Co-Fermentation of Oil Palm Empty Fruit Bunch with Pig Manure in Anaerobic Leach Bed Reactor for Volatile Fatty Acid Production

Kanyarat Saritpongteeraka

A Thesis Submitted in Fulfillment of the Requirements for the Degree of
Doctor of Philosophy in Environmental Management
Prince of Songkla University
2015

Co-Fermentation of Oil Palm Empty Fruit Bunch with Pig Manure in Anaerobic Leach Bed Reactor for Volatile Fatty Acid Production

Kanyarat Saritpongteeraka

A Thesis Submitted in Fulfillment of the Requirements for the Degree of
Doctor of Philosophy in Environmental Management
Prince of Songkla University
2015

Thesis Title	Co-Fermentation of Oil Palm Empty Fruit Bunch with Pig Manure in Anaerobic Leach Bed Reactor for Volatile Fatty Acid Production
Author	Miss Kayarat Saritpongteeraka
Major	Environmental Management

Major Advisor :

……..
(Assoc. Prof. Dr. Sumate Chaiprapat)

Co-adivisor :

……..
(Prof. Dr. Shihwu Sung)

……..
(Asst. Prof. Dr. Piyarat Boonsawang)

Examining Committee :

……Chairperson
(Assoc. Prof. Dr. Duangporn Kantachote)

……
(Assoc. Prof. Dr. Veerasak Thonglimp)

……
(Asst. Prof. Dr. Thunwadee Suksaroj)

……..……..
(Asst. Prof. Dr. Piyarat Boonsawang)

……
(Assoc. Prof. Dr. Sumate Chaiprapat)

The Graduate School, Prince of Songkla University, has approved this Thesis as fulfillment of the requirements for the Degree of Doctor of Philosophy in Environmental Management

……

(Assoc. Prof. Dr. Teerapol Srichana)

Dean of Graduate School

ชื่อวิทยานิพนธ์ การหมักร่วมระหว่างทะลายปาล์มเปล่ากับมูลสุกรในถังหมักไร้อากาศลิชเบดสำหรับการผลิตกรดไขมันระเหยง่าย

ผู้เขียน นางสาวกัญญารัตน์ สฤษฏ์พงศ์ทีรฆ

สาขาวิชา การจัดการสิ่งแวดล้อม

ปีการศึกษา 2557

บทคัดย่อ

วัตถุประสงค์หลักของงานวิจัยครั้งนี้เพื่อศึกษาผลของปัจจัยที่ส่งผลต่อการย่อยสลายทะลายปาล์มเปล่าที่หมักร่วมด้วยมูลสุกรที่มีปริมาณไนโตรเจนสูงภายใต้การย่อยสลายเบื้องต้นในสภาวะไร้อากาศ โดยศึกษาปัจจัยสัดส่วนการผสมของมูลสุกร (%PM) ได้แก่ 0PM:100EFB, 25PM:75EFB, 50PM:50EFB and 100PM:0EFB (ชุดควบคุม), รอบการหมุนเวียนน้ำชะ (FI) ได้แก่ 12, 24 และ 48 ชั่วโมง และระยะเวลาการหมัก (FT) จนสิ้นสุดการหมัก 0-60 วัน ด้วยระบบถังปฏิกรณ์ไร้อากาศแบบลิชเบดโดยออกแบบการทดลองด้วยแฟกทอเรียล

ผลการทดลองพบว่าการเติมมูลสุกรส่งเสริมการย่อยสลายทะลายปาล์มเปล่าและส่งผลให้การผลิตกรดไขมันระเหยง่ายจากทะลายปาล์มเปล่าเพิ่มขึ้นอย่างรวดเร็วเมื่อสัดส่วนการผสมมูลสุกรเพิ่มขึ้นจากร้อยละ 0-25 อย่างไรก็ตามการเพิ่มมูลสุกรส่งเสริมสัมประสิทธิ์ปริมาณผลผลิตการย่อยสลาย (η_h) และสัมประสิทธิ์ปริมาณผลผลิตของการสร้างกรด (η_a) เนื่องจากในมูลสุกร มีสารอินทรีย์ที่ย่อยสลายง่ายและธาตุอาหาร โดยมีค่า η_h และ η_a เฉลี่ยเท่ากับร้อยละ 23.28±2.2 และ 51.7±2.5 ตามลำดับ ความเข้มข้นของกรดไขมันระเหยง่ายสะสมมีค่าเฉลี่ยสูงสุดเท่ากับ 152.3 ±0.6 กรัมกรดไขมันระเหยง่ายต่อกิโลกรัมวัสดุแห้ง ของชุดการทดลอง 50PM:50EFB ที่ FI 48 ชั่วโมง ของวันที่ 60 แบบจำลองสมการถดถอยแสดงถึงความสัมพันธ์ของพารามิเตอร์ต่อการผลิตกรดไขมันระเหยง่าย η_h และ η_a มีค่าความเชื่อมั่นทางสถิติเท่ากับเท่ากับ 0.87, 0.95 และ 0.80 ตามลำดับ มีค่าแตกต่างกันทางสถิติอย่างมีนัยสำคัญที่ระดับ <0.0001 แบบจำลองสามารถอธิบายถึงความสัมพันธ์ระหว่างปัจจัย %PM, FI และ FT ต่อการย่อยทะลายปาล์มเปล่าได้อย่างน่าพึงพอใจ สำหรับผลจากการวิเคราะห์อนุพันธ์ของกรดไขมันระเหยง่ายโดยเฉพาะอย่างยิ่งอนุพันธ์กรดโพรพิโอนิค กรดบิวทีริค และกรดวาราริค ปรากฏที่ FI มีระยะเวลานาน บ่งชี้ได้ว่าอนุพันธ์ของกรดไขมันระเหยง่ายเกิดจากการย่อยสลายทะลายปาล์มเปล่า ของแข็งระเหยง่ายถูกย่อยสลายหลังจากผ่านกระบวนการย่อยเบื้องต้นเพิ่มขึ้น 6.9 เท่า เมื่อ %PM เพิ่มขึ้นจากร้อยละ 0 ถึง ร้อยละ 50 และ FI เพิ่มขึ้นจาก 12 ชั่วโมงถึง 48 ชั่วโมง ตามลำดับ

นอกจากนี้กรดไขมันระเหยง่ายหรือกรดอ่อนที่ผลิตขึ้นจากกระบวนการหมักไร้อากาศสามารถช่วยทำลายโครงสร้างของเส้นใยทะลายปาล์มเปล่าได้ การเดินระบบถังปฏิกรณ์ไร้อากาศแบบลิชเบดสามารถกำจัดเฮมิเซลลูโลสจากเส้นใยได้สูงถึงร้อยละ 56.9 และสามารถชะโพแทสเซียมออกจากเส้นใยทะลายปาล์มเปล่าได้เฉลี่ยร้อยละ 83.5±4.0 ส่งผลให้องค์ประกอบของโพแทสเซียมลดลงสามารถช่วยลดการเกิดตะกรันในห้องเผาไหม้ นอกจากนี้คุณสมบัติของทะลายปาล์มเปล่าเมื่อสิ้นสุดกระบวนการหมักแบบกะ ให้ค่าความร้อนเพิ่มขึ้นจาก 7.7 เมกะจูนต่อกิโลกรัมแห้ง (วันที่ 0) ถึง 19.5 จูนต่อกิโลกรัมแห้ง ของชุดการทดลอง 50PM:50EFB ที่ FI 48 ชั่วโมง ผลจากการเพิ่มมูลสุกรยังช่วยปรับปรุงธาตุอาหารสำหรับพืช (ไนโตรเจน:ฟอสฟอรัส:โพแทสเซียม) ของวัสดุหลังหมักได้สามารถใช้เป็นวัสดุปรับปรุงดินได้ อย่างไรก็ตามการหมักร่วมโดยการเพิ่มมูลสุกรสามารถทำให้เพิ่มระดับของการผลิตกรดไขมันระเหยง่ายและเพิ่มประสิทธิภาพการย่อยทะลายปาล์มเปล่าขั้นต้นด้วยถังปฏิกรณ์ไร้อากาศแบบลิชเบด นำไปใช้จัดการทะลายปาล์มเปล่าที่วัสดุเหลือทิ้งจากอุตสาหกรรมผลิตน้ำมันปาล์มที่มีปริมาณมากก่อนนำไปแปรรูปให้เป็นประโยชน์ต่อไป

Thesis Title Co-Fermentation of Oil Palm Empty Fruit Bunch with Pig Manure in Anaerobic Leach Bed Reactor for Volatile Fatty Acid Production

Author Miss Kanyarat Saritpongteeraka

Major Program Environmental Management

Academic Year 2014

ABSTRACT

The main objective of this study was to investigate the parameters affecting the digestibility of empty fruit bunch (EFB) co-fermenting with the pig manure (PM) which is a nitrogen rich material under anaerobic pretreatment. Effects of pig manure mixing ratio (%PM) at 0PM:100EFB, 25PM:75EFB, 50PM:50EFB and 100PM:0EFB (as control), flushing interval (FI) at 12, 24 and 48 h and fermentation time (FT) over 60d were studied using anaerobic leach bed reactor (ALBR) in batch-wise operation at full factorial experimental design.

It was found that PM addition could help the degradation of EFB. Results showed specific total volatile fatty acid (TVFA) production from EFB increased rapidly as PM mixing went from 0 to 25 %PM. However, addition of PM promoted higher hydrolysis yield (η_h) and acidification yield (η_a) due to the easily soluble organics in PM and nutrients it supplemented. The highest η_h and η_a found were 23.3±2.2% and 51.7±2.5%, respectively. The 60-d cumulative TVFA production reached the highest level at 152.3 ±0.6 gTVFA/kg $_{\text{dry substrate added}}$ in 50PM:50EFB at FI 48 h treatment. Multiple regression models revealed the interactive relationship of the parameters on TVFA production, η_h and η_a, The model R^2 of 0.87, 0.95 and 0.80 of TVFA production, η_h and η_a respectively was obtained with *p*-value <0.0001 suggesting a good fit to the experimental data. The model was able to satisfactorily explain the relationship between factors %PM, FI and FT in pretreating EFB. VFA species were analyzed where the longer chain acids particularly propionic acid, butyric acid and valeric acid were found with longer FI indicating the prospect of higher EFB degradation. Volatile solid (VS) release from the EFB fiber after 60-day fermentation was enhanced by 6.9 times when *%PM* and *FI* increased from 0 and 12 h

to 50 and 48 h, respectively. VFAs, the mild acids produced, when in contact with lignocellulosic fiber helped loosen the structure of EFB. ALRB operation could remove hemicellulose from the fiber as high as 56.9% and leach potassium (K) off up to 83.5±4.0%. The EFB digestate at the end of the batch had an elevated heating value from 7.7 MJ/kg_{dry} (at day 0) to 19.5 MJ/kg_{dry} at 50PM:50EFB and FI 48h. The lowering K content could help avoid the formation of slag in boilers. The addition of PM also improved plant nutrients (N, P, K) in the digestate. It, however, can be used as a better source for soil amendment humus material. Thus, addition of PM induced higher degree of VFA production that helped degrade the co-substrate. ALBR performed well in pretreating EFB residue and improved its properties more suitable for further conversion and uses.

ACKNOWLEDGEMENTS

First of all, this thesis could not have been accomplished without my advisor, Assoc. Prof. Dr. Sumate Chaiprapat. He has been invaluable on academic and a personal level and he gave me the opportunity and good experience. He trained me how to do the best research, commented for this work and supported me until this thesis is completed. Special thanks to Professor Shihwu Sung who helped me when I was doing research at Iowa State University, USA, and sharing many research ideas. I am also grateful to Assistant Professor Piyarat Boonsawang who is my co-advisor for her advice and suggestion.

I would like to thank the supports from The Royal Golden Jubilee Ph.D. Program (RGJ), Thailand Research Fund Grant, the Office of National Research Council of Thailand, Prince of Songkla University, Graduate School of Prince of Songkla University (PSU), SPM Feed Meal Co., Ltd. Lam Soon Public Company Limited, Thailand. The authors would also like to recognize the Biogas Research Laboratory, Faculty of Engineering and Energy System Research Institute of Prince of Songkla University (PERIN) for their research facility.

I also wish to thank my family: my parents: Supat Saritpongteeraka and Katunyoo Saritpongteeraka for their moral support all the time. I wish to express my gratitude to all members at the Biogas Research Laboratory and all my friends at the Faculty of Environmental Management of Prince of Songkla University for their help, encouragement, cheerfulness and friendship. Additionally, I am really thankful my friends in USA and my best friends in Thailand for their encouraging, listening to my complaint and frustration.

Kanyarat Saritpongteeraka

TABLE OF CONTENTS

CONTENTS (Cont.)

CONTENTS (Cont.)

LIST OF TABLE

LIST OF TABLE (Cont.)

LIST OF FIGURES

LIST OF FIGURES (Cont.)

LIST OF FIGURES (Cont.)

LIST OF ABBREVIATIONS

PF	Palm oil fronts
PT	Palm oil trunk
PPF	Palm oil press fiber
PS	Palm oil shell
EFB	Empty fruit bunch
AD	Anaerobic digestion
MSW	Municipal solid waste
ALBR	Anaerobic leach bed reactor
ASBR	Anaerobic sequencing batch reactor
AF	Anaerobic filter
AA	Atomic absorption spectroscopy method
UASB	Up-flow anaerobic sludge blanket
CSTR	Continuous stirrer tank reactor
C:N ratio	Cabon:Nitrogen ratio
PM	pig manure
%PM	%PM mixing
FI	Flushing or flooding interval
FT	Fermentation time
TVFA	Total volatile fatty acid
ALK	Alkalinity
BOD	Biochemical oxygen demand
COD	chemical oxygen demand
SCOD	soluble COD
TS	Total solid
VS	Total volatile solid
NH4+N	Ammonia nitrogen
TKN	Total Kjeldahl nitrogen
NDF	Detergent fiber and acid
ADF	Acid detergent fiber

LIST OF ABBREVIATIONS (CONC.)

ADL	Detergent lignin
HRT	Hydraulic retention time
SRT	Solids retention time
SSF	solid state fermentation
ISR	Inoculum to substrate ratio
DI	Deionized Water
C2	Acetic acid (HAc)
C3	Propionic acid (HPr)
C4	Butyric acid (i-HBu+HBu)
C5	Valeric acid (i-HVa+Hva)
C6	Hexanoic acid or Caproic acid (i-HCap+HCap)
η_h	Hydrolysis yield
η_a	Acidification yield
S_S	The cumulative SCOD production
S_I	The initial total COD
S_{TVFA}	Cumulative TVFAs expressed as g COD
X_1	PM mixing (%PM)
X_2	Flushing interval (*FI*)
X_3	Fermentation time (*FT*)
Y	The interested response; TVFA production
Y_i	The predicted response
β_0	A constant
β_i	The linear coefficient
β_{ii}	The squared coefficient
β_{ij}	The cross-product coefficient
X_i	The actual values of the studied independent variables.
ANOVA	One-way analysis of variance
C.V.	Coefficient of variance
HHV	Higher heating value

CHAPTER I
INTRODUCTION

1.1 Background

At present, the world is confronting energy problem. Rising energy prices and trans-broader global warming regulatory requirements have forced businesses to be more efficient in energy expenditure in order to stay competitive in the marketplace. The production of energy from biomass, wastes and other renewable resources is regarded as key to success in green and sustainable development. Production of methane via anaerobic digestion of energy crop and organic waste will benefit the society by providing a clean fuel from renewable feedstock (Ladanai and Vinterbäck, 2009). This should replace fossil fuel energy and reduce environmental impacts particularly global warming and acid rain. Although the cost of biomass energy, depending on economic restrictions and advances in technology is always more expensive than fossil fuel energy and only energy subsidy policy could increase the biomass energy competitiveness (Geng, 2013). Key by-products of anaerobic digestion include digested solids and liquids, which may be used as soil amendments and liquid fertilizers.

In 2013, the renewable energy is expected to get a bigger share for years to come which currently occupies approximately 19% of the world total energy use with various forms of renewable energies according to Figure 1-1 (REN21, 2014). Feedstock materials useable as substrate include maize, grass silage, corn silage, whole crop, algae biomass, weed, oil palm waste and others that are biodegradable. These biological materials can be converted to many kinds of fuels such as ethanol, biodiesel, vegetable oil, liquefied fuel, and biogas. However, it is interesting to note that per one hectare of agricultural land, the energy conversion to biogas gave the highest distance about 67.6 km. When compared with other renewable energies, biomass to biogas is the most efficient (Envitech-Biogas, 2009). This is due to the high conversion efficiency of the process.

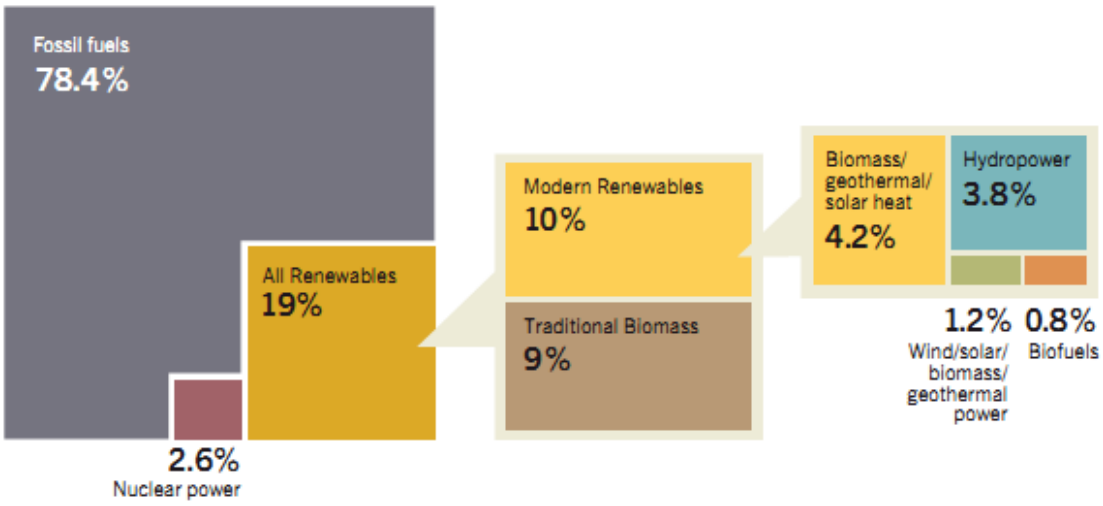


Figure 1-1 Renewable energy share of global final energy consumption in the World (REN21, 2014)

Palm oil is the second most traded economic crops of Southern Thailand, after rubber. Palm oil is mostly used in the manufacture of food products. Consequently there are tremendous amount of biomass residues from oil palm plantation and palm oil mill namely palm oil fronts (PF), palm oil trunk (PT) palm oil press fiber (PPF), palm oil shell (PS) and empty fruit bunch (EFB). These materials amount for 4.85 million ton in 2012 (Office of Agricultural Economic, 2012). EFB waste residual is a lignocellulose fiber which composed of 49.95% cellulose, 18.91% hemicellulose and 25.17% lignin (Piñeros-Castro and Velásquez-Lozano, 2014). EFB contains high carbon at 49.07% with low nitrogen of 0.7% (Geng, 2013). Due to its large quantity, EFB biomass is a potential source of renewable resource for energy production in Thailand. Unfortunately, high potassium content makes it difficult for combustion application (Obernberger and Thek, 2004). Burning biomasses is not the only way to release their energy, but biological conversions could achieve the same tasks mostly in forms of biodiesel, bio-ethanol, and biogas. However, burning EFB will destroy the nitrogen nutrient in the material and it can produce excessive slack accumulating in boilers causing frequent shutdowns and loss of efficiency and make air pollution as emit dust and tar and greenhouse gas (GHG) as NO_x, CO, CO_2, and SO_2 into atmosphere, especially CO_2 is the effect to global warming (Alkarimiah and

Rahman, 2014). Nowadays, most of this EFB was taken to oil palm plantation and let it disintegrate as soil amendment. This method releases methane to the atmosphere.

Plant cell is composed of three main substances: cellulose, hemicellulose, and lignin. Within the plant cell wall, chains of cellulose molecules associate with other polymers such as parcrystalline celluslose and hemicullulose to form linear structures of high tensile strength known as microfibril. Cellulose and hemicellulose are sugar polymer of about 75% (Qian, 2014). In addition to cross-linking individual microfibrils, hemicellulose also forms covalent in association with lignin, a rigid aromatic polymer (Taherzadeh and Karimi, 2008). Lignin structure and organization within the cell wall are poorly understood.

Pretreatment of feedstock can increase solubilization of the material and enhance biogas production and volatile solid destruction (Dogan et al., 2008; Uke and Stentiford, 2013). The use of pretreatment is particularly useful when the materials to be digested are resistant to further conversion, i.e. improving digestibility of lignin-rich biomass waste. Pretreatment methods include physical, chemical and biological means. Physical pretreatments focus on breaking down the structure with forces (Taherzadeh and Karimi, 2008), such as mechanical size reduction, ultrasonic pretreatment, temperature either heat or cold (Hendriks and Zeeman, 2009; Qian, 2014; Riau et al., 2015). Chemical pretreatments use acid or alkali chemical to loosen or solubilize the structure (Agbor et al., 2011).

Biological pretreatment seems to be the cheapest method by employing microbial enzymatic activity to degrade different substances in the materials (Taherzadeh and Karimi, 2008) to make it more accessible for further conversions. This method could be carried out mostly by lactic acid bacteria and other facultative microorganisms which could proliferate in moist environment, i.e. moisture content 30-70% (Insam et al., 2010). Since lignocellulosic materials are very complicated depending on the types of materials, their pretreatment may, therefore, require different effort levels. Pretreatment of biomass or oil palm waste with enzymes or acids is necessary to remove the surrounding matrix of hemicellulose and lignin from

the cellulose core prior to hydrolysis (Kumar et al., 2009). Within each pretreatment, conditions should be optimized for the effective transformation that follows.

In anaerobic digestion (AD), biogas production of the methane producing reactor will be improved if the materials are pretreated prior to feeding the digester in acidogenesis stage. This will certainly fasten the start up speed, the processing rate of a reactor, and the overall throughput of the system. However, their additional cost must always be balanced against resultant improvements in efficiency. Anaerobic dry digestion has features and advantages over other systems, that is low space requirement by a reduced volume of the bioreactor and low wastewater to be handled because no or very low water consumption as well as fertilizer production, and high VS degradation (Bollon et al., 2013; Dogan et al., 2008). A disadvantage of anaerobic dry digestion is a requirement for material pretreatment before feeding to the bioreactor. Dry digestion does not need special techniques but mechanical device for bioreactor operation such as slurry pump, shredders, scraper, and etc. (Kothari et al., 2014). In Germany, dry fermentation has been applied to municipal solid waste (MSW).

Anaerobic leach bed reactor (ALBR) is the reactor design that is capable of digesting high solids or dry digestion (20-40% TS) (Kothari et al., 2014). ALBR is usually operated in a semi-batch fed manner. Reactor operation will dictate functions of the ALBR but it normally utilized as the first stage for hydrolysis and acid production that will require the subsequent biogas production reactor. These modules still has many aspects to study particularly for various kinds of substrates. Due to missing agitation inside the leach-bed reactor, a liquid leachate formed is used for circulation (Myint and Nirmalakandan, 2009; Parawira et al., 2005). This leachate circulates through the leach-bed reactor and through a high rate anaerobic digester. ALBR can be considered a high rate anaerobic fermenter since it promotes immobilization of bacteria in the reactor (Stabnikova et al., 2008). Operational parameters of ALBR include packing density, liquid flooding/circulation interval, percolation, C:N ratio and temperature, moisture content and feed cycle (Bollon et al., 2013; Bollon et al., 2011; Cysneiros et al., 2012; Michele et al., in press; Shewani et al., in press). Leachate recirculation methods are of interest because these factors

could directly improve the performance of ALBR. However Babel et al. (2004) was found that intermittent flooding gave higher methane production per gram of volatile solids destroyed at approximately 9.23%. Control of pH in the system must be maintained at optimal to maximize volatile fatty acid production. Slightly acidic environment was reportedly more effective in hydrolysis and acid production for pineapple peel fermentation.

It is known that pig manure (PM) contains abundant nutrients such as nitrogen and phosphorus in particular with low C:N ratio approximately of 13:1 (Dechrugsa et al., 2013). Majority of the pig manure will be sold to farmers for applying as fertilizer for vegetables, plants, orchards, and etc. In contrast, with high C:N ratio, EFB has a low potential for digestion as the optimal range for anaerobic digestion is 25:1-30:1 (Ward et al., 2008) for methane formation and C:N ratio of 10:1-45:1 for hydrolysis (Deublein and Steinhauser, 2008). Co-digestion is a simultaneous digestion of a homogenous mixture of two or more substrates in order to balance digestion environmental condition as such C:N ratio (Mata-Alvarez et al., 2014; Sawatdeenarunat et al., 2015), which should lead to higher biogas yields. EFB (C:N 99.55:1) (Kerdsuwan and Laohalidanond, 2011) in mix with pig manure to balance C:N ratio should improve hydrolysis and acidogenesis prior to the methanogenic reactor.

However, Thai government has long been giving financial support to anaerobic digestion projects in manure farms as animal manures are rich in organic materials and can also harm the aquatic environment due to its high nitrogen and phosphorus. Anaerobic digestion (AD) can treat the organic pollutant while producing biogas as a renewable energy. These factors typically play an important role in an owner's decision to install AD system. One of the strategies to improve biogas production is through co-digesting of multiple materials available in the area. Agricultural residues are the main target as they are abundant in Thailand. EFB has a vast potential as a co-substrate but it is still difficult for biological transformation. Co-digestion of EFB with pig manure can be a match if the degradation of EFB could be enhanced and volatile fatty acids can be derived from this fermentation. This should release the potential of this material for energy production.

The objective of this study is to evaluate the effect of %PM mixing, flushing or flooding interval (FI) and fermentation time (FT) on the performance of ALBR co-fermentating EFB and PM. This work focused on improving hydrolysis and acidogenesis steps for pretreating of the lignocellulosic EFB for organic acid recovery and EFB fiber improvement. The EFB resource can then be utilized at its full potential for the production of renewable chemicals, biomass fuel, and fertilizer. This approach can also strategically help stabilize the price of oil palm in the country and benefit the palm oil farmers as a whole.

1.2 Objectives

1. To optimize fatty acid generation, hydrolysis yield and acidification yield from EFB pretreatment by co-fermenting with pig manure.
2. To study the effects of %PM mixing, flooding/flushing interval (FI) and fermentation time (FT) on the performance of ALBR in EFB pretreatment.
3. To study EFB degradation properties and properties of the material from the co-fermentation.

1.3 Benefits expected

To improve hydrolysis and acidification yield production and pretreatment the EFB fiber by anaerobic dry fermentative pretreatment of EFB using co-substrate from pig manure. The results of factors affecting ALBR and its performance could be used to develop a better EFB pretreatment process. This approach will strategically help stabilize the price of oil palm in the country and benefit the oil palm industry and oil palm farmers all together.

1.4 Scope of study

Study of the effects of 3 factors; percentage of pig manure; *%PM* (*X1*) (0PM:100EFB, 25PM:75EFB, 50PM:50EFB and 100PM:0EFB), flooding/flushing interval; *FI* (*X2*) (12h, 24h and 48h) and fermentation time; *FT* (*X3*) (0-60 d) on the performance ALBR for pretreating the EFB and sequentially improving its hydrolysis and acidification. A total of 12 treatments were run triplicate over 60 d. The

experimental design was conducted using full factorial design for 3 response factors with TVFA production (*Y1*), hydrolysis yield (*Y2*) and acidification yield (*Y3*) as the interested responses of the model. Additionally, analyses of solid digesteate from the fermentative pretreatment in ALBR were performed at various times to monitor changes in fiber properties in terms of fuel and fertilizer.

REFERENCE

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., Levin, D.B. 2011. Biomass pretreatment: Fundamentals toward application. Biotechnology Advances, 29(6), 675-685.

Alkarimiah, R., Rahman, R.A. 2014. Co-composting of EFB and POME with the role of nitrogen-fixers bacteria as additives in composting process-A review. International Journal of Engineering Science and Innovative Technology, 3(2), 132-145.

Babel, S., Fukushi, K., Sitanrassamee, B. 2004. Effect of acid speciation on solid waste liquefaction in an anaerobic acid digester. Water Research, 38(9), 2417-2423.

Bollon, J., Benbelkacem, H., Gourdon, R., Buffière, P. 2013. Measurement of diffusion coefficients in dry anaerobic digestion media. Chemical Engineering Science, 89(0), 115-119.

Bollon, J., Le-hyaric, R., Benbelkacem, H., Buffiere, P. 2011. Development of a kinetic model for anaerobic dry digestion processes: Focus on acetate degradation and moisture content. Biochemical Engineering Journal, 56(3), 212-218.

Cysneiros, D., Banks, C.J., Heaven, S., Karatzas, K.-A.G. 2012. The role of phase separation and feed cycle length in leach beds coupled to methanogenic reactors for digestion of a solid substrate (Part 1): Optimisation of reactors' performance. Bioresource Technology, 103(1), 56-63.

Dechrugsa, S., Kantachote, D., Chaiprapat, S. 2013. Effects of inoculum to substrate ratio, substrate mix ratio and inoculum source on batch co-digestion of grass and pig manure. Bioresource Technology, 146(0), 101-108.

Deublein, D., Steinhauser, A. 2008. Biogas from waste and renewable resources : An introduction. WILEY-VCH Verlag GmbH & Co. KGaA,Weinheim, Germany.

Dogan, E., Dunaev, T., Erguder, T.H., Demirer, G.N. 2008. Performance of leaching bed reactor converting the organic fraction of municipal solid waste to organic acids and alcohols. Chemosphere 74, 797-803.

EnviTech-Biogas. 2009. Tailored biogas systems –an investment for the future., p. 4.

Geng, A. 2013. Conversion of oil palm empty fruit bunch to biofuels. in: Liquid, Gaseous and Solid Biofuels - Conversion Techniques, (Ed.) P.Z. Fang, Intech, 479-490.

Hendriks, A.T.W.M., Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technology, 100(1), 10-18.

Insam, H., Franke-Whittle, I., Goberna, M. 2010. Microbes in aerobic and anaerobic waste treatment. in: Microbes at work from wastes to resources, (Ed.) F.-W.I. In: Insam H, Goberna M (eds), Springer. Heidelberg, 1-34.

Kerdsuwan, S., Laohalidanond, K. 2011. Renewable Energy from Palm Oil Empty Fruit Bunch. in: Renewable Energy - Trends and Applications, (Ed.) D.M. Nayeripour, InTech. http://www.intechopen.com/books/renewable-energy-trends-and-applications/renewable-energy-from-palm-oil-empty-fruit-bunch.

Kothari, R., Pandey, A.K., Kumar, S., Tyagi, V.V., Tyagi, S.K. 2014. Different aspects of dry anaerobic digestion for bio-energy: An overview. Renewable and Sustainable Energy Reviews, 39(0), 174-195.

Kumar, P., Barrett, D.M., Delwiche, M.J., Stroeve, P. 2009. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. Journal of Industrial & Engineering Chemistry Research, 48(8), 3713–3729.

Ladanai, S., Vinterbäck, J. 2009. Global potential of sustainable biomass for energy Department of Energy and Technology.

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M., Astals, S. 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renewable and Sustainable Energy Reviews, 36(0), 412-427.

Michele, P., Giuliana, D.I., Carlo, M., Sergio, S., Fabrizio, A. in press. Optimization of solid state anaerobic digestion of the OFMSW by digestate recirculation: A new approach. Waste Management(0).

Myint, M.T., Nirmalakandan, N. 2009. Enhanceing anaerobic hydrolysis of cattle manure in leach bed reactors. Bioresource Technology, 100, 1695-1699.

Obernberger, I., Thek, G. 2004. Physical characterisation and chemical composition of densified biomass fuels with regard to their combustion behaviour. Biomass and Bioenergy, 27(6), 653-669.

Office of Agricultural Economic. 2012. Data of agricultural economic crop (in Thai). Ministry for Agricultural and Coperatives.

Parawira, W., Murto, M., Read, J.S., Mattiasson, B. 2005. Profile of hydrolases and biogas production during two-stage mesophilic anaerobic digestion of solid potato waste. Process biochemistry, 40(9), 2945-2952.

Piñeros-Castro, Y., Velásquez-Lozano, M. 2014. Biodegradation kinetics of oil palm empty fruit bunches by white rot fungi. International Biodeterioration & Biodegradation, 91, 24-28.

Qian, E.W. 2014. Chapter 7 - Pretreatment and Saccharification of Lignocellulosic Biomass. in: Research Approaches to Sustainable Biomass Systems, (Ed.) S.T. Hirasawa, Academic Press. Boston, 181-204.

REN21. 2014. Renewables 2014 Gobal Status Report. Police Network for 21st century.

Riau, V., De la Rubia, M.A., Pérez, M. 2015. Upgrading the temperature-phased anaerobic digestion of waste activated sludge by ultrasonic pretreatment. Chemical Engineering Journal, 259(0), 672-681.

Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H., Khanal, S.K. 2015. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. Bioresource Technology, 178, 178-186.

Shewani, A., Horgue, P., Pommier, S., Debenest, G., Lefebvre, X., Gandon, E., Paul, E. in press. Assessment of percolation through a solid leach bed in dry batch anaerobic digestion processes. Bioresource Technology(0).

Stabnikova, O., Liu, X.-Y., Wang, J.-Y. 2008. Anaerobic digestion of food waste in a hybrid anaerobic solid–liquid system with leachate recirculation in an acidogenic reactor. Biochemical Engineering Journal, 41(2), 198-201.

Taherzadeh, M.J., Karimi, K. 2008. Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. International Journal of Molecular Sciences, 9(9), 1621–1651.

Uke, M.N., Stentiford, E. 2013. Enhancement of the anaerobic hydrolysis and fermentation of municipal solid waste in leachbed reactors by varying flow direction during water addition and leachate recycle. Waste Management, 33(6), 1425-1433.

CHAPTER II

LITERATURE REVIEW

2.1 Feed stocks

A wide range of materials can be used in a biogas plant. Energy crops can be grown specifically as a feedstock or by-products and waste materials are normally used at competitive price. Mesophilic and thermophilic bacteria responsible for the anaerobic digestion process are only able to work and multiply if their substrates are sufficiently dilute making slurry an ideal substrate for the process (Regueiro et al., 2012). But recently, some dry digestion processes are developed for solid substrate in a drier environment. Various municipal, agricultural and industrial wastes are frequency treated using anaerobic digestion. The feedstock ranging from readily degradable wastewater to complex high-solid waste can be converted to methane by anaerobic bacteria at various degrees and difficulties. Even toxic compounds may be degraded. One requirement is that a given organic waste/wastewater should contain a substantial amount or concentration of organic matter that to make enough biogas at a reasonable cost. The classification in Figure 2-1 shows an overview of the various feedstocks assigned to the three major sources. Nevertheless, agriculture is the highest potential feedstock and widely used.

Feedstock is a comprehensive term representing the substrate input to the anaerobic system. It interacts with various aspects in anaerobic digestion that includes reactor configuration and operation as well as microbial physiology and community. Feedstock will also dictate type and quality of the end-products and their utilization to suit economic and legal conditions that differ from place to place. Among the agricultural wastes, manure from animals majorly from pig, cow and chicken are of primary importance since they contain abundant organics and nutrients. Harvested residues and garden wastes in most cases are treated through traditional routes for composting, soil conditioning and fertilizer purposes but they can be a significant source of substrate to anaerobic digestion. Agricultural feedstock materials useable as substrate include maize, grass silage, corn silage, whole crop, algae

biomass, weed, oil palm waste and any others biodegradable. These biological materials can be converted to many kinds of fuels such as ethanol, biodiesel, vegetable oil, liquefied fuel, and biogas. However, it is interesting to note that per one hectare of agricultural land, the energy conversion to biogas gave the highest energy in terms of driving distance at about 67.6 km (Figure 2-2). When compared with other renewable energies, biomass to biogas is the most efficient (Envitech-Biogas, 2009). Various kinds of materials can be used in anaerobic digestion as shown in Table 2-1

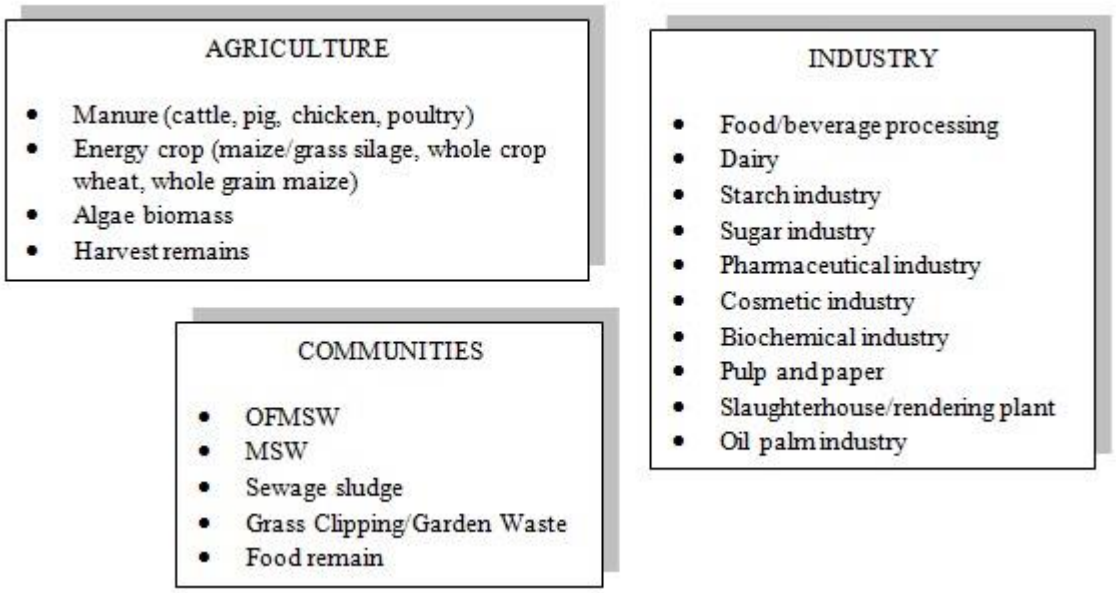


Figure 2-1 Survey of the various feed stocks from different source (Steffen et al., 1998b)

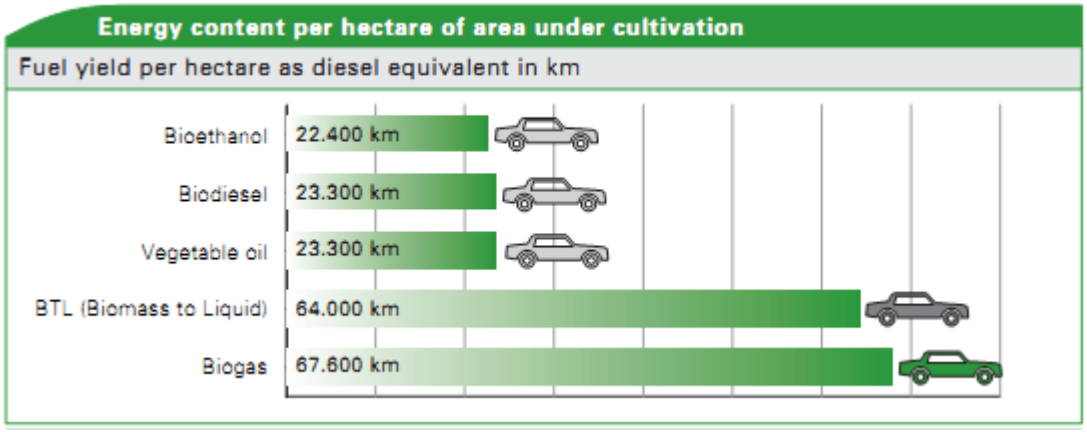


Figure 2-2 Compared fuel yield produced from biomass (Envitech-Biogas, 2009)

Table 2-1 Evaluation of organic wastes and by – products for the use in anaerobic digestion (NPR – No pre-treatment required)

Material	Excellent	Good	Poor	Remarks
Animal manure				
Chicken manure		+		Inhibiting NH_3^-contents can occur
Cow manure		+		Chopping of bedding straw
Animal manure from other animals		+		Chopping of bedding straw
Liquid piggery manure	+			NPR
Sludge from wine production	+			NPR; Increased H_2S – formation can occur
Biogenic materials from agriculture				
Straw and other fibrous plant residues			+	Chopping or grinding required
Green plant material, crops, grain, silages		+		Chopping required, disturbing sand, stones, scum layer formation can occur
Harvest residues		+		Chopping required, disturbing sand, stones
Silage leachate	+			High COD loading can result
Garden- and yard wastes			+	Chopping and impurities separation
Industrial and trade waste and Food waste				
Expired food		+		Expensive unpacking required
Whey	+			NPR
Residues from canning & frozen foods		+		Expensive unpacking required
Residues from fruit juice production		+		Chopping advisable
Pulp- and paper industry wastes		+		High fibre (cellulose) content, bactericidal agents from pulp additives
Residues from potato starch production		+		NPR
Residues from maize starch production		+		NPR
Residues from rice starch production		+		NPR
Primary sludge	+			NPR
Oil- and fat trap wastes		+		Scum layers and fat hardening can occur
Food leftovers from restaurants, large kitchens, refectories		+		Impurities separation (metals, plastics, bones) and sterilization required
Market wastes		+		Chopping and impurities separation

(Braun, 2003)

2.1.1 Pig manure (PM)

Livestock manure can produce greenhouse gas emission through the release of both methane and nitrous oxide. Livestock waste from animal farm is a source of classical pollution problems including air pollutant, water quality, disease vectors, insects, and etc. Livestock waste is comprised of excreta, hair or feathers, spilled water and feed, process-generated wastewater (water used for flushing gutters, etc.), bedding (sand, sawdust, wood shavings, peanut hulls, composted manure, and other substances.) and mortality, which contain high organic concentration. Livestock manure also contain high ammonia which could cause inhibition to anaerobic bacteria in the digester (Sawatdeenarunat et al., 2015). In some cases, manure still contain organic fiber as some animal eat grass, making it a bit more difficult to digest in AD (Mata-Alvarez et al., 2014). Chicken manure on the other hand contains small grits and stones that are mixed in the feed, some pretreatment may be needed prior to feeding it to AD.

Animal production, especially commercial pig production, is increasing rapidly in Thailand and tending to be concentrated on larger production units. This increases the risk of air, water and soil pollutions. Pig manure is high in nutrients particularly nitrogen and phosphorous. Pig manure also has a large biochemical oxygen demand (BOD) about 3,000 mg/L or be equal to wastewater quantity to 2-3 persons (40-50 g/person/day) (PCD, 2010). In 2012, Thailand has a produced about 7,824,421 pigs, but in 2014 had increased to about 7,909,670 pigs (OAE, 2014). It is alarming that such tendency to increase occurred every year. This will inevitably be the cause of increased waste quantity to be taken care of. If no proper management of pig wastes is in place, serious environmental problem will arise.

For animal farms in Thailand, manure and water are the major feedstock for digester to produce energy. The amount of water required to mix is controlled by the solids content of the manure and the type of digester installed (Figure 2-3). Ideally, manure should be free of foreign materials such as soil, sand, stones, or even fibrous bedding material for ease of digestion. The quality of the

manure is largely affected by animal diet, manure handling, and storage method. Animals fed with higher energy feed (e.g. grain-based diets) normally excrete manure with higher potential for methane gas generation as compared to that from animals fed with a fibrous diet (Ogejo et al., 2002).

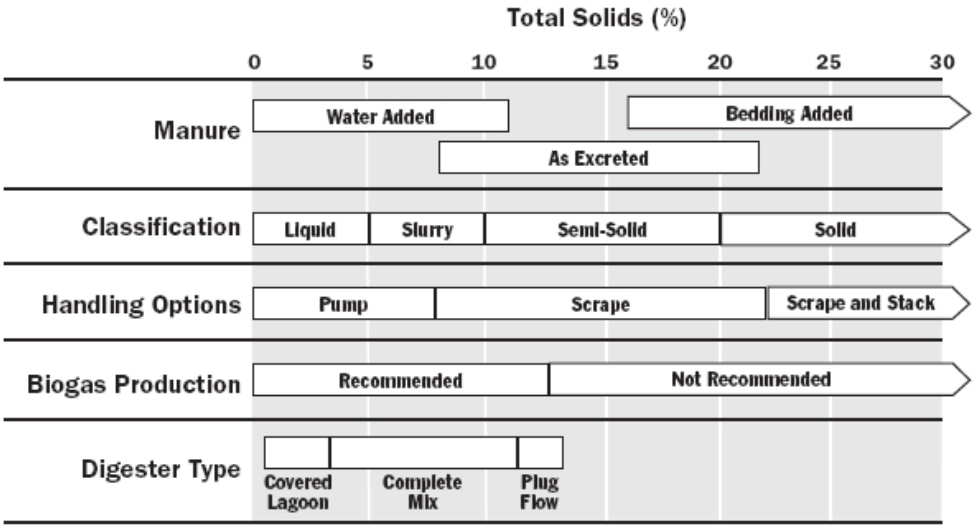


Figure 2-3 Appropriate manure characteristics and handling systems for specific types of biogas digester systems (Ogejo et al., 2002)

Usually one pig will excrete per day about 2% of weight, manure content with water about 65-85%, organic materials about 10-20% and inorganic 5-15%, majority of the materials will be sold to farmers for applying as fertilizer for vegetables, plants, orchards, and etc. Characteristics of the raw pig manure are shown in Table 2-2.

Table 2-2 Characteristics of the raw pig manure

Parameter	Unit	Average value±SD
Moisture	% of fresh waste	74±2
TS	g/kg fresh waste	235±4
VS	g/kg TS	716±10
COD	g/kg VS	1,400±200
VFA	gCOD/kg VS	67±5
Cellulose	g/kg VS	108±15
Hemicellulose	g/kg VS	54±7
Lignin	g/kg VS	83±18
NH_4^+N	g/kg TS	6±1
TKN	g/kg TS	44.23±1
Protein	g/kg VS	277±5
Lipid	g/kg VS	124±6
Carbon	% of TS	35
Nitrogen	% of TS	3
C:N ratio		14

(Panichnumsin et al., 2010)

2.1.2 Empty fruit bunch (EFB) biomass

EFB is lignocellulosic fiber and it has become a major source of renewable biological resource derived from the palm oil industry. Its quantity has been increased since palm oil has and continues to have the biggest share of consumable oils and the arable plantation is expanding due to the biodiesel production from palm oil. Palm oil is mostly used in the manufacture of major food products because of its comparatively low price. The palm biomass residues in Thailand totals about 8,160 kg/ha/year (Nimmanterdwong et al., 2015) that includes oil palm front (PF), and oil palm trunk (PT) and oil palm press fibre (PPF), oil palm shell (PS) and empty fruit bunch (EFB) are variable daily throughout the year when the palm are pruned during the harvest of fresh fruit bunch, PT is obtained during re-plantation of the oil palm tree. Plantation period of palm crop is about 25-30 years, when fruit yield drops and the trees are cut down for re-plantation. Burning biomass is not the only way to release its energy. Oil palm biomass can be converted to other usable forms of energy like methane gas or transportation fuels like ethanol and biodiesel. However in Malaysia, oil palm biomass usability to particleboard, cultivating material for mushroom culture, bioethanol, biohydrogen, composting and combustion for steam

and electricity at the mill. Summary of oil palm biomass utilization is shown in Table 2-3.

Table 2-3 Comparison of oil palm biomass utilization for various value added products.

Usability	Type of oil palm biomass				Remark
	EFB	PPF	PF	PT	
Energy conversion by Combustion[1,2]	+	+		+	- Incinerated to obtain oil palm ash that can be used as a source of fertilizer due to its high potassium content, but oil palm ash to synthesis absorbent for toxic gas removal (NO_x, SO_x)
Paper-making pulp[1]	+	+			- Waste from biomass residual - Water pollution and air pollution
Bioethanol[1]	+	+		+	- Waste from biomass residual, but low organic content
Biohydrogen[2]	+	+			- Waste from biomass residual, low organic content but can utilized to combustion
food for ruminants[1,3]		+	+		- PPF must be pretreatment due to improve of quality of food for ruminants
Furniture[1]	+	+		+	- Waste from biomass residual - Furniture cannot building structure because its low specific density
Particle board[1]	+	+		+	- Waste biomass residual - Water pollution and air pollution
Fertilizer[1,4]	+	+	+		- Increase the fertility of the soil - Provide a source of nutrient to growing oil palm trees, N:P:K; 1:0.7:1.3
Methane Production[5]					- Biogas 434.3 MJ/m^3 of oil palm wastewater are generated at the mill - Sludge form digester are used as soil conditioners or fertilizer

[1]Sumathi et al. (2008), [2]Kong et al. (2014), [3]Sinjermsiri et al. (2006), [4]Baharuddin et al. (2009), [5]Prasertsan and Sajjakulnukit (2006)

EFB is one of the largest organic waste residues that is now becoming available with the rise in biodiesel, cheap consumable oil and other purposes. Most of this valuable waste-stream was not fully recovered. It can be transformed to composting (Stichnothe and Schuchardt, 2010), biochar (Harsono et al., 2013), pellets and biofiber composite profiles (Eria, 2013). Utilization of EFB in Malaysia includes brown paper making, combust to generate electricity, binding agent for activated

carbon pellets production, cushion filling material, briquettes, biofuel production (pyrolysis oil), biomass as a fuel to be burnt in power stations for electricity generation or steam to be used in the milling process. Plywood or lumber for manufacturing furniture, fertilizer, mushroom culture and animal feedstock are also possible (Kong et al., 2014). For EFB (Figure 2-4) consists of high carbon content in forms of lignocelluloses; cellulose, hemicellulose and lignin. Table 2-4 shows characteristics of the oil palm empty fruit bunch.

Figure 2-4 EFB biomass from oil palm mill industry (a), and lignocellolisic fibre of EFB (b)

The chemical composition of plant cell is composed of three main substances: cellulose, hemicellulose, and lignin. Within the plant cell wall, chains of cellulose molecules linked with other polymers such as parcrystalline celluslose and hemicullulose to form linear structures of high tensile strength of microfibril. Multiple layers of microfibril stacks make up the cell wall (Figure 2-5). Each microfibril has a diameter of about 10 to 20 nm and could consist up to 40 cellulose chains. Although individual microfibrils cross-link to each other, hemicellulose also forms covalent in association with lignin, a rigid aromatic polymer. Lignin structure and organization within the plant cell wall are not well understood. Pretreatment of biomass or lignocellolosic wastes with enzymes or chemicals (alkaline or acid) is needed to disrupt the surrounding matrix of hemicellulose and lignin from the cellulose core prior to hydrolysis (Saha et al., 2005). Some conversion products of plant fiber are shown in Figure 2-6.

Table 2-4 Characteristic of empty fruit bunch

Proximate analysis	**Characteristics of EFB**
Quantity[1] (million ton/year in 2012)	4.85
Proximate analysis[1]	
Moisture (%)	38.4
Volatile Matter (%)	66.1
Fixed carbon (%)	28.4
Ash%	5.5
Ultimate Analysis[1]	
Carbon (%)	40.7
Nitrogen (%)	0.3
Hydrogen (%)	5.4
Oxygen (%)	47.0
Sulfur (%)	1.1
C:N ratio	135.7
Other chereacteristics[2]	
Higher heating Value (KJ/kg)	9,043
Lower heating value (KJ/kg)	7,109
Chemical components[3,4]	
Cellulose (%dry wt.)	38.3-50.0
Hemicellulose (%dry wt.)	18.9-35.3
Lignin (%dry wt.)	22.1-25.2

[1](Madhiyanon et al., 2012), [2](Kaewmai et al., 2012), [3](Kong et al., 2014) and [4](Kong et al., 2014)

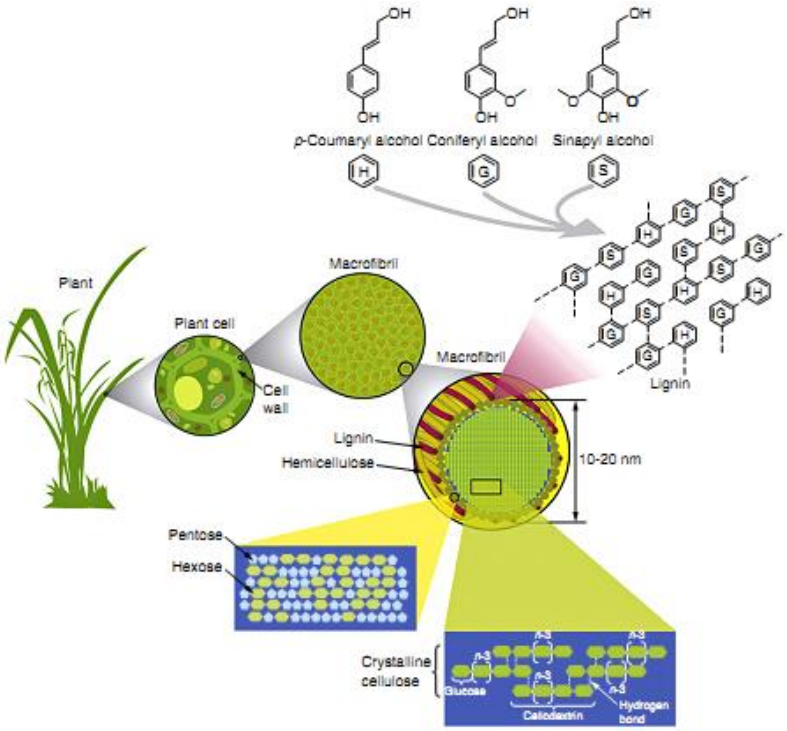


Figure 2-5 Plant and cellulose Structure (Meng and Ragauskas, 2014)

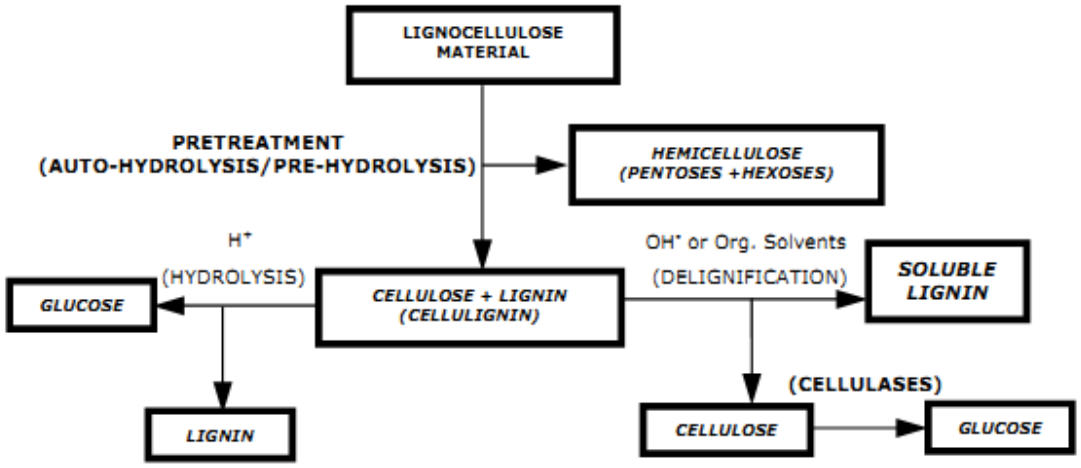


Figure 2-6 Some possible lignocellulose, hemicellulose and lignin conversion products (Pereira, 2008)

2.2 Pretreatment methods

Pretreatment of feedstock can increase solubilization of the material and enhance volatile solid destruction and any ensuing conversions (Tiehm et al., 2001). The use of pretreatment is particularly useful when the materials to be digested are resistant to further processing as it improves digestibility of lignin-rich biomass waste. Pretreatment methods include physical, chemical and biological means. Physical pretreatments focus on breaking down the structure with forces such as mechanical size reduction, ultrasonic pretreatment, temperature either heat or cold (Kondusamy and Kalamdhad, 2014). Chemical pretreatments use acid or alkali chemical to loosen or solubilize the structure. Biological pretreatment seems to be the cheapest method by employing microbial enzymatic activity to degrade different substances in the materials to make it more accessible for further conversions. The pretreatment methods are summarized in Table 2-5

In anaerobic digestion, biogas production of the methane producing reactor will be improved if the materials are pretreated prior to feeding the digester. This will certainly fasten the start up speed, the processing rate of a reactor, and the overall throughput of the system (Ariunbaatar et al., 2014). However, their additional cost must always be balanced against resultant improvements in efficiency. All physical, chemical, and biological pretreatment methods could be used to achieve complete hydrolysis to sugars or as partial step for breaking certain links in the hemicellulose-lignin polymeric system that will increase diffusivity of hydrolytic enzyme to the material. Alkali pretreatment has shown high potential and been adopted by several researchers since it has a capability to achieve increased production of volatile fatty acid and biogas in anaerobic digestion (Steffen et al., 1998a).

Pretreatment of cellulose and hemicellulose by biological method could be carried out mostly by lactic acid bacteria and other facultative microorganisms which could proliferate in moist environment, i.e. moisture content 30-70% (Insam *et al*., 2010). These microorganisms could release enzymes to attack and hydrolyze cellulose and hemicellulose extracellularly. Since lignin will not be biologically degraded by anaerobic bacteria, a group of facultative microorganisms is required for lignin degradation. Lignin metabolizing microorganisms comprise of different genera of bacteria such as *Pseudomonas*, *Bacillus*, *Steptomyses*, *Arthrobacter*, *Aeromonas*, *Xanthomonas*, *Flavobacterium* and Fungi (Schulze and Mooney, 1999). Manganese peroxidase, laccase, and lignin peroxidase produced extracellularly by aerobic white rot fungi can be involved in the degradation of lignin (Tamagawa et al., 2006). Since lignocellulosic materials are very complicated depending on the types of biomass, their pretreatment may, therefore, require different effort levels. Within each pretreatment, conditions should be optimized for the effective transformation that follows.

Rahman et al. (2006) studied pretreatment of EFB by thermochemical method using 6% H_2SO_4 at 120 °C, 15 min. It was found that under optimum conditions, xylose glucose, furfural and acetic acid were 29.4, 2.34, 0.87 and 1.25 g/L, respectively. Sinjermsiri et al. (2006) used urea and molasses to add to palm oil press fiber (PPF) and compared to the biological pretreatment with *Bacillus* and *Lactic* acid bacteria in 21-day fermentation. It was found that neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were decreased by 19.66%, 14.84% and 6.81%, respectively with urea plus molasses. In addition, that method could increase crude protein from 7.28% to 9.94%. In contrast, the fermentation by *Bacillus* did not improve NDF, ADF and ADL in PPF fiber.Umikalsom et al. (1997) reported that the treatment of EFB with dilute (0-5%) nitric acid followed by autoclaving at 121°C, 15 psi for 5 min was sufficient to remove hemicellulose from 21.9±1.4% (untreated) to 4.6±0.2% to break the cellulose-lignin complex. And *Chaetomium globosum* Kunze was able to grow using the treated EFB and produce high amounts of cellulese which assisted for rapid hydrolysis of the materials.

Table 2-5 Pretreatment process of lignocellulosic materials

Type of pretreatment method	Method	Possible changes in biomass	Notable remarks	References
Physical pretreatments	- Milling	- Affect rate of AD as it affects availability of a substrate to hydrolysis enzyme - Increase methane yield with decreasing particle size from 100 mm to 2 mm		Ward et al. (2008) Hendriks and Zeeman (2009) Qian (2014) Liu et al. (2013)
	- Microwave irradiation - High pressure steaming - Steam explosion - Hydrothermal - Ultrasonic	- Increase in accessible surface area and pore size - Decrease in cellulose crystallinity - Decrease in degrees of polymerization - Improve anaerobic stabilization - enhance the efficiency of hydrolysis and subsequently increase the sugar yield	- Most of the methods are highly energy-demanding - Most of them cannot remove the lignin - It is preferable not to use these methods for industrial applications - No chemicals are generally required for these methods	Tiehm et al. (2001) Oleskowicz-Popiel et al. (2008) Umikalsom et al. (1997) Liu et al. (2012) Karimi et al. (2014)
Chemical pretreatments	Acid - H_2SO_4 - HCl Alkaline - $CaCO_3$ - NaOH - H_2O_2 - Urea - molasses - $Ca(OH)_2$	- Increase methane production - Increase biodegradability - Increase in accessible surface area of lignocellulosic fiber - Partial or nearly complete delignification - Decrease in cellulose crystallinity - Decrease in degrees of polymerization - Partial or complete hydrolysis of hemicelluloses - Breakdown of cellulose to glucose	- These methods are among the most effective and include the most promising processes for industrial applications - Usually rapid treatment rate - Typically need harsh conditions - There are chemical requirements - More expensive	Sinjermsiri et al. (2006) Misson et al. (2009) Ortíz and Quintero (2014) Zheng et al. (2014) Mosier et al. (2005) Behera et al. (2014) Singh et al. (2015)

Table 2-5 (cont.)

Type of pretreatment method	Method	Possible changes in biomass	Notable remarks	References
Biological Pretreatments	- *Penicillium restrictum* (fungi) - *Plurotus ostreatus* (fungi) - *Lactic* - *Bacillus* - *Clostridiuum* - *Cellulimonas* - *Streptomyces* - *Acetovibrio* - *Clostridium thermocellom* - *Bacteroides callulosovens* - *etc.*	- Reduction in degree of polymerization of cellulose - Partial hydrolysis of hemicellulose	-Low energy requirement -No chemical requirement - Mild environmental conditions -Very low treatment rate - Did not consider for commercial application	Wan and Li (2012) Yang et al. (2014) Gupta and Verma (2015)

2.3 The principle of anaerobic digestion (AD)

In the past, anaerobic digestion (AD) was a single substrate, single purpose treatment. For example manure was digested to reduce organic content, sewage sludge is stabilized, or industrial wastewater is pre-treated. At present, AD is better known and easier to control. The confidence in the technology has increased and consequently become a multi-purpose process that includes waste upgrading, energy production, and improvement of fertilizer quality (Comino et al., 2009; Steffen et al., 1998b). Recently, it has been realized that digestion could be more in balance when a variety of substrates are supplied in mix and this co-digestion strategy also improves biogas production potential.

Originally, anaerobic digestion (AD) process is essential for treatment of waste with high organic strength from various industries and animal raising units. Concentrated latex wastewater, palm oil mill wastewater, municipal solid waste, soybean processing wastewater, animal manure, starch processing wastewater are just a few that could be treated by anaerobic digestion with success (Browne et al., 2013; Dechrugsa et al., 2013; Kaparaju et al., 2009; Liu et al., 2009). More recently, agricultural biomasses are good candidates as substrate material. In AD process, degradation of organic content in waste is accomplished by anaerobic metabolism carried by various groups of microorganisms. This type of microorganisms is present in the oxygen free environment. In an uncontrolled situation, this process is simply organic decomposition occurring naturally giving off bad smells and leachate. With a controlled environment, the off-gas is captured and used as fuel while the waste is being treated. The operating cost of anaerobic treatment is minimal because no energy is required for oxygen delivery.

Most anaerobic biological unit processes have low operating cost when compared with physical or chemical unit processes. The growth rate of cell mass in this process is, however, slower than that in the aerobic process about 6-8 times (Metcalf and Eddy, 2004). By this characteristic, detainment of the biomass within the system is of great importance. Also, nutrients requirement is usually low due to slower growth, making minimal biomass or sludge to be disposed of.

The anaerobic digestion of waste is a complex biochemical reaction carried out in a number of steps with several types of anaerobic microorganisms. During the process, a gas principally composed of methane (CH_4) and carbon dioxide (CO_2) is produced. The amount of gas produced varies with the amount of organic waste fed to the digester. Also, the temperature greatly influences the rate of decomposition and gas production. Anaerobic degradation (AD) occurs in four distinct steps (Deublein and Steinhauser, 2008a; Haandel and Lubbe, 2007; Speece, 2008), as shown in the Figure 2-7 and Table 2-6.

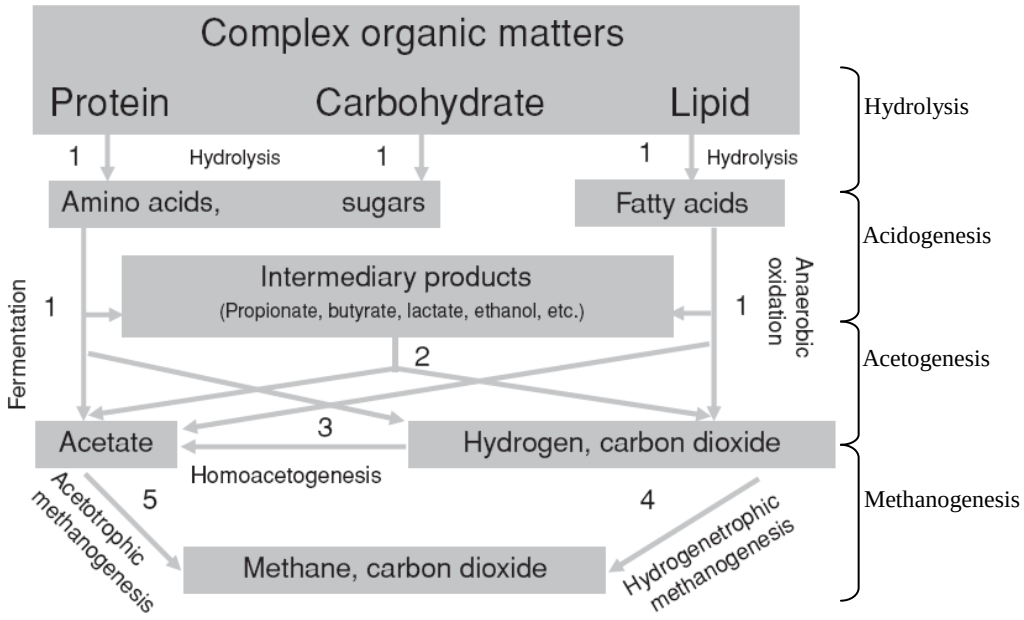


Figure 2-7 Conversion steps in anaerobic digestion of complex organic matter. (Khanal, 2008a)

Table 2-6 Some of the important microbial reactions in methanogenic ecosystems

Reaction			
1. Hydrolysis reaction			
1)	$(C_6H_{12}O_6)n + H_2O$	→	$nC_6H_{12}O_6$
2)	Sucrose + H_2O	→	Glucose + Fructose
3)	Lipid	→	Fatty acids
4)	Protein	→	Amino acid
2. Acidogenic reaction			
1)	$C_6H_{12}O_6 + 3H_2O$	→	$3CH_4 + 3HCO_3^- + 3H^+$
2)	$C_6H_{12}O_6 + 2H_2O$	→	2 Ethanol + $2HCO^- + 2H^+$
3)	$C_6H_{12}O_6 + 3H_2O$	→	Butyrate + $2H_2$+ $2HCO_3^- + 2H^+$
4)	$C_6H_{12}O_6$	→	2 Lactate + $2H^+$
5)	$C_6H_{12}O_6$	→	3 Acetate + $3H^+$
6)	3 Lactate	→	2 Propionate + Acetate + $HCO_3^- + H^+$
3. Acetogenic reaction			
1)	Lactate + $2H_2O$	→	Acetate + $HCO_3^- + H^+$+ $2H_2$
2)	Ethanol + $2HCO_3^-$	→	Acetate + 2 Formate + $H_2O + H^+$
3)	Ethanol + $2H_2O$	→	Acetate + $2H_2 + H^+$
4)	Butyrate + $2H_2O$	→	2 Acetate + $2H_2 + H^+$
5)	Propionate + $3H_2O$	→	Acetate + $3HCO_3^- + H^+$
4. Methanogenic reaction			
1)	Acetate + H_2O	→	$CH_4 + HCO_3^-$
2)	$H_2 + HCO_3^-$	→	$CH_4 + 3H_2O$
3)	Acetate	→	$CH_4 + CO_2$
4)	$CO_2 + 4H_2$	→	$CH_4 + 2H_2O$
5)	4 Formate + $H_2O + H^+$	→	$CH_4 + 3HCO_3^-$

(Gerardi, 2006; Stams et al., 2003; Thiele, 1991)

- Hydrolysis : This is the first step of AD. The complex organic matter is decomposed into simple soluble organic molecules or liquefaction. Hydrolytic bacteria convert the organic complex polymers to their respective organic monomers. For example, celluloses are transformed to glucose or alcohols, proteins to amino acids, and lipids to fatty acids. These are carried out by several hydrolytic enzymes such as cellulases, hemicelluase, amylases, lipases, proteases and etc. (Metcalf and Eddy, 2004; Speece, 2008).

- Acidogenesis : In this acid forming phase, acidogenic bacteria convert intermediary products such as simple sugars, fatty acids, and amino acid from hydrolysis phase into simple compounds such as short chain volatile fatty acids (propionic, formic, lactic, butyric, succinic acid), alcohol, ketone, ethanol, methanol, glycerol, acetone (Rittmann and McCarty, 2001). Typical reactions in the acid forming phase are show in Table 2-6.

- Acetogenesis or acetoclastic : The intermediates from the acidogenic products, i.e. the propionate, butyrate and alcohols are transformed by acetogenic bacteria into hydrogen, carbon dioxide and acetic acid. A number of different microbial species, e.g., *Syntrophobacter wolinii*, a propionate decomposer, *Syntrophomonas wolfei*, a butyrate decomposer are involved in this process. The final products of fermentation are the precursors of methane formation mainly H_2, CO_2, and acetate.

- Methanenogenesis : In the last stage, methane is produced by a group of archaea called methane formers or methanogens in two different pathways: either by means of cleavage of acetic acid molecules to generate carbon dioxide and methane, or by reduction of carbon dioxide with hydrogen. Methanogens are divided in two subcategories;

i) Acetotrophic methanogens are also called acetate splitting methanogens. Seventy two percent of the COD is converted into methane by this pathway. This group comprises two main genera: *Methanosarcina* and *Methanosaeta* (Khanal, 2008a).

ii) *Hydrogenetrophic methanogens* convert formic acid, hydrogen and carbon dioxide into methane which accounts for about 28 percent of the COD flow (Figure 2-8). The hydrogen utilizing methanogen helps maintain very low partial pressure ($<10^{-4}$ atm) (Gerardi, 2006) necessary for the continuous conversion of volatile fatty acids and alcohol to acetate.

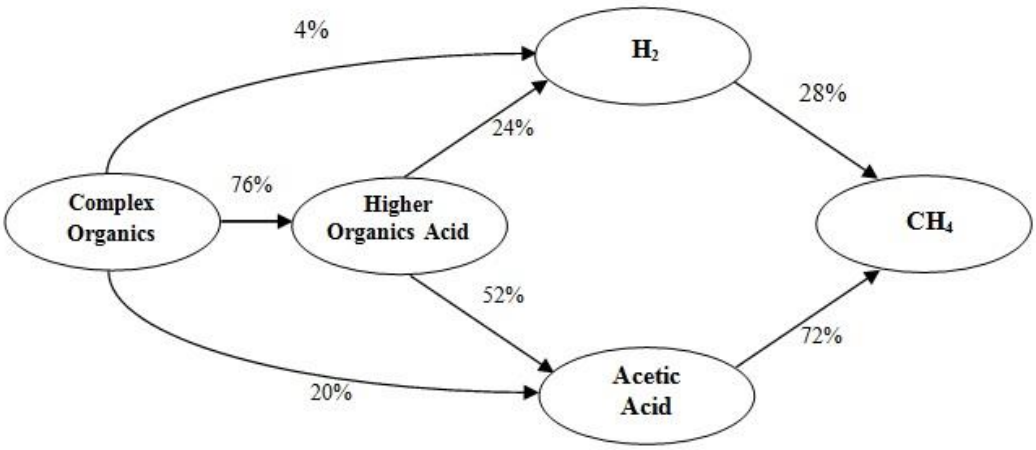


Figure 2-8 Carbon and hydrogen flow in anaerobic digestion process (percentages are based on COD) (Metcalf and Eddy, 2004)

The four steps of anaerobic digestion dictate transformation of complex materials into simple molecules such as methane and carbon dioxide. The bacteria in all 4 steps have a synergistic relationship. Although some bacteria, fungi and protozoa may be found in AD, bacteria are undoubtedly the dominant microorganism. Large number of strict and facultative anaerobic bacteria that include species such as *Bacteroides, Bifidobacterium, Clostridium, Lactobacillus,* and *Streptococcus* are involved in hydrolysis and acidogenesis in the digestion of organic compounds (Boonapatchchroen, 2003; Deublein and Steinhauser, 2008a; Jupraputtasri et al., 2005). The products from this group of microbes are acetate, propionate, butyrate, lactate, hydrogen. All intermediary products from acidogenic and acetogenic phase are substrates for methanogens (Achaea). The methanogenic bacterial consortium includes species, e.g., *Methanococcus, Methanobacterium, Methanobacillus,* and *Methanosarcina spp*. These bacteria are very sensitive to oxygen. The anaerobic treatment process relies on a balanced symbiotic relationship between many metabolically distinct microbial populations such as acidogens and methanogens (Lastella et al., 2002). The methanogens are dependent on intermediary acetogens for the supply of their substrates (acetate, hydrogen and formate). The acetogens in turn depend on the methanogens for the conversion or removal of their intermediary products to prevent product inhibition. Accumulation of these acids can

lead to digester failure, so methanogenic reactions are important for the stable operation of methane producing reactor.

2.4 Co-digestion

Co-digestion is a simultaneous digestion of a homogenous mixture of two or more substrates in order to balance digestion environmental condition as such C:N ratio and moisture. The most common situation is when an amount of main basic substrate (e.g. wastewater, manure or cow slurry, biomass, and etc.) is mixed and digested together with minor amounts of another or a variety of additional substrates. The expression "co-digestion" does not specify the ratio of the respective substrates in use simultaneously.

The stability of anaerobic digesters depends largely on the balance of nutrients within the system. C:N ratio of the reactor content is controlled by the feedstocks. Microorganisms generally favor C:N ratio of 25-30:1 (Ward et al., 2008) for methane formation and C:N ratio of 10-45 for hydrolysis. Co-digestion of low C:N with a high C:N feedstocks such as biomass can adjust the ratio closer to ideal. For optimum degradation, a C:N:P ratio of 100:5:1 is recommended (Steffen et al., 1998a).

Co-digestion is a mean to improve yield of a single substrate digestion. The use of a co-substrate in most cases should increase the product and biogas/methane yields due to positive synergisms established in the digestion medium resulted from the supply of lacking nutrients by the co-substrates. The mixing ratio of the co-substrates either wastes or agricultural materials is of importance for the effectiveness of the co-digestion strategy. It should also be realized that, it might not be possible always to operate with the optimum blend of wastes particularly in a large scale co-digestion facility (Misi and Forster, 2001). However, co-digestion offers several possible ecological, economical and technology advantages. Advantages and limitations of the anaerobic co-digestion are shown in Table 2-7.

Table 2-7 Advantages and limits of anaerobic co-digestion

Advantages	Known Limits
1. Improved nutrient balance and digestion reaction[1]	1. Increased digester effluent COD[1]
2. Increased biogas production and methane yield [2,3,4,7]	2. Additional pretreatment requirements[1]
3. Higher income tank to gate fees for waste treatment[2,5]	3. Increased mixing requirements[5,6,7]
4. Additional fertilizer (soil conditioner)[1]	4. Restrictions of land use for digestate[1]
5. Recycling of biomass back to agriculture[2]	5. Economically critical dependent on crop costs and yield[3]
6. A source of carbon neutral energy is produced in the form of biogas[2]	

(Braun and Wellinger, 2003; Comino et al., 2009; Dechrugsa et al., 2013; Deublein and Steinhauser, 2008b; Mata-Alvarez et al., 2014; Regueiro et al., 2012; Sawatdeenarunat et al., 2015)

2.5 Factors affecting anaerobic digestion in acidification phase

There are several factors involved in successful startup and operation of an anaerobic digestion system in acidification phase. Depending on the quality and nature of the wastes, some additional digester equipment will help achieve a reliable digestion. Normally, the additional equipment is required for pre-treatment, homogenisation and mixing of co-substrates, prevention of excessive foaming and scum layer formation, removal of sediments from the digester, digestion control and monitoring (Braun and Wellinger, 2008). The key factors in anaerobic digestion are as follows.

- pH and buffering capacity. In anaerobic environment, pH is known to affect enzymatic activity because only a specific and a narrow pH range is often suitable for the maximum activity. A pH range between 6.7-7.4 is reported suitable for most methanogenic bacteria to function (Sawyer et al., 2003) and 5.5-7 for acidogenesis bacteria (Mtz.-Viturtia et al., 1995). The rate of methanogenesis may decrease if the pH is lower than 6.3 or higher than 7.8. The main reason for the absence of biodegradation is a rapid acidification of the waste (Verma, 2002). Under normal conditions, this pH reduction is buffered by bicarbonate produced by

methanogens. Under adverse environmental conditions, the buffering capacity of the system can be upset, eventually stopping methane production. Adequate alkalinity in an anaerobic digester can be maintained by providing an acceptable VFA-to-alkalinity ratio (VFA/ALK). The range of the acceptable VFA/ALK is 0.1 to 0.2. The rise of this ratio is generally due to the over production of VFA by the activity of hydrolytic acidogenic bacteria capable of degrading the waste in the first step. Variations in pH of material in the feedstocks can easily upset the operation of anaerobic digester. In a stuck or sour digester, methane production is interrupted, VFA and other fermentation products accumulate, and sometimes it is difficult and takes a long time to restore to normal operation. Once that occurred, it is usually necessary to clean out the digester and recharge it with large volumes of anaerobic sludge from an operational unit, which is a costly and time consuming task (Bitton, 2005; Sawyer et al., 2003).

- Volatile Fatty Acid (VFAs). The long chain fatty acids were found to be inhibitory to the several kinds of essential reactions in the anaerobic digestion because of their toxicity to the bacteria (Metcalf and Eddy, 2004; Rittmann and Mccarty, 2001; Speece, 2008). If the pH is maintained near neutrality, volatile acids such as acetic or butyric acid appear to exert little toxicity toward methanogens. Propionic acid, however, displays toxicity to both acid-forming bacteria and methanogens (Bitton, 2005). Pind *et al.* (2003) studied the effects of VFA on the anaerobic process and showed that the high concentrations of propionate affected the degradation of all VFAs. Any change in the loading of the digester must be gradual in order to ensure that the concentration of volatile acids does not exceed the normal buffering capacity of the system. Normal volatile acids concentrations in sewage sludge digesters are between 250–1,000 mg/L, but values in excess of 1,800–2,000 mg/L indicate some problems. Yang (2009) found that the lignocellulosic structure damage was caused by the effect of VFAs from anaerobic process. It increased accessibility of enzyme into structure, and caused an improved rate of hydrolysis.

- Seeding. The first and second steps of anaerobic digestion dictate transformation of complex materials into simple molecules to volatile fatty acid (VFA), hydrogen and carbon dioxide. The bacteria in all 2 steps have a synergistic relationship. Although some bacteria fungi and protozoa may be found in acid phase

of AD, bacteria are undoubtedly the dominant microorganism. Large number of strict and facultative anaerobic bacteria includes, for example, *Bacteroides, Bifidobacterium, Clostridium, Lactobacillus, Streptococcus* are participating in hydrolysis and acidogenesis in the digestion of organic compounds. The products from this group of microbes are acetate, propionate, butyrate, lactate, hydrogen. Cow manure is containing bacterial consortium with a variety of microbial population such as hydrolytic, acidogenic, e.g. *Enterobacter, Bifidobacterium Thermacidophilum* and *Caloramator* (Yan et al., 2014). Hydrolysis bacteria released extracellular enzyme able to degrade the lignocellulosic biomass (cellulose hemicellulose and lignin) (Yue et al., 2013), and it produces VFAs as intermediates from anaerobic pathway.

- Retention time. The number of days the reactor content stays in the digester is defined as the retention time. Two types of retention times are involved in an anaerobic digester: solids retention time (SRT) and hydraulic retention time (HRT). The solids retention time (SRT) represents the average time microorganisms spend in the system (Gerardi, 2006). The HRT is the time the liquid stays in the anaerobic digester. SRT should correctly be determined because it indicates the potential of bacterial biomass loss. If there is a significant washout of bacteria, the digester will fail. Longer SRT value in anaerobic digesters will maximize the gas production and provide buffering capacity against the effects of shock loadings and toxic compounds in feedstock. It can as well permit the bacteria to acclimate to toxic compounds.

Retention time can be viewed as the average period that a given quantity of input remains in the digester to be acted upon by the microorganisms. The retention time is also dependent on the temperature. The higher the temperature, the lower the retention time is required because of higher microbial and enzymatic activities. The length of time that volatile solids remain in an anaerobic digester is an important factor in the digestion process. In completely mixed anaerobic digesters with no sludge recycling, the SRT is equal to the hydraulic retention time (HRT). Hydraulic retention times usually vary from 10 to 30 days depending on the substrate and temperature. If solid retention time is too short the microbes are “washed out” of

the digester and digestion process fails, while a long retention time requires a larger digester (Deublein and Steinhauser, 2008a)

- Loading rate or solids content. Typically there are different operational parameters associated with the solids content of the feedstock to the digesters.
  - High-solids digestion or dry digestion (20-40% TS)
  - Medium-solids (15-20% TS)
  - Low-solids (<15% TS)

Digesters can either be designed to operate in a high solids content, e.g. with a total solids (TS) in digester at 20-40% also called dry digestion, medium solid concentration between 15-20% and low solid concentration at <15% TS (Kothari et al., 2014). The thickness of the material may also lead to associated problems with abrasion to piping and machine parts. High-solids digesters will typically have a lower land requirement due to the lower volumes resulted from the low moisture. In low-solids digesters, materials can be transported through the system using standard pumps which need low energy input. Low-solids digesters have a larger footprint as a result of the increased liquid-to-feedstock ratio of the digesters. Nevertheless, there are benefits in the operation of a liquid or wet environment as a thorough mixing and circulation of materials are achieved and the contact between the bacteria and their food is ensured. The bacteria have a more readily access to the substrates and increases the rate of gas evolution (Deublein and Steinhauser, 2008a).

2.6 Anaerobic dry digestion

Dry anaerobic digestion is a process in which anaerobic fermentation is carried out at a total solid content of the materials about 20-40%. Dry digestion has some advantages over other systems. It has lower wastewater to be handled because no or very low water consumption and the digestate is relatively dry suitable for making fertilizer or compost. It demands lower energy input because of the reduced volume compared to the wet process (with liquid around 90%) while still has high solid destruction with low operating cost. A disadvantage of anaerobic dry digestion is a requirement for material pretreatment before feeding to the bioreactor and

material handling and mixing are difficult (Dogan et al., 2008; Kothari et al., 2014; Liang et al., 2014).

In Germany, dry fermentation has been applied to municipal solid waste (MSW) (Figure 2-9). Dry fermentation process is carried out in gas tight fermenters in a dry fermentation plant. This fermenter comprises multiple air tight chambers which will accept the solid waste in rotation. The door of the chamber must be specific, since it must prevent air from entering to the fermenting piles inside the chamber (Figure 2-9b). The liquid is sprayed onto the pile to percolate and the liquid leachate is then recirculated through the MSW pile, keeping pile moist. And the leachate is brought to a separate anaerobic digester tank for biogas production. The biogas produced could have the methane component of about 80% (Deublein and Steinhauser, 2008b).

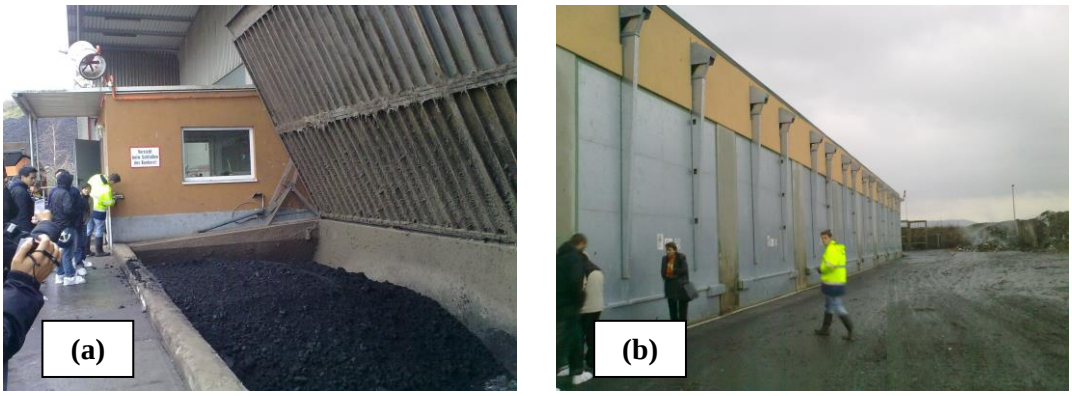


Figure 2-9 Dry fermentation plant (a) solid loading dock and (b) dry fermentation chambers

After MSW pile is digested, it will be taken off the chamber and made to window composting piles (Figure 2-10a). The pile will be mixed and aerated frequently with the scrapper (Figure 2-10b). This method will yield compose as a product.

Figure 2-10 Composing pile of the solid digestate (a), and scraper (b)

2.7 Bio-digester

The bio-digester can be classified as low rate or high rate as shown in Table 2-8. The low rate systems usually do not control of the environment inside the digester tank. The substrate enters and lets stand in the tank as the anaerobic organisms can work their way to the substrate by diffusion or natural movement of the digester content. No temperature control is embedded to stimulate biochemical reactions. In high rate systems, mixing is administered to promote contact of microbial cells with the substrate, or attach media is placed inside the reactor. Heating is usually provided particularly in cold climatic regions.

Table 2-8 Classification of anaerobic reactors

Low rate anaerobic digester	High rate anaerobic digester
• Anaerobic pond/covered lagoon • Septic tank • Imhoff tank • Standard rate anaerobic digester	• Suspended growth - High rate anaerobic digester - Anaerobic contact process - Up-flow anaerobic sludge blanket (UASB) - Anaerobic sequencing batch reactor (ASBR) - Continuous stirrer tank reactor (CSTR) - Anaerobic leach bed reactor (ALBR) • Attach growth - Anaerobic filter (AF) - Fluidized/Expended bed reactor • Other - Hybrid reactor - Static granular bed reactor

(Khanal, 2008b)

Methods to operate reactors can be divided into 3 modes as batch, semi-continuous, and continuous for feeding organic substrate into bioreactor. The selection of these three modes depends on the method of the anaerobic systems design (Ahring, 2003; Deublein and Steinhauser, 2008b).

- Batch mode feeding -- Organic substrate is fed only once to a bioreactor and let the spontaneous degradation by microorganisms in bioreactor proceeds. A batch system is the simplest form of digestion. Biomass is added to the reactor at the beginning in one batch and the reactor starts until the end of the process. Batch reactors face odor issues when they are emptied. Typically, biogas production will follow a bell shape distribution pattern over time. The operator can somehow estimate the end of the process as the substrate conversion has finished. Batch digestion requires less equipment and lower levels of design work making it typically a cheaper form of digester.

- Semi-continuous mode feeding -- A substrate will be fed to a reactor periodically. The reactions will proceed and continue until all or most of the substrate is converted into end products. A part of the reactor's content is then withdrawn and the new substrate is fed to the reactor. And the process will be repeated at time intervals.

- Continuous mode feeding -- In continuous digestion processes, organic matter is constantly added to the bioreactor. The reactor's content will be replaced by the substrate fed to the system. There will also be effluent coming out of the reactor continuously. The end products are constantly or periodically removed, resulting in constant production of the end product.

2.8 Anaerobic Leach bed Reactor (ALBR)

ALBR is a high solid digester operated in a semi-batch fed manner. The solid materials are fed through the bottom the reactor and the fermentation proceeds while the materials move up to the top. Other analogous operations may vary with the principle of the fresh material is to be fed to the digestate pile full of microorganisms. In Figure 2-11, the digested residue will be removed from the top.

Gas bubbles generated by the bacteria adhere to solid particles and thus, naturally induce floatation like in common digesters. Due to missing agitation inside the leach-bed reactor, a liquid phase is formed and used as leachate to circulate to the separate digester, which can improve volumetric methane yield (Browne et al., 2013; Jagadabhi et al., 2011). This leachate circulates upwards through the leach-bed reactor and through a high rate anaerobic digester with immobilized bacteria (Stabnikova et al., 2008). By this configuration, ALBR can separate to two stages for acidogenic and methanogenic. The organic substrate such as lignocellulosic material, municipal solids waste, food waste, are hydrolyzed in the first stage reactor by leachate recirculation on materials (Xu et al., 2012), and the leachate contains soluble organics will be converted to methane in the second stage.

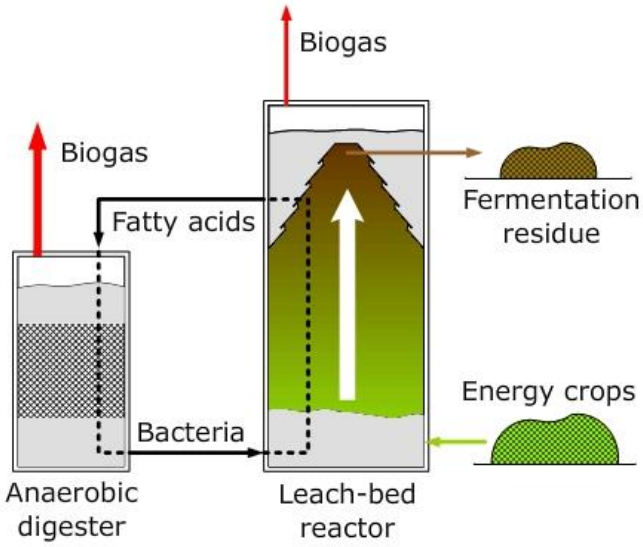


Figure 2-1 Schematic diagram of two stage process comprising anaerobic leach bed reactor (right) with leachate anaerobic digester (left) (ATB, 2011)

Beside the typical anaerobic digestion parameters such as temperature, pH, mixing, and etc. as previously mentioned, other operating parameters also play an important role. Packing density of the bed and leachate recirculation rate are of interest because these factors could directly improve the performance of ALBR. Packing density in the bed will affect hydraulic conductivity or the ability of liquid to permeate through the bed. Chen and Chynoweth (1995) studied the effects of packing density of compacted municipal solid waste at 160, 320 and 480 kg/m^3 to ALBR operation. The leachate was recirculated by means of periodic flooding. It was found that hydraulic conductivity varied positively with packing density. The packing density of 160 kg/m^3 yielded the highest hydraulic conductivity of 9.6×10^{-2} cm/s. It is notable that higher density of material would develop over time as the large size particles are decomposed into smaller ones. In this work, only characteristics of the hydraulic conductivity of compacted municipal solid waste were studied. No digestion test had been performed. Resistance to leachate movement and biogas release might have some effects on the waste digestability. Myint and Nirmalakandan (2009) reported the increased performance of the ALBR by having a more porous bed. Adding pistachios-half-shell in mix with cattle manure caused improved VFA and COD production from 0.132 to 0.152 g VFA/g dry manure, and 0.172 to 0.185 g COD/ g dry manure, respectively. More active acidogenesis in the bed was achieved. Also, the dilution of leachate reportedly affected ALBR performance positively.

Browne et al. (2013) studied the rate of degradation of food waste by using ALBR 50L at different leachate flowrate rate 17 and 102 L/d, digestion time 24 days. It was found that increase in leachate recirculation flowrate could improve VS degradation. The leachate flowrate rate of 17 and 102 L/d raised VS degradation from 51.5% to 72.2%. Furthermore, inoculum to substrate ratio (ISR) was found to be insignificant to increase hydrolysis rate of food waste in ALBR although it showed protein hydrolysation increase at higher ISR 0.8 (w/w basis) (Xu et al., 2012). Lower ISR was recommended for hydrolytic-acidogenic acitivity.

Percolating and flooding modes of leachate recirculation mechanism have been of importance to the operation of ALBR because the leachate from solid fermentation reactor removes intermediates from the bed allowing continuous reactions while the leachate could be used to produce biogas. Approach in percolating the leachate has a limitation since the liquid could not thoroughly be in contact with the solid particles. Although flooding could be better in this respect, difficulties related to flooding particularly the large liquid volume pumping and drainage still persist. Kusch et al. (2008) studied the biogas production from horse dung digestion by comparing between percolated and flooded of leachate for 50-L ALBR at 37°C. Leachate was recirculated 2 times per day for 15-20 min. It was found that within 42 days, the flooded operation achieved an average methane production of 160.9 L CH_4/kgVSadded and VS removal of 50.7%, which were higher than the percolated operation (147.3 L CH_4/kgVS added and 40.5% VS removal).

REFERENCE

Ahring, B.K. 2003. Advances in Biochemical Engineering/Biotechnology. Springer-Verlag Berlin Heidelberg, 2204.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L. 2014. Pretreatment methods to enhance anaerobic digestion of organic solid waste. Applied Energy, 123(0), 143-156.

ATB. 2011. Innovations : Novel high rate system for biogas production from energy crops, The Leibniz Institute for Agricultural Engineering Potsdam-Bornim (ATB). Avaliable in : http://www2.atb-potsdam.de/Hauptseite-englisch/research/Innovations_e/Aufstrom_07/biogas_aufstrom_mehr_e.htm.

Baharuddin, A.S., Wakisaka, M., Shirai, Y., Adb-Aziz, S. 2009. Co-Composting of Empty Fruit Bunched and Partially Treated Palm Oil Mill Effluents in Pilot Scale. International Journal of Agricultural Research, 4(2), 69-78.

Behera, S., Arora, R., Nandhagopal, N., Kumar, S. 2014. Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. Renewable and Sustainable Energy Reviews, 36(0), 91-106.

Bitton, G. 2005. Wastewater Microbiology. John Wiley & Sons, Inc., New Jersey.

Boonapatchchroen, N. 2003. Characterization of Microbial Community in Granular Sluudge Treating Cassava Starch Wastewater by 16S rRNA Approach. in: Biotechnology, Vol. M. Sc. Thesis, School of Bioresource and Technology, King Mongkut's University of Technology Thonburi.

Braun, R. 2003. Energy from Biogas and Landfill Gas, Vol. Task 37, Department for Agrobiotechnology

University of Natural Resources and Applied Life Sciences, Vienna. Avaliable in : http://www.limpiemoselagua.com.mx/archivos/Presentaciones%20y%20catalogos/47_POTENTIAL%20of%20Co-DIGESTION.pdf.

Braun, R., Wellinger, A. 2008. Potential of Co-digestion. IEA Bioenergy Task 37. (online).

Braun, R., Wellinger, A. 2003. Task 37 : Energy from Biogas and Landfill Gas. IEA Bioenergy, Avaliable in : www.iea-biogas.net.

Browne, J.D., Allen, E., Murphy, J.D. 2013. Improving hydrolysis of food waste in a leach bed reactor. Waste Management, 33(11), 2470-2477.

Chen, T.-h., Chynoweth, D.P. 1995. Hydraulic conductivity of compacted municipal solid waste. Bioresource Technology, 51(2–3), 205-212.

Comino, E., Rosso, M., Riggio, V. 2009. Development of a pilot scale anaerobic digester for biogas production from cow manure and whey mix. Bioresource Technology 100, 5072-5078.

Dechrugsa, S., Kantachote, D., Chaiprapat, S. 2013. Effects of inoculum to substrate ratio, substrate mix ratio and inoculum source on batch co-digestion of grass and pig manure. Bioresource Technology, 146(0), 101-108.

Deublein, D., Steinhauser, A. 2008a. Biogas from Waste and Renewable Resources. WILEY-VCH Verlag GmbH & Co. KGaA., Germany.

Deublein, D., Steinhauser, A. 2008b. Biogas from waste and renewable resources : An introduction. WILEY-VCH Verlag GmbH & Co. KGaA,Weinheim, Germany.

Dogan, E., Dunaev, T., Erguder, T.H., Demirer, G.N. 2008. Performance of leaching bed reactor converting the organic fraction of municipal solid waste to organic acids and alcohols. Chemosphere 74, 797-803.

EnviTech-Biogas. 2009. Tailored biogas systems –an investment for the future., p. 4.

ERIA. 2013. Sustainability Assessment of Biomass Utilisation in East Asia Working Group Extending the ERIA WG Methodology for Sustainability Assessment of Biomass Utilization in East Asian Countries

Economic Research Institute for ASEAN and East Asia.

Gerardi, M.H. 2006. Wastewater Bacteria. John Wiley & Sons, Inc., New Jersey, 267.

Gupta, A., Verma, J.P. 2015. Sustainable bio-ethanol production from agro-residues: A review. Renewable and Sustainable Energy Reviews, 41(0), 550-567.

Haandel, V., A., Lubbe, V., J. . 2007. Handbook Biological Wastewater Treatment. Leidschendam Publishing, Netherlands.

Harsono, S.S., Grundman, P., Lau, L.H., Hansen, A., Salleh, M.A.M., Meyer-Aurich, A., Idris, A., Ghazi, T.I.M. 2013. Energy balances, greenhouse gas emissions

and economics of biochar production from palm oil empty fruit bunches. Resources, Conservation and Recycling, 77(0), 108-115.

Hendriks, A.T.W.M., Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technology, 100(1), 10-18.

Jagadabhi, P.S., Kaparaju, P., Rintala, J. 2011. Two-stage anaerobic digestion of tomato, cucumber, common reed and grass silage in leach-bed reactors and upflow anaerobic sludge blanket reactors. Bioresource Technology, 102(7), 4726-4733.

Jupraputtasri, W., Boonapatcharoen, N., Cheevadhanarak, S., Chaiprasert, P., Tanticharoen, M., Techkarnjanaruk , S. 2005. Use of an Alternative Archaea-Specific Probe for Methanogen Detection. Journal of Microbiological Methods, 61, 95- 104.

Kaewmai, R., H-Kittikun, A., Musikavong, C. 2012. Greenhouse gas emissions of palm oil mills in Thailand. International Journal of Greenhouse Gas Control, 11(0), 141-151.

Kaparaju, P., Serrano, M., Angelidaki, I. 2009. Effect of reactor configuration on biogas production from wheat straw hydrolysate. Bioresource Technology 100, 6317-6323.

Karimi, M., Jenkins, B., Stroeve, P. 2014. Ultrasound irradiation in the production of ethanol from biomass. Renewable and Sustainable Energy Reviews, 40(0), 400-421.

Khanal, S. 2008a. Anaerobic biotechnology for bioenergy production: principles and applications. JohnWiley & Sons, Ltd., USA.

Khanal, S.K. 2008b. Anaerobic Biotechnology for Bioenergy Production : Principles and Applications. First Edition ed. John Wiley & Sons, Inc., Singapore.

Kondusamy, D., Kalamdhad, A.S. 2014. Pre-treatment and anaerobic digestion of food waste for high rate methane production – A review. Journal of Environmental Chemical Engineering, 2(3), 1821-1830.

Kong, S.-H., Loh, S.-K., Bachmann, R.T., Rahim, S.A., Salimon, J. 2014. Biochar from oil palm biomass: A review of its potential and challenges. Renewable and Sustainable Energy Reviews, 39(0), 729-739.

Kothari, R., Pandey, A.K., Kumar, S., Tyagi, V.V., Tyagi, S.K. 2014. Different aspects of dry anaerobic digestion for bio-energy: An overview. Renewable and Sustainable Energy Reviews, 39(0), 174-195.

Kusch, S., Oechsner, H., Jungbluth, T. 2008. Biogas production with horse dung in solid-phase digestion systems. Bioresource Technology, 99(5), 1280-1292.

Lastella, G., Testa, C., Cornacchia, G., Notornicola, M., Voltasio, F., Sharma, V.K. 2002. Anaerobic digestion of semi-solid organic waste: Biogas production and its purification. *Energy Conversion and Management*, 43, 63-75.

Liang, Y.-g., Yin, S.-s., Si, Y.-b., Zheng, Z., Yuan, S.-j., Nie, E., Luo, X.-z. 2014. Effect of pretreatment and total solid content on thermophilic dry anaerobic digestion of Spartina alterniflora. Chemical Engineering Journal, 237(0), 209-216.

Liu, K., Tang, Y.-Q., Matsui, T., Morimura, S., Wu, X.-L., Kida, K. 2009. Thermophilic anaerobic co-digestion of garbage, screened swine and dairy cattle manure. Journal of Bioscience and Bioengineering, 107(1), 54-60.

Liu, X., Wang, W., Gao, X., Zhou, Y., Shen, R. 2012. Effect of thermal pretreatment on the physical and chemical properties of municipal biomass waste. Waste Management, 32(2), 249-255.

Liu, Z.-H., Qin, L., Pang, F., Jin, M.-J., Li, B.-Z., Kang, Y., Dale, B.E., Yuan, Y.-J. 2013. Effects of biomass particle size on steam explosion pretreatment performance for improving the enzyme digestibility of corn stover. Industrial Crops and Products, 44(0), 176-184.

Madhiyanon, T., Sathitruangsak, P., Sungworagarn, S., Pipatmanomai, S., Tia, S. 2012. A pilot-scale investigation of ash and deposition formation during oil-palm empty-fruit-bunch (EFB) combustion. Fuel Processing Technology, 96(0), 250-264.

Mata-Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M., Astals, S. 2014. A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renewable and Sustainable Energy Reviews, 36(0), 412-427.

Meng, X., Ragauskas, A.J. 2014. Recent advances in understanding the role of cellulose accessibility in enzymatic hydrolysis of lignocellulosic substrates. Current Opinion in Biotechnology, 27(0), 150-158.

MetCalf, Eddy. 2004. Wastewater Engineering Treatment and Reuse. McGraw-Hill, Inc., New York.

Misi, S.N., Forster, C.F. 2001. Batch co-digestion of multicomponent agro-wastes. *Bioresource Technology*, 80, 19-28.

Misson, M., Haron, R., Kamaroddin, M.F.A., Amin, N.A.S. 2009. Pretreatment of empty palm fruit bunch for production of chemicals via catalytic pyrolysis. Bioresource Technology, 100, 2867–2873.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology, 96(6), 673-686.

Mtz.-Viturtia, A., Mata-Alvarez, J., Cecchi, F. 1995. Two-phase continuous anaerobic digestion of fruit and vegetable wastes. Resources, Conservation and Recycling, 13(3–4), 257-267.

Myint, M.T., Nirmalakandan, N. 2009. Enhanceing anaerobic hydrolysis of cattle manure in leach bed reactors. Bioresource Technology, 100, 1695-1699.

Nimmanterdwong, P., Chalermsinsuwan, B., Piumsomboon, P. 2015. Emergy evaluation of biofuels production in Thailand from different feedstocks. Ecological Engineering, 74(0), 423-437.

OAE. 2014. Statistic of agricultrual of Thailand 2014, Office of Agricultural Economics (OAE). Avaliable in : http://www.oae.go.th/download/journal/trends_FEB2557.pdf.

Ogejo, A.J., Wen, Z., Ignosh, J., Bendfeldt, E., Collins, E., R. . 2002. Biomethane Technology. Virginia Polytechnic Institute and Iowa State University.

Oleskowicz-Popiel, P., Lisiecki, P., Holm-Nielsen, J.B., Thomsen, A.B., Thomsen, M.H. 2008. Ethanol production from maize silage as lignocellulosic biomass in anaerobically digested and wet-oxidized manure. Bioresource Technology 99, 5327-5334.

Ortíz, I., Quintero, R. 2014. Chapter 4 - Recent Advancements in Pretreatment Technologies of Biomass to Produce Bioenergy. in: Bioenergy Research: Advances and Applications, (Eds.) V.K. Gupta, M.G.T.P. Kubicek, J.S. Xu, Elsevier. Amsterdam, 57-69.

Panichnumsin, P., Nopharatana, A., Ahring, B., Chaiprasert, P. 2010. Production of methane by co-digestion of cassava pulp with various concentrations of pig manure. Biomass and Bioenergy, 34(8), 1117-1124.

PCD. 2010. Agriculture wastewater management. 6 August 2010 ed, Pollution control department, Ministry of natural resource end environment. Avaliable in: http://www.pcd.go.th/info_serv/water_Agricultural.htm.

Pereira, J.N. 2008. Lignocellulosic biomass for the production of fuels and chemicals within the context of Biorefinery. in: Series on Biotechnology (Ed.) N.P. Jr, Vol. 2, Brazilian National Library Avaliable in : http://www.ladebio.org.br/download/series-em-biotecnologia-vol-ii-lignocellulosic-biomass.pdf, 22.

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

Qian, E.W. 2014. Chapter 7 - Pretreatment and Saccharification of Lignocellulosic Biomass. in: Research Approaches to Sustainable Biomass Systems, (Ed.) S.T. Hirasawa, Academic Press. Boston, 181-204.

Rahman, S.H.A., Choudhury, J.P., Ahmad, A.L. 2006. Production of xylose from oil palm empty fruit bunch fiber using sulfuric acid. Biochemical Engineering Journal 30, 97-103.

Regueiro, L., Carballa, M., Álvarez, J.A., Lema, J.M. 2012. Enhanced methane production from pig manure anaerobic digestion using fish and biodiesel wastes as co-substrates. Bioresource Technology, 123(0), 507-513.

Rittmann, B.E., McCarty, P.L. 2001. Environmental Biotechnology. McGraw-Hill, Inc., New York.

Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V. 2005. Dilute Acid Pretreatment, Enzymatic Saccharification and Fermentation of Rice Hulls to Ethanol. Biotechnology Progress, 21(3), 816-822.

Sawatdeenarunat, C., Surendra, K.C., Takara, D., Oechsner, H., Khanal, S.K. 2015. Anaerobic digestion of lignocellulosic biomass: Challenges and opportunities. Bioresource Technology, 178, 178-186.

Sawyer, C.N., McCarty, P.L., Parkin, G.F. 2003. Chemistry for Environmental Engineering. McGraw-Hill, Inc., New York.

Schulze, D., E., Mooney, A., H. 1999. Biodiversity and Ecosystem Function: Function Group of Microorganism. Springer, New York

Singh, J., Suhag, M., Dhaka, A. 2015. Augmented digestion of lignocellulose by steam explosion, acid and alkaline pretreatment methods: A review. Carbohydrate Polymers, 117(0), 624-631.

Sinjermsiri, D., Hanbunchong, A., Thanomwongwattana, R. 2006. Quality improvement of oil palm press fibre by the chemical and biological. Animal Science Conference, Kasartsart University'14, Bangkok, Thailand. 307-315.

Speece, R., E. 2008. Anaerobic Biotechnology for Industrial Wastewater. Archae Press, Tennessee Nashville.

Stabnikova, O., Liu, X.-Y., Wang, J.-Y. 2008. Anaerobic digestion of food waste in a hybrid anaerobic solid–liquid system with leachate recirculation in an acidogenic reactor. Biochemical Engineering Journal, 41(2), 198-201.

Stams, A.J.M., Elferink, S.J.W.H.O., Westermann, P. 2003. Metabolic Interactions Between Methanogenic Consortia and Anaerobic Respiring Bacteria. in: Biomethanation I, (Eds.) B. Ahring, I. Angelidaki, E.C. de Macario, H.N. Gavala, J. Hofman-Bang, A.J.L. Macario, S.J.W.H.O. Elferink, L. Raskin, A.J.M. Stams, P. Westermann, D. Zheng, Vol. 81, Springer Berlin Heidelberg, 31-56.

Steffen, R., O., S., Braun, R. 1998a. Feedstocks for anaerobic Digestion (Project report). Institute for Agrobiotechnology Tulln, University of Agriculture Science Vienna.

Steffen, R., Szolar, O., Braun, R. 1998b. Feedstocks for Anaerobic Digestion. Institute for Agrobiotechnology Tulln, University of Agricultural Sciences Vienna.

Stichnothe, H., Schuchardt, F. 2010. Comparison of different treatment options for palm oil production waste on a life cycle basis. The International Journal of Life Cycle Assessment, 15(9), 907-915.

Sumathi, S., Chai, S.P., Mohamed, A.R. 2008. Utilization of oil palm as a source of renewable energy in Malaysia. Renewable and Sustainable Energy Reviews, 12(9), 2404-2421.

Tamagawa, Y., Yamaki, R., Hirai, H., Kawai, S., Nishida, T. 2006. Removal of estrogenic activity of natural steroidal hormone estrone by ligninolytic enzymes from white rot fungi. Chemosphere, 65, 97-101.

Thiele, J.H. 1991. In Mixed Cultures in Biotechnology. in: Mixed-culture interactions in methanogenesis., (Eds.) G. Zeikus, E.A. Johnson, McGraw-Hill. New York.

Tiehm, A., Nickel, K., Zellhorn, M., Neis, U. 2001. Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. Water Research, 35(8), 2003-2009.

Umikalsom, M.S., Ariff, A.B., Zulkifli, H.S., Tong, C.C., Hassan, M.A., Karim, M.I.A. 1997. The Treatment of Oil Palm Empty fruit bunch fibre for Subsequent use as Substrare fir Cellulase Production by Cheatomium Globosum Kunze. Bioresource Technology 62, 1-9.

Verma, S. 2002. Anaerobic Digestion of Biodegradable Organics in MSW (A report).

Wan, C., Li, Y. 2012. Fungal pretreatment of lignocellulosic biomass. Biotechnology Advances, 30(6), 1447-1457.

Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L. 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresource Technology, 99(17), 7928-7940.

Xu, S.Y., Karthikeyan, O.P., Selvam, A., Wong, J.W.C. 2012. Effect of inoculum to substrate ratio on the hydrolysis and acidification of food waste in leach bed reactor. Bioresource Technology, 126(0), 425-430.

Yan, B.H., Selvam, A., Wong, J.W.C. 2014. Application of rumen microbes to enhance food waste hydrolysis in acidogenic leach-bed reactors. Bioresource Technology, 168(0), 64-71.

Yang, T.C., Kumaran, J., Amartey, S., Maki, M., Li, X., Lu, F., Qin, W. 2014. Chapter 5 - Biofuels and Bioproducts Produced through Microbial Conversion of Biomass. in: Bioenergy Research: Advances and Applications, (Eds.) V.K. Gupta, M.G.T.P. Kubicek, J.S. Xu, Elsevier. Amsterdam, 71-93.

Yue, Z.-B., Li, W.-W., Yu, H.-Q. 2013. Application of rumen microorganisms for anaerobic bioconversion of lignocellulosic biomass. Bioresource Technology, 128(0), 738-744.

Zheng, Y., Zhao, J., Xu, F., Li, Y. 2014. Pretreatment of lignocellulosic biomass for enhanced biogas production. Progress in Energy and Combustion Science, 42(0), 35-53.

CHAPTER III

CO-FERMENTATION OF OIL PALM LIGNOCELLULOSIC RESIDUE WITH PIG MANURE IN ANAEROBIC LEACH BED REACTOR FOR FATTY ACID PRODUCTION

3.1 Abstract

Batch experiments were performed to investigate the co-fermentation of palm oil empty fruit bunch (EFB) and pig manure (PM) at various PM mixing ratios (*%PM*) and flooding intervals (*FI*) over 60-day fermentation time (*FT*) using anaerobic leach bed reactors. Addition of PM promoted hydrolysis yield (η_h) and acidification yield (η_a) due to the more degradable and soluble nature of PM that gave out organic acids, and nutrients it supplemented. The highest η_h and η_a found were 27.9±0.3% and 51.7±2.6%, respectively. Longer *FI* that delayed bed flushing prolonged the dry condition where hydrolytic reaction could be enhanced. The developed multiple regression model with R^2=0.87 and p<0.0001 suggested a good fit to the data and able to describe the interactive relationship of the parameters on total volatile fatty acids (VFA) production. The short chain acids, i.e. propionic (C3), butyric (C4), and valeric (C5) acids, were found in higher concentrations with longer *FI* and higher pig manure mixing ratios. The mixing of easier biodegradable pig manure as a co-substrate could help induce higher degree of fermentation of the recalcitrant EFB.

Key Words: oil palm; empty fruit bunch; pig manure; volatile fatty acid; leach bed reactor; flooding

3.2 Introduction

Palm oil is mostly used in the production of chemicals and food products particularly as fat and cream ingredient because of its relatively low price compared to other oils and dairy product. In tropical countries such as Malaysia, Indonesia, Papua New Guinea, Columbia, and Thailand, oil palm is a major economic crop that generates large revenues for their economy. When the palm fruits are taken apart for oil extraction, its remaining empty fruit bunch (EFB) is left to be disposed. Its gigantic volume is deemed to be a major renewable resource when fully utilized. The EFB residues in Thailand amounts for about 4.85 million tons in year 2012 (Office of Agricultural Economic, 2012) and on the rise due to the expansion of palm plantation. EFB has high carbon content in a form of lignocellulose, comprising cellulose, hemicellulose and lignin at 39.1±0.8, 22.0±1.2 and 23.0±0.7%, respectively (O-Thong et al., 2012). Since EFB possesses high carbon to nitrogen ratio (C:N), 47.6:1 - 107.9:1 (Jamari and Howse, 2012), biological activity on EFB would face some difficulties from the lack of nutrients apart from the already tough, high lignin fiber.

Research works on EFB utilization have focused on treatment and conversion of this lignocellulose by chemical and physical means to enhance or produce various intermediate products such as hydrolysate chemicals and sugars (Bahrin et al., 2012; Kim et al., 2012; Lau et al., 2010; Misson et al., 2009; Umikalsom et al., 1997). Nevertheless, they typically could accomplish only partial conversion by breaking certain links in the hemicellulose-lignin polymeric system, which would provide increased diffusivity to hydrolytic enzymes. Physical pretreatment focuses on breaking down the structure with forces to increase active sites for enzymatic attack, while treatment with hydrolytic enzyme, alkali and acid aim to remove the surrounding matrix of hemicellulose and lignin from the cellulose core to enhance subsequent hydrolysis (Kim et al., 2012; Vavouraki et al., 2014). Unfortunately, cost of chemicals often becomes prohibitively high unless high value products are generated. Biological treatment has many advantages as it is the least expensive, requires lower energy, and produces minimal chemical waste. Although fungi were widely studied for aerobic degradation of lignocelluloses, there is always an inevitable

carbon loss, particularly from a non-selective degradation (Isroi et al., 2011). Biological pretreatment is carried out mostly by lactic acid bacteria as well as hydrolytic and acidogenic bacteria which could proliferate in moist environment at 30-70% moisture (Insam et al., 2010). However, matching pretreatment methods to different types of substrate are still a challenge.

Anaerobic degradation of bagasse and maize barn by rumen microorganisms could achieve maximum fiber degradation at 49% and 52% of the total fiber fractions, respectively after 168 h, while volatile fatty acids were produced as a result of these conversions (Kivaisi and Eliapenda, 1995). A decrease in lactic acid bacteria population after the experiment which lasted only 21 days was nevertheless reported in the oil palm press fiber (PPF) treated with bacillus and lactic acid bacteria (Sinjermsiri et al., 2006). Biological treatment of lignocellulosic biomass still has rooms for improvement. Anaerobic leach bed reactor (ALBR) is the reactor designed to ferment solid biomasses in a batch wise operation. By allowing liquid to percolate through the layer of static biomass bed packed to the vessel, the substrate is hydrolyzed in a relatively dry environment which gives an advantage by having small amount of liquid to handle and intense hydrolysis, as suggested by previous studies (Demirer and Chen, 2008; Jagadabhi et al., 2011; Lehtomaki et al., 2008; Singh et al., 2011). Although some biogas is produced during this stage, the majority is produced in the subsequent methanogenic reactor to which the percolating liquid is collected and sent. Tough fibrous biomass such as woody materials or lignocellulosic biomasses are a challenge in this dry fermentation since there are little nutrients available and the plant cell structure was tightly bonded. External nutrient addition is usually required for EFB bioconversion in order to improve the microbial activity (Boonsawang and Wongsuvan, 2010). A co-substrate that is more biodegradable and rich in nutrients could help balance the microbial nutritional requirement and might induce higher degradation rate of the other co-substrates.

Use of ALBR for fatty acids production was very limited (Bable et al., 2004; Myint and Nirmalakandan, 2009; Xie et al., 2012) and none has been done for fermentation of the lignocellulosic EFB. The product in the forms of fatty acids in the leachate drained out of the reactor becomes a valuable source for further conversions

to different products (Parawira et al., 2005; Weimer et al., 2009). Traditionally, intermittent liquid spray onto the substrate pile is often used in ALRB although uniform distribution and percolation into the bed is hard to ensure. Liquid flooding, typically complete submergence can be used as a mean to solubilize the substrate but the enzymatic activity is diluted with the bulk liquid. The intermittent flooding and flushing of the bed could have an advantage by allowing the bed to be at semi-dry environment which could intensify enzymatic activity.

The main objective of this study was, thus, to investigate the co-fermentation of palm empty fruit bunch and a nutrient rich, more biodegradable substrate, pig manure. The parameters selected in this study were essential in the operation of ALBR, including liquid flushing interval (*FI*), pig manure mixing ratio (*%PM*) and the fermentation time (*FT*). Interactive relationship of these parameters on the fermentation aiming to optimize fatty acids generation was examined. Focus was given on improving hydrolysis and acidification of the lignocellulosic EFB material as part of the front end treatment prior to the methanogenic biogas production process.

3.3 Material and methods

3.3.1 Inoculum and seeding

The cow manure from Tepa Livestock Research Training Station, Tepa District, Songkhla Province, Thailand was used as inoculum. The inoculum was analyzed for moisture content, total solids (TS) and volatile solids (VS) within 2 h after collection. It was then stored at 4 °C no longer than 48 h prior to use in the experiments to maintain freshness and active rumen microorganisms. The inoculum was then mixed with the substrates to obtain an initial inoculum concentration of 20% w/w on dry basis of the mixture before loading to the reactors.

3.3.2 Substrate

Oil palm fresh fruit bunch (FFB) of species *Eleaeis guineensis* from plantation in Sikao Destrict, Trang Province (N99^{o}19’, E7^{o}42’) was harvested seasonally in August 2012 and transferred to Lam Soon Palm Oil Mill located in the vicinity for oil extraction. The FFB was steam cooked at 140 ^{o}C for 15 min followed by mechanical threshing to release the fruits off the bunch. The freshly rejected oil

palm empty fruit bunch (EFB) was then collected within 24 h to prevent fungal contamination. After that, EFB was dried at 60 °C and reduced in size to 5 cm. The prepared EFB was stored at 4 ^{o}C until use. The co-substrate in this experiment was pig manure (PM) which was collected from a large scale pig farm in Phattalung Province in southern Thailand. Both were measured for moisture content, TS and VS.

Prior to use, the predetermined weight of EFB was soaked with DI water overnight to restore its moisture. Then, the predetermined quantity of PM was mixed with EFB at ratios 50PM:50EFB, 25PM:75EFB, and 0PM:100EFB. These ratios were based on dry mass (TS). The inert material, nylon fiber, cut to 5 cm in length was used in place of EFB in the control set of 100PM:0EFB. The fresh inoculum was brought to mix to make up a total weight of 1000 g on dry basis before loading into their respective labeled reactors. The wet weight of 100PM:0EFB, 50PM:50EFB, 25PM:75EFB, and 0PM:100EFB mixtures were 5758, 4877, 4437 and 3996 kg wet, respectively. The substrates and inoculum were manually homogenized to achieve uniform samples for use in all experiments. Characteristics of the substrates and the inoculated mixtures are shown in Table 3-1.

3.3.3 Reactor configuration

The anaerobic leach bed reactors (ALBR) used in this study was made of 40-L cylindrical PVC drums. Each reactor has a diameter and height of 30 and 55 cm, respectively, making an effective bed volume of approximately 22.6 L. A gas port was located on top of the reactor and connected to a gas balloon at all times to provide extra volume displacement from the cyclic flooding and flushing operation. A drain pipe at the bottom was used for liquid flooding and drainage. This drain pipe was connected to the liquid holding tank at all times to ensure no excessive liquid accumulation in the reactor.

The leachate was stored in an enclosed holding tanks made of 40 L plastic container with a storage volume of 35 L. There was another gas balloon connected to this tank to allow volume displacement in the cyclic operation. A sampling port for the flushed leachate in the holding tank was installed at liquid mid depth.

Table 3-1 Characteristics of pig manure (PM), palm empty fruit bunch (EFB) and the inoculated mixtures at the beginning of experiments

Parameter	Unit	Seed	EFB	PM	0PM:100EFB*	25PM:75EFB*	50PM:50EFB*	100PM:0EFB*
Total solid, TS	g TS/kg wet	104.8±17.8	383.1±61.4	270.8±10.3	211.1±3.7	232.5±16.2	244.3±58.8	252.4±8.39
Volatile solids, VS	g VS/kg dry	805.4±99.9	938.2±25.0	802.3±6.1	968.5±12.9	955.3±1.8	902.4±16.0	875.6±36.9
Carbon (C)	% dry wt.	43.0±0.14	42.9±0.36	42.4±0.23	42.5	42.4	42.3	42.0
Nitrogen (N)	% dry wt.	1.7±0.04	0.9±0.02	3.1±0.04	1.3	1.7	2.2	3.1
Hydrogen (H)	% dry wt.	5.4±0.05	5.7±0.11	5.5±0.06	5.6	5.6	5.5	5.4
Oxygen (O)	% dry wt.	23.5±0.34	30.7±0.30	26.2±0.20	29.0	28.1	27.2	25.4
Sulfur (S)	% dry wt.	0.2±0.01	< 0.01	0.2±0.00	0.1	0.1	0.2	0.2
Phosphorus	% dry wt.	0.5	0.1	2.3	0.2	0.6	1.1	2.0
Potassium	% dry wt.	0.1	1.7	1.2	1.4	1.3	1.2	1.0
C:N ratio	-	25.3	47.7	13.8	32.7	24.9	19.4	13.8

* The values for elemental composition (CHONS) were calculated by weighted average of individual substances including seed according to the specified ratios TS and VS values were from direct measurement of the mixtures.

3.3.4 System operations

The batch-wise experiments were designed to investigate fermentation efficiency of EFB co-fermenting with PM. Initially, the prepared mixtures of substrate and inoculum were randomly loaded into each labeled reactor. Nitrogen gas was used to purge the air in all reactors to ensure anaerobic environment. Thirty five liters of deionized water was pumped to flood the solid bed in ALBRs overnight (approximately 12 h) and then drained off. Subsequently, the cyclic operation started by the liquid being fed to the ALBR within the first 10 min. It was left to stay for 20 min, and then the drainpipe valve was switched open allowing the leachate to flow back to the holding tank within the next 10 min. After that, the reactor was left to react in dry environment until the next cycle arrived. That completed one flushing interval (*FI*) in our study, which comprised *FI* 12h, 24h, and 48h. The ALBRs were operated for 60 d where the production of TVFA ceased to minimal.

3.3.5 Analytical methods

The performance of ALBRs was evaluated by determination of the leachate of each treatment for soluble COD (SCOD) according to the Standard Methods (APHA et al., 1998). Volatile fatty acid (VFA) species were determined using a Hewlett-Packard gas chromatography (Model 7890) with an Inowax capillary column (30m×0.25mm×0.25μm) and a flame ionization detector. Helium was used as carrier gas with the injector temperature of 260°C. The column temperature was increased at the rate of 20^{o}C/min to 120°C, after that the temperature was decreased at 10^{o}C/min to 205°C. The elemental analyses for C, H, O, N and S were performed using Thermoquest Flash 1112 EA series EA elemental analyzer.

The hydrolysis yield (η_h) was defined as the ratio of SCOD of leachate to the initial COD of the substrate. It is calculated according to Eq.1 (Xie et al., 2012).

$$\eta_h = \frac{S_S}{S_I} \times 100\% \quad (1)$$

where S_I is the initial total COD (g) of substrate (0PM:100EFB, 25PM:75EFB, 50PM:50EFB, and 100PM:0EFB), and S_S is the cumulative SCOD production (g/kg substrate) in the leachate. Acidification yield (η_a) was defined as the ratio between the

cumulative TVFA in COD equivalence (S_{TVFA}) (gCOD/kg substrate) and SCOD (gCOD/kg substrate) in the leachate, according to Eq. 2.

$$\eta_a = \frac{S_{TVFA}}{S_S} \times 100\% \quad (2)$$

where S_{TVFA} is the cumulative TVFAs expressed as g COD calculated from the theoretical COD equivalents for each VFA species. The theoretical COD equivalence of acetic acid, propionic acid, butyric acid, valeric acid, and caproic acid are 1.066, 1.512, 1.816, 2.036, and 2.204 gCOD/g substrate, respectively (Demirel and Yenigun, 2004). In order to determine the overall substrate conversion in terms of acidification yield, S_{TVFA} and S_S were calculated from the final cumulative TVFA and SCOD values (at day 60) subtracted with their initial value at day 0, which was zero since no COD was present in deionized water used at the beginning.

3.3.6 Multiple regression and statistical analysis

The experimental design was conducted using full factorial design. The first variable X_1 is PM mixing (*%PM*) was tested at 4 levels, and the second variable X_2 is flushing interval (*FI*) was studied at 3 levels, while the third variable X_3 is fermentation time (*FT*) of 60 d was divided into 18 levels. The zero levels are the midpoint of the parameter range that have natural values of 50 for *%PM*, 24 for *FI*, and 30 for *FT*. A total of 12 treatments were run in triplicate over 60 d (18 samplings throughout the runs) with TVFA production (*Y*) as the interested response of the model. For parameter relationship and optimization purpose, a second order polynomial equation was employed to fit the experimental data using the Design Expert Trial Version 8.0.7.1. A quadratic model (Eq. 3) derived was used to describe the correlation of our operating parameters *FT*, *FI* and *%PM*. Coefficients with statistical significance were indicated at $p<0.05$. Response contour plots were generated with the said software.

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1^{\,2} + \beta_5 X_2^{\,2} + \beta_6 X_3^{\,2} + \beta_7 X_1 X_2 + \beta_8 X_1 X_3 + \beta_9 X_2 X_3 \quad (3)$$

where Y_i is the predicted response, β_0 is a constant, β_i is the linear coefficient, β_{ii} is the squared coefficient, β_{ij} is the cross-product coefficient, and X_i is the actual values of the studied independent variables. The response of the TVFA production was regressed with respect to *%PM* (%), *FI* (h) and *FT* (d). Mean and the standard deviation were calculated and used to compare the effects of our independent variables. Comparison of means was carried out with SPSS software version 11.0 by one-way analysis of variance (ANOVA) and Duncan's multiple-range test.

3.4 Results and discussion

3.4.1 SCOD production and hydrolysis yield

The substrates were hydrolyzed in anaerobic conditions in ALBR, which produced soluble organic compounds represented by SCOD concentration in the leachate. In order to fairly compare the organics leaching from each substrate mixture, SCOD in the leachate was normalized by the initial mass of the substrate mixture. The cumulative results over 60 d a shown in Figure 3-1. The SCOD profiles were clearly separable into four groups according to the substrate mix ratios of 0PM:100EFB, 25PM:75EFB, 50PM:50EFB and 100PM:0EFB (control). All four groups exhibited the linear relationship with time although the flat tail or saturation curve type was expected when the rate of hydrolysis ceased. It showed that hydrolysis was not inhibited and could still continue beyond 60 days unless other factors could become limiting for hydrolytic bacteria such as volatile fatty acid concentration that reportedly impeded hydrolytic rate (Llabrés-Luengo and Mata-Alvarez, 1988). However, once flushed, the hydrolysis products were removed from the substrate bed, allowing the bacteria to hydrolyze effectively again.

Slopes of the data sets in each group were averaged at 2.06±0.43, 2.74±0.15, 3.74±2.10 and 3.06±0.17 gSCOD/kg·d for 0PM:100EFB, 25PM:75EFB, 50PM:50EFB and 100PM:0EFB, respectively. An increase of around 33 and 36 percent were gained with the increasing mixture of pig manure from zero to 25 and 50 percent, in order, but dropped 18% with PM alone in the control set. Nevertheless, highest SCOD productivity was found in the control set at 424±46 gSCOD/kg $_{\text{dry substrate add}}$ on average at 60 d. This was a direct result of the pig manure portion in the

substrate, which consisted of the more biodegradable organics. Over the course of 60 days, SCOD productivity at flushing interval (*FI*) 48 hours had shown some slight advantage over the others although it was not so distinctive. This would be tested and discussed in the section of acidification yield.

Addition of PM to EFB corresponded to an increased SCOD production rate. Such SCOD may include other fermentation intermediates such as fatty acids and enzymes. During the dry period in ALBR, approximately 94-99% in each cycle, the enzymes released by the immobilized microbes on the fibers could be locally concentrated without the effect of dilution by the leaching liquid. That ALBR could retain or immobilize the microbes within the reactor made the ratio of substrate to microbe (F/M) lower than the wet process (Stabnikova et al., 2008). This condition favored the early steps of lignocellulose degradation by breaking down the linkage of biomass structure and hydrolyzing cellulose and hemicellulose to acetate, H_2, and H_2CO_3, respectively (Qu et al., 2009) by extracellular enzyme from hydrolytic bacteria (Geng, 2013).

It is of interest to examine the first step of anaerobic digestion by the degree of hydrolysis or so-called hydrolysis yield (η_h) which indicates the relative amount of substrate becoming soluble compared to the original biomass on COD basis (Eq.1). First, the COD equivalence of the substrate mixture must be known. Elemental compositions (carbon, hydrogen, oxygen, nitrogen and sulfur) of each mixture from Table 1 were used to calculate the chemical formula, noting that ash was excluded from these formulations. The oxidation equations of each mixture was calculated according to Eq. 4 (Rish, 1963) to find the COD equivalent, which are shown in Eq. 5-8.

$$C_aH_bO_cN_d + \left(\frac{4a+b-2c-3d}{4}\right)O_2 \rightarrow aCO_2 + \left(\frac{b-3d}{2}\right)H_2O + dNH_3 \qquad (4)$$

The initial COD of the solid substrate calculated from the stoichiometric equation of 0PM:100EFB, 25PM:75EFB, 50PM:50EFB and 100PM:0EFB were 1.600, 1.618, 1.629 and 1.679 gCOD/g $_{\text{dry substrate}}$, respectively.

$$C_{38}H_{60}O_{20}N + 42.3O_2 \rightarrow 38CO_2 + 28.5H_2O + NH_3 \quad (5)$$

$$C_{28}H_{44}O_{14}N + 31.3O_2 \rightarrow 28CO_2 + 20.5H_2O + NH_3 \quad (6)$$

$$C_{23}H_{35}O_{11}N + 25.5O_2 \rightarrow 23CO_2 + 16.0H_2O + NH_3 \quad (7)$$

$$C_{16}H_{25}O_7N + 18.0O_2 \rightarrow 16CO_2 + 11.0H_2O + NH_3 \quad (8)$$

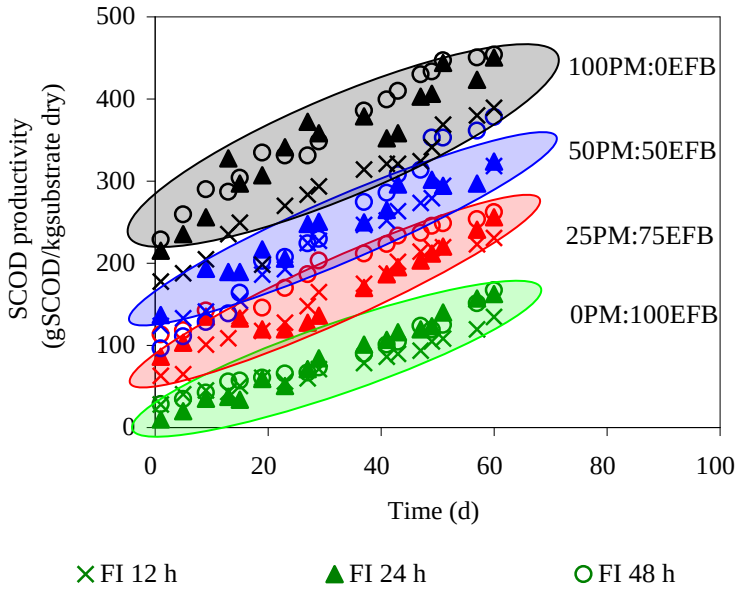


Figure 3-1 SCOD productivity of pig manure (PM) and palm empty fruit bunch (EFB) co-fermentation at different mixing ratios in the ALBR and flooding interval (FI)

Results indicated that PM mixing ratio (*%PM*) had a positive correlation with hydrolysis yield (η_h) from the beginning (Figure 3-2). This was associated with the easily soluble organics in the pig manure. It was seen that at *FI* 48 h, the initial η_h in the first 10 days were lower than those of *FI* 24 and 12 h. Longer *FI* had delayed the flushing and leaching of the substrates to the leachate while the enzymatic hydrolysis were more intense. This phenomenon was explained by the slope of the hydrolysis yield over time, designated as hydrolysis rate which represented a speed of the hydrolysis reaction of the substrate. At 50PM:50EFB, the hydrolysis rates for *FI* 48 h was 0.31, which was higher than those of *FI* 24 and 12 h at 0.17, and 0.19, respectively. As a result, η_h at day 60 of *FI* 48 h reached 23.3±2.2 % compared to 19.9±0.4 % for *FI* 24 h, and 19.6±1.7 % for *FI* 12 h, despite the lower starting points. This trend was similar for other mixing ratios (*%PM*) but at a lesser degree. The increases of η_h from 0PM:100EFB to 25PM:75EFB and 0PM:100EFB to 50PM:50EFB were 6.14 and 11.25 % for *FI* 12 h, 6.58 and 11.24 % for *FI* 24 h, and 5.97 and 12.92 % for *FI* 48 h. Larger increase was observed at *FI* 48 h. A longer flushing interval could also reduce operational cost of the system. In addition, the lower macro and micro nutrients (Table 1) at 0PM:100EFB could have also taken a toll in low hydrolytic activity. Deublein and Steinhauser (2008a) suggested that hydrolytic and acidogenic bacteria required C:N:P:S at 500:15:5:3 but the substrate without pig manure had a lower level of phosphorus and sulfur (C:N:P:S = 500:15:2:1.2).

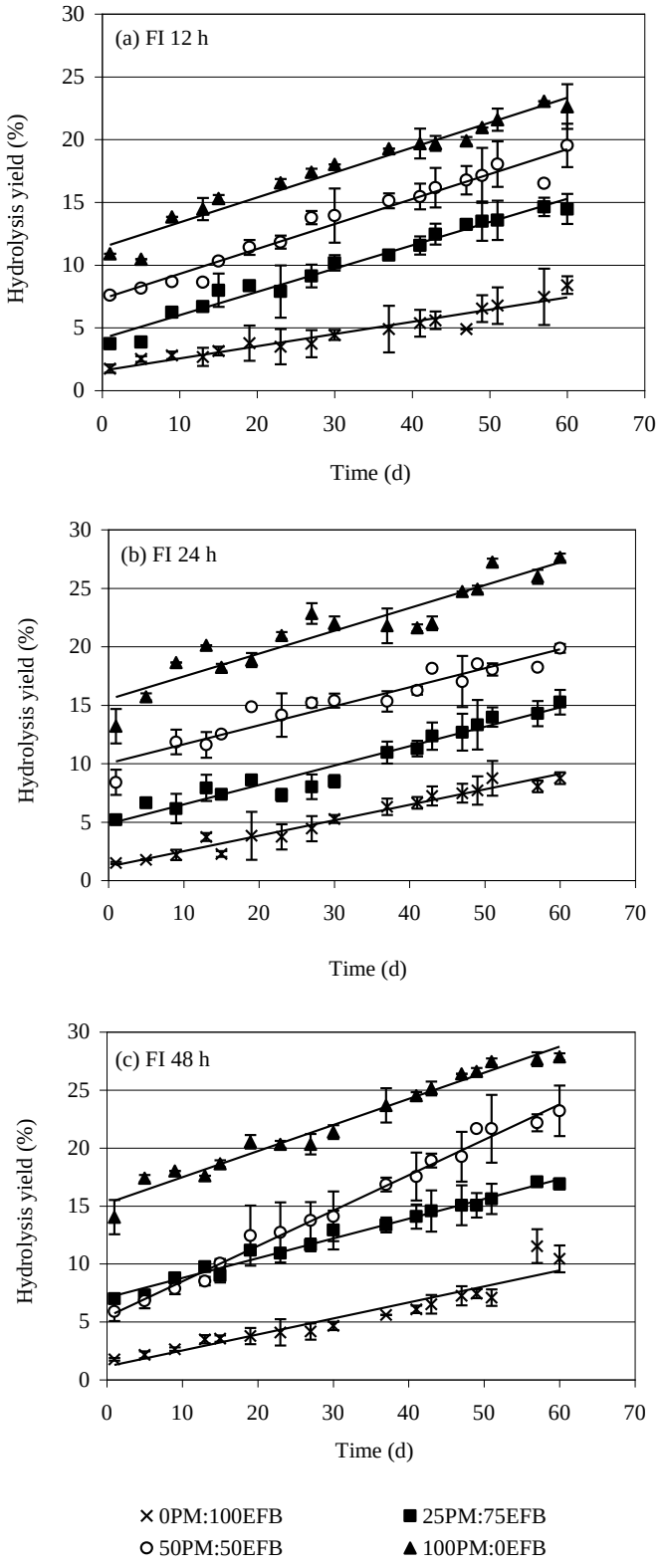


Figure 3-2 Hydrolysis yield from co-fermentation of pig manure (PM) and palm empty fruit bunch (EFB) at different mixing ratios in the ALBR operated at flooding interval (FI) (a) 12 h, (b) 24 h, and (c) 48 h

3.4.2 Acidification yield (η_a)

The acidification yield is the ability of acidophiles to convert the soluble substrate (SCOD) to fatty acids, represented by TVFA:SCOD ratio. It was obvious that PM mixing had positive impact on the acidification yield (Figure 3-3) while effects from flushing interval were not so evident. Higher *%PM* gave a higher hydrolysis yield (η_h) which will be a starting substance for acidification. The higher conversion efficiency to fatty acids was resulted from the additional nutrients necessary for the acidogenic bacteria and the alkalinity to buffer the pH of the leachate. Higher alkalinity from ammonia nitrogen in the pig manure helped maintain pH level in the leachate (data not shown) even with the high fatty acid production in higher %PM treatments. On the other hand, the comparison of the acidification yield (Table 3-2 and Figure 3-3) showed that the 60-day acidification yields were equivalent statistically at 50PM:50EFB across *FI* values. Within the same flushing interval, addition of PM enhanced acidification yield while the mixture of PM at 25 and 50% did not make a clear difference statistically ($p<0.05$). The acidification yield of EFB with no PM (0PM:100EFB) was lowest at all *FI*'s with the averages of 15.9±1.7, 35.5±3.3 and 33.3±0.9%, respectively. These results coincided with the pH data where leachate pH of the treatments with PM mixing dropped continuously in the first 20 days before rising whereas pH level slowly rose in no PM treatment from the beginning. The statistical test revealed that addition of PM contributed to the higher η_a. However, it was observed that the control set of 100PM:0EFB still had lower η_a than the co-fermentation treatments. This appeared to be the limitation of acidification in this operation. Although 100PM:0EFB generated significantly higher SCOD in the leachate, our data indicated that the VFA production was only slightly higher than the co-fermentation treatments.

Table 3-2 Acidification yield and TVFA production of pig manure (PM) and palm empty fruit bunch (EFB) co-fermentation at different mixing ratios in the ALBR at the end of 60 days

Flooding Interval (h)	Mixing ratio (PM:EFB)	Acidification yield (%)	TVFA production ($g/kg_{dry\ substrate\ added}$)
12	0:100	15.9 ± 1.7^{a}	22.6 ± 1.3^{a}
	25:75	40.2 ± 0.8^{bc}	118.9 ± 4.2^{b}
	50:50	49.5 ± 7.5^{c}	134.5 ± 1.4^{bc}
	100:0	32.1 ± 1.1^{b}	162.7 ± 8.4^{c}
24	0:100	35.5 ± 3.3^{b}	41.8 ± 0.9^{a}
	25:75	41.8 ± 1.9^{bc}	121.7 ± 19.7^{b}
	50:50	48.6 ± 3.8^{c}	144.4 ± 1.0^{bc}
	100:0	38.8 ± 1.4^{bc}	174.6 ± 2.8^{c}
48	0:100	33.3 ± 0.9^{b}	42.1 ± 1.5^{a}
	25:75	43.7 ± 3.5^{bc}	129.6 ± 5.3^{b}
	50:50	51.7 ± 2.5^{c}	152.3 ± 0.6^{bc}
	100:0	43.3 ± 0.3^{bc}	96.5 ± 3.6^{c}

The development of η_a profiles showed some lag time before the sharp increase, more noticeably in 25PM:75EFB and 50PM:50EFB treatments. Lag phase approximately 10 days was taken for the acidogenic bacteria to effectively build fatty acids. After approximately 20 days, η_a started to level off as the SCOD production (hydrolysis) and TVFA production (acidogenesis) proceeded at the proportionally similar rates. This phenomenon corresponded to the modified Monod's model bacterial growth where high substrate concentration would give fast growth and reaction rate, but also the inhibition developed from the soluble compounds produced from the reaction (De La Rubia et al., 2009). At this stage, it was suggested to partially replace the flooding liquid to lower SCOD and TVFA to accelerate the rate of biochemical reactions (Babel et al., 2004). In particular, such liquid could be the digestate from an anaerobic reactor digesting the leachate itself (Nizami et al., 2010). The acidification yields achieved from the present study under different factors *%PM*, *FI* and *FT* varied in a range of 15.9±1.7% to 51.7±2.6%, which the high side is comparable to the grass silage fermentation in ABR that gave 57-60% acidification yield (Xie et al., 2012). While hydrolysis relying on extracellular enzymatic reaction from various organisms depends on the substrate type, acidogenesis with its product

variety is dependent more on the reactor operation and the microbial consortia within the system employed (Xu et al., 2012; Zhang et al., 2005; Zhang et al., 2012).

It would be wise to evaluate the effect of PM addition to the degradation of EFB. Results from the control set were used to assess the VFA production per dry mass of PM at each condition. It was found that at 100PM:0EFB, TVFA was produced at an average of 0.163, 0.175 and 0.169 $gTVFA/g_{dry\ substrate\ added}$ at FI 12, 24 and 48 h, respectively. By subtracting the TVFA produced from PM off the overall production, TVFA from EFB could be determined. Specific VFA production from EFB increased rapidly as PM mixing went from 0 to 25%PM and was quite static beyond that point (Figure 3-4). The weak acids generated from hydrolysis-acidogenic process when in contact with the fiber could loosen the structure of lignocellulose and increase accessibility of extracellular enzymes from hydrolytic bacteria of cow manure in the seed and fresh PM as co-substrate. Altogether will result in an improved overall rate of EFB degradation through hydrolysis but the acidogenesis became limited by the product (VFAs) inhibition where there is no further production of VFAs (Babel et al., 2004).

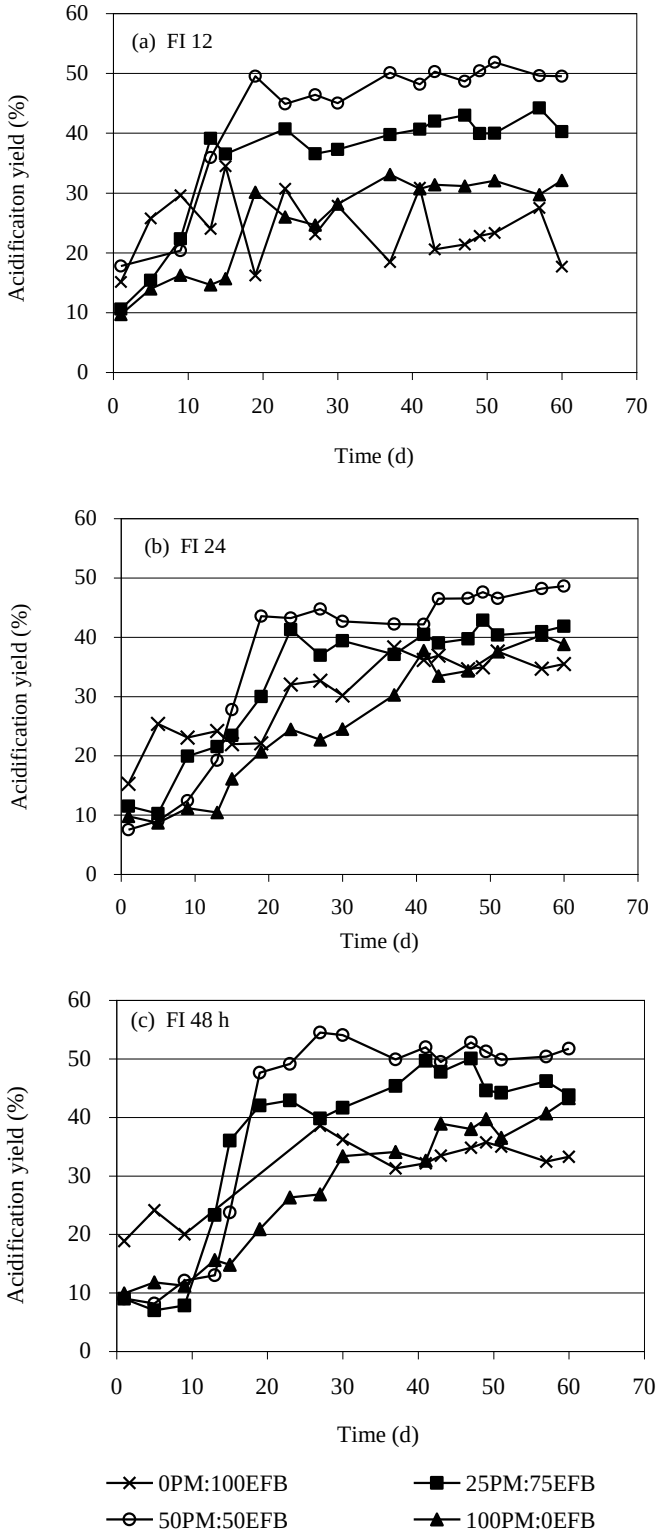


Figure 3-3 Acidogenesis yield (TVFA:SCOD) in leachate from co-fermentation of pig manure (PM) and palm empty fruit bunch (EFB) at different mixing ratios in the ALBR operated at flooding interval (FI) (a) 12 h, (b) 24 h, and (c) 48 h

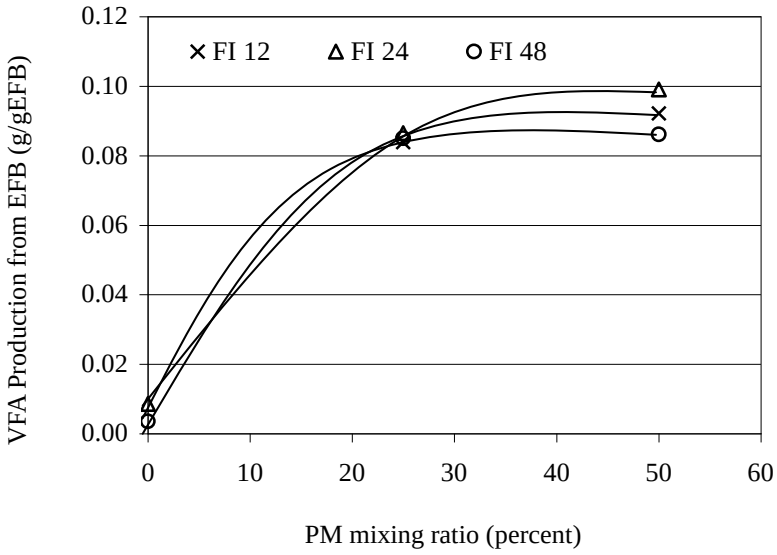


Figure 3-4 VFA production form palm empty fruit bunch (EFB) at different pig manure (PM) mixing ratios in the ALBR operated at flooding interval (FI) 12 h, 24 h, and 48 h

3.4.3 Effects of *%PM*, *FI* and *FT* on TVFA production

Full factorial design was used to find the relationship of PM mixing (*%PM*), flushing interval (*FI*) and fermentation time (*FT*) to the TVFA production with 12 experiments and 18 samplings throughout the fermentation period, comprising a total of 216 data sets. The goodness of fit of the TVFA production regression model (Eq.9) was checked by F-test, and the analysis of variance (ANOVA) for quadratic model is shown in Table 3-3. The model F-test gave the value of less than 0.0001, indicating that the model was statistically significant (p<0.05) and had high predictability within the studied range. The good fit of the model was expressed by the high determination coefficient R^2 of 0.87 by which 87% of the variability in the response could be explained. Figure 3-5 shows the relationship between the predicted values and the observed data evidencing an obvious correlation over the range of the model outputs while the low coefficient of variance (CV) suggests the precision and reliability of the experiments performed. All terms in the model are statistically highly significant except FI^2 which has p-value higher than 0.05. To retain high accuracy, all terms were included in the model (Eq. 9) that used to generate contour plots (Figure 3-6).

$$\text{TVFA production} = -8.4680 + 0.8886*\%PM + 0.0501*FI + 1.7174*FT - 0.0098*\%PM^{2} - 0.0032*FI^{2} - 0.0149*FT^{2} + 0.0045*\%PM*FI + 0.0182*\%PM*FT + 0.0103*FI*FT \quad (9)$$

Table 3-3 Estimated regression coefficient and ANOVA of the fitting model for TVFA production

Source	TVFA production (g/kg $_{\text{dry substrate added}}$)	
	Coefficient Estimate	Probability
b_0	-8.4680	<0.0001
$b_1 \times \%PM$	0.8886	<0.0001
$b_2 \times FI$	0.0501	0.0030
$b_3 \times FT$	1.7174	<0.0001
$b_4 \times \%PM \times \%PM$	-0.0098	<0.0001
$b_5 \times FI \times FI$	-0.0032	0.7271
$b_6 \times FT \times FT$	-0.0149	0.0004
$b_7 \times \%PM \times FI$	0.0045	0.0479
$b_8 \times \%PM \times FT$	0.0182	<0.0001
$b_9 \times FI \times FT$	0.0103	0.0188
F-significant	<0.0001	
R^2	0.8681	
R^2 adjusted	0.8622	
Coefficient of variance	27.14	

In order to achieve the main purpose of eventual energy production, the total yield of VFA in the leachate should be maximized as they not only helped attack the EFB fiber but also be converted into biogas. The interactive effects of *%PM*, *FI*, and *FT* on the TVFA production (Figure 3-6) were displayed pair-wise whereas the third variable was fixed; *FI*=48h, *FT*=60d and *%PM*=50%. Individually, the regression model confirmed that higher proportion of PM mixing has positive result on TVFA production either in relation to *FT* or *FI* as seen by a drastic shift of profiles in Figure 3-6b and 3-6c, respectively. Simultaneously, increase in *%PM* and *FT* synergistically improved the TVFA production (Figure 3-6a). This observation revealed the impacts of these two parameters on the overall bioconversion through the combined hydrolysis and acidogenesis reactions. It was also confirmed by a large

coefficient with high statistical significance (p<0.0001) in the model compared to those of the quadratic and interaction terms. Although quadratic FI^2 term was not highly significant, the impact of *FI* was noticeable at high *%PM* (Figure 3-6b). This was investigated in more details in the analysis of the VFA speciation in a following section.

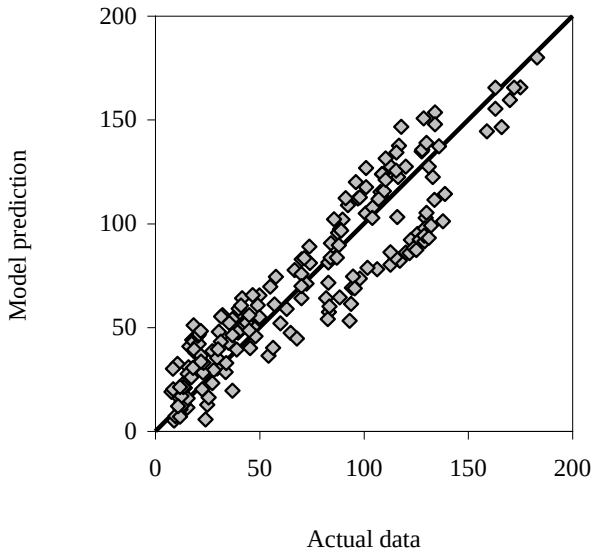


Figure 3-5 Predicted versus observed values of the TVFA production response function (the line represents actual = modeled values)

Figure 3-6c also reveals that the effect of *FT* was diminishing, evidenced by slower paces of TVFA generation after approximately 30 days. Longer *FT* practically implies the longer time for feedstock holding time and loss of the overall TVFA productivity (Nizami et al., 2010). This observation coincided with the results of acidification yield shown in Figure 3-3 that *FT* of approximately 30 days could probably be suitable, and the TVFA concentration in the leachate should be reduced to prevent the microbial product inhibition as previously mentioned.

It is interesting to also note that no local optimal or maximum value of TVFA production was found within our contour plots although the selected ranges of all studied independent variables; pig manure mixing ratio (*%PM*) 0-100, fermentation time (*FT*) 0-60 days, and flushing interval (*FI*) 12-48, obviously cover most practical operational values of this ALBR system. This circumstance is rather

typical in this kind of problem since the setup of this experiment was intended mainly to study the characteristics of this particular system with specific substrates at the realistic operational parameters, and secondly try to optimize within such limits. There is always a chance that the maximum outcome of dependent variable would not show up in this situation.

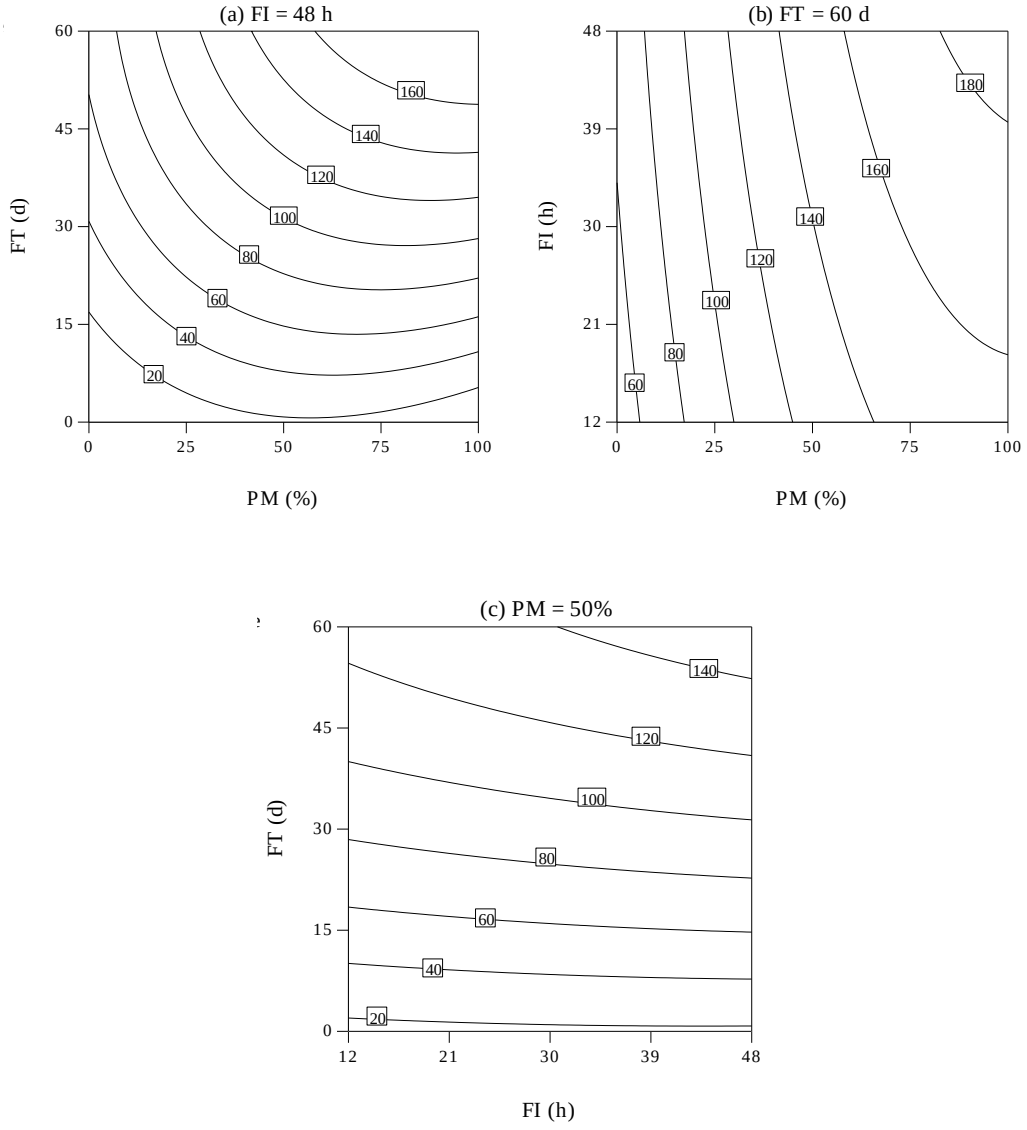


Figure 3-6 Two-dimensional contours plots of TVFA production as a function of (a) PM vs. FT, (b) PM vs. FI, (c) FI vs. FT

3.4.4 VFA species

The VFAs are intermediary products as a result of acidogenesis. The cumulative TVFAs production at the end of 60-d batch with statistical comparison is shown in Table 3-2 and the variations of VFA species produced with different *%PM* and *FI* over the 60-d fermentation are shown in Figure. 3-7. VFA species were categorized by the carbon number as C2 (HAc), C3 (HPr), C4 (i-HBu+HBu), C5 (i-HVa+Hva) and C6 (i-HCap+HCap). VFAs were mostly preserved as the levels of acetic acid were not reduced due to the acidification that brought pH value down below 6.0 in all experiments.

Increase in TVFA production occurred at highest PM mixing ratio (Figure 3-7) as the pig manure was easier to be hydrolyzed and transformed into fatty acids. It was confirmed by statistical analysis shown in Table 3-2 that higher *%PM* co-digestion gave higher overall fatty acids production ($p<0.05$). It was interesting to find that acetic acid was predominant early but the larger molecule fatty acids started to rise as the experiment progressed. The C2 proportion was, for instance, approximately 42-49% at day 30 and went down to 32-39% at day 60 in 50PM:50EFB treatment. Acetogenesis reaction which the bacteria cleaved the larger molecule fatty acids into acetate was not active probably due to the product inhibition since methanogenation from the acetic acid, also known as acetoclastic methanogenesis, was not taking place.

Addition of PM, which consisted of a rather high protein content left over from the pig feed, obviously caused the production of higher molecular weight VFAs, C5 and C6, that were commonly found in protein fermentation (Mcinerney, 1988; Parawira et al., 2005) although C5 could also found from lignocellulose fermentation such as cattail *T. latifolia* (Chen et al., 2012). Without PM addition, a longer *FI* (compared at 0PM:100EFB in Figure 3-7) caused the appearance of C4. With PM addition, the higher proportion of butyric and valeric acids (C4 and C5) also emerged at longer *FI*. This seemed conclusive that the longer dry period had allowed more time for the microbial enzymatic reaction to proceed as opposed to the frequent flushing. Higher TVFA production, resulted from the higher hydrolysis and acidogenesis, together with the larger proportion of C4 and C5 at longer *FI* suggested

that the EFB was more likely to degrade better in such condition. The effect of adding PM as co-substrate was also positive since the mild acids formed from the easier degradable substrate by hydrolytic and acidogenic bacteria would help loosen the lignocellusic structure and increase enzyme accessibility resulting in an improved overall hydrolysis rate (Figure 3-2). This is in agreement with Yang et al. (2009) who reported lignocellulosic structural changes on the surface of cordgrass *S. alterniflora* co-digesting with potato, which was a more biodegradable substrate, as a result of higher TVFA production from co-digestion compared to mono-digestion of only *S. alterniflora*. Effect of the weak acids induced from one substrate to disrupt the lignicellulosic structure of the other substrates played an important role in this co-fermentation scheme.

C:N ratios of the mixtures may also play a role in this fermentation process. The mixtures of 0PM:100EFB, 25PM:75EFB, 50PM:50EFB and 100PM:0EFB had C:N ratios of 32.7:1, 24.9:1, 19.4:1 and 13.8:1, respectively. Differences in TVFA production and VFA species distribution were evident at different C:N (Table 3-2 and Figure 3-7). The results with C:N ratio can be described in a similar trend to the EFB in the mixture, i.e. high C:N at high EFB. Deublein and Steinhauser (2008b) reported that the C:N requirement for hydrolysis and acidogenesis process is 10-45:1 which covered the initial C:N ratio used in our experiments of 13.8-32.7:1. However, high C:N tended to induce lower TVFA production from the lignocellulosic material fermentation (Lay et al., 2013), possibly related to the interference of electron flow caused by the presence of high nutrient concentration in the culture (Lay et al., 2010).

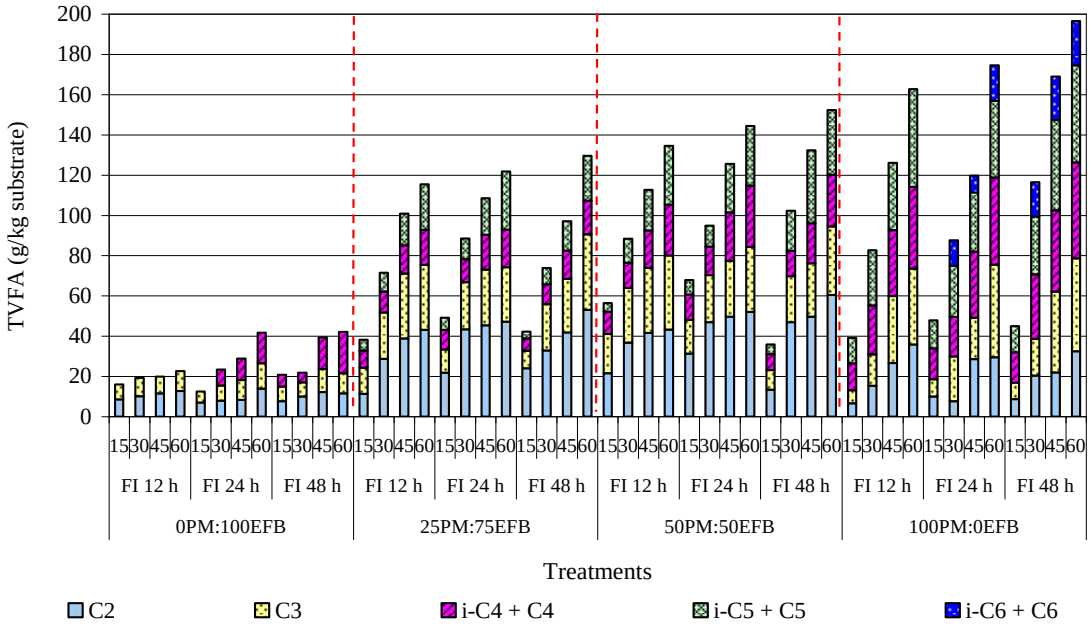


Figure 3-7 VFA production and composition at different co-substrate mixing ratios in the ALBR operated at flooding interval (FI) 12 h, 24 h, and 48 h

3.3 Conclusion

The co-substrate of pig manure (PM) and oil palm empty fruit bunch (EFB) was converted to VFAs using anaerobic leach bed reactor in intermittent liquid flushing mode. Higher PM mixing ratio and longer fermentation time had statistically significant impacts on the hydrolysis and acidogenesis efficiencies. It was found that co-digestion with pig manure could give acidification yield better than mono EFB digestion. The quadratic regression model developed was able to describe the relationship of the studied parameters on the overall TVFA production well. Although the liquid flushing interval did not show obvious impact on TVFA production, it influenced the speciation of VFAs produced. Adding pig manure as co-substrate could initiate hydrolysis and acidogenesis, which the mild acids produced, could then help loosen the lignocellulosic structure and increase enzyme accessibility. Hence, the mixing of a more easily biodegradable co-substrate could help facilitate the early stage of fermentation comprising hydrolysis and acidogenesis.

REFERENCE

APHA, AWWA, WEFF. 1998. Standard methods for the examination of water and wasterater. 20 ed, Springer. Berlin.

Babel, S., Fukushi, K., Sitanrassamee, B. 2004. Effect of acid speciation on solid waste liquefaction in an anaerobic acid digester. Water Research, 38(9), 2417-2423.

Bable, S., Fukushi, K., B., S. 2004. Effect of acid speciation on solid waste liquid faction in an anaerobic acid digester. Water Research, 38(9), 2417-2423.

Bahrin, E., Baharuddin, A., Ibrahim, M., Razak, M., Sulaiman, A., Abd-Aziz, S., Hassan, M., Shirai, Y., Nishida, H. 2012. Physicochemical property changes and enzymatic hydrolysis enhancement of oil palm empty fruit bunches treated with superheated steam. Bioresour Technolgy, 7, 1784-1801.

Boonsawang, P., Wongsuvan, T. 2010. Nutrient optimization of polyhydroxyalkanoate production from palm oil fiber by *Ralstonia eutropha* TISTR 1095 using response surface methodology. Songklanakarin Journal of Science and Technology, 32(1), 9-16.

Chen, Y., Wen, Y., Zhou, J., Xu, C., Zhou, Q. 2012. Effects of pH on the hydrolysis of lignocellulosic wastes and volatile fatty acids accumulation: The contribution of biotic and abiotic factors. Bioresource Technology, 110(0), 321-329.

De La Rubia, M.A., Raposo, F., Rincón, B., Borja, R. 2009. Evaluation of the hydrolytic–acidogenic step of a two-stage mesophilic anaerobic digestion process of sunflower oil cake. Bioresource Technology, 100(18), 4133-4138.

Demirel, B., Yenigun, O. 2004. Anaerobic acidogenesis of dairy wastewater: the effects of variations in hydraulic retention time with no pH control. Journal of Chemical Technology and Biotechnology, 79, 755-760.

Demirer, G.N., Chen, S. 2008. Anaerobic biogasification of undiluted dairy manure in leaching bed reactors. Waste Management, 28(1), 112-119.

Deublein, D., Steinhauser, A. 2008a. Biogas from waste and renewable resources. WILEY-VCH Verlag GmbH & Co. KGaA., Germany, 89-122.

Deublein, D., Steinhauser, A. 2008b. Biogas from waste and renewable resources : An introduction. WILEY-VCH Verlag GmbH & Co. KGaA,Weinheim, Germany.

Geng, A. 2013. Conversion of oil palm empty fruit bunch to biofuels. in: Liquid, Gaseous and Solid Biofuels - Conversion Techniques, (Ed.) P.Z. Fang, Intech, 479-490.

Insam, H., Franke-Whittle, I., Goberna, M. 2010. Microbes in aerobic and anaerobic waste treatment. in: Microbes at work from wastes to resources, (Ed.) F.-W.I. In: Insam H, Goberna M (eds), Springer. Heidelberg, 1-34.

Isroi, Millati, R., Syamsiah, S., Niklasson, C., Cahyanto, M.N., Lundquist, K., Taherzadeh, M.J. 2011. Biological pretreatment of lignocelluloses with white-rot fungi and its applications: A review. Bioresources Technology, 6(4), 5224-5259.

Jagadabhi, P.S., Kaparaju, P., Rintala, J. 2011. Two-stage anaerobic digestion of tomato, cucumber, common reed and grass silage in leach-bed reactors and upflow anaerobic sludge blanket reactors. Bioresource Technology, 102(7), 4726-4733.

Jamari, S.S., Howse, J.R. 2012. The effect of the hydrothermal carbonization process on palm oil empty fruit bunch. Biomass and Bioenergy, 47, 82-90.

Kim, S., Park, J.M., Seo, J.-W., Kim, C.H. 2012. Sequential acid-/alkali-pretreatment of empty palm fruit bunch fiber. Bioresource Technology, 109, 229-233.

Kivaisi, A.K., Eliapenda, S. 1995. Appication of ruman microorganisms for enhance anaerobic degradation of bagasse and maize bran. Biomass and Bibenergy, 8(1), 45-50.

Lau, M.J., Lau, M.W., Gunawan, C., Dale, B.E. 2010. Ammonia fibre expansion (AFEX) pretreatment, enzymatic hydrolysis, and fermentation on empty palm fruit Bunch fibre (EPFBF) for cellulosic ethanol production. Applied Biochemistry and Biotechnology, 162, 1847-1857.

Lay, C.-H., Chen, C.-C., Lin, H.-C., Lin, C.-Y., Lee, C.-W., Lin, C.-Y. 2010. Optimal pH and substrate concentration for fermentative hydrogen production from preserved fruits soaking solution. Journal of Environmental Engineering and Management, 20(1), 35-41.

Lay, C.-H., Sen, B., Chen, C.-C., Wu, J.-H., Lee, S.-C., Lin, C.-Y. 2013. Co-fermentation of water hyacinth and beverage wastewater in powder and pellet form for hydrogen production. Bioresource Technology, 135(0), 610-615.

Lehtomaki, A., Huttunen, S., Lehtinen, T.M., Rintala, J.A. 2008. Anaerobic digestion of grass silage in batch leach bed processes for methane production. Bioresource Technology 99, 3267-3278.

Llabrés-Luengo, P., Mata-Alvarez, J. 1988. The hydrolytic step in a dry digestion system. Biological Wastes, 23(1), 25-37.

McInerney, M.J. 1988. Anaerobic hydrolysis and fermentation of fats and proteins. Wiley, New York, 373-415.

Misson, M., Haron, R., Kamaroddin, M.F.A., Amin, N.A.S. 2009. Pretreatment of empty palm fruit bunch for production of chemicals via catalytic pyrolysis. Bioresource Technology, 100, 2867-2873.

Myint, M.T., Nirmalakandan, N. 2009. Enhanceing anaerobic hydrolysis of cattle manure in leach bed reactors. Bioresource Technology, 100, 1695-1699.

Nizami, A.S., Thamsiriroj, T., Singh, A., Murphy, J.D. 2010. Role of leaching and hydrolysis in a two-phase grass digestion system. Energy Fuels, 24, 4549-4559.

O-Thong, S., Boe, K., Angelidaki, I. 2012. Thermophilic anaerobic co-digestion of oil palm empty fruit bunches with palm oil mill effluent for efficient biogas production. Applied Energy 93, 648-654.

Office of Agricultural Economic. 2012. Data of agricultural economic crop (in Thai). Ministry for Agricultural and Coperatives.

Parawira, W., Murto, M., Read, J.S., Mattiasson, B. 2005. Profile of hydrolases and biogas production during two-stage mesophilic anaerobic digestion of solid potato waste. Process biochemistry, 40(9), 2945-2952.

Qu, X., Vavilin, V.A., Mazéas, L., Lemunier, M., Duquennoi, C., He, P.J., Bouchez, T. 2009. Anaerobic biodegradation of cellulosic material: Batch experiments and modelling based on isotopic data and focusing on aceticlastic and non-aceticlastic methanogenesis. Waste Management, 29(6), 1828-1837.

Rish, L.G. 1963. Unit of process of engineering Wiley, New York.

Singh, A., Nizami, A.-S., Korres, N.E., Murphy, J.D. 2011. The effect of reactor design on the sustainability of grass biomethane. Renewable and Sustainable Energy Reviews, 15(3), 1567-1574.

Sinjermsiri, D., Hanbunchong, A., Thanomwongwattana, R. 2006. Quality improvement of oil palm press fibre by the chemical and biological. Animal Science Conference, Kasartsart University'14, Bangkok, Thailand. 307-315.

Stabnikova, O., Liu, X.-Y., Wang, J.-Y. 2008. Anaerobic digestion of food waste in a hybrid anaerobic solid–liquid system with leachate recirculation in an acidogenic reactor. Biochemical Engineering Journal, 41(2), 198-201.

Umikalsom, M.S., Ariff, A.B., Zulkifli, H.S., Tong, C.C., Hassan, M.A., Karim, M.I.A. 1997. The Treatment of Oil Palm Empty fruit bunch fibre for Subsequent use as Substrare fir Cellulase Production by Cheatomium Globosum Kunze. Bioresource Technology 62, 1-9.

Vavouraki, A.I., Volioti, V., Kornaros, M.E. 2014. Optimization of thermo-chemical pretreatment and enzymatic hydrolysis of kitchen wastes. Waste Management, 34(1), 167-173.

Weimer, P.J., Russell, J.B., Muck, R.E. 2009. Lessons from the cow: What the ruminant animal can teach us about consolidated bioprocessing of cellulosic biomass. Bioresource Technology, 100(21), 5323-5331.

Xie, S., Lawlor, P.G., Frost, J.P., Wu, G., Zhan, X. 2012. Hydrolysis and acidification of grass silage in leaching bed reactors. Bioresource Technology, 114, 406-413.

Xu, S.Y., Karthikeyan, O.P., Selvam, A., Wong, J.W.C. 2012. Effect of inoculum to substrate ratio on the hydrolysis and acidification of food waste in leach bed reactor. Bioresource Technology, 126(0), 425-430.

Yang, S., Li, J., Zheng, Z., Meng, Z. 2009. Lignocellulosic structural changes of sparitina alterniflora after anaerobic mono- and co-digestion. International Biodeterioration & Biodegradation, 63, 569-575.

Zhang, B., Zhang, L., Zhang, S., Shi, H., Cai, W. 2005. The influence of pH on hydrolysis and acidogenesis of kitchen wastes in two-phase anaerobic digestion. Environmental Technology, 26, 329-340.

Zhang, X., Qiu, W., Chen, H. 2012. Enhancing the hydrolysis and acidification of steam-exploded cornstalks by intermittent pH adjustment with an enriched microbial community. Bioresource Technology, 123(0), 30-35.

CHAPTER IV

SOLIDS STATE CO-FERMENTATION OF LIGNOCELLULOSIC PALM OIL EMPTY FRUIT BUNCH FOR ORGANIC ACID RECOVERY AND FIBER PROPERTY IMPROVEMENT

4.1 Abstract

Batch wise co-fermentation of lignocellulosic oil palm empty fruit bunch (EFB) fiber and pig manure (PM) was carried out using anaerobic leach bed reactor (ALBR) in intermittent flushing mode for organic acid production and possible fiber property improvement. Higher PM addition (*%PM*), long fermentation time (*FT*), and long leachate flushing interval (*FI*) showed positive effects on hydrolysis yield and acidification yield of the substrate leading to a higher total volatile fatty acid production. Their relationships were described with multiple regression model and three-dimensional response surface plots. VS release from the EFB fiber after 60-day was improved by 6.9 times when *%PM* and *FI* increased from 0 and 12 h to 50 and 48 h, respectively. Mild acids produced were able to remove hemicellulose from the fiber as high as 56.9% and leach potassium (K) off 83.5±4.0%. The fiber fuel properties were improved by increasing its heating value and lowering K content that caused slag in boilers. Addition of PM also increased plant nutrients in the EFB digestate but still lower than organic fertilizer standard. This study demonstrated that co-fermentation could be one of the pretreatment methods to recovery some products from lignocellolosic biomass and may ease their subsequent transformations.

Keywords: Palm empty fruit bunch; lignocellulose; pretreatment; fermentation; digestate; leach bed reactor

4.2 Introduction

Oil palm is the most productive crop that generates consumable oil at the most competitive price. Compared to other oilseeds, oil palm plantation, at an average production of nearly 4 tons per hectare per year, can produce approximately 5.2 times higher than the second place rapeseed, leaving behind the sunflower and soybean in that range (OPEC, 2013). Many tropical countries have cultivated oil palm plantation as their major economic crop, particularly Indonesia ($33.5x10^6$ tones/yr), Malaysia ($21.3x10^6$ tons/yr), and Thailand ($2.3x10^6$ tons/yr), whose total amounts for about 90 percent of the world production (USDA, 2014). In palm oil mills, crude palm oil is extracted from the fruit leaving the tremendous amount of oil palm empty fruit bunch (EFB) to be disposed of. Per ton of oil produced, there is 4.3 tons of EFB generated (Prasertsan and Prasertsan, 1996). This is an enormous biomass resource that requires effective valorizations to various products. In the past, EFB was typically transported back to the palm plantation and let disintegrate and mineralize to soil, or in some cases be left to decompose elsewhere. This method releases methane to the atmosphere. More recently, it has been put through thermal conversion as fuel for energy generation. Its high potassium content unfortunately makes it difficult for combustion due to the slack formation in the combustion chamber (Mohammed et al., 2012; Obernberger and Thek, 2004). Alternative biological conversion is another path as it could also lead to the generation of energy such as bio-ethanol and biogas, or other more valuable chemicals.

Biodegradability of EFB is slow due to its high lignin content 28.8% (Kim et al., 2012). Obstruction of enzymatic attack to cellulose, which is simply a glucose chain, is known to be a function of lignin and hemicellulose in nature. This lignocellulosic EFB also contains low nutrients at C:N:P of 100:2.0:0.2 (Saritpongteeraka et al., 2014). Its recalcitrant nature needs to be modified before EFB can be efficiently used for further transformations. Previous works on pretreatments of EFB have focused on the physical and chemical approaches aiming to extract fermentable sugars in combination with the disruption of the fiber structure as to improve its biodegradability. Those include grinding, milling, high temperature, high pressure, steam explosion, alkaline and acid, or any combination (Hendriks and

Zeeman, 2009; Kumar et al., 2009; Mosier et al., 2005). Biological pretreatment typically is the most economical method since the enzymes to degrade different substances in the materials are produced by microorganisms immobilized with the biomass and no chemical waste is generated. However, very limited works in this area had been done with EFB. Sinjermsiri et al. (2006) had pretreated oil palm press fiber (PPF), derived from palm fruit, by bacillus and lactic bacteria and found a decline in lactic bacteria population after their 21-day fermentation. This could be associated with the imbalanced nutrients of the fibrous substrate. Aerobic white rot fungi had been trialed to delignify EFB but still faced some carbon loss of polysacharides (Piñeros-Castro and Velásquez-Lozano, 2014) due to the non-selective degradation (Isroi et al., 2011) of fungal enzymes. To date, no studies on the fermentative pretreatment of EFB have been reported.

In this work, anaerobic solid state fermentation (SSF) was employed as a pretreatment to liquefy and leach the biomaterials from EFB. Co-fermentation strategy of EFB with pig manure, which is rich in nutrients, to induce the degradability of the lignocellulosic materials are of interest. The mixture of EFB of C:N 47.6:1 – 107.9:1 (Jamari and Howse, 2012; Kerdsuwan and Laohalidanond, 2011) was balanced with the pig manure of C:N 13.0:1 (Dechrugsa et al., 2013). The hydrolytic enzymes released during the hydrolysis stage together with the organic acids from the acidogenic stage could help loosen the fiber structural crystallinity making it more accessible for enzymatic attack and overall improved conversion rate. Levels of hydrolysis and acidogenesis of EFB would be the key indicators to monitor the effectiveness of this SSF pretreatment process. Enhancement of the fiber degradability shall lead to the changes of its properties and suitability for subsequent treatments or uses.

Anaerobic leach bed reactor (ALBR) is a type of solid state fermentation processes operated in a semi-batch fed manner. The moisture in the substrate pile is maintained by the liquid spray-percolation or submerge-flushing mode. Both operations promote immobilization of bacteria in contact with the substrate (Stabnikova et al., 2008). The intermittent submerge-flushing cycle could better ensure the thoroughness of liquid-solid contact, while the semi-dry period

allows the enzymes released to intensify on the surface of the solid substrate. Limited studies on the use of ALBR for fatty acids production have been done (Babel et al., 2004; Myint and Nirmalakandan, 2009; Xie et al., 2012) and none was reported as a pretreatment of the lignocellulosic biomass with EFB in particular.

In our study, EFB and pig manure (PM) were co-fermented in order to pretreat EFB. The key objective of this study was to determine the conversion efficiency of EFB with the use of pig manure as co-substrate. The aqueous fermentable products released from EFB lignocellulose were measured in batch test ALBR in intermittent submerge-flushing mode with the designed variables pig manure mix ratio (*%PM*), flushing interval (*FI*) and fermentation time (*FT*). The changes in properties of the material during fermentation were also monitored and compared against possible EFB utilization purposes as fuel and fertilizer.

4.3 Material and methods

4.3.1 Substrate and seeding

The inoculum used was fresh cow manure from Tepa Livestock Research Training Station, Tepa District, Songkhla Province, Thailand. The co-substrates in this experiment were a fresh pig manure (PM) collected from a pig farm in Phattalung Province in southern Thailand, and oil palm empty fruit bunch (FFB) of species *Eleaeis guineensis* collected from Lam Soon Palm Oil Mill in Sikao District, Trang Province, Thailand (N99°19´,E7°42´). The palm fruit was harvested seasonally in August. The collected EFB was dried at 60°C and cut to 5 cm in size, then stored at 4°C until use. The substrates and inoculum were homogenized manually and analyzed separately for moisture content, total solids (TS) and volatile solids (VS) within 2 h after mixing. They were then stored at 4 °C no longer than 48 h prior to use in the experiments to maintain material freshness and active rumen microorganisms.

The EFB sample was divided by weight into different portions according to different treatments in the experiment. They were then soaked in tap water to moisten the fiber overnight. The predetermined quantity of PM was brought to mix with the moist EFB to make up 800 g of the mixture at ratios 0PM:100EFB, 25PM:75EFB 50PM:50EFB and 100PM:0EFB on dry weight basis (TS). Two

hundred grams (dry weight basis) of the inoculum, 20% w/w dry basis, was added in each treatment. The nylon fiber, cut to 5 cm in length was used in place of EFB in the control set (100PM:0EFB). Each mixture was loaded to separate reactors. Table 4-1 shows characteristics of substrates and the inoculum used in the experiment. The analytical methods of the samples are similar to those stated in section 2.3.

Table 4-1 Characteristics of PM, EFB and the inoculum used in the experiments

Parameter	Inoculum	EFB	PM
Total solid, TS (g/kg wet)	104.8±17.8	383.1±61.4	270.8±10.3
Volatile solids, VS (g/kg dry)	805.4±99.9	938.2±25.0	802.3±6.1
Cellulose (% dry wt.)	50.3±5.4	37.6±5.6	41.6±3.0
Hemicellulose (% dry wt.)	39.1±0.9	38.8±1.7	38.6±4.0
Lignin (% dry wt.)	10.7±1.3	23.6±4.0	17.0±2.1
Carbon (% dry wt.)	43.0±0.14	42.9±0.36	42.4±0.23
Nitrogen (% dry wt.)	1.7±0.04	0.9±0.02	3.1±0.04
Hydrogen (% dry wt.)	5.4±0.05	5.7±0.11	5.5±0.06
Oxygen (% dry wt.)	23.5±0.34	30.7±0.30	26.2±0.20
Sulfur (% dry wt.)	0.2±0.01	< 0.01	0.2±0.00
Phosphorus (P) (% dry wt.)	0.52	0.08	2.31
Potassium (K) (% dry wt.)	0.08	1.71	1.20
C:N ratio	25.3	47.7	13.8
COD initial ($gCOD/g_{sub.dry}$)*	1.08	0.89	0.71
Higher heating value, HHV (MJ/kg)	12.5	7.7	16.5

* Initial COD was calculated from the values of elemental composition (CHONS) according to Saritpongteeraka et al. (2014)

4.3.2 Reactor configuration and system operation

Anaerobic leach bed reactor (ALBR) was employed in this experiment. They were made of 40-L cylindrical PVC drums with 30 cm in diameter and 55 cm in height. A sampling port for solid sample withdrawal was located on top of the reactor beside a biogas port which was connected to a gas balloon to provide extra gas volume replacement from flooding and flushing operation (Figure 4-1). A drain pipe at the bottom was used for liquid flooding and drainage. This drain pipe was connected to the liquid holding tank to prevent liquid accumulation inside the reactor. The leachate was stored in a sealed holding tank of 40 L. A leachate sampling port was installed on container top with a ball valve which was opened only when taking

the leachate sample. There was a balloon connected to the headspace of the tank to allow volume displacement corresponding to the main reactor operating cycle.

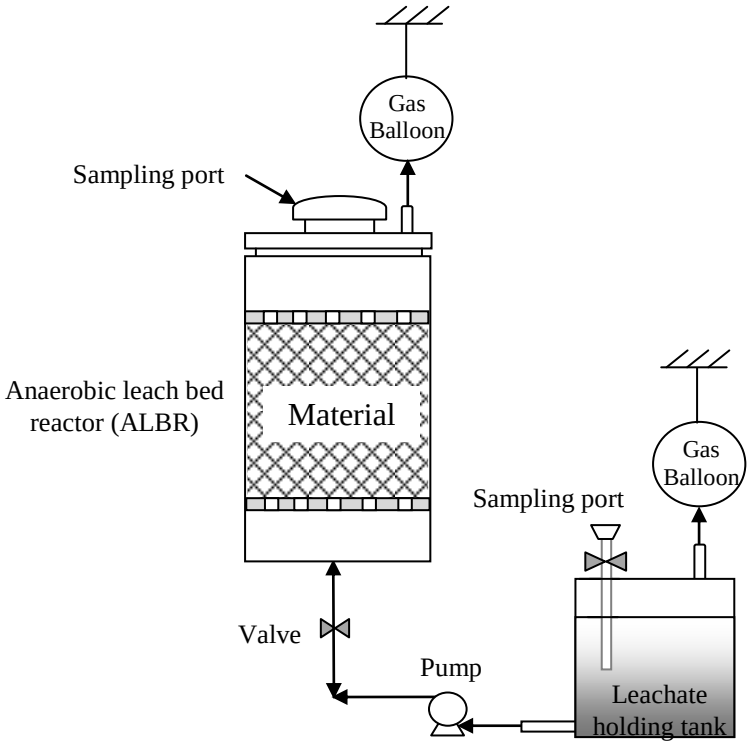


Figure 4-1 Schematic diagram of the anaerobic leach bed reactor (ALBR) and ALBRs in this study

Batch assays were used in this study, where the substrate mixture was fermented in ALBR system over 60-day period. Initially, 35 L of deionized water was pumped from the holding tank to flood the solid bed in the ALBR and stayed overnight (approximately 12 h) before draining off. The leachate was then sampled as day 1 data. After that, the cyclic operation started by the liquid being pumped to the ALBR within the first 10 minutes and left to stay for another 20 minutes. Then the drainpipe valve was switched open allowing the leachate to run back to the holding tank within the next 10 minutes before closing the drain valve. Subsequently, the main reactor was left to react until the next cycle arrived. These actions constituted one flushing interval (*FI*). The ALBRs were under such operation for 60 days where the production of TVFA was minimal.

4.3.3 Sample and data analyses

Leachate from each treatment was collected and analyzed for pH, soluble chemical oxygen demand (SCOD) according to the Standard Methods (APHA et al., 2005). Volatile fatty acid (VFA) composition was determined using a Hewlett-Packard Gas Chromatography Model 7890 with an Inowax capillary column (30m×0.25mm×0.25µm) and a flame ionization detector. The injection port temperature was set at 260 °C with helium as carrier gas. The column temperature was increased at 20 ^{o}C/min to 120 °C, then increased at 10 ^{o}C/min to 205 °C. In preparation of sample for VFA test, the liquid was centrifuged at 10,000 rpm for 10 min to separate the supernatant which was then acidified with phosphoric acid (0.1%) to pH below 2. It was filtered with 0.22 µm membrane (APHA et al., 2005).

At the end of each batch experiment (60 days), the total solid digestate was taken out of the reactor, mixed well, and sampled to determine nitrogen (N) using macro Kjeldahl method. Digestion of sample was performed by persulfate digestion method followed by vanadomolybdophospohric acid colorimetric method (APHA et al., 1998) for the analysis of phosphorus (P). Potassium (K) was determined by atomic absorption spectroscopy (AA) method. The samples were then tested for heating values by combusting in an adiabatic oxygen-bomb calorimeter following the ASTM D240 method (ASTM, 2009).

The EFB fibers in the total solid digestate were sampled, rinsed well with DI water and gently oven dried at 60 °C to observe its surface deformation using scanning electron microscopy (SEM) model Quanta 400, FEI, Czech Republic with an Everhart Thornley detector at voltage 15 kV. The samples were also tested for volatile solids content (VS) according to the Standard Methods (APHA et al., 2005) while determination of cellulose, hemicellulose and lignin contents were carried out following AOAC method 973.18 (AOAC, 1995).

The hydrolysis yield (η_h) was defined as the ratio between the cumulative SCOD production (g/kg substrate) in the leachate and initial total COD of the substrate, while acidification yield (η_a) was defined as the ratio between the cumulative TVFA in COD equivalence ($gCOD/kg_{dry\ substrate\ added}$) and SCOD

(gCOD/$kg_{dry\ substrate\ added}$). These two parameters were calculated according to Eq. 1 and 2, in order (Xie et al., 2012).

Hydrolysis yield $$\eta_h = \frac{S_S}{S_I} \times 100\% \quad \text{(Eq. 1)}$$

Acidification yield $$\eta_a = \frac{S_{TVFA}}{S_S} \times 100\% \quad \text{(Eq. 2)}$$

where S_I is the initial total COD (g) of substrate (0PM:100EFB, 25PM:75EFB, 50PM:50EFB, and 100PM:0EFB), and S_S is the cumulative SCOD production (g) in the leachate. S_{TVFA} is the cumulative TVFAs expressed as g COD calculated from the theoretical COD equivalents for each VFA species. The theoretical COD equivalence of acetic acid, propionic acid, butyric acid, valeric acid, and caproic acid are 1.066, 1.512, 1.816, 2.036, and 2.204 gCOD/g, respectively (Demirel and Yenigun, 2004)

4.3.4 Experimental design and statistical analysis

The experiment was conducted in full factorial matrix. Variables X_1 (*%PM*), X_2 (*FI*), and X_3 (*FT*) were tested respectively at 4, 3, and 18 levels. X_1 is a percentage of pig manure in the mixture covering a range of 0-100 as previously described. X_2 is flooding interval tested at 12h, 24h and 48h, and X_3 is fermentation time from 0 to 60 days. The zero levels are the midpoint of the parameter range that have natural values of 50 for *%PM*, 24 for *FI*, and 30 for *FT*. Twelve treatments (4 *%PM* X 3 *FI*) were run in triplicate for 60 days. The interested responses of the model were hydrolysis yield (η_h) and acidification yield (η_a). A second order polynomial model (Eq. 3) was employed to fit the experimental data using the software Design Expert Version 8.0.7.1. Parameters *%PM*, *FI* and *FT* were regressed to describe their correlation in the model. Statistical significance of the coefficients within the model were indicated at *p-value* <0.05. Contour plots and overlay plots of multiple responses were generated to reflect the possible cross relationships.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1,<j}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \qquad \text{(Eq. 3)}$$

where Y is the predicted response, β_0 is the constant of the model, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the coefficient for the ij interaction effect when $i<j$, k is the number of studied variables, and X_i and Xj are the independent variables (natural values). The response Y_1; hydrolysis yield (η_h) (%) and Y_2; acidification yield (η_a) (%) were regressed with respect to *%PM*, *FI* and *FT*. Mean and the standard deviation were calculated and used to compare the effects of the independent variables.

4.4 Results and discussion

4.4.1 pH profiles and TVFA production

Change of pH was caused by the production of TVFA from degradation of organics in the substrate. pH of the starting water was 6.80, and the first data point of each treatment was taken from the leachate after the first flushing. All exhibited slightly pH drop to within 6.7-6.8 as the fermentation had just started. Over the course of 60-day fermentation, pH of the leachate fluctuated from 5.5 to 7.0 in all experiments, which was in an optimal range for acidogenesis (Cho et al., 1995; Mtz.-Viturtia et al., 1995).

It was noticeable that treatment 0PM:100EFB had experienced a sharp drop of pH in the first 24 hours (Figure 4-2a) but later climbed slowly back to 6.7-7.0 in all 3 *FI*'s (12 h, 24 h, and 48 h) over 60 days. With PM (25PM:75EFB, 50PM:50EFB and 100PM:0EFB), pH; however, decreased gradually due to the alkalinity it contained (Figure 4-2b, 4-2c and 4-2d). This buffer capacity was resulted predominantly from ammonia in PM which reacted with carbon dioxide to produce ammonium bicarbonate (Gerardi, 2006; Sawyer et al., 2003). It was also observed that with PM, pH drop occurred from the beginning until approximately 20 days before started to rise. It corresponded to the progressive rise in TVFA production rate. Intense hydrolysis-acidogenesis of the substrate in ALBR took place during this stage. The TVFA production rate declined with the corresponding rise of pH. However, over time the system should be able to buffer itself from the release of alkalinity from

substrate digestion (Dogan et al., 2008). Although the hydrolytic and acidogenic organisms could tolerate fluctuations in environmental condition well and could stay active in pH range 3-7 (Wu et al., 2006), the TVFA produced could become self (product) inhibition. Production rate of TVFA slowed down as the accumulation of TVFA was higher towards the end of fermentation period.

Flushing interval also played a role in TVFA production. Shorter flushing interval, i.e. *FI* 12 h, generally gave lower TVFA production compared to the longer ones (Figure 4-2). In PM mix treatments (Figure 4-2b, 4-2c), the daily TVFA production at *FI* 48 h reached a higher peak with a faster pace before beginning to drop rather sharply. This characteristic was favorable since it could produce the same amount of TVFA in a shorter period. Nonetheless, at equal time frame of 60 days, results of TVFA production in the leachate in terms of gram TVFA per gram dry substrate revealed that *FI* 48 h generated at the highest level when compared in each mixing ratio; 0PM:100EFB (42.1±1.5, 41.8±0.9, 22.6±1.3 $g/kg_{dry\ substrate\ added}$), 25PM:75EFB (129.6±5.3, 121.7±19.7, 118.9±4.2 $g/kg_{dry\ substrate\ added}$), 50PM:50EFB (152.3±0.6, 144.4±1.0, 134.5±1.4 $g/kg_{dry\ substrate\ added}$), and 100PM:0EFB (196.5±3.6, 174.6±2.8, 162.7±8.4 $g/kg_{dry\ substrate\ added}$) for FI 48 h, 24 h, and 12 h, in order. Longer dry period had benefited the hydrolysis and acidogenesis of the solid substrate in the pile, due largely to the concentrated enzymatic activity by the immobilized microbes in direct contact to the solid biomass bed for an extended period. Moreover, there were abundant macro and micro nutrients available at the solid biomass. At higher flushing frequency, the dry environment was disturbed more often and generally diluted the enzymatic activity on the substrate surface.

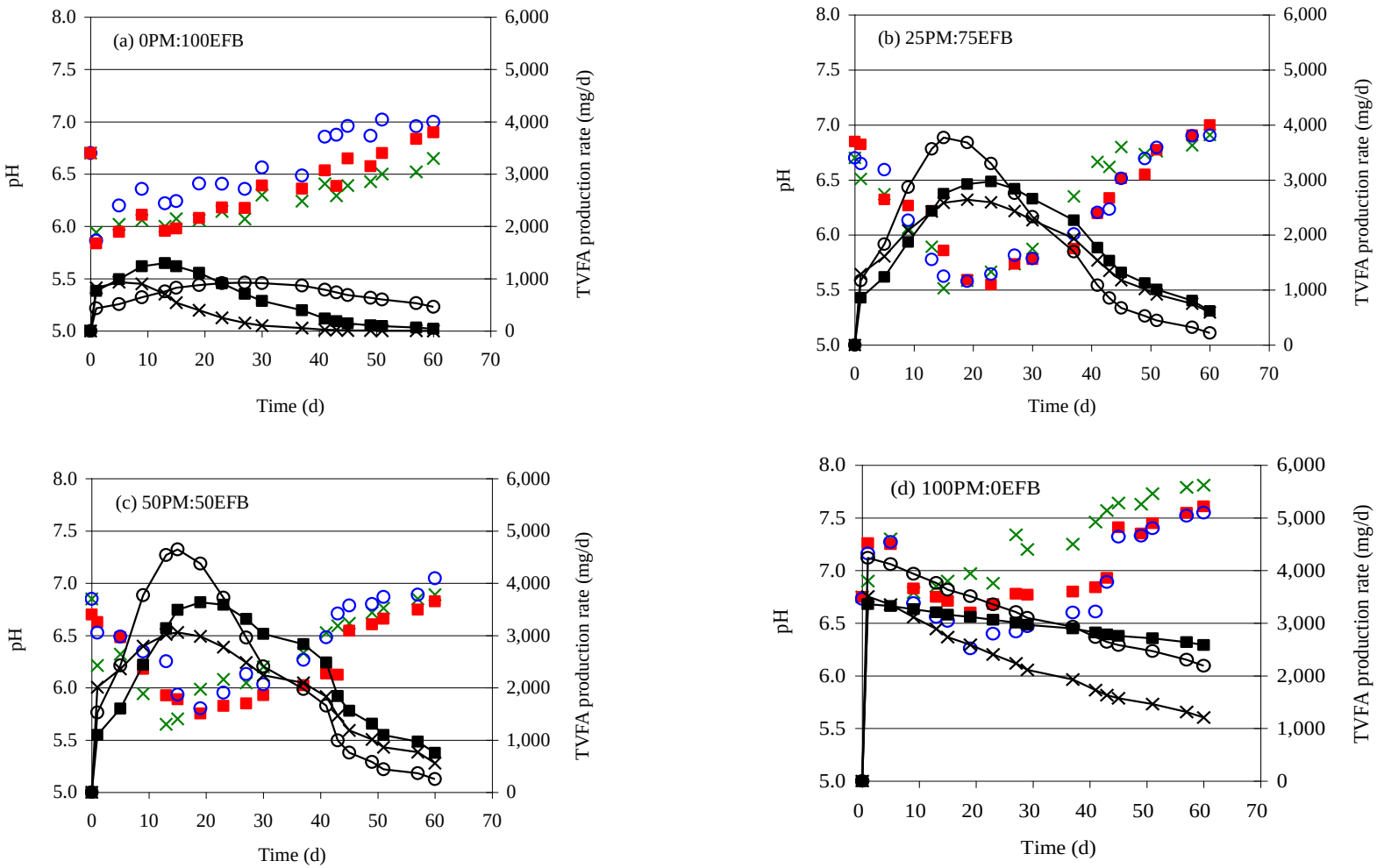


Figure 4-2 pH (× FI 12h , ■ FI 24 h and ○ FI 48 h) and TVFA production rate (-×- FI 12h ,-■- FI 24 h and -○- FI 48 h) when operating ALBRs with different mixing ratios (a) 0PM:100EFB, (b) 25PM:75EFB and (c) 50PM:50EFB at FI 12, 24 and 48 h

4.4.2 Effect of %PM, FI and FT on hydrolysis yield and acidification yield

In order to maximize the ALBR efficiency, the relationships between three key variables were evaluated. Hydrolysis yield (η_h) and acidification yield (η_a) were regressed as a response function by *F-test*, and the resulted analysis of variance (ANOVA) is shown in Table 4-2. Given the low *F* value (<0.0001), the ANOVA of both models were highly significant, indicating that these quadratic models possessed good predictability. A good fit to the experimental data by the studied variables *%PM*, *FI* and *FT* was expressed by the high determination coefficient (R^2) of 0.9509 and 0.8064 for η_h and η_a, respectively. In addition, the value of the coefficient of variance (C.V.) was 13.26 for η_h and 20.53 for η_a confirmed a satisfactory precision and reliability for the experiments performed. All 9 terms in the models (Eq. 4 and 5) were used to generate three-dimensional surface plots in Figure 4-3.

Table 4-2 Estimated regression coefficients and ANOVA of the fitting model for hydrolysis yield (ηh) and acidification yield (ηa)

Source	Hydrolysis yield (%)		Acidification yield (%)	
	Coefficient Estimate	Probability	Coefficient Estimate	Probability
b_0	-1.9763	<0.0001	9.9053	<0.0001
$b_1 \times$ *%PM*	0.1661	<0.0001	0.3603	<0.0001
$b_2 \times$ *FI*	0.1240	<0.0001	-2.9881	0.0329
$b_3 \times$ *FT*	0.2242	<0.0001	1.2675	<0.0001
$b_4 \times$ *%PM* $\times$ *%PM*	-0.0006	<0.0001	-0.0048	<0.0001
$b_5 \times$ *FI* $\times$ *FI*	-0.0022	0.0103	0.0037	0.2594
$b_6 \times$ *FT* $\times$ *FT*	-0.0015	<0.0001	0.0169	<0.0001
$b_7 \times$ *%PM* $\times$ *FI*	0.0007	0.0009	-0.0008	0.2953
$b_8 \times$ *%PM* $\times$ *FT*	0.0008	<0.0001	0.0026	<0.0001
$b_9 \times$ *FI* $\times$ *FT*	0.0012	0.0030	0.0056	0.0004
F-significant	<0.0001		<0.0001	
R^2	0.9509		0.8064	
R^2 adjusted	0.9487		0.7976	
Coefficient of variance	13.26		20.53	

$$\eta_h\ (\%) = -1.9763 + 0.1661*\%PM + 0.1240*FI + 0.2242*FT - 0.0006*\%PM^2 - 0.0022*FI^2 - 0.0015*FT^2 + 0.0007*\%PM*FI + 0.0008*\%PM*FT + 0.0012*FI*FT \quad \text{(Eq. 4)}$$

$$\eta_a\ (\%) = 9.9053 + 0.3603*\%PM - 0.9881*FI + 1.2675*FT - 0.0048*\%PM^2 + 0.0037*FI^2 + 0.0169*FT^2 - 0.0008*\%PM*FI + 0.0026*\%PM*FT + 0.0056*FI*FT \quad \text{(Eq. 5)}$$

The contour plots demonstrated the effects of the proposed variables on hydrolysis and acidification yields at three combinations: (1) *FI* and *%PM*, (2) *FT* and *%PM* and (3) *FT* and FI (Figure 4-3). The contours were generated at the third variable FI of 48 hr, *FT* of 60 d and %*PM* of 50. The studied variables showed the influence on performance of ALBR at varying degrees. It was clearly shown in Figure 3a that *FT* and %*PM* had strong positive impacts on hydrolysis yield. Simultaneous increase in *FT* and %*PM* rapidly improved hydrolysis. *FI*, on the other hand, had a subtle influence particularly at lower PM mixture and fermentation time (Figure 4-3c, 4-3e). Effect of *FI* required the established acidic environment which showed up at the higher %*PM* and longer *FT*. Without or minimal acids to begin, the length of dry period, which the produced mild acids could be in contact with EFB, could not provide benefit on fiber attack. However, the regression model confirmed that *%PM*, *FI* and *FT* had positive effect on hydrolysis yield.

Regarding ηa, a model optimal of 50.7% was found at FT 49.7 h, %PM 52.6 and FI 48 h (Figure 4-3b). Higher *FT* gave higher acidification yield but some loss of acids occurred when *FT* went above 49.7 h. Soluble COD derived from the hydrolysis continued to rise while the production rate of acids could not keep up. This was caused by some conversion of the intermediate acids to other end products such as carbon dioxide and cell synthesis, as well as the product inhibition in the culture. This led to a small drop in η_a (Eq. 2). Another observation from Figure 4-3b was the larger incremental step (each at 5% acidification yield) toward the optimum. The relative increase in SCOD was smaller than that of TVFA causing acidogenic

yield to increase at fast pace at early stage. Figure 4-3d and 4-3f showed minor saddle shape response surfaces, which the high *FI* and *FT* created a slightly larger variations in acidification yield. PM between 50% and 75% delivered optimal acidification yield. Higher PM would not further promote acids production as their concentration in the leachate reached a certain level that could have entered an acidifying limitation by product self-inhibition while hydrolysis still benefited by the work of those acids created. Too low PM mix just could not create acids fast enough to realize the full benefit of hydrolyzing the substrate.

In addition, Pearson's correlation analysis was performed to determine the association degree of hydrolysis and acidification yields of EFB degradation in the semi-dry environment. The analysis had also expanded to cover TVFA production. Results showed that there was a significant correlation between η_h and η_a at 0.752 with 0.01 level (2-tailed test). These two parameters have strong positive synchronization effect where one could assist another. Different kinds of microorganisms can perform hydrolysis by releasing the extracellular enzymes whereas the majority of acidogens could also complete the same task (Vavilin et al., 1994). Both η_h and η_a also had significant correlations (0.01 level, 2-tailed) with the TVFA production (g/kg dry substrate) at 0.814 and 0.793, respectively. A slightly higher degree of correlation with η_h might reiterate the bottle neck of anaerobic fermentation particularly of solid biomass that the hydrolysis is always a rate limiting step.

Not only a shift in C:N ratio has a strong effect for anaerobic digestion, the difference in the amount of fresh pig manure addition to each treatment may also play a role in the effectiveness of the fermentation. This is due to the viable microbial consortia in the pig manure that assisted the process. If a dried pig manure were used in this experiment, the impact of different microbial population could be eliminated. However, the difference should be small because the pig manure was screened in open air and left the liquid to dry up for a few minutes prior to placing in the sealed bag until use. Major reduction in the viable microbial population occurred.

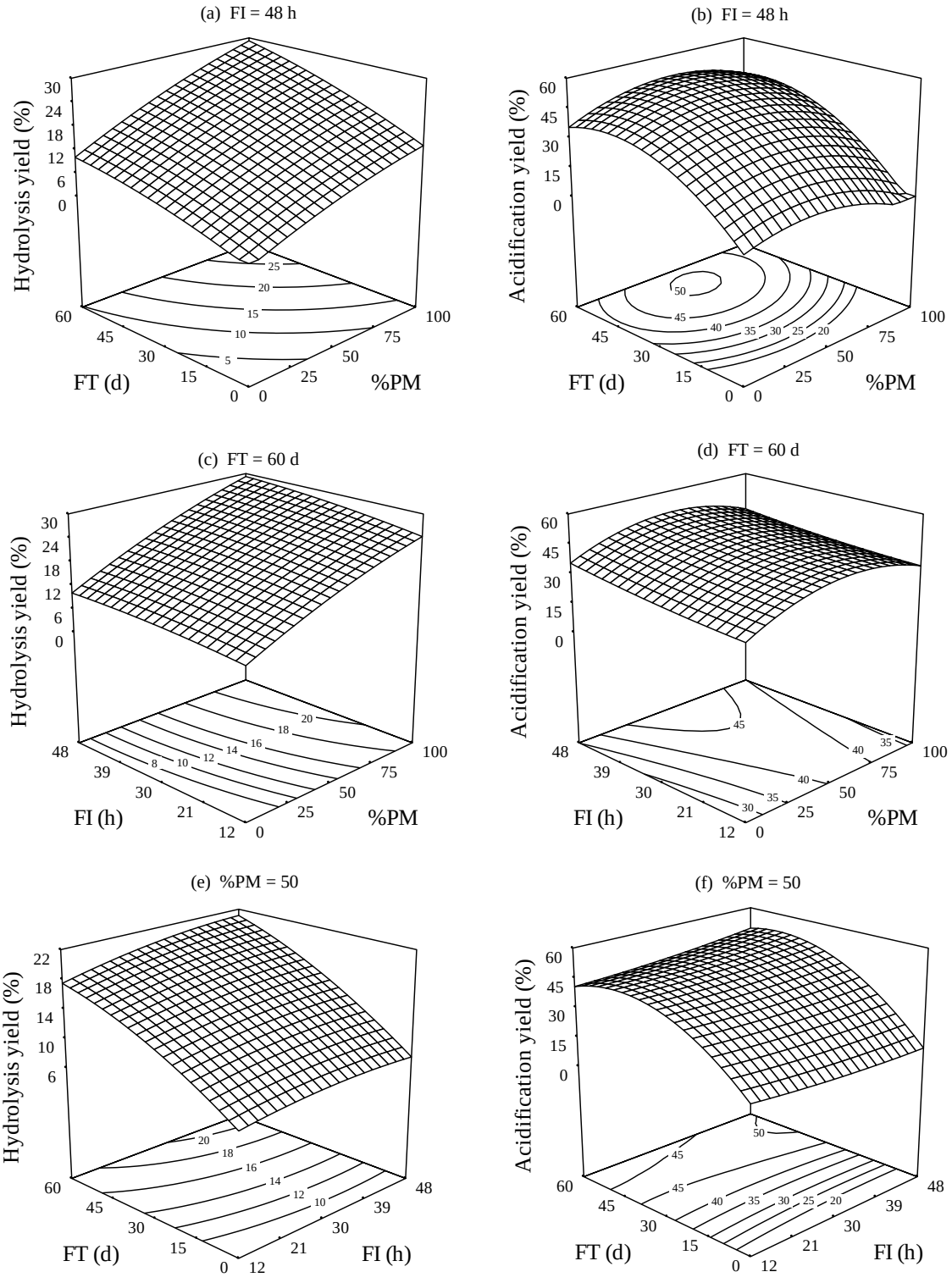


Figure 4-3 Three-dimensional response surface plots of the hydrolysis and acidification yields as a function of (a, b) FT vs. %PM, (c, d) FI vs. %PM and (e, f) FT vs. FI

4.4.3 Fiber degradation

Volatile solids (VS) in organic biomasses is attributed to its ultimate biodegradable fraction. High VS of an organic biomass indicated higher possibility to be biodegraded; hence, it could be used to track the extent of biodegradability as its biodegradable fraction was released or solubilized in the ALBR operation. Volatile solid leaching was brought about by multiple anaerobe enzymatic attack to the cellulose and hemicellulose exposed region on the fiber by multiple anaerobe enzymes and increase solubilization of various substances with the creation of low pH environment by acidogenic bacteria.

Figure 4-3 shows the volatile solids released from the EFB fiber at 60 days of experiment. It was clearly seen that *FI* and *%PM* had positive effect on VS leaching from the EFB. These results were in line with the hydrolysis and acidogenesis data in Figure 4-3 as well as the TVFA production. The lowest 60-day VS release was 5.1 percent at *FI* 12 h, *%PM* 0, although it increased by 6.9 times when *%PM* and *FI* reached 50 and 48 h, respectively. Frequent flushing did not benefit the leachability of the EFB fiber and even disturbed the biological pretreatment. This was in contrast with Browne et al. (2013) where volatile solids in ground food waste in the leach bed reactor was removed better at higher liquid circulation in 25-day period experiment. This probably due to the fact that the easy-to-degrade-and-solubilize part which was the majority in the ground food waste would leach quickly and certainly with subject to higher liquid wash out. In EFB+PM substrate, the easy part leaching out first was coming from the pig manure but over time more liquid application just could not benefit to the tough EFB fiber. The nature of the substrates appeared to influence the degradation characteristics even in same reactor type of ALBR.

Table 4-3 Characteristics of EFB fiber by the fermentative pretreatment in ALBR at different conditions

Parameter	Day 0	0PM:100EFB			25PM:75EFB			50PM:50EFB		
		12h	24h	48h	12h	24h	48h	12h	24h	48h
VS (g/kgEFB dry wt.)	938.2±25.0	882.8±6.2^{b} (-5.1)	823.5±2.9^{a} (-13.2)	789.5±20.0^{a} (-15.9)	819.1±30.2^{b} (-13.0)	780.4±24.9^{ab} (-16.8)	720.4±9.6^{a} (-23.2)	736.0±47.8^{b} (-21.6)	690.0±23.4^{ab} (-26.4)	609.0±24.9^{a} (-35.1)
Cellulose (g/kgEFB dry wt.)	375.9±56.6	373.3±51.0 (-3.9)	369.6±53.9 (-4.8)	N/A (N/A)	404.6±12.3 (+7.6)	459.0±65.4 (+22.1)	435.9±44.6 (+16.0)	450.9±55.0 (+20.0)	515.1±95.0 (+37.0)	511.9±33.3 (+36.2)
Hemicellulose (g/kgEFB dry wt.)	388.2±16.7	370.4±62.5 (-1.5)	366.6±55.1 (-2.5)	435.9±37.4 (-32.2)	365.0±71.6 (-2. 9)	281.7±47.2 (-25.1)	254.7±37.4 (-32.2)	312.4±62.0 (-16. 9)	232.5±11.9 (-38.1)	162.2±20.0 (-56.9)
Lignin (g/kgEFB dry wt.)	235.9±39.8	256.3±11.5 (+8.7)	263.8±48.5 (+11.8)	309.4±11.7 (+31.2)	230.5±59.6 (-2.3)	259.3±17.4 (+9.9)	309.4±11.7 (+31.2)	236.7±12.7 (+0.3)	252.4±24.5 (+7.0)	325.9±18.4 (+38.2)

Note: Value in parenthesis represents percentage change (%) of the respective parameter in relation to day zero. “+” and “-” represent increase and decrease of the component, respectively. And mean in VS row followed by a difference letter are significantly by Duncan’s multiple-range test ($p < 0.05$)

In Table 4-3, the disappearing VS was associated with the decrease in hemicellulose and the rise in cellulose and lignin contents of the EFB fiber up to 36.2% to 38.2%, respectively. The trend of hemicellulose leaching was parallel to VS release which was positively affected by *%PM* and *FI*. As high as 56.9% of hemicellulose was removed from the EFB fiber even though it resided mostly at the inner layer of the fiber (Hsu, 1996). Hydrolysis of hemicellulose into monosaccharides occurred mainly in dilute acid pretreatment (Hendriks and Zeeman, 2009). The mild acids produced in the co-fermentation facilitated this process. Moreover, hemicellulose has a molecular weight lower than cellulose and lignin making it a more hydrolysable polymer (Fengel and Wegener, 1983). When losing hemicellulose, the fiber loosened up and became more venerable to further processing.

The heightened lignin and cellulose contents were the direct result of the shift in distribution of the three components in the fiber. The higher the lignin or cellulose content remained in the EFB, the lesser they were leached. Even there might have been some leaching, their contribution was comparatively much smaller than hemicellulose. This could be reasoned by the characteristic of lignin that is not biodegraded anaerobically since it required free oxygen for fragmentation (Komilis and Ham, 2003; Strauber et al., 2012). It acted as a structural reinforcement of the crystalline complex and protected the inner buddle of hemicellulose-cellulose microfibrils. The accessible surface of the cellulose still close to the enzymatic attack granting the hemicellulose was partly removed hypothetically through the cross sectional area of the fiber cut and on the exposed surface of EFB fiber. Physical treatment such as milling could help expose more surface area but with the additional investment on energy input.

Figure 4-4 shows the SEM surface morphology of the EFB at different treatments compared to the virgin fiber. Outer lignin layer was smooth (Figure 4-4a) before being attacked by the mild acids and enzymes produced through the co-fermentative pretreatment. Inoculum alone (Figure 4-4b) could not cause much damage to the EFB fiber compared to when pig manure was co-fermented (Figure 4-4c and 4-4d). Nonetheless, these were still at a subtle degree. The small holes

appeared over the surface showing the weak spots that some hemicellulose exposed to the external surface. Hydrolytic enzymes might have penetrated into the inner layer of the microfibrils through these holes and as mentioned earlier the cross section from fiber cut. It was quite conclusive that the mild acids produced had worked in favor in fiber degradation but only at a limited capability. Although heat (120-190 ^{o}C) was able to enhance the pretreatment of lignocellulose with the dilute sulfuric acid (Saha et al., 2005), such temperature was prohibitive in this present approach since the organic acids must be formed biologically and the intense heat would kill the acidogens. However, this could certainly help reduce requirement of hemicellulose enzyme mixture for degrading EFB, and the weakening structure should be easier to enter further treatments with higher efficiency.

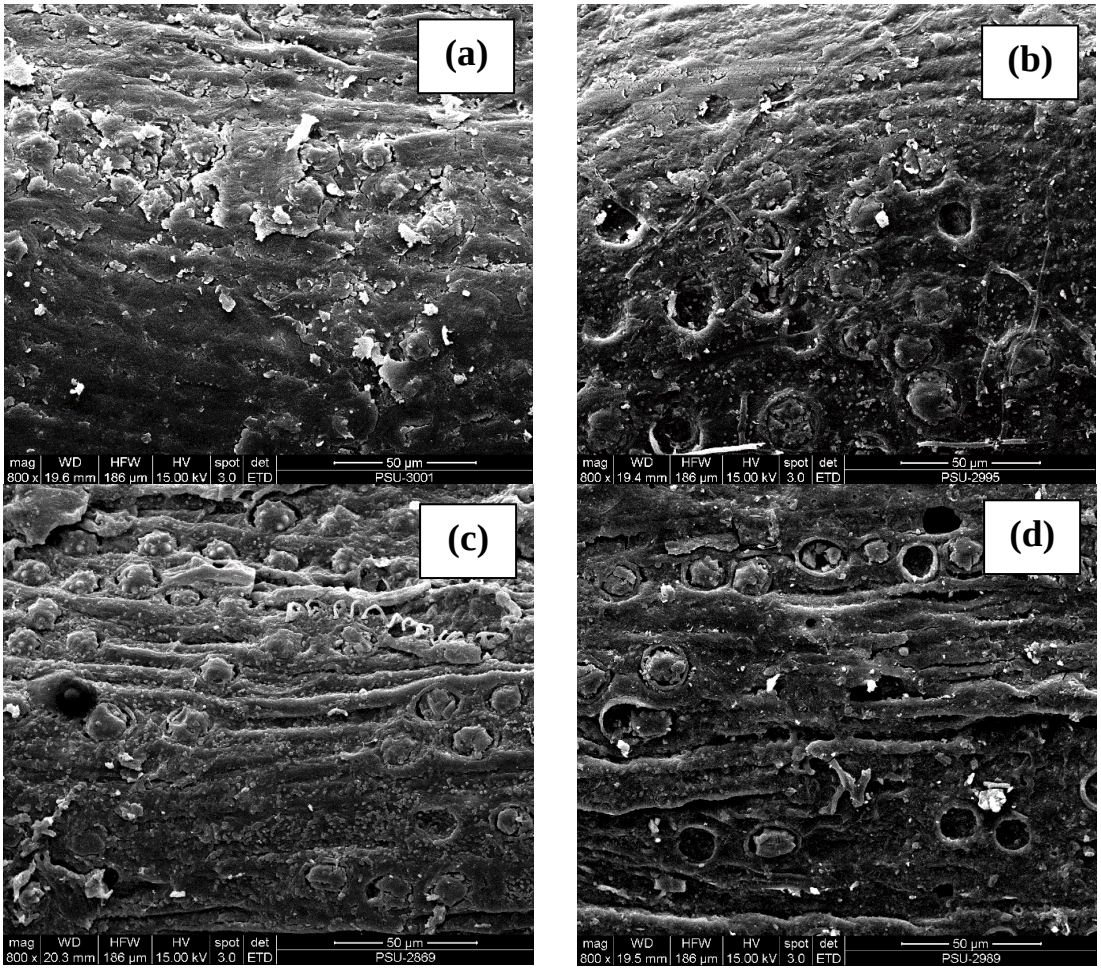


Figure 4-4 Scanning electron microscope images (SEM) of (a) EFB at day 0, (b) 0PM:100EFB, (c) 25PM:75EFB and (d) 50PM:50EFB at day 60 and FI 48 h

4.4.4 Heating value of the EFB digestate

Direct combustion, gasification, and palletization of EFB are among the applications for managing this biomass in large quantity. Table 4-4 demonstrates that thermal characteristic of the solid digestate changed over time based on kg dry substrate added, which subjected to the pretreatment conditions. Heating value in terms of higher heating value (HHV) of the fiber increased with fermentation time (*FT*) and flushing interval (*FI*). EFB itself has a rather low heating value of 7.65 MJ/kg (Table 4-1). After co-fermentation, it was found that HHV of the solid digestate from all treatments were increased from 9.0, 12.9 and 15.9 of 0PM:100EFB, 25PM:75EFB and 50PM:50EFB, respectively, to 17.2-20.2 MJ/kg. It was interesting to note that these data correlated well with the heightened lignin content in the EFB (Table 4-3). Heating content of both cellulose and hemicellulose equal to 17 MJ/kg while lignin's is 25 MJ/kg (Arshadi, 2011). Once the hemicellulose was released from the fermented EFB, its lignin content was enriched so as its HHV. Not only the digestate possessed elevated HHV, the potassium content was greatly reduced from 11.8-13.8 g/kg to 1.4-2.8 g/kg. High potassium content in EFB is detrimental to boiler since it lowered the ash melting point that resulted in the bed agglomeration and formation of slag deposit on the heat transfer surface at high temperatures (Obernberger and Thek, 2004). Thus, the co-fermentative pretreatment of EFB with PM could improve EFB fuel properties both heating value and potassium content. Nevertheless, one must realize that the fiber still must pass through the screw press again to get rid of the left over moisture if it is intended to be used as fuel. Such option is practical; for instance, a biomass power plant in Thailand uses screw press to remove liquid from EFB before sending it to its combustion chamber.

Although %VS reduction of solid digestate occurred as shown in Table 3-4, the degraded portion was lost to the liquid phase in forms of particulate and soluble COD, VFA included. The heating value of the solid digestate increased while the total mass decreased, making the total heating value of the solid digestate reduced. However, the total energy derived both in the forms of solid digestate and COD should be rather stable when they are converted to energy through burning and anaerobic fermentation, respectively.

Table 4-4 Thermal and agricultural characteristics of total solid digestate from the fermentative pretreatment in ALBR

% PM	*FI* (h)	Heating value, HHV (MJ/kg)					Nitrogen (g/kg dry wt.)		Phosphorus (g/kg dry wt.)		Potassium (g/kg dry wt.)	
		0 d	15 d	30 d	45 d	60 d	0 d	60 d	0 d	60 d	0 d	60 d
0PM:100EFB	12	9.0	13.0	14.6	15.5	17.5	10.6	7.6	1.7	1.0	13.8	2.0
	24		13.6	14.9	15.7	18.6		7.1		0.8		1.9
	48		14.4	16.4	18.7	20.2		6.8		0.7		1.4
25PM:75EFB	12	12.9	15.0	15.6	17.2	18.5	15.0	9.7	6.3	2.3	12.8	1.9
	24		15.6	15.9	17.0	18.9		10.8		2.1		2.2
	48		16.4	17.8	18.6	19.5		10.6		2.0		2.0
50PM:50EFB	12	15.9	16.4	15.6	16.5	17.2	19.4	11.8	10.7	3.2	11.8	2.8
	24		16.7	15.9	16.8	18.5		12.1		2.9		2.4
	48		16.0	16.4	17.5	19.5		11.5		2.1		2.2

4.4.5 Plant nutrients in EFB digestate

Another possible use of the solid digestate is as compost for agricultural purposes. Nitrogen (N), phosphorus (P) and potassium (K) contents in the digestate were shown in Table 4-4. Addition of pig manure (N 31 g/kg, P 9 g/kg) had improved nutritional values to EFB (N 23 g/kg, P 0.8 g/kg) although the whole substrate pile had leached N and P to the leachate during 60 days of experiment at an average of 35.0±5.3% and 70.3±5.9% in 25PM:75EFB and 50PM:50EFB treatments, respectively. This leached nutrients were mainly from the pig manure. As mentioned in section 3.4, a significant loss of K in all treatments at 83.5±4.0% on average to the level below that of the virgin EFB (17 g/kg). It was noted that K in PM was 12 g/kg dry wt. basis. The virgin EFB fiber had comparatively higher content of potassium which was an attributional characteristic to the palm biomass since the plant requires a lot of potassium for kernel and bunch as well as the trunk biomasses synthesis (Corley and Tinker, 2003). At lower pH, the mild acids produced effectively helped solubilize this element in the loosened fiber. Overall, the data showed no significant impact of *FI* on the nutrient contents in the digestate. Although data of nutrient content over time was not measured, it is anticipated that it would change upon the length of fermentative time. The digestate could be put to the field as to increase humus material in soil. Nevertheless, these plant nutrients were trapped in the leachate that could still be used for agricultural purposes preferably after going through anaerobic digestion for biogas production. Thus, if the purpose of the pretreatment of EFB biomass was for fuel alone, pig manure addition would not be necessary because HHV and potassium could be taken care of even in 0PM:100EFB treatment.

4.5 Conclusion

Addition of PM as co-substrate in the fermentation of EFB induced higher mild acids production in the anaerobic leach bed reactor. The quadratic regression models of hydrolysis yield (η_h) and acidification yield (η_a) developed were able to describe the effect of the studied variables of flushing interval (*FI*), PM mixing ratio (*%PM*) and fermentation time (*FT*) as well as their interaction. All 3 variables showed positive influence to η_h while optimal points were found for η_a. There was a significant correlation between η_h and η_a, signifying the synchronistic effect of the two reactions during the semi-dry fermentative EFB pretreatment. Longer *FI* caused higher EFB fiber degradation in terms of VS destruction due to the more intense acid with the extended period of contact to the biomass surface. The fuel properties of the EFB digestate were improved by having higher heating value and lower potassium content, while its fertilizer properties were better by the addition of pig manure. Co-fermentation of EFB with PM not only could produce VFA for use in energy production or biorefinery, but also could enhance the quality of EFB digestate for various uses.

REFERENCE

AOAC. 1995. Official Methods of Analysis of AOAC International. in: method 973.18, AOAC International. Virginia.

APHA, AWWA, WEFF. 2005. Standard methods for the examination of water and wasterater. 21 ed. American Public Health Association, Washington, DC.

APHA, AWWA, WEFF. 1998. Standard methods for the examination of water and wasterater. 20 ed, Springer. Berlin.

Arshadi, M. 2011. Handbook of Biofuels Production: Processes and Technologies. Woodhead Publishing Limited, United Kongdom, 206.

ASTM. 2009. ASTM D240-09. in: Standard Test Method for Heat of Combustion of Liquid Hydrocarbon Fuels by Bomb Calorimeter, ASTM International. West Conshohocken PA USA.

Babel, S., Fukushi, K., Sitanrassamee, B. 2004. Effect of acid speciation on solid waste liquefaction in an anaerobic acid digester. Water Research, 38(9), 2417-2423.

Browne, J.D., Allen, E., Murphy, J.D. 2013. Improving hydrolysis of food waste in a leach bed reactor. Waste Management, 33(11), 2470-2477.

Cho, J.K., Park, S.C., Chang, H.N. 1995. Biochemical Methane Potential and Solid State Anaerobic Digestion of Korean food wastes. Bioresource Technology, 52(3), 245–253.

Corley, R.H.V., Tinker, P.B. 2003. The Oil Palm. Fourth Edition ed. Blackwell Science Ltd., Oxford, 316, 303.

Dechrugsa, S., Kantachote, D., Chaiprapat, S. 2013. Effects of inoculum to substrate ratio, substrate mix ratio and inoculum source on batch co-digestion of grass and pig manure. Bioresource Technology, 146(0), 101-108.

Demirel, B., Yenigun, O. 2004. Anaerobic acidogenesis of dairy wastewater: the effects of variations in hydraulic retention time with no pH control. Journal of Chemical Technology and Biotechnology, 79, 755-760.

Deublein, D., Steinhauser, A. 2008. Biogas from waste and renewable resources : An introduction. WILEY-VCH Verlag GmbH & Co. KGaA,Weinheim, Germany.

Dogan, E., Dunaev, T., Erguder, T.H., Demirer, G.N. 2008. Performance of leaching bed reactor converting the organic fraction of municipal solid waste to organic acids and alcohols. Chemosphere 74, 797-803.

Fengel, D., Wegener, G. 1983. Wood: Chemistry, Ultrastructure, Reactions. Walter De Gruyter&Co., Berlin, 632.

Gerardi, M.H. 2006. Wastewater Bacteria. John Wiley & Sons, Inc., New Jersey, 267.

Hendriks, A.T.W.M., Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technology, 100(1), 10-18.

Hsu, T.-A. 1996. prettreatment of biomass. in: Handbook on Bioethanol: Production and Utilization, (Ed.) C. Wyman, Taylor&Francis. Washington, 179-212.

Isroi, Millati, R., Syamsiah, S., Niklasson, C., Cahyanto, M.N., Lundquist, K., Taherzadeh, M.J. 2011. Biological pretreatment of lignocelluloses with white-rot fungi and its applications: A review. Bioresources Technology, 6(4), 5224-5259.

Jamari, S.S., Howse, J.R. 2012. The effect of the hydrothermal carbonization process on palm oil empty fruit bunch. Biomass and Bioenergy, 47, 82-90.

Kerdsuwan, S., Laohalidanond, K. 2011. Renewable energy from palm oil empty fruit bunch in: Trends and applications, (Ed.) M. Nayeripour, InTech. Shanghai, Chaina, 123-150.

Kim, S., Park, J.M., Seo, J.-W., Kim, C.H. 2012. Sequential acid-/alkali-pretreatment of empty palm fruit bunch fiber. Bioresource Technology, 109, 229-233.

Komilis, D.P., Ham, R.K. 2003. The effect of lignin and sugars to the aerobic decomposition of solid wastes. Waste Management, 23(5), 419-423.

Kumar, P., Barrett, D.M., Delwiche, M.J., Stroeve, P. 2009. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. Journal of Industrial & Engineering Chemistry Research, 48(8), 3713–3729.

Mohammed, M.A.A., Salmiaton, A., Wan Azlina, W.A.K.G., Mohamad Amran, M.S. 2012. Gasification of oil palm empty fruit bunches: A characterization and kinetic study. Bioresource Technology, 110, 628-636.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M., Ladisch, M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology, 96, 673-686.

Mtz.-Viturtia, A., Mata-Alvarez, J., Cecchib, F. 1995. Two-Phase Continuous Anaerobic Digestion of Fruit and Vegetable Wastes. Resources, Conservation and Recycling, 13 (3-4), 257-267.

Myint, M.T., Nirmalakandan, N. 2009. Enhanceing anaerobic hydrolysis of cattle manure in leach bed reactors. Bioresource Technology, 100, 1695-1699.

Obernberger, I., Thek, G. 2004. Physical characterisation and chemical composition of densified biomass fuels with regard to their combustion behaviour. Biomass and Bioenergy, 27(6), 653-669.

OPEC. 2013. World oil outlook, Organization of the Petroleum Exporting Countries. Avaliable in: http://www.opec.org/opec_web/static_files_project/media/downloads/publications/WOO_2013.pdf.

Piñeros-Castro, Y., Velásquez-Lozano, M. 2014. Biodegradation kinetics of oil palm empty fruit bunches by white rot fungi. International Biodeterioration & Biodegradation, 91, 24-28.

Prasertsan, S., Prasertsan, P. 1996. Biomss Residual from palm oil mill in Thailand : An Overview on Quantity and Pothential Usage. Biomass and Bioenergy, 11(5), 387-395.

Saha, B.C., Iten, L.B., Cotta, M.A., Wu, Y.V. 2005. Dilute Acid Pretreatment, Enzymatic Saccharification and Fermentation of Rice Hulls to Ethanol. Biotechnology Progress, 21(3), 816-822.

Saritpongteeraka, K., Boonsawang, P., Sung, S., Chaiprapat, S. 2014. Co-fermentation of oil palm lignocellulosic residue with pig manure in anaerobic leach bed reactor for fatty acid production. Energy Conversion and Management, 84, 354-362.

Sawyer, C.N., McCarty, P.L., Parkin, G.F. 2003. Chemistry for Environmental Engineering. McGraw-Hill, Inc., New York.

Sinjermsiri, D., Hanbunchong, A., Thanomwongwattana, R. 2006. Quality improvement of oil palm press fibre by the chemical and biological. Animal Science Conference, Kasartsart University'14, Bangkok, Thailand. 307-315.

Stabnikova, O., Liu, X.-Y., Wang, J.-Y. 2008. Anaerobic digestion of food waste in a hybrid anaerobic solid–liquid system with leachate recirculation in an acidogenic reactor. Biochemical Engineering Journal, 41(2), 198-201.

Strauber, H., Schroder, M., Kleinsteuber, S. 2012. Metabolic and microbial community dynamics during the hydrolytic and acidogenic fermentation in a leach-bed process. Energy, Sustainability and Society, 2(1), 13.

USDA. 2014. Agricultural Marketing Service United States Department of Agriculture. Avaliable in: www.ams.usda.gov, 15 September 2014.

Vavilin, V.A., Vasiliev, V.B., Ponomarev, A.V., Rytow, S.V. 1994. Simulation model 'methane' as a tool for effective biogas production during anaerobic conversion of complex organic matter. Bioresource Technology, 48(1), 1-8.

Wu, M.-C., Sun, K.-W., Zhang, Y. 2006. Influence of temperature fluctuation on thermophilic anaerobic digestion of municipal organic solid waste. Journal Zhejiang Univiversity Scince B, 7(3), 180-185.

Xie, S., Lawlor, P.G., Frost, J.P., Wu, G., Zhan, X. 2012. Hydrolysis and acidification of grass silage in leaching bed reactors. Bioresource Technology, 114, 406-413.

CHAPTER V

CONCLUSION

The substrate of PM and EFB were co-fermented in order to convert EFB lignocellulosic biomass to fatty acids and to improve EFB fuel property using anaerobic leach bed reactor (ALBR). The study was carried out by full factorial methodology with three important operating parameters; pig manure mixing ratio (%PM), flushing interval (FI) and fermentation time (FT). The result showed that addition of PM could help stimulate the degree of hydrolysis (η_h) and acidification (η_a) as well as promoted TVFA production in the co-fermentation of the mixed substrate. Higher PM mixing ratio and longer fermentation time has statistically significant impacts on η_h and η_a. It was also found that co-digestion with PM could give η_a better than mono EFB digestion (0PM:100EFB). There was a significant correlation between η_h and η_a, as there was a synchronistic effect of the two reactions in the fermentative digester. Higher EFB fiber degradation (VS destruction) was enhanced by longer *FI* because of a more intense acid with the prolong period of contact to the biomass. EFB fuel properties were improved as the digestate had higher heating value and lower potassium content. The fertilizer properties were also improved with the addition of pig manure. Co-fermentation of EFB with PM not only could produce VFA for use in energy production or biorefinery, but also could enhance the quality of EFB digestate for various uses.

Response surfaces and contour plots constructed from the quadratic regression model were used to describe the relationship of three variables satisfactorily. The results showed the interaction effect of %PM and FT on the TVFA production. Although FI did not show obvious impact on TVFA production, it influenced the speciation of VFAs produced from high %PM and long FI. The quadratic regression model developed was able to describe the relationship of the studied parameters on the overall TVFA production well. Multiple regression models revealed the interaction of the parameters (%PM,FI and FT) on TVFA production, ηh and ηa. Addition of PM could help to enhance the ηh and ηa promote ($P<0.05$) in the

co-fermentation of the mixed substrate, which the mild acids produced could then help loosen the lignocellulosic structure and increase enzyme accessibility. Hence, the mixing of a more easily biodegradable co-substrate could help facilitate the early stage of fermentation comprising hydrolysis and acidogenesis.

Addition of PM as co-substrate in the fermentation of EFB induced higher mild acids production in the anaerobic leach bed reactor. The quadratic regression models of hydrolysis yield (η_h) and acidification yield (η_a) developed were able to describe the effect of the studied variables of flushing interval (*FI*), PM mixing ratio (*%PM*) and fermentation time (*FT*) as well as their interaction. All 3 variables showed positive influence to η_h while optimal points were found for η_a. There was a significant correlation between η_h and η_a, signifying the synchronistic effect of the two reactions during the semi-dry fermentative EFB pretreatment. Longer *FI* caused higher EFB fiber degradation in terms of VS destruction due to the more intense acid with the extended period of contact to the biomass surface. The fuel properties of the EFB digestate were improved by having higher heating value and lower potassium content, while its fertilizer properties were better by the addition of pig manure. Co-fermentation of EFB with PM not only could produce VFA for use in energy production or biorefinery, but also could enhance the quality of EFB digestate for various uses.

The leachate contains VFA as soluble organics which can be used for biochemical products or methane production. Conversion of the biomass will be improved if the materials are pretreated prior to feeding the energy conversion apparatus. While this method can improve quality of digestate, increased nitrogen content it good for plant nutrient and reduced potassium content, decrease quantity of slag is problem in combustion system. Furthermore, it will be helpful in solving the EFB disposal via recycling plant nutrient and great optimization of benefit from EFB are environmentally valuable and give more sustainable EFB waste management.

Co-fermentation of EFB with PM using ALBR under anaerobic dry process for VFA production as intermediate substance for methane production and residual from digestion process for organic fertilizer and combustion. This method

can help reduce environmental problems, as it means to economically reuse an otherwise wasted resource to a source of additional revenue. In addition, ALBR has a lower land requirement, low energy requirement and low water operation and this operation can apply to reduction in degree of polymerization of lignocellulosic EFB fiber by hydrolysis and acidogenesis bacteria. Our method purposed can pretreat the EFB at low operation cost. The fresh substrate can then be utilized at its full potential. Ultimately, it can help stabilize the price of oil palm in the country and increase profit for the palm oil industry.

Future research needs

There are many ideas and some specific suggestion arising from the current studies as shown below.

1. Effect of temperature, size of substrate, percolate, and recirculation time should be studied to increase the TVFA production, η_h and η_a

2. Biogas production should be studied for a combination between ALBR for acid production followed by the biogas production by anaerobic digester.

3. Digestate of the experiment at the end of batch should be studied for humus property, biofertilizer and microbial community identification.

4. Dewatering of solid digestate for fuel preparation.

5. Economic analysis for solid digestate fuel preparation in combination with biogas production.

6. Other waste from oil palm industry such as palm fiber, palm trunk, and palm press fiber should be investigated.

APPENDIX

A. Data for chapter III

Figure 3-1 SCOD productivity (gSCOD/$kg_{substrate\ dry}$) of pig manure (PM) and palm empty fruit bunch (EFB) co-fermentation at different mixing ratios in the ALBR and flooding interval (FI)

Time	0PM:100EFB			25PM:75EFB			50PM:50EFB			100PM:0EFB		
(day)	FI 12 h	FI 24 h	FI 48 h	FI 12 h	FI 24 h	AI 48 h	FI 12 h	FI 24 h	FI 48 h	FI 12 h	FI 24 h	FI 48 h
1	27.93	9.86	28.34	62.70	85.99	113.10	123.82	136.75	96.10	177.45	214.99	228.64
5	40.25	19.71	34.50	64.92	103.24	118.03	133.06	#N/A	110.88	187.69	235.46	259.35
9	44.84	34.99	42.38	100.78	135.03	142.17	141.19	192.93	128.25	204.75	255.94	290.06
13	42.93	37.33	56.00	108.36	#N/A	#N/A	#N/A	189.00	138.60	235.46	327.60	286.65
15	50.40	33.60	56.93	135.24	132.72	#N/A	154.40	189.00	163.80	249.11	296.89	303.71
19	60.48	59.00	60.48	117.60	119.19	145.60	185.92	216.72	202.72	197.93	307.13	334.43
23	56.00	50.40	65.54	127.68	119.28	169.68	192.76	205.35	207.20	269.59	341.25	331.01
27	59.73	70.93	66.71	147.84	127.68	186.48	224.36	247.56	224.00	283.24	371.96	331.01
30	70.93	84.23	72.80	164.64	136.08	203.28	227.22	250.53	229.60	293.48	358.31	348.08
37	78.40	100.80	89.60	174.72	169.68	211.68	246.38	249.77	274.40	313.95	378.79	385.61
41	85.87	106.40	100.80	187.04	186.48	223.44	251.86	264.73	285.60	320.78	351.93	399.26
43	89.60	115.73	104.45	201.60	194.88	233.52	263.34	295.58	308.00	320.78	358.31	409.50
45	93.33	119.47	123.59	213.92	203.28	236.88	273.09	#N/A	313.60	324.19	402.68	429.98
49	104.53	123.20	118.99	218.40	211.68	245.28	279.25	301.72	352.80	341.25	406.09	433.39
51	108.27	140.00	124.65	219.52	220.08	248.64	294.00	294.02	352.80	368.55	443.63	447.04
57	119.47	156.80	151.20	222.88	240.71	253.68	#N/A	296.84	361.20	379.93	423.15	450.45
60	134.40	162.40	166.99	230.58	255.96	262.08	318.08	323.48	378.00	389.03	450.45	453.86

Figure 3-2 Hydrolysis yield from co-fermentation of pig manure (PM) and palm empty fruit bunch (EFB) at different mixing ratios in the ALBR operated at flooding interval (FI) (a) 12 h, (b) 24 h, and (c) 48 h

Time	0PM:100EFB			25PM:75EFB			50PM:50EFB			100PM:0EFB		
(day)	FI 12 h	FI 24 h	FI 48 h	FI 12 h	FI 24 h	AI 48 h	FI 12 h	FI 24 h	FI 48 h	FI 12 h	FI 24 h	FI 48 h
1	1.75	1.49	1.77	3.73	5.20	6.99	7.60	8.39	5.90	10.89	13.20	14.04
5	2.52	1.78	2.16	3.87	6.65	7.29	8.17	#N/A	6.81	10.47	15.71	17.39
9	2.80	2.19	2.65	6.23	6.15	8.79	8.67	11.84	7.87	13.83	18.64	18.02
13	2.68	3.72	3.50	6.70	7.92	9.76	8.64	11.60	8.51	14.45	20.11	17.60
15	3.15	2.28	3.56	8.00	7.37	9.00	10.31	12.52	10.06	15.29	18.23	18.64
19	3.78	3.83	3.78	8.36	8.59	11.19	11.41	14.85	12.44	#N/A	18.85	20.53
23	3.50	3.73	4.10	7.89	7.31	10.93	11.83	14.15	12.72	16.55	20.95	20.32
27	3.73	4.43	4.17	9.14	8.01	11.64	13.77	15.20	13.75	17.39	22.83	20.32
30	4.43	5.26	4.65	10.18	8.49	12.92	13.95	15.38	14.09	18.02	22.00	21.37
37	4.90	6.30	5.60	10.80	10.94	13.36	15.12	15.32	16.84	19.27	21.79	23.67
41	5.37	6.65	6.07	11.56	11.28	14.09	15.46	16.25	17.53	19.69	21.60	24.51
43	5.60	7.23	6.53	12.46	12.35	14.57	16.17	18.14	18.91	19.69	22.00	25.14
45	4.90	7.47	7.25	13.22	12.69	15.06	16.76	17.02	19.25	19.90	24.72	26.40
49	6.53	7.70	7.44	13.50	13.32	15.06	17.14	18.52	21.66	20.95	24.93	26.60
51	6.77	8.75	7.09	13.57	13.97	15.61	18.05	18.05	21.66	21.58	27.23	27.44
57	7.47	8.05	11.53	14.64	14.28	17.07	16.50	18.22	22.17	23.04	25.98	27.65
60	8.40	8.75	10.44	14.47	15.25	16.90	19.53	19.86	23.20	22.62	27.65	27.86

Table 3-1 Statistic of Acidification yield (a) and TVFA production (b) of pig manure (PM) and palm empty fruit bunch (EFB) co-fermentation at different mixing ratios in the ALBR at the end of 60 days

a. Statistic of Acidification yield

Oneway ANOVA

ACIDYIEL

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11070.763	8	1383.845	70.934	.000
Within Groups	351.159	18	19.509		
Total	11421.922	26			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: ACID YIEL
Scheffe

(I) FI	(J) FI	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
100efb12	75efb12	-45.6100(*)	3.60637	.000	-61.7709	-29.4491
	50efb12	-45.3467(*)	3.60637	.000	-61.5076	-29.1858
	100efb24	-13.6067	3.60637	.148	-29.7676	2.5542
	75efb24	-56.2833(*)	3.60637	.000	-72.4442	-40.1224
	50efb24	-55.1867(*)	3.60637	.000	-71.3476	-39.0258
	100efb48	-14.9033	3.60637	.087	-31.0642	1.2576
	75efb48	-58.1000(*)	3.60637	.000	-74.2609	-41.9391
	50efb48	-36.5967(*)	3.60637	.000	-52.7576	-20.4358
	0efb12	-12.0433(*)	3.60637	.040	-23.7306	-.3561
	0efb24	-28.9867(*)	3.60637	.000	-40.6739	-17.2994
	0efb48	-24.4567(*)	3.60637	.000	-36.1439	-12.7694
75efb12	100efb12	45.6100(*)	3.60637	.000	29.4491	61.7709
	50efb12	.2633	3.60637	1.000	-15.8976	16.4242
	100efb24	32.0033(*)	3.60637	.000	15.8424	48.1642
	75efb24	-10.6733	3.60637	.410	-26.8342	5.4876
	50efb24	-9.5767	3.60637	.550	-25.7376	6.5842
	100efb48	30.7067(*)	3.60637	.000	14.5458	46.8676
	75efb48	-12.4900	3.60637	.226	-28.6509	3.6709
	50efb48	9.0133	3.60637	.625	-7.1476	25.1742
	0efb12	-33.6033(*)	3.60637	.000	-45.2906	-21.9161
	0efb24	-25.3833(*)	3.60637	.000	-37.0706	-13.6961
	0efb48	-10.7300	3.60637	.089	-22.4173	.9573

(Cont.)

50efb12	100efb12	45.3467(*)	3.60637	.000	29.1858	61.5076
	75efb12	-.2633	3.60637	1.000	-16.4242	15.8976
	100efb24	31.7400(*)	3.60637	.000	15.5791	47.9009
	75efb24	-10.9367	3.60637	.379	-27.0976	5.2242
	50efb24	-9.8400	3.60637	.515	-26.0009	6.3209
	100efb48	30.4433(*)	3.60637	.000	14.2824	46.6042
	75efb48	-12.7533	3.60637	.205	-28.9142	3.4076
	50efb48	8.7500	3.60637	.660	-7.4109	24.9109
	0efb12	-35.7933(*)	3.60637	.000	-47.4806	-24.1061
	0efb24	-27.7800(*)	3.60637	.000	-39.4673	-16.0927
	0efb48	-12.0433(*)	3.60637	.040	-23.7306	-.3561
100efb24	100efb12	13.6067	3.60637	.148	-2.5542	29.7676
	75efb12	-32.0033(*)	3.60637	.000	-48.1642	-15.8424
	50efb12	-31.7400(*)	3.60637	.000	-47.9009	-15.5791
	75efb24	-42.6767(*)	3.60637	.000	-58.8376	-26.5158
	50efb24	-41.5800(*)	3.60637	.000	-57.7409	-25.4191
	100efb48	-1.2967	3.60637	1.000	-17.4576	14.8642
	75efb48	-44.4933(*)	3.60637	.000	-60.6542	-28.3324
	50efb48	-22.9900(*)	3.60637	.002	-39.1509	-6.8291
	0efb12	33.6033(*)	2.60806	.000	21.9161	45.2906
	0efb24	8.2200	2.60806	.332	-3.4673	19.9073
	0efb48	22.8733(*)	2.60806	.000	11.1861	34.5606
75efb24	100efb12	56.2833(*)	3.60637	.000	40.1224	72.4442
	75efb12	10.6733	3.60637	.410	-5.4876	26.8342
	50efb12	10.9367	3.60637	.379	-5.2242	27.0976
	100efb24	42.6767(*)	3.60637	.000	26.5158	58.8376
	50efb24	1.0967	3.60637	1.000	-15.0642	17.2576
	100efb48	41.3800(*)	3.60637	.000	25.2191	57.5409
	75efb48	-1.8167	3.60637	1.000	-17.9776	14.3442
	50efb48	19.6867(*)	3.60637	.010	3.5258	35.8476
	0efb12	-2.1900	3.60637	.999	-13.8773	9.4973
	0efb24	5.8233	3.60637	.748	-5.8639	17.5106
	0efb48	21.5600(*)	3.60637	.000	9.8727	33.2473
50efb24	100efb12	55.1867(*)	3.60637	.000	39.0258	71.3476
	75efb12	9.5767	3.60637	.550	-6.5842	25.7376
	50efb12	9.8400	3.60637	.515	-6.3209	26.0009
	100efb24	41.5800(*)	3.60637	.000	25.4191	57.7409
	75efb24	-1.0967	3.60637	1.000	-17.2576	15.0642
	100efb48	40.2833(*)	3.60637	.000	24.1224	56.4442
	75efb48	-2.9133	3.60637	.999	-19.0742	13.2476
	50efb48	18.5900(*)	3.60637	.016	2.4291	34.7509
	0efb12	4.6167	3.60637	.911	-7.0706	16.3039
	0efb24	9.1467	3.60637	.213	-2.5406	20.8339
	0efb48	25.3833(*)	3.60637	.000	13.6961	37.0706

(Cont.)

100efb48	100efb12	14.9033	3.60637	.087	-1.2576	31.0642
	75efb12	-30.7067(*)	3.60637	.000	-46.8676	-14.5458
	50efb12	-30.4433(*)	3.60637	.000	-46.6042	-14.2824
	100efb24	1.2967	3.60637	1.000	-14.8642	17.4576
	75efb24	-41.3800(*)	3.60637	.000	-57.5409	-25.2191
	50efb24	-40.2833(*)	3.60637	.000	-56.4442	-24.1224
	75efb48	-43.1967(*)	3.60637	.000	-59.3576	-27.0358
	50efb48	-21.6933(*)	3.60637	.004	-37.8542	-5.5324
	0efb12	-8.2200	3.60637	.332	-19.9073	3.4673
	0efb24	14.6533(*)	3.60637	.007	2.9661	26.3406
	0efb48	-10.4100	3.60637	.107	-22.0973	1.2773
75efb48	100efb12	58.1000(*)	3.60637	.000	41.9391	74.2609
	75efb12	12.4900	3.60637	.226	-3.6709	28.6509
	50efb12	12.7533	3.60637	.205	-3.4076	28.9142
	100efb24	44.4933(*)	3.60637	.000	28.3324	60.6542
	75efb24	1.8167	3.60637	1.000	-14.3442	17.9776
	50efb24	2.9133	3.60637	.999	-13.2476	19.0742
	100efb48	43.1967(*)	3.60637	.000	27.0358	59.3576
	50efb48	21.5033(*)	3.60637	.004	5.3424	37.6642
	0efb12	-10.4100	3.60637	.107	-22.0973	1.2773
	0efb24	-2.3967	3.60637	.999	-14.0839	9.2906
	0efb48	13.3400(*)	3.60637	.018	1.6527	25.0273
50efb48	100efb12	36.5967(*)	3.60637	.000	20.4358	3.60637
	75efb12	-9.0133	3.60637	.625	-25.1742	3.60637
	50efb12	-8.7500	3.60637	.660	-24.9109	7.4109
	100efb24	22.9900(*)	3.60637	.002	6.8291	39.1509
	75efb24	-19.6867(*)	3.60637	.010	-35.8476	-3.5258
	50efb24	-18.5900(*)	3.60637	.016	-34.7509	-2.4291
	100efb48	21.6933(*)	3.60637	.004	5.5324	37.8542
	75efb48	-21.5033(*)	3.60637	.004	-37.6642	-5.3424
	0efb12	4.6167	2.60806	.911	-7.0706	16.3039
	0efb24	9.1467	2.60806	.213	-2.5406	20.8339
	0efb48	25.3833(*)	2.60806	.000	13.6961	37.0706
0efb12	100efb12	10.7300	2.60806	.089	-.9573	22.4173
	75efb12	-22.8733(*)	2.60806	.000	-34.5606	-11.1861
	50efb12	-14.6533(*)	2.60806	.007	-26.3406	-2.9661
	100efb24	-25.0633(*)	2.60806	.000	-36.7506	-13.3761
	75efb24	-17.0500(*)	2.60806	.002	-28.7373	-5.3627
	50efb24	-1.3133	2.60806	1.000	-13.0006	10.3739
	100efb48	-18.2567(*)	2.60806	.001	-29.9439	-6.5694
	75efb48	-13.7267(*)	2.60806	.014	-25.4139	-2.0394
	0efb12	35.7933(*)	2.60806	.000	24.1061	47.4806
	0efb24	2.1900	2.60806	.999	-9.4973	13.8773
	0efb48	10.4100	2.60806	.107	-1.2773	22.0973

(Cont.)

0efb24	100efb12	12.0433(*)	2.60806	.040	.3561	23.7306
	75efb12	-21.5600(*)	2.60806	.000	-33.2473	-9.8727
	50efb12	-13.3400(*)	2.60806	.018	-25.0273	-1.6527
	100efb24	1.3133	2.60806	1.000	-10.3739	13.0006
	75efb24	-23.7500(*)	2.60806	.000	-35.4373	-12.0627
	50efb24	-15.7367(*)	2.60806	.004	-27.4239	-4.0494
	100efb48	-16.9433(*)	2.60806	.002	-28.6306	-5.2561
	75efb48	-12.4133(*)	2.60806	.032	-24.1006	-.7261
	0efb12	28.9867(*)	2.60806	.000	17.2994	40.6739
	0efb24	-4.6167	2.60806	.911	-16.3039	7.0706
	0efb48	3.6033	2.60806	.978	-8.0839	15.2906
0efb48	100efb12	1.2067	2.60806	1.000	-10.4806	12.8939
	75efb12	16.9433(*)	2.60806	.002	5.2561	28.6306
	50efb12	4.5300	2.60806	.919	-7.1573	16.2173
	100efb24	24.4567(*)	2.60806	.000	12.7694	36.1439
	75efb24	-9.1467	2.60806	.213	-20.8339	2.5406
	50efb24	-.9267	2.60806	1.000	-12.6139	10.7606
	100efb48	13.7267(*)	2.60806	.014	2.0394	25.4139
	75efb48	-11.3367	2.60806	.062	-23.0239	.3506
	0efb12	-3.3233	2.60806	.987	-15.0106	8.3639
	0efb24	12.4133(*)	2.60806	.032	.7261	24.1006
	0efb48	-4.5300	2.60806	.919	-16.2173	7.1573

* The mean difference is significant at the .05 level.

Homogeneous Subsets

ACID YIEL

Scheffe

FI	N	Subset for alpha = .05		
		1 (a)	2 (b)	3 (c)
100efb12	3	15.0733		
100efb24	3	28.6800		
100efb48	3	29.9767		
75efb12	3		51.6700	51.6700
75efb24	3		52.1246	52.1246
75efb48	3		54.2218	54.2218
50efb12	3			70.2600
50efb24	3			71.3567
50efb48	3			73.1733
0efb12	3		60.4200	60.4200
0efb24	3		60.4200	60.4200
0efb48	3		60.6833	60.6833
Sig.		.087	.625	.205

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

b. Statistic of TVFA production yield

Oneway ANOVA

Descriptives

Treatments		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
VFA12h	100EFB	3	618.00	25.87	14.93	553.75	682.25	590.00	641.00
	75EFB	3	1004.00	30.27	17.47	928.82	1079.18	980.00	1038.00
	50EFB	3	2523.67	147.28	85.03	2157.81	2889.52	2365.00	2656.00
	0EFB	3	3746.33	170.69	98.55	3322.31	4170.36	3552.00	3872.00
	Total	12	7892.00	374.10	215.99	6962.68	8821.32	7487.00	8207.00
VFA24h	100EFB	3	1263.33	121.58	70.20	961.31	1565.36	1175.00	1402.00
	75EFB	3	1116.67	75.16	43.39	929.95	1303.38	1030.00	1164.00
	50EFB	3	2731.67	247.21	142.72	2117.58	3345.76	2452.00	2921.00
	0EFB	3	3957.67	155.99	90.06	3570.15	4345.18	3782.00	4080.00
	Total	12	9069.33	599.94	346.38	7578.99	10559.68	8439.00	9567.00
VFA48h	100EFB	3	1205.00	46.86	27.06	1088.59	1321.41	1175.00	1259.00
	75EFB	3	1117.00	69.20	39.95	945.09	1288.91	1060.00	1194.00
	50EFB	3	2708.33	143.67	82.95	2351.44	3065.22	2549.00	2828.00
	0EFB	3	3964.67	278.06	160.54	3273.93	4655.40	3644.00	4139.00
	Total	12	8995.00	537.79	310.49	7659.06	10330.94	8428.00	9420.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
VFA12h	Between Groups	17455857.556	4	8727928.778	852.300	.000
	Within Groups	61442.667	8	10240.444		
	Total	17517300.222	12			
VFA24h	Between Groups	15352224.222	4	7676112.111	514.416	.000
	Within Groups	89532.000	8	14922.000		
	Total	15441756.222	12			
VFA48h	Between Groups	15732709.556	4	7866354.778	279.937	.000
	Within Groups	168602.667	8	28100.444		
	Total	15901312.222	12			

.

Post Hoc Tests

Multiple Comparisons

Scheffe

Dependent Variable	(I) EFB12	(J) EFB12	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
VFA12h	100EFB	75EFB	-386.0000(*)	82.62544	.010	-651.0011	-120.9989
		50EFB	-2011.3333(*)	82.62544	.000	-2293.2641	-1729.4025
		0EFB	-3128.3333(*)	82.62544	.000	-3393.3345	-2863.3322
	75EFB	75EFB	386.0000(*)	82.62544	.010	120.9989	651.0011
		50EFB	-2011.3333(*)	82.62544	.000	-2293.2641	-1729.4025
		0EFB	-2742.3333(*)	82.62544	.000	-3007.3345	-2477.3322
	50EFB	75EFB	3128.3333(*)	82.62544	.000	2863.3322	3393.3345
		50EFB	1875.0000(*)	82.62544	.000	1593.0692	2156.9308
		0EFB	2742.3333(*)	82.62544	.000	2477.3322	3007.3345
	0EFB	75EFB	-2107.0000(*)	82.62544	.000	-2513.4852	-1700.5148
		50EFB	*4107.0000(*)*	*82.62544*	*.000*	1700.5148	*2513.4852*
		0EFB	*5053.6667(*)*	*82.62544*	*.000*	1647.1814	*2460.1519*
VFA24h	100EFB	75EFB	146.6667	99.73966	.397	-173.2242	466.5575
		50EFB	1850.0000(*)	80.33218	.000	1592.3539	2107.6461
		0EFB	-2694.3333(*)	99.73966	.000	-3014.2242	-2374.4425
	75EFB	100EFB	-146.6667	99.73966	.397	-466.5575	173.2242
		50EFB	-1850.0000(*)	80.33218	.000	-2107.6461	-1592.3539
		0EFB	2841.0000(*)	99.73966	.000	-3160.8909	-2521.1091
	50EFB	100EFB	2694.3333(*)	99.73966	.000	2374.4425	3014.2242
		75EFB	2875.0000(*)	87.90399	.000	-2156.9308	-1593.0692
		0EFB	2841.0000(*)	99.73966	.000	2521.1091	3160.8909

(Cont.)

VFA48h	100EFB	75EFB	88.0000	136.87085	.819	-350.9802	526.9802
		50EFB	2107.0000(*)	126.73916	.000	1700.5148	2513.4852
		0EFB	2759.6667(*)	136.87085	.000	-3198.6468	-2320.6865
	75EFB	100EFB	-88.0000	136.87085	.819	-526.9802	350.9802
		50EFB	2053.6667(*)	126.73916	.000	1647.1814	2460.1519
		0EFB	-2847.6667(*)	136.87085	.000	-3286.6468	-2408.6865
	50EFB	100EFB	2759.6667(*)	136.87085	.000	2320.6865	3198.6468
		75EFB	2107.0000(*)	126.73916	.000	1700.5148	2513.4852
		0EFB	3847.6667(*)	136.87085	.000	2408.6865	3286.6468

* The mean difference is significant at the .05 level.

Homogeneous Subsets

VFA12h

Scheffe

EFB12	N	Subset for alpha = .05		
		1	2	3
100EFB	3	618.0000		
75EFB	3		1004.0000	
50EFB	3		2434.5480	2434.5480
0EFB	3			3746.3333
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

VFA24h

Scheffe

EFB12	N	Subset for alpha = .05		
		1	2	3
100EFB	3	1116.6667		
75EFB	3		1263.3333	
50EFB	3		2341.3333	2341.3333
0EFB				3957.6667
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

VFA48h

Scheffe

EFB12	N	Subset for alpha = .05		
		1	2	3
100EFB	3	1186.6667		
75EFB	3		1938.6667	
50EFB	3		2605.0000	2605.0000
0EFB	3			3964.6667
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 3.000.

Figure 3-3 Acidogenesis yield (TVFA:SCOD) in leachate from co-fermentation of pig manure (PM) and palm empty fruit bunch (EFB) at different mixing ratios in the ALBR operated at flooding interval (FI) (a) 12 h, (b) 24 h, and (c) 48 h

Time	0PM:100EFB			25PM:75EFB			50PM:50EFB			100PM:0EFB		
(day)	FI 12 h	FI 24 h	FI 48 h	FI 12 h	FI 24 h	AI 48 h	FI 12 h	FI 24 h	FI 48 h	FI 12 h	FI 24 h	FI 48 h
1	15.13	15.28	18.84	10.60	11.49	8.97	17.78	7.51	9.06	9.7	9.8	9.9
5	25.75	25.34	24.15	15.39	10.26	7.03	#N/A	9.01	8.18	13.9	8.7	11.8
9	29.61	23.04	20.02	22.34	19.90	7.85	20.34	12.41	12.07	16.2	11.1	11.2
13	23.99	24.18	#N/A	39.11	21.54	23.30	35.91	19.22	13.01	14.6	10.4	15.6
15	34.48	21.95	#N/A	36.52	23.43	36.00	#N/A	27.77	23.73	15.7	16.1	14.8
19	16.21	22.10	#N/A	#N/A	30.00	42.04	49.52	43.52	47.63	30.1	20.6	20.9
23	30.72	32.00	#N/A	40.70	41.28	42.88	44.86	43.20	49.11	26.0	24.4	26.3
27	23.07	32.67	38.58	36.56	36.96	39.78	46.39	44.73	54.47	24.6	22.7	26.8
30	27.85	30.14	36.23	37.28	39.37	41.63	45.02	42.67	54.04	28.1	24.5	33.4
37	18.46	38.30	31.27	39.74	37.07	45.37	50.11	42.19	49.90	33.1	30.3	34.1
41	30.88	36.15	32.13	40.64	40.44	49.67	48.14	42.17	51.98	30.7	37.7	32.6
43	20.55	36.87	33.45	41.99	39.00	47.78	50.28	46.47	49.45	31.4	33.4	38.9
45	21.39	34.57	34.82	42.98	39.72	50.02	48.66	46.55	52.78	31.1	34.3	38.0
49	22.82	34.91	35.72	39.91	42.86	44.55	50.40	47.60	51.26	#N/A	#N/A	39.7
51	23.30	37.59	34.98	39.95	40.35	44.21	51.85	46.52	49.87	32.1	37.5	36.5
57	27.52	34.67	32.43	44.19	40.90	46.19	49.59	48.17	50.37	29.7	40.3	40.6
60	17.69	35.48	33.27	40.24	41.81	43.73	49.50	48.62	51.75	32.1	38.8	43.3

Figure 3-4 VFA production form palm empty fruit bunch (EFB) at different pig manure (PM) mixing ratios in the ALBR operated at flooding interval (FI) 12 h, 24 h, and 48 h

Mixing	VFA Production (gVFA/gEFB)		
	FI 12 hr	FI 24 hr	FI 48 hr
0PM:100EFB	0.00	0.01	0.00
25PM:75EFB	0.08	0.09	0.08
50PM:50PM	0.09	0.10	0.09
100PM:0EFB	0.16	0.17	0.20

Figure 3-5 Predicted versus observed values of the TVFA production ($g/kg_{substrate\ dry}$) response function (the line represents actual = modeled values)

No.	Actual value	Model Predicted value	No.	Actual value	Model Predicted value	No.	Actual value	Model Predicted value
1	10.65	17.00	26	14.15	20.92	51	11.77	21.37
2	7.76	19.02	27	15.45	15.93	52	74.15	81.08
3	15.41	11.38	28	12.47	16.99	53	84.10	90.58
4	12.04	10.60	29	93.79	61.42	54	70.22	82.88
5	8.50	20.26	30	83.15	57.41	55	66.69	77.64
6	15.92	30.75	31	82.64	54.00	56	94.18	69.10
7	29.58	30.22	32	27.94	29.50	57	81.86	64.10
8	33.75	28.55	33	22.78	28.18	58	83.45	60.20
9	8.91	5.20	34	17.20	26.12	59	29.65	35.36
10	9.69	7.35	35	72.45	71.09	60	23.07	33.05
11	11.82	7.03	36	49.92	65.38	61	18.09	30.50
12	10.67	32.39	37	57.14	61.13	62	57.75	74.37
13	8.48	30.16	38	93.13	53.25	63	22.44	20.20
14	18.32	51.00	39	46.91	50.24	64	117.25	82.18
15	39.66	47.49	40	64.87	47.33	65	39.00	55.86
16	45.40	40.11	41	27.28	23.15	66	40.79	49.08
17	27.37	38.58	42	13.29	22.83	67	19.17	44.30
18	54.22	36.42	43	13.43	21.26	68	108.57	123.82
19	24.83	12.74	44	49.10	60.63	69	97.07	112.15
20	12.34	13.90	45	32.19	55.92	70	100.85	104.92
21	13.44	13.08	46	38.17	52.16	71	125.60	95.07
22	17.63	44.05	47	67.89	44.61	72	120.07	86.10
23	16.29	40.82	48	35.06	42.59	73	112.68	80.21
24	19.25	37.81	49	56.39	40.18	74	37.01	54.06
25	15.50	27.64	50	25.56	16.33	75	39.40	47.78

Figure 3-5 (Cont.)

No.	Actual value	Model Predicted value	No.	Actual value	Model Predicted value	No.	Actual value	Model Predicted value
76	18.42	43.24	101	30.01	39.45	126	41.00	60.54
77	96.09	120.08	102	21.86	36.40	127	43.83	52.27
78	92.29	108.91	103	108.24	113.18	128	20.88	46.74
79	89.71	101.93	104	129.58	102.84	129	115.52	134.31
80	122.51	92.24	105	126.68	92.37	130	110.37	121.16
81	114.96	83.77	106	121.85	85.75	131	49.87	55.05
82	106.36	78.13	107	40.00	59.10	132	21.80	48.40
83	35.55	52.14	108	42.85	51.33	133	117.82	146.63
84	36.98	46.36	109	20.45	46.05	134	110.38	131.49
85	20.75	42.07	110	112.86	127.43	135	116.39	122.53
86	91.22	112.25	111	21.83	33.47	136	133.72	111.52
87	85.70	102.07	112	104.12	107.80	137	132.11	99.07
88	87.56	95.59	113	108.02	115.27	138	138.78	114.28
89	112.73	86.23	114	129.22	97.78	139	137.89	101.09
90	101.70	78.74	115	123.89	88.31	140	131.12	93.10
91	98.06	73.61	116	128.99	91.45	141	128.61	150.76
92	30.43	47.94	117	41.63	64.14	142	127.60	134.87
93	31.47	43.15	118	44.75	54.39	143	46.61	65.54
94	18.61	39.36	119	21.58	48.12	144	31.56	55.23
95	88.53	97.39	120	116.79	137.57	145	115.41	125.54
96	73.85	88.95	121	108.77	123.92	146	55.00	69.62
97	71.44	83.34	122	109.61	115.70	147	10.89	12.05
98	94.80	74.55	123	130.02	105.18	148	60.00	51.94
99	95.61	68.81	124	128.86	94.23	149	70.00	75.72
100	88.32	64.54	125	125.14	87.35	150	101.00	126.81

Figure 3-5 (Cont.)

No.	Actual value	Model Predicted value
151	128.43	135.60
152	101.21	117.55
153	83.33	81.37
154	98.21	112.74
155	104.33	102.76
156	70.54	64.07
157	39.47	39.33
158	34.11	32.85
159	37.65	19.53
160	24.21	5.72
161	134.11	153.62
162	134.04	147.88
163	175.65	165.65
164	170.11	159.54
165	166.48	146.51
166	136.19	137.23
167	120.43	127.47
168	133.47	122.42
169	107.05	111.94
170	88.27	89.55
171	84.43	83.66
172	83.11	71.51
173	63.43	58.89
174	48.07	45.78
175	183.38	180.06

No.	Actual value	Model Predicted value
176	163.09	165.55
177	172.11	165.55
178	163.43	155.27
179	159.04	144.52
180	130.21	138.97
181	131.11	127.50
182	116.54	103.13
183	89.20	96.74
184	87.42	83.60
185	70.03	69.98
186	45.11	55.89
187	45.04	48.66
188	34.21	39.05
189	34.55	25.23
190	33.21	33.85
191	22.54	10.93
192	33.19	18.56
193	23.21	2.79
194	196.48	186.92

Figure 3-6 Two-dimensional contours plots of TVFA production as a function of (a) PM vs. FT, (b) PM vs. FI, (c) FI vs. FT

No.	%PM	FI (h)	FT (d)	TVFA
1	0	12	0	0
2	0	24	0	0
3	0	48	0	0
4	25	12	0	0
5	25	24	0	0
6	25	48	0	0
7	50	12	0	0
8	50	24	0	0
9	50	48	0	0
10	0	12	1	4
11	0	24	1	5
12	0	48	1	5
13	25	12	1	15
14	25	24	1	11
15	25	48	1	12
16	50	12	1	11
17	50	24	1	8
18	50	48	1	8
19	0	12	5	10
20	0	24	5	9
21	0	48	5	6
22	25	12	5	22
23	25	24	5	12
24	25	48	5	14
25	50	12	5	15

No.	%PM	FI (h)	FT (d)	TVFA
26	50	24	5	8
27	50	48	5	11
28	0	12	9	12
29	0	24	9	10
30	0	48	9	9
31	25	12	9	34
32	25	24	9	30
33	25	48	9	16
34	50	12	9	19
35	50	24	9	16
36	50	48	9	18
37	0	12	13	13
38	0	24	13	12
39	0	48	13	25
40	25	12	13	54
41	25	24	13	27
42	25	48	13	45
43	50	12	13	40
44	50	24	13	18
45	50	48	13	32
46	0	12	15	15
47	0	24	15	12
48	0	48	15	26
49	25	12	15	56
50	25	24	15	35

No.	%PM	FI (h)	FT (d)	TVFA
51	25	48	15	68
52	50	12	15	38
53	50	24	15	32
54	50	48	15	49
55	0	12	19	13
56	0	24	19	13
57	0	48	19	27
58	25	12	19	65
59	25	24	19	47
60	25	48	19	93
61	50	12	19	57
62	50	24	19	50
63	50	48	19	72
64	0	12	23	17
65	0	24	23	23
66	0	48	23	28
67	25	12	23	83
68	25	24	23	83
69	25	48	23	94
70	50	12	23	55
71	50	24	23	58
72	50	48	23	74
73	0	12	27	18
74	0	24	27	23
75	0	48	27	30

Figure 3-6 (Cont.)

No.	%PM	FI (h)	FI (d)	TVFA
76	25	12	27	83
77	25	24	27	82
78	25	48	27	94
79	50	12	27	67
80	50	24	27	70
81	50	48	27	84
82	0	12	30	22
83	0	24	30	22
84	0	48	30	30
85	25	12	30	88
86	25	24	30	96
87	25	48	30	95
88	50	12	30	71
89	50	24	30	74
90	50	48	30	89
91	0	12	37	19
92	0	24	37	31
93	0	48	37	30
94	25	12	37	98
95	25	24	37	102
96	25	48	37	113
97	50	12	37	88
98	50	24	37	86
99	50	48	37	91
100	0	12	41	21

No.	%PM	FI (h)	FI (d)	TVFA
101	0	24	41	37
102	0	48	41	36
103	25	12	41	106
104	25	24	41	115
105	25	48	41	123
106	50	12	41	90
107	50	24	41	92
108	50	48	41	96
109	0	12	43	18
110	0	24	43	39
111	0	48	43	37
112	25	12	43	113
113	25	24	43	120
114	25	48	43	126
115	50	12	43	101
116	50	24	43	97
117	50	48	43	109
118	0	12	45	19
119	0	24	45	41
120	0	48	45	39
121	25	12	45	117
122	25	24	45	124
123	25	48	45	129
124	50	12	45	104
125	50	24	45	108

No.	%PM	FI (h)	FI (d)	TVFA
126	50	48	45	113
127	0	12	49	20
128	0	24	49	43
129	0	48	49	40
130	25	12	49	122
131	25	24	49	127
132	25	48	49	130
133	50	12	49	108
134	50	24	49	110
135	50	48	49	116
136	0	12	51	21
137	0	24	51	44
138	0	48	51	41
139	25	12	51	125
140	25	24	51	129
141	25	48	51	130
142	50	12	51	110
143	50	24	51	109
144	50	48	51	117
145	0	12	57	22
146	0	24	57	45
147	0	48	57	42
148	25	12	57	129
149	25	24	57	132
150	25	48	57	134

Figure 3-6 (Cont.)

No.	%PM	FI (h)	FI (d)	TVFA
151	50	12	57	116
152	50	24	57	110
153	50	48	57	118
154	0	12	60	22
155	0	24	60	50
156	0	48	60	47
157	25	12	60	131
158	25	24	60	138
159	25	48	60	139
160	50	12	60	115
161	50	24	60	128
162	50	48	60	129
163	100	12	1	17
164	100	12	5	24
165	100	12	9	37
166	100	12	13	34
167	100	12	15	39
168	100	12	19	60
169	100	12	23	70
170	100	12	27	70
171	100	12	29	83
172	100	12	37	104
173	100	12	41	98
174	100	12	43	101
175	100	12	47	101

No.	%PM	FI (h)	FI (d)	TVFA
176	100	12	51	128
177	100	12	57	134
178	100	12	60	134
179	100	24	1	21
180	100	24	5	22
181	100	24	9	34
182	100	24	13	34
183	100	24	15	48
184	100	24	19	63
185	100	24	23	83
186	100	24	27	84
187	100	24	29	88
188	100	24	37	107
189	100	24	41	133
190	100	24	43	120
191	100	24	47	136
192	100	24	51	166
193	100	24	57	170
194	100	24	60	175
195	100	48	1	23
196	100	48	5	33
197	100	48	9	33
198	100	48	13	45
199	100	48	15	45
200	100	48	19	70

No.	%PM	FI (h)	FI (d)	TVFA
201	100	48	23	87
202	100	48	27	89
203	100	48	29	116
204	100	48	37	131
205	100	48	41	130
206	100	48	43	159
207	100	48	47	163
208	100	48	51	172
209	100	48	51	163
210	100	48	57	183
211	100	48	60	196

Figure 3-7 VFA production and composition at different co-substrate mixing ratios in the ALBR operated at flooding interval (FI) 12 h, 24 h, and 48 h

VFA species ($g/_{kg substrate dry}$)	0PM:100EFB											
	FI 12 h				FI 24 h				FI 48 h			
	15d	30d	45d	60d	15d	30d	45d	60d	15d	30d	45d	60d
C2	8.64	10.16	11.55	12.72	7.08	7.98	8.31	13.93	7.67	10.03	12.23	11.53
C3	7.34	9.08	8.34	9.85	5.40	7.48	9.87	12.56	7.21	7.04	11.44	10.08
i-C4 + C4	-	-	-	-	-	7.95	10.62	15.29	5.89	4.80	15.72	20.51
i-C5 + C5	-	-	-	-	-	-	-	-	-	-	-	-
i-C6 + C6	-	-	-	-	-	-	-	-	-	-	-	-
VFA species ($g/_{kg substrate dry}$)	25PM:75EFB											
	FI 12 h				FI 24 h				FI 48 h			
	15d	30d	45d	60d	15d	30d	45d	60d	15d	30d	45d	60d
C2	11.30	28.76	38.83	43.21	21.80	43.38	45.39	47.14	23.99	32.85	41.90	53.07
C3	13.06	22.90	32.32	32.15	11.55	23.38	27.69	27.01	8.79	22.99	26.53	37.56
i-C4 + C4	8.55	10.48	13.94	17.43	9.80	11.44	17.28	18.77	6.14	9.93	14.13	16.52
i-C5 + C5	5.25	9.30	15.76	22.62	5.95	10.32	18.21	28.81	3.27	8.09	14.52	22.41
i-C6 + C6	-	-	-	-	-	-	-	-	-	-	-	-
VFA species ($g/_{kg substrate dry}$)	100PM:0EFB											
	FI 12 h				FI 24 h				FI 48 h			
	15d	30d	45d	60d	15d	30d	45d	60d	15d	30d	45d	60d
C2	6.69	15.40	26.65	35.88	9.99	7.65	28.63	29.53	8.67	20.41	21.92	32.50
C3	6.42	15.59	33.27	37.72	8.55	22.27	20.52	45.99	8.26	18.22	40.14	46.15
i-C4 + C4	13.46	24.20	32.77	40.52	15.41	19.54	32.90	43.32	15.04	32.06	40.47	47.61
i-C5 + C5	12.50	27.41	33.42	48.62	13.85	25.42	29.31	38.08	13.01	28.68	44.79	48.26
i-C6 + C6	-	-	-	-	-	12.67	8.37	17.64	-	17.13	21.60	21.97

B. Data for chapter IV

Figure 4-2 pH (× FI 12h , ■ FI 24 h and ○ FI 48 h) and TVFA production rate (× FI 12h , ■ FI 24 h and ○ FI 48 h) when operating ALBRs with different mixing ratios (a) 0PM:100EFB, (b) 25PM:75EFB and (c) 50PM:50EFB at FI 12, 24 and 48 h

0PM:100EFB						
t (d)	VFA Production rate (mg/d)			pH		
	12 h	24 h	48 h	12 h	24 h	48 h
1	0	0	0	6.70	6.70	6.70
5	826	765	432	5.94	5.86	5.84
9	935	991	515	6.02	6.20	5.95
13	900	1238	644	6.06	6.36	6.11
15	707	1295	756	6.00	6.22	5.96
19	541	1235	825	6.07	6.24	5.98
23	398	1111	875	6.06	6.41	6.08
27	251	911	915	6.14	6.41	6.18
30	154	710	925	6.07	6.36	6.18
37	104	574	915	6.30	6.56	6.39
41	57	397	869	6.24	6.49	6.36
43	25	240	787	6.41	6.86	6.54
45	17	185	738	6.29	6.88	6.39
49	11	143	686	6.39	6.96	6.65
51	7	110	634	6.43	6.87	6.58
57	6	92	600	6.50	7.02	6.70
60	3	65	532	6.52	6.96	6.84
1	2	43	461	6.65	7.00	6.90

25PM:75EFB						
t (d)	VFA Production rate (mg/d)			pH		
	12 h	24 h	48 h	12 h	24 h	48 h
1	0	0	0	6.70	6.85	6.70
5	1288	860	1173	6.51	6.82	6.65
9	1611	1235	1833	6.37	6.32	6.59
13	2082	1871	2868	6.06	6.27	6.13
15	2428	2435	3564	5.89	6.22	5.78
19	2587	2749	3769	5.52	5.86	5.63
23	2641	2923	3682	5.59	5.59	5.58
27	2593	2973	3301	5.67	5.55	5.65
30	2433	2840	2758	5.74	5.74	5.82
37	2266	2660	2330	5.88	5.78	5.79
41	1938	2266	1696	6.35	5.88	6.01
43	1536	1769	1085	6.67	6.20	6.21
45	1348	1533	856	6.62	6.34	6.24
49	1175	1319	675	6.80	6.51	6.52
51	1016	1124	527	6.74	6.55	6.70
57	920	1008	447	6.76	6.77	6.80
60	753	807	322	6.82	6.91	6.90
1	592	619	218	6.91	7.00	6.91

Figure 4-2 (Cont.)

50PM:50EFB							100PM:0EFB						
t (d)	VFA Production rate (mg/d)			pH			t (d)	VFA Production rate (mg/d)			pH		
	12 h	24 h	48 h	12 h	24 h	48 h		12 h	24 h	48 h	12 h	24 h	48 h
1	0	0	0	6.85	6.70	6.85	1	0	0	0	6.75	6.75	6.73
5	2011	1096	1525	6.21	6.63	6.53	5	3508	3361	4241	6.90	7.26	7.16
9	2363	1601	2427	6.32	6.48	6.49	9	3351	3323	4122	7.30	7.25	7.27
13	2797	2439	3772	5.94	6.18	6.35	13	3113	3263	3938	6.79	6.83	6.69
15	3023	3138	4539	5.65	5.93	6.25	15	2892	3205	3763	6.85	6.75	6.56
19	3060	3489	4646	5.70	5.89	5.93	19	2736	3162	3636	6.90	6.71	6.52
23	2985	3636	4374	5.99	5.75	5.80	23	2589	3119	3514	6.97	6.60	6.26
27	2775	3587	3726	6.08	5.83	5.95	27	2406	3063	3358	6.88	6.68	6.40
30	2483	3317	2960	6.05	5.85	6.13	30	2235	3008	3208	7.34	6.78	6.42
37	2238	3029	2408	6.20	5.93	6.03	37	2114	2967	3100	7.20	6.77	6.47
41	2092	2832	1977	6.33	6.02	6.27	41	1930	2901	2930	7.25	6.80	6.60
43	1830	2480	1660	6.53	6.14	6.48	43	1727	2823	2735	7.46	6.84	6.61
45	1467	1842	991	6.59	6.12	6.71	45	1634	2785	2643	7.57	6.93	6.89
49	1184	1560	758	6.62	6.55	6.79	49	1575	2759	2584	7.64	7.41	7.32
51	1013	1313	581	6.72	6.61	6.80	51	#N/A	#N/A	#N/A	7.63	7.35	7.33
57	860	1095	440	6.76	6.66	6.87	57	1464	2710	2469	7.73	7.45	7.40
60	770	967	366	6.85	6.75	6.89	60	1311	2637	2306	7.79	7.55	7.52
1	558	755	255	6.89	6.83	7.05	1	1206	2584	2190	7.81	7.61	7.55

Figure 4-3 Three-dimensional response surface plots of the hydrolysis and acidification yields as a function of (a, b) *FT* vs. *%PM*, (c, d) *FI* vs. *%PM* and (e, f) *FT* vs. *FI*

No.	%PM	FI (h)	FT (d)	Hydrolysis yield (%)	Acidificaiton yield (%)
1	0	12	0	0.00	0.00
2	0	24	0	0.00	0.00
3	0	48	0	0.00	0.00
4	25	12	0	0.00	0.00
5	25	24	0	0.00	0.00
6	25	48	0	0.00	0.00
7	50	12	0	0.00	0.00
8	50	24	0	0.00	0.00
9	50	48	0	0.00	0.00
10	0	12	1	1.73	15.13
11	0	24	1	1.48	15.28
12	0	48	1	1.76	18.84
13	25	12	1	3.73	10.60
14	25	24	1	5.20	11.49
15	25	48	1	6.99	8.97
16	50	12	1	7.62	17.78
17	50	24	1	8.42	7.51
18	50	48	1	5.92	9.06
19	0	12	5	2.50	25.75
20	0	24	5	1.77	25.34
21	0	48	5	2.14	24.15
22	25	12	5	3.87	15.39
23	25	24	5	6.65	10.26
24	25	48	5	7.30	7.03
25	50	12	5	8.19	#N/A

No.	%PM	FI (h)	FT (d)	Hydrolysis yield (%)	Acidificaiton yield (%)
26	50	24	5	#N/A	9.01
27	50	48	5	6.83	8.18
28	0	12	9	2.78	29.61
29	0	24	9	2.17	23.04
30	0	48	9	2.63	20.02
31	25	12	9	6.23	22.34
32	25	24	9	6.15	19.90
33	25	48	9	8.79	7.85
34	50	12	9	8.69	20.34
35	50	24	9	11.64	12.41
36	50	48	9	7.90	12.07
37	0	12	13	2.66	23.99
38	0	24	13	2.69	24.18
39	0	48	13	3.47	29.76
40	25	12	13	6.70	39.11
41	25	24	13	7.93	21.54
42	25	48	13	9.77	23.30
43	50	12	13	#N/A	35.91
44	50	24	13	12.16	19.22
45	50	48	13	8.53	13.01
46	0	12	15	3.13	34.48
47	0	24	15	2.26	21.95
48	0	48	15	3.53	#N/A
49	25	12	15	8.00	36.52
50	25	24	15	7.38	23.43

Figure 4-3 (Cont.)

No.	%PM	FI (h)	FT (d)	Hydrolysis yield (%)	Acidificaiton yield (%)
51	25	48	15	9.00	36.00
52	50	12	15	10.34	#N/A
53	50	24	15	12.56	27.77
54	50	48	15	10.09	23.73
55	0	12	19	3.75	16.21
56	0	24	19	3.80	22.10
57	0	48	19	3.75	28.12
58	25	12	19	8.36	#N/A
59	25	24	19	8.60	30.00
60	25	48	19	11.19	42.04
61	50	12	19	11.45	49.52
62	50	24	19	11.79	43.52
63	50	48	19	12.48	47.63
64	0	12	23	3.47	30.72
65	0	24	23	3.71	32.00
66	0	48	23	4.07	34.39
67	25	12	23	7.90	40.70
68	25	24	23	7.31	41.28
69	25	48	23	10.93	42.88
70	50	12	23	11.87	44.86
71	50	24	23	14.20	43.20
72	50	48	23	12.76	49.11
73	0	12	27	3.71	23.07
74	0	24	27	4.40	32.67
75	0	48	27	4.14	38.58

No.	%PM	FI (h)	FT (d)	Hydrolysis yield (%)	Acidificaiton yield (%)
76	25	12	27	9.14	36.56
77	25	24	27	8.02	36.96
78	25	48	27	11.65	39.78
79	50	12	27	13.82	46.39
80	50	24	27	15.24	44.73
81	50	48	27	13.79	54.47
82	0	12	30	4.40	27.85
83	0	24	30	5.23	30.14
84	0	48	30	4.61	36.23
85	25	12	30	10.18	37.28
86	25	24	30	8.50	39.37
87	25	48	30	12.92	41.63
88	50	12	30	13.99	45.02
89	50	24	30	15.43	42.67
90	50	48	30	14.14	54.04
91	0	12	37	4.86	18.46
92	0	24	37	6.25	38.30
93	0	48	37	5.56	31.27
94	25	12	37	10.81	39.74
95	25	24	37	10.95	37.07
96	25	48	37	13.37	45.37
97	50	12	37	15.17	50.11
98	50	24	37	15.37	42.19
99	50	48	37	16.90	49.90
100	0	12	41	5.33	30.88

Figure 4-3 (Cont.)

No.	%PM	FI (h)	FT (d)	Hydrolysis yield (%)	Acidificaiton yield (%)
101	0	24	41	6.60	36.15
102	0	48	41	6.03	32.13
103	25	12	41	11.57	40.64
104	25	24	41	11.28	40.44
105	25	48	41	14.09	49.67
106	50	12	41	15.51	48.14
107	50	24	41	16.30	42.17
108	50	48	41	17.59	51.98
109	0	12	43	5.56	20.55
110	0	24	43	7.18	36.87
111	0	48	43	6.48	33.45
112	25	12	43	12.47	41.99
113	25	24	43	12.36	39.00
114	25	48	43	14.58	47.78
115	50	12	43	16.22	50.28
116	50	24	43	18.20	46.47
117	50	48	43	18.97	49.45
118	0	12	45	4.86	21.39
119	0	24	45	7.41	34.57
120	0	48	45	7.20	34.82
121	25	12	45	13.23	42.98
122	25	24	45	12.69	39.72
123	25	48	45	15.07	50.02
124	50	12	45	16.82	48.66
125	50	24	45	17.07	46.55
126	50	48	45	19.31	52.78
127	0	12	49	6.48	22.82
128	0	24	49	7.64	34.91
129	0	48	49	7.38	35.72
130	25	12	49	13.51	39.91
131	25	24	49	13.33	42.86
132	25	48	49	15.07	44.55
133	50	12	49	17.20	50.40
134	50	24	49	18.58	47.60
135	50	48	49	21.72	51.26
136	0	12	51	6.72	23.30
137	0	24	51	8.68	37.59
138	0	48	51	7.04	34.98
139	25	12	51	13.58	39.95
140	25	24	51	13.98	40.35
141	25	48	51	15.62	44.21
142	50	12	51	18.10	51.85
143	50	24	51	18.10	46.52
144	50	48	51	21.72	49.87
145	0	12	57	7.41	27.52
146	0	24	57	7.99	34.67
147	0	48	57	11.45	32.43
148	25	12	57	13.78	44.19
149	25	24	57	14.29	40.90
150	25	48	57	16.10	46.19

Figure 4-3 (Cont.)

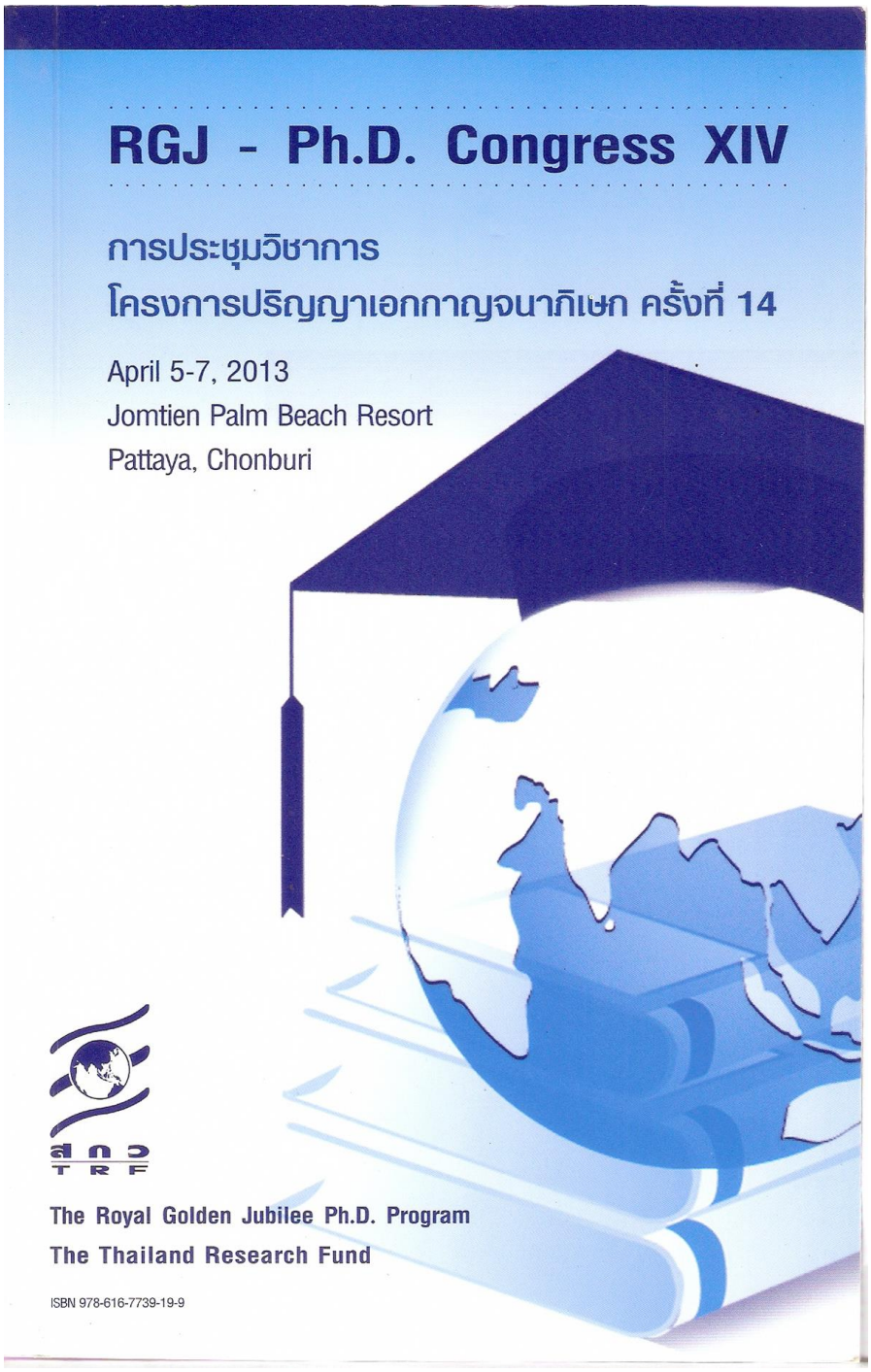
No.	%PM	FI (h)	FT (d)	Hydrolysis yield (%)	Acidificaiton yield (%)
151	50	12	57	16.55	49.59
152	50	24	57	18.28	48.17
153	50	48	57	22.24	50.37
154	0	12	60	8.34	17.69
155	0	24	60	8.68	35.48
156	0	48	60	10.36	33.27
157	25	12	60	14.48	40.24
158	25	24	60	15.26	41.81
159	25	48	60	16.33	43.73
160	50	12	60	19.59	49.50
161	50	24	60	19.92	48.62
162	50	48	60	23.28	51.75
163	100	12	1	10.89	9.69
164	100	12	5	10.47	13.94
165	100	12	9	13.83	16.24
166	100	12	13	14.45	14.64
167	100	12	15	15.29	15.69
168	100	12	19	16.55	30.13
169	100	12	23	17.39	25.96
170	100	12	27	18.02	24.60
171	100	12	29	19.27	28.15
172	100	12	37	19.69	33.06
173	100	12	41	19.69	30.72
174	100	12	43	19.90	31.36

No.	%PM	FI (h)	FT (d)	Hydrolysis yield (%)	Acidificaiton yield (%)
176	100	12	51	21.58	32.06
177	100	12	57	23.04	29.72
178	100	12	60	22.62	32.11
179	100	24	1	13.20	9.79
180	100	24	5	15.71	8.69
181	100	24	9	18.64	11.15
182	100	24	13	20.11	10.42
183	100	24	15	18.23	16.10
184	100	24	19	18.85	20.64
185	100	24	23	20.95	24.42
186	100	24	27	22.83	22.68
187	100	24	29	22.00	24.49
188	100	24	37	21.97	30.28
189	100	24	41	21.60	37.71
190	100	24	43	22.00	33.44
191	100	24	47	24.72	34.31
192	100	24	51	27.23	37.55
193	100	24	57	25.98	38.77
194	100	24	60	27.65	40.33
195	100	48	1	14.04	9.91
196	100	48	5	17.39	11.79
197	100	48	9	18.02	11.24
198	100	48	13	17.60	15.60
199	100	48	15	18.64	14.81

Figure 4-3 (Cont.)

No.	%PM	FI (h)	FT (d)	Hydrolysis yield (%)	Acidificaiton yield (%)
200	100	48	19	20.53	20.87
201	100	48	23	20.32	26.31
202	100	48	27	20.32	26.82
203	100	48	29	21.37	33.37
204	100	48	37	23.67	34.06
205	100	48	41	24.51	32.60
206	100	48	43	25.14	38.92
207	100	48	47	26.40	38.01
208	100	48	51	26.60	39.70
209	100	48	51	27.44	36.53
210	100	48	57	27.65	40.64
211	100	48	60	27.86	43.29

C. Conference



Oral presentation

Engineering and Technology/Environmental Technology

S1B-O3

Co-Fermentation of Oil Palm Empty Fruit Bunch and Pig Manure for Volatile Fatty Acids Production Using Leach Bed Reactor

Kanyarat Saritpongteeraka[a], Sumate Chaiprapat[b], Piyarat Boonsawang[c] and Shihwu Sung[d]

[a]*Faculty of Environmental Management, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
[b]*Department of Civil Engineering, Faculty of Engineering, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand*
[c]*Faculty of Agro-Industry, Department of Industrial Biotechnology, Prince of Songkla University, Hat Yai Campus, Hat Yai, Songkhla 90110, Thailand*
[d]*Department of Civil, Construction & Environmental Engineering, Collage of Engineering, Iowa State University, USA*

Introduction and Objective

Oil palm empty fruit bunch (EFB) is a lignocellulosic residual with high C:N. It amounted for 2.01 million ton in year 2011, thus, utilization of this waste is of our interest. The main objective of this study was to investigate the parameters affecting the digestibility of EFB co-fermenting with the pig manure which is a nitrogen rich material. Effects of pretreating time (PT), flooding interval (FI), and pig manure mixing ratio (%PM) were tested using Response Surface Methodology (RSM).

Methods

Anaerobic leach bed reactors (ALBR) used in this study (Fig. 1) were fed with the different mixtures of EFB and pig manure (PM). Three levels of PT, PM, and FI were engaged in the experiments.

Results

Biodegradation of the mixture of 50PM:50EFB at FI 24 h gave the highest cumulative TVFA production at 152.7±7.2 gTVFA/$kg_{substrate\ dw.}$ Acetate and propionate were the major aqueous products at all experiments. Hydrolysis yield was highest at 23.3±2.2% for 50PM:50EFB at FI 48 h while acidification yield was highest at 51.7±2.5% for 50PM:50EFB at FI 48 h. %PM mixing, FI and PT could promote degree of hydrolysis and acidification. Effect of %PM, FI and PT were modelled using quadratic equation. The generated response surface and contour plot showed the maximum TVFA production of 152.3 g/$kg_{substrate\ dw}$ at %PM 50PM:50EFB, FI 48 h, and PT 60 d.

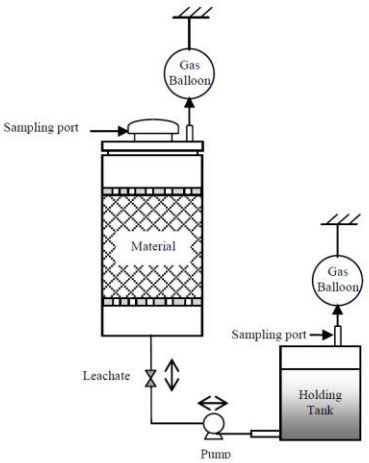


Figure 1 Anaerobic leach bed reactor set up used in this study

Conclusion

Addition of pig manure could help stimulate the degree of hydrolysis and TVFA production in the co-fermentation of the mixed substrate. Response surface and contour plot constructed from the quadratic regression model were able to describe the relationship of three variables satisfactorily.

Keywords: oil palm; empty fruit bunch; pig manure; VFA; leach bed reactor

Selected References:

1. Xie, S., Lawlor, P.G., Frost, J.P., Wu, G., Zhan, X. 2012. Hydrolysis and acidification of grass silage in leaching bed reactors. *Bioresource Technology*, 114(0), 406-413

Kanyarat Saritpongteeraka (กัญญารัตน์ สฤษฎ์พงศ์ทีรฆ) **RGJ-industry 11**
Prince of Songkla University, Environmental Management, M.Sc. 2004
Research field: Anaerobic digestion and Biogas production

Poster presentation

Engineering and Technology/Environmental Technology

(S1-P29)

Co-Fermentation of Oil Palm Empty Fruit Bunch and Pig Manure for Volatile Fatty Acids Production Using Leach Bed Reactor

Kanyarat Saritpongteeraka[a], Sumate Chaiprapat[b], Piyarat Boonsawang[c] and Shihwu Sung[d]

[a]Faculty of Environmental Management, [b]Department of Civil Engineering, Faculty of Engineering, [c]Faculty of Agro-Industry, Department of Industrial Biotechnology, Prince of Songkla University, Hat Yai Campus , Thailand and [d]Department of Civil, Construction & Environmental Engineering, Collage of Engineering, Iowa State University, USA

Introduction and Objective

Oil palm empty fruit bunch (EFB) is a lignocellulosic residual with high C:N. It amounted for 2.01 million ton in year 2011, thus, utilization of this waste is of our interest. The objective of this study was to investigate the parameters affecting the digestibility of EFB co-fermenting with the pig manure which is a nitrogen rich material. Effects of pretreating time (PT), flooding interval (FI), and pig manure mixing ratio (%PM) were tested using Response Surface Methodology (RSM).

Method

Anaerobic leach bed reactors (ALBR) used in this study (Fig. 1) were fed with the different mixtures of EFB and pig manure (PM). Three levels of PT, PM, and FI were engaged in the experiments

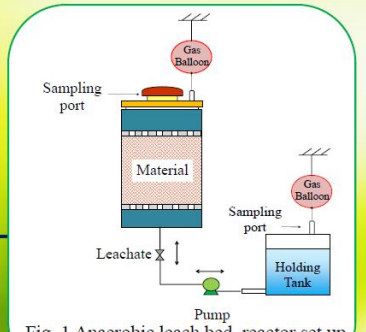


Fig. 1 Anaerobic leach bed reactor set up used in this study

Results

Biodegradation of the mixture of 50PM:50EFB at FI 24 h gave the highest cumulative TVFA production at 152.7±7.2 gTVFA/$kg_{substrate\ dry}$. Hydrolysis yield and acidification yield were highest at 23.2±2.2% and 51.7±2.5 % for 25PM:75EFB at FI 12 h. The generated response surface and contour plot (Fig 2) showed the maximum TVFA production of 152.7±7.2 g/$kg_{substrate\ dry}$ at %PM 50PM:50EFB, FI 48 h, and PT 60 d.

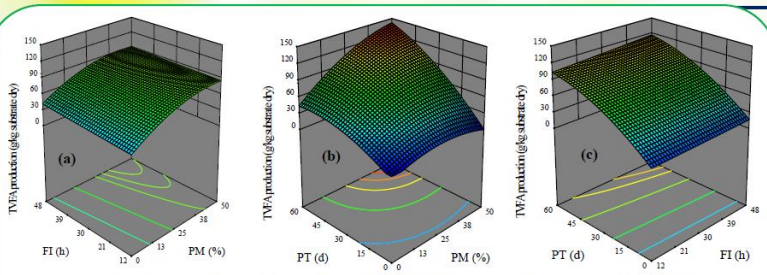


Fig. 2 Response surfaces and contours of TVFA production as a function of (a) FI vs PM, (b) PT vs PM, (c) PT vs FI

Conclusion

Addition of pig manure could help stimulate the degree of hydrolysis and TVFA production. Response surface and contour plot constructed from the quadratic regression model were able to describe the relationship of three variables satisfactorily.

Acknowledgments

This research was financially supported by The Royal Golden Jubilee Ph.D. Program: RGJ, Thailand Research Fund, SPM Feed Meal Co., Ltd., Graduate Studies Grant, Graduate School and Biogas Research Laboratory, Energy System Research Institute of Prince of Songkla University (PERIN).

References: Xie, S., Lawlor, P.G., Frost, J.P., Wu, G., Zhan, X. 2012. Hydrolysis and Acidification of Grass Silage in Leaching Bed Reactors. *Bioresource Technology*, 114 (0), 406-413

D. LIST OF PAPERS

Saritpongteeraka, K., Boonsawang, P., Sung, S., Chaiprapat, S. 2014. Co-fermentation of oil palm lignocellulosic residue with pig manure in anaerobic leach bed reactor for fatty acid production. Energy Conversion and Management, 84(0), 354-362.

Saritpongteeraka, K., Boonsawang, P., Sung, S., Chaiprapat, S. (2015). Solid state co-fermentation as pretreatment of lignocellulosic palm empty fruit bunch for organic acid recovery and fiber property improvement. International Biodeterioration & biodegradation, 100, 172-180.

VITAE

Name Miss Kanyarat Saritpongteeraka

Student ID 5210930001

Educational Attainment

Degree	Name of Institution	Year of Graduation
B.Sc. Biotechnology	Rajamangala Institute of Technology	2003
M.Sc. Environmental Management	Prince of Songkla University	2007
B.P.H. Occupational Health and Safety	Sukhothai Thammathirat Open University	2010

E-mail address: kanya.sarit@gmail.com

Scholarship Awards during Enrolment

- The Royal Golden Jubilee Ph.D. Program: RGJ, Thailand Research Fund Grant No 2.O.PS/51/A.1
- The Office of National Research Council of Thailand, Prince of Songkla University, Graduate Studies Grant, Graduate School of Prince of Songkla University (PSU)
- Biogas Research Laboratory, Energy System Research Institute of Prince of Songkla University (PERIN).
- SPM Feed Meal Co., Ltd.
- Lam Soon (Thailand) Public Company Limited. Sikao District, Trang, Thailand

List of Conference and Publications

Saritpongteeraka, K., Boonsawang, P., Sung, S., Chaiprapat, S., 2515 Solid state co-fermentation as a pretreatment of lignocellulosic oil palm empty fruit bunch for organic acid recovery and fiber property improvement. International Biodeterioration & Biodegradation, 100, 172-180.

Saritpongteeraka, K., Boonsawang, P., Sung, S., Chaiprapat, S., 2014. Co-fermentation of oil palm lignocellulosic residue with pig manure in anaerobic leach bed reactor for fatty acid production. Energy Conversion and Management 84, 354-362.

Saritpongteeraka, K., Chaiprapat, S., 2008. Effects of pH adjustment by parawood ash and effluent recycle ratio on the performance of anaerobic baffled reactors treating high sulfate wastewater. Bioresource Technology 99, 8987-8994.

Conference

Saritpongteeraka, K., Boonsawang, P., Sung, S., Chaiprapat, S., 2013. Co-fermentation of oil palm empty fruit bunch and pig manure for volatile fatty acids production using leach bed reactor. RGJ-Ph.D congress 14th Conference, The Thailand Research Fund. 5-7 April 2013, Jomtien Beach Resort, Pattaya, Chonburi, Thailand.

Thesis

Saritpongteeraka, K., Chaiprapat, S., 2007. Effect of pH adjustment by parawood ash and effluent recycle ratio on the performance of anaerobic buffled reactors treating concentrated rubber latex wastewater. Master's thesis, Faculty of Environmental Management, Prince of Songkla University, Thailand.