultry farm soil water

Table 3.4: Experimental table for PMW treatment (coded value)

Run	Agitation (rpm)	Reaction time (h)	Type of soil water	Soil: water	PMW: SW
1	+1	-1	+1	+1	-1
2	+1	-1	+1	+1	+1
3	-1	+1	+1	+1	-1
4	+1	+1	+1	-1	-1
5	-1	-1	-1	-1	-1
6	-1	-1	-1	+1	-1
7	+1	-1	-1	+1	-1
8	+1	+1	-1	-1	+1
9	+1	-1	+1	-1	+1
10	+1	+1	-1	+1	+1
11	-1	+1	+1	-1	+1
12	+1	-1	+1	-1	-1
13	+1	+1	+1	+1	-1
14	-1	+1	+1	+1	+1
15	+1	-1	-1	+1	+1
16	-1	-1	+1	+1	-1
17	+1	-1	-1	-1	+1
18	+1	-1	-1	-1	-1
19	-1	-1	+1	-1	-1
20	-1	+1	-1	+1	-1
21	-1	-1	-1	+1	+1

22	-1	-1	-1	-1	+1
23	+1	+1	+1	+1	+1
24	-1	+1	-1	-1	+1
25	+1	+1	-1	-1	-1
26	-1	+1	-1	+1	+1
27	+1	+1	+1	-1	+1
28	-1	-1	+1	+1	+1
29	-1	+1	-1	-1	-1
30	+1	+1	-1	+1	-1
31	-1	-1	+1	-1	+1
32	-1	+1	+1	-1	-1
	-1= 0 +1= 200	-1= 2 +1= 5	1= SSW +1= PFW	-1= 1:6 +1= 1:1	-1= 1:4 +1= 2:3

*PMW = poultry manure wastewater, *SW = soil water, *SSW= Sandy soil water *PFW= Poultry farm soil water

3.4 Biogas production

3.4.1 Collection of soil

The collected soil was poultry soil (PSL). The PSL was collected besides the poultry barn area namely Site D (Appendix B1.4). Soil was taken in a bulk plastic container and then stored at 4°C, prior to use.

3.4.2 Characterization of substrates

Both PMW and treated PMW (from section 3.3) were characterized based on test method summarized in Table 3.5.

Table 3.5: Test method for characterization of PMW and treated PMW

No.	Parameter	Unit	Test method (Appendix B4)
1	pH	-	Standard Methods APHA, 1998
2	Suspended solid (SS)	mg/L	Standard Methods APHA, 1998

3	Biological oxygen demand (BOD)	mg/L	Standard Methods APHA, 1998
4	Chemical oxygen demand (COD)	ppm	HACH Spectrophotometer Method 8000
5	Ammoniacal nitrogen (AN)	mg/L	HACH Spectrophotometer Method 8155
6	Nitrate	mg/L	HACH Spectrophotometer Method 8171
7	Nitrite	mg/L	HACH Spectrophotometer Method 8153
8	Phosphorus	mg/L	HACH Spectrophotometer Method 10127

3.4.3 Preparation of soil mixed culture

The PSL was characterized similarly with Section 3.3.2. The tests methods for soil characterization were summarized in Table 3.1. Soil mixed culture (SMC) was prepared by mixing PSL thoroughly with distilled water for 5-10 minutes. Soil to water ratio of 1:6 (v/v) was used.

3.4.4 Acclimatization of soil mixed culture

SMC was acclimatized with treated PMW (from section 3.3), producing inoculum. The acclimatization was developed by using five liters of digester (Appendix B5.1). The conditions for the acclimatization were SMC to PMW ratio of 1:4 (v/v), no agitation, ambient temperature and hydraulic retention time (HRT) of 30 days.

The acclimatization set up was illustrated in Appendix B5.1. The water displacement unit functions as to monitor and measure the biogas production volume. The reading of biogas volume was taken daily for 30 days.

3.4.5 Preliminary study

The factors chosen in the biogas production was based on other previous researches such as Chamy and Ramos (2011), Angelidaki et al., (2006), and Fernandez et al., (2001). Preliminary study was first conducted to determine the ranges of the factors.

The preliminary study was conducted by using control and treated PMW. Two shake flasks with working volume of 250 ml were filled with PMW (control), and another one with treated PMW. After that, inoculum was added to each flask and reaction time started. The conditions for this study were fixed at mixing ratio (v/v) of 1:4 (SMC to PMW or treated PMW), reaction time of 13 days, and no agitation. The response of preliminary study was biogas yield.

3.4.6 Experimental design for factorial analysis by using TFD

Based on the preliminary results, the ranges for treatment were suggested (Table 3.6). The design of experiment (DOE) of factorial analysis was generated by using two-level factorial design (TFD) in RSM. A total of 16 runs were made with five factors two-level TFD (2^5).

Table 3.6: Ranges of factors for factorial analysis

Factor	Symbol	Type	Low coded (-1)	High coded (+1)
Agitation (rpm)	A	Numerical	0	120
Reaction time (days)	B	Numerical	3	7
Substrate : inoculum	C	Categorical	3:2	4:1
Process system	D	Categorical	Batch	Continuous
Type of substrate	E	Categorical	PMW	Treated PMW

*PMW = poultry manure wastewater

3.4.7 Experimental set up for factorial analysis

Experiments were performed at Environmental Laboratory of Civil Engineering and Earth Resources, UMP. Anaerobic digestion process was carried out in 250 ml shake flasks. Each flask was run based on conditions in Table 3.7 (actual factor) and Table 3.8 (coded value). Both tables can represent the experimental condition, but in term of engineering, Table 3.8 was much preferable. The shake flasks were then covered with silicone seal with gas line to the biogas collector (Figure 3.2). After covered, the flasks will be sealed with parafilm to avoid contamination.

Table 3.7: Experimental table for factorial analysis (actual factor)

Run	Agitation (rpm)	Reaction time (days)	Substrate: inoculum	Process system	Type of substrate
1	0	7	4:01	Continuous	PMW
2	120	7	4:01	Batch	PMW
3	0	7	3:02	Continuous	Treated PMW
4	120	3	4:01	Batch	Treated PMW
5	0	3	4:01	Batch	PMW
6	120	3	3:02	Batch	PMW
7	120	7	3:02	Continuous	PMW
8	0	7	4:01	Batch	Treated PMW
9	120	7	4:01	Continuous	Treated PMW
10	120	3	4:01	Continuous	PMW
11	0	3	4:01	Continuous	Treated PMW
12	0	3	3:02	Continuous	PMW
13	0	7	3:02	Batch	PMW
14	120	3	3:02	Continuous	Treated PMW
15	0	3	3:02	Batch	Treated PMW
16	120	7	3:02	Batch	Treated PMW

*PMW= poultry manure wastewater

Table 3.8: Experimental table for factorial analysis (coded value)

Run	Agitation (rpm)	Reaction time (days)	Substrate : inoculum	Process system	Type of substrate
1	-1	+1	+1	+1	-1
2	+1	+1	+1	-1	-1
3	-1	+1	-1	+1	+1
4	+1	-1	+1	-1	+1
5	-1	-1	+1	-1	-1
6	+1	-1	-1	-1	-1
7	+1	+1	-1	+1	-1
8	-1	+1	+1	-1	+1
9	+1	+1	+1	+1	+1
10	+1	-1	+1	+1	-1
11	-1	-1	+1	+1	+1
12	-1	-1	-1	+1	-1
13	-1	+1	-1	-1	-1
14	+1	-1	-1	+1	+1
15	-1	-1	-1	-1	+1
16	+1	+1	-1	-1	+1
	-1= 0 rpm +1= 120 rpm	-1= 3 days +1= 7 days	-1= 3:2 +1= 4:1	-1= Batch +1= Continuous	-1= PMW +1= Treated PMW

*PMW= poultry manure wastewater

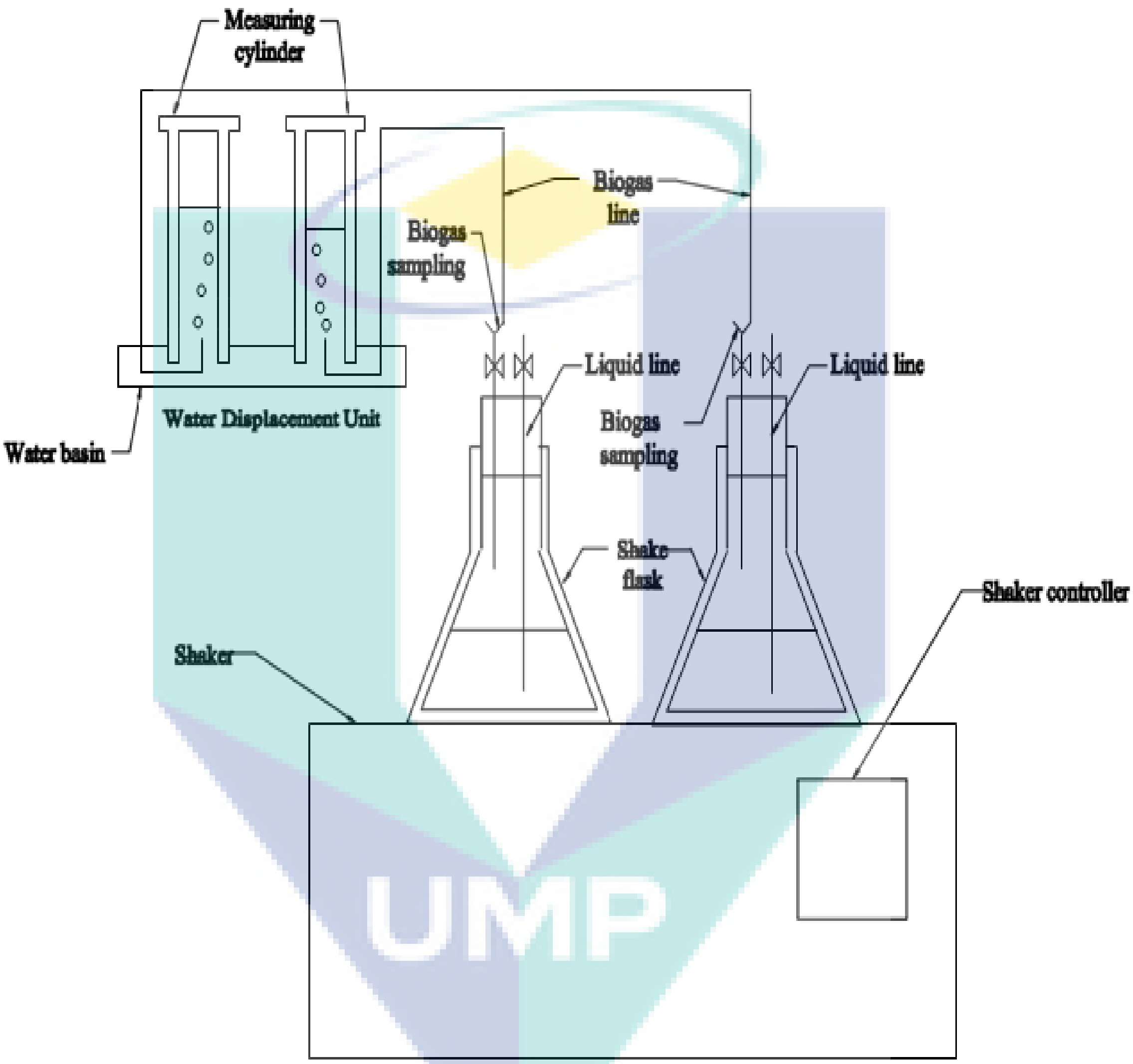


Figure 3.2: Experimental set up for biogas production

3.4.8 Experimental design for process optimization by using CCD

The design of experiment (DOE) of process optimization was generated by using CCD in RSM. Factors with their ranges (Table 3.9) were based on the result of factorial analysis. All factors were studied in 5-levels coded values which -2, -1, 0, +1 and +2. Thus, there were a total of 13 runs.

Table 3.9: Ranges of factors for process optimization

Factor	Symbol	Unit	Coded values				
			-2	-1	0	+1	+2
Agitation	A	rpm	100	110	120	130	140
Reaction time	B	days	1	2	3	4	5

3.4.9 Experimental set up for process optimization

Experiments were performed at Environmental Laboratory of Civil Engineering and Earth Resources, UMP. Anaerobic digestion process was carried out using 250 ml shake flasks. The fixed conditions of flasks were based on the result of factorial analysis study at substrate: inoculum at 1:4 (v/v), batch system and PMW as substrate. The variable conditions were based on Table 3.10 (actual factor) and Table 3.11 (coded factor). Firstly, substrate was mixed with inoculum. Next, the flasks were covered with silicone seal. After covered, the flasks were sealed with parafilm to avoid contamination. Experimental set up was illustrated in Figure 3.2.

Table 3.10: Experimental table for process optimization (actual factor)

Run	Agitation (rpm)	Reaction time (day)
1	120	3
2	130	4
3	130	2
4	120	5
5	120	1
6	110	2

7	140	3
8	120	3
9	120	3
10	110	4
11	120	3
12	120	3
13	100	3

Table 3.11: Experimental table for process optimization (coded value)

Run	Agitation (rpm)	Reaction time(days)
1	0	0
2	+1	+1
3	+1	-1
4	0	-2
5	0	-2
6	-1	-1
7	+2	0
8	0	0
9	0	0
10	-1	+1
11	0	0
12	0	0
13	-2	0
	-2= 100 rpm, -1= 110 rpm, 0= 120 rpm, +1= 130 rpm, +2= 140 rpm	-2= 1 day, -1= 2 days, 0= 3 days, +1= 4 days, +2= 5 days

3.4.10 Analysis for biogas production

Response for both factorial analysis and process optimization was biogas yield. Biogas yields were calculated as Eq. 3.2 and based on biogas production from initial concentration of Chemical Oxygen Demand (COD). The concentration of COD was tested by using HACH Spectrophotometer DR5000 following Method 8000 (Appendix

B4.4). Biogas was collected in water displacement unit, filled with water. Reading of biogas volume was taken daily until end of reaction time.

The responses were analyzed using Design Expert Software. In this software, the goodness of fit was determined by coefficient of determination, R-squared (R^2) while the statistical significance of the regression experimental design was checked by the Fisher statistical test (F-test) in analysis of variance (ANOVA) (Chauhan et al., 2013). Effects with a confidence level higher than 95 % (p-value less than 0.05) were selected to represent the reliability of result from this study (Azami et al., 2011).

$$Biogas\ yield\ (Y) = \left(\frac{biogas\ \ production\ \ volume}{initial\ \ concentration\ \ \ of\ COD}\right)\left(\frac{L}{g}COD\right) \qquad \text{(Eq. 3.2)}$$

Composition of methane in biogas produced was measured using Gas Chromatograph (Agilent 6980N, Agilent Technology, USA) equipped with a capillary column (Agilent 19095P-Q40, 30m x 530µm x 40µm) and a thermal conductivity detector (Agilent, USA) with inject temperature 110ºC. The carrier gas was helium operated with a flow rate of 20 ml/min at 50ºC.

3.4.11 Validation of optimum condition

Validation experiment was performed after obtaining the suggested optimum condition. Suggested optimum conditions with predicted biogas yield were listed in Table 3.12.

Table 3.12: Suggested optimum conditions for process optimization

Run	Agitation (rpm)	Reaction time (days)	Predicted biogas yield (L/g COD)
1	120	3.00	0.00370
2	120	3.30	0.00375

Experimental set up for this experiment was illustrated in Figure 3.2. The percentage error from the predicted and actual biogas yield was calculated using Eq. 3.3.

$$Error\ (\%) = \left(\frac{Predicted\ response - Actual\ response}{Predicted\ response}\right) \times 100\% \qquad \text{(Eq. 3.3)}$$

3.5 Application of PMW treatment to biogas production

Two sets of experiments were conducted to evaluate the requirement of PMW treatment using Flask A and Flask B. Flask A was filled with PMW while Flask B with treated PMW. After that, inoculum was added to each flask, and reaction time started. The conditions of these two flasks were constant at substrate to inoculum ratio of 1:4 (v/v), no agitation and at room temperature. The flasks were then covered with silicone seal with gas line to the water displacement unit (Appendix B5.2). Reading of biogas volume was taken daily until biogas production stopped. Response of this experiment was biogas yield as calculated using Eq. 3.2.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Characterization of soils and poultry manure wastewater (PMW)

4.1.1 Characterization of soils

Soil is defined as the uncombined mineral or organic matter on the surface of the earth, based on parent material over a period of time (Soil Science Society of America, 2008). According to Miles (2008), soils can acts as filter, exchanger, and absorber, in many physical, chemical or biological treatment of wastewater. It can treat and degrade organic materials because organic matter and bacteria are food for soils organisms.

The characteristics of soils used in this study were listed in Table 4.1. The characterization was divided into chemical and physical properties. The chemical properties included the percentage of nitrogen, moisture content, carbon, conductivity, available phosphorus and cations exchange capacity (CEC). The physical properties included the percentage of coarse sand, fine sand, silt and clay. There were two types of soil characterized; sandy soil (SS) and poultry soil (PSL). SS was used in the treatment of PMW while PSL in biogas production.

From Table 4.1, the percentage of nitrogen (N) in SS and PSL was 0.003% and 0.46%, respectively. Based on Sawyer (2008), nitrogen in soils is a complex mixture of chemical and biological processes. The majority of N in soils is contained in organic matter. The N content of soils varied greatly as the organic matter varies. This indicated that PSL comprises more organic matter compared to SS as the percentage of N in PSL much higher than that in SS. So, PSL was more suitable to be used in biogas production compared to SS due to high organic content.

Moisture content for SS and PSL were 0.23% and 27.325%, respectively. Moisture content in soil indicates the water contained in it. The water is held within the soil pores. The water in soil serves as solvent and nutrient itself. In this study, soil was used in both treatment and biogas production from PMW. It is crucial to know the moisture content in soil because microorganisms involved in both processes require water for their metabolic activities. So, it helps in chemical and biological activities of soil (Soil Science Society of America, 2008).Based on the result, moisture content in PSL much higher than SS, so it make PSL more suitable to be used in the biogas production process compared to treatment.

By referring to Table 4.1, the percentage of carbon in PSL was much higher than in SS. This result was supported by the fact that carbon content in soil is according to soil type. Gershenson et al., (2009) reported that soil with higher clay content increases the carbon content in it. Based on the result, it is proven that PSL contained higher carbon content because it have more clay content compare to SS. So, PSL more suitable for biogas production compared to SS. In biogas production, the carbon content in PSL was expected to lost through microbial decomposition, which largely dependent on temperature, moisture and substrate availability (Gershenson et al., 2009).

Based on Table 4.1, conductivity of SS was higher than PSL. Conductivity indicates the total ion and the ability of a material to transmit electrical current (Barbosa and Overstreet, 2011). Higher conductivity would result in better treatment of PMW because ions would removed AN by binding with it. Conductivity depends on the particle size and soil textures of the soil because according to Barbosa and Overstreet, (2011) higher sand percentage resulted in higher conductivity. This was supported by the result that SS contained higher sand percentage compared to PSL. So, SS was much suitable to be used in treatment of PMW compared to PSL.

Another chemical test for soil was available phosphorus (P). Based on Table 4.1, available P in PSL was higher than SS. This related to Busman et al., (2009), which reported that phosphate in soils is associated more with fine particles than coarse particles. This is because when soil erosion occurs, more fine particles are removed than coarse particles, causing the sediment to be enriched of P. So, PSL contained more fine particles than SS. So, PSL was much more suitable to be used in biogas production compared to SS. In biogas production, the microorganisms in the digester do not consume P and some P can be converted to ortho P (a soluble form) in the digester, but the total mass remains constant (Topper et al., 1993).

From Table 4.1, the cations exchange capacity (CEC) in SS and PSL was 0.05 and 17.74 cmol/kg, respectively. Cations are positively charged ions and the capacity of the soil to hold these cations are called CEC. These cations are held by the negatively charged clay and organic matter particle in the soil through electrostatic forces. Thus, the CEC of a soil represents the total amount of exchangeable cations that the soil can absorb. According to Masunaga et al., (2012), the lower the CEC, the higher the AN removal in the treatment of PMW. This because, if the CEC are low, more negatively charged particle available, and they can attract and hold positively charged AN and removed it. Since the CEC of SS was much lower than PSL, so, SS was more suitable to be used in the treatment than in biogas production.

Other than chemical properties, physical properties of soil were also important. The soil textures analysis was listed in Table 4.2. Based on study by Miles (2008), the size of the soil particle less than 2 mm can be divided into sand, silt and clay. Based on Table 4.2, the chemical activities are largely occurring in clay with the most surface area compared to greater particle size as sand and silt. PSL contained more clay particle than in SS (Table 4.2). So, this makes PSL much suitable for biogas production as its contained larger surface area than SS. More, particles greater than 2 mm are called coarse sand and it is able to modify texture while increasing the treatment of PMW. SS contained more coarse sand compared to PSL (Table 4.1). So, this was the reason of SS much suitable to be used in the treatment compared to PSL. Based on both chemical and physical properties, SS was used in the treatment of PMW while PSL in biogas production.

Table 4.1: Characteristics of soils

No.	Parameter	Unit	SS	PSL
1	Nitrogen (N)	%	0.003	0.46
2	Moisture content (MC)	%	0.23	27.32
3	Carbon (C)	%	0.11	2.12
4	Conductivity		72.65	3.44
5	Available phosphorus	ppm	5.55	8701
10	CEC	cmol/kg	0.05	17.74
6	Coarse sand	%	92	24
7	Fine sand	%	5	37
8	Silt	%	1	16
9	Clay	%	7	28

*CEC= cations exchange capacity ; S= sandy soil S; PSL = poultry soil

Table 4.2: Soil textures analysis (Miles, 2008)

Type of soil	Size (mm)	Surface area	Chemical activity
Sand	2.000-0.050	Least	Small
Silt	0.050-0.002	Intermediate	Intermediate
Clay	< 0.002	Most	Large

4.1.2 Characterization of poultry manure wastewater (PMW)

The characteristics of poultry manure wastewater (PMW) and treated PMW were listed in Table 4.3. In a study conducted by Yetilmezsoy and Sakar (2008), the characteristics of poultry manure wastewater were almost similar compared to this study. In that study, the pH, chemical oxygen demand (COD), suspended solid and phosphorus concentration, were 7.30, 21,100 mg/L, 446 mg/L, respectively. In this study, the pH, COD, suspended solid and phosphorus concentration were 8.1, 35,600 mg/L, >750 mg/L, 710 mg/L, respectively.

From Table 4.3, the pH for PMW was higher than pH for treated PMW at 8.1 and 7.5, respectively. Although the pH for PMW was much higher, but both pHs were in good range as the biogas production microorganisms are less sensitive and can function in a wider range of pH between 4.0 to 8.5 (Hwang et al., 2004). In fact, each group of microorganisms has a different optimum functional pH range between 6.5 and 7.2 (Turovskiy and Mathai, 2006).

In this study, the initial chemical oxygen demand (COD) concentration and biological oxygen demand (BOD) for PMW was 35,600 mg/L and 18300 mg/L, respectively. This was differ with the initial COD and BOD value for treated PMW which was 4985 mg/L and 2300 mg/L, respectively. This indicates that initial COD and BOD for PMW were higher than treated PMW. Cakir and Stenstrom (2003) reported that wastewater having wide range of COD and BOD concentration of 2000 to 20,000 mg/L. COD and BOD are water quality analyses commonly used to indicate the amount of organic matter present in wastewater. From the results, more amount of organic matter consists in PMW, thus have more potential to be degraded and produce more biogas than treated PMW. An experimental study was conducted by Demirci and Demirer, (2004) to investigate biogas generation potential of poultry manure. They reported biogas yields of 180-270 mg/L COD for initial COD concentrations of 12,000 mg/L.

Other characteristic tested was suspended solids (SS) content.SS content will affect the mixing, process dynamics and digester feeding method. The SS value for PMW and treated PMW was above 750 mg/L. The exact value could not be obtained due to equipment limitation. However, both of the values were in a good range for biogas production (Yetilmezsoy and Sakar, 2008). Yetilmezsoy and Sakar, (2008) conducted a study on treatment of PMW with SS value of 5020 mg/L and 1130 mg/L for PMW and treated PMW respectively. Anaerobic digester must be operated in suitable range (>750mg/L) of SS to ensure stabilization in the process and increase of biogas production (Chamy and Ramos, 2011).

The ammoniacal nitrogen (AN) concentration of PMW reduced after treatment process from 1490 mg/L to 440 mg/L. The AN content reduced after the PMW treatment using soil water. The treatment using soil water was proven were able to decrease the AN content and avoiding AN inhibition. This may due to some reaction between the soil water and PMW because soil can reduce ultimate sludge quantity, destroys most of pathogens present in the sludge, and eliminates unpleasant smell problems (Zabranka et al., 2000). More, it was estimated that microorganisms with more than 100 millions in population and several thousands of species live in 1 g of soil (Trosvik et al., 2007). For more understanding regarding to this matter, further mechanism study required. In this research, the focus was on biogas production while treatment was study to help improving biogas production only. If AN inhibition occurs, Bujoczek et al., (2000) reported that nearly no biogas production, even after 120 days of reaction time. Based on Liu and Sung (2002), AN concentration below 200 mg/L are beneficial to anaerobic process. However, AN inhibition can start at AN content up to 1000 mg/L (Liu et al., 2012). A few previous studies dealt with higher initial AN concentration compared to this study, such as at 1500 mg/L (Lei et al., 2007) and also 2250-3000 mg/L (Rao et al., 2008). A few more studies, have demonstrated that acclimatization at high AN concentration was effective to raise AN tolerance for biogas production (Abouelenien et al., 2010; Demirci and Demirer, 2004).

The two compounds of concern in poultry manure wastewater are nitrogen and phosphorus. Inappropriate application of these compounds on ground can lead to eutrophication of surface water resources and pollution of soil and ground water (Demirci and Demirer, 2004). Nitrogen starts as ammonia. In an aerobic environment it is transformed to nitrate. Nitrate and nitrite content may occur through several key mechanisms such as nitrogen fixation, ammonification, synthesis, nitrification and denitrification (Gustafson, 1987). In this research, the concentration of nitrate, nitrite and phosphorus for PMW was noticeably higher than in treated PMW. Thus PMW have more potential to be degraded and produce more biogas than treated PMW.

According to the characteristics of both treated PMW and PMW, it showed that more potential in biogas production using treated PMW compared to untreated PMW. Further result in treatment, factorial analysis and optimization needed to validate the biogas production potential based on the characteristics. However, either way, conversion of PMW or treated PMW through anaerobic digestion will produce biogas in return while reducing the adverse impact on environment.

Table 4.3: Characteristics of PMW and treated PMW

No	Parameter	Unit	PMW	Treated PMW
1	pH	-	8.1	7.5
2	BOD	mg/L	18300	2300
3	COD	mg/L	35600	4985
4	Suspended solids	mg/L	More than 750	More than 750
5	Ammoniacal nitrogen	mg/L	1490	440
6	Nitrate	mg/L	2270	1210
7	Nitrite	mg/L	58	20
8	Phosphorus	mg/L	710	140

*PMW = poultry wastewater,manure; BOD = biological oxygen demand; COD = chemical oxygen demand

4.2 Treatment of PMW

4.2.1 Preliminary study

Result of preliminary study was listed in Table 4.4. From the table, treatment of poultry manure using soil water (SW) gave 4% higher of AN removal compared to control. This indicated that treatment by using poultry farm soil water (SSW) was much preferable compared to using distilled (DI) water. According to (Miles, 2008), using soil can destroy pathogen because wastewater bacteria do not survive long in aerobic conditions. Viruses will be attached to clay and eventually deactivated. It was estimated that microorganisms with more than 100 millions in population and several thousands of species live in 1 g of soil (Trosvik et al., 2007). For this reason, the treatment was proven better by using soil water compared to by using distilled water.

Table 4.4: Result of preliminary study

Run	Treatment	Initial AN (mg/L)	Final AN (mg/L)	% AN removal
1	Poultry manure + DI water (control)	1250	300	76
2	Poultry manure + soil water (SSW)	1750	350	80

*AN = ammonical nitrogen; DI= distilled water; SSW= sandy soil water

4.2.2 Statistical analysis for treatment

Five selected parameters were agitation, reaction time, type of soil water, soil to water ratio (soil: water) and PMW to soil water ratio (PMW: SW). A total of 32 runs were made with five factors at two-level factorial design (TFD). The response was percentage of AN removal in term of Response 1 (R1). The ranges of factors and results were listed in Table 4.5 and Table 4.8, respectively. The percentage of AN removal for this treatment study varied between 31.03% to 81.90%.

Responses were analyzed using analysis of variance (ANOVA) combined with the F-test to evaluate significant factors at the level of 5% ($p<0.05$). The ANOVA of treatment study was listed in Table 4.6. Each factor that having p-value values less 0.05 were considered as significant. From Table 4.6, three factors were significant. The factors were agitation, soil: water and PMW: SW, with all p-value less than 0.05. Reaction time was slightly significant with p-value 0.0547. Type of soil was not a significant factor.

From Table 4.6, the sum of squares for the model F-value was 5061.54 which was the summation of regression sum of squares for the quadratic regression model. Each regression source has a corresponding degrees of freedom (DF) of one and hence contribute a total DF of 14 for the model source. The mean squares of the model was 5061.54, which was the division of sum of squares by the corresponding DF. The model F-value of 24.64 in F-test implies the significant of the model. There was only a 0.01 % probability that a model F-value this large could occur due to noise.

The best suggested condition for treatment by Design Expert software was agitation (0 rpm), reaction time (5 hours), type of soil water (SS), soil: water (1:6) and PMW: SW (1:4). The coded mathematical model for two-level TFD (2^5) was in Eq. 4.1. The ANOVA proved the reliability of this model with the coefficient of determination (R^2) value at 0.9132. Table 4.7 provides a scale for strength of correlation for evaluating the correlation coefficient (Zady, 2000). Compared Table 4.7 to the R^2 obtained, showed that the value interpreted in very high correlation. Regression was used for prediction (which does not extrapolate beyond the data used in the analysis) whereas, correlation was used to determine the degree of association (Asuero et al., 2006).

$$\% AN\ removal\ (R1) = 59.12 - 8.15A - 1.94B - 0.79C + 2.89D - 7.18E - 2.40AB - 0.75AC - 0.62AE + 0.007188BC + 0.29BD + 1.46CD + 1.00CE - 3.45ACE - 2.36BCD \qquad \text{(Eq. 4.1)}$$

Table 4.5: Ranges of factors for treatment

Factor	Symbol	Type	Low coded (-1)	High coded (+1)
Agitation (rpm)	A	Categorical	0	200
Reaction time (hour)	B	Numerical	2	5
Type of soil water	C	Categorical	SSW	PFW
Soil: water	D	Categorical	1:6	1:1
PMW: SW	E	Categorical	1:4	2:3

*SSW = sandy soil water; PFW = poultry farm soil water

Table 4.6: Analysis of variance for treatment of PMW

Source	Sum of squares	DF	Mean squares	F-value	p-value	R^2
Model	5061.54	14	5061.54	12.78	< 0.0001	0.9132
Agitation	2126.34	1	2126.34	75.14	< 0.0001	
Reaction time	120.47	1	120.47	4.26	0.0547	
Type of soil water	19.96	1	19.96	0.71	0.4127	
Soil : water	267.90	1	267.90	9.47	0.0068	
PMW : SW	1649.53	1	1649.53	58.29	< 0.0001	

*PMW = poultry manure wastewater; SW = soil water; DF = degrees of freedom;

* R^2= coefficient of determination

Table 4.7: Strength of correlation

Size of R^2	Interpretation
0.90 to 1.00	Very high correlation
0.70 to 1.89	High correlation
0.50 to 0.69	Moderate correlation
0.30 to 0.49	Low correlation
0.00 to 0.29	Little if any correlation

*R^2= coefficient of determination

Table 4.8: Experimental result for treatment of PMW (coded value)

Run	Agitation (rpm)	Reaction time (h)	Type of soil water	Soil: water	PMW: SW	% AN removal
1	+1	-1	+1	+1	-1	55.00
2	+1	-1	+1	+1	+1	41.05
3	-1	+1	+1	+1	-1	61.11
4	+1	+1	+1	-1	-1	54.78
5	-1	-1	-1	-1	-1	76.00
6	-1	-1	-1	+1	-1	78.18
7	+1	-1	-1	+1	-1	61.33
8	+1	+1	-1	-1	+1	47.10
9	+1	-1	+1	-1	+1	49.47
10	+1	+1	-1	+1	+1	44.00
11	-1	+1	+1	-1	+1	64.76
12	+1	-1	+1	-1	-1	70.59
13	+1	+1	+1	-1	-1	58.33
14	-1	+1	+1	+1	+1	66.00
15	+1	-1	-1	+1	+1	50.48
16	-1	-1	+1	+1	-1	66.25
17	+1	-1	-1	-1	+1	47.06
18	+1	-1	-1	-1	-1	67.50
19	-1	-1	+1	-1	-1	70.00
20	-1	+1	-1	+1	-1	77.22

21	-1	-1	-1	+1	+1	62.31
22	-1	-1	-1	-1	+1	52.00
23	+1	+1	+1	+1	+1	31.03
24	-1	+1	-1	-1	+1	64.44
25	+1	+1	-1	-1	-1	54.78
26	-1	+1	-1	+1	+1	46.43
27	+1	+1	+1	-1	+1	35.17
28	-1	-1	+1	+1	+1	53.08
29	-1	+1	-1	-1	-1	81.90
30	+1	+1	-1	+1	-1	47.83
31	-1	-1	+1	-1	+1	76.67
32	-1	+1	+1	-1	-1	80.00
	-1= 0 +1= 200	-1= 2 +1= 5	-1= SSW +1= PFW	-1= 1:6 +1= 1:1	-1= 1:4 +1= 2:3	

*PMW = poultry manure wastewater, *SW = soil water, *AN = ammoniacal nitrogen, *SSW= sandy soil water *PFW= poultry farm soil water

4.2.3 Main effect analysis

Effects list (Table 4.9) showed the percentage contribution of each main factor and their interaction. Percentage contribution indicates the percentage of effect of each factor or interaction between factors to treatment of PMW. Based on Table 4.9, agitation gave the highest contribution at 38.36%. In this study, the AN removal runs at aerobic conditions and according to Yetilmezsoy and Sapci-Zengin (2009), air flows play an important role in AN volatization. As air introduced, it begins to agitate the solution, creating a removal pathway for dissolved free ammonia to volatize and leave the solution. Based on Hygnstrom et al., (2011), agitation is the ability of the soil to treat wastes as in the amount of accessible soil particle surface area.

The second highest contribution factor was PMW: SW at 29.76% (Table 4.9). The PMW: SW was considered as food-to-microorganism (F/M). In this case, PMW were considered the food while SW was the microorganism. F/M refers to the balance between the food supply and the mass of microorganism in the system (Liu et al., 2012).

It is important operational factor affecting removal efficiency (Li et al., 2011 and Tay and Yan, 1996). High F/M ratio provides a high driving force for metabolic activity, microbial growth and high overall conversion rates of wastewater to biogas (Lobos et al., 2008). However, too high or too low F/M ratio may disturb the balance in the treatment process (Ghangrekar et al., 2005).

The contribution of factors soil: water was 4.83% (Table 4.9). Significant different in the percentage contribution compared to other main factors can be credited to the difference in environmental conditions and also reaction time (Chen et al., 2008). For factor soil: water, the amount of water content in soil water was crucial because microorganisms involved in the treatment process would require water for their metabolic activities. It helps in biological activities of soil in treatment study (Forster-Carneiro, 2008). So, higher water content in soil: water, would resulted in higher AN removal.

From Table 4.9, factor reaction time was worth to discuss because only contributed by 2.17%. Based on Dumas et al., (2010), one day of reaction time were taken for good treatment process and ensured high biogas yield in return. In a treatment study conducted by Abouelenien et al., (2010), different reaction time was implemented, ranges 4-10 days. They were able to improved anaerobic digestion by AN removal. Factor reaction time plays an important role to other researchers compared to this research. This might be due to the higher effect to AN removal by other main factor of this research.

The factor type of soil water was not significant with only 0.36% contributions (Table 4.9). Type of soil water was not significant might be due the nature of the particular substances in the wastewater (Cogger, 1995). This mean that type of soil should gave effect in the treatment according how the soil water reacts to PMW. Soil water developed from soil that contained higher coarse particle should contribute to higher AN removal (Miles, 2008).

Table 4.9: Percentage contribution of each main factor and interactions

Factor	Percentage contribution (%)
A- Agitation	38.36
B- Reaction time	2.17
C- Type of soil water	0.36
D- Soil: water	4.83
E- PMW: SW	29.76
AB	3.33
CD	1.22

*PMW= poultry manure wastewater; SW= soil water; *AB: agitation and reaction time; CD: Type of soil water and soil: water

4.2.4 Interactions between factors

There were two interactions discovered in this treatment study with high effect among other interactions. The first one with 3.33% contribution was the interaction between factors agitation (A) and reaction time (B). The interaction graph between these two factors was illustrated in Figure 4.1. Based on Figure 4.1, as agitation increased, the percentage AN removal decreased. More, as reaction time increase, the percentage AN removal also decreased. So, lower agitation and reaction time resulted in higher percentage of AN removal. According to Yetilmezsoy and Sapci-Zengin (2009), air flows play an important role in AN volatization. As air introduced, it begins to agitate the solution, creating a removal pathway for dissolved free ammonia to volatize and leave the solution. However, based on Gustafson (1987), when wastewater was put in soil water, biomat forms creating unsaturated flow. This mean more contact with the soil particles and the aerobic organisms present in the soil will treat the wastewater. So, due to the nature of soil water providing natural biological treatment requires lower agitation and reaction time for AN removal in PMW.

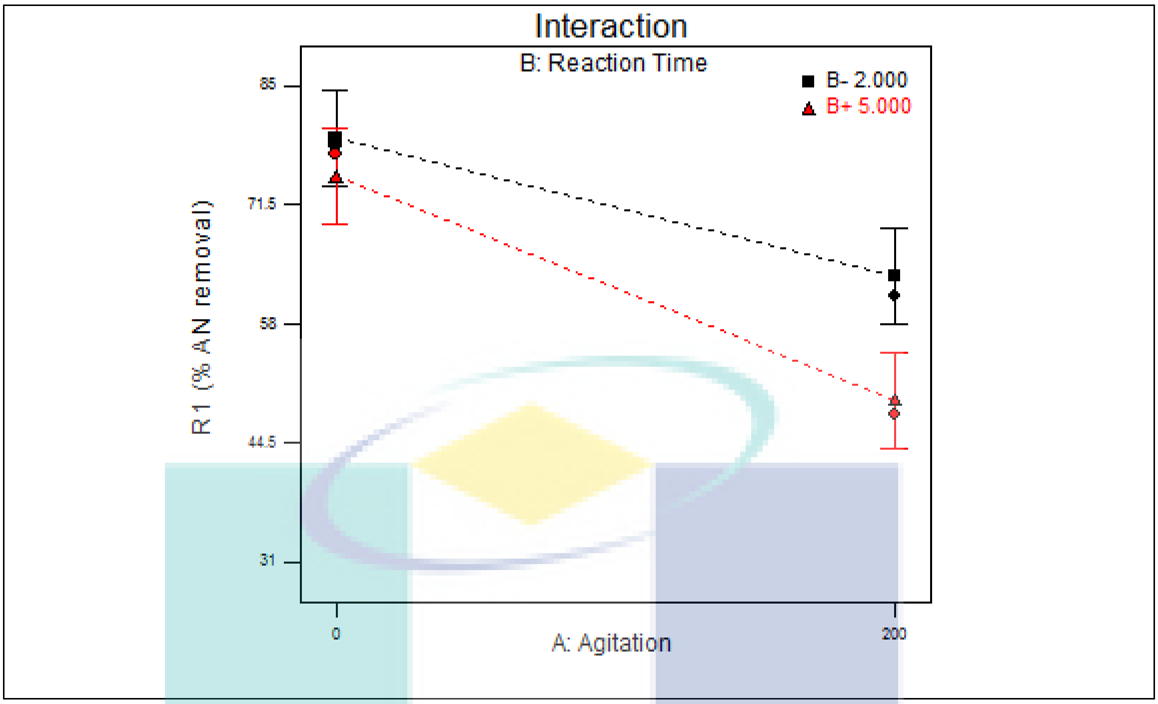


Figure 4.1: The interaction graph between factors agitation (A) and reaction time (B)

The other interaction was in between factor type of soil water (C) and soil: water (D), with 1.22 % contributions. The interaction graph between these two factors was illustrated in Figure 4.2. Based Figure 4.2, using sandy soil water (SSW) as soil water in the treatment, resulted in higher AN removal, compared to poultry farm soil water (SSW). More, 1:6 of soil: water gave higher AN removal compared to 1:1. Water content in soil water was crucial because microorganisms involved in the treatment process require water for their metabolic activities. So, it helps in biological activities of soil (Forster-Carneiro, 2008). Based on Hygnstrom et al., (2011), soil contains complex microscopic organisms and some of these organisms feed on the organic matter in the poultry manure wastewater. The ability of a soil to treat wastes depends of four factors which are the amount of accessible soil particle surface area, the chemical propertied of the surfaces, the soil environment conditions, and the nature of the particular substances in the wastewater (Gustafson, 1987). This mean, type of soil water also greatly affect the wastewater treatment. So, in order for soil water to treat the PMW, the soil: water and type of soil play an important role because they related to each other.

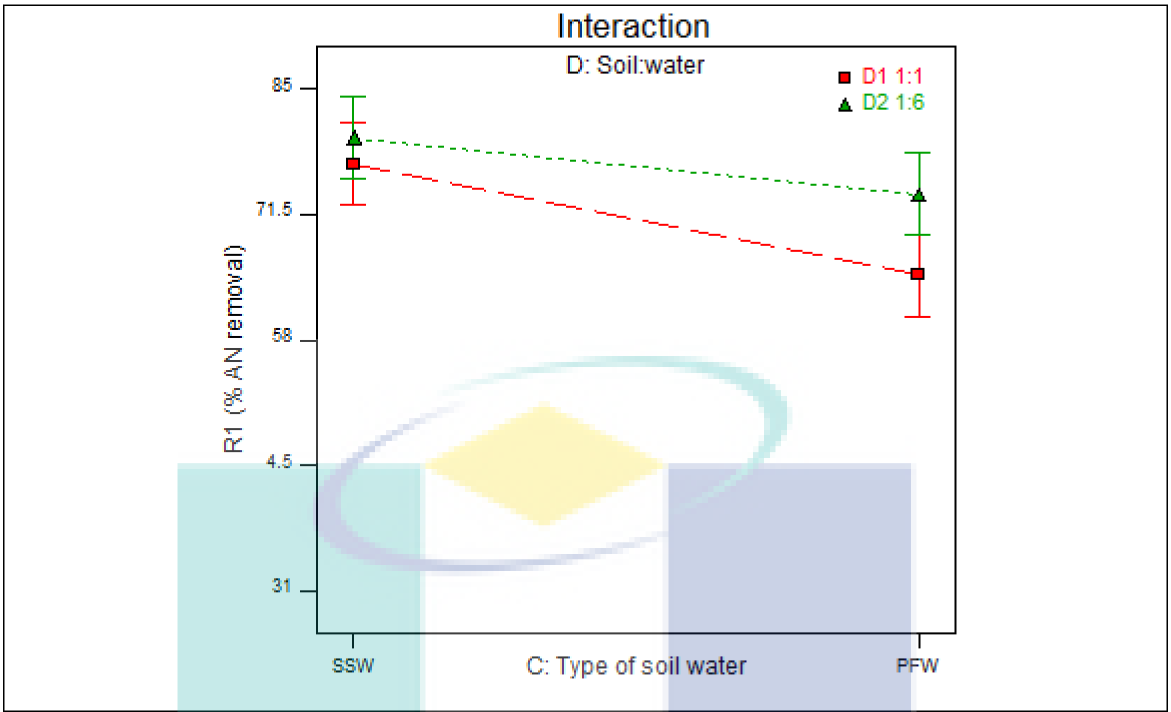


Figure 4.2: The interaction graph between type of soil water (C) and soil: water (D)

4.3 Biogas production from PMW

4.3.1 Statistical analysis for factorial analysis

In this design of experiment, two-level factorial design (TFD) was implemented for factorial analysis. The five factors involved were agitation, reaction time, substrate to inoculum ratio (substrate: inoculum), process system and type of substrate using symbol A, B, C, D and E, respectively. The range of factors was listed in Table 4.10. From TFD, a total of 16 runs were generated with different condition. The results were listed in Table 4.11. The goodness of fit was able to be determined by coefficient of determination, R-squared (R^2) while the statistical significance of the regression model was checked by the Fisher statistical test (F-test) in analysis of variance (ANOVA) (Chauhan et al., 2013). The ANOVA for factorial analysis was listed in Table 4.11. Each factor that having p-value values less 0.05 were considered as significant (Azami et al., 2011). From Table 4.12, four factors were significant. The factors were agitation, substrate: inoculum, process system and type of substrate, with all p-value less than 0.05. Reaction time was slightly significant with p-value 0.1970.

Table 4.10: Ranges of factors for factorial analysis

Factor	Symbol	Type	Low coded(-1)	High coded(+1)
Agitation (rpm)	A	Numerical	0	120
Reaction time (days)	B	Numerical	3	7
Substrate: inoculum	C	Categorical	3:2	4:1
Process system	D	Categorical	Batch	Continuous
Type of substrate	E	Categorical	PMW	Treated PMW

*PMW = Poultry manure wastewater

Table 4.11: Experimental result for factorial analysis (coded value)

Run	Agitation (rpm)	Reaction time (days)	Substrate : inoculum	Process system	Type of substrate	Biogas yield (L/g COD)
1	-1	+1	+1	+1	-1	0.00174
2	+1	+1	+1	-1	-1	0.00342
3	-1	+1	-1	+1	+1	0.00209
4	+1	-1	+1	-1	+1	0.00996
5	-1	-1	+1	-1	-1	0.00000
6	+1	-1	-1	-1	-1	0.00362
7	+1	+1	-1	+1	-1	0.00130
8	-1	+1	+1	-1	+1	0.00053
9	+1	+1	+1	+1	+1	0.00623
10	+1	-1	+1	+1	-1	0.00034
11	-1	-1	+1	+1	+1	0.00210
12	-1	-1	-1	+1	-1	0.00105
13	-1	+1	-1	-1	-1	0.00225
14	+1	-1	-1	+1	+1	0.00073
15	-1	-1	-1	-1	+1	0.00003
16	+1	+1	-1	-1	+1	0.00431
	-1= 0 +1= 120	-1=3 +1= 7	-1= 3:2 +1= 4:1	-1= batch +1=continuous	-1= PMW +1= treated PMW	

*PMW= poultry manure wastewater

Table 4.12: Analysis of variance for factorial analysis

Source	Sum of squares	DF	Mean square	F-value	p-value	R^2
Model	1.007×10^{-4}	11	9.158×10^{-6}	24.64	0.0036	0.9855
A	2.463×10^{-5}	1	2.463×10^{-5}	66.27	0.0012	
B	8.883×10^{-7}	1	8.883×10^{-7}	2.39	0.1970	
C	4.698×10^{-6}	1	4.698×10^{-6}	12.64	0.0237	
D	4.851×10^{-6}	1	4.851×10^{-6}	13.05	0.0225	
E	9.81×10^{-6}	1	9.81×10^{-6}	26.40	0.0068	

*DF = degrees of freedom; R^2= coefficient of determination

From Table 4.12, the sum of squares for the model F-value was 24.64 which was the summation of regression sum of squares for the quadratic regression model. Regression was used for prediction (which does not extrapolate beyond the data used in the analysis). Each regression source has a corresponding degrees of freedom (DF) of one and hence contribute a total DF of 11 for the model source. The mean squares of the model was 9.158×10^{-6}, which was the division of sum of squares by the corresponding DF. The model F-value of 24.64 in F-test implies the significant of the model. There was only a 0.36 % probability that a model F-value this large could occur due to noise.

The best suggested condition by Design Expert software were type of substrate (treated PMW), substrate: inoculum (4:1), agitation (120 rpm), reaction time (3 days) and process system (batch). The coded mathematical model for two-level factorial design was in Eq. 4.2. The ANOVA proved the reliability of this model with the coefficient of determination (R^2) value at 0.9855. Based on Zady (2000), the R^2 obtained interpreted in very high correlation. Correlation was used to determine the degree of association (Asuero et al., 2006).

$$R1 = 0.002498 + 0.001241\,A + 0.0002356\,B + 0.0005419\,C - 0.0005506\,D + 0.0007831\,E + 0.0007069\,AC - 0.001038\,AD + 0.0007856\,AE - 0.0002956\,BC + 0.0006569\,BD + 0.0008819\,CE \qquad \text{(Eq. 4.2)}$$

4.3.2 Main effect analysis for factorial analysis

Effects list (Table 4.13) showed the percentage contribution of each main factor and their interaction. Percentage contribution indicates the percentage of effect of each factor or interaction between factors to biogas production. From Table 4.13 factor agitation gave the highest contribution at 24.09%. In a review by Ward et al., (2008), agitation was applied to basic reactor for anaerobic digestion. This to ensure efficient transfer of organic material for the active microbial biomass, to release gas bubbles trapped in the medium and to prevent sedimentation of denser particulate material. However, excessive mixing can reduce biogas production. It has been shown that low speed mixing conditions allowed a digester to better absorb the disturbance of shock loading than did high speed mixing conditions (Gomez et al., 2006). Reducing the mixing level however, could improve performance and could also stabilize a continuously-mixed unstable digester (Stroot et al., 2001).

The second highest contribution was factor type of substrate at 9.60% contribution (Table 4.13). Overall performance of anaerobic digestion is strongly dependent on the substrates used because different substrates gave different biogas yields (Borjesson and Berglund, 2005). The type of substrate studied in this research was PMW and treated PMW. Treatment was applied to remove AN in PMW because AN was considered as inhibitory substances. Inhibitory substance often found to be the leading cause of anaerobic reactor upset and failure (Demirel and Yenigun, 2002). According to Liu and Sung, 2002, AN concentrations below 200 mg/L are beneficial to anaerobic process. However, AN inhibition can start at concentration up to 1000 mg/L (Liu et al, 2012).

The third highest contribution was contributed by factor process system with 4.75% contribution (Table 4.13). A variety of process systems are being used for biogas production from poultry manure wastewater. Callaghan et al., (2002) was able to established biogas production from cattle slurry, fruit and vegetable wastes and chicken manure by continuous system. They concluded that methane yield was improved from 0.23 to 0.45 L/g VS added. Suitable process system was required in biogas production

(Yadvika et al., 2004). Production of biogas could be in continuous process. However, its use is limited near to the site of the biogas plant (Kapdi et al., 2005).

The next highest contribution was with 4.60% contribution was factor substrate: inoculums (Table 4.13). The substrate: inoculum was crucial for the assessment of anaerobic biodegradability of solid wastes (Lopes et al., 2004). When substrate: inoculum was high, it allowed a higher rate of biogas production than when the ratio was lower (Angelidaki et al., 2006; Fernandez et al., 2001). Based on Forster-Carneiro et al., (2008), the best performance for was the reactor substrate: inoculums of 0.30. In this study, inoculum used was already acclimatized to the substrate, thus it is highly active. The use of highly active anaerobic inoculum will reduce the amount of inoculum required (Obaja et al., 2003). Failure to maintain the balance in substrate: inoculum was the primary cause of digester instability (Demirel and Yenigun, 2002).

The last factor with 0.87% contribution was factor reaction time (Table 4.13). The major drawbacks of anaerobic digestion are its long reaction time and low degradation efficiency for organic matter (Appels et al., 2011). Anaerobic digestion of poultry waste was preferably to operate at shorter reaction time so as to meet the requirement of economics and environmental beneficial extent. This is because under short reaction time, the decomposition of organic matter can be achieve efficiently without accumulation of excessive residual and intermediate products like volatile fatty acids (Ndon and Dague, 1997).

Table 4.13: The percentage contribution of each factor and their interaction

Factor	Contribution (%)
A-Agitation	24.09
B-Reaction time	0.87
C-Substrate: inoculums	4.60
D- Process system	4.75
E- Type of substrate	9.60
AD	16.87
CE	12.17

*AD: Agitation and process system; CE: Substrate: inoculums and type of substrate

4.3.3 Interaction between factors for factorial analysis

In term of interaction, factors agitation (A) and process system (D) gave the highest contribution by 16.87%. The interaction graph was illustrated in Figure 4.3. Based on the figure, when batch process system was applied, biogas yield was higher at 120 rpm. This was contradicted with continuous process system application which gave lower biogas yield at 120 rpm. More, there was interaction between these two factors at agitation of 30 rpm. Based on Hygnstrom et al., (2011), factor agitation affects the ability of the reaction between inoculum and substrates. Since microorganisms in PMW are very sensitive to any sudden change (Yadvika et al., 2004), continuous system was not suitable if agitation being applied. In continuous system, sudden change might interrupt the microorganism performance in biogas production. This might be the reason of such result. Production of biogas could be in continuous process. However, its use is limited near to the site of the biogas plant (Kapdi et al., 2005).

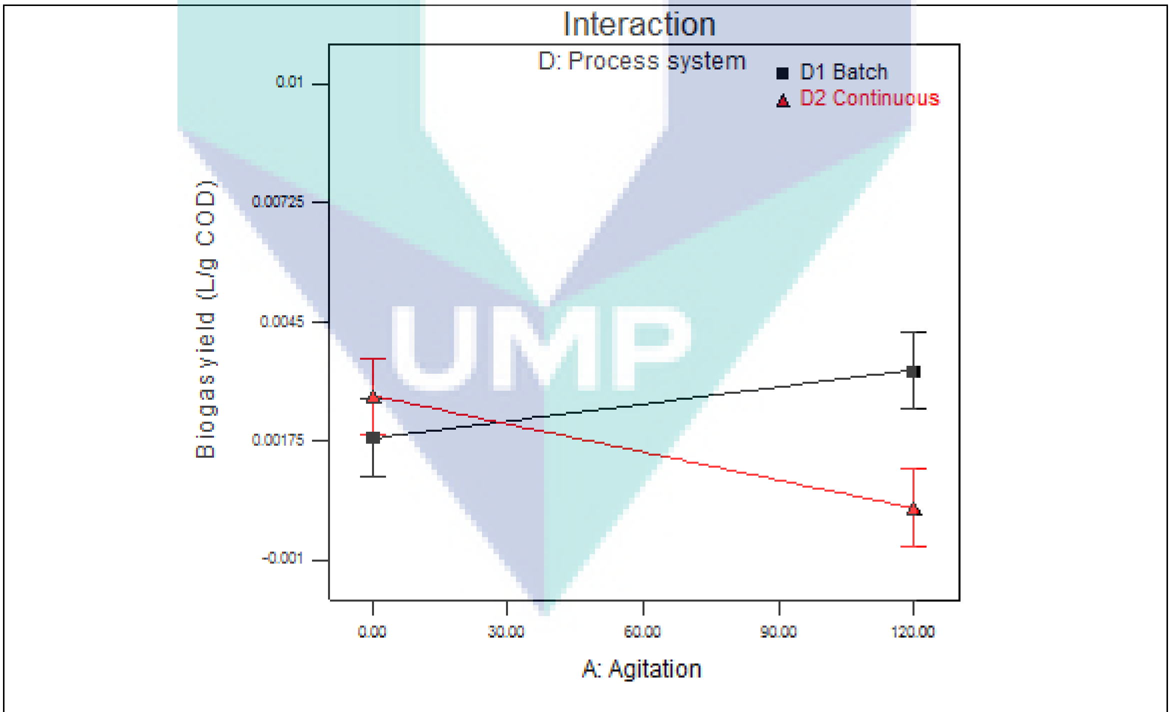


Figure 4.3: The interaction graph between factors agitation (A) and process system (D)

The second highest interaction was between factors substrate: inoculum (C) and type of substrate (E) with 12.17% contribution. The interaction between these two factors must be given attention for their suitability to alter the structure and composition of biogas production process. The interaction was illustrated in Figure 4.4. Based on Figure 4.4, application of treated PMW as substrate compared to PMW at substrate: inoculum of 4:1 resulted higher biogas yield. More, the two factors interacted with each other at substrate: inoculums of 3:2. The substrate: inoculum influence was investigated in the literature for different substrate (Chen and Hashimoto, 1996; Gunaseelan, 1995; Raposo et al., 2009). Many authors showed the importance of the present of inoculum, in order to have a fast start up of the process and a right equilibrium of microorganism population. Charles et al., (2009) reported that anaerobic digestion process did not start in absence of inoculum.

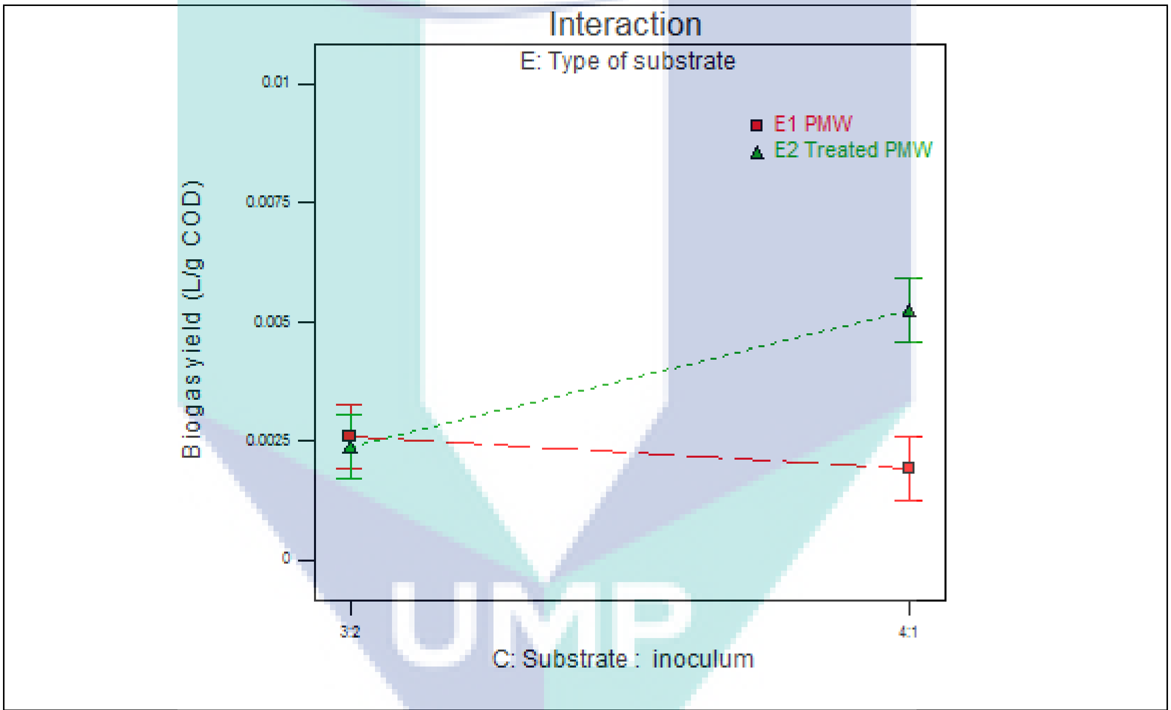


Figure 4.4: Interaction graph between factors substrate: inoculum (C) and type of substrate (E)

4.3.4 Statistical analysis for process optimization

In this design of experiment, central composite design (CCD) was implemented for the optimization of biogas production. Only two factors involved in this study which were agitation and reaction time which represented by symbol A and B, respectively. Ranges of factors used were listed in Table 4.14. By using CCD, a total of 13 runs were generated with different set up condition (Table 4.15). The result for process optimization was listed in Table 4.15. The response was biogas yield (L/g COD) in term of Response 1 (R1). The goodness of fit was able to be determined by coefficient of determination, R-squared (R^2) while the statistical significance of the regression model was checked by the Fisher statistical test (F-test) in analysis of variance (ANOVA) (Chauhan et al., 2013). The ANOVA for factorial analysis was listed in Table 4.15. Each factor that having p-value values less 0.05 were considered as significant (Azami et al., 2011).

From Table 4.16, the sum of squares for the Model source was 2.125×10^{-5} which was the summation of regression sum of squares for the quadratic regression model. Regression was used for prediction (which does not extrapolate beyond the data used in the analysis). Each regression source has a corresponding degrees of freedom (DF) of one and hence contribute a total DF of 5 for the model source. The mean squares of the model was 4.259×10^{-6}, which was the division of sum of squares by the corresponding DF. The model F-value of 7.86 in F-test implies the significant of the model. There was only a 0.86 % probability that a model F-value this large could occur due to noise. The lack of fit F-value of 14.39 indicates the significant relative to the pure error. There was only a 1.31 % chance that it could occur due to noise.

The ANOVA proved the reliability of this model with the coefficient of determination (R^2) value at 0.8489. Based on Zady (2000), the R^2 obtained interpreted in high correlation. Correlation was used to determine the degree of association (Asuero et al., 2006). The final equation was as in Eq. 4.3.

$$Biogas\ yield = (3.701 \times 10^{-3}) + (6.917 \times 10^{-5} \times A) + (3.3308 \times 10^{-4} \times B) - (1.250 \times 10^{-5} \times AB) - (8.799 \times 10^{-4} \times A^2) - (5.424 \times 10^{-4} \times B^2) \quad \text{(Eq. 4.3)}$$

Table 4.14: Ranges of factors for process optimization

Factor	Symbols	Units	Coded values				
			-2	-1	0	+1	+2
Agitation	A	rpm	100	110	120	130	140
Reaction time	B	days	1	2	3	4	5

Table 4.15: Experimental result for process optimization (coded value)

Run	Agitation (rpm)	Reaction time(days)	Biogas yield (L/g COD)
1	-2	0	0.00361
2	-2	-2	0.00204
3	-2	-1	0.00125
4	0	0	0.00250
5	0	+2	0.00133
6	0	+1	0.00096
7	0	0	0.00064
8	0	0	0.00437
9	0	0	0.00395
10	0	0	0.00180
11	-1	-1	0.00395
12	-1	-2	0.00416
13	+2	0	0.00049
	-2=100, -1=110,0=120, +1=130, +2=140	-2=1, -1=2, 0=3, +1=4, +2=5	

Table 4.16: Analysis of variance for process optimization

Source	Sum of squares	DF	Mean square	F-value	p-value	Lack of Fit	R^2
Model	2.125×10^{-5}	5	4.259×10^{-6}	7.86	0.0086	14.39	0.8489
A	5.741×10^{-8}	1	5.741×10^{-8}	0.11	0.7541		
B	1.313×10^{-6}	1	1.313×10^{-6}	2.43	0.1632		
AB	6.250×10^{-10}	1	6.250×10^{-10}	1.155×10^{-3}	0.9738		
A^2	1.774×10^{-5}	1	1.774×10^{-5}	32.8	0.0007		
B^2	6.741×10^{-6}	1	6.741×10^{-6}	12.46	0.0096		

*A= agitation; B= reaction time; R^2= coefficient of determination

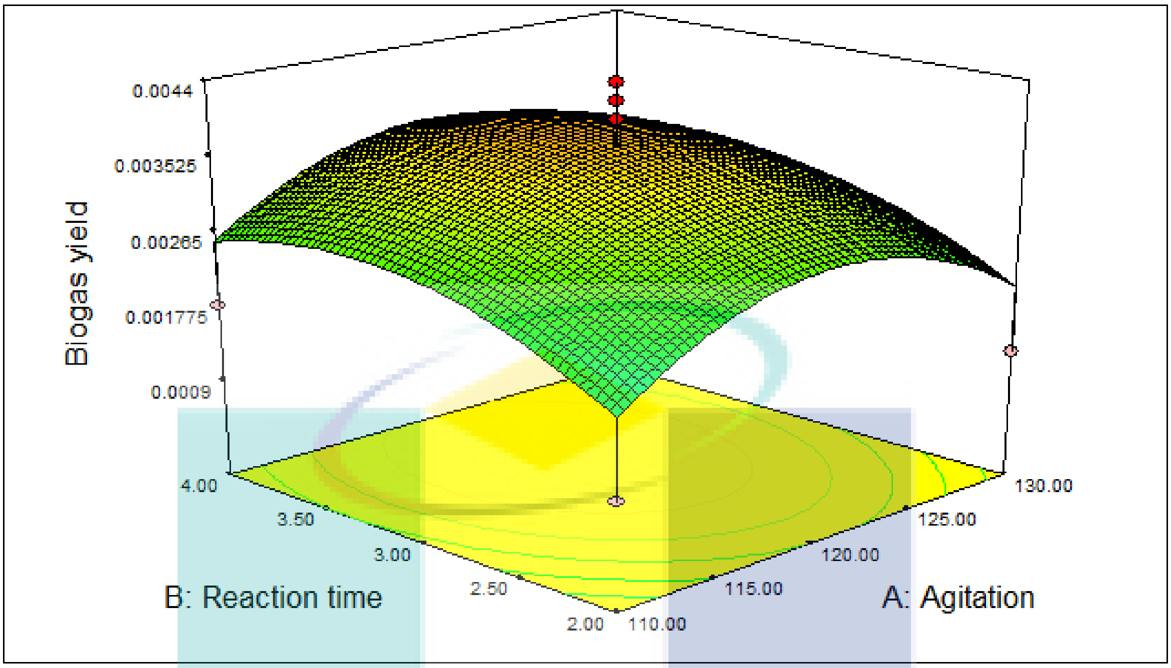


Figure 4.5: Model graph of process optimization

From Figure 4.5, the three-dimensional surface graph generated was in a dome shape. Maximum point yielded 0.00437 L/g COD of biogas (Table 4.14). This result in the best suggested optimum condition at agitation (120 rpm) and reaction time (3 days). However, the suggested optimum conditions need to be validated.

In this study, agitation enhances the efficiency of substrate conversion to biogas by provided contact between poultry manure wastewater and its inoculum (EPA, 1999). Mass and heat transfer can be fostered by agitation which can improve biogas production (Chen and Louge, 2008). Besides, it avoids both the scum layers formation on the surface and the sedimentation of sludge on the bottom of the digester (Veeken et al., 2000). In addition, there will be occurrence of natural mixing in the digester due to gas bubbles rise when the wastewater is added with inoculums. The addition will generate reaction once combined (Appels et al., 2008). Inadequate mixing will results in foam production due to overloaded (WEF, 1995). Nevertheless, the structure of microbial substrate will be disrupted by vigorous continuous mixing (Kaparaju et al., 2008).

Other than agitation, there was factor reaction time. Anaerobic digestion of poultry wastewater was preferably to operate at shorter reaction time so as to meet the requirement of economics and environmental beneficial extent (Ndon and Dague, 1997). This is because under short reaction time, the decomposition of organic matter can be achieve efficiently without accumulation of excessive residual and intermediate products like volatile fatty acids (Ndon and Dague, 1997). Reaction time depends on other factors, such as feed stock and operational temperature (Sakar et al., 2009).

4.3.5 Interaction between factors in process optimization

In this study the interaction of process optimization were between agitation and reaction time (Figure 4.6). From Figure 4.6, at agitation of 120 rpm, biogas yielded highest compared to other agitation speed. Same trend was seen for factor reaction time. Reaction time at 3 days gave highest biogas yield compared to other reaction time.

According to Yetilmezsoy and Sapci-Zengin (2009), agitation plays an important role in anaerobic digestion. Agitation enhances microorganisms and substrate contact and distribution. Reaction time started as soon as inoculum was added to the substrate. Since the inoculum used was already acclimatized with the substrate, lower reaction time was required to obtained high biogas yield. Similar material is often used to acclimatize new digester to reduce the start-up time (Ward et al., 2008). As mixing applied, it begins to agitate the solution, creating a removal pathway for biogas bubbles to leave the solution.

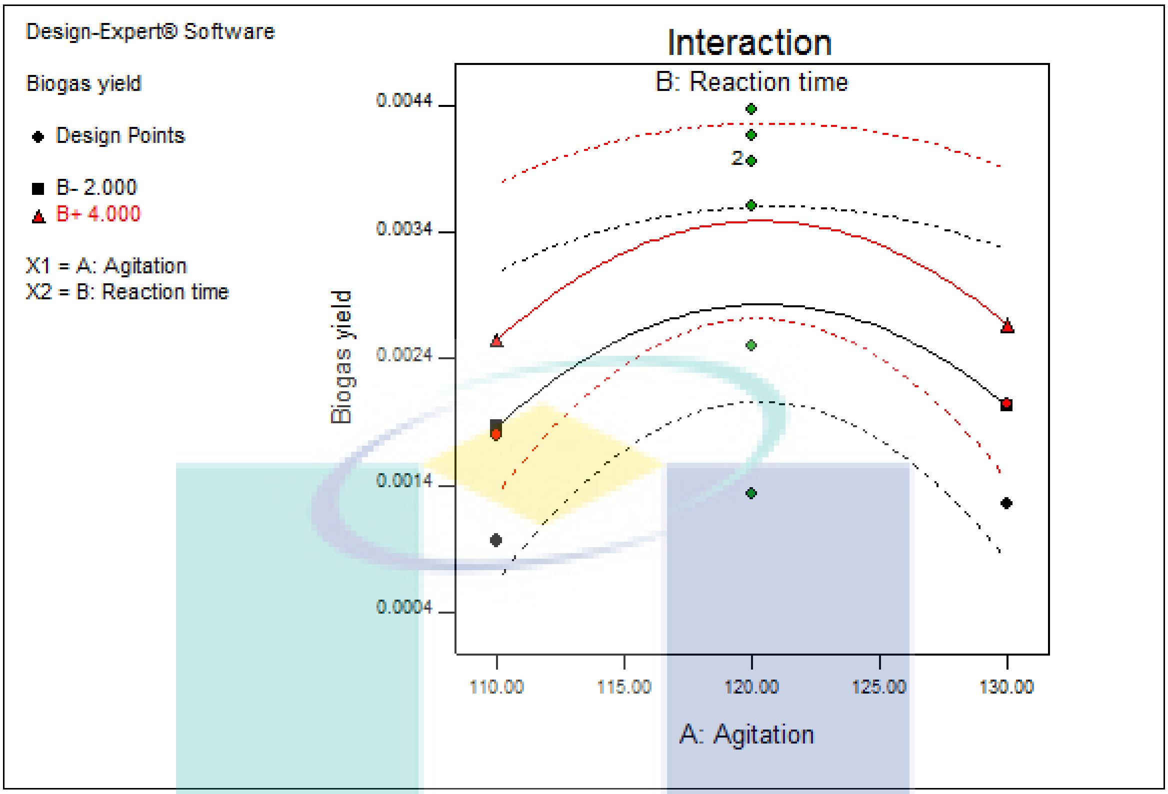


Figure 4.6: Interaction graph of process optimization

4.3.6 Validation of suggested optimum condition

The suitability of the model equation for predicting the optimum response values was validated using the best suggested optimum conditions by CCD. Table 4.17 showed biogas yield according to predicted and actual experimental values.

From the result, the experimental values were closed to the predicted values and it confirmed the validity and adequacy of the predicted models. The average percentage error for experimental values was 5.82% error from predicted value. The validation result confirmed that the model equation was adequate for reflecting the expected optimization.

Conclusively, the suggested optimum conditions by Design Expert software were validated at agitation (120 rpm) and reaction time (3.3 days). Under this condition, 0.00397 L/g COD of biogas yield was obtained. Methane content in the biogas was 30%.

Table 4.17: Suggested optimum conditions for process optimization

Run	Agitation (rpm)	Reaction time (days)	Predicted biogas yield (L/g COD)	Actual biogas yield (L/g COD)	Error (%)
1	120	3	0.00370	0.00339	8.54
2	120	3.30	0.00375	0.00397	5.82

4.4 Application of treatment to biogas production

Two sets of experiments were conducted to evaluate the requirement of PMW treatment using Flask A and Flask B. Flask A was filled with PMW while Flask B with treated PMW. After that, inoculum was added to each flask, and reaction time started. Reading of biogas volume was taken daily until 13 days of reaction time.

Response of this experiment was biogas yield over initial COD concentration. The initial COD concentration for PMW and treated PMW were 180,000 ± 14,200 mg/L and 15,200 ± 3400 mg/L., respectively. The result was in Figure 4.9. The ammoniacal nitrogen (AN) concentration of PMW and treated PMW used in this study was 440 and 1490 mg/L, respectively. The initial AN value was differ with Liu et al., (2012) at range of 400-3000 mg/L. According to Liu and Sung, 2002, AN concentrations below 200 mg/L are beneficial to biogas production process. However, AN inhibition can start at AN content up to 1000 mg/L (Liu et al., 2012).

From the result, the average biogas production was 1.12×10^{-3} L/g.d COD. This was slight lower than the biogas yield according to the suggested optimum condition. The biogas yield according to the suggested optimum condition was 1.20×10^{-3} L/g.d COD. Based on Figure 4.9, the biogas yield for treated PMW was noticeable much higher than PMW. The total biogas yield for PMW and treated PMW was 0.0045 L/g and 0.0248 L/g COD, respectively. PMW gave higher biogas yield after treatment by AN removal. This means that the removal AN was required to obtained higher biogas yield. This was due to the fact that high AN concentration could inhibit anaerobic digestion process (Demirel and Yenigun, 2002. Inhibitory substances are often found to be the leading cause of anaerobic reactor upset and failure since they are present in substantial concentrations in wastewater.

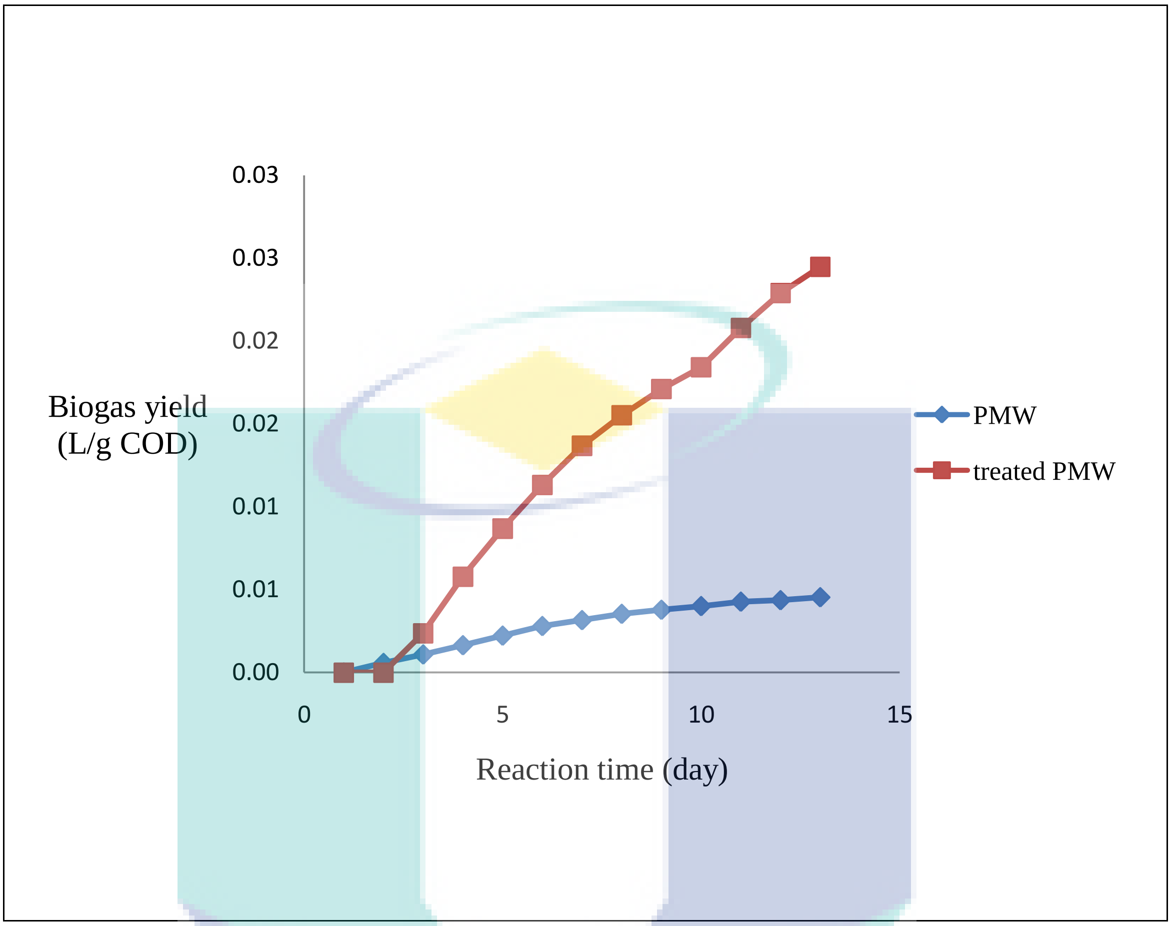


Figure 4.7: Biogas yield graph

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Characterization of soils and poultry manure wastewater (PMW)

The first objective of the study, to characterize the soils and manure wastewater (PMW), was achieved. For the characterization of soils, there were two types of soil characterized; sandy soil (SS) and poultry soil (PSL). After characterization, in term of chemical and physical properties, SS was found much suitable to be used in the treatment of PMW due to it high conductivity and lower cations exchange capacity (CEC) compared to PSL. On the other hand, PSL was found much suitable to be used in biogas production while SS in PMW treatment. PSL was recorded higher in nitrogen, moisture, carbon and available phosphorus content. Conclusively, based on both chemical and physical properties, SS was used in the treatment of PMW while PSL in biogas production. For the characterization of PMW, both PMW and treated PMW were tested for several chemical properties. The pH for PMW and treated PMW was found to be in good range for biogas production process. The nitrate, nitrite and phosphorus for PMW were noticeably higher than that in treated PMW. More, the initial chemical oxygen demand (COD) and biological oxygen demand (BOD) concentration for PMW was higher than treated PMW. This indicate that more amount of organic matter consist in PMW, thus have more potential to be degraded and produce more biogas than treated PMW. However, PMW produced less biogas than treated PMW. This was due to initial ammoniacal nitrogen (AN) concentration of PMW ranged up to 1490 mg/L and more than 1000 mg/L AN will caused AN inhibition to the biogas production process. Conclusively, according to the characteristics of both treated PMW and PMW, it showed that biogas production potential was more by using treated PMW compared to untreated PMW.

5.2 Treatment of poultry manure wastewater

The second objective of the study, to determine the best suggested condition for treatment of PMW, was achieved. Agitation gave highest contribution at 38.36% followed by PMW to soil water ratio (PMW: SW) at 29.76% contribution. In term of interaction, agitation and reaction gave the highest contribution to the treatment process at 3.33% contribution. The ANOVA proved the reliability of this model with the coefficient of determination (R^2) value at 0.9132.

The best suggested condition for treatment by Design Expert software was agitation (0 rpm), reaction time (5 hours), type of soil water (SSW), soil: water (1:6) and PMW: SW (1:4). Utilization of this suggested treatment conditions resulted in 81.90% of AN removal.

5.3 Factorial analysis of poultry manure wastewater

The third objective of the study, to analyze the factors affecting biogas production from PMW, was achieved. From the result, agitation gave the highest contribution at 24.09%, followed by type of substrate with 9.60% contribution. In term of interaction, the agitation and process system gave the highest contribution at 16.87% compared to interaction between substrate to inoculum ratio (substrate: inoculum) and type of substrate with 12.17% contribution. The ANOVA proved the reliability of this model with the coefficient of determination (R^2) value at 0.9855.

The best suggested condition for factorial analysis of PMW by Design Expert software was agitation (120 rpm), reaction time (3 days), substrate: inoculum (4:1), process system (batch), and type of substrate (treated PMW).

5.4 Process optimization of poultry manure wastewater

The fourth objective of the study, to optimize the process of biogas production from PMW was achieved. Center composite Design (CCD) was used to determine the optimum condition for the biogas production from PMW. Quadratic model was used in predicting all the responses. The suggested optimum conditions by Design Expert software were determined at agitation (120 rpm) and reaction time (3.3 days). Under this condition, 0.00397 L/g COD of biogas yield was obtained with 30-40% of methane content. This counts for 5.82% percentage error from predicted models. The ANOVA proved the reliability of this model with the coefficient of determination (R^2) value at 0.8489.

5.5 Recommendations

As this study achieved its objectives, several recommendations were proposed to improve the quality of work and thus yield better results. The recommendations were listed below.

5.5.1 Using bio-reactor with sensor and data logger

The rates of biogas production could be improved by considering the sensitiveness of anaerobic digestion microorganisms for pH and temperature changes. The sensors will detect the value of parameter selected and data logger is able records the changes in the process over time automatically for 24 hours. Any slight changes over time could be well understandable by applying this technology.

5.5.2 The mechanism of biogas production from PMW

In mechanism study, the role of each factor contribution towards biogas production can be better analyzed. Understanding the mechanism of biogas production, would greatly improve the optimization process. The factors that posses higher role in biogas production will be prioritized during anaerobic digestion process.

5.5.3 Kinetic study of biogas production from PMW

Kinetic study is important to clarify the reaction mechanism of a process. The reaction kinetic equation of the biogas production process could be developed in kinetic study. This study includes how different experimental conditions can influence the biogas production. The determination of kinetic parameter would allow biogas production from PMW to another level. This proves that kinetic study is suitable for future work.

REFERENCES

Abbasi, T., Tauseef, S.M., and Abbasi, S.A., 2012. Anaerobic digestion for global warming control and energy generation-An overview. Renew. And Sustain. Energy Reviews 16, pp. 3228-3242.

Abouelenien, F., Fujuwara, W., Namba, Y., Kosseva, M., Nishio, and Nakashimada, Y., 2010. Improved methane fermentation of methane manure via ammonia removal by biogas recycle. Bioresour. Technol. 101, pp. 6368-6373.

Abouelenien, F., Nakashimada, Y., and Nishio, N., 2009. Dry mesophillic fermentation of poultry manure for production of methane by repeated batch culture. J. Biosc. and Bioeng. 3, pp. 293-295.

Adderley, A.E., Smith, I.E., and Probert, S.D., 1976. Anaerobic fermentation of poultry manure. Appl. Energy 2, pp. 163-173.

Adinarayana, K., and Ellaiah, P., 2002. Response surface optimization of the critical medium components for the production of alkaline protease by a newly isolated Bacillus sp. J. Pharm. Sci., 5, 281-287.AgriInfo, 2014. Soil moisture. Resource document: My Agriculture Information Bank. Retrieved May 2014. http://www.agriinfo.in/?page=topic&superid=4&topicid=274

Angelidaki, I., Chen, X.,Cui, J., Kaparaju, P., and Ellegaard, L., 2006. Thermophillic anaerobic digestion of source-sorted organic fraction of house municipal solid waste: start-up procedure for continuous stirred tank reactor. Water Res. 40, pp. 2621-2628.

APEC, 2006. APEC energy demand and supply outlook. http://www.ieej.or.jp/aperc/2006pdf/Outlook2006/Whole_Report.pdf.

Appels, L., Baeyens, J., Degrève, J., and Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science, 34(6), pp. 755-781.

Appels, L., Lauwers, J., Degreve, J., Helsen, L., Lievens, B., Willems, K., Impe, J.V., and Dewil, R., 2011. Anaerobic digestion in global bio-energy production: Potential and research challenges. Renew. Sust. Energy Rev. 15, pp. 4295-4301.

Asuero, A.G., Sayago, A., and Gonzalez, A.G., 2006. The correlation coefficient: An overview. Critical reviews in Analytical Chemistry 36, pp. 41-59.

Atuanya, E.I., and Aigbirior, M., 2002. Mesophillic biomethanation and treatment of poultry wastewater using pilot scale UASB reactor. Environ. Monit. Assess. 77, pp. 139-147.

Azami, M., Bahram M., Nouri S., and Naseri A., 2011. A Central Composite Design for the. Optimization of the Removal of the Azo Dye, Methyl Orange, from Waste Water Using the Fenton Reaction. Journal of Serbian Chemical Society, 77 (2), pp. 235--246.

Barbosa, R,N., and Overstreet, C.,2011. What is soil electrical conductivity?. Resource document: LSU Ag Center. www.LSUAgCenter.com. Retrieved May 2014.

Badariah, A.M., 2010. Renewable energy development in Malaysia. Proceedings of the 34th Meeting of APEC Expert Group on New and Renewable Energy Technologies, April 26-28, 2010, Kuala Lumpur, Malaysia.

Bas,.D and Boyaci, H., 2007. Modeling and optimization I: Usability of response surface methodology. Journal of Food Engineering, 78 (3), pp. 836--845.

Beccari, M., Bonemazzi, F., Majone, M., and Riccardi, C., 1996. Interaction between acidogenesis and methanogenesis in anaerobic treatment of olive-oil mill effluents. Water Res. 30(1), pp. 183-189.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S. and Escaleira, L. A. 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Pure and Applied Analyt. Chem. 76 (5), pp. 965--977.

Borjesson and Berglund, 2005. Environmental system analysis of biogas systems-Part 1: Fuel-cycle emissions. Biomass and Bioenergy 30, pp. 469-485.

Bradley, N., 2007. The Response Surface Methodology. Dissertation, Indiana University South Bend. Retrieved from website: https://www.iusb.edu/math-compsci/_prior-thesis/NBradley_thesis.pdf.

Bujoczek, G., Oleszkiewicz, J.A., Sparling, R.R., and Cenkowski, S., 2000. High solid anaerobic digestion of poultry manure. J. Agric. Eng. Resour. 76, pp. 51-60.

Busman, L., Lamb, J., Randall, G., Rehm, G., and Schmitt, M., 2009. The nature of phosphorus in soils. Resource document: University of Minnesota. http://www.extension.umn.edu/agriculture/nutrient management/phosphorus/the-nature-of-phosphorus/.

Cakir, F. K., and Stenstrom, M. K. 2003. A Dynamic Model for Anaerobic Filter. J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng., 38 ,pp. 2069-2076.

Callaghan, F. J., Wase, D.A.J., Thayanithy, K., and Forster, C.F., 2002. Continuous co-digestion of cattle slurry with fruit and vegetable wastes and chicken manure. Biomass and Bioenergy 27, pp. 71-77.

Chamy, R., and Ramos, C., 2011. Factors in the determination of methanogenic potential of manure. Bioresour. Technol. 102, pp. 7673-7677.

Chen, T.H., and Hashimoto, A.G., 1996. Effect of pH and substrate: inoculums ratio on batch methane fermentation. Bioresour. Technol. 56, pp. 179-186.

Chen, X., and Louge, M., 2008. Heat transfer enhancement in dense suspensions of agitated solids. Part I: theory. International journal of heat and mass transfer, 51(21), pp. 5108-5118.

Chen, Y., Cheng, J.J., and Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99, pp. 4044-4064.

Charles, W., Walker, L., and Ruwisch, R.C., 2009. Effect of pre-aeration and inoculums on the start-up of batch thermophillic anaerobic digestion of municipal solid waste. Bioresour. Technol. 100, pp. 5103-5108.

Chauhan, V., Bhardwaj, N., and Chakrabarti, S., 2013. Application of response surface methodology and central composite design for the optimization of talc filler and retention aid in paper making. Indian Journal of Chemical Technology, 20(2), pp.121-127.

Chynoweth, D.P., Owens, J.M., and Legrand, R., 2001. Renewable methane from anaerobic digestion of biomass. Renew. Energy 22, pp. 1-8.

Cogger, C.G., 1995. Clean Water for Washington. Issued by Washington State Cooperative Extension, and the U.S Department of Agriculture, United State. http://cru.cahe.wsu.edu/CEPublications/eb1475/eb1475.html.retrieved 20/12/2012.

Czitrom, V., 1999. One-factor-at-a-time versus designed experiments. Resource document: http://www.amstat.org/publications/tas/czitrom.pdf. Retrieved May 2014.

Davies, R.M., 2006. Anaerobic Digestion and Biogas. www.face-online.org.uk/resources/factsheets/discovering/pdf. Retrieved 12/7/2011.

Demirci, G.G., and Demirer, G.N., 2004. Effect of initial COD cocncentration, nutrient addition, temperature and microbial acclimation on anaerobic treatability of broiler and cattle manure. Bioresour. Technol. 93, pp. 109-117.

Demirel, B., and Yenigun, O., 2002. Two-phase anaerobic digestion processes: A review. J. Tech. Biotechnol. 77, pp. 743-755.

Demirer, G.N., and Othman, M., 2008 Two-phase thermophillic acidification and mesophillic methanogenesis anaerobic digestion of waste-activated sludge. Environ. Eng. Sci. 25, pp. 1291-1300.

Department of Veterinary Services (DVS), 2014. Malaysia: Output of Livestock Product, 2004-2013. Retrieved from Official Portal of DVS, DVS Resources Statistics, May 2014. http://www.dvs.gov.my/en/.

Deublein and Steinhauser, 2008. Biogas from waste and renewable resources. Wiley-VCH, Weinheim.

Dornburg, V., Faaij, A.P.C., and Meuleman, B., 2006. Optimising waste treatment systems Part A: Methodology and technological data for optimizing energy roduction and economic evaluation. Resour. Conserv. And Recycling. 49, pp. 68-88.

Dumas, C., Perez, S., Paul, E., and Lefebvre, X., 2010. Combined thermophillic aerobic process and conventional anaerobic digestion: Effect on sludge biodegradation and methane production. Bioresour. Technol. 101(8), pp. 2629-2636.

Environmental Protection Agency (EPA), 1999.Wastewater Technology Fact SheetFine Bubble Aeration.US.EPA 832-F-99-065.

Eppinger, T., Wehinger, G., and Kraume, M., 2014. Parameter optimization for the oxadive coupling of methane in a fixed bed reactor by combination of response surface methodology and computational fluid dynamics. Article in press: Chemical engineering research and design.p.11.

Fernandez, B., Porrier, P., and Chamy, C., 2001. Effect of inoculums-substrate ratio on the start-up of solid waste anaerobic digesters.Water Sci. Technol. 44(4), pp. 103-108.

Forster-Carneiro, T., Peres, M., and Romero, L.I., 2008. Influence of total solid and inoculums content on performance of anaerobic reactors treating food waste. Bioresour. Technol. 99, pp. 6994-7002.

Gangagni, R.A., Sasi, K.R., Surya, P.S., Vanajakshi, J., Joseph, J., Jetty, A., Rajashekhara, R.A., and Sarma, P.N., 2008. Biomethanation of poultry litter leachate in UASB reactor coupled with ammonia stripper for enhancement of overall performance. Bioresour. Technol. 99 (18), pp. 8679-8684.

Gershenson, A., and Barsimantov, J., and EcoShift Consulting, LLC., 2009. Accounting for carbon in soils. Resource document: Climate action reserve. http:// www.ecoshift.com. Retrieved May 2014.

Ghangrekar, M.M., Asolekar, S.R., and Joshi, S.R., 2005. Characteristics of sludge developed under different loading conditions during UASB reactor start-up and granulation. Water Res. 39, pp. 1123-1133.

Gomez, X., Cuetos, M.J., Cara, J., Moran, A., and Garcia, A.I., 2006. Anaerobic co-digestion of primary sludge and the fruit and vegetable fraction of the municipal solid wastes – condition for mixing and evaluation of the organic loading rate. Renew. Energy 31, pp. 2017-2024.

Gonzalez-Fernandez, C., Leon-Cofreces, C., and Garcia-Encina, P.A., 2008. Different pretreatments for increasing the anaerobic biodegradability in swine manure. Bioresour Technol. 99, pp. 8710-8714.

Gunaseelan, V.N., 1995. Effect of inoculums/substrate ratio and pretreatments on methane yield from parthenium. Biomass Bioenergy 8, pp. 39-44.

Gunst, R. F., 1996. Response surface methodology: process and product optimization using designed experiments. Statistics, 38 (3), pp. 284-286.

Gustafson, A., 1987. Nitrate leaching from arable land in Sweden under four cropping systems. Swedish Journal of Agricultural research 17; pp. 169-177.

Harikishan, S., and Sung, S., 2003. Cattle waste treatment and Class A biosolid production using temperature-phased anaerobic digester. Adv. Environ. Resour. 7, pp. 701-706.

Hills, D.J., and Roberts, D.W., 1982. Conversion of tomato, peach and honeydew solid waste into methane gas. Transaction of Asae 25, pp. 820-826.

Husted, S., 1994. Seasonal-variation in methane emission from stored slurry and solid manures. Jour. of Envi. Quality 23, pp. 585-592.

Hwang, M.H., Jang, N.J., Hyum, S.H, and Kim, I.S., 2004. Anaerobic bio-hydrogen production from ethanol fermentation: the role of pH. Jour. Biotechnol. 111, pp. 297-309.

Hygnstrom, J.R., Skipton, S.O., and Woldt, W., 2011. Residential onsite wastewater treatment: the role of soil. Resource document, University of Nebraska-Lincoln Extension.http://www.ianrpubs.unl.edu/epublic/pages/publicationD.jsp?publicationId=249. Retrieved May 2014.

Jamaludin, M.F., and Zainol, N., 2011. Isolation and Identification of Acetic Acid Producer from Mixed Culture of Soil and Banana Stem Waste in Anaerobic Condition. International Journal of Chemical and Environmental Engineering.

Joanta, H.G., 1996. Renewable Energy Systems in Southeast Asia. PennWell Publishing Company, Tulsa, Oklahoma, ISBN: 0878144641, Pages: 167.

Kana, E.B.G., Oloke, J.K., Lateef, A., and Adesiyan, M.O., 2012. Modeling and optimization of biogas production on saw dust and other co-substrate using Artificial Neural network and Genetic Algorithm. Renew. Energy 46, pp. 276-281.

Kaparaju, P., Buendia, I., Ellegaard, L. and Angelidaki, I., 2008. Effects of mixing on methane production during thermophilic anaerobic digestion of manure: Lab-scale and pilot-scale studies. Bioresource Technology, 99 (11), pp. 4919-4928.

Kapdi, S. S., Vijay, V. K., Rajesh, S. K., and Prasad, R., 2005. Biogas scrubbing, compression and storage: Perspective and prospectus in Indian context. Renewable Energy, 30(8), pp. 1195-1202.

Kelleher, B.P., Leahy, J.J., Henihan, A.M., O'Dwyer, T.F., Sutton, D., and Leahy, M.J., 2002. Advances in poultry litter disposal technology- a review. Bioresour. Technol. 83, pp. 27-36.

Kleerebezem, R., and Loosdrecht, M.C.M.V., 2007. Mixed culture biotechnology for bioenergy production. Rev. Energy Biotechnol. 18, pp. 207-212.

Knol, W., Vandermost, M.M., and Waart, J.D., 1978. Biogas production by anaerobic digestion of fruit and vegetable waste-preliminary study. Journal of the Science of Food and Agricultrue 29, pp. 822-830.

Lei, X., Sugiura, N., Feng, C., and Maekawa, T., 2007. Pretreatment of anaerobic digestion effluent with ammonia stripping and biogas prorification. J. Hazard. Mater. 145, pp. 391-397.

Li, A.J., Li, X.Y., and Yu, H.Q., 2011. Effect of the food-to-microorganism (F/M) ratio on the formation and size of aerobic sludge granules. Process Biochem. 46, pp. 2269-2276.

Liu, T., and Sung, S., 2002. Ammonia inhibition on thermophilic aceticlastic methanogens. Water Sci. Technol. 45, pp. 113–120.

Liu, Z.G., Zhou, X.F., Zhang, Y.L., and Zhu, H.G., 2012. Enhanced anaerobic treatment of CSTR-digested effluent from poultry manure: the effect of ammonia inhibition. Waste Manage. 32, pp. 137-143.

Lobos, J., Wisniewski, C., Heran, M., and Grasmick, A., 2008. Sequencing versus continuous membrane bioreactors: effect of substrate to biomass ratio (F/M) on process performance. J. Membr. Sci. 317, pp. 71-77.

Lopes, W.S., Leite, V.D., and Prasad, S., 2004. Influence of inoculums on performance of anaerobic reactors for treating municipal solid waste. Bioresour. Technol. 94, pp. 261-266.

Magbanua, B.S., Adams, T.T., and Johnston, P., 2001. Anaerobic co-digestion of hog and poultry waste. Bioresour. Technol. 76, pp. 165-168.

Mahanty, B., Zafar, M., Han, M.J., and Park, H.S., 2014. Optimization of co-digestion of various industrial sludges for biogas production and sludge treatment: Methane production potential experiments and modeling. Waste. Manage. 34, pp. 1018-1024.

Markidis, I., Komilis, D., Tsagas, F., and Petalas, A., 2013. A fractional factorial field experiment to study the decomposition of municipal solid wastes stored in wrapped bales. J. of Envi. Manage. 115, pp. 32-41.

Masunaga, T., Sato, K., and Wakatsuki, T., 2012. Soil's environment purifying function- polluted water treatment by multi soil-layering system. Retrieved May 27, 2014 from wwwwec.web.ntut.edu.tw/ezfiles/31/1031/img/155/169121271.pdf.

Miles, R., 2008. Soils and onsite wastewater treatment for Texas settings. University of Missouri.16th Annual Texas On-Site Wastewater Treatment Research Council, Waco, Texas.

Mohd Salleh, N. H., Mohamed Daud, M. Z., Arbain, D., Ahmad, M. S., and Ku Ismail, K. S. 2011. Optimization of alkaline hydrolysis of paddy straw for ferulic acid extraction.Industrial Crops Production 34, pp. 1635-1640.

Molinuevo-Salces, B., Garcia-Gonzalez, M.C., Gonzalez-Fernandez, C., Cuetos, M.J., Moran, A., and Gomez, X., 2010. Anaerobic co-digestion of livestock wastes with vegetables processing wastes: A statistical analysis. Bioresour. Technol. 101, pp. 9479-9485.

Montgomery, D. C. 2001. Design and Analysis of Experiments, 5th Ed. New York: John Wiley & Sons, Inc.

Mussnug, J.H., Klassen, V., Schluter, A., and Kruse, O., 2010. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. J. Biotechnol. 150, pp. 51-56.

Ndon, U., and Dague, R. 1997. Effects of temperature and hydraulic retention time on anaerobic sequencing batch reactor treatment of low-strength wastewater. Water Research, 31(10), pp. 2455-2466.

Obaja, D., Mace, S., Costa, J., Sans, C., and Mata-Alvarez, J., 2003. Nitrification-denitrification and biological phosphorus removal in piggery waster using sequencing batch reactor. Bioresour. Technol. 87, pp. 103-111.

Olayiwola, O.M., Amahia, G.N. and Adewara, A.A., 2011 Application of Response Surface Methodology for Capturing Optimum Response in a Longitudinal Survey, International Journal of Applied Science and Technology, 1(5). pp. 121-124.

Othman, M.Y.H., Yatim, B., and Salleh, M.M., 1996. Chicken dung biogas power generating system in Malaysia. WREC, pp. 930-933.

Peavy, H.S., Donald, R.R., and George, T., 1985. Environmental Engineering-Engineered Systems For Wastewater Treatment And Disposal. MC.Graw-Hill Internatianal Editions. Civil Engineering Series,1985. ISBN 0-07-100231-6.

Poh, P.E., and Chong, M.F., 2009. Development of Anaerobic Digestion Methods For Palm Oil Mill Effluent (POME) Treatment. Bioresource Technology 100, pp. 1-9.

Prajapati, S.K., Kaushik, P., Malik, A., and Vijay, V.K., 2013. Phycoremediation and biogas potential of native alga isolates from soil and wastewater. Bioresour. Technol. 135, pp. 232-238.

Prasertsan, S., and Sajjakulnukit, B., 2006. Biomass and biogas energy in Thailand: Potential, opportunity and barriers. Renew. Energy 31, pp. 599-610.

Rao, A.G., Reddy, T.S.K., Prakash, S.S., Vanajakshi, J., Joseph, J., Jetty, A., Reddy, A.R., and Sarma, P.N., 2008. Biomethanation of poultry litter lechate in UASB reactor coupled with ammonia stripper for enhancement of overall performance. Bioresour. Technol. 99, pp. 8679-8684.

Raposo, F., Borja, R., Martin, M.A., Martin, A., de la Rubia, M.A., and Rincon, B., 2009. Influence of inoculums-substrate ratio on the anaerobic digestion of sunflower oil cake in batch mode: Process stability and kinetic evaluation. Chem. Eng. J. 149, pp. 70-77.

Riano, B., Molinuevo, B., and Garcia-Gonzalez, M.C., 2011. Potential for methane production from anaerobic co-digestion of swine manure with winery wastewater. Bioresour. Technol. 102, pp. 4131-4136.

Santos, L.O., Alegre, R. M., Garcia-Diego, C., and Cuellar, J., 2010. Effects of magnetic fields on biomass and glutathione production by the yeast *Saccharomyces cerevisiae*. Process Biochem. 45, pp. 1362-1367.

Sakar, S., Yetilmezsoy, K. and Kocak, E., 2009. Anaerobic digestion technology in poultry and livestock waste treatment-a literature review. Waste Management & Research, 27 (1), pp. 3-18.

Salminen, E., and Rintala, J., 2002. Anaerobic digestion of organic solid poultry slaughterhouse waste-a review. Bioresour. Technol. 83, pp. 13-26.

Sawyer, J., 2008. Soil Fertility: Current topic: Importance of nitrogen in soils. Resource document: Agronomy Extension Soil Fertility Web Site. http://www.agronext.iastate.edu/soilfertility/.

Shariffadeen, M.A., 1980. Workshop II: Short-term Development of Appropriate Solar, Biomass and Biogas Application for Malaysia, Proc Tenaga ’80. The Malaysia Nationa Committee of The World Energy Conference, pp. 137-138.

Shiota, N., Akashi, A., and Hasegawa, S., 2002. A strategy in wastewater treatment process for significant reduction of excess sludge production. Water Sci. Technol. 45(12), pp. 127-134.

Singh, J., and Gu, S., 2010. Commercialization potential of microalgae for biofuels production. Renew. Sustain. Energy. Rev. 14, pp. 2596-2610.

Soil Science Society of America, 2008. What is soil?.Retrieved from Soil Science Glossary, May 2014. https://www.soils.org/publications/soils-glossary/.

Stroot, P.G., McMahon, K.D., Mackie, R.I., and Raskin, L., 2001. Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions- digester performance. Water Res. 35, pp. 1804-1816.

Sung, S., and Liu, T., 2003. Ammonia inhibition on thermophillic anaerobic digestion. Chemosphere 53, pp. 43-52.

Taleghani, G., and Kia, A.S., 2005. Technical-economical analysis of the Saveh biogas power plant. Renew. Energy. 30, pp. 441-446.

Tay, J.H., and Yan, Y.G., 1996. Influence of substrate concentration on microbial selection and granulation during start-up of upflow anaerobic sludge blanket reactors, Water Environ. Res. 68, pp. 1140–1150.

Themelis, N.J., and Verma, S., 2004. The better opinion, anaerobic digestion of organic wastes in MSW. Waste Management World. www.foe.co.uk. Retrieved 12/7/2011.

Topper, K.F., T.A. Tindall and D.W. James. 1993. Field Crops. In Utah Fertilizer Guide, D.W. James and K.F. Topper. Extension Circular No. 431. Utah State University Cooperative Extension Service. Available online at ext.usu.edu.

Trosvik, P., Skanseng, B., Jakobsen, K.S., Stensen, N.C., Naes, T., and Rudi, K., 2007. Multivariate analysis of compex DNA sequence electropherograms for high-throughput quantitative analysis of mixed microbial populations. Applied and Envi. Microb. 73(15), pp. 4975-4983.

Turovskiy, I.S., and Mathai, P.K., 2006. Wastewater sludge processing. Publisher: Wiley. ISBN: 0471791601, 9780471791607.

Veeken, A.H.M., Hamelers, B.V.M., 2000. Effect of substrate-seed mixing and leachate recirculation on solid state digestion of biowaste. Water Sci. Technol. 41, pp. 255-262.

Vindis, P., Mursec, B., Janzekovic, M., Cus, F., 2009. The impact of mesophillic and thermophillic anaerobic digestion on biogas production. J. Achiev. Mater. Manuf. Eng. 25, pp. 192-198.

Walid, A.L., Khaled, M., Ehab, R., and El-Helow, 2007. Citric acid production by a novel *Aspergillus niger* isolate: II. Optimization of process parameters through statistical experimental designs. Bioresour. Technol. 98, pp. 3470-3477.

Ward, A.J., Hobbs, P.J., Holliman, P.J., and Jones, D.L., 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresour. Technol. 99, pp. 7928-7940.

Water Environment Federation (WEF), 1995.Wastewater Residuals Stabilization, MOP No.FD-9. Alexandria, VA: Water Environment Federation.

Wikandari, R., Gudipudi, S., Pandiyan, I., Millati, R., and Taherzadeh, M.J., 2013. Inhibitory effects of fruit flavors on methane production during anaerobic digestion. Bioresour. Technol. 145, pp. 188-192.

Yadvika, Santosh, Sreekrishnan, T.R., Kohli, S., and Rana, V., 2004. Review paper: Enhancement of biogas production from solid substrates using different techniques-a review. Bioresour. Technol. 95, pp. 1-10.

Yetilmezsoy, K., and Sakar, S., 2008. Development of empirical models for performance evaluation of UASB reactors treating poultry manure wastewater under different operational conditions. J. Hazard. Mater. 153, pp. 532-543

Yetilmezsoy, K., and Sapci-Zengin, Z., 2009. Recovery of ammonium nitrogen from the effluent of UASB treating poultry manure wastewater by MAP precipitation as a slow release fertilizer. J. Hazard. Mater. 166, pp. 260-269.

.

Yusoff, S., 2006. Renewable energy from palm oil-innovation on effective utilization of waste. J. Cleaner Prod., 14, pp. 87-93.

Zabranska, J., Dohanyos, M., Jenicek, P., and Kutil, J., 2000. Thermophilic process and enhancement of excess activated sludge degradability- two ways of intensification sludge treatment in Prague central wastewater treatment plant. Water Science and Technology 41, pp. 265-272.

Zady, M.F., 2000. Correlation and simple least squares regression. Available online, October 2000, http://www.westgard.com/lesson44.htm.

Zamalloa, C., Boon, N., and Verstraete, W., 2012. Anaerobic digestibiity of *Scenedesmus obliquus* and *Phaeodactylum tricornutum* under mesophilic and thermophiic conditions. Appl. Energy 92, pp.733-738.

Zinatizadeh, A.A.L., Mohamed, A.R., Mashitah, M.D., Abdullah, A.Z., and Isa, M.H., 2007. Optimization of pre-treated palm oil mill effluent digestion in an up-flow anaerobic sludge fixed film bioreactor: A comparative study. Biochem. Eng. Jour. 35, pp. 226-237.

Zinatizadeh, A.A.L., Pirsaheb, M., Bonakdari, H., and Younesi, H., 2010. Response surface analysis of effects of hydraulic retention time and influent feed concentration on performance of an UASFF bioreactor. Waste Manage. 30, pp. 1798-1807.

APPENDICES

Appendix A Supporting details

A1 Details for Figure 1.1 and Figure 1.2

A1: Malaysia output of poultry products. 2004-2013 (DVS, 2014)

Commodity	Region	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Poultry Meat (‘000 M.Tan)	P.Malaysia	839.73	891.01	907.72	998.17	1058.97	1096.25	1180.29	1214.37	1237.05	1273.30
	Sabah	44.47	44.28	73.96	45.42	45.92	45.65	50.53	53.38	61.85	60.55
	Sarawak	43.29	44.77	53.72	56.41	57.68	60.10	64.78	66.72	75.60	81.89
Total		927.49	980.05	1035.40	1100.00	1162.57	1202.00	1295.60	1334.47	1374.50	1415.74
Poultry Eggs *(‘000 M.Tan)	P.Malaysia	389	383	390	423	449	480	509	538	555	573
	Sabah	28	25	33	25	29	32	34	36	38	39
	Sarawak	37	35	44	44	45	44	47	49	51	52
Total		434	443	465	492	523	556	590	621.48	642.6	664.42

P.Malaysia: Peninsular Malaysia

* Estimated average weight of poultry eggs = 60 gm/egg

Appendix B Experimental procedures and data

B1 Sampling

B1.1: Site A

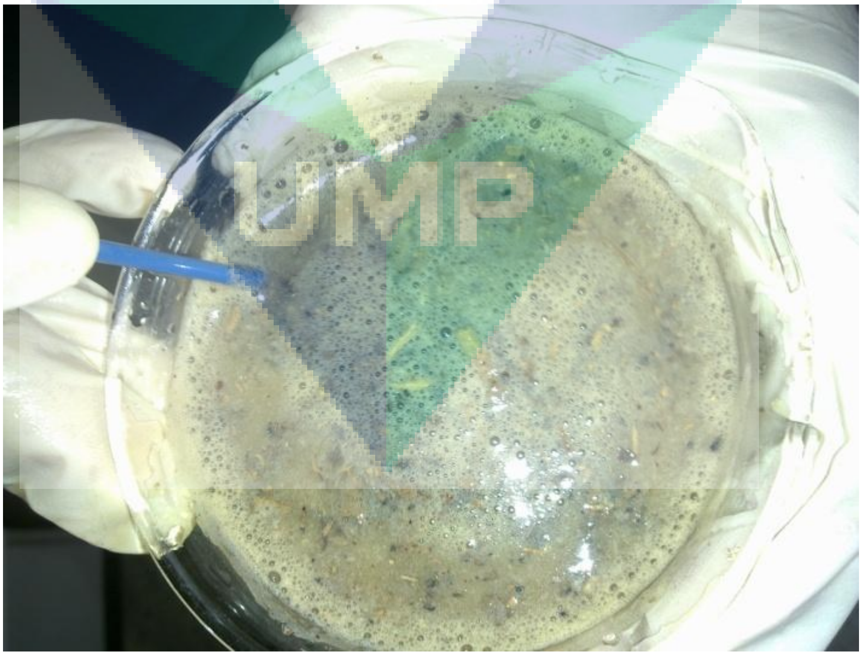
B1.2: Site B

B1.3: Site C

B1.4: Site D

B2 Preparation of poultry manure wastewater and soil water

B2.1: Poultry manure wastewater



B2.2: Soil water

B3 Soil characterization method

B3.1 Determination of total nitrogen by Micro Kjedahl Method

Apparatus:

1. Digester (Kjeldahl digestion tubes in heating block)
2. Steam distillation unit (fitted to accept digestion tubes)
3. Burette 25 ml

Reagents:

1. Sulphuric acid-selenium digestion mixture
2. Hydrogen peroxide 30%
3. Sodium hydroxide solution (NaOH), 38%
4. Mixed indicator solution
5. Baric acid indicator solution, 1%
6. Hydrochloric acid (HCI), 0.010 M standard

Procedure:

Digestion

1. Approximately 5 g of fine particle to pass a 0.25 mm sieve.
2. 1 g of this material was weighed into a digestion tube. In each batch, two blanks and a control sample were included.
3. 2.5 ml digestion mixture was added.
4. 3 aliquots of 1 ml hydrogen peroxide. The next aliquot was added when frothing has subsided. If frothing excessive, cool the tube in water.
5. The tubes was placed on the heater and heated for about 1 hour at moderate temperature (200ºC).
6. The temperature was turned up to approximately 330 ºC and heating was continued until mixture was transparent.
7. The tubes were removed from heater and were allowed to cool and approximately 10 ml of water was added to wash bottle while swirling.

Distillation

1. 20 ml boric acid indicator solution was added to a 250 ml beaker and was placed on stand beneath the condenser tip.
2. 20 ml of sodium hydroxide solution was added to digestion tube and distilled for about 7 minutes.
3. Beaker was removed from distiller, condenser tip was rinsed and distillate was titrated with 0.01 M hydrochloric acid until color changes.

Calculation:

$$\% N = \frac{a-b}{s}\ x\ M\ x\ 1.4\ x\ mcf$$

Where

a = ml HCI required for titration sample

b = ml HCI required for titration blank

s = air-dry sample weight in gram

M = molarity HCI

1.4 = 14 x 10^{-3} x 100% (14 = atomic weight of nitrogen)

mcf = moisture correction factor

B3.2 Moisture content and loss of ignition

Calculation of the results of soil analysis was done of "oven-dry soil". The moisture content of the sample should be determined shortly before soil analysis. The apparatus need were moisture tins or flasks with fitting lid and also drying oven. The procedure:

1. Approximately 5g of fine earth was transferred to a tared moisture tin and weighed (A gram).
2. Dried overnight at 105°C (lid removed).
3. Tin was removed from the oven, and closed with lid and cooled in desiccator and weight (B gram).

4. Moisture content in wt% was obtained by calculation:

$$\text{Moisture content } (\%) = \frac{A - B}{B - tare\ tin} x\ 100$$

B3.3 Determination of carbon (Walkley-Black Method)

Equipment:

1. 500-mL Erlenmeyer flasks.
2. 10-mL pipette.
3. 10-and 20-mL dispensers.
4. 50-mL burette.
5. Analytical balance.
6. Magnetic stirrer.
7. Incandescent lamp.

Reagents:

1. H_3PO_4, 85%.
2. H_2SO_4, concentrated (96%).
3. NaF, solid.
4. Standard 0.167*M* $K_2Cr_2O_7$: Dissolve 49.04 g of dried (105°C) $K_2Cr_2O_7$ in water and diluted to 1 L.
5. 0.5*M* Fe^{2+} solution: Dissolve 196.1 g of $Fe(NH_4)_2(SO_4)\bullet 6H_2O$ in 800 mL of water containing 20 mL of concentrated H_2SO_4 and dilute to 1 L. The Fe^{2+} in this solution oxidizes slowly on exposure to air so it must be standardized against the dichromate daily.
6. Ferroin indicator: Slowly dissolve 3.71 g of o-phenanthroline and 1.74 g of$FeSO_4\bullet 7H_2O$ in 250 mL of water.

Procedure:

1. 0.10 to 2.00 g dried soil (ground to <60 mesh) was weighed and transferred to a 500-mLErlenmeyer flask. The sample contains 10 to 25 mg of organic C (17 to 43 mg organic matter). Two gram of sample was used for light coloured soils and 0.1 g for organic soils.

2. 10 mL of 0.167 M$K_2Cr_2O_7$ was added by means of a pipette.
3. 20 mL of concentrated $H_2SO_4$was added by means of dispenser and was swirled gently to mix. Avoid excessive swirling. This result in organic particles adhering to the sides of the flask out of the solution.
4. The flask was allowed to stand for 30 minutes. The flask was placed on an insulation pad to avoid rapid heat loss.
5. The suspension was diluted with 200 mL of water to provide a clearer suspension for viewing the endpoint.
6. 10 mL of 85% H_3PO_4 and 0.2 g of NaF were added. The $H_3PO_4$and NaF were added to complex Fe^{3+}which interferes with the titration endpoint.
7. 10 drops of ferroin indicator was added. The indicator was added prior to titration to avoid deactivation by adsorption onto clay surfaces.
8. 0.5 M$Fe^{2+}$was titrated to a burgundy endpoint. The colour of the solution at the beginning was yellow-orange to dark green, depending on the amount of unreacted$Cr_2O_7^{2-}$remaining. The colour was shifted to a turbid gray before the endpoint and then changed sharply to a wine red at the endpoint. Magnetic stirrer and incandescent light were used to make the endpoint easier to seen in the turbid system (fluorescent lighting gives a different endpoint color).
9. Reagent blank was done using the above procedure without soil. The blank was used to standardize the Fe^{2+} solution daily.
10. Calculate %C and % organic matter:

a. % Easily Oxidizable Organic C

$$\%C = \frac{(B - S) x\ M\ of\ Fe^{2+} x\ 12\ x\ 100}{g\ of\ soil\ x\ 4000}$$

where:

B = mL of Fe2+ solution used to titrate blank

S = mL of Fe2+ solution used to titrate sample

12/4000 = milliequivalent weight of C in g.

Easily oxidizable organic C was converted to total C by divided with 0.77 (or multiply by 1.30) or other experimentally determined correction factor.

b. % Organic Matter

$$\% \, OM = \frac{\% \, total \, C \, x \, 1.72}{0.58}$$

B3.4 Determination of conductivity in soil

Apparatus:

Conductivity meter with dip cell and pipette cell

10 ml, 50 ml and 100 ml beaker

1 L volumetric flask

Reagent:

Standard potassium chloride solution (0.1M). Standard analytical concentrate ampoule of 0.100 M KCI was diluted according to instruction. 10 ml standard 0.100 M KCI solution was pipette into a 100 ml volumetric flask and make to volume with water. Alternatively, dissolve 0.7456 g of oven-dried (105ºC) in water in a 1 L volumetric flask and make to volume with water.

Procedure:

1. About 30 ml standard 0.01 M KCI solution was added to a 50 ml beaker and the temperature was measured.
2. Pipette cell was rinsed and filled with the standard KCI solution or cell was directly dipped in the solution.
3. Compensation dial was set at measured temperature and reading of the meter was adjusted to 1.412 mS/cm with cell constant-dial (This is the specific conductivity of the standard 0.01 M KCI solution at 25ºC)
4. The temperature of the extract was measured and compensation dial was set at this temperature. The reading automatically corrected to 25ºC.
5. Pipette cell was filled with extract or insert dip cell into extract and conductivity was read.

B3.5 Available phosphorus in soil

Equipment:

1. Soil scoop calibrated to hold 1.5 g of light-colored silt loam soil.
2. Erlenmeyer flasks (50-ml)
3. Pipette banks (3-ml)
4. Time-controlled oscillating shaker set at 160 excursions per minute.
5. Filter paper
6. Funnel tubes (15-ml)
7. Matched colorimetric tubes (10-ml)
8. UV-Vis spectrophotometer

Reagents:

1. Stock P-A solution (1.25 N HCl, 1.5 N NH_4F): 54 ml of 48% HF was added to 700 ml of deionized water. The pH was neutralised to 7.0 with NH_4OH. 108 ml of concentrated HCl (11.6 N) was added and diluted to 1 L.
2. Diluted P-A solution (0.025 N HCl, 0.03 N NH_4F): 20 ml of stock P-A solution was diluted to 1 L with deionized water.
3. P-B solution (0.87 N HCl, 0.38% ammonium molybdate, 0.5%H_3BO_3): 3.8 g of ammonium molybdate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ was dissolved in 300 ml of deionized water at 60°C. The solution then was cooled to room temperature. 5.0 g boric acid, H_3BO_3 was dissolved in 500 ml of deionized water and 75 ml concentrated HCl (11.6 N) was added. The molybdate solution was added and dilute to 1 L with deionized water.
4. P-C powder: 2.5 g 1-amino-2- napthol-4 sulfonic acid fine powder, 5.0 g sodium sulfite (Na_2SO_3), and 146 g of sodium metabisulfite ($Na_2S_2O_5$) were mixed and grinded thoroughly.
5. P-C solution: 8 g of dry P-C powder was dissolved in 50 ml of warm deionized water.

Procedure:

1. 1.5 g scoop of soil was placed into a 50-ml Erlenmeyer flask.
2. 15 ml of P-A solution was added.
3. The suspension on oscillating shaker was shaken for 5 minutes.
4. The sample was filtered through filter paper into a 15-ml funnel tube.
5. 3.0-ml aliquot of filtrate was pipette with constant suction pipette apparatus and transferred to a 10-ml colorimeter tube.
6. 3.0 ml of P-B solution was added with the same pipette apparatus and mixed well.
7. Add 3 drops of P-C solution was added, and mixed immediately.
8. The colour was readable after 15 min with a UV-Vis spectrophotometer.
   Note: UV – Vis spectrophotometer should be set at 645 nm.

B3.6 Determination of Exchange Cations by ICP & CEC by distillation method

Equipment:

1. 250 mL beaker
2. Balance to weigh to the nearest 0.01 g
3. 7.0 cm Buchner funnel
4. Filter paper
5. 250 mL suction flask connected to vacuum pump
6. 250 mL volumetric flasks
7. Balance, stir plate, stir bars and container for reagents
8. Apparatus and instrumentation for NH_4^+ analysis.

Reagents:

1. 1 M NH_4OAc at pH 7: The solution was prepared in fume hood to avoid breathing vapors of ammonia and acetic acid. 580 mL of glacial acetic acid (99.5%) was added to 5 L of water. 680 mL of concentrated ammonium hydroxide (58% NH_4OH) was added. Water was added to yield a volume approximately 1900 mL.the pH was adjusted to 7. The solution was diluted to 10 L.
2. Ethyl alcohol (95%).

3. 1 M KCl: 745 g KCl was dissolved in 8 L of water. The solution was diluted to 10 L.

Procedures:

1. 10 grams of air-dried soil ground was weighed and placed into a 250 ml beaker.
2. 25 mL NH4OAc was added to the soil. The solution was covered and let overnight.
3. A 7 cm Buchner funnel was prepared by fitting it with a filter paper. The filter was wetted with a minimum amount of NH4OAc. The funnel was inserted into a 250 mL suction flask. The vacuum pump was turned on to seat the moistened filter. The soil-NH4OAc mixture was stirred and transferred into the filter.
4. 75 mL NH4OAc was measured for each sample into a plastic squirt bottle with one bottle for each sample. 10 mL of NH4OAc in the bottle was used to transfer all the soil to the Buchner funnel.
5. The soil was covered with filter paper to keep the soil moist between leachings.
6. The soil was leached five to seven times with 10 to 15 increments of NH4OAc.
7. The leachate was transferred into a 250 mL volumetric flask and brings to volume with 1 M NH4OAc.
8. The soil was leached with ethanol to remove excess NH4OAc. The soil was leached with 25 mL portions of ethanol five to six times for a total volume of about 150 mL.
9. The soil was leached with 1 M KCl to remove adsorbed NH_4. The soil was leached with 25 mL portions of 1 M KCl four to five times for a total volume of about 125 mL.
10. The leachate was transferred to a 250 mL volumetric flask and brings to volume using 1 M KCl. The solution for NH_4 concentration was analysed using calorimetry, distillation or ion- selective electrode potentiometry.

Calculations

1. If mg/L of NH_4-N is quantified in the leachate, use the following to calculate CEC.

$$\text{CEC (cmol}_c\text{/kg)} =$$
$$\text{(mg NH}_4\text{-N / L) (0.25 L / 10 g soil) (1 meq NH}_4\text{-N / 14 mg NH}_4\text{-N) x 100}$$

If mg/L of NH_4 is quantified in the leachate use 18 mg NH_4 instead of 14 mg NH_4-N.

B3.7 Determination of soil texture

Coarse sand

1. Soil samples were transfer to a plastic tray for air-drying.
2. Proper labeling was to avoid identification errors during transfer. Large cods were break up to speed up drying.
3. Large plant residues were removed. Avoid placing in direct sunlight. After drying, total weight was weighted.
4. Then, the soils were sieved through a 2 mm sieve. Clods, not passing through the sieve are carefully crushed by pestle and mortar and sieved again. Gravel, rock fragments etc. not passing through the sieve, after removal of any adhering finer particles, was weighed and their content was reported as fraction of the whole.
5. Coarse sand was picked out as quantitatively as possible and the content was determined separately at fraction > 2mm. The fraction < 2mm (oven-dry soil) was homogenized and constituted the sample subjected to the usual laboratory procedures.

Fine sand

1. The soil was passed through a 50 µm sieve which was placed in a funnel positioned above a sedimentation cylinder with a stand and clamp.
2. This was making to 1 L mark with water.

3. The sand fraction in the remaining on the sieve was washed, evaporates on water bath and dried at 105ºC for at least an hour.
4. Sand fraction was weighted.

Silt (fraction<50μm)

1. After adding material <50μm possibly collected during sieving, the sedimentation was closed with a rubber stopper and shake well.
2. The cylinder was placed on the table, stopper was removed and 20 m was immediately pipette from the centre of the cylinder.
3. The aliquot was transferred to a tared moisture tin, evaporated in water bath and dried overnight at 105ºC.
4. The tin was removed from drying oven and closed with lid and cooled in desiccators.
5. Silt fraction was weighted.

Clay (fraction<2μm)

1. A cylinder was placed on a vibration-free table under the pipette assembly.
6. Exactly after 5 and half an hour, 20 ml was pipette and transferred to tared moisture tin, evaporate on water bath and dry overnight at at 105ºC.
7. The tin was removed from drying oven and closed with lid and cooled in desiccators.
8. Silt fraction was weighted.

Calculation

The basis of the calculation was obtained by summation of the individual fractions.

B4 Poultry manure wastewater characterization method

B4.1 pH

pH was measured directly in the undiluted sample using a combination electrode. Before each measurement, the electrode was rinsed and wiped with soft tissue paper.

B4.2 Suspended solid (SS)

Solids suspended in water may consist of inorganic and organic particles or of immiscible liquids. Inorganic solids such as clay, silt, and other soil constituents are common in surface water. Organic material such as plant fibers and biological solids (algal cells, bacteria, etc.) are also common constituents of surface waters.

These materials are often natural contaminants resulting from the erosive action of water flowing over surfaces. Because of the filtering capacity of the soil, suspended material is seldom a constituent of groundwater.

Other suspended material may result from human use of the water. Domestic wastewater usually contains large quantities of suspended solids that are mostly organic in nature. Industrial wastewater may result in a wide variety of suspended impurities of either organic or inorganic nature. Immiscible liquids such as oils and greases are often constituents of wastewater.

Suspended solids, where such material is likely to be organic and/or biological in nature, are an important parameter of wastewater. The suspended solids parameter is used to measure the quality of wastewater influent, to monitor several treatment processes, and to measure the quality of the effluent. Environmental Protection Agency (EPA) has set a maximum suspended solids standard of 30 mg/L for most treated wastewater discharges.

A well-mixed measured sample is filtered through a weighed standard glass-fiber filter and the residue retained on the filter is dried to a constant weight at 103°C to 105°C. The increase in weight of the filter represents the total suspended solids. If the suspended material clogs the filter and prolongs filtration, it may be necessary to increase the diameter of the filter or decrease the sample volume.

Materials and methodology

Glass fiber filter disk, 47 mm @ 70 mm – pre dry in the oven
Measuring cylinder, 100 mL
Pipette, 10 mL
Analytical balance
Oven – preheated to 103°C to 105°C
Desiccator
Buchner flask and funnel
Vacuum pump
Aluminum weighing dishes/Crucible dish

1. The filter disk was dried in the oven at 103°C to 105°C for 1 hour, cooled in a desiccator and weigh.
2. Filtering apparatus was assembled and suction began. The filter was wet with a small volume of distilled water to seat it.
3. 50 mL of water sample (mixed to ensure homogeneity) was pipette onto centre of filter disk in a Buchner flask, using gentle suction (under vacuum).
4. Filter was washed with three successive 10 mL volumes of distilled water, allowing complete drainage between washings, and suction was continued for about 3 min after filtration completed.
5. Filter was carefully removed from filtration apparatus and transfered to aluminum weighing dish/crucible dish as a support.
6. It was dried at least 1 hour at 103°C to 105°C in an oven, cooled in a desiccator to balance temperature, and weigh.
7. the cycle of drying, cooling, desiccating, and weighing was repeated until a constant weight obtained.
8. The Total Suspended Solids (TSS) in the water and wastewater samples was calculated using the following equation:

$$mg\frac{TSS}{L} = \frac{((A-B)x\ 1000)}{Sample\ volume, mL}$$

where;

A = weight of filter + dried residue, mg

B = weight of filter, mg

References

Andrew, D. E., Lenore, S. C., Eugene, W. R. & Arnold, E. G. 2005. Standard Methods For The Examination of Water And Wastewater. 21st Edition. USA.

Howard, S. P., Donald, R. R. & George, T. 1985. Environmental Engineering. McGraw-Hill. Singapore.

Kasmawati, M. & Lee, K. K. 2007. Methods of Analysis for Water and Wastewater. UiTM. Shah Alam.

B4.3 Biological oxygen demand (BOD)

Biochemical oxygen demand (BOD) test measures the ability of naturally occurring microorganisms to digest organic matter, usually in 5 days incubation at 20°C by analyzing the depletion of oxygen. BOD is the most commonly used parameter for determining the oxygen demand on the receiving water of a municipal or industrial discharge. BOD can also be used to evaluate the efficiency of treatment processes, and is an indirect measure of biodegradable organic compounds in water. The BOD test is normally required by a regulatory program. For this experiment, BOD_5 will be examined by dilution method (Standard Method 5210B).

Materials and methodology

Incubation bottles: 300 mL bottles

BOD incubator

Volumetric flask, 1L.

Beaker, 500mL.

Dissolved oxygen meter.

Reagents were prepared in advanced but discard if there is any sign of precipitation or biological growth in the stock bottles. Use reagents grade or better for all chemicals and use distilled or equivalent water.

a. Phosphate buffer solution

Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g NH_4CI in about 500 mL distilled water and dilute to 1L. The pH should be 7.2 without further adjustment.

b. Magnesium sulfate solution

Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1L.

c. Calcium chloride solution

Dissolve 27.5 g $CaCI_2$ in distilled water and dilute to 1L.

d. Ferric chloride solution

Dissolve 0.25 g $FeCI_3 \cdot 6H_2O$ in distilled water and dilute to 1L.

e. Acid and alkali solutions, 1N for neutralization of caustic or acidic waste samples.

i. Acid-Slowly and while stirring, add 28 mL concentrated sulfuric acid to distilled water. Dilute to 1L.

ii. Alkali-Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1L.

1. *Preparation of dilution water*: 1mL each of phosphate buffer, magnesium sulfate, calcium chloride, ferric chloride solution was added into 1L volumetric flask. Add distilled water to 1L.
2. 10mL wastewater sample was into a 500mL beaker.
3. Dilution water up to 300mL was added into the same beaker.
4. pH value was adjusted to 6.5 to 7.5 by adding acid/alkali.
5. 300mL dilution water was prepared as control in another 500mL beaker.
6. All prepared samples and control was put in 300mL-incubation bottle each.
7. Dissolved oxygen (DO) concentration for each sample was measured and recorded using Dissolved Oxygen Meter.

8. Water was added to the flared mouth of bottle and covered with an aluminum foil.
9. All the bottles was put in BOD Incubator for five days. The temperature was set at 20°C.
10. Final DO value was measured after five days.
11. BOD_5 was calculated according to the formula.

$$BOD_5, mg/L = (D_1 - D_2) / P$$

Where;
D_1 = DO value in initial sample
D_2 = DO value in final sample
P = Decimal volumetric fraction of sample used

Or;

BOD_5, mg/L = $(D_1 - D_2)$ x Dilution factor
Dilution factor = Bottle volume (300mL) / Sample volume

References

Andrew, D. E., Lenore, S.C., Eugene, W. R. & Arnold, E. G. 2005. Standard Methods For The Examination Of Water & Wastewater. 21st Edition. APHA. AWWA. WEF. United States of America.

Howard, S. P., Donald, R. R. & George, T. 1985. Environmental Engineering. McGraw Hill. Singapore.

Fahid, R. Biological Wastewater Treatment-Lecture Notes in Advanced Sanitary Engineering. Faculty of Engineering. Islamic University of Gaza. 2005-2006.

B4.4 Chemical oxygen demand (COD)

Chemical oxygen demand (COD) is used as a measure of oxygen requirement of a sample that is susceptible to oxidation by strong chemical oxidant. The dichromate reflux method is preferred over procedures using other oxidants (e.g. potassium permanganate) because of its superior oxidizing ability, applicability to a wide variety of samples and ease of manipulation. Oxidation of most organic compounds is 95-100% of the theoretical value.

The mg/L COD results are defined as the mg of O_2 consumed per liter of sample under conditions of this procedure. In this procedure, the sample is heated for two hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion ($Cr_2O7_2^-$) to green chromic ion (Cr_3^+). When the 3-150 mg/L colorimetric or titrimetric method is used, the amount of Cr_6^+ remaining is determined. When the 20-1,500 mg/L or 200-15,000 mg/L colorimetric method is used, the amount of Cr_3^+ produced is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences. Test results for the 3 to 150 mg/L range are measured at 420 nm. Test results for the 20 to 1, 5000 and the 200 to 15,000 mg/L range are measured at 620 nm.

Materials and methodology

COD Digestion Reactor
Spectrophotometer, HACH DR/5000
COD Digestion Reagent Vial LR @ HR
COD rack
Volumetric pipette, 2 mL
Paper towel/Tissue

1. 100 mL of sample was homogenized for 30 seconds in a blender.
   *For samples containing large amounts of solids, increase the homogenization time.
2. For the 200-15,000 mg/L range or to improve accuracy and reproducibility of the other ranges, the homogenized sample was poured into a 250-mL beaker and gently stirred with a magnetic stir plate.
   *If the sample does not contain suspended solids, omit step 1 and step 2.
3. COD Reactor was turn on and preheated to 150°C. The safety shield was placed in front of the reactor.

4. The caps were removed from two COD Digestion Reagent Vials.
   *Be sure to use vials for the appropriate range.
5. One vial was hold at a 45-degree angle. A clean volumetric pipette was used to add 2.00 mL of sample to the vial. This was the prepared sample.
6. A second vial was hold at a 45-degree angle. A clean volumetric pipette was used to add 2.00 mL de-ionized water to the vial. This was the blank.
7. The vials were cap tightly, rinsed with de-ionized water and wiped with a clean paper towel.
8. The vials were hold by the cap over a sink and inverted gently several times to mix. The vials were placed in the preheated COD Reactor.
   *The sample vials will become very hot during mixing.
9. The vials were heated for two hours.
10. The reactor was turned off. 20 minutes was let for the vials to cool to 120°C or less.
11. Each vial was inverted several times while still warm and then placed into a rack to cool to room temperature.
12. “Hach Programs” was touched. Program “430 COD LR (Low Range) or 435 COD HR (High Range/High Range Plus)” was touched “Start”.
13. The outside of the vials was cleaned with a damp towel followed by a dry one to remove fingerprints or other marks.
14. The 16-mm adapter was installed and the blank was placed into the adapter.
15. “Zero” was touched and the display showed: 0 mg/L COD.
16. The sample vial was paced into the adapter when the timer beeps. “Read” was touched and results will appear in mg/L COD.

References

Andrew, D. E., Lenore, S. C., Eugene, W. R. & Arnold, E. G. 2005. Standard Methods For The Examination of Water And Wastewater. 21st Edition. USA.

Howard, S. P., Donald, R. R. & George, T. 1985. Environmental Engineering. McGraw-Hill. Singapore.

Kasmawati, M. & Lee, K. K. 2007. Methods of Analysis for Water and Wastewater. UiTM. Shah Alam.

B4.5 Determination of ammoniacal nitrogen (AN) and nitrate

In ammoniacal nitrogen test, ammonia compounds combine with chlorine to form monochloride. Monochloride reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green-colored solution. Test results are measured at 655 nm. Meanwhile, in nitrate test, cadmium metal reduces nitrates in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber colored solution. Test results are measured at 430 nm.

Materials and methodology

Ammonia Cyanurate Reagent Powder Pillows-2
Ammonia Salicylate Reagent Powder Pillows-2
NitraVer 5 Nitrate Reagent Powder Pillows-1
HACH Spectrophotometer DR/5000
Rounded/Square sample cell, 10 mL
Measuring cylinder, 25 mL
Beaker, 50 Ml

Ammoniacal nitrogen (Method 8155, 0.01 to 0.50 mg/L NH_3-N)

1. “Hach Programs” was touched. Program “385 N, Ammonia, Salic” was selected. “Start” touched.
2. A round sample cell was filled to the 10 mL mark with sample.
3. Another round sample cell was filled to the 10 mL mark with deionized water (the blank).
4. The contents of one Ammonia Salicylate Powder Pillow were added to each cell. Stopper and shake was applied to dissolve the powder.
5. The timer icon was touched “OK”. A three-minute reaction period began.
6. The contents of one Ammonia Cyanurate Reagent Powder Pillow were added when the timer beeps. Stopper and shake was applied to dissolve the reagent.

7. The timer icon was touch “OK”. A 15-minute reaction period began.
   *A green color will develop if ammonia-nitrogen is present.
8. The blank was placed into the cell holder when the timer beeps,
9. “Zero” touched. The display showed: “0.00 mg/L NH_3-N”.
10. The sample was wiped and placed into the cell holder.
11. “Read” touched. Results appeared in mg/L NH_3-N.

Nitrate (Method 8171, MR 0.1 to 10.0 mg/L NO_3-N)

1. “Hach Programs” was touched. Program “353 N, Nitrate MR” was selected. “Start” was touched.
2. A round sample cell was filled with 10 mL of sample.
3. One NitraVer 5 Nitrate Reagent Powder Pillow was added to the sample and cap tightly.
4. The timer icon was touch “OK”. A one-minute reaction period will begin. The cell was shaked vigorously until the timer beeps.
5. When the timer beeps, the timer icon touched “OK”. A five-minute reaction period began.
   *An amber color will develop if nitrate is present.
6. When the timer beeps, a second round sample cell with 10 mL of sample was filled (the blank).
7. The blank was placed into the cell holder.
8. “Zero” touched. The display showed: “0.0 mg/L NO_3–N”.
9. The prepared sample was placed into the cell holder. “Read” touched. Results appeared in mg/L NO_3^--N.

References

Howard, S. P., Donald, R. R. & George, T. 1985. Environmental Engineering. McGraw-Hill. Singapore.

Kasmawati, M. & Lee, K. K. 2007. Methods of Analysis for Water and Wastewater. UiTM. Shah Alam.

DR/5000 Spectrophotometer Procedure Manual. 2004. Hach Company.

B4.6 Nitrite

Materials and methodology

Spectrophotometer, HACH DR/5000
NitriVer 2 Nitrite Reagent Powder Pillow
10 ml sample cell

1. “Hach Programs” was touched. Program “373 N, Nitrite HR” was selected and “Start” was touched.
2. A round sample was filled with 10 ml of sample. The contents of one NitriVer 2 Nitrite Reagent Powder Pillow were added. The cell was cap and shake to dissolve (the prepared sample).
3. The timer icon was touched “OK”. A ten-minute reaction period began. To prevent low results, leave the sample on a flat surface and do not disturb it during the reaction period.
4. Another sample cell was filled with 10 ml of sample (the blank).
5. The blank was wiped and placed into the cell holder.
6. “Zero” was touched”. The display showed 0 mg/L NO_2^-.
7. Invert the prepared sample twice after the beeps. Avoid excessive mixing, or low results may occur.
8. The prepared sample was wiped and placed into cell holder. Results appeared in mg/L NO_2^-.

References

Howard, S. P., Donald, R. R. & George, T. 1985. Environmental Engineering. McGraw-Hill. Singapore.
Kasmawati, M. & Lee, K. K. 2007. Methods of Analysis for Water and Wastewater. UiTM. Shah Alam.
DR/5000 Spectrophotometer Procedure Manual. 2004. Hach Company.

B4.7 Phosphorus

Materials and methodology

COD Digestion Reactor
Spectrophotometer, HACH DR/5000
Total Phosphorus Test ‘N Tube Vial HR
Polyethylene dropper
Molybdovanadate Reagent
Potassium Persulfate Porder Pillow
1.54N Sodium Hydroxide
Deionized water
Test tube rack
Volumetric pipette, 5 mL
Funnel
Paper towel/Tissue

9. COD reactor was turn on and heated to 150ºC. Safety shield was paced in front of the reactor.
10. “Hach Programs” was touched. Program “541 P Total HR TNT” was selected. “Start” was touched.
11. Volumetric pipette was used to add 5 ml of deionized water to a Total Phosphorus Test ‘N Tube Vial (the blank).
12. Volumetric pipette was used to add 5 ml of sample to a Total Phosphorus Test ‘N Tube Vial (the sample).
13. Funnel was used to add the contents of one Potassium Persulfate Porder Pillow for Phosphonate to each via. The vials were cap tightly and shake to dissolve.
14. The vials were placed in the COD reactor.
15. Timer icon was touched and then “OK” was touched. A 30 minutes heating period began.
16. After the timer beeps, hot vials were carefully removed from the reactor and were placed in test tube rack to allow to room temperature (18-25ºC).

17. Volumetric pipette was used to add 2.0 ml of 1.54N sodium hydroxide to each vial. Cap was inverted to mix.
18. Polyethylene dropper was used to add 0.5 ml of Molybdovanadate Reagent. Vials were cap and inverted to mix.
19. Timer icon was touched "OK". A 7-minute reaction period will begin. The sample was read within seven to nine minutes after adding the Molybdovanadate Reagent.
20. The vials were wiped with damp towel, followed by a dry one, to remove fingerprints or other marks.
21. The blank was placed in the cell holder once the timer beeps.
22. "Zero" was touched and display showed 0.0 mg/L PO_4^{3-}.
23. The prepared sample was place into the cell holder and the results appeared in mg/L PO_4^{3-}.

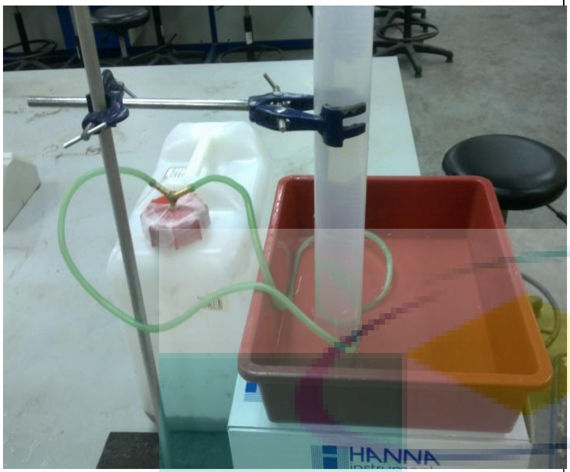
References

Howard, S. P., Donald, R. R. & George, T. 1985. Environmental Engineering. McGraw-Hill. Singapore.

Kasmawati, M. & Lee, K. K. 2007. Methods of Analysis for Water and Wastewater. UiTM. Shah Alam.

DR/5000 Spectrophotometer Procedure Manual. 2004. Hach Company.

B5 Experimental set up

 B5.1 Acclimatization set up	 B5.2 Water displacement set up

B5.3 Biogas production set up

B6 Treatment of PMW

B6.1 Experimental results for treatment of PMW

Run	Agitation (rpm)	Reaction time (h)	Type of soil water	Soil: water	PMW: SW	AN removal (%)
1	200	2.00	PFW	1:1	1:4	55.00
2	200	2.00	PFW	1:1	2:3	41.05
3	0	5.00	PFW	1:1	1:4	61.11
4	200	5.00	PFW	1:6	1:4	54.78
5	0	2.00	SSW	1:6	1:4	76.00
6	0	2.00	SSW	1:1	1:4	78.18
7	200	2.00	SSW	1:1	1:4	61.33
8	200	5.00	SSW	1:6	2:3	47.10
9	200	2.00	PFW	1:6	2:3	49.47
10	200	5.00	SSW	1:1	2:3	44.00
11	0	5.00	PFW	1:6	2:3	64.76
12	200	2.00	PFW	1:6	1:4	70.59
13	200	5.00	PFW	1:1	1:4	58.33
14	0	5.00	PFW	1:1	2:3	66.00
15	200	2.00	SSW	1:1	2:3	50.48
16	0	2.00	PFW	1:1	1:4	66.25
17	200	2.00	SSW	1:6	2:3	47.06
18	200	2.00	SSW	1:6	1:4	67.50
19	0	2.00	PFW	1:6	1:4	70.00
20	0	5.00	SSW	1:1	1:4	77.22
21	0	2.00	SSW	1:1	2:3	62.31
22	0	2.00	SSW	1:6	2:3	52.00
23	200	5.00	PFW	1:1	2:3	31.03
24	0	5.00	SSW	1:6	2:3	64.44
25	200	5.00	SSW	1:6	1:4	54.78
26	0	5.00	SSW	1:1	2:3	46.43
27	200	5.00	PFW	1:6	2:3	35.17
28	0	2.00	PFW	1:1	2:3	53.08
29	0	5.00	SSW	1:6	1:4	81.90
30	200	5.00	SSW	1:1	1:4	47.83
31	0	2.00	SFW	1:6	2:3	76.67
32	0	5.00	SFW	1:6	1:4	80.00

Selection: Manual Order: Modified

	Term	Stdized Effects	Sum of Squares	% Contribution
M	A-Agitation	-16.30	2126.34	38.36
M	B-Reaction Time	-3.88	120.47	2.17
M	C-Type of soil water	-1.58	19.96	0.36
M	D-Soil:water	5.79	267.90	4.83
M	E-PMW:SW	-14.36	1649.53	29.76
M	AB	-4.80	184.46	3.33
M	AC	-1.50	18.08	0.33
e	AD	-1.11	9.89	0.18
M	AE	-1.24	12.26	0.22
M	BC	0.014	1.653E-003	2.983E-005
M	BD	0.59	2.74	0.050
e	BE	-0.27	0.58	0.010
M	CD	2.91	67.83	1.22
M	CE	2.01	32.18	0.58
e	DE	-0.50	2.01	0.036
e	ABC	-0.53	2.26	0.041
e	ABD	-2.60	54.11	0.98
e	ABE	1.26	12.71	0.23
e	ACD	-1.44	16.52	0.30
M	ACE	-6.90	381.23	6.88
e	ADE	-1.11	9.93	0.18
M	BCD	-4.72	178.56	3.22
e	BCE	-1.69	22.93	0.41
e	BDE	0.13	0.14	2.463E-003
e	CDE	0.53	2.24	0.040
e	ABCD	0.88	6.26	0.11
e	ABCE	-2.26	40.84	0.74
e	ABDE	2.44	47.80	0.86
e	ACDE	1.22	11.82	0.21
e	BCDE	-3.27	85.51	1.54
e	ABCDE	4.41	155.54	2.81
	Lenth's ME	4.89		
	Lenth's SME	9.30		

B6.2 The percentage contribution of each main factor and their interaction in treatment of PMW

Response 1 R1 (% AN removal)

ANOVA for selected factorial model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	5061.54	14	361.54	12.78	< 0.0001	significant
A-Agitation	*2126.34*	*1*	*2126.34*	*75.14*	*< 0.0001*	
B-Reaction Tir	*120.47*	*1*	*120.47*	*4.26*	*0.0547*	
C-Type of soil	*19.96*	*1*	*19.96*	*0.71*	*0.4127*	
D-Soil:water	*267.90*	*1*	*267.90*	*9.47*	*0.0068*	
E-PMW:SW	*1649.53*	*1*	*1649.53*	*58.29*	*< 0.0001*	
AB	*184.46*	*1*	*184.46*	*6.52*	*0.0206*	
AC	*18.08*	*1*	*18.08*	*0.64*	*0.4352*	
AE	*12.26*	*1*	*12.26*	*0.43*	*0.5192*	
BC	*1.653E-003*	*1*	*1.653E-003*	*5.842E-005*	*0.9940*	
BD	*2.74*	*1*	*2.74*	*0.097*	*0.7593*	
CD	*67.83*	*1*	*67.83*	*2.40*	*0.1400*	
CE	*32.18*	*1*	*32.18*	*1.14*	*0.3012*	
ACE	*381.23*	*1*	*381.23*	*13.47*	*0.0019*	
BCD	*178.56*	*1*	*178.56*	*6.31*	*0.0224*	
Residual	481.08	17	28.30			
Cor Total	5542.63	31				

Std. Dev.	5.32	R-Squared	0.9132
Mean	59.12	Adj R-Squared	0.8417
C.V. %	9.00	Pred R-Squared	0.6925
PRESS	1704.60	Adeq Precision	13.812

B6.3 Analysis of variance (ANOVA) for treatment

B7 Factorial analysis study

B7.1 Result for factorial analysis

Run	Agitation (rpm)	Reaction time (days)	Substrate: inoculum	Process system	Type of substrate	Biogas yield (L/g COD)
1	0	7	4:01	Continuous	PMW	0.00174
2	120	7	4:01	Batch	PMW	0.00342
3	0	7	3:02	Continuous	Treated PMW	0.00209
4	120	3	4:01	Batch	Treated PMW	0.00996
5	0	3	4:01	Batch	PMW	0.00000
6	120	3	3:02	Batch	PMW	0.00362
7	120	7	3:02	Continuous	PMW	0.00130
8	0	7	4:01	Batch	Treated PMW	0.00053
9	120	7	4:01	Continuous	Treated PMW	0.00623
10	120	3	4:01	Continuous	PMW	0.00034
11	0	3	4:01	Continuous	Treated PMW	0.00210
12	0	3	3:02	Continuous	PMW	0.00105
13	0	7	3:02	Batch	PMW	0.00225
14	120	3	3:02	Continuous	Treated PMW	0.00073
15	0	3	3:02	Batch	Treated PMW	0.00003
16	120	7	3:02	Batch	Treated PMW	0.00431

Selection: Manual Order: Modified

Term	Stdized Effects	Sum of Squares	% Contribution
Intercept			
A-Agitation	-16.30	2126.34	38.36
B-Reaction Time	-3.88	120.47	2.17
C-Type of soil water	-1.58	19.96	0.36
D-Soil:water	5.79	267.90	4.83
E-PMW:SW	-14.36	1649.53	29.76
AB	-4.80	184.46	3.33
AC	-1.50	18.08	0.33
AD	-1.11	9.89	0.18
AE	-1.24	12.26	0.22
BC	0.014	1.653E-003	2.983E-005
BD	0.59	2.74	0.050
BE	-0.27	0.58	0.010
CD	2.91	67.83	1.22
CE	2.01	32.18	0.58
DE	-0.50	2.01	0.036
ABC	-0.53	2.26	0.041
ABD	-2.60	54.11	0.98
ABE	1.26	12.71	0.23
ACD	-1.44	16.52	0.30
ACE	-6.90	381.23	6.88
ADE	-1.11	9.93	0.18
BCD	-4.72	178.56	3.22
BCE	-1.69	22.93	0.41
BDE	0.13	0.14	2.463E-003
CDE	0.53	2.24	0.040
ABCD	0.88	6.26	0.11
ABCE	-2.26	40.84	0.74
ABDE	2.44	47.80	0.86
ACDE	1.22	11.82	0.21
BCDE	-3.27	85.51	1.54
ABCDE	4.41	155.54	2.81
Lenth's ME	4.89		
Lenth's SME	9.30		

B7.2 The percentage contribution of each main factor and their interaction

Response	1	Biogas yield (L/g COD)				
ANOVA for selected factorial model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	1.007E-004	11	9.158E-006	24.64	0.0036	significant
A-Agitation	*2.463E-005*	*1*	*2.463E-005*	*66.27*	*0.0012*	
B-Reaction tim	*8.883E-007*	*1*	*8.883E-007*	*2.39*	*0.1970*	
C-Substrate :	*4.698E-006*	*1*	*4.698E-006*	*12.64*	*0.0237*	
D-Process sys	*4.851E-006*	*1*	*4.851E-006*	*13.05*	*0.0225*	
E-Type of sub:	*9.813E-006*	*1*	*9.813E-006*	*26.40*	*0.0068*	
AC	*7.995E-006*	*1*	*7.995E-006*	*21.51*	*0.0097*	
AD	*1.724E-005*	*1*	*1.724E-005*	*46.40*	*0.0024*	
AE	*9.875E-006*	*1*	*9.875E-006*	*26.57*	*0.0067*	
BC	*1.398E-006*	*1*	*1.398E-006*	*3.76*	*0.1244*	
BD	*6.904E-006*	*1*	*6.904E-006*	*18.58*	*0.0125*	
CE	*1.244E-005*	*1*	*1.244E-005*	*33.48*	*0.0044*	
Residual	1.486E-006	4	3.716E-007			
Cor Total	1.022E-004	15				

Std. Dev.	6.096E-004	R-Squared	0.9855
Mean	2.498E-003	Adj R-Squared	0.9455
C.V. %	24.40	Pred R-Squared	0.7673
PRESS	2.378E-005	Adeq Precision	18.123

B7.3 Analysis of variance (ANOVA) for factorial analysis

B8 Process optimization study

B8.1 Experimental results for process optimization

Run	Agitation (rpm)	Reaction time (day)	Biogas yield (L/g COD)
1	120	3	0.00361
2	130	4	0.00204
3	130	2	0.00125
4	120	5	0.00250
5	120	1	0.00133
6	110	2	0.00096
7	140	3	0.00064
8	120	3	0.00437
9	120	3	0.00395
10	110	4	0.00180
11	120	3	0.00395
12	120	3	0.00416
13	100	3	0.00049

Sequential Model Sum of Squares [Type I]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Mean vs Total	7.416E-005	1	7.416E-005			
Linear vs Mean	1.371E-006	2	6.854E-007	0.29	0.7546	
2FI vs Linear	6.250E-010	1	6.250E-010	2.377E-004	0.9880	
Quadratic vs 2FI	1.987E-005	2	9.937E-006	18.37	0.0016	Suggested
Cubic vs Quadra	5.933E-008	2	2.967E-008	0.040	0.9613	Aliased
Residual	3.727E-006	5	7.455E-007			
Total	9.919E-005	13	7.630E-006			

Lack of Fit Tests						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Linear	2.334E-005	6	3.890E-006	48.74	0.0011	
2FI	2.334E-005	5	4.668E-006	58.49	0.0008	
Quadratic	3.467E-006	3	1.156E-006	14.48	0.0129	Suggested
Cubic	3.408E-006	1	3.408E-006	42.70	0.0028	Aliased
Pure Error	3.193E-007	4	7.982E-008			

B8.2 Fit summary for process optimization

Response	1	Biogas yield				
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	2.125E-005	5	4.249E-006	7.85	0.0087	significant
A-Agitation	*5.741E-008*	*1*	*5.741E-008*	*0.11*	*0.7541*	
B-Reaction tim	*1.313E-006*	*1*	*1.313E-006*	*2.43*	*0.1632*	
AB	*6.250E-010*	*1*	*6.250E-010*	*1.155E-003*	*0.9738*	
A^2	*1.774E-005*	*1*	*1.774E-005*	*32.80*	*0.0007*	
B^2	*6.741E-006*	*1*	*6.741E-006*	*12.46*	*0.0096*	
Residual	3.787E-006	7	5.410E-007			
Lack of Fit	*3.467E-006*	*3*	*1.156E-006*	*14.48*	*0.0129*	*significant*
Pure Error	*3.193E-007*	*4*	*7.982E-008*			
Cor Total	2.503E-005	12				

Std. Dev.	7.355E-004	R-Squared	0.8487
Mean	2.388E-003	Adj R-Squared	0.7407
C.V. %	30.79	Pred R-Squared	0.0293
PRESS	2.430E-005	Adeq Precision	7.321

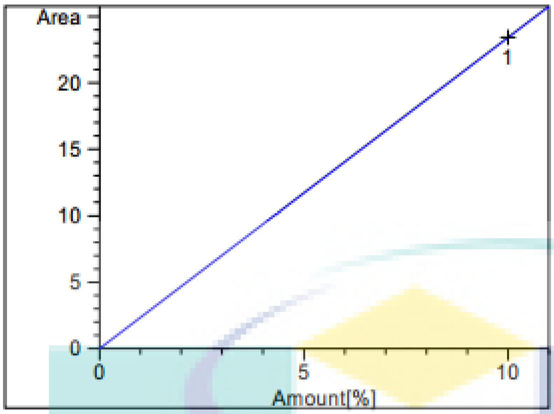
B8.3 Analysis of variance (ANOVA) for process optimization

B9 Determination of methane

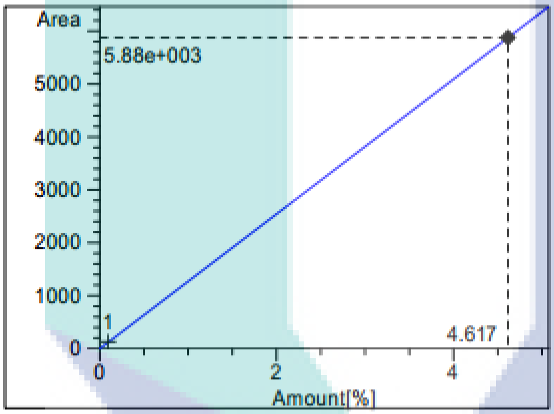
B9.1 Equipment information

```
=====================================================================
Module                                      Type   Firmware rev.   Serial number
--------------------------------------|------|---------------|---------------
68GC                                        68GC   N.05.05         CN10524020
7673                                        7673   G1530N.05.05

Software Revision: Rev. B.01.03 [204] Copyright © Agilent Technologies
=====================================================================

Column Description :  HP-PLOT/Q
Product#           :  19095P-Q04        Batch#:  Agilent
Serial#            :  AB003
Diameter           : 530.00 µm          Length :   30.0 m
Film thickness     : 40.00 µm        Void time :   0.825 min
Maximum Pressure   :    0 bar       Maximum pH :     0
Maximum Temperature:    0 °C
Comment            :

Column Description :
Product#           :                    Batch#:
Serial#            :
Diameter           : 250.00 µm          Length :   30.0 m
Film thickness     :  0.25 µm        Void time :   0.000 min
Maximum Pressure   :    0 bar       Maximum pH :     0
Maximum Temperature:    0 °C
Comment            :
=====================================================================
```

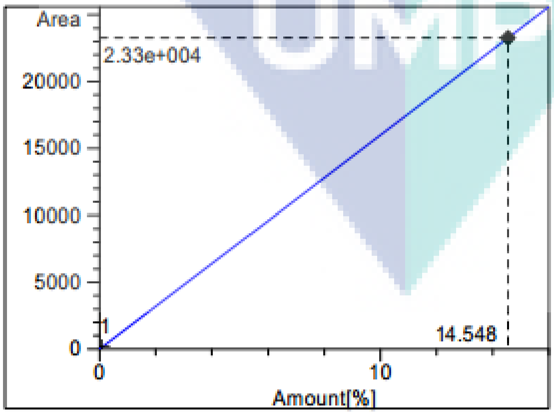
B9.2 Calibration curve



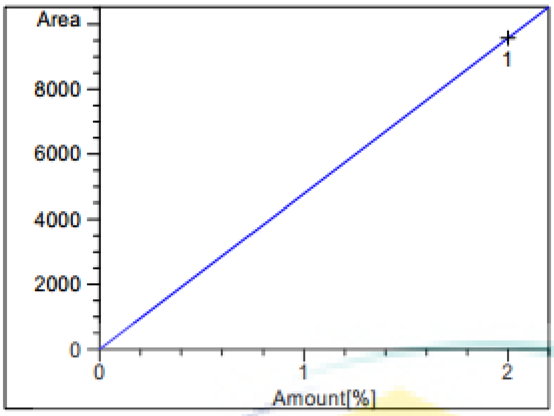

```
H2 at exp. RT: 5.483
TCD2 B,
Correlation:              1.00000
Residual Std. Dev.:       0.00000
Formula: y = mx + b
       m:         2.34358
       b:         0.00000
       x: Amount[%]
       y: Area
```



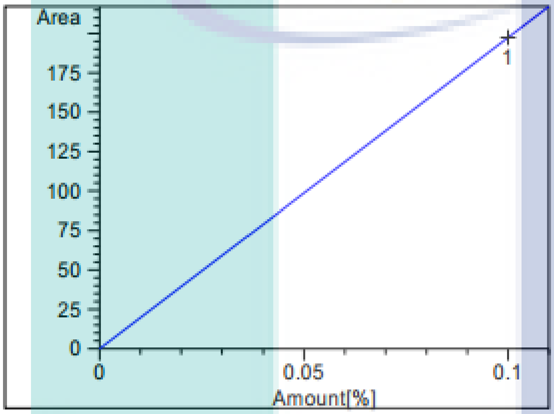

```
CH4 at exp. RT: 7.305
TCD2 B,
Correlation:              1.00000
Residual Std. Dev.:       0.00000
Formula: y = mx + b
       m:    1273.25943
       b:         0.00000
       x: Amount[%]
       y: Area
```




```
CO2 at exp. RT: 10.235
TCD2 B,
Correlation:              1.00000
Residual Std. Dev.:       0.00000
Formula: y = mx + b
       m:    1600.44769
       b:         0.00000
       x: Amount[%]
       y: Area
```

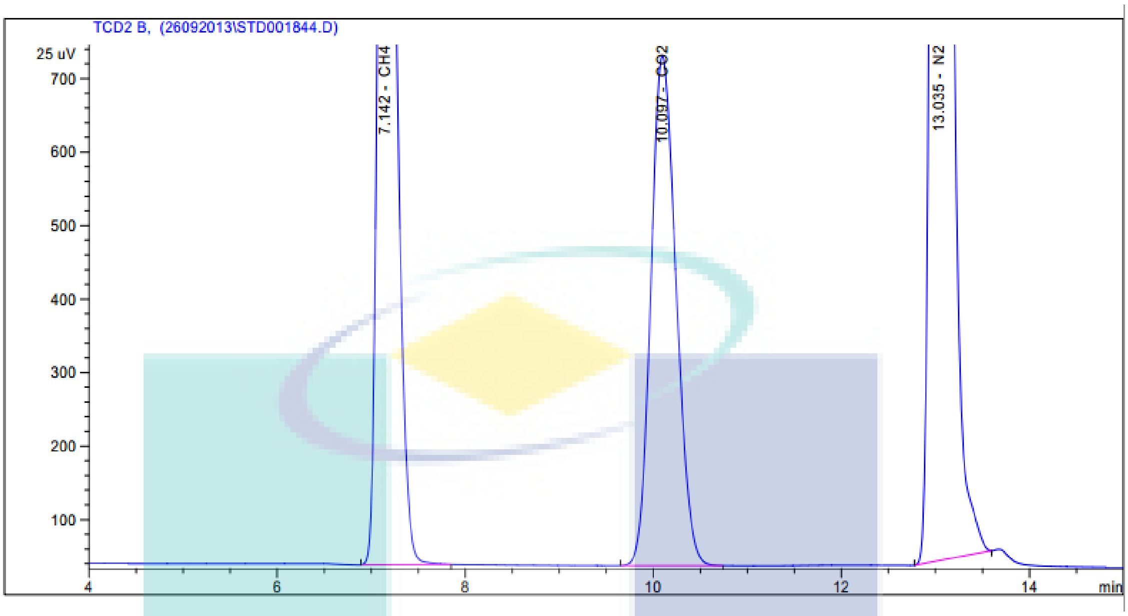


N2 at exp. RT: 13.358
TCD2 B,
Correlation: 1.00000
Residual Std. Dev.: 0.00000
Formula: y = mx + b
m: 4789.75586
b: 0.00000
x: Amount[%]
y: Area



CO at exp. RT: 14.193
TCD2 B,
Correlation: 1.00000
Residual Std. Dev.: 0.00000
Formula: y = mx + b
m: 1975.07004
b: 2.22045e-14
x: Amount[%]
y: Area

B9.3 Result



RetTime [min]	Type	Area [25 uV*s]	Amt/Area	Amount [%]	Grp	Name
5.483		-	-	-		H2
7.142	BB	1.97219e4	7.85386e-4	15.48932		CH4
10.097	BB	1.28755e4	6.24825e-4	8.04492		CO2
13.035	BB	3.87577e4	2.08779e-4	8.09178		N2
14.193		-	-	-		CO
Totals :				31.62602		

Appendix C List of publications

1. Nina Farhana Mohd Jamaludin. Screening of Factors Affecting Pre-treatment by Ammonia-N Removal from Poultry Manure Wastewater by Using Soil Water to Improve Biogas Production. 27th Regional Conference on Solid State Science & Technology, The Magellan Sutera Resort and Spa, Kota Kinabalu, Sabah, Malaysia. 20-22 December 2013.

2. Nina Farhana Mohd Jamaludin and Norazwina Zainol, 2014. Factorial Analysis on Biomethanation of Poultry Manure Wastewater. *International Journal of Applied Environmental Sciences (IJAES)*, 9 (4), pp. 1663-1672, ISSN 0973-6077. Published.

3. Nina, F. M. Jamaludin and Norazwina Zainol, 2104. Effect of Agitation and Reaction Time to Biological Treatment of Poultry Manure Wastewater. *Pollution Research*, EM International, ISSN: 0257-8050. Published.

4. Choo Wei Chun, Nina Farhana Mohd Jamaludin, Norazwina Zainol, 2015. Optimization of Biogas Production from Poultry Manure Wastewater in 250 ml Flasks. *Jurnal Teknologi (Scinces & Engineering)*, 75:1 (2015), pp. 275-285, ISSN 2180-3722. Published.