PRODUCTION OF BIOGAS FROM POULTRY MANURE WASTEWATER: OPTIMIZATION

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PRODUCTION OF BIOGAS FROM POULTRY MANURE WASTEWATER: OPTIMIZATION

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Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JANUARY 2015

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SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

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STUDENT'S DECLARATION

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

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Dedication

I would like to dedicate my thesis to my beloved supervisor, friends and family who supported me each step of the way.

ACKNOWLEDGEMENT

I am using this opportunity to express my gratitude to everyone who supported me throughout my Undergraduate Research Project (URP) in University Malaysia Pahang (UMP). I would like to express my deepest appreciation to all those who provided me the possibility to complete this research. First and foremost, I would like to thank UMP for giving me this opportunity to undergo my Undergraduate Research Project (URP) in UMP. Nevertheless, a special and sincere gratitude I give to my final year project supervisor, Dr. Norazwina binti Zainol, whose contribution in stimulating suggestions and encouragement, helped me to coordinate my project especially in writing this report.

Next, I express my warm thanks to Ms. Nina Farhana binti Mohd Jamaludin (Postgraduate student), whose aspiring guidance and persistent help for laboratory works make my URP completed. Furthermore I would also like to acknowledge with much appreciation the crucial role of the dean of Faculty of Civil Engineering and Earth Resources (FKASA), Engr Fadzil bin Hj Mat Yahaya, who gave the permission to use all required equipment and the necessary material to complete the laboratory experiment.

After that, a special thanks goes to Faculty Chemical and Natural Resources Engineering (FKKSA) in UMP has provided the support and equipment I have needed to produce and complete my research. My gratitude is also referred to all my friends for their friendly and spiritual support when I am depressed.

Last but not least, many thanks go to my parents and siblings for being so supportive along the way. Their presence is the most important driving force for me to keep striving for the best. Thus, I dedicate this thesis to all these people mentioned who had made my life and URP a wonderful experience.

ABSTRACT

An exaggeration increment of population has led to depletion of energy resources. One of the most effective solutions is to implement the uses of renewable energy sources. In particular, biomass, which can be further converted into biofuel, is normally derived from plants. However, it brings several drawbacks which may directly threaten the sensitive species. To alleviate these, biogas production from poultry manure wastewater was explored in the current work. Its usage to produce biogas was considered as a triumph to the concept of waste-to-wealth. The main objective of this research study was the optimization of biogas production from poultry manure wastewater by soil mixed culture. The poultry manure collected was gone through characterization and pre-treatment processes to remove excessive ammonia-N which cause inhibition to the biogas production. The optimization was analyzed by central composite design (CCD). Previous studies have screened out five processing parameters, which were agitation speed, reaction time, substrate to inoculum ratio, process system and type of substrate. Significantly, it had been identified that agitation speed and reaction time were the most crucial parameters. The best screening condition obtained from previous studies was 120 rpm agitation speed and 3 days of reaction time. Consequently, there were two factors involved in current research study, which are agitation speeds ranged from 100 rpm to 140 rpm; and reaction time ranged from 2 days to 5 days. The biogas production was collected by water displacement experimental set up. In addition, chemical oxygen demand (COD) value was determined using HACH DR5000 spectrophotometer with the aid of COD digestion reactor. Lastly, the experiment was designed and analyzed by Design Expert V7.0 software using response surface methodology (RSM). The biogas production performance was evaluated on the basis of biogas yield from initial COD and was found ranging from 0.49 to 4.37 mL/g COD. Quadratic model was well fitted (R-squared>0.80) with a confidence level higher than 95 %. For validation run, the optimum biogas production was using agitation: 120 rpm and reaction time: 3.3 days. Under this condition, 4.45 mL/g COD of biogas yield was obtained. This counted for 5.82 % error from predicted models. It is recommended to construct a pilot study of scale-up experiment for the optimization of biogas production under optimum conditions obtained from this study.

ABSTRAK

Populasi penduduk yang semakin meningkat telah menyebabkan penyusutan sumber tenaga. Salah satu cara penyelesaian yang paling berkesan adalah dengan melaksanakan penggunaan sumber tenaga boleh diperbaharui. Khususnya, biojisim, yang boleh terus ditukar menjadi bahan api bio, biasanya diperolehi daripada tumbuhan. Walau bagaimanapun, ia membawa beberapa kelemahan yang secara langsung boleh mengancam beberapa spesies tumbuhan. Untuk mengatasi masalah ini, penghasilan biogas daripada najis ayam akan diterokai dalam kerja kajian ini. Penggunaannya untuk menghasilkan biogas akan dianggap sebagai kemenangan kepada konsep sisa kepada kekayaan. Objektif utama kajian penyelidikan ini adalah pengoptimuman penghasilan biogas daripada najis ayam oleh kultur tanah campuran. Najis ayam yang diperolehi telah melalui proses pencirian dan pra-rawatan untuk mengurangkan ammonia-N yang berlebihan yang akan menyebabkan perencatan untuk pengeluaran biogas. Pengoptimuman telah dianalisis oleh Reka Bentuk Komposit Pusat (CCD). Kajian sebelum ini telah disaringkan dengan lima parameter pemprosesan, iaitu kelajuan pergolakan, masa tindak balas, nisbah substrat kepada inokulum, sistem proses dan jenis substrat. Dengan ketara, ia telah dikenal pasti bahawa kelajuan pergolakan dan masa tindak balas merupakan parameter yang paling penting. Keadaan penyaringan terbaik yang diperolehi daripada kajian sebelum ini ialah kelajuan pergolakan dengan 120 rpm dan masa tindak balas dengan 3 hari. Oleh yang demikian, terdapat dua faktor yang terlibat dalam kajian penyelidikan semasa, iaitu kelajuan pergolakan antara 100 rpm hingga 140 rpm; dan masa tindak balas antara 2 hari hingga 5 hari. Penghasilan biogas telah dikumpulkan dengan teknik eksperimen anjakan air. Selain itu, nilai permintaan oksigen kimia (COD) telah ditentukan dengan menggunakan spektrofotometer HACH DR5000 dengan bantuan COD penghadaman reaktor. Akhir sekali, eksperimen telah direka dan dianalisis oleh perisian Design Expert versi 7.0 menggunakan metodologi permukaan tindak balas (RSM). Prestasi penghasilan biogas telah dinilai atas dasar hasil biogas daripada COD awal dan didapati dari 0.49 untuk 4.37 mL/g COD. Model kuadratik telah dipasang dengan baik (R-kuasa dua> 0.80) dengan tahap keyakinan yang lebih tinggi daripada 95%. Untuk eksperimen pengesahan, penghasilan biogas optimum adalah menggunakan pergolakan: 120 rpm dan tindak balas masa: 3.3 hari. Di bawah keadaan ini, 4.45 mL/g COD hasil biogas diperolehi. Ini diambil kira untuk kesilapan 5.82% daripada model yang diramalkan. Adalah disyorkan untuk membina satu kajian perintis eksperimen meningkatkan skala untuk mengoptimumkan pengeluaran biogas di bawah keadaan optimum yang diperolehi dari kajian ini .

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LIST OF ABBREVIATIONS

AD	Anaerobic digestion
AN	Ammoniacal Nitrogen
ANOVA	Analysis of variance
ASBR	Anaerobic sequencing batch reactor
BOD	Biochemical Oxygen Demand
CCD	Central Composite Design
CHP	Combined heat and power
CM	Chicken manure
COD	Chemical Oxygen Demand
DF	Degree of freedom
FFD	Full Factorial Design
F/M	Food-to-microorganism ratio
GHG	Greenhouse gases
HRT	Hydraulic retention time
LSD	Least Significant Difference
MSW	Municipal solid waste
NPK	Nitrogen: Phosphorus: Potassium ratio
OHPA	Obligatory Hydrogen-Producing acetogens
OLR	Organic Loading Rate
PMW	Poultry Manure Wastewater
PS	Peat soil
RSM	Response Surface Methodology
S/I	Substrate to inoculum ratio
SMC	Soil mixed culture
SS	Suspended solid
SW	Soil water

Greek

k	parameter number
c_p	replicate number of central point
α	coded factor
R^2	coefficient of determination
e_i	residuals
y_i	difference between the actual individual values

1 INTRODUCTION

1.1 Motivation and statement of problem

Biogas, a gas mixture produced by the decomposition of organic matter in anaerobic condition, is introduced to act as an alternative renewable energy source (Ahn *et al,* 2010). It contains on an average distribution of 50-70% methane, 30-40% carbon dioxide, 1-2% nitrogen, 5-10% hydrogen, trace amounts of hydrogen sulfide and water vapor (Grant and Marshalleck, 2008).

The annual production of poultry and livestock manure on centralized farms (216,000 pigs per year, 15,000 beef per years and up to 4 million chickens per year) in Russia overreached 700 million m^3 and led to severe environmental issues such as foul odor problems due to high levels of ammonia, attraction of rodents and pathogenic microorganisms, runoff of phosphorus into water source and groundwater contamination due to nitrate leaching (Kalyuzhnyi *et al.,* 1998; Atuanya and Aigbirior, 2002). According to the Polish Act of 10 July 2007 on fertilizers and fertilizing, the Construction Law (Journal of Laws of 2006, No. 156, item 1118), poultry farmers are obliged to dispose a minimum of 70% of poultry manure on farms and store the manure in sealed containers with capacity of at least four months of fertilizer production (Borowski *et al*., 2014).The utilization of poultry manure wastewater to produce biogas via anaerobic digestion can help to resolve the overwhelmed manure in farms (Sakar *et al.,* 2009).

The motivation of utilization of poultry manure for anaerobic digestion (AD) instead of other manures to produce biogas is due to its high nutrient contents (Roeper *et al.,* 2005). According to FFTC Annual Report 2011 (2012), the approximate ratio value of Nitrogen: Phosphorus: Potassium (NPK) on dry basis matter of dairy cow dung is 2.19: 1.37: 0.67%; for swine is 2.91: 2.85: 1.38%; and highest for poultry which is 4.34: 4.41: 2.24%, respectively. Although poultry manure can be characterize as one of the best organic fertilizer sources, excessive implementation may lead to severe environmental issues (Kalyuzhnyi *et al.,* 1998).

In addition, the AD of biomass requires lower capital and operating cost as compared to other renewable energy sources (Rao *et al.,* 2010). This technology having great

potential in pathogen levels reduction, odor regulation and fertilizer value improvement to alleviate both economic and environmental issues (Demirer and Chen, 2005). The end products of AD are biogas and digestate. The methane concentrations contain in biogas yield from AD of manure or biodegradable waste can be up to 80% in volume whereas the digestate is a moist solid bringing fertilizer values (Demirbas *et al.,* 2011). As a bio-renewable energy source, biogas produced can be used to generate heat and electricity (Angelidaki and Ahring, 1997).

In order to optimize this model with a number of interrelated parameters in one time, RSM conducted by process modeling and optimization has been introduced (Rastegar *et al.,* 2011). RSM is an optimization method which collects a group of mathematical and statistical techniques to define the relationships between the response and the independent variables. It is divided into three major stages which are preliminary determination of independent parameters and levels, selection of experimental design, and graphical presentation of result analysis (Ba ş and Boyacı, 2007). Lastly, the significance of the design model will be analysed by the coefficient of determination (R^2) from the analysis of variance (ANOVA) which determine the quality of the fit of quadratic correlations (Amani *et al.,* 2012).

1.2 Objectives

The main objective of this study is to optimize the production of biogas from poultry manure wastewater.

1.3 Scope of this research

Chicken manure (CM) wastewater sample will be collected from local poultry manure farm, before the pre-treatment process using soil mixed culture for Ammonia-N removal. Then, sample will be diluted for COD testing. The optimum conditions for the production of biogas from poultry manure wastewater will be determined using RSM. CCD will be applied to investigate the effects of two independent variables, namely agitation speed (rpm) and reaction time (days). Design Expert V7.0 will be used to design the experiment and analyse the experimental data. Spectrophotometer namely HACH DR5000 will be used to detect the COD of CM wastewater sample. The overall process performance will be measured by the yield of the biogas produced.

1.4 Main contribution of this work

The significant contribution of this research was to minimize the problem of inappropriate poultry manure wastewater management which cause negative impacts to the environment by foul odour transmissions, attraction of infectious microorganisms and runoff of nutrients into water sources (Kalyuzhnyi *et al.,* 1998; Atuanya and Aigbirior, 2002). Besides, biogas produced from AD is utilised worldwide to supply secure and economical energy.

In households, utilization of methane-rich biogas is initially contributed to cooking and lighting purpose in developing countries. The production of this renewable energy source is of household-scale common digesters with 2-10 m^3 volume which can only accommodate household energy consumption (Surendra *et al.,* 2014).

In industry, large-scale institutional biogas plants produce and purify biogas into bio-methane for other end-use purposes. The burning of biogas in combined heat and power (CHP) plants can generate electricity power supply for industrial and commercial areas. The waste heat also can be used for heating, drying or in refrigeration machines.

Moreover, biogas can be used as fuel for natural gas vehicles by the decoupling of production and utilisation (Lagerkvist *et al.,* 2012). The leftover digestate waste generated from AD can be used as bio-fertilizer for crops and plants in agricultural areas to improve the soil fertility (Zhang *et al*., 2007).

1.5 Organisation of this thesis

The organization structure of the thesis after introductory part was outlined as follow:

Chapter 2 provided the importance and application together with its background description on biogas and its applications in steam production, electricity generation, vehicle fuel and chemical production. Furthermore, this chapter delineated the potential of poultry manure wastewater in biogas production. Next, few potential feedstock for biogas production were reviewed. Besides, the AD technique used for biogas production from poultry manure wastewater includes hydrolysis, acidogenesis, acetogenesis and methanogenesis were briefly explained. Moreover, the modeling technique, RSM, used for optimization process with the aid of CCD in Design Expert Version 7.0 was described in detail. The mathematical analysis, ANOVA which aims to check the

adequacy of model proposed also being discussed in the chapter. The factors affecting biogas production, which including agitation speed, reaction time, substrate-to-inoculum ratio, process system and type of substrate, were discussed in details with justification. This chapter ended with the selection of factors for optimization in this study.

Chapter 3 showed the methodology of the research work. It included the characterization and pre-treatment method of substrates. Besides, this chapter reviewed on the processes flow in AD experiment executed for biogas production from poultry manure wastewater by using RSM experimental design. In addition, COD-vial method analysis was described in details in this chapter. Next, validation run process also was being included in this chapter.

Chapter 4 devoted to the results data obtained from the experiment with the discussion as well as the comparison with other researchers. Besides, the characterization and pre-treatment results were discussed in the beginning of this chapter. The adequacy of proposed model was proved by mathematical analysis.

Chapter 5 summarized the research works covered in the body of this thesis includes a comprehensive summary of the findings. The thesis ended with recommendations which forecasting future works which might be developed in this work.

2 LITERATURE REVIEW

2.1 Introduction to Biogas

Biogas is a combustible mix of gases produced by AD of various forms of organic matter such as energy crops biomass (i.e., sugarcane and cassava) and waste materials (i.e., manure and sewage). Biogas is mainly composed of methane (CH_4) and carbon dioxide (CO_2) (Cvetković *et al.,* 2014). The characteristics of biogas such as it odor, exposure limits and impact on environment are shown in **Table 2.1**.

Table 2.1: Biogas characteristics (Grant and Marshalleck, 2008)

Biogas	**Odor**	**Exposure Limit (ppm)**	**Environmental Impact**
Methane (CH_4)	None	1000	GHG; explosive at 15 % mixture with air
Carbon dioxide (CO_2)	None	5000	GHG
Ammonia (NH_4)	Pungent	10	Acid rain when oxidized
Hydrogen sulphide (H_2S)	Rotten eggs	10	Highly flammable; acid rain when oxidized to sulfur

2.1.1 Importance of biogas

Biogas, a potential renewable energy source converts from biomass by AD, which acts a vital role to regulate the crisis of energy deficit and negative environmental effects (Schröder *et al.,* 2008; Rao *et al.,* 2010). The usage of biogas as a renewable energy source has great potential to minimize the emission of methane gas into environment (Cvetković *et al.*, 2014). Biogas has great potential to reduce global climate change. Since, the greenhouse effect for methane is 23 times higher than that of carbon dioxide (Gerlach *et al.,* 2013). The recovery of this significant energy by anaerobic treatment helps to reduce fossil fuel and greenhouse gases (GHG) (Gupta *et al.,* 2012). Biogas production has been paid close attention because of its potential as renewable and versatile energy source for heat and electricity generation, and transportation fuel (Lagerkvist *et al.,* 2012).

2.1.2 Application of Biogas

Biogas can be utilized for four main areas *viz.*, heat and steam production, electricity generation, vehicle fuel, and ultimately as feedstock for chemicals production. Primarily, the utilization of biogas in some developing countries only limited to cooking and lighting purpose because of moderate biogas digester size (Surendra *et al.,* 2014). Biogas provides higher energy content in cooking than fire fueled by traditional solid fuel resources. Lighting ranked second common usage of biogas right after cooking. In some regions out of electrical grid connection, biogas is introduced using special gas mantle lamps for lighting purpose (Singh and Sooch, 2004).

Besides, injection of upgraded biogas, named as biomethane, which meets stringent quality standards into the electrical grid helps to prevent contamination of the grid. The only biogas component that contributes as energy carrier is CH_4 (Surendra *et al.,* 2014). The energy content for pure biomethane is approximate to be 10 kWh/m^3 while that for biogas with assumption of 60 % methane content is 6 kWh/Nm^3 (Appels *et al*., 2008).

Biogas acts as an ideal fuel in CHP applications all around the world. The mechanism of these combine engines is generated by the conversion of mechanical power into electricity. Biogas can replace fossil fuels to drive natural gas vehicles after it is upgraded to have same quality as natural gas. For example, in Pura, India, a biogas project was implemented by the community in which a modified diesel engine and an electrical generator were successfully powered by a biogas digester (Reddy, 2004).

2.2 Feedstock for biogas production

For biogas production from organic matter, its appropriate raw material must be suitable for AD process. There are a variety of feedstock such as animal manure, municipal wastewater and agricultural crops residues. The sustainability of biogas production must consider on a few aspects include energy potential, environment, and economic feasibility (Cvetković *et al.*, 2014). In the following section, the review of various feedstock was discussed.

2.2.1 Poultry Manure Wastewater

Poultry manure wastewater is one of the most abundant biodegradable materials accumulating in local poultry farming area and has good potential to produce biogas. Generally, poultry manure includes faeces and urine excreted by chicken, which contains high organic nutrients and has high fertilizer value on crops in agricultural areas. The CM production in one poultry farm at California is illustrated in Figure 2.1

Figure 2.1: CM production on California poultry farm (Mullens et al., 2001)

A good management of this waste can augment high cost commercial fertilizers (Moreki and Chiripasi, 2011). The tremendous expansion of poultry population is due to the increasing demand for chicken products. As a side effect, the amount of poultry excrement is also rising. An inappropriate disposal and treatment of these poultry manure can cause spread of diseases, soil and groundwater pollution and risks the health and environment (Roeper *et al.,* 2005). Poultry manure is mainly categorized in solid, slurry and wastewater. Collection of CM is commonly the mixture of solid form and other chicken production residues (Haga, 2001).

Poultry manure is richer in biodegradable organic nutrient than other animal wastes (Hill, 1983; Morris *et al*., 1975). The AD of fresh poultry manure will decrease the process efficiency due to ammonia accumulation in high solid content, therefore the treatment of poultry manure in its semi-solid state has been experimented (Bujoczek *et al*., 2000).

2.2.2 Municipal Solid Waste

Municipal solid waste (MSW) is waste collected mainly from households, non-hazardous solid waste from commerce and trade, offices and institutional establishment including hospitals, wastes from market and yard, and even sweepings from streets (Ogwueleka, 2009). Composition and quantity of MSW can be determined from the living habits and standard of community. The quantification unit used for MSW is expressed in kg/person/year, which indicating the waste generated per person in a year (Cvetković *et al.*, 2014).

Kiely (1997) defined solid wastes to include activities from human and animal and also liquid wastes such as paints, old medicines and spent oils. This shows the possibility of intermixing between both solid and liquid wastes. However, the study found the MSW as largely static which emerged as one of the greatest challenges in its handling and management. A proper disposal management can prevent many environmental problems such as unpleasant odours and blockage of water drain ways which might further lead to pollution and flooding respectively (Igoni *et el*., 2008). The example of one disposal area of MSW in Malaysia is shown in **Figure 2.2**.

Figure 2.2: Municipal solid waste in Malaysia in 2013 (Eckard, 2013)

Conventionally, MSW disposal has been mainly managed by land filling. However, the anthropogenic methane emission from the landfills waste has been identified as essential contributor to global warming (Stocker *et al.,* 2013). AD of MSW has been

emphasized as one of the acceptable treatment to reduce and stabilize solid waste volume for biogas production (Stroot *et al.*, 2001).

2.2.3 Agricultural wastes

Agricultural waste describes both organic and non-organic wastes produce on an agriculture farm through various farming activities. Horticulture, dairy farming, seed growing, grazing land, livestock breeding, nursery plots and woodlands are among the examples of agricultural activities (Ashworth and Azevedo, 2009). Agricultural wastes such as crop residues, wood and other plant residues are highly energy rich and inexpensive for fermentation. Some of the famous substrate used for AD to produce biogas includes sugarcane bagasse, rice straw, cassava waste, palm oil mill waste, and wheat bran (Ezejiofor et al., 2014). The sugarcane bagasse is shown in **Figure 2.3**. The agricultural wastes production in Indonesia in year 1989 has shown in **Table 2.2**.

Figure 2.3: Sugarcane bagasse (Openpr.com, 2008)

Table 2.2: Production of agricultural wastes in year 1989 (Dewi and Siagian, 1992)

Type of Agricultural wastes	Production (ton/year)
Rice straw	44,723,000
Sugarcane bagasse	8,561,606
Cassava waste (root shell and stalk)	6,713,000

The potentiality of agricultural wastes for biogas production can reduce environmental pollution and also minimize the utilization of commercial energy source such as kerosene and firewood. These can be proved via some examples of the process applications for biogas production. Firstly is the cassava waste treatment to reduce polluted river near tapioca starch industry. Secondly, the utilization of water hyacinth as substrate for AD can solve Curug dam problem in Indonesia (Ishizuka *et al.,* 2010).

The biogas production by AD of agricultural wastes is done via the synergistic action of a consortium of hydrogenic, acidogenic, acetogenic and methanogenic bacteria (Amigun *et al.*, 2008). Although agricultural wastes are one of the potential feedstock for AD to produce biogas, but it still possesses some limitations. The main problem with AD of agricultural wastes is that it contains high cellulose levels, hemicellulose, starch, lipids and proteins (Oliveira and Franca, 2009). This speciality and complexity structure makes cellulose resistant to both biological and chemical treatments (Taherzadeh and Karimi, 2008). The lignocelluloses degradation makes the hydrolysis stage slower and rate limiting. Therefore, agricultural wastes substrate needs to practice chemically or mechanically pre-treatment to ease the accessibility for microbial growth in AD process (Ezejiofor et al., 2014).

2.3 Technique used for Biogas Production

AD is one of the advantageous and beneficial processes used for biogas production from poultry manure (Sakar *et al.,* 2009). Generally, the biogas production from AD using the concept of biomethanation of animal manure yield principal gases *viz.*, methane and carbon dioxide (Rao *et al.,* 2010). The biogas yield will depends on substrate mix and several operating conditions such as incubation time and temperature (Olsson and Fallde, 2014). Biogas production can reduce nuisance odors in agricultural farms (Schröder *et al.,* 2008). Beside the function of stabilization and deodorization of poultry manure, AD also turns poultry manure, which initially acts as natural fertilizers into easy-disposable organic fertilizers (Borowski *et al*., 2014). Biogas produced from AD can be utilized as cooking gas and fuel, the digestate become bio-fertilizer and the sludge component can used as a soil conditioner after dried (Zhang *et al*., 2007). AD is a natural established bioconversion technology which follows a sequence of reactions which are hydrolysis, acidogenesis, acetogenesis and methanogenesis (Poh and Chong,

2009). These interdependent reactions are occurs simultaneously and synergistically as shown in **Figure 2.4**.

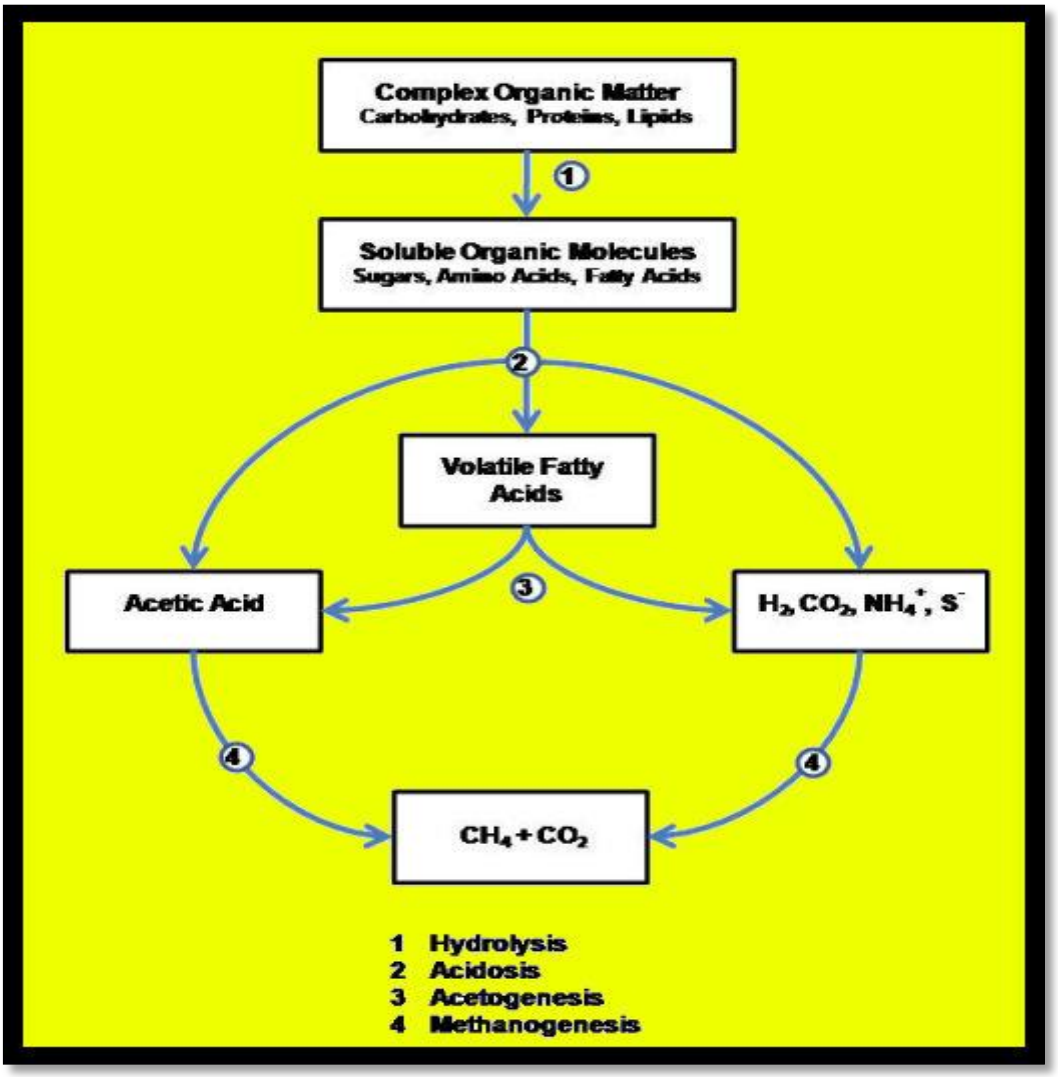

Figure 2.4: Step in AD (Hamilton and Ciolcosz, 2010).

2.3.1 Hydrolysis

In AD, hydrolysis breaks down insoluble and complex organic molecules such as lipids, carbohydrates and proteins into water soluble, simpler organic molecules such as sugars and amino acids by enzyme (Hamilton and Ciolcosz, 2010). Proteins provide a source of carbon, nitrogen and energy for the growth of bacteria in anaerobic digesters (Palmisano & Barlaz, 1996).

Hydrolysis is considered as the time limiting step for solid matter degradation. This is because some solid matter such as lignin and cellulose might be non-degradable during AD. Consequently, woody waste such as straw residues which is rich in lignin content

is not an ideal feedstock to undergo AD process as its phenolic groups might be inhibitory to the enzymes (Arsova, 2010). The other way round, acid development will be fastening up if major content of easily degradable carbohydrates like glycogen or starch in the substrate.

In this stage, the enzymes involved such as lipases, cellulase and protease are produced by fermentative and hydrolytic bacteria (Arsova, 2010). These bacteria play important role to depolymerize organic matter towards their monomer compounds. Basically, extracellular lipases and phospholipases take the role to hydrolyze fats into glycerine, alcohols and fatty acids (Dornack, 2012). Besides, proteolytic enzymes produced by anaerobic bacteria are responsible to hydrolyze proteins to peptides, amino acids and carbon dioxide (Palmisano & Barlaz, 1996).

An example of hydrolysis break down of organic waste into simple sugar, in this case glucose is shown in equation (2.1) in which n indicates the stoichiometric coefficient of respective compounds. On the other hand, degradation of proteins into their constituent amino acid and lipids into long chain fatty acids are occur under similar reactions.

$$[C_6H_{10}O_4]n + 2nH_2O \rightarrow n[C_6H_{12}O_6] + nH_2 \quad (2.1)$$

2.3.2 Acidogenesis

Acidogenesis then convert soluble organic molecules into volatile fatty acids. The anaerobic fermentation happens by the combination of hydrolysis and acidogenesis (Hamilton and Ciolcosz, 2010). In this stage, the acid formers microorganisms transform hydrolytic products such as sugars, amino acids, alcohols and fatty acids into simple organic acids. The fermentation products varying by the concentration of intermediary bound hydrogen. A high pH values lead to reduced end products such as propionic acid and butyric acid whereas a low pH values lead to an intensified production of acetate, carbon dioxide and hydrogen (Palmisano & Barlaz, 1996).

Equation (2.2) and (2.3) shows the examples of typical acidogenesis reactions, where glucose is transformed into ethanol and propionic acid, respectively (Ostrem, 2004).

Conversion of glucose to ethanol

$$C_6H_{12}O_6 \leftrightarrow 2\ CH_3CH_2OH + 2CO_2 \quad (2.2)$$

Conversion of glucose to propionic acid

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3CH_2COOH + 2\ H_2O \quad (2.3)$$

2.3.3 Acetogenesis

After that, in acetogenesis, the intermediate volatile fatty acids are further converted into acetate and a gas composition of hydrogen, carbon dioxide, ammonia and hydrogen sulfide by acetogenic bacteria (Hamilton and Ciolcosz, 2010). Basically, this pathway of single acid forming stage aims to reduce biochemical oxygen demand (BOD) and COD values. Generally, high pH values inhibit the growth rate of acetogenic bacteria. Acetogenic bacteria are also known as obligatory hydrogen-producing acetogens (OHPA) as they exhibit a metabolism of proton reduction and are mandatory dependent on hydrogen removal (Arsova, 2010). Therefore, there is a close special symbiosis between acetogenesis and methanogenesis to ensure the direct utilization of the hydrogen developed.

Practically, some fermentation products such as alcohols, propionic acid and butyric acid formed within this stage as a mechanism to remove accumulating electrons and hydrogen. In this stage, some of the important reactions for the formation of acetic acid are as follow, which are mainly from the conversion of glucose (equation 2.4), ethanol (equation 2.5) and propionic acid (equation 2.6).

Conversion of glucose to acetic acid

$$C_6H_{12}O_6 + 2H_2O \leftrightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (2.4)$$

Conversion of ethanol to acetic acid

$$CH_3CH_2OH + H_2O \leftrightarrow CH_3COOH + 2H_2 \quad (2.5)$$

Conversion of propionic acid to acetic acid

$$CH_3CH_2COOH + 2H_2O \leftrightarrow CH_3COOH + CO_2 + 3H_2 \quad (2.6)$$

Hydrogen plays a crucial intermediary role in acetogenesis as all the acids conversion happen under low hydrogen partial pressure condition. The thermodynamic feasibility of acetogenesis is sustained by the presence of hydrogen scavenging bacteria (hydrogenotrophs) which lowering the partial pressure of hydrogen (Ostrem, 2004).

Hydrogenotrophs are pH sensitive bacteria. Whenever the conditions within the anaerobic digester result in a pH drop, hydrogen will be stored within propionic acid by ecology response. This reversal of the reaction to achieve equilibrium can be explained in Le Chatelier's principle.

The health condition of an anaerobic digester is indicated by its low hydrogen concentration. This is also the reason in which hydrogen only appears as a trace component in biogas. Acetogenesis along with acidogenesis represents the transition from soluble organic molecules to the methanogenic substrate.

2.3.4 Methanogenesis

In last stage which is methanogenesis, then substrate for methanogenic microorganisms release biogas, which include methane and carbon dioxide as principal products (Hamilton and Ciolcosz, 2010). Methane, the main component in biogas, is produced through a syntrophic relationship between acetate-oxidizing bacteria and hydrogen-utilizing methanogens (Arsova, 2010). Acetotrophic or acetoclastic methanogens convert acetic acids to methane and carbon dioxide via decarboxylation of acetic acid as shown in equation (2.7). The second type of anaerobic archaea is called hydrogenotrophic or hydrolytic methanogens reduce carbon dioxide and hydrogen into methane and water using H_2 as electron donor as shown in equation (2.8) (O'Flaherty *et al.*, 2006; Hamilton and Ciolcosz, 2010).

Acetic acid cleavage

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2.7)$$

Carbon dioxide reduction

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2.8)$$

Ordinarily, methanogenesis process occurs naturally in manures, agricultural fields and aquatic sediments, and plays a vital role for the carbon cycle to sustain the ecosystem (Arsova, 2010). Stabilization is said to be achieved when methane and carbon dioxide are produced. The archeabacter genus methanogenic bacteria are mainly categorized according to their shape. According to Eggeling *et al.* (1986), Methanosarcina genus is in spherically shaped, Methanothrix bacteria is in long and tubular shaped and bacteria

that catabolize furfural and sulfates appeared as short and curved rods as illustrated in **Figure 2.5**.

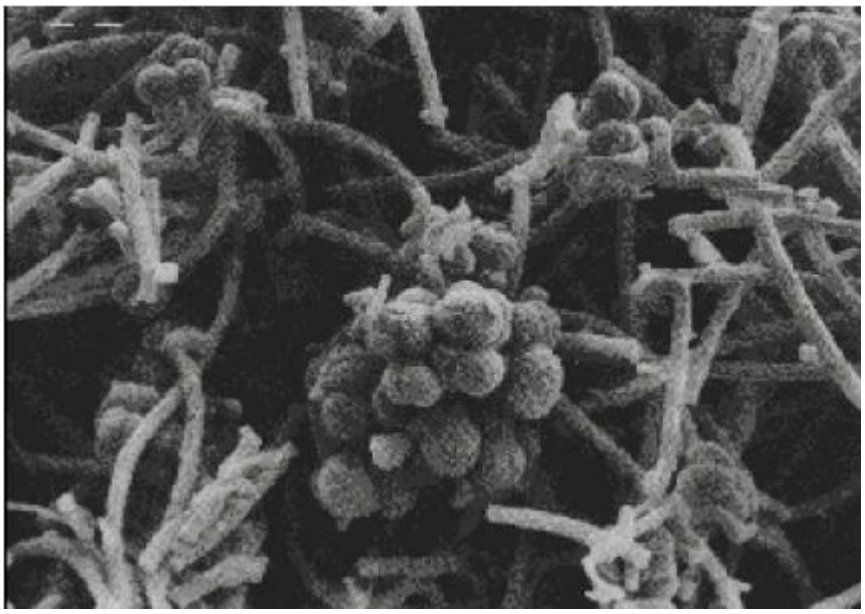

Figure 2.5: Common methanogenic bacteria in methane formation process (Eggeling et al., 1986).

2.4 Optimization Using RSM

RSM is a widely used modelling technique functioned to develop, improve and optimize the response variable in the statistical design of experiments (Ba ş and Boyacı, 2007). RSM is applicable when a response of interest is influenced by several parameters or variables and the objective is to optimize this response. It can be expressed as

$$y = f(x_1, x_2) + e \tag{2.9}$$

where the response *y* depends on independent variables x_1 and x_2, and the experimental error denoted as e.

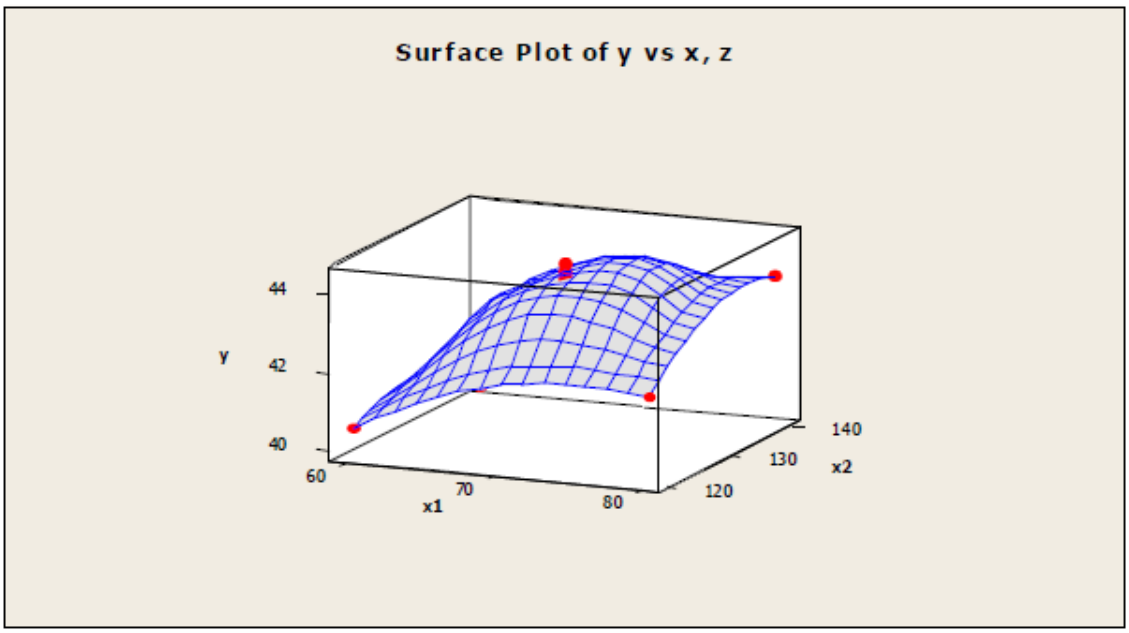

Figure 2.6: Response surface plot.

From the example of three-dimensional response surface plot in **Figure 2.6**, 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).

Design Expert Version 7.0 is software which applies important statistical and mathematical methods to find the best model to describe the response data. A three dimensional surface graph for the responses will be modelled out where the optimization point can be easily obtained from (Ba ş and Boyacı, 2007). There are several types of design of RSM such as three-level factorial, Box-Behnken, D-Optimal and CCD.

2.4.1 Central Composite Design

According to Bezerra *et al.* (2008), CCD is the most employed design of optimization for the development of analytical procedures compared to the others as their low efficiency of the latter especially for a numbers of variables. CCD is a second order factorial design utilized in RSM since full factorial design (FFD) possessed too large number of runs which is less practical (Box and Wilson, 1951).

The design composed of a full factorial, an additional design in which experimental points are located at a distance α from center point and a centre point. The experiment number is based on the number of parameters as expressed as

$$N = k^2 + 2k + c_p \quad (2.10)$$

where k is the parameter number and c_p represents the replicate number of central point. The replication of central point is to estimate experimental error while axial points are to ensure the rotate ability of the design. All design factors are codified in 5-levels which are – α, -1, 0, +1, + α. In this study, the independent variables number are two, thus there are 13 experiments to be completed including five centre points. 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). CCD for two variables and three variables optimization are shown in **Figure 2.7** in (a) and (b), respectively.

Salleh *et al.* (2011) carried out the optimization process by comparing CCD and FFD. The R^2 obtained were 0.998 and 0.96 for CCD and FFD respectively. This implies that CCD has the higher accuracy compared to others such as FFD.

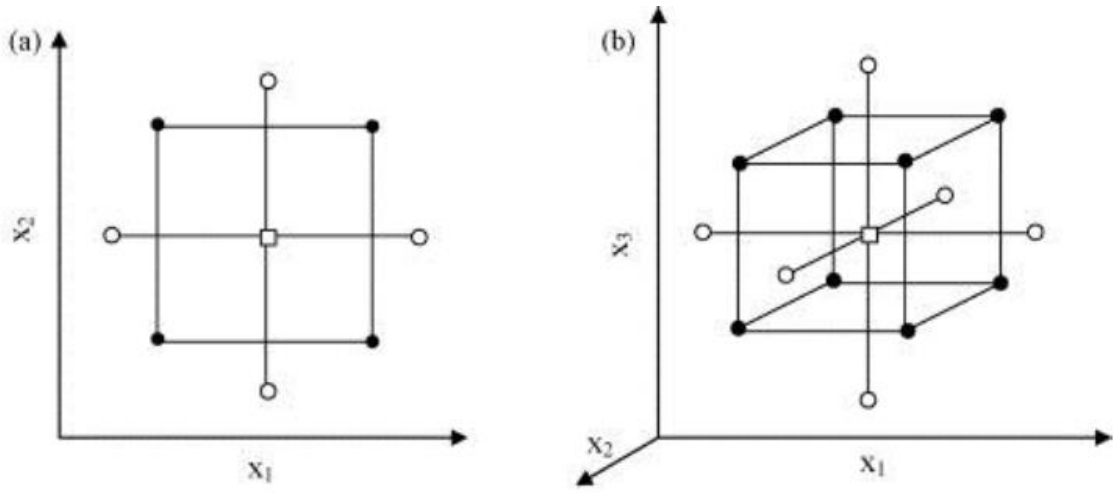

Figure 2.7: Central composite designs for the optimization of: (a) two variables and (b) three variables. (●) Points of factorial design, (○) axial points and (□) central point.

2.4.2 Analysis of variance (ANOVA)

ANOVA is a statistical technique used to analyse relationship and variation between quantitative dependent variable and qualitative independent variables. It was used to estimate the significance of model coefficients. Sir Ronald Fisher pioneered the development of ANOVA for analysing experimental results (Fisher, 1974). The objective of ANOVA is to test whether the response means are identical across factor levels. A replication means that when two or more independent experimental units are utilized for a factor level (Larson, 2008).

From the statistical analysis using ANOVA, the program suggests the best fitted model and provides a response graph for the measured response. The quality of the polynomial regression model was expressed by the coefficient of determination (R^2) and its adjusted value (Adj R^2). The R^2 coefficients value is 0 to 1, indicating the range of percentage of the variability in the response that could be explained by the model. The Fisher, *F*-ratio, is the ratio of the regression mean square to the mean square error. It is a variance ratio performed to test the significance of the regression model under investigation with respect to the variance of all the terms included the error at the desired significance level (Montgomery, 2001).

The probability value, *P*-value determination is to test the risk of falsely rejecting a given hypothesis. A "Prob. > *F*" value on the *F*-test indicates the expected time proportion to get the stated *F*-value if no significant effects of factor. A statistical test can be performed to indicate the significance of the replicate error in comparison to the dependent error of the model as the replicate measurements are available (Montgomery, 2001). In this test, the residual or error of sum of squares is divided into two portions, first which is due to pure error based on replicate measurements and the second is due to lack-of-fit based on performance of model. The lack-of-fit test is a ratio of lack-of-fit mean square to the pure error mean square. An insignificant lack-of-fit is desired as it indicates there is no contribution in the regressor-response relationship accounted by the model (Noordin *et al.*, 2004).

Additionally, the model adequacy is investigated by the residuals examination which shows the difference between the observed and predicted responses using the normal probability plots of the residuals and residuals versus the predicted response plot. A straight line is generated on the normal probability plots in an adequate model while the

residuals versus predicted response plot should contain no obvious patterns (Montgomery, 2001).

2.5 Factors Affecting Biogas Production

Despite there are several factors affecting the biogas production in AD, there are five parameters were selected based on the most straightforward factors for the screening process. Therefore, agitation speed, reaction time, substrate-to-inoculum ratio, process system and type of substrate were chosen in the previous study. Results obtained from previous studies of screening have indicated that agitation speed (rpm) and reaction time (days) were the two most significant parameters that influenced the production of biogas from poultry manure wastewater via AD. Any drastic changes in these can adversely affect the biogas production. So these parameters should be optimized within a desirable range to operate the experiment efficiently.

2.5.1 Effects of Mixing

Substrate agitation or mixing is crucial to maintain the fermentation process stability within the digester. Auxiliary mixing enhances the efficiency of substrate conversion in digester by provides intimate contact between poultry manure wastewater and its inoculum. Besides, mixing plays the role to ensure organic material being transferred efficiently for the active microbial biomass inside an anaerobic digester (Ward *et al.*, 2008). It avoids both the scum layers formation on the surface and the sedimentation of sludge on the bottom of the digester (Igoni *et al*., 2008). In addition, there will be occurrence of natural mixing in the anaerobic digester due to gas bubbles rise and the currents of thermal convection when the sludge is added with inoculum which generate reaction once combined (Appels *et al*., 2008). Moreover, mixing provide a uniform bacterial population density and prevent the formation of dead spaces that would reduce the effective digester volume.

Although mixing system is essential factor for biogas production, but there is still a certain mixing degree which is necessary for introducing substrate to the bacteria during AD process. On the other hand, an excessive mixing can cause reduction in biogas production (Ward *et al.*, 2008). It has been proved that reducing mixing conditions provide a better performance and stabilization for a continuously-mixed anaerobic digester (Stroot *et al.*, 2001). This is because excessive agitation can lead to anaerobic

granule structure disruption, and thus slowing down the rate of oxidation of fatty acids which can lead to instability of digester (McMahon *et al.*, 2001). 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). Typically, there are three methods of mixing *viz.*, mechanical mixing, hydraulic mixing and gas recirculation (EPA, 2006).

2.5.2 Effects of Reaction Time

The period of time that substrate resides in the anaerobic digester is defined as retention time or residence time. Retention time in AD process is the average period for degradation of organic material. The appropriate retention time period depends on technologies, process temperature and the variety of feedstock undergoing AD process.

An optimum hydraulic retention time (HRT) is crucial to the treatment of liquid poultry manure. Ho *et al.* (2013) investigated that AD under thermophilic conditions (40 - 60 ℃) able to generate biogas in a shorter retention time than that operates under mesophilic conditions (25 – 40 ℃). However, the quantity of ammonia content in an anaerobic digester also rises with the increasing temperature and this has a known inhibitory effect on methanogenic bacteria which lead to reduction of biogas yield. Besides, higher gross energy is required to maintain the thermophilic conditions within the anaerobic digester (Biogasaustralia.com.au, 2012). Hence, the operating temperature in this research was set at ambient temperature of 25 ℃.

Additionally, AD depends on the biological activity of relatively slowly reproducing methanogenic bacteria. These bacteria must be provided with sufficient reproduction period of time, so that they can substitute cells lost with the effluent sludge, and adjust their population size to follow fluctuations in organic loading rate (OLR). AD of poultry waste is preferably to operate at shorter HRT so as to meet the requirement of economics and environmental beneficial extent for biogas production (Sakar *et al.*, 2009).

In bioengineering studies, Doran (2013) defined the dilution rate for the reverse of HRT. When the dilution rate is greater than the growth rate of the microbial cells in the reactor, wash out will occurs, and otherwise the microbe will be accumulated in the reactor. In this case, too low retention times might cause a significant washout of biomass (Polo and Biswas, 2006). This washout can be avoided by maintaining a sufficient retention

time for substrate to ensure that the bacterial cells remain in optimal concentration within the anaerobic digester. When operating AD under short HRT, there is insufficient contact time available for CM sludge granules to mineralize organic matters and the intermetabolites (Zhang *et al.*, 2014). Thus, devising suitable HRT for AD of poultry manure wastewater for biogas production is a topic of major interest.

2.5.3 Effects of Ratio of Substrate to Inoculum

Eskicioglu and Ghorbani (2011) studied that the ratio of substrate to inoculum (S/I) is essential to ensure an optimum control of biogas production. Inoculum is commonly obtained from AD fed with similar type of substrate to ease adapted microbial species. In addition, the content of inhibitors, such as ammonia and heavy metals should be in minimum amount. This is due to the AD of high strength poultry manure wastewater will be inhibited significantly by free ammonia (González-Fernández and García-Encina, 2009).

The research conducted by González and García resulted that the biogas yield decreased with increasing S/I after its optimized condition as the higher ratios will require longer HRT to decompose organic matter, which also lead to increase in anaerobic digester volumes. The study conducted by Hashimoto (1989) resulted that S/I below 4.0 yielded drastically low amount of methane production from AD of straw.

Generally, an exorbitant S/I value may lead to toxicity whereas a meager S/I value may inhibit the induction for biodegradation of enzyme in the reaction (Prashanth *et al.*, 2006). Chen and Hashimoto (1996) proposed that the low S/I will shorten the lag phase in methane production.

2.5.4 Effects of Reactor Mode

Laboratory reactors process system can be either batch, fed-batch, continuously or intermittently mixed (Wu, 2007). Ndegwa *et al.* (2008) suggested that anaerobic sequencing batch reactor (ASBR) remarked higher potential to improve the economics in AD of animal waste. Operating principle of ASBR follows four phases *viz.*, feed, react, settle, and decant in cyclic at fixed HRT. The studies proved that AD of dilute manure slurries in ASBR is more effective at room temperature than at 35 ℃.

Generally, the two reactor models which always utilized in AD process technology include batch process and continuous process. In the batch process, the feedstock

substrate is initially feed into the digester and sealed properly during the degradation period. After the completion of predetermined retention time, the digester is opened to remove the effluent. On the other hand, fresh substrate is continuously feed into the digester in the continuous process. In this process, the digested effluent is removed in an equal amount (Ostrem, 2004). The reactor mode being employed in this research work for biogas production was a batch process.

2.5.5 Effects of Type of Substrate

There are different types of biomass appropriate to be used as substrate in AD for biogas production such as food crops, plants, animal manure and phototrophic microorganisms. Among the agricultural wastes, animal manure such as cow and pig slurry, CM and farmyard manure are of primary importance. Food crops have not gained large significance in AD as it has more competitive edge on energy source provision. In addition, there is limited land for crop production on the earth. Harvest residues and garden wastes are rather preferable to undergo traditional treatments for composting, soil conditioning and fertilizer purposes (Fantozzi and Buratti, 2009).

Generally, selection of substrate for AD depends on their nutrient contents of chemical composition. The main nutrient components of the substrate include fats, proteins, carbohydrates and cellulose. As previously mentioned, the typical biogas contains approximately 50 % to 70 % CH_4 and 30 % to 40 % CO_2. The theoretical ratio of CH_4 to CO_2 for various substrates components were determined using equation (2.11) developed by McCarty (1964).

$$C_xH_yO_z + \left(x - \frac{y}{4} - \frac{z}{2} \right) H_2O \rightarrow \left(\frac{x}{2} - \frac{y}{8} + \frac{z}{4} \right) CO_2 + \left(\frac{x}{2} + \frac{y}{8} - \frac{z}{4} \right) CH_4 \quad (2.11)$$

The theoretical CH_4 content of biogas for major components in substrate are shown in Table 2.3. Substrates which consist of high readily degradable components such as fats, protein and carbohydrate contribute to highest biogas yield (Hobson *et al.*, 1974). Although fats provide the highest biogas production, however it required highest retention time for AD due to poor bioavailability. Proteins and carbohydrates reported fastest conversion rates.

Table 2.3: Theoretical Methane content of biogas.

Substrate Components	**Chemical Composition**	**CH_4 (% of total biogas)**
Fat	$C_{15}H_{31}COOH$	72
Protein	C_4H_6ON	63
Carbohydrate	$C_6H_{12}O_6$	50

The bioconversion of animal manure into methane gas considered as a mature technology to recover pollutants into energy resource as compared to other biomass (Rao *et al.,* 2010). Besides, the digestate residues produced from AD of poultry droppings can be used as cleaner bio-fertilizers (Möller and Müller, 2012).

2.5.6 Selection of factors for optimization

The two major factors being studied in this research work were agitation speed and reaction time. It is important to obtain an optimal condition with suitable agitation speed and reaction time for the operation of AD of poultry manure wastewater for biogas production. An optimized biogas yield can be obtained at suitable agitation speed and reaction time as this can sustain the feasibility on AD of poultry manure wastewater by provide sufficient intimate contact between substrate and the inoculum.

3 MATERIALS AND METHODS

3.1 Overview

This chapter discusses the materials and methods that were adopted in the experimental work. A schematic process flow was constructed and illustrated in Figure 3.1. The process flow explains the biogas production from poultry manure wastewater in laboratory scale experiment and also the analysis of biogas produced. The subchapter covers in this chapter are collection of sample, characterization and pre-treatment of substrates, preparation of sample, design of experiment, laboratory experimental set up and COD-vial method analysis. These methodologies were used thoroughly in current study. The operating condition and the factors screened for the optimization of biogas production from poultry manure wastewater was obeyed the continuation of research in the same group.

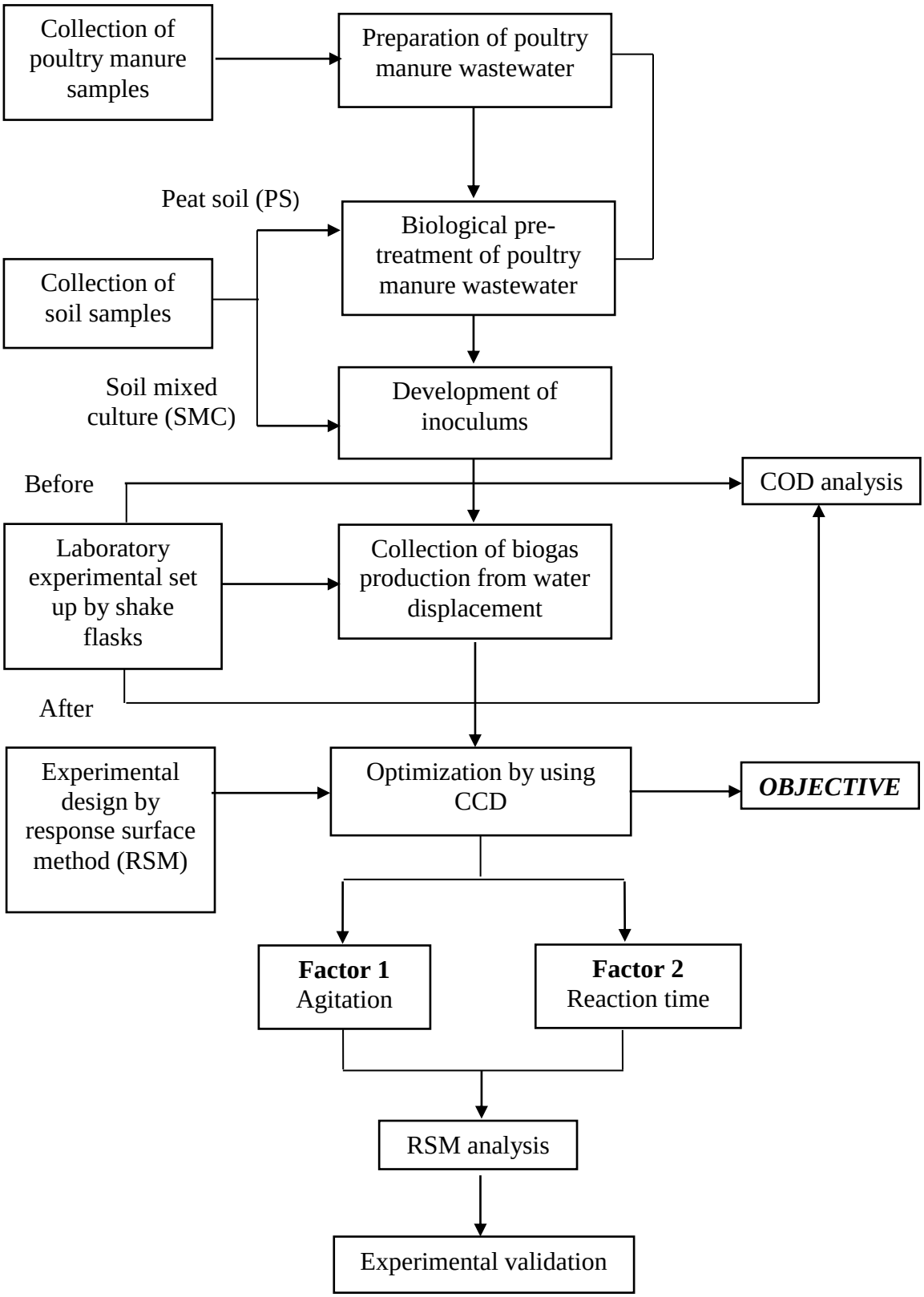

Figure 3.1: Schematic process flow of the experiment.

3.2 Collection of Sample

CM sample and soil for pre-treatment and inoculum purposes were collected from three sampling sites in a poultry manure farm in Gambang, Pahang. Firstly, the CM sample was collected directly under the chicken barn area as shown in Figure 3.2. Next, the soil sample for pretreatment purpose was collected at area nearby to chicken barn as shown in Figure 3.3. After that, the soil sample for inoculum purpose was collected nearby the CM collection point as illustrated in Figure 3.4.

Figure 3.2: Chicken manure sample collection sites.

Figure 3.3: Soil sample collection sites for pre-treatment purpose.

Figure 3.4: Soil sample collection sites for inoculum purpose.

3.3 Preparation of Poultry manure wastewater

In order to maintain the moisture consistency, the CM sample collected was mixed thoroughly with distilled water at a feed ratio of 1:1 for 5 to 10 minutes to produce poultry manure wastewater (PMW). After that, it was kept in freezer at 4 ℃ to avoid any initial fermentation (Davies *et al*., 2000). Demirci and Demirer (2004) reported that nutrients content in the manure can be sufficient for anaerobic microbial growth if sufficient amount of water is present.

3.4 Characterization and Pre-treatment of substrates

The PMW used as model substrate was tested for its biochemical characteristics as presented in Table 3.1. After characterization, PMW was gone through pre-treatment processes to remove excessive ammonia-N which might cause inhibition on biogas production.

The type of soil collected for pre-treatment purpose, namely peat soil (PS) was kept frozen just prior to use. Upon pre-treatment, PS was mixed thoroughly for 5 to 10 minutes with distilled water to produce soil water (SW). Previous screening study suggested that the best pre-treatment condition using PS to distilled water of 1:6 ratio to produce SW, and SW to PMW at 1:4 ratios without agitation for 5 hours reaction time (Jamaludin and Zainol, 2013).

The pre-treatment experiment was conducted at laboratory scale study by using 250 ml conical flask under aerobic condition. The flask was filled with poultry manure wastewater first, and then reaction time started as soon as the SW was added. Ammonia-N and COD concentration were determined by using HACH Spectrophotometer DR/2800 following Method 8155 and Method 8000, respectively with suitable dilution factor.

Table 3.1: Test method for characterization of poultry manure wastewater.

No.	Parameter	Unit	Test method
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

3.5 Preparation of inoculum

Soil sample used in soil mixed culture (SMC) for inoculum development was different from the soil used for pre-treatment purpose. Poultry soil was mixed thoroughly with distilled water at a ratio of 1:6 for 5 to 10 minutes to produce SMC. Treated PMW was acclimatized with SMC anaerobically at a substrate inoculum ratio (S/I) of 1:4 in 5 litres plastic digester, producing seeding to be used in AD for biogas production. The HRT for acclimatization was 30 days under ambient temperature. The inoculation of the fresh material with either digested material or the liquid fraction from the reactor was used by most reactors to minimize washout of microorganisms (Ward *et al.*, 2008).

3.6 Preparation of Sample

Substrates, treated PMW were poured into three labeled 250 ml conical flasks followed S/I of 4:1 obtained from the continuation of research in the same group. This fact is supported in which high instantaneous food-to-microorganism ratio (F/M) favored the metabolic activity and microbial growth to produce biogas from poultry manure wastewater (Lobos *et al.*, 2008). The flask was shook gently and 1 ml sample was took out into labeled 50 ml beakers for further dilution follow a dilution factor of 10. Then

the flasks were added with inoculum and closed with rubber stopper together with gas line piping. After closing, the flasks were sealed perfectly with parafilm to avoid unwanted contamination.

3.7 Design of Experiment (RSM)

The two factors of agitation speed (A) and reaction time (B) were used to optimize the production of biogas. RSM using Design Expert V7.0 was introduced for analysis of optimization in the experiment. Under RSM, CCD was selected to insert response results. These two independent variables involved in CCD with preset range and levels are shown in Table 3.2. Next, an experimental design table was constructed. Then, experimental run was sorted in standard order to avoid bias as illustrated in **Table 3.2**. All the experiments were run in triplicate. Data obtained from lab experiment were inserted into the response column and were analyzed statistically using ANOVA. After the suggested optimum conditions has obtained, validation run was conducted.

Table 3.2: Independent variables involved in Central Composite Design (CCD)

Independent variable	Units	Range and level				
		2.00(-α)	-1	0	+1	2.00(+α)
Agitation speed	rpm	100	110	120	130	140
Reaction time	days	1	2	3	4	5

Table 3.3: Preliminary optimization design of CCD in Design Expert V7.0 software.

Standard	Run	Factor 1: Agitation speed (rpm)	Factor 2: Reaction time (days)
1	12	110	2
2	2	130	2
3	5	110	4
4	8	130	4
5	4	100	3
6	13	140	3
7	11	120	1
8	1	120	5
9	6	120	3
10	7	120	3
11	10	120	3
12	3	120	3
13	9	120	3

3.8 Laboratory Experimental Set Up

The laboratory scale biogas production was conducted in batch mode under ambient temperature via AD in shake flask experiments (Zakarya *et al*., 2008; Cone *et al*., 1996). Standard 250 ml Erlenmeyer flask was used in shake flask AD as laboratory scale shakers are mostly designed to use these flasks. The New Brunswick Scientific Shaker was utilized for agitation purpose in this research study. The medium was kept uniform by constant agitation during incubation (Rymer *et al*., 2005). Each experimental run was performed in triplicate. Retort stands with clamps were assembled to ensure the mechanical stability of the experiment.

The concept using water displacement was used to collect biogas production (Beuvink and Spoelstra, 1992). Firstly, the measuring cylinders were filled with pipe water and were placed inversely into a tray which was filled with pipe water. In this step, bubble was strictly avoided to enter when setting up. Measuring cylinders were clipped to lock firmly on a retort stand before all respective gas pipelines being inserted. Extra water in the tray was transferred out to avoid unnecessary pressure act to slow down the bubble production. The gas pipelines were observed from time to time and the biogas production were recorded according to HRT of that particular run. **Figure 3.5** shows the water displacement experimental set up for shake flasks analysis which was carried in Environmental laboratory.

Figure 3.5: Laboratory experimental set up.

3.9 Chemical Oxygen Demand (COD)-Vial Method Analysis

Firstly, 2 ml of diluted sample was inserted into COD HR-PLUS vials and cap tightly. Secondly, the vials were inverted gently for several times to mix. Thirdly the vials were preheated in 150 ℃ COD digestion reactor namely HACH DRB 200 for two hours. After two hours, the vials were placed into a rack and cooled down to room temperature. Then the vials were wiped using tissue paper to clean off fingerprint before being put into HACH Spectrophotometer with wavelength of 435 nm to obtain the COD value for respective samples. This HACH Spectrophotometer namely DR5000 following Method 10212 can detect a reading range of 0 – 15,000 mg/L COD. A labeled blank vial was injected with 2ml of distilled water as a standard blank to obtain more accurate COD readings for each sample. Finally, the results reading obtained were multiplied with the dilution factor of 10. Both of the equipment used for sample analysis, spectrophotometer HACH DR5000 and COD digestion reactor HACH DRB200 are illustrated in **Figure 3.6** and **Figure 3.7** respectively.

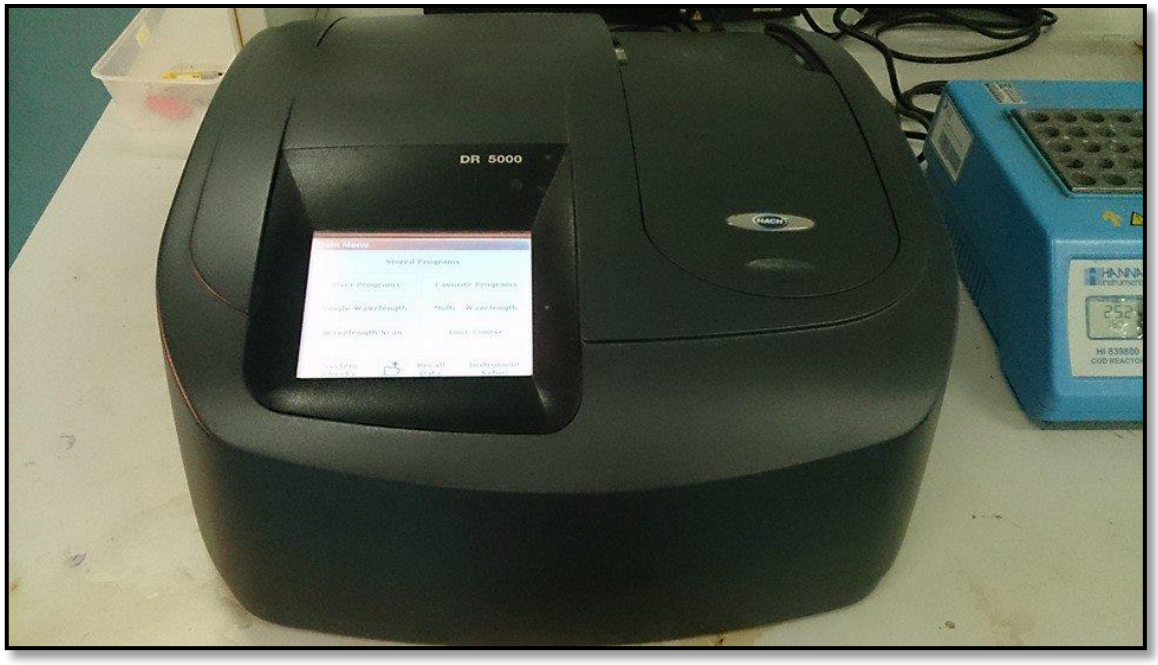

Figure 3.6: Spectrophotometer HACH DR5000.

Figure 3.7: COD digestion reactor HACH DRB200.

3.10 Validation experimental set up

The optimum condition for the biogas production from poultry manure wastewater depended on the agitation speed and reaction time were obtained from the predictive equations of CCD. By using the optimize parameter set points, the experiment was carried out once again under two different reaction time to obtain the experimental response of biogas production. The optimum condition predicted by CCD in Design Expert V7.0 was agitation speed of 120 rpm and 3.3 days of reaction time for the biogas production. The experimental and predicted values were compared in Table 3.4 in order to determine the validity of the model.

Table 3.4: Validation experiment condition

Factor 1: Agitation speed (rpm)	**Factor 2: Reaction time (days)**
120	3
120	3.3

4 RESULTS AND DISCUSSION

4.1 Characterization of substrates

The characteristics of PMW and treated PMW were listed in **Table 4.1**. The characteristics of poultry manure wastewater studied by Yetilmezsoy and Sakar (2008) were almost similar compared to this study. In that study, the pH, COD, suspended solid and phosphorus concentration, were 7.30, 21,100 mg/L, 446 mg/L, respectively.

From **Table 4.1**, the pHs for PMW before and after treated were at 8.1 and 7.5, respectively. These pHs were in good range as the anaerobic microorganisms for biogas production are less sensitive and can function in a wider range of pH between 4.0 to 8.5 (Hwang *et al.*, 2004). When pH is below 4.0 or above 8.5, AD will be inhibited. When pH is below 4.0, the activity of the methanogens is completely suppressed. Only when pH value is strictly regulated in the range of 4.0 to 8.5, methanogens can grow healthily and play a role of biocatalyst. If pH is out of optimized range, the amount of soluble organic matter and other sulphur-contained organic compounds will increase greatly in the AD. These will then lead to growth inhibition of methanogenic bacteria which yield biogas (Sung *et al.*, 2014).

In this study, the initial COD concentration for PMW of 35,600 mg/L was about 7 times higher than treated PMW at 4985 mg/L. Cakir and Stenstrom (2004) reported that wastewater having wide range of COD concentration of 2000 to 20,000 mg/L. Biological and chemical oxygen demand (BOD and COD), are water quality analyses commonly used to indicate the amount of organic matter present in wastewater. BOD and COD are biodegradable and could degrade readily in soil (Cogger, 1998).

An important characteristic was suspended solids (SS) content, which 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 treatment using soil water was proven were able to decrease the AN content to avoid inhibition. The AN content reduced after the PMW treatment using soil water. It was estimated that microorganisms with more than 100 million in population and several thousands of species live in 1 g of soil (Trosvik et al., 2008). 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. 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 Sung and Liu (2003), AN concentration below 200 mg/L are beneficial to anaerobic process. However, AN inhibition can start at AN content up to 1000 mg/L. 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.*, 2010). 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).

Table 4.1: 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

4.2 Optimization studies with CCD

In this design of experiment, CCD was implemented for the optimization of biogas production. The two factors involved in this study were agitation speed and reaction time. By using CCD, a total of 13 runs were generated with different set up condition. The response of biogas yield attained from the experiment was tabulated in **Table 4.2**. These results data were input into the Design Expert V7.0 software for further analysis. By employing multiple regression analysis on the experimental data, the optimization result data generated from RSM was fitted with a second order polynomial equation as shown in equation (4.1).

$$\text{Biogas yield (L/g COD)} = -0.13039 + 2.12578 \times 10^{-3} A + 3.75473 \times 10^{-3} B - 1.38744 \times 10^{-6} AB - 8.81116 \times 10^{-6} A^2 - 5.42927 \times 10^{-4} B^2 \quad (4.1)$$

where A and B represent agitation speed and reaction time respectively.

Table 4.2: Result of optimization of biogas yield in CCD.

Run	Agitation (rpm)	Reaction time (days)	Biogas yield Experimental (L/g COD)
1	120	3	0.003607
2	130	4	0.002040
3	130	2	0.001249
4	120	5	0.002497
5	120	1	0.001332
6	110	2	0.000957
7	140	3	0.000638
8	120	3	0.004370
9	120	3	0.003954
10	110	4	0.001804
11	120	3	0.003954
12	120	3	0.004162
13	100	3	0.000486

4.3 Statistical Analysis

In order to analyze the results obtained, there are three tests need to be performed, which are test for significance of the regression model, test for significance on individual model coefficients and test for lack-of-fit.

From ANOVA result summarized in Table 4.3, the Model F-value of 7.86 and the *p*-value of 0.0086 imply the significant of model. There is only a 0.86 % probability that a Model F-value this large could occur due to noise. This is desirable as it indicates the significant effect on the response of the model. In the same manner, both of the second-order effects which are agitation (A^2) and reaction time (B^2) categorized as significant model terms with *p*-value less than 0.05. Other insignificant model terms can be terminated to generate an improved model. However, those models required to support the hierarchy are not counting.

The Sum of Squares for the Model source was 2.125×10^{-5}, which represented the summation of Regression Sum of Squares for the quadratic regression model. Each regression source has corresponding degrees of freedom (DF) of one and hence contributes 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 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. This means that there was some significant effect that has been neglected and that effect was a function of the factors which already existed in the model. A little change in the parameters might affect the fit of model. It was advisable to add more factors such as temperature and S/I ratio in order to make the lack of fit to become desirably insignificant. Apart from that, it was recommended to widen the range of the parameters so that outliers can be included.

This model having a satisfactory R-Squared value of 0.8489 which implies the model was adequate for the design space navigation. The adequate precision measures the signal to noise ratio which compares the predicted values range at points of design to the average prediction error. A ratio greater than 4 is desirable for an adequate model. In this particular case, the ratio of 7.327 indicates adequate signal discrimination.

Table 4.3: Result for ANOVA.

Source	Sum of Squares	DF	Mean Square	F-value	*p*-value
Model	2.125×10^{-5}	5	4.259×10^{-6}	7.86	0.0086
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
Lack of fit	3.470×10^{-6}	3	1.157×10^{-6}	14.39	0.00131

4.4 Residuals Analysis and Diagnostic Plots

Residual analysis is necessary to ensure that the assumptions for the ANOVA are met. From the least squares fit, the residuals (e_i) play a crucial role in judging the adequacy of the model and are defined by equation (4.2). The difference between the actual individual values is indicated as y_i while the predicted value from the model is indicated as $\hat{y}_i$.

$$e_i = y_i - \hat{y}_i \qquad \text{where } i = 1, 2, 3, \ldots, n \qquad (4.2)$$

Diagnostic plots generated from CCD using Design Expert V7.0 are reviewed in residuals analysis to determine the feasibility of the model. The normality assumption may be checked by a normal probability plot of the residuals. The experimenter handbook by Kraber *et al.* (2002) stated that a good normal probability plot should shows a linear straight line whereas an S shape indicating a bad normal plot. The handbook also mentioned that good residuals versus predicted response plot should be random scatter whereas a bad plot of the kind will shows a megaphone shape. If the variance of the response depends on the mean level of *y*, then this plot will often exhibit a funnel-shaped pattern. This is also suggestive of the need for transformation of the response variable *y*. A review on the normal probability plot for biogas yield as

illustrated in **Figure 4.1** revealed that the residuals generally fall on a straight line implying that the errors are distributed normally. On the other hand, the residuals versus predicted response as shown in **Figure 4.2** revealed that they are random scattered without obvious pattern and unusual structure. This general impression implies that the model proposed was adequate and there was no reason to suspect any violation of the independence or constant variance assumption.

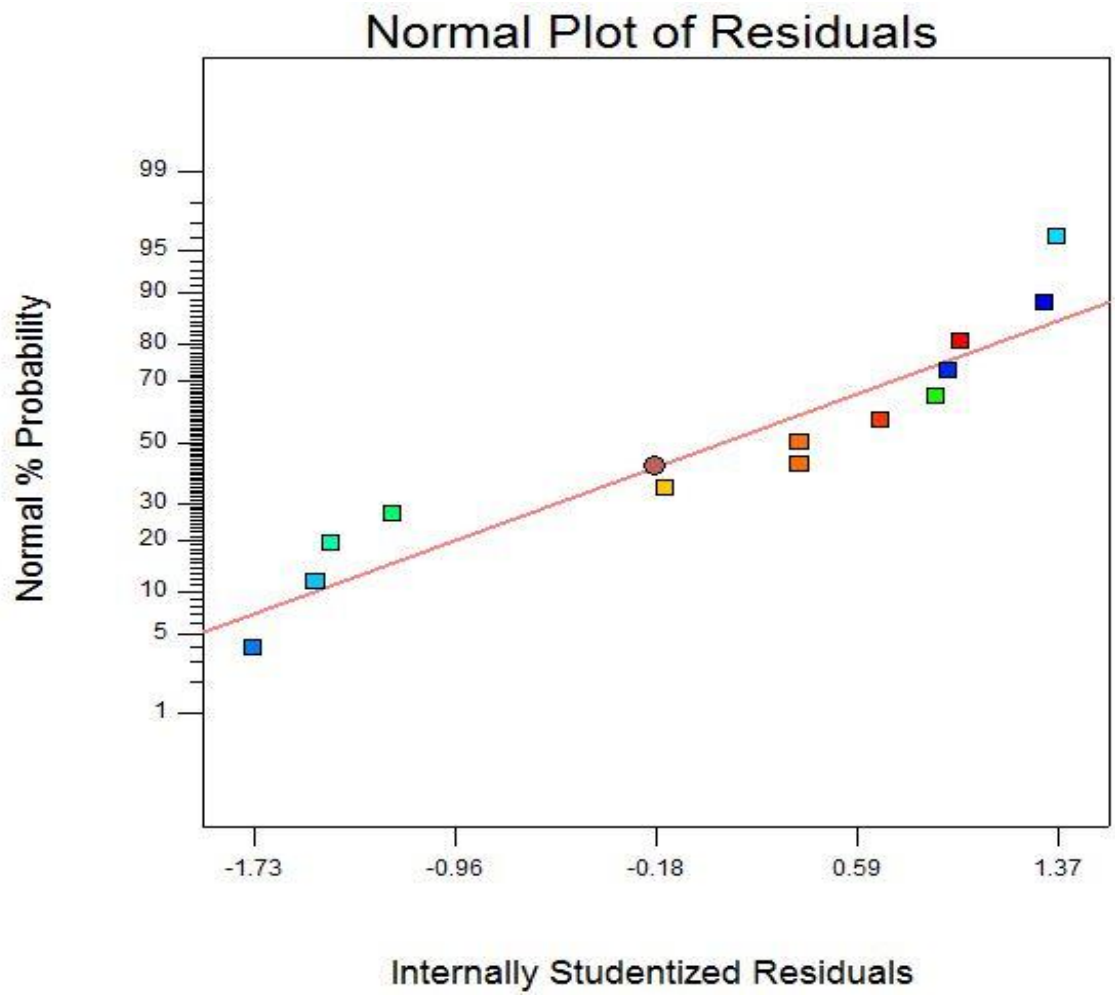

Figure 4.1: Normal probability plot of residuals for biogas yield data.

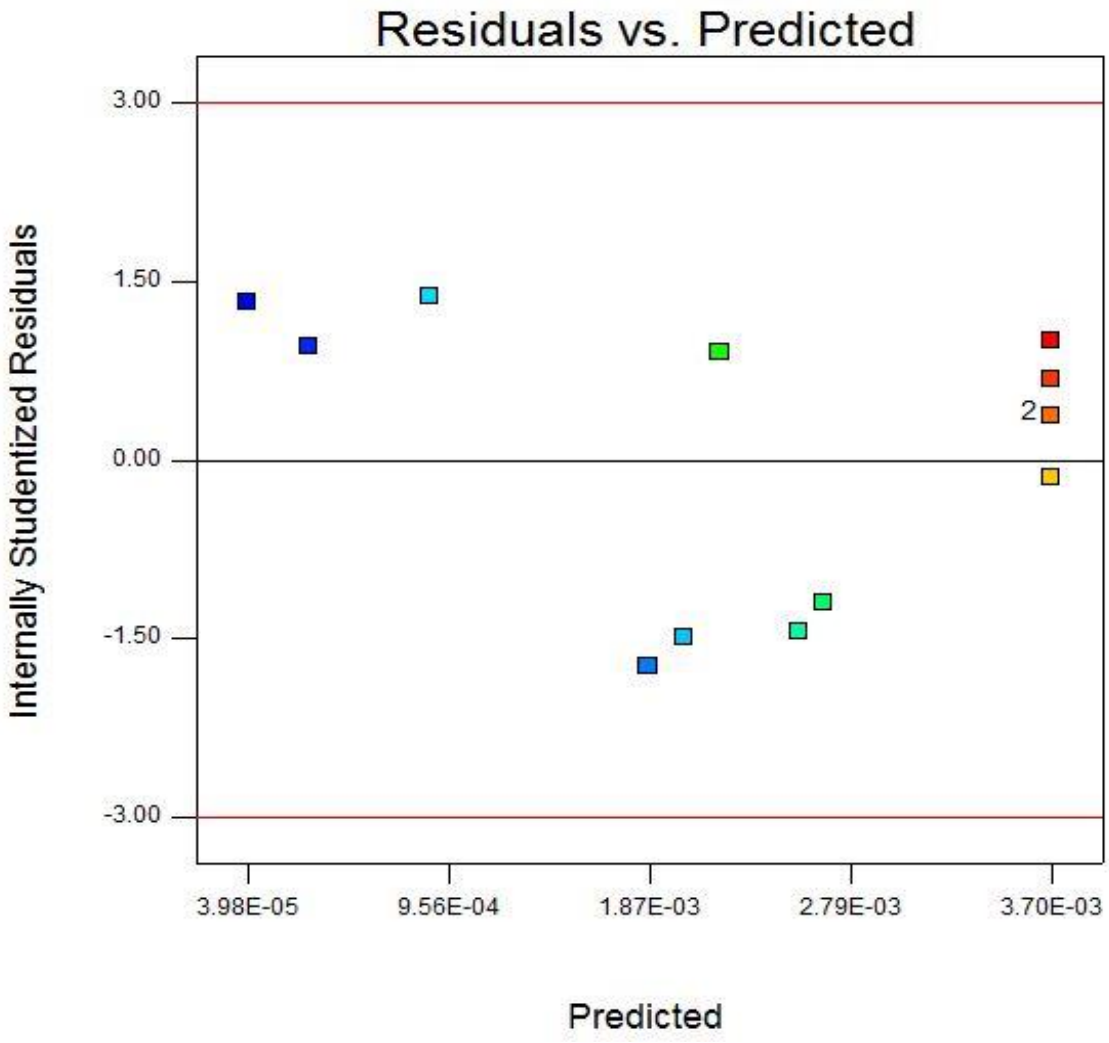

Figure 4.2: Residuals versus predicted response plot for biogas yield data.

4.5 Main Effect Contribution

The contour plot graph of the effects of agitation and reaction time on the biogas yield is illustrated as in **Figure 4.3**. The units for the response biogas yield, agitation and reaction time were L/g COD biogas, rpm and days, respectively. **Figure 4.3** clearly show that the agitation of 120 rpm and reaction time of 3 days yield highest biogas production. The yield of biogas decreased when agitation and reaction time were out of this condition. From the contour plot, the elliptical profile proved an extraordinary interaction between agitation and reaction time. It can be explained that as agitation increased, the yield of biogas was increased. This also happened to another parameter as the proportional relationship between reaction time and biogas yield. Nevertheless, once the agitation and reaction time were greater than the centre point value, the reverse trend was observed.

The three-dimensional response surface graph generated in a perfect dome shape in which maximum points yield 0.00437 L/g COD biogas as shown in **Figure 4.4**. This indicated the result obtained is optimized. This result in optimal conditions was at agitation speed of 120 rpm and reaction time of 3 days. Therefore, the optimization result data was used for this research study to be further validated.

In order to get a better understanding of the results, the response function for RSM data was assessed graphically by the use of perturbation plot. The perturbation plot helps to compare the effect of all the factors at a particular point in the RSM design space. It displays the effect of changing one factor from the reference point while holding the other factor constant. As can be seen from **Figure 4.5**, both agitation (A) and reaction time (B) affected the biogas yield in an almost similar trend of curvature. This indicates that both agitation and reaction time factors showed significant quadratic effects that contributed to the biogas yield.

For factor A, the biogas yield increased up to a certain point, which is at coded unit of 0.000, and dropped when the agitation speed increasing. Tailing of biogas yield peak reduces due to higher agitation than the 0 coded units which might cause substrate disruption. In this study, the effect of agitation to the optimization of biogas production was crucial because agitation provides auxiliary mixing which enhances the efficiency of substrate conversion in digester by provides intimate contact between poultry manure wastewater and its inoculums (EPA, 1999). Mass and heat transfer also can be fostered by agitation which can improve efficiency of mixing (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 (Igoni *et al*., 2008). In addition, there will be occurrence of natural mixing in the anaerobic digester due to gas bubbles rise and the currents of thermal convection when the sludge is added with inoculums which 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).

On the other hand, for factor B, the biogas yield showed an upward trend when the reaction time increased. However, the tailing of growing trend started to slow down

after the coded unit range of 0.000 to 0.500. Reaction time can be considered as another vital factor in the determination of optimum condition for biogas production. This due to the fact that an optimum HRT is crucial to the treatment of liquid poultry manure. AD of poultry waste is preferably to operate at shorter HRT so as to meet the requirement of economics and environmental beneficial extent. This is because under short HRT, the decomposition of organic matter can be achieve efficiently without accumulating excessive residual and other intermediate products such as volatile fatty acids (Ndon and Dague, 1997). HRT depends on other factors, such as feed stock and operational temperature (Sakar *et al.*, 2009). Based on Sakar *et al.* (2009), the HRT of AD of poultry manure studied were between 13.2 h and 91 days under mesophilic conditions which maintained between 25 and 35 ℃.

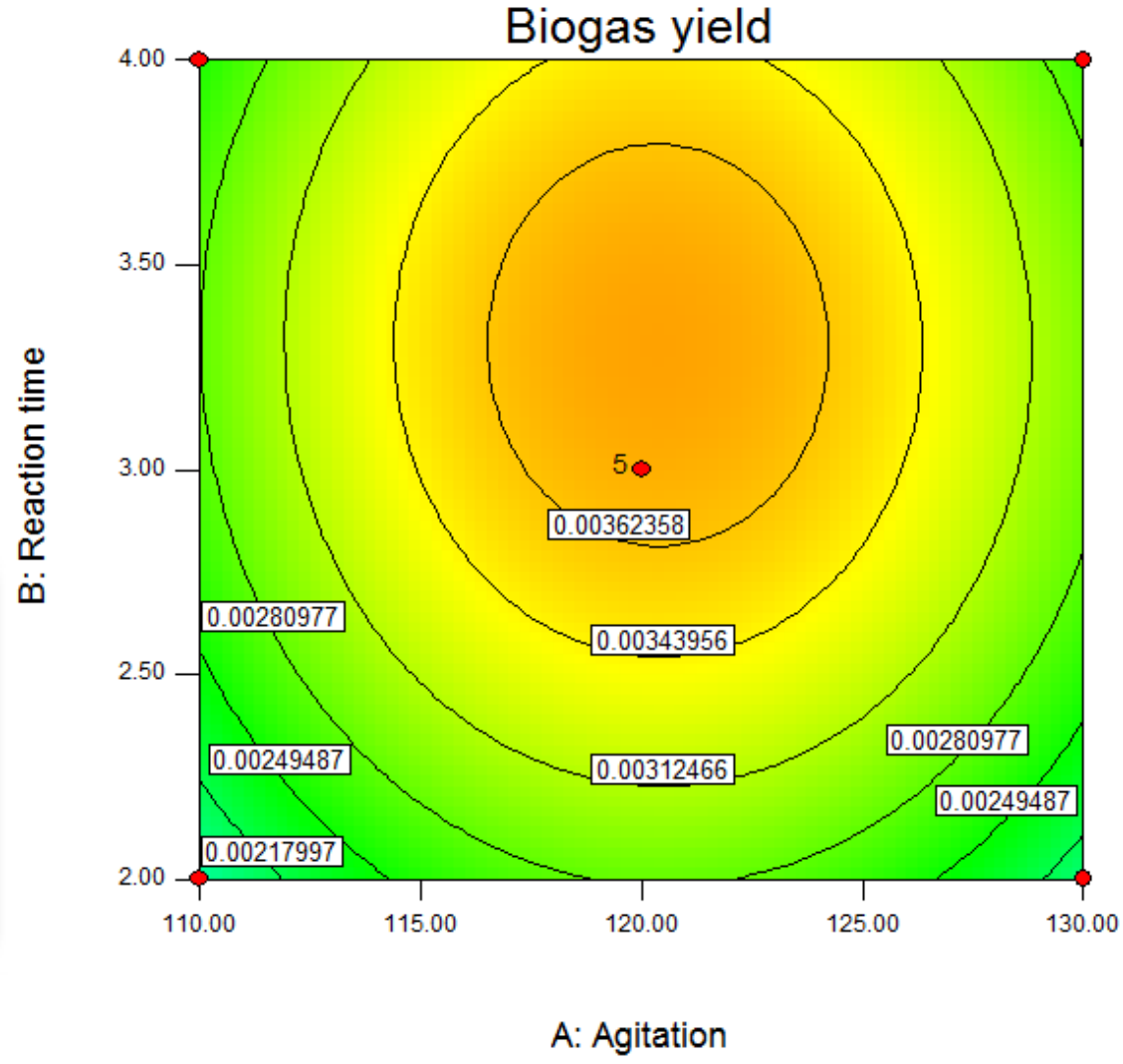

Figure 4.3: Contour plot graph of optimization.

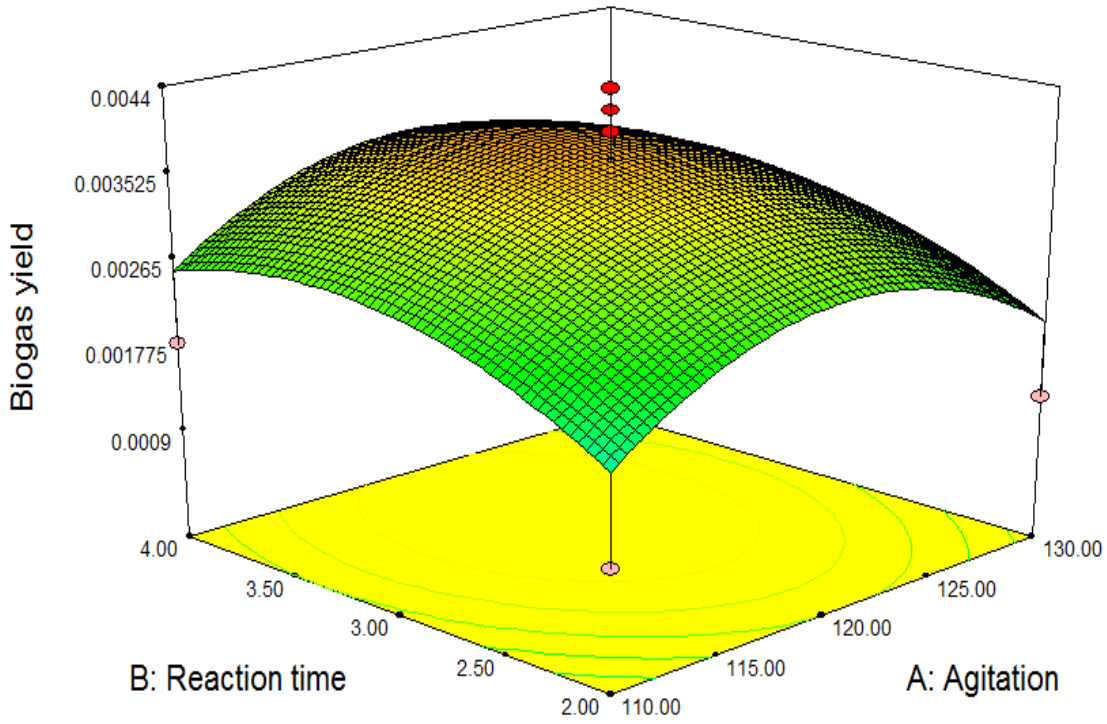

Figure 4.4: Model graph of optimization.

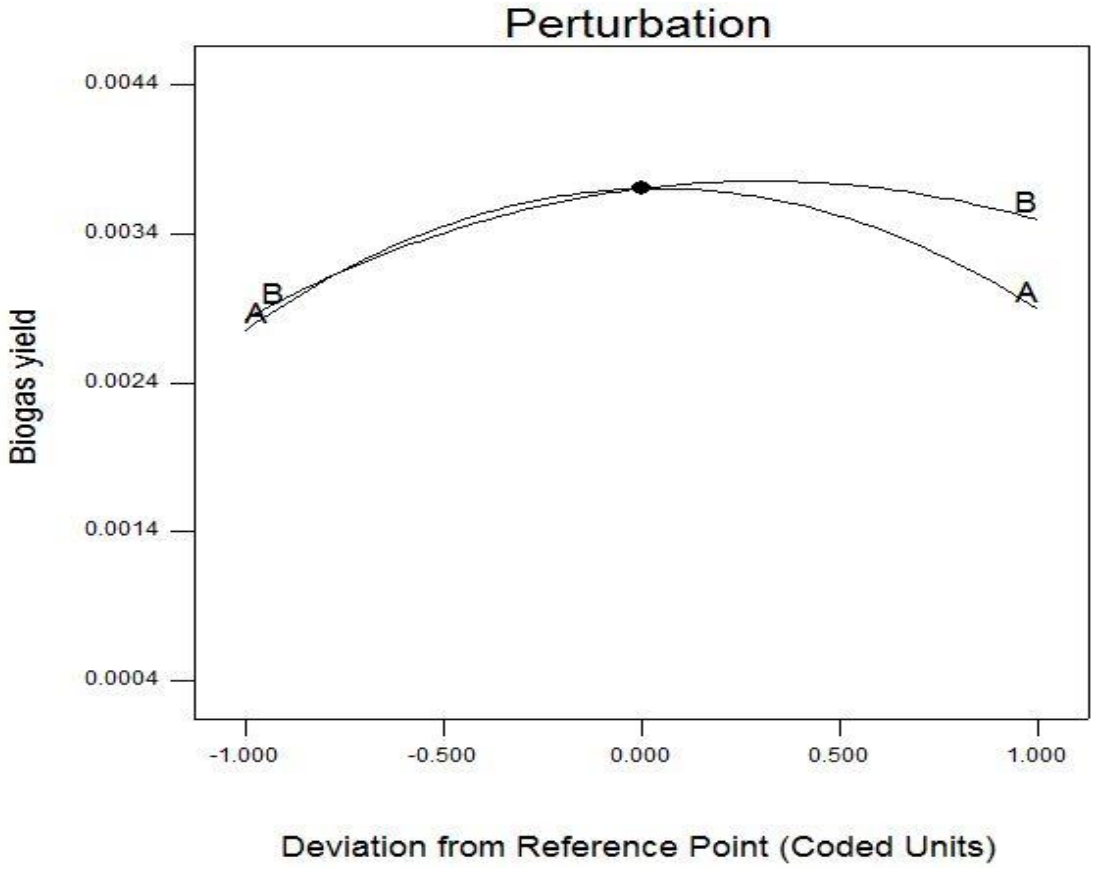

Figure 4.5: RSM Perturbation plot for biogas yield.

4.6 Interaction of factors

The interactive effect of agitation and reaction time on biogas production from poultry manure wastewater is plotted as in **Figure 4.6**. The non-parallel lines displayed in the interaction plot indicated that there was an interaction effect between agitation (A) and reaction time (B) on biogas production. According to Bakeman (2005), the less parallel the lines are, the most likely there is to be a significant interaction. In **Figure 4.6**, the lines are not parallel and there is no cross-over interaction, but an interaction would be expected. The biogas yield response grows curvilinear when the agitation increasing at a fixed level of reaction time factor.

At lower coded time factor (B-) which is 2 days of reaction time, agitation had a significant effect on biogas production. This was because during limited reaction time period, the agitation became the crucial factor in biogas production. In such short reaction time, the capability of biogas yield from AD of poultry manure wastewater was relatively lower compared to longer reaction time. Higher agitation at 120 rpm can supply adequate mixing which hence enhances the efficiency of substrate conversion in anaerobic digester by provides intimate contact between poultry manure wastewater and its inoculum. However, too high agitation (over 120 rpm) will cause cell disruption to microbial methanogens. This will directly lead to reduction of biogas production.

Similarly, at higher coded time factor (B+) which is 4 days of reaction time, agitation also showed a significant effect on biogas yield. In such case, the biogas yield response also affected in the same manner by the agitation as in lower coded time factor. In this longer reaction time, the biogas production was slightly increase because the poultry manure wastewater substrates were given longer duration of intact with the inoculum. This longer duration of reaction time supplied with high agitation of 120 rpm may definitely promise a higher yield of biogas from poultry manure wastewater as compared to short reaction time. However, too long period of reaction is tried to be prevented due to economical factor and the extent to the beneficial of environment.

The Least Significant Difference (LSD) bars act as the visual aids in assisting to interpret effect on interaction plots. As shown in **Figure 4.6**, the overlapping of the LSD bars for 2 means indicated that both lower coded time factor (2 days) and higher coded time factor (4 days) cover the same range of biogas yield. In the other words, it defines that the difference in those means is not large enough to be declared significant using a

t-test. The overlaps between pairs of LSD bars indicate that the associated means differ is not lie on 95 % confidence levels.

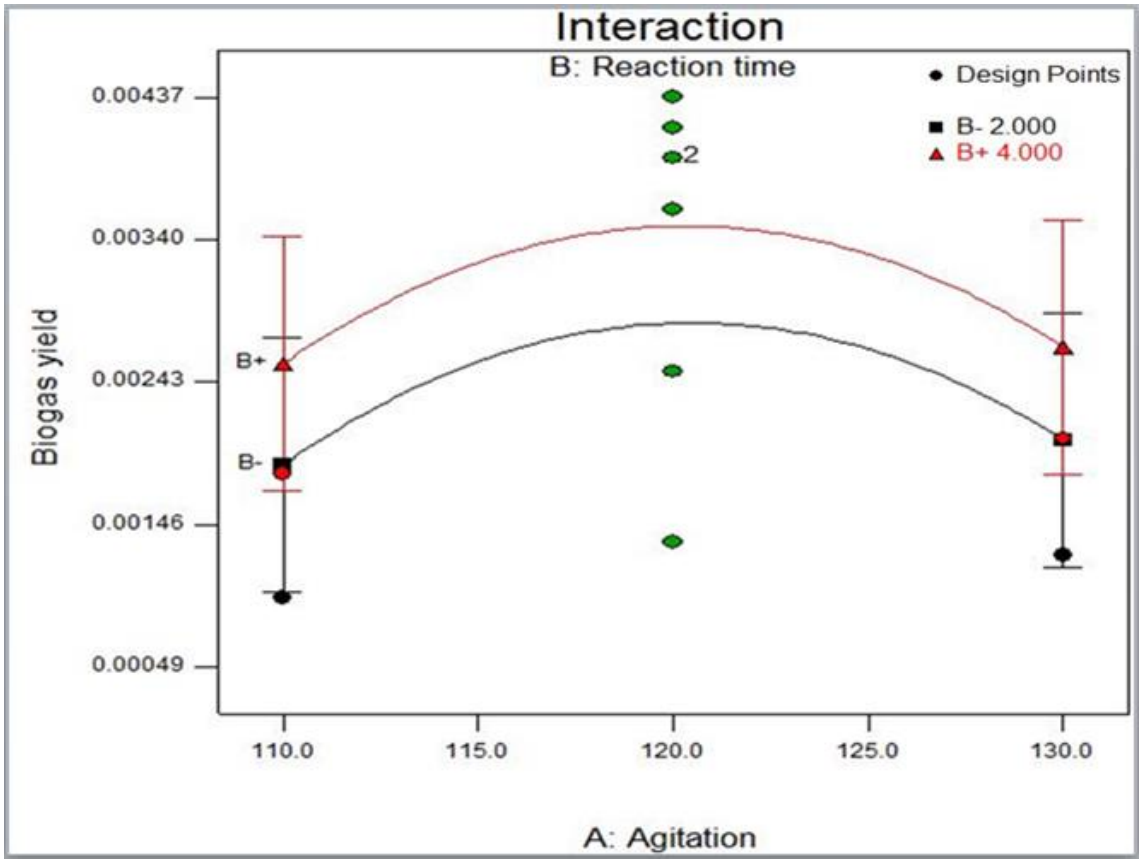

Figure 4.6: Interaction plot of agitation and reaction time on biogas yield.

4.7 Validation Experimental Results

In order to verify the adequacy of the model developed, six confirmation experimental runs were performed. The suitability of the model equation for the prediction of the optimum response values was validated using the optimal conditions suggested by CCD. **Table 4.4** shows the biogas yield according to the suggested agitation and reaction time based on predicted and experimental values. The error deviations lower than 30 % can be accepted in the validation run. From the result obtained, the experimental values were closed to the predicted values and it confirmed the validity and adequacy of the predicted models. Under condition with 120 rpm and reaction time of 3 days, the percentage error for experimental values was 8.50 % from the predicted value. On the other hand, the percentage error for experimental values was 5.82% from the predicted value under suggested optimal condition of 120 rpm and 3.3 days. The result of analysis proved that the response model was adequate for reflecting the expected optimization and the model of equation (4.1) was satisfactory and accurate.

Table 4.4: Predicted and experimental values of the optimization parameter.

No	Agitation (rpm)	Reaction time (days)	Biogas yield (L/g COD)	Predicted biogas yield (L/g COD)
1	120	3	0.00425	0.00370
2	120	3	0.00283	0.00370
3	120	3	0.00308	0.00370
4	120	3.3	0.00391	0.00375
5	120	3.3	0.00354	0.00375
6	120	3.3	0.00445	0.00375

4.8 Comparison of Biogas Yield from other researchers

The comparison of biogas yield in this study with other researchers is shown in **Table 4.5**. The biogas yield from poultry manure wastewater in this study was 0.00445 L/g COD. In daily biogas production basis, AD process of poultry manure wastewater in this study yield 1.48×10^{-3} L/g COD day^{-1}. Kafle and Kim (2013) utilized swine manure as substrate to undergo AD for biogas production had yielded 0.95×10^{-3} L/g COD day^{-1}. Under their same study by replaced substrate with apple waste, the biogas yield was slightly lower which were only 0.75×10^{-3} L/g COD day^{-1}. Studies of Syaichurrozi *et al.* (2013) and Vlassis *et al.* (2013) produced 2.21×10^{-3} L biogas/g COD vinasse day^{-1} and 1.11×10^{-3} L biogas/g COD glycerol day^{-1}, respectively.

The operating temperature for all researchers including this study was set in mesophilic range between 25 - 38 ℃. The HRT for AD of poultry manure wastewater recorded the lowest value of 3.3 days only. The reactor used in this study was with 250 ml and operated in batch mode. The result obtained proved that poultry manure wastewater was a potentially substrate for biogas production. It recorded the highest biogas yield compared to other substrate of swine manure, apple waste and glycerol, except for vinasse.

In experiments of Kafle and Kim (2013), the AD under batch mode operation took place in 1.2 L glass bottles (liquid volume of 0.8 L). The substrate of swine manure took 22 days for highest biogas yield while the substrate of apple waste took 146 days to achieve highest biogas production. The low biogas yield from AD of swine manure might due to its high ammonia content which is a major limitation that has plagued anaerobic digesters for many years (Hansen *et al.,* 1998; Kaparaju and Rintala, 2005; Strik *et al.,* 2006). Similarly, fruit and vegetable waste such as apple waste also has major limitations to its usefulness in AD because of its characters that rapidly acidifies, stressing and activity inhibition by methanogens (Misi and Forster, 2001; Bouallagui *et al.*, 2005).

Syaichurrozi *et al.* (2013) who employed vinasse as substrate for AD yield the highest amount of biogas within the comparison among researchers listed in **Table 4.5**. The HRT for his batch AD experiment was 60 days used 5 L polyethylene digesters. It produced higher biogas than in this study because vinasse contained sufficient nitrogen sources which were needed by bacteria to build cell structure (Speece, 1996). However, too high amount of nitrogen sources might inhibit bacteria activity.

Vlassis *et al.* (2013) conducted AD experiments with substrate of glycerol under continuous stirred tank reactor mode of operation. Within a HRT of 378 days, the AD yield biogas of 1.11×10^{-3} L /g COD day^{-1}, this is only slightly lower than that obtained in this study.

Table 4.5: Comparison of biogas yield with other researchers.

Study	Substrate	HRT	Temperature	Biogas yield (10^{-3})
This study	Poultry manure wastewater	3.3 days	25 ℃	1.48 L/g COD day^{-1}
Kafle and Kim (2013)	Swine manure	22 days	36.5 ℃	0.95 L/g COD day^{-1}
Syaichurrozi *et al.* (2013)	Vinasse	60 days	25 ℃	2.21 L/g COD day^{-1}
Kafle and Kim (2013)	Apple waste	146 days	36-38 ℃	0.75 L/g COD day^{-1}
Vlassis *et al.* (2013)	Glycerol	378 days	35 ℃	1.11 L/g COD day^{-1}

5 CONCLUSION

This final chapter is written to summarize all the results and discussion of the data presented in chapter 4. Recommendation for further study is also suggested for biogas production using poultry manure wastewater.

5.1 Conclusion

This research focuses on experimental investigation into the effect of agitation speed and reaction time on biogas production from poultry manure wastewater using AD. The RSM with CCD was used to determine the optimum condition for the production of biogas from poultry manure wastewater. The ANOVA showed that the effect of agitation and reaction time for biogas yield was significant. Quadratic model was used in predicting all the responses. The biogas production performance was evaluated on the basis of biogas yield from initial COD and was found ranging from $0.49x10^{-3}$ to $4.37x10^{-3}$ L/g COD.

There was a fairly strong correlation between the interaction of agitation and reaction time to the biogas production from poultry manure wastewater. With an agitation of 120 rpm, the maximum biogas production was obtained at reaction time of 3 days. Beyond this reaction time and agitation, biogas production began to decrease and a reverse trend occurred.

A validation experiment was carried out to validate the reliability and sustainability of this model. The optimal conditions determined were agitation of 120 rpm and 3.3 days of reaction time. Under this condition, 4.45×10^{-3} L/g COD of biogas yield was obtained. This counts for 5.82% error from predicted models which was within the acceptable range (0-30%). Therefore, it is suggested the models obtained by using CCD can be used to optimize the biogas production from poultry manure wastewater.

The yield of biogas from poultry manure wastewater in this research with 1.48×10^{-3} L/g COD day^{-1} was compatible with other researchers. The study of Kafle and Kim (2013) yielded 0.95×10^{-3} L/g COD day^{-1} from swine manure. Under their same study by replaced substrate with apple waste, the biogas yield was slightly lower which were only 0.75×10^{-3} L/g COD day^{-1}. On the other hand, studies of Syaichurrozi *et al.* (2013) and Vlassis *et al.* (2013) produced 2.21×10^{-3} L biogas/g COD vinasse day^{-1} and 1.11 x

10^{-3} L biogas/g COD glycerol day^{-1}, respectively. The operating temperature for all researchers including this study was set in mesophilic range between 25 - 38 ℃. The HRT for AD of poultry manure wastewater recorded the lowest value of 3.3 days only. After all, the technology employed in this study was simple which used AD for biogas production and performed biogas collection by water displacement method.

From current study, it can be concluded that animal wastes such as poultry manure wastewater can be a potentially promising source of biogas production. The implementation of this technology can produce a highly potential alternative energy to replace non-renewable energy sources. Besides, this can fulfill the concept of waste-to wealth to sustain a green environment.

5.2 Future Research Recommendation

The research carried in this project to generate biogas from poultry manure wastewater can really add value to the global bioenergy chain. It is recommended to construct a pilot study of scale-up experiment for the optimization of biogas production under optimum conditions obtained from this study. Further studies are also required to more thoroughly assess product quality than was done in this work.

A paradigm shift and a more holistic biogas development model that considers energy, products and wastes including the environment will make it more sustainable. Besides, a combination of different technologies can be implemented in future research on biogas production. As to fulfil the concept of waste-to-wealth, biogas research that utilizes waste and non-food energy crops should be focused. For this the national government should enact biogas policies and regulations that cater agricultural industries on waste management.

Next, there is need for an efficient and effective way of collecting and utilizing poultry manure resource to ensure sustained biogas sufficiency and reliability. Furthermore, awareness campaigns and sensitization on the benefits of biogas production can be highlighted to agricultural industry owners. This is aiming not only to improve hygiene in the countryside and provide alternative bioenergy, but also generating rich low-cost fertilizer (slurry) in agricultural production.

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APPENDICES

Table A1: Raw optimization experimental result data.

Std	Run	Factor 1 A: Agitation (rpm)	Factor 2 B: Reaction time (days)	Gas volume (ml)			Gas yield (L/g COD)			R1
				1	2	3	1	2	3	Average
5	13	100	3	1	1	1.5	0.000416	0.000416	0.000624	0.000486
1	6	110	2	1	2.7	3.2	0.000416	0.001124	0.001332	0.000957
3	10	110	4	5.5	2.4	5.1	0.002289	0.000999	0.002123	0.001804
10	1	120	3	12.5	8.5	5	0.005203	0.003538	0.002081	0.003607
8	4	120	5	2	7	9	0.000832	0.002914	0.003746	0.002497
7	5	120	1	2.2	3.2	5.4	0.000916	0.001332	0.002248	0.001498
13	8	120	3	10.5	7	4	0.00437	0.002914	0.001665	0.002983
9	9	120	3	5	14.5	9	0.002081	0.006035	0.003746	0.003954
12	11	120	3	5.5	9.5	18.5	0.002289	0.003954	0.0077	0.004648
11	12	120	3	6	25	10	0.002497	0.010406	0.004162	0.005689
4	2	130	4	3.5	7	4.2	0.00146	0.002914	0.001748	0.00204
2	3	130	2	1.2	2.6	3	0.000499	0.001082	0.001249	0.000943
6	7	140	3	1	1.6	2	0.000416	0.000666	0.000832	0.000638

Study Type	Response Surface		**Runs**	13							
Initial Design	Central Composite		**Blocks**	No Blocks							
Design Model	Quadratic										
Factor	**Name**	**Units**	**Type**	**Low Actual**	**High Actual**	**Low Coded**	**High Coded**	**Mean**	**Std. Dev.**		
A	Agitation	rpm	Numeric	110.00	130.00	-1.000	1.000	120.000	9.608		
B	Reaction time	days	Numeric	2.00	4.00	-1.000	1.000	3.000	0.961		
Response	**Name**	**Units**	**Obs**	**Analysis**	**Minimum**	**Maximum**	**Mean**	**Std. Dev.**	**Ratio**	**Trans**	**Model**
Y1	Biogas yield	L/g COD	13	Polynomial	0.000485605	0.00437045	0.00238854	0.00144588	9	None	Quadratic

Figure A1: CCD design summary using Design Expert V 7.1.6.

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Mean vs Total	7.417E-005	1	7.417E-005			
Linear vs Mean	1.370E-006	2	6.849E-007	0.29	0.7552	
2FI vs Linear	7.700E-010	1	7.700E-010	2.922E-004	0.9867	
Quadratic vs 2FI	1.993E-005	2	9.963E-006	18.39	0.0016	Suggested
Cubic vs Quadra	6.048E-008	2	3.024E-008	0.041	0.9606	Aliased
Residual	3.731E-006	5	7.461E-007			
Total	9.925E-005	13	7.635E-006			

"Sequential Model Sum of Squares [Type I]": Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Linear	2.340E-005	6	3.899E-006	48.52	0.0011	
2FI	2.339E-005	5	4.679E-006	58.22	0.0008	
Quadratic	3.470E-006	3	1.157E-006	14.39	0.0131	Suggested
Cubic	3.409E-006	1	3.409E-006	42.42	0.0029	Aliased
Pure Error	3.215E-007	4	8.037E-008			

Figure A2: Model fit summary generated using Design Expert V 7.1.6.

Response 1	Biogas yield					
ANOVA for Response Surface Quadratic 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	2.130E-005	5	4.259E-006	7.86	0.0086	significant
A-Agitation	*5.775E-008*	*1*	*5.775E-008*	*0.11*	*0.7536*	
B-Reaction tim	*1.312E-006*	*1*	*1.312E-006*	*2.42*	*0.1635*	
AB	*7.700E-010*	*1*	*7.700E-010*	*1.422E-003*	*0.9710*	
A^2	*1.779E-005*	*1*	*1.779E-005*	*32.85*	*0.0007*	
B^2	*6.754E-006*	*1*	*6.754E-006*	*12.47*	*0.0096*	
Residual	3.791E-006	7	5.416E-007			
Lack of Fit	*3.470E-006*	*3*	*1.157E-006*	*14.39*	*0.0131*	*significant*
Pure Error	*3.215E-007*	*4*	*8.037E-008*			
Cor Total	2.509E-005	12				

Figure A3: ANOVA test summary table in RSM.

Constraints						
Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Agitation	is equal to 120.00	110	130	1	1	3
Reaction time	is in range	2	4	1	1	3
Biogas yield	maximize	0.000485605	0.00437045	1	1	3

Solutions					
Number	Agitation	Reaction time	Biogas yield	Desirability	
1	120.00	3.30	0.00375339	0.841	Selected

Figure A4: Optimization solution suggested from Design Expert V 7.1.6.

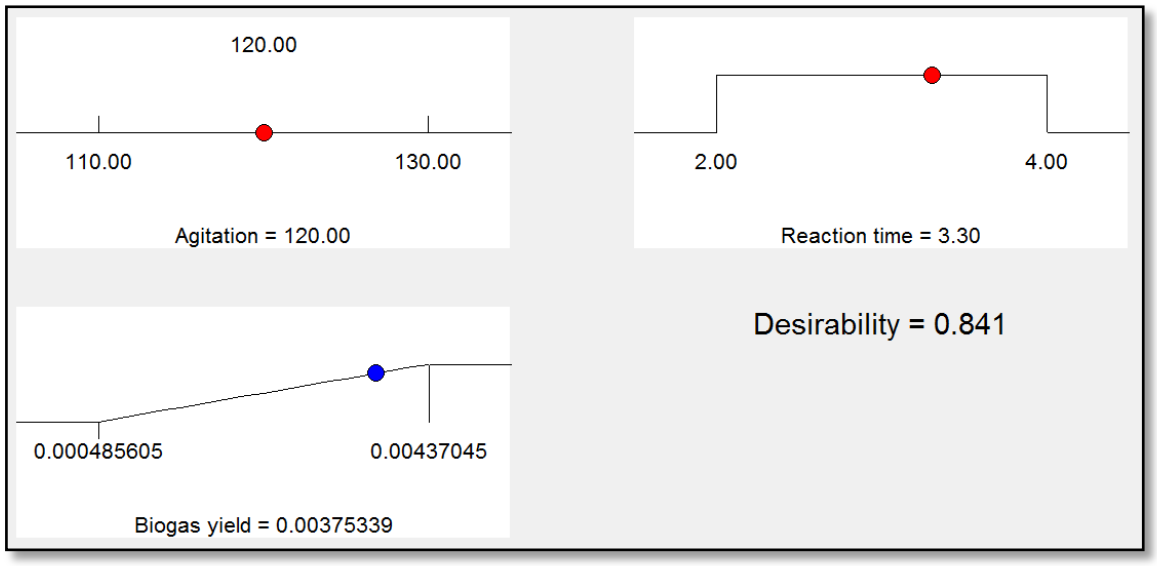

Figure A5: Ramps for parameters and response using Design Expert V 7.1.6.

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	Agitation	120.00	110.00	130.00	0.000	Actual
B	Reaction time	3.30	2.00	4.00	0.000	Actual

Response	Prediction	SE Mean	95% CI low	95% CI high	SE Pred	95% PI low	95% PI high
Biogas yield	0.00375339	3.043E-004	3.034E-003	4.473E-003	7.963E-004	1.870E-003	5.636E-003

Figure A6: Point prediction from suggested solution in Design Expert V 7.1.6.

Characterization Analysis Procedures

B1: Chemical Oxygen Demand (COD)

1. Homogenize 100 mL of sample 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, pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate.
   *If the sample does not contain suspended solids, omit step 1 and step 2.
3. Turn on the COD Reactor. Preheat to 150 ℃. Place the safety shield in front of the reactor.
4. Remove the caps from two COD Digestion Reagent Vials.
   *Be sure to use vials for the appropriate range.
5. Hold one vial at a 45-degree angle. Use a clean volumetric pipette to add 2.00 mL of sample to the vial. This is the prepared sample.
6. Hold a second vial at a 45-degree angle. Use a clean volumetric pipette to add 2.00 mL de-ionized water to the vial. This is the blank.
7. Cap the vials tightly. Rinse them with de-ionized water and wipe with a clean paper towel.
8. Hold the vials by the cap over a sink. Invert gently several times to mix. Place the vials in the preheated COD Reactor.
   *The sample vials will become very hot during mixing.
9. Heat the vials for two hours.
10. Turn the reactor off. Wait about 20 minutes for the vials to cool to 120 ℃ or less.
11. Invert each vial several times while still warm. Place the vials into a rack and cool to room temperature.
12. Touch Hach Programs. Select program 430 COD LR (Low Range) or 435 COD HR (High Range/High Range Plus). Touch Start.
13. Clean the outside of the vials with a damp towel followed by a dry one to remove fingerprints or other marks.
14. Install the 16-mm adapter. Place the blank into the adapter.
15. Touch Zero. The display will show: 0 mg/L COD.
16. When the timer beeps, place the sample vial into the adapter. Touch Read. Results will appear in mg/L COD.

B2: Biochemical Oxygen Demand (BOD_5)

Prepare reagents 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*: Add 1mL each of phosphate buffer, magnesium sulfate, calcium chloride, ferric chloride solution into 1L volumetric flask. Add distilled water to 1L.
2. Add 10mL wastewater sample (refer Appendix) into a 500mL beaker.
3. Add dilution water up to 300mL into the same beaker.
4. Adjust pH value to 6.5 to 7.5 by adding acid/alkali.
5. Prepare 300mL dilution water as control in another 500mL beaker.
6. Put all prepared samples and control in 300mL-incubation bottle each.
7. Measure and record dissolved oxygen (DO) concentration for each sample using Dissolved Oxygen Meter.
8. Add water to the flared mouth of bottle and cover with an aluminum foil.

9. Put all the bottles in BOD Incubator for five days. Set the temperature at 20°C.
10. Measure final DO value after five days.
11. Calculate BOD_5 according to the formula below;

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

B3: Ammonia Nitrogen (Method 8155, 0.01 to 0.50 mg/L NH_3-N)

1. Touch **Hach Programs**. Select program **385 N, Ammonia, Salic**. Touch **Start**.
2. Fill a round sample cell to the 10 mL mark with sample.
3. Fill another round sample cell to the 10 mL mark with deionized water (the blank).
4. Add the contents of one Ammonia Salicylate Powder Pillow to each cell. Stopper and shake to dissolve the powder.
5. Touch the timer icon. Touch **OK**. A three-minute reaction period will begin.
6. When the timer beeps, add the contents of one Ammonia Cyanurate Reagent Powder Pillow. Stopper and shake to dissolve the reagent.
7. Touch the timer icon. Touch **OK**. A 15-minute reaction period will begin.

    *A green color will develop if ammonia-nitrogen is present.
8. When the timer beeps, place the blank into the cell holder.
9. Touch **Zero**. The display will show: **0.00 mg/L NH_3-N**.
10. Wipe the sample and place it into the cell holder.
11. Touch **Read**. Results will appear in mg/L NH_3-N.

B4: Nitrate (Method 8171, MR 0.1 to 10.0 mg/L NO_3-N)

1. Touch **Hach Programs**. Select program **353 N, Nitrate MR**. Touch **Start**.
2. Fill a round sample cell with 10 mL of sample.
3. Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow (the prepared sample) and cap the cell.
4. Touch the timer icon. Touch OK. A one-minute reaction period will begin. Shake the cell vigorously until the timer beeps.
5. When the timer beeps, touch the timer icon. Touch **OK**. A five-minute reaction period will begin.

    *An amber color will develop if nitrate is present.
6. When the timer beeps, fill a second round sample cell with 10 mL of sample (the blank).
7. Place the blank into the cell holder.
8. Touch **Zero**. The display will show: **0.0 mg/L NO_3-N.**
9. Place the prepared sample into the cell holder. Touch Read. Results will appear in mg/L NO_3^--N.

B5: Total Suspended Solids (TSS)

1. Dry the filter disk (47 mm @ 70 mm) in the oven at 103°C to 105°C for 1 hour, cool in a desiccator and weigh.
2. Assemble filtering apparatus and filter and begin suction. Wet the filter with a small volume of distilled water to seat it.
3. Pipette 50 mL of water sample (mixed to ensure homogeneity) onto centre of filter disk in a Buchner flask, using gentle suction (under vacuum).
4. Wash filter with three successive 10 mL volumes of distilled water, allowing complete drainage between washings, and continue suction for about 3 min after filtration is complete.
5. Carefully remove filter from filtration apparatus and transfer to aluminum weighing dish/crucible dish as a support.
6. Dry at least 1 hour at 103°C to 105°C in an oven, cool in a desiccator to balance temperature, and weigh.
7. Repeat the cycle of drying, cooling, desiccating, and weighing until a constant weight is obtained.

Calculate the Total Suspended Solids (TSS) in the water and wastewater samples using the following equation:

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

where;

A = weight of filter + dried residue, mg

B = weight of filter, mg