INTEGRATED UTILISATION OF *Pennisetum purpureum* cv. NAPIER PAK CHONG1 GRASS FOR RENEWABLE ENERGY PRODUCTION

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DOCTOR OF ENGINEERING
IN ENVIRONMENTAL ENGINEERING

GRADUATE SCHOOL
CHIANG MAI UNIVERSITY
JUNE 2019

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A THESIS SUBMITTED TO CHIANG MAI UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF ENGINEERING IN ENVIRONMENTAL ENGINEERING

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THIS THESIS HAS BEEN APPROVED TO BE A PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
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IN ENVIRONMENTAL ENGINEERING

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ACKNOWLEDGMENTS

The dissertation has been accomplished with kindliness of Asst. Prof. Dr. Patiroop Pholchan, Advisor and Asst. Prof. Dr. Thanongsak Chaiyaso, Co-advisor. I appreciate them to bring up the useful advices and consultative with carefulness. I feel deeply grateful and realize to their cordially intention and dedicate themselves to the purpose.

I am thankful for the Energy Policy and Planning Office: the Ministry of Energy, Department of Environmental Engineering: Faculty of Engineering, and The Graduate School Chiang Mai University to support research funding. I appreciate to Chiang Mai Fresh Milk to provide Napier Pak Chong1 grass along the study and I am really thankful Asst. Prof. Dr. Pruk Aggarangsi, Director of Energy Research and Development Institute-Nakornping, Chiang Mai University to contribute the apparatus and laboratory for proceeding the experiment including all my colleagues have supported any information until this dissertation absolutely complete.

This is truly grateful to my parents and relatives to cheer up me including appreciate professors in department of environmental engineering, faculty of engineering Chiang Mai University to cordially advise the helpful suggestion for the research. Eventually, to be really grateful to all participants and other not mention name herein who involve the achievement of this dissertation.

Pitchaya Suaisom

หัวข้อดุษฎีนิพนธ์	การใช้ประโยชน์อย่างบูรณาการในการผลิตพลังงานทดแทนจากหญ้าเนเปียร์ปากช่อง 1	
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บทคัดย่อ

งานวิจัยนี้เป็นการศึกษาการผลิตพลังงานทดแทนจากหญ้าเนเปียร์ปากช่อง 1 แบบบูรณาการ โดยเป็นการผลิตพลังงานทดแทนในรูปแบบก๊าซชีวภาพร่วมกับเชื้อเพลิงชีวภาพในรูปของบิวทานอล เพื่อเป็นทางเลือกเพิ่มหรือทดแทนการผลิตพลังงานทดแทนจากหญ้าเนเปียร์ปากช่อง 1 แบบดั้งเดิม ในการทดลองนี้ได้แบ่งออกเป็นสามส่วนดังนี้

ส่วนที่หนึ่งเป็นการศึกษาสภาวะที่เหมาะสมต่อการผลิตน้ำคั้นหญ้า เพื่อนำไปใช้ในเป็นวัตถุดิบเริ่มต้นในการผลิตก๊าซชีวภาพด้วยการประยุกต์ใช้กระบวนการผลิตเชื้อเพลิงแข็งร่วมกับการผลิตก๊าซชีวภาพของชีวมวล (IFBB) โดยการนำหญ้าเนเปียร์ปากช่อง 1 ผ่านการปรับสภาพด้วยวิธีไฮโดรเทอร์มอล และทำการแยกน้ำคั้นหญ้าด้วยกระบวนการทางกล จากนั้นทำการศึกษาศักยภาพการผลิตก๊าซชีวภาพ (BMP) ของน้ำคั้นหญ้า ส่วนกากหญ้าสามารถนำไปใช้เป็นเชื้อเพลิงแข็งได้ การศึกษาสภาวะที่เหมาะสมต่อการผลิตน้ำคั้นหญ้าได้ออกแบบการทดลองแฟคทอเรียลแบบเต็มจำนวน (Full Factorial Design) และการออกแบบการทดลองแบบส่วนผสมกลาง (Central Composite Design: CCD) โดยศึกษาถึงปัจจัยดังนี้ อายุการเก็บเกี่ยวหญ้าเนเปียร์ อัตราส่วนของหญ้าต่อน้ำ อุณหภูมิและระยะเวลาของการแช่หญ้า ต่อปริมาณสารอินทรีย์ในน้ำคั้นหญ้า จากการศึกษาพบว่า สภาวะที่เหมาะสม คือ อายุเก็บเกี่ยวหญ้าเนเปียร์ 75 วัน อัตราส่วนของหญ้าต่อน้ำเท่ากับ 1:6 โดยน้ำหนัก ทำการแช่หญ้าที่อุณหภูมิบรรยากาศ (อุณหภูมิน้ำแช่ประมาณ 25 องศาเซลเซียส) เป็นระยะเวลา 355 นาที ได้ค่าปริมาณสารอินทรีย์ในรูปซีโอดีเท่ากับ 226.42 กรัม คิดเป็นร้อยละ 71.5 ของค่าทำนาย (316.68 กรัม) และค่า

ของสัมประสิทธิ์จลนศาสตร์และศักยภาพการผลิตก๊าซมีเทนของน้ำคั้นหญ้ามีความสอดคล้องกับสมการ modified Gompertz ที่ระดับความเชื่อมั่น (R^2) 0.995 โดยมีค่าศักยภาพการผลิตก๊าซมีเทนเท่ากับ 412.18 มิลลิลิตรก๊าซมีเทนต่อกรัมสารอินทรีย์ในรูปของแข็งระเหยที่ป้อนเข้าสู่ระบบ มีค่าอัตราการผลิตก๊าซมีเทนสูงสุดเท่ากับ 51.47 มิลลิลิตรก๊าซมีเทนต่อกรัมสารอินทรีย์ในรูปของแข็งระเหยที่ป้อนเข้าสู่ระบบต่อวัน และ มีระยะเวลาพักของเชื้อจุลินทรีย์ เท่ากับ 4.36 วัน

สำหรับการทดลองในส่วนที่สองนั้น เพื่อศึกษาสภาวะที่เหมาะสมต่อการผลิตก๊าซชีวภาพของน้ำคั้นหญ้า โดยใช้ระบบเอบีอาร์ โดยปัจจัยที่มีผลต่อประสิทธิภาพการผลิตก๊าซมีเทนของน้ำคั้นหญ้าที่ได้ดำเนินการศึกษาได้แก่ อัตราภาระบรรทุกสารอินทีย์ รูปแบบการเติมน้ำคั้นหญ้า การเติมสารอาหารรอง และอัตราการหมุนเวียนน้ำกลับมาที่ระบบบำบัด จากผลการศึกษาพบว่า ค่าอัตราภาระบรรทุกสารอินทรีย์และอัตราการหมุนเวียนน้ำกลับมาที่ระบบเอบีอาร์ที่ค่าสูงกว่าค่าเหมาะสม จะส่งผลให้ประสิทธิภาพการผลิตก๊าซมีเทนมีค่าลดลง เนื่องจากน้ำออกจากระบบมีการปนเปื้อนของเชื้อตะกอนเพิ่มมากขึ้น และพบว่าเมื่อทำการเติมน้ำคั้นหญ้าเข้าระบบเอบีอาร์แบบกึ่งต่อเนื่องร่วมกับการเติมสารอาหารรองพบว่า ระบบเอบีอาร์มีความเสถียร โดยสภาวะที่เหมาะสมต่อการผลิตก๊าซมีเทนของน้ำคั้นหญ้า คือ ที่อัตราภาระบรรทุกสารอินทรีย์เท่ากับ 4 กิโลกรัมซีโอดีต่อลูกบาศ์กเมตรต่อวัน โดยมีรูปแบบการการเติมน้ำคั้นหญ้าเข้าระบบเอบีอาร์แบบกึ่งต่อเนื่องร่วมกับการเติมสารอาหารรอง และมีอัตราการหมุนเวียนน้ำกลับมาที่ระบบ (Q_R/Q) เท่ากับ 0.5 ซึ่งค่าอัตราการผลิตก๊าซมีเทนของน้ำคั้นหญ้าที่สภาวะมาตรฐานจะมีค่าเท่ากับ 0.49 ± 0.05 ลูกบาศ์กเมตรต่อกิโลกรัมของแข็งระเหยที่ป้อนเข้าสู่ระบบ ดังนั้นค่าอัตราภาระบรรทุกสารอินทรีย์ที่เหมาะสมต่อการการผลิตก๊าซชีวภาพจากน้ำคั้นหญ้าด้วยระบบเอบีอาร์ มีค่าเท่ากับ 4 กิโลกรัมซีโอดีต่อลูกบาศ์กเมตรต่อวัน

สำหรับการศึกษาในส่วนที่สาม เป็นการศึกษาสภาวะที่เหมาะสมต่อการผลิตบิวทานอลจากกากหญ้าที่ผ่านการปรับสภาพด้วยด่างโซเดียมไฮดรอกไซด์ โดยศึกษาถึงปัจจัยของพีเอชและความเข้มข้นของน้ำตาลต่อผลผลิตของบิวทานอล โดยใช้การออกแบบการทดลองแฟคทอเรียลแบบเต็มจำนวน (Full Factorial Design) และการออกแบบการทดลองแบบส่วนผสมกลาง (Central Composite Design: CCD) จากการทดลองพบว่า การปรับสภาพด้วยสารละลายด่างโซเดียมไฮดรอกไซด์ที่ความเข้มข้นร้อยละ 3 (โดยน้ำหนักต่อน้ำหนัก) สามารถกำจัดลิกนินได้ถึงร้อยละ 77 นอกจากนี้ยังพบว่า ปริมาณเอนไซม์ที่เหมาะสมต่อการผลิตน้ำตาลมีค่าเท่ากับ 2 มิลลิลิตรต่อกรัมหญ้าที่ปรับสภาพแล้ว โดยจะให้ค่าอัตราการผลิตน้ำตาลรีดิวซ์เท่ากับ 672 ± 23 มิลลิกรัมต่อกรัมหญ้าปรับสภาพ และเมื่อนำสารละลายไฮโดรไลเซทนี้ไปเป็นสารตั้งต้นในการผลิตบิวทานอลด้วยเชื้อคลอสตริเดียมสายพันธุ์ TISTR 1462 พบว่า สารละลายไฮโดรไลเซทที่พีเอชและความเข้มข้นน้ำตาลรีดิวซ์เริ่มต้นเท่ากับ 6.08 และ 43 กรัม

ต่อลิตร ตามลำดับ มีผลผลิตบิวทานอลสูงสุด โดยมีค่าเท่ากับ 0.135 + 0.002 กรัมต่อกรัมน้ำตาลรีดิวซ์ที่ถูกใช้ คิดเป็นร้อยละ 77.33 ของค่าทำนาย

Dissertation Title	Integrated Utilisation of *Pennisetum Purpureum* cv. Napier Pak Chong1 Grass for Renewable Energy Production	
Author	Miss Pitchaya Suaisom	
Degree	Doctor of Engineering **(**Environmental Engineering)	
Advisory Committee	Asst. Prof. Dr. Patiroop Pholchan	Advisor
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ABSTRACT

This research is the study of the integrated renewable energy production from *Pennisetum Purpureum* cv. Pakchong1 (Napier Pak Chong 1 grass). The produced renewable energies are biogas and biobutanol. The proposed method is an alternative or replacement for the conventional renewable energy production from Napier Pak Chong 1 grass. Experiments are divided into 3 parts as follow;

The first section was focused on the optimization of hydrothermal conditioning conditions for Napier PakChong1 grass to produce press fluid for biogas production. The integrated generation of solid fuel and biogas from biomass (IFBB) process was adopted to separate press fluid from the biomass. Napier PakChong1 grass was hydrothermally pretreated and then mechanically pressed. The press fluid was used for biochemical methane potential (BMP) test while the press cake could be utilized as the solid fuel. The full factorial design of experiment with center points and the Central Composite Design (CCD) were developed to obtain the best possible combination of harvesting time, grass to water ratio, temperature and soaking time for the maximum organic substance (as COD) in press fluid. It was found that the obtained model could satisfactorily predict the mass of COD in press fluid used as the model response. The optimum hydrothermal conditioning conditions were as follows; harvesting time 75 d, ratio of grass to water of 1:6 (by weight), ambient temperature (about 25°C) of the water and the soaking time of

355 min. The mass of COD obtained in these conditions was 226.42 g equating to 71.5% of the value predicted by the model (316.68 g). The microbial kinetic coefficients and biogas yield potential of press fluid at these optimum conditions were properly fitted with the modified Gompertz equation (adjusted $R^2 = 0.995$). The methane yield potential (P), the maximum methane production rate (R_m) and lag phase time (λ) were 412.18 $mlCH_4/gVS_{added}$, 51.47 $mlCH_4/gVS_{added}/d$ and 4.36 days, respectively.

The experiment in the second section was determined the suitable conditions for biogas production from press fluid using the anaerobic baffled reactor (ABR). Effects of factors, i.e. organic loading rates (OLRs), feeding schemes, trace element additions and effluent recirculation rates, on ABR performance were systematically investigated. The result shows that increase of OLRs and effluent recirculation rates adversely affected methane yields when capability of ABR in containing microorganisms was deteriorated. High stability of ABR performance was detected under the semi-continuous feeding scheme with trace element additions. The suitable condition for ABR was found at the OLR of 4.0 kg $COD/m^3.d$ under the semi-continuous feeding scheme with trace element additions at the effluent recirculation rate of 0.5 (Q_R/Q). At this condition, high methane yield (0.49 ± 0.05 $Nm^3/kg\ VS_{added}$) could be achieved using the economical ABR at relatively high OLR of 4.0 kg $COD/m^3.d$.

The third section was conducted in order to optimize the initial pH and sugar concentration of enzymatic hydrolysate from NaOH-pretreated Napier Pak Chong 1 grass press cake for butanol fermentation using 2-level full factorial design with center points and the Central Composite Design (CCD) of experiment. Up to 77% of lignin removal was observed when grass press cake was pretreated with 3% (w/w) NaOH. The suitable enzyme loading volume of 0.20 mL/g pretreated biomass was found when the pretreated press cake was hydrolyzed by commercial enzyme cellulose with the maximum reducing sugar yield of 627 ± 23 mg/g pretreated biomass. The enzymatic hydrolysate fermented in batch cultures of *C. beijerinckii* TISTR 1461 rendered the maximum butanol yield of 0.135 ± 0.002 $g/g_{reducing\ sugar\ utilized}$. The optimum initial pH and sugar concentration for butanol production were 6.08 and 43 g/L, respectively. At these conditions butanol production was 77.33% of the estimated butanol yield.

Keywords*:* Anaerobic baffled reactor, Butanol production, Hydrothermal conditioning, Napier grass, Pretreatment

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LIST OF ABBREVIATIONS

ABE	Acetone-Butanol-Ethanol
ABR	Anaerobic Baffled Reactor
Alk	Alkalinity
BBD	Box Behnken Design
BMP	Biochemical Methane Potetial
BOLR	Biological Organic Loading Rate
CCD	Central Composite Design
C/N	Carbon/Nitrogen
CSTR	Continuous Stirred Tank Reactor
FCOD	Filtered Chemical Oxygen demand
HRT	Hydraulic Retention Time
IFBB	Integrated Generation of Solid Fuel and Biogas from Biomass
LIHD	Low Input High Diversity Biomass
NDF	Neutral Detergent Fiber
NFE	Nitrogen Free Extract
OD	Optical density
OLR	Organic Loading Rate
RSM	Response Surface Methodology
RTD	Residence Time Distribution
SEM	Scanning Electron Microscope
SRT	Solid Retention Time
SS	Suspended Solids
STP	Standard condition of Temperature and Pressure
TCOD	Total Chemical Oxygen demand
TEs	Trace Elements
TKN	Total Kjeldahl Nitrogen
TP	Total Phosphorus
TS	Total Solids

UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acid
VOLR	Volumetric Organic Loading Rate
VS	Volatile Solids
VSS	Volatile Suspended Solids
WCD	Whole Crop Digestion

ข้อความแห่งการริเริ่ม

1) วิทยานิพนธ์นี้ได้ดำเนินการศึกษาการผลิตพลังงานทดแทนจากหญ้าเนเปียร์ปากช่อง 1 อย่างบูรณาการ โดยนำหญ้าเนเปียร์ปากช่อง 1 มาลดความชื้นด้วยเครื่องจักรกล เพื่อได้ผลผลิตเป็น 2 ส่วนได้แก่ ส่วนที่ 1 เรียกว่า น้ำคั้นหญ้า (Press fluid) ส่วนนี้จะมีส่วนประกอบของคาร์โบไฮเดรตและไนโตรเจนละลายอยู่ สามารถนำไปผลิตพลังงานทดแทนในรูปแบบก๊าซชีวภาพ โดยสามารถนำมาใช้ประโยชน์เป็นพลังงานไฟฟ้าหรือความร้อนได้ ส่วนที่ 2 เรียกว่า กากหญ้า (Press cake) ซึ่งส่วนนี้มีคุณลักษณะที่เป็นเซลลูโลส สามารถนำมาผลิตเป็นบิวทานอล ซึ่งเป็นพลังงานทดแทนในรูปแบบเชื้อเพลิงชีวภาพสำหรับยานยนต์ โดยบิวทานอลมีคุณสมบัติที่กัดกร่อนน้อยกว่า และมีค่าพลังงานสูง จึงส่งผลให้บิวทานอลมีคุณสมบัติที่โดดเด่นกว่าเอทานอล โดยกระบวนการนี้เป็นการผลิตพลังงานทดแทนที่เป็นมิตรต่อสิ่งแวดล้อม และลดการปล่อยของเสีย อีกทั้งยังเป็นการเพิ่มมูลค่าให้กับหญ้าเนเปียร์ปากช่อง 1 ซึ่งสามารถช่วยลดปัญหาของกระบวนการผลิตพลังงานทดแทนจากหญ้าเนเปียร์ปากช่อง 1 แบบใช้ทั้งต้นได้ ซึ่งแนวทางนี้สามารถนำไปประยุกต์ใช้กับวัสดุการเกษตรรวมถึงวัสดุเหลือทิ้งจากกระบวนการอุตสาหกรรมแปรรูป เป็นต้น

2) ผลการศึกษาของงานวิจัยทำให้ได้ค่าสภาวะที่เหมาะสมต่อการผลิตก๊าซชีวภาพจากน้ำคั้นหญ้า ได้แก่ ค่าอัตราภาระบรรทุกสารอินทรีย์ รูปแบบการป้อนน้ำเสีย ผลของสารอาหารรอง และค่าอัตราการหมุนเวียนน้ำทิ้ง สามารถใช้ในการออกแบบและเดินระบบก๊าซชีวภาพของน้ำคั้นหญ้า

3) ได้สมการการทำนายการผลิตเชื้อเพลิวชีวภาพไบโอบิวทานอลจากกากหญ้าด้วยเชื้อ *C.beijerinckii* TISTR 1461 ซึ่งสามารถใช้เป็นแนวทางในการพัฒนาการผลิตบิวทานอลต่อไปได้

STATEMENT OF ORIGINALITY

1. This thesis investigates the integrated renewable energy production from Napier Pak Chong 1 grass. The studied grass moisture is mechanically reduced in order to obtain 2 products, i.e. press fluid and press cake. The press fluid contains carbohydrate and nitrogen and can be used as the feedstock for biogas production for electricity or thermal energy generation. On the other hand, the press cake which is high in cellulose can be used for butanol production, which is the renewable vehicle fuel. This fuel is less corrosive and has higher energy than ethanol. The proposed concept for renewable energy production from Napier Pak Chong 1 grass is environmentally friendly, which is capable of reducing waste while offering the added value for the grass and is superior to the whole crop digestion concept. This novel concept can also be applied for the utilization of agricultural materials or residues from industrial processes.

2. The research results reveal optimum conditions, i.e. organic loading rate, feeding scheme, addition of trace elements and rate of effluent recirculation, for biogas production from the press fluid. These optimum conditions can be used for the design and operation of the biogas production system from the press fluid.

3. The equation for prediction of biobutanol production from the press cake by *C.beijerinckii* TISTR 1461 is attained. This is a fundamental basis required for biobutanol production development.

CHAPTER 1

Introduction

1.1 Research rationale

The energy consumption in Thailand has increased continuously by economic growth. According to Thailand energy situation during January-September 2018, consumption of refined petroleum products is the top demand comparing the other energy sources. The consumption proportions of refined petroleum products are 48.9%, with electricity (20%), renewable energy (9.3%), coal/lignite (8.5%), natural gas (6.8%) and conventional renewable energy (6.5%) added up to the total consumption (Ministry of Energy, 2019). These volumes are contrary to the energy from fossil fuel and crude oil available as the major source of energy production in Thailand. To tackle energy situations in the country, the Ministry of Energy develops Alternative Energy Development Plan 2015 (AEDP 2015) for minimizing energy deficiency and to ensure the security and stability of country's energy. This plan focuses on promoting renewable energy from regional raw material. The targeted consumption of renewable energy is 30% of the final energy consumption in 2036. The AEDP 2015 covers energy usages in forms of electricity, thermal, and biogas at percentages of 15-20, 30-35, and 20-25, respectively.

Targets of electricity generating using biogas from energy crop and wastewater or waste are set at 680 and 600 MW respectively. This target is equivalent to 6.52% of electricity generating from the entire renewable energy source within 2036. Furthermore, government also promotes the generation of biomass fuels for transportation sector, such as biodiesel, ethanol, compressed biomethane gas or CBG. It is obvious that renewable energy is the solution to reduce amounts of imported energy, lower the energy generating cost and comply with the carbon dioxide mitigation agreement. Plant or biomass is the smart alternative to be used as raw material for alternative energy generation. The growth of plant is activated by carbon dioxide gas and sunlight through photosynthesis process. The main product of photosynthesis was glucose which are collected in form of starch.

Thus, plants are able to be utilized directly as energy resources by chemical process and biochemical process. In addition, plant consumes carbon dioxide as the primary raw material in photosynthesis, which is beneficial for GHG mitigation. Renewable energy generation has been studied using various kinds of energy crop and biomass, such as biogas production from grass (Bedoić et al., 2019; Huang et al., 2019; Sawatdeenarunat et al., 2018; Rodriguez et al., 2017; Sawasdee and Pisutpaisal, 2014); biogas production from maize (Feng et al., 2018; Samarappuli and Berti, 2018; Ustak and Munoz, 2018; Arodudu et al., 2017; Maw et al., 2017; Chen et al., 2014; Oslaj et al., 2010); ethanol production from grass (Kou et al., 2017; Cardona et al., 2016; Menegol et al., 2016); ethanol production from maize (Eckert et al., 2018; Arodudu et al., 2017); ethanol production from sugarcane (Pina et al., 2017); buthanol production from grass (Nan et al., 2019; He et al., 2017a); or buthanol production from cassava (He et al., 2017b).

Napier Pak Chong1 grass (*Pennisetum purpureum* cv. Pakchong1) is a promising grass species in Thailand. This species of grass has prominent yield as harvesting gains approximately 70 tons per rai per year of fresh grass which is higher than other crops used as the animal food. This grass can also be easily harvested throughout the year and adapted to grow in various soil types. After planting, this grass can be harvested for the period of 6-7 years. Moreover, Napier Pak Chong1 grass contains more nutrition in forms of protein (8.64-12.64%), cellulose (24.91-40.05%), hemicellulose (21.57-35.69%) and lignin (5.38-7.14%) depending on the harvesting period (Lounglawan et al., 2014). According to its advantages, the government has promoted Napier Pak Chong1 grass as an energy crop particularly to generate biogas. However, process of biogas generating from Napier Pak Chong1 grass requires preparation of the large stacking area for silage, which is the pre-treatment step. To produce biogas from silage, the Continuous Stirred Tank Reactor (CSTR) installed with the agitating mechanism is normally used. This type of reactor is rather complicated and costly in the phases of both reactor construction and operation. Moreover, the digestate generally contains the cellulose component which is relatively recalcitrant. Napier Pak Chong1 grass can be used as solid fuel, known as briquette, after drying to produce thermal energy. However, the drying process requires both time and energy. In addition, under the combustion process, high nitrogen content

contained in Napier Pak Chong1 grass cannot be beneficially utilized but to produce some undesirable gases, e.g. NO_x.

Butanol is a product from fermentation process of *Clostridium* bacteria. This process is known as Acetone, Butanol, and Ethanol (ABE) fermentation. Butanol has been produced from agricultural biomass or agricultural waste, such as grass (Nan et al., 2019; He et al., 2017a), cassava (He et al., 2017b), palm oil waste (Razali et al., 2018; Shukor et al., 2014). Butanol is one of the most appropriate alternative fuels for utilizing as substitute liquid fuel for ethanol thanks to its higher quality. Butanol can be mixed with gasoline better than ethanol as the mixture does not require engine modification but still provide comparably good engine efficiency. The combustion of mixture of butanol and gasoline emits exhaust gas without carbon monoxide, hydrocarbon and nitrous oxide. Moreover, butanol is less corrosive so it can be distributed through the commonly used pipeline whereas ethanol cannot be stored for the long period owing to its high vapor pressure (Luo et al., 2017; Wackett, L. P., 2008). Besides, butanol is the main chemical used in several industries, such as paint industry, automotive industry, glass production industry, and detergent industry.

Even though renewable energy generating from various forms of biomass has been investigated, some efforts are done using only the single type of biomass. This practice, more often than not, fails to completely utilize biomass or provides relatively low yield. As the consequence, the integrated utilization of biomass to improve the conversion efficiency and provide some added values are required. The concept of this integrated utilization has been proposed in Germany by Buhle et al. (2001). Comparisons of life cycle analysis of the Integrated Generation of Solid Fuel and Biogas from Biomass (IFBB) and the Whole Crop Digestion (WCD) were made. IFBB extracted biomass into two components. The first component is in form of juice, which was used as the feedstock to produce biogas for the generation of thermal energy and electrical energy. The second component is in solid form or press cake, which was combusted to produce thermal energy. On the other hand, WCD process used entire crop for producing biogas which was turned to be thermal energy and electrical energy. Efficiencies of the entire process, energy products, GHG mitigation issues and acidity and eutrophication issues were

considered in Buhle et al. (2001) study. Buhle et al. (2001) reported that the gross energy of IFBB (91. 2- 94. 4 MWh/ ha) was greater than that of WCD (63. 4 MWh/ ha) corresponding to 57% and 50% of the internal energy of biomass, respectively. In addition, WCD contributed to more acidity and eutrophication problems than IFBB, owing to its ammonia emission in form of fertilizer and high viscosity residue.

In this work, the modified concept of IFBB (**Figure 1-1**) is applied for the utilization of Napier Pak Chong1 grass. The process produces two products, i.e. press fluid and press cake. The press fluid containing high amounts of carbohydrate and nitrogen is utilized to generate renewable energy in form of biogas. Instead of producing thermal energy from combustion process as suggested in the IFBB concept, the press cake containing mostly solids in forms of cellulose is used for producing butanol. This proposed modified IFBB process is expected to increase the benefit and provide the more sustainable utilization method for Napier Pak Chong1 grass. Moreover, it can be considered as the novel concept as there have been no similar studies conducted to investigate the utilization of biomass, especially grass, to produce renewable energy.

1.2 Objectives

1.2.1 To determine the optimum condition for producing press fluid from Napier Pak Chong1 grass using hydrothermal conditioning and mechanical dehydration process

1.2.2 To investigate effects of organic loading rates, feeding scheme, trace element additions and effluent recirculation rates on the performance of ABR for biogas production from press fluid of Napier Pak Chong1 grass

1.2.3 To determine the suitable bacterial strain and investigate effects of initial sugar concentrations and pH values on the efficiency of biobutanol production from the press cake of Napier Pak Chong1 grass

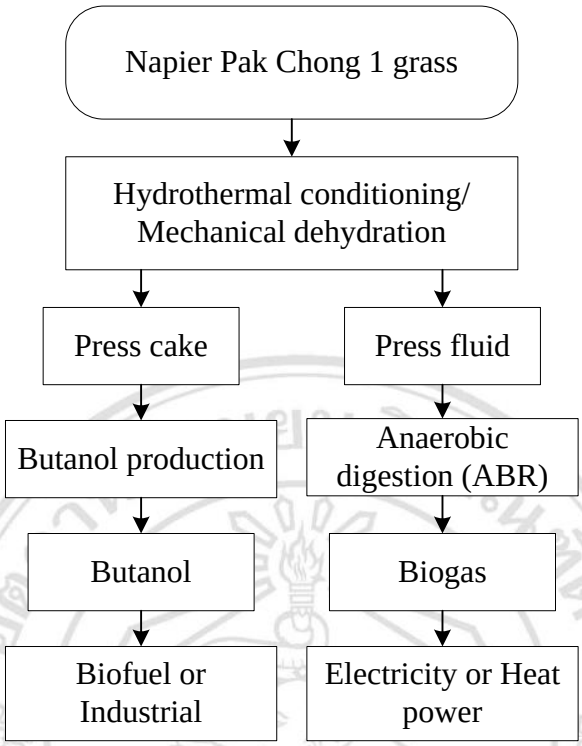


Figure 1-1: The integration utilization concept for producing renewable energy from Napier Pak Chong1 grass

1.3 Scopes of study

1.3.1 Part I: Determination of the optimum condition for hydrothermal conditioning and mechanical dehydration

In this part, optimization of the hydrothermal conditioning condition was investigated. Studied factors were harvesting time (30-60 day), solid: water ratio (1:3 to 1:6 kg:L), temperature (37-80°C) and mixing time (10-240 min). The conditioned grass was mechanical dehydrated by a screw press. The full factorial design of experiment with center points and the Central Composite Design (CCD) were utilized for determining optimum conditions.

1.3.2 Part II: Investigation of effects of organic loading rates, feeding scheme, trace element addition and effluent recirculation rates on the performance of an anaerobic baffled reactor (ABR)

In this part, suitable conditions for producing biogas from the press fluid obtained in part I were determined. Effects of organic loading rates (1-8 kg COD/m^3.d) and effluent recirculation rates (0.25 - 2.00) on the performance of an anaerobic baffled reactor were investigated using 37.5 L lab-scale ABRs with the dimension of 0.30 × 1.25 × 0.10 m.

1.3.3 Part III: Determination of the suitable bacterial strain and investigation of effects of initial sugar concentrations and pH values on the efficiency of biobutanol production

In this part, suitable bacterial strain and optimum conditions for biobutanol production from the press cake obtained in part I were determined. The studied bacterial strains were *Clostridium beijerinckii* TISTR 1461, *Clostridium acetobutylicum* TISTR 1462, *Clostridium beijerinckii* JCM 1390, *Clostridium acetobutylicum* JCM 1419 and *Clostridium beijerinckii* DSM 791. Effects of sugar concentrations (40 -60 g/L) and pH (5.5 – 6.5) on the efficiency of butanol production were investigated. The full factorial design of the experiment with center points and the Central Composite Design (CCD) were utilized for determining the optimum conditions.

1.4 Expected Results

1.4.1 The novel concept of integrated renewable energy production, i.e. biogas, solid fuel and biobutanol from Napier Pak Chong1 grass

1.4.2 The suitable organic loading rate and operational conditions for biogas production from grass liquor using anaerobic baffled reactor

1.4.3 The suitable bacterial strain and optimum conditions for butanol production from hydrolysate of cellulosic residues press cake

CHAPTER 2

Literature review

2.1 Anaerobic digestion

Anaerobic digestion is the process by which microorganisms break down biodegradable material under the anaerobic condition. Biogas (methane and carbon dioxide gas) is major products from anaerobic digestion. The process is illustrated in **Figure 2-1** and can be summarized as following;

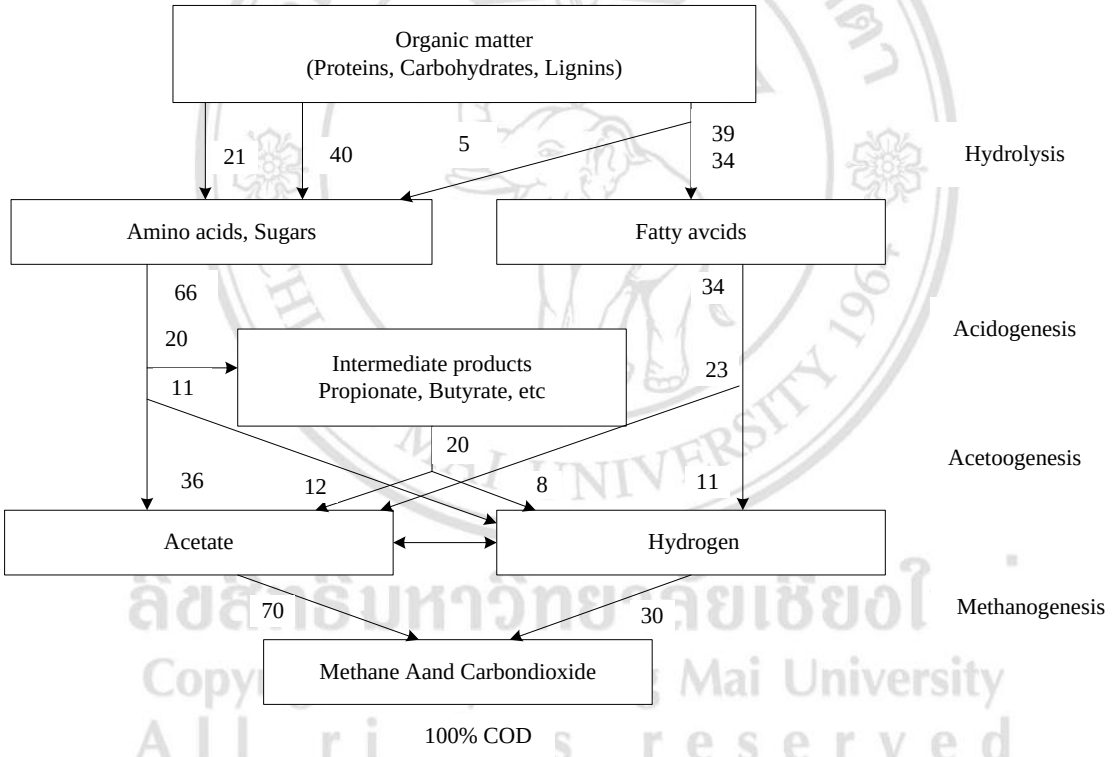


Figure 2-1: Reaction process for anaerobic digestion of organic matter (numbers mention to percentages, indicated as COD) (Gujer and Zehnder, 1983)

1. Hydrolysis

Hydrolysis is the first reaction in biogas process that large organic molecule or polymer such carbohydrate protein, fat and low decomposition rate of carbohydrate such Cellulose and fiber from crop such lignin and hemicellulose. These large molecules are decomposed by bacteria enzyme as extracellular enzyme and turn to small organic molecule. Group of Facultative Bacteria works for decomposition process and this bacteria group is classified by different enzyme such cellulitic, lipolytic, proteolytic. Decomposition process rate depends upon enzyme from bacteria. This enzyme is most specific in reaction by selecting reaction type and reactant type. The factors of enzyme reacting depend upon several factors such organic matter concentration, enzyme concentration, temperature, and enzyme and organic matter combination. The decomposition products from large molecule to small molecule or monomer are single sugar molecule, amino acid, and fat (Li et al., 2011). Breaking large molecule to smaller molecule is making better solubility in water and absorb into microorganism cell in saprophytes type. This step is called solubiliztion. Step of hydrolysis always happens as rate limiting step of total reaction due to this step has slower reaction rate than other that hydrolytic bacteria is to react (Ma et al., 2013; Luo et al., 2012). The optimal condition keeps pH around 6 and temperature range around 15- 20º C. At low temperature, hydrolytic bacteria has low digestion rate.

2. Acidogenesis

In the liquefaction step would produce dissolved compounds that supply in fermented bacteria cell after acidogenesis step. So, dissolved compounds are excreted as simple form of organic compounds such as volatile fatty acids, alcohols, lactic acid and mineral compounds such as carbon dioxide, hydrogen, ammonia and hydrogen sulphide gas (Meegoda et al., 2018). The various group of bacteria that mostly are anaerobes conduct in acidogenesis fermentation process. Nevertheless, some bacteria groups are able to do metabolise organic matter by the oxidative method. This is significant as dissolved oxygen probably become toxic to control anaerobic digestion such as the methanogens.

3. Acetogenesis

Acidogenesis products are transformed to the final products in methane production process such as acetate, hydrogen and carbon dioxide. As shown in **Figure 2-1**, a fraction COD approximate 70% originally appear in the influent that transformed to acetic acid. The electron donor remnants capability is intense in the formed hydrogen. Base on the oxidation phase of the original organic matter, the acetic acid formation probably accompanied by the formation of carbon dioxide or hydrogen.

4. Methanogenesis

Methanogenesis is frequently the rate limiting step in the entire digestion process even though at lower temperatures probably process hydrolysis. Methane is formed from acetate or carbon dioxide dropping by hydrogen that utilizing acetotrophic and hydrogenotrophic bacteria (.

Acetotrophic methanogenesis transformation:

$$CH_3COOH \longrightarrow CH_4 + CO_2 \qquad (2.1)$$

Hydrogenotrophic methanogenesis transformation:

$$4H_2 + CO_2 \longrightarrow CH_4 + 2H_2O \qquad (2.2)$$

The bacteria that produce methane from hydrogen and carbon dioxide is able to grow more rapid than the bacteria utilizing acetate (Henzen and Harremoues, 1983). Therefore, the acetotrophic methanogens are regularly rate limiting with considering to the transformation of complex macromolecules in wastes to biogas.

The different groups of bacteria are getting involved in the transformation of influent organic matter all active anaerobic and catabolic activity. Thus, compare to the releasing of the various fermentation products, new biomass is formed involved with the four transformation products as described above. As the first three processes are probably together and defined as acid fermentation whereas the fourth step is mentioned as methanogenetic fermentation.

Two crucial points have to focus to the different processes that occur during anaerobic digestion:

a) The removal of organic matter- COD during the acid fermentation is restricted to the releasing of hydrogen .As shown in **Figure 2-1**, only 30% of the organic matter is transformed to methane by the hydrogenotrophic method . Therefore, the vital condition for organic matter removal in an anaerobic treatment system is to apply an adequate number of acetotrophic methanogens developed.

b) Acid fermentation tends to lead pH dropping due to the production of volatile fatty acids and other intermediates that not involve in proton producing. Methanogenesis would get good improvement at neutral pH and instability might occur. As some reason, the acid removal rate by methane production is fallen after the acid production rate. The net acid production tends to reduce pH thus it probably decreases the methanogenic activity. This is called "souring" of the anaerobic treatment systems. The risk of souring could avoid by keeping the optimal balance between acidic and methanogenic fermentation. In fact, the system would rather have sufficient high of the methanogenic fermentation capacity and the buffer capacity.

2.1.1 Theoretical biogas production

The reaction of the anaerobic digestion process could be derived as in equation 2.3 (Kwietniewska and Tys, 2014).

$$C_aH_bO_cN_d + {}^1/_4(4a - b - 2c + 3d)H_2O \rightarrow {}^1/_8(4a + b - 2c - 3d)CH_4 + {}^1/_8(4a - b + 2c + 3d)CO_2 + dNH_3 \quad (2.3)$$

Nevertheless, it has to be focused that this equation does not take cell metabolism into account. If the composition of the organic material is known and provided that the entire material is transformed to biogas. The theoretical methane yield potential is able to calculated from Buswell's equation (as shown in eq.2.4). This uses to assess the specific methane yield in standard temperature and pressure (STP) and it is usually expressed as STP LCH_4/g VS.

$$B_{0,th} = \frac{\left(\frac{a}{2}+\frac{b}{8}-\frac{c}{4}\right)22.4}{12a+b+16c}\left(STP\,\frac{lCH_4}{gVS}\right) \quad (2.4)$$

Where, 22.4 is the volume of 1 mole of methane gas at STP

The theoretical methane yield could be calculated from the chemical oxygen demand (COD) of the substrate. Biogas production as associated to COD is approximate 0.5 L/g COD removed and accordant to a methane production as approximate 0.35 LCH_4 per g of COD removed (as shown in equation 2.5).

$$COD_{substrate} \times 0.35 \left(STP \frac{lCH_4}{gCOD_{substrate}}\right) = theoretical\ methane\ yield\ (lCH_4\ gVS^{-1}) \quad (2.5)$$

The COD of methane shows as 4 mg/mg CH_4 and independent energy is released from the methane oxidation per 1 g COD as 12.52 kJ. COD that such a good indicator of the degradation during process.

2.1.2 Significant environmental factors

The significant environmental factors affecting anaerobic digestion are following;

2.1.2.1 Temperature

Anaerobic digestion strongly depends upon temperature. The anaerobic degradation process is strongly influenced by temperature and the microorganisms are able to classified into psychrophilic (5-15˚C), mesophilic (35-37˚C) and thermophilic (50-55˚C). Anaerobic reactors are regularly operated in the mesophilic and thermophilic condition (Meegoda et al., 2018). Mesophilic processes are necessary working in extended hydraulic retention and unable to eliminate pathogenic microorganisms. Thermophilic anaerobic digestion becomes an interesting option in mesophilic digestion due to the high growth rates of the related bacteria. However, the effect of temperature on the performance of anaerobic digestion is essential to consider with the economic assessment. The influence of temperature on anaerobic digestion is not restricted to the rate of the process.

2.1.2.2 pH

The optimal pH for methane producing microbes is 6.8-7.2 whereas the optimal value for acid-forming bacteria is approximate 6 (Speece, 1996). The growth rate of methanogenic microbes dropped absolutely below pH 6.6. The aceticlastic methanogens have more sensitive to low pH than the hydrogenotrophic methanogens. In

mono stage of anaerobic treatment process, the pH is generally kept in better optimal conditions for methanogens to prevent the predominance of acid-forming bacteria that causes VFAs accumulation.

2.1.2.3 Alkalinity

Alkalinity is the significant parameter in anaerobic digestion systems that is used for measuring of the chemical buffering capacity of the aquatic solution. It is essential that the reactor contents provide sufficient buffering capacity to neutralize VFA accumulation in the reactor and to maintain pH (6.8-7.2) for stability the operation. Bicarbonate or carbonic acid, hydrogen sulphide, dihydrogen phosphate and ammonia are the chemical compounds that provide a significant buffering capacity in the useful region around pH 7. The presence and concentration of a buffering compound depends upon the composition of the substrate and the total organic load. As if pH in an anaerobic bioreactor has dropped, that feeding would be stopped and the buffering capacity would increase. The optimal bicarbonate alkalinity range should be in 2,500-5,000 mg/L as $CaCO_3$. It is explained that the ratio of volatile fatty acids and alkalinity lower than 0.4 contributes good buffering capacity in anaerobic system.

2.1.2.4 Volatile fatty acid

The concentration of VFAs is one of the most significant parameters to monitor the anaerobic digestion process. VFA build-up is the result of unbalanced digestion conditions. So, pH dropping accompanying the VFAs accumulation is the sign of toxicity and reactor failure in the anaerobic digestion process. As the reason the toxicity of VFAs has depended upon pH since only the nonionized forms are toxic to microorganisms. VFAs are toxic at they exist in protonated forms, as they are able to penetrate in cell membrane. When the cells are attacked at pH around 7, they are ionized and the hydrogen ion released causing a reduction in the intracellular pH (Björnsson et al., 2000).

2.1.2.5 Nutrient

All organisms need vital ingredients for their growth, macronutrients and trace elements, and a lack of these nutrients negatively affect their growth. Nutrients such as nitrogen and phosphorus, and trace elements (sulphur, potassium, calcium, magnesium, iron, nickel, cobalt, zinc, manganese and copper) are required for efficient process in anaerobic degradation. They usually need in sufficient amounts in wastes that are treated in anaerobic digesters (Rajeshwari et al., 2000).

2.1.2.6 C/N ratio

The factor that is inseparable with pH is carbon and nitrogen ratio (C/N ratio). The optimal C/N ratio for anaerobic digestion of organic waste is presented as 20-35. A high C/N ratio indicates a rapid consumption of nitrogen by the methanogens and results to produce lower gas yield. Otherwise, low C/N ratio causes ammonia accumulation and pH values go exceeding 8.5 that is toxicity to methanogenic bacteria. The optimal C/N ratio of the feedstock substrates could be attained by mixing waste in low and high C/N ratio, such as organic solid waste mixed with sewage or animal manure.

2.1.2.7 Organic loading rate

The organic loading rate (OLR) is the quantity of organic matter fed per unit volume of the digester per unit time, (e.g., kg VS/L/day). OLR plays the significant role in anaerobic wastewater treatment in continuous systems and is a useful criteria for evaluating reactors performance. Feeding the system with sustainable OLR results in low biogas yield due to of inhibitor matter accumulation in the digester slurry (i.e. fatty acids) and the feeding rate of the system would be reduced.

2.1.2.8 Retention time

Retention time is the required time to achieve the absolutely degradation of the organic matter. The retention time varies with process parameters, such as process temperature and waste composition.

2.1.2.9 Mixing

Mixing would assist to improve the contact between the microorganisms and substrate and bring up the ability of bacterial to obtain nutrients. Mixing also prevents the formation of scum and temperature gradients enhancement in the digester. Nevertheless, excessive mixing could ruin the microorganisms and the system prefers slow mixing. In case of co-digestion, the different feedstocks should be premixed before feeding into the digester to ensure sufficient homogeneity.

2.1.2.10 Toxic substances

Inhibition of the anaerobic digestion process could interfere to varying degrees by toxic materials in the system. These substances are probably components of the influent wastewater, or by products of the metabolic activities of the digester bacteria. The toxic inhibitory compound that affect to the anaerobic microorganisms was illustrated in **Table 2-1**

Table 2-1: The highest concentration of toxic inhibitory compound that are not inhibit to the anaerobic microorganisms (McCarty, 1964)

Toxic substances	Concentration (mg/L)	Toxic substances	Concentration (mg/L)
Cu	1.0	Na^{+}	3,500
Zn	5.0	K^{+}	2,500
Cr^{6+}	5.0	Ca^{2+}	2,500
Chloride	15,000	Mg^{2+}	1,000
Cr^{3+}	2,000	Averylonitrite	5.0
Total chromium	5.0	Benzene	5.0
Ni	2.0	CCl_4	10
Cd	0.02	Chloroform	0.1
S^{2-}	100	Pentachlorophenol	0.4
SO_4^{2-}	500	Cyanide	1.0
Ammonia	1,500		

2.1.3 Anaerobic baffled reactor (ABR)

The ABR has been developed since early 1980s by McCarty and co-worker at Stanford University, USA (Barber and Stuckey, 1999). The ABR can be described as a series of Upflow Anaerobic Sludge Blanket (UAS) for it can be divided into a few compartments.

2.1.3.1 Design of the Anaerobic baffled reactor

The ABR is a reactor design which uses a series of baffles to force a wastewater containing organic matter to flow under and over (or through) the baffles as it passes from the inlet to the outlet (McCarty and Buchmann, 1992). Bacteria within the reactor gradually arise and settle by flow pattern and gas production. However, as if there are more activities of settlement, the reactor would be a slow rate. The variation of the ABR as shown in **Figure 2-2**.

2.1.3.2 Hydrodynamics of ABR

1) Flow patterns

Grobicki and Stuckey (1992) studied the hydrodynamic characteristics of the ABR by using tracer studies (Li^+). The study is shown that the empty reactor had the hydraulic dead space approximate 8% by volume whereas with 8 g VS/L affected to increase to 18% and the dead space did not vary with HRT.

2) Effect of effluent recycle

The effluent recycling tends to reduce removal efficiency due to the reactor meets a completely mixed system. Therefore, the mass transfer and driving force for substrate removal have reduced even a few enlargements in the loading rate. Chynoweth et al. (1980) illustrated that methane yield expanded more than 30% when 20% of effluent was recycled. The reused effluent assisted to solve the low pH issues that cause by high volatile fatty acids level at the front of the reactor, and break gelatinous bacterial growth at the reactor inlet for the treatment of a complex protein carbohydrate wastewater (Bachmann et al., 1983). The other beneficial of recycle is to dilute some toxicants and decrease substrate inhibition in the influent (Bachmann et al., 1985;

Grobicki and Stuckey, 1991). Nachaiyasit (1995) illustrated that dead space was increased double approximate 40% when the recycle ratio was raised up from zero to 2. The beneficial of recycle depended on the type of waste being treated. Advantages and disadvantages of effluent recycle were shown in Error! Reference source not found.**-2**

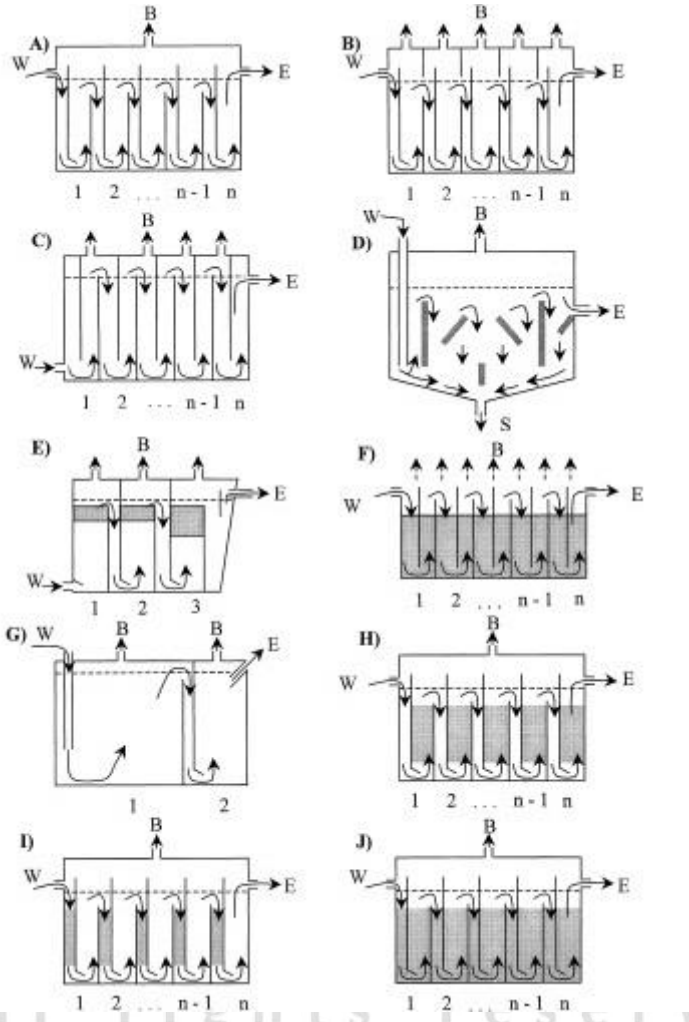


Figure 2-2: Variations of the anaerobic baffled reactor. (A) Single gas headspace, (B) Individual gas headspace, (C) Vertical, (D) Horizontal, (E) Hybrid with settling zone, (F) Open top, (G) Enlarged first compartment, (H-J) various packing arrangements: (H) Up-corner, (I) Down-corner, (J) Entire reactor. Key: W=Wastewater, B=Biogas, E=Effluent, S=Solids, (shaded areas represent random packing) (Barber and Stuckey, 1999)

Table 2-2: Advantages and disadvantages of effluent recycle (Liu et al., 2010a)

Advantages	Disadvantages
1. pH raise up	1.The entire efficiency dropped
2. To drop the influent toxicity and substance inhibition	2. Increasing of solids loss
3. Possibly higher loading rates	3. Increasing of hydraulic dead space
4. Better substrate/biomass contact	4.To interrupt of bacterial communities and bioflocs
	5. Encourages one-phase digestion

2.1.3.3 Advantages and Disadvantages of the ABR

The ABR shows various advantages surpass other high-rate reactor system. The major significant advantage is to separate acidogenesis and methanogenesis longitudinally and to allow the reactor behave as two-phase system independent control and high costs.

The reactor is simply designed without remove parts or mechanical mixing that make it is affordable and no requirement unusual settling properties for biomass. Sludge generation is low and the SRT is high. This attained without to be fixed to media particles of biomass or a solid-settling chamber especially gas separation is not required.

Since the HRT and SRT are separate, increased volumes of wastewater can be treated, relative to a continuous stirred tank reactor (CSTR) where the HRT equals the SRT. Intermittent operation is possible, which would facilitate treatment of seasonal wastewater. ABR is applied for making stable to hydraulic and organic shock loads and the reactor configuration utilizes to protect toxic to interrupt the biomass compounds in the influent (Barber and Stuckey, 1999).

The disadvantages of the ABR are similar to the other anaerobic digestion. One possible disadvantage of full scale of ABR is the requirement to build shallow reactors to maintain acceptable liquid and gas upflow (Barber and Stuckey, 1999).

2.1.4 Literature Reviews of Hydrothermal conditioning and mechanical dehydration and biogas production from press fluid

Corton et al. (2014) studied the optimization of press fluid pre-treatment on the integrated generation of solid fuel and biogas from biomass (IFBB) process. IFBB process is an innovative approach to maximize energy conversion from low input high diversity (LIHD) biomass. This study compared the effects of pre-treatment and non pre-treatment on the press fluid in IFBB process. To use water pre-treatment by pouring 5°C of water on the silage through pipeline on the top of the screw press augur. Two treatment processes were designed to concentrate the press fluid that used to evaluate on productivity in an IFBB system. The results illustrated the concentrated press fluid and water were not added during the process, energy production from methane was increased by 75% per unit time. In addition, the productivity of the solid fuel was increased by 80% per unit of fluid product.

Jia et al. (2013) studied the efficient extraction method to gather sugar from sweet sorghum for ethanol production. Sweet sorghum comprises of a sugar-rich juice that enable to produce ethanol. The conventional extracting method sugar from sweet sorghum is to press the stalks through a roller mill. Their work provided an efficient water extraction method that could collect as much available sugar. The result exposed that operating parameters i.e. temperature, stalk size and solid-liquid ratio influenced to the rate of sugar release and the maximal amount recovered with low water consuming. The most desirable conditions included 30°C, 0.6 ratio of solid to liquid (w/w), which collected 90% of the available sugar.

King et al. (2012) studied the influence of hydrothermal conditioning, detergent and mechanical pressing in separating the rich fiber in press cake from grass silage. The results illustrated that to do washing up to couple steps and mechanical dehydration up to 3.0 MPa would attain the best treatment for removing soluble stuff from grass silage and also separate rich fiber press cake. Furthermore, mechanical pressure raised up that affect to high quantity of pressed juice from grass silage and the DM concentration of the press-cake also expanded. While hydrothermal conditioning, water temperature was raising in the range of 20-60°C that showed a few effect on soluble removal from grass silage. Most

of (0.85) solubles excite to remove at 20°C. Moreover, adding detergent while hydrothermal conditioning would gain a few positive responses on proportion of removed solubles.

Abu-Dahrieh et al. (2011) studied batch and continuous biogas production from grass silage liquor. The grass silage liquor was prepared by Irish Agricultural Institute 'Teagasc', Grange Research Centre, Dunsany, Co., Meath Ireland. The results of BMP experiment illustrated that the optimal substrate/inoculum ratio is approximately 1 with a maximum methane yield of 0.385 m^3/kg COD. The continuous process was carried out in armfield digester (upward-flow packed bed reactor) with an OLR ranging from 0.851 to 1.77 kg COD/m^3.d. The result of continuous process explained that when the OLR has increased, the methane yield would decrease in the reactor with silage influent only. Whereas in the reactor with recirculation of the effluent, methane yield would increase. Consequently, the COD removal from both digesters was equally at the same OLR. Therefore, grass silage liquor could be successfully converted to biogas by using anaerobic digestion.

Kuila et al. (2011) studied the optimal process for fluid extraction of reducing sugar from cashew apple bagasse by varying the ratio of liquid: solid (mL/g), pH, incubation time and temperature. Response Surface Methodology (RSM) based on Box Behnken Design (BBD) was applied in the extraction process. The optimal conditions were shown as following liquid: solid ratio 3.25 (mL/g), pH 6.42, incubation time 6.30 h and temperature 52.27°C and the result of the reducing sugar yield was 56.89 (g/100 g dry substrate)

Hensgen et al. (2011) studied integrated solid fuel and biogas production from landscape chopped green material in private households (IFBB). IFBB is to subject biomass to hydrothermal conditioning and use a screw press for dehydration. A press fluid was used for biogas production and a press cake was used as solid fuel for combustion. After chopping and ensiling the green material, the sample material involved in the hydrothermal conditioning process at proportion of solid to water as 1:4 (raw material: water) and ongoing stirred for 15 minutes. Temperatures at 40 and 60°C were investigated in hydrothermal condition. The results illustrated that mass transfer in press

fluid at 40 and 60°C was not different. The IFBB process produced a high biogas substrate as well as a good quality of solid fuel with reduced content of K, Cl, Mg and P. Hydrothermal conditioning at 40°C showed higher energy efficiencies compared to 60°C. The net energy yield of IFBB at 40°C range between 1.96 and 2.85 kWh/kg dry matter and for directly combustion between 1.75 and 2.65 kWh/kg dry matter.

Richter et al. (2011) studied grass silage and IFBB process regarding the effect of sward stuff maturity and pre-conditioning in fermentation temperature (10-90°C). The result illustrated that raising up temperature of hydrothermal conditioning let mass flows of press fluid grow up including to reduce concentration of hazardous matter for press cake combustion. So, element concentration would be reduced by increasing of sward stuff maturation. The properties of press cake such mass flows and element concentration were intensely influenced by temperature adjustment likewise concentration of neutral detergent fiber (NDF) and dry matter in the silage (R2 from 0.70 to 0.99). The most optimal for combustion specific considered the late sampling date press cake.

Kaparaju et al. (2009) the biorefinery concept for producing biogas, bioethanol, and biohydrogen from wheat straw. The initial process conducted to pretreat wheat straw hydrothermally to break cellulose that rich of fiber fraction and hemicellulose rich liquid fraction (hydrolysate). Then to do enzymatic hydrolysis and obtained cellulose yielded 0.41 g-ethanol/g-glucose whereas hydrolysate as dark fermentation produced 178 mL-H_2/g-sugars. Methane yield from the effluent in batch experiment at 55°C was produced as 0.324 and 0.381 m^3 CH_4/kg VS by bioethanol and biohydrogen process respectively.

Richter et al. (2009) studied the anaerobic digestion of press fluids and the influential condition in hydrothermal conditioning and mechanical dehydration. The whole-crop silages from five locations of grassland were collected for the experiment. Hydrothermal conditioning at temperatures of 5, 60 and 80°C was undergone for press fluid production that would be supplied as substrates in anaerobic digestion. At the condition in batch experiment in a mesophilic temperature (37±1°C) with 15 minutes of mixing time cycle every 3 hours. Consequently, the concentration of crude protein from press fluids was higher than the silage whereas the crude fiber concentration was lower. The methane yield of press fluid from batch digestion test contributed double level as

397-426 NL CH_4/kg VS after 13 days and for the whole crop silage from grassland showed 218 NL CH_4/kg VS after 27 days. The average of OM in press fluid was 0.89 would be transformed to biogas and there was no effect of larger temperature during condition process. The degradation degree of whole-crop silage was range 0.36 to 0.73 significantly.

Wachendorf et al. (2009) studied the influence of hydrothermal conditioning and mechanical dehydration in organic mass flows, plant's mineral component, and nutrient balances of semi-natural grassland. Including integrated generation of solid fuel and biogas from biomass (IFBB). As this study, to chop the five species of semi-natural grass into 5 cm. and ensiling for 3 months. Hydrothermal conditioning attained by combing in the ratio of silages and water was 1:4 (w/w) in vary temperature (5, 60, and 80°C) in the 200 L of concrete container. The admixture was stored at the constant temperature controlling by gas burner and stirred by 15 minutes until silage rinse water. The process of mechanical dehydration, silage was undergone a screw press for producing press fluid and press cake. The result explained that 0.18 of crude fiber would be turned into fluid whereas the digestible organic substance as crude protein and nitrogen-free extract (NFE) provided mass flows as 0.40 and 0.31 respectively. The hazardous element in combustion such K and Cl were transformed into press fluid with average mass flows 0.77 and 0.86 respectively. This to enhance beneficial in combustion. Mass flows of press fluid and mineral compound evolved when conditioning temperature raised up from 5 to 60°C or from 5 to 80°C.

Reulein et al. (2007) studied biomass utilization efficient in mechanical dehydration of silages. The result illustrated that mechanical dehydration conducted to maize and wheat silages by screw press, this would decrease ash and N contents in press fluid whereas dry matters of stuff material were raised up. More adding fluid prior the dehydration enabled to evolve press fluid quality that beneficial for solid fuel production. This to contribute to reduce ash and N content in press solid. The result illustrated the high fermentable rate of organic matter in press fluids that occurred in short period. The organic matter in press fluid was decomposed 80-90% within the first three days. Press

fluid from whole crops of maize and wheat straw brought methane yield as 360 and 500 NL CH_4/kg VS, respectively.

2.1.5 Literature review of Anaerobic baffled reactor (ABR)

Xu et al. (2014) studied the optimal compartment and flow patterns of ABR. Four reactors with each effective volume as 56.4 L had been designed individual with 3, 4, 5 and 6 compartments. They were used for investigating the plow pattern in ABR and to proceed the tracer pulse stimulus-response technique and cold-model test. The result illustrated that the dead space decreased whereas the ABR compartments increased. The dead space was excited by biomass and hydraulic aspect in 3-, 4-, 5- and 6-compartment ABRs were 6.40, 5.00, 3.30 and 3.00% respectively. ABR compartment increasing affected to back-mix reducing and 1/Pez values (from 0.118 to 0.060). So, this condition let fluid in reactor approached to the plug flow state. The theoretical optimal number of ABR compartments was explained that the series number (N) of compartments would be kept at $4<N<5$ when the removal efficiency of the reaction was 50% and when the removal efficiency of the reaction system was 90% the number was $4<N<6$. The series value N of the ABR compartments would be retained at 4 or 5 when the operating performance and economic factors of the reactor were considered.

Krishma G.G.V.T. (2013) studied ABR utilization for low capability soluble wastewater treatment. Wastewater was produced as synthetic waste that comprised of 450 mg of sucrose COD/L, and 50 mg of peptone COD/L and operated in 10 L liquid volume of ABR. The reactor located in the wooden chamber with temperature controlling at $30\pm1^{\circ}C$. The operating was at hydraulic retention times (HRTs) of 20, 16, 12, 8, 6, 10, 8 and 6 hours. Organic loading rates (OLRs) were 0.60, 0.75, 1.0, 1.5, 2.0, 1.2, 1.5 and 2.0 kg COD/m^3.d. At HRT 10, 8 and 6 hours achieved Pseudo steady state (PSS). COD and BOD removal were attained at least 89% at OLRs range 1.2 to 2.0 kg COD/m^3.d. Raw wastewater COD more than 55% derived by mass balance would be anticipated to recover in gas phase. Due to acidogenesis and acetogenesis, pH was fallen 7.8 to 6.7 and VFA 54 to 98 mg/L was produced in the first compartment. The concentration of VFA dropped in vertical direction deep in the reactor conversely pH raised up in vertical direction deep in

the reactor. The study of residence time distribution (RTD) exposed that the flow pattern in ABR behaved neither completely plug-flow nor perfectly mixed.

Motteran et al. (2013) studied the biological effluent treatment of swine farm and ABR aspect in the first stage of treatment. Total effective volume of 6.06 m^3 ABR combined three chambers. To define the average of VOLR was 17.8 kg COD/m^3.d., the biological organic loading rate (BOLR) based on total and COD_f were 1.3 kg COD_{total}/kg TVS.d and 0.98 kg $COD_{filtered}$/kg VS.d respectively, and the hydraulic loading rate (HLR) were approximate 1.4 m^3/m^3.d. The results illustrated that the average removal efficiency for TCOD was 80% at 18 hours of hydraulic retention time (HRT). The average removal efficiencies for oil/grease and total soluble solids were 41% and 78% respectively. To find the difference of sludge and biogas production in ABR in the first and the third compartment that each chamber had different microbial consortium.

Tawfik et al. (2013) studied the utilization of mesophillic ABR for producing hydrogen and methane from starch wastewater. The reactor condition operated at varied HRT 72, 48, and 24 hours and followed by OLRs 7.4, 7.6 and 22 kg COD/m^3.d respectively. The results explained that COD and BOD_5 removals enlarged as 40% and 50% respectively in the condition of OLR 22 kg COD/m^3.d. At HRTs 24 and 72 hours derived the percentage of COD recovering gas phase of methane showed as 56.7% and 43% including obtained average of methane yield as 0.29 and 0.30 m^3 CH_4/kg $COD_{removal}$ respectively. As ratio of BOD_5/COD was increased from 0.59 to 0.66 that affected to the removal efficiency of BOD_5 from 40% to 54% respectively.

Malakahmad et al. (2011) studied a modified anaerobic baffled reactor and performance in high intense wastewater treatment. Due to uncertain determination of HRT and OLR, the major issue was the management of the high strength stuff that hard to break fat, protein, and hydrocarbon molecules in the primary phase of anaerobic decomposition in ABR. The 50 L of modified 4-compartment ABR was designed to define the system efficiency and methane production rate of severe intense wastewater at the varied HRT (5, 4, 3 and 2 days) and OLR (2, 3, 4, 5 and 6 kg/m^3.d). The first compartment was designed as double in size to support the extension of solid retention time. As the condition C/N ratio 30, a mixture as 62% kitchen waste and 38% sewage

sludge was input as substrate. The result illustrated that the most COD removal as 74.5 and 75.4% were monitored at HRT 3 days and OLR 2 kg/m^3.d respectively. The best production of biogas derived 7.40 and 9.10 L/day were observed at HRT 5 days and OLR 6 kg/m^3.d respectively.

Ferraz et al. (2009) studied ABR performance in cassava wastewater treatment. ABR was designed as separation in four individual compartment in total volume 4 L in operating condition at 35°C. Wastewater COD was varied from 2,000 to 7,000 mg/L and it was beneficial as the most optimal hydraulic retention time (HRT) for COD removal. The buffering potentiality of ABR worked for reducing acidity in the compartment causing alkalinity and pH were raised up. Cassava wastewater could be treated by removing 92% of COD in ABR and working in the condition of COD concentration varied from 2,000 to 5,000 mg/L at HRT 3.5 days. When COD was 7,000 mg/L in the similar HRT the system efficiency would drop to 83%.

Movahedyan et al. (2007) studied the assessment of system performance of industry wastewater of wheat starch process by anaerobic baffled reactor treatment. The supernatant of the wastewater after the simple gravity sedimentary was used as feeding. The ratio of COD: N: P as 300:5:1 was maintained in the operation by adding NH_4Cl and K_2HPO_4. The working volume of ABR 13.5 L with 5-compartment was applied in the experiment. The reactor had working condition at hydraulic retention time of 72 h at 35°C and primary organic loading rate of 1.2 kg COD/m^3.d contributed 61% COD removal efficiency. The greatest reactor performance got ahead with an organic loading rate of 2.5 kg COD/m^3.d whereas hydraulic retention time of 2.45 days and the COD conversion 67% was accomplished.

2.2 Butanol production

Butanol (butyl alcohol and 1-butanol) is a primary alcohol with a 4 carbon structure and having the molecular formula of C_4H_9OH (MW 74.12). It is sometimes called biobutanol when produced biologically. It is the component of the higher alcohols and branched-chain alcohols. It is heated at boiling point 117.7°C and melts at -89.3°C, a specific gravity of 0.8098 g/cm^2 at 20°C. Even more it is soluble in water (**Table 2-3**).

There are four possible isomeric structures for butanol from a straight-chain primary alcohol to a branched-chain tertiary alcohol. Butanol is a colorless liquid with a different odor and its vapor has an irritant effect on mucous membranes and a narcotic effect in higher concentrations.

Table 2-3: Properties of butanol (Mužíková et al., 2014)

Properties	1-Butanol
Melting point (˚C)	-88.6
Boiling point (˚C)	117.7
Viscosity at 25ºC (mm^2/s)	2.6
Flash point (˚C)	36.17
Density at 15ºC (kg/m^3)	809
Reid vapour pressure (kPa)	2.3
Solubility of water at 30ºC (w/w, %)	287

Butyl acrylate and methacrylate esters as kind of butanol were used in latex surface coating, enamels and lacquers and also butanol are butyl glycol ether, butyl acetate and plasticizers. Butanol is also an excellent diluent for breaking fluid and use as solvent for manufacturing process of antibiotics, vitamins and hormones. The significant application of butanol that use as replacement of gasoline or as fuel additives. Butanol occupies similar characteristics to gasoline and directly use in any gasoline engine without modification and/or substitution (**Table 2-4**). From the Error! Reference source not found., butanol is superior to ethanol as a fuel additive that higher energy content, lower volatility, less hydroscopic and less corrosive.

Table 2-4: Comparison fuel properties (Mužíková et al., 2014)

Properties	Butanol	Fuels		
		Gasoline	Ethanol	Methanol
Energy density (MJ/L)	29.2	32	19.6	16
Air-fuel ratio	11.2	14.6	9	6.5
Heat of vaporization (MJ/kg)	0.43	0.36	0.92	1.2
Research octane number	96	91-99	129	136
Motor octane number	78	81-89	112	104

2.2.1 Butanol Production

2.2.1.1 Chemical synthesis of butanol

There are three most significant processes for the chemical butanol synthesis in industry part: oxo systhesis, reppe systhesis and crotonaldehyde hydrogenation (**Figure 2-3**).

In oxo synthesis as hydroformylation, carbon monoxide and hydrogenare added to a carbon-carbon double bond using catalysts such as Co, Rh or Ru substituted hydrocarbonyls. Aldehyde mixtures are derived in the first reaction that is followed by hydrogenation for the production of butanol. Propylene, carbon monoxide and water are reacted in the presence of a catalyst in the Reppe process (Bochman et al., 1999). The Reppe process directly produces butanol at low temperature and pressure. This process is not commercially successful because of process technology is costly. The crotonaldehyde hydrogenation has been occurred for 20 years and the process consists of aldol condensation, dehydration and hydrogenation (Bochman et al., 1999).

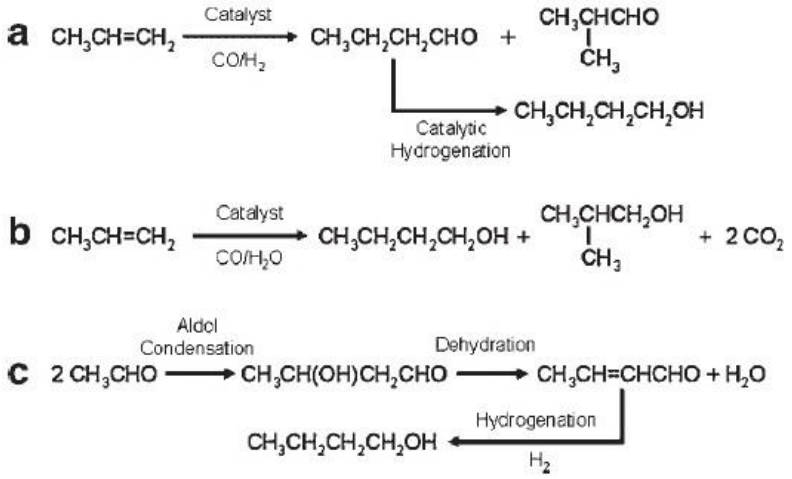


Figure 2-3: Chemical synthesis of butanol, (a) Oxo synthesis, (b) Reppe synthesis and (c) crotonaldehyde hydrogenation (Lee et al., 2008)

2.2.1.2 Biological butanol production

Biological production of butanol has described a long history. Acetone-butanol-ethanol (ABE) fermentation process is available. In 1945, two thirds of industrially used butanol was derived by fermentation in the U.S. However, the ABE fermentation process had lost competitiveness by 1960s due to feedstock costs raising up and development of the petrochemical industry except Russia and in South Africa where the substrate and labor costs were low. The ABE fermentation processes in South Africa and Russia continued to operate until the late 1980s to early 1990s (Zverlov et al., 2006). In the late 1990s to early 2000s has been reported that the Russian fermentation industry focused on agricultural biomass transform to butanol (Zverlov et al., 2006).

1) Microorganism

Butanol as acetone, ethanol and isopropanol are naturally formed by a number of clostridia. Normally, clostridia enable to create chiral products that are difficult to make by chemical synthesis and lessen a number of toxic chemicals. Clostridia forms in rod-shaped, spore-forming Gram positive bacteria and typically strict anaerobes. Solventogenic clostridia is able to apply as variety substrates from monosaccharides including many pentoses and hexoses to polysaccharides. Complex nitrogen source such

as yeast extract are commonly required for proper growth and solvent production. **Figure 2-4** shows the ABE fermentation by clostridia. As many solventogenic clostridia, *C. beijerinckii*, *C. saccharobutylicum* and *C. saccharoperbutylacetonicum* are primary solvent producers.

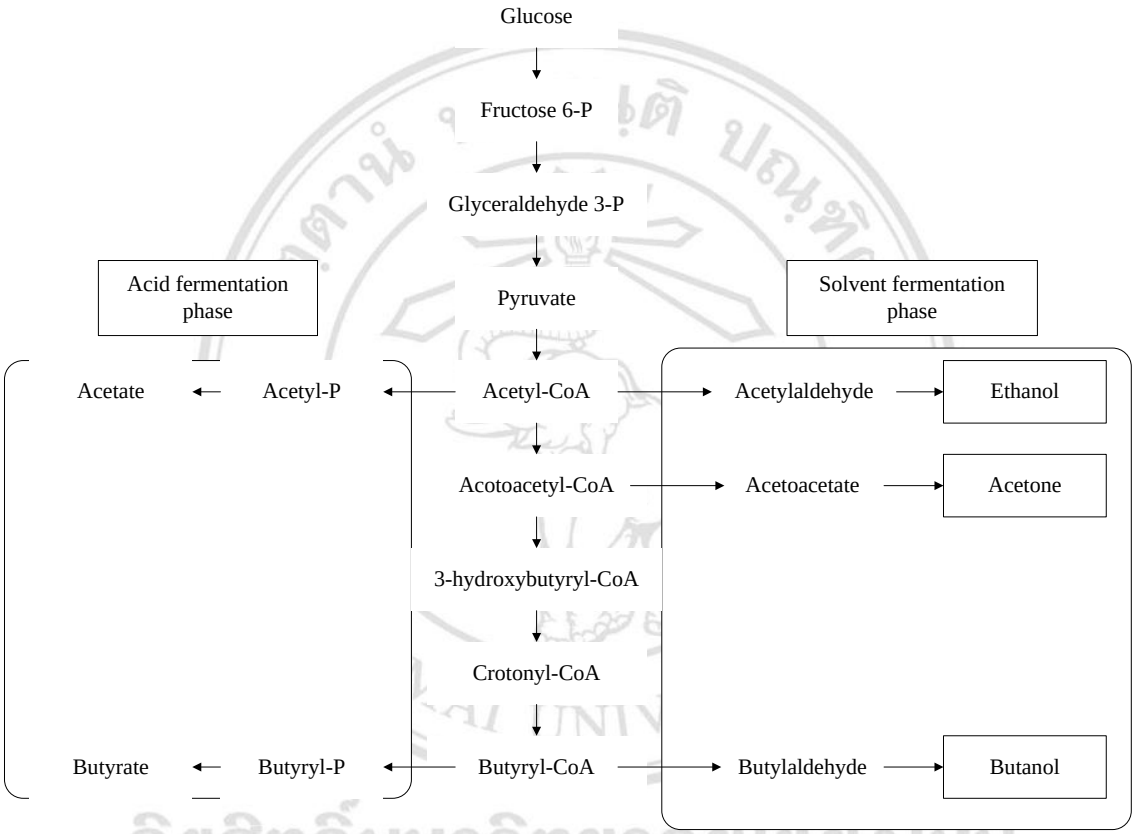


Figure 2-4: Acetone-Butanol-Ethanol fermentation by Clostridium

Figure 2-4 illustrates a typical feature of the clostridial solvent production is biphasic fermentation. The acidogenic phase is the first phase which the acids forming pathways are activated. Acetate, butyrate, hydrogen, and carbon dioxide are produced as major products. This acidogenic phase usually occurs during the exponential growth phase (Andersch et al., 1983; Hartmanis and Gatenbeck, 1984.). The second phase is the solventogenic phase during which acids are reassimilated and used in the production acetone, butanol and ethanol. The transition from acidogenic to

solventogenic phase is the result of a dramatic change in gene expression pattern (Dürre et al., 1987).

2) Fermentation

Butanol fermentation including upstream and downstream processing were evaluated by Qureshi and Blaschek (2001). Using the excessive carbon under nitrogen limitation is required to attain the high level of solvent production (Madihah et al., 2001). Iron is significant mineral supplement since the conversion of pyruvate to acetyl-CoA involves a ferredoxin oxidoreductase iron-sulfur protein (Kim et al., 1988).

The pH of the medium is very important to the biphasic acetone-butanol fermentation. In acidogenesis, rapid formation of acetic and butyric acids causes in pH reduction. Solventogenesis starts when pH meets a critical point which acids are reassimilated and butanol and acetone are produced. Thus, low pH is a prerequisite for solvent production (Kim et al., 1984). However, if the pH is going below 4.5 before sufficient acids are produced, solventogenesis would be summarized as unproductive. Increasing the buffering capacity of the medium is a simple method to raise up growth and carbohydrate utilization as well as butanol production (Bryant and Blaschek, 1988).

It takes 2-6 days to complete a batch fermentation depending upon the condition and the type of substrate. In batch fermentation the final total concentration of solvents produced ranges 12-20 g/L which could be separated from the fermentation broth by distillation. Classical fed-batch and continuous cultivation do not work to be economic feasible because of solvent toxicity and the biphasic nature of acetone-butanol fermentation respectively. To overcome this issue, fed-batch culture has been coupled with as in situ recovery process and multistage continuous fermentation has been proceeded (Godin and Engasser, 1990).

3) Substrate

Butanol has been produced from various raw materials such as Napier grass stem, switchgrass, sweet sorghum stem juice, corn cob, corn stover. Substrate cost is a major factor influencing economics of butanol production.

Lignocellulose or cellulosic biomass is the most plentiful renewable resource and it is recognized as providing great potential as a substrate for fermentation. The potential of utilizing of lignocellulosic materials for conversion to useful fermentation products such as fuel butanol has generated extensive interest in the past decades. Cellulosic biomass comprises of plant fibers as cellulose that are inedible by humans. These fibers would be hydrolyzed to yield a variety of sugars that can be fermented by microorganisms. It is affordable renewable resources and available in bulk quantities. Obviously, Butanol could be produced from various cellulosic biomass feedstocks including agricultural waste such corn stover, sugarcane bagasse, rice straw, and plant waste from industrial process like paper pulp and energy crops.

4) Solvent toxicity

One of the most critical problems in ABE fermentation is solvent toxicity. Clostridial cellular metabolism ends in the presence of 20 g/L or more solvents. This limits the concentration of carbon substrate could use for fermentation resulting in low final solvent concentration and productivity. The lipophilic solvent butanol is more toxic than others as it disrupts the phospholipid components of the cell membrane causing an increasing in membrane fluidity (Bowles and Ellefson, 1985). Increasing membrane fluidity causes destabilization of the membrane and disruption of membrane-associated functions such as various transport process, glucose uptake, and membrane-bound ATPase activity (Bowles and Ellefson, 1985). Butanol is the only solvent produced to the level that becomes toxic to the cells during the fermentation of clostridia (Jones and Wood, 1986). The addition of 7-13 g/L of butanol to culture medium results in a 50% inhibition of growth whereas the addition of acetone and ethanol up to 40 g/L would reduce growth by 50% (Jones and Woods, 1986).

2.2.2 Butanol production from lignocellulosic material

Lignocellulosic material consist of major three different types of polymers as cellulose, hemicellulose and lignin which are associated which each other.

There are three steps to produce butanol from lignocellulosic materials by using a biological approach. First of all, pretreatment step to make the lignocellulosic materials

amenable to hydrolysis. Second, hydrolysis step to break down the molecules into sugars. Third, fermentation of sugars to produce butanol.

2.2.2.1 Pretreatment

The pretreatment step is crucially required for efficiency hydrolysis of cellulose to its consistent sugars. Pretreatment strategies have been developed the reactivity of cellulose and to increase the yield of fermentable sugars. The pretreatment process is to remove lignin and hemicellulose, decrease the crystallinity of cellulose, and expand the porosity of the lignocellulosic material. The targets of pretreatment are following (1) production of highly digestible solids that supplys sugar yields during enzyme hydrolysis, (2) avoiding the degradation of sugar especially pentose including sugar derived from hemicellulose, (3) minimizing the formation of inhibitors in fermentation steps, (4) recovery of lignin for conversion into valuable coproducts, and (5) to be prominent cost effective by operating in moderate size reactors and to reduce heat and power consumption.

Pretreatment methods could be classified into different categories such physical as milling and grinding, physiochemical such steam pretreatment/ autohydrolysis, hydrothermolysis, and wet oxidation, chemical as alkali, dilute acid, oxidizing agents, and organic solvents, biological, electrical or combination methods. The advantages and disadvantages of the pretreatment technologies are compared in **Table 2-5**.

Table 2-5: Advantages and disadvantages of different pretreatment technologies of lignocellulosic biomass (Brodeur et al., 2011)

Pretreatment methods	Advantages	Disadvantages
Alkali	(i) Efficient removal of lignin (ii) Low inhibitor formation	(i) High cost of alkali catalyst (ii) Alternation of lignin structure
Acid	(i) High glucose yield (ii) Solubilized hemicellulose	(i) High costs of acids and need for recover

Table 2-5: Advantages and disadvantages of different pretreatment technologies of lignocellulosic biomass (Brodeur et al., 2011) (continued)

Pretreatment methods	Advantages	Disadvantages
Acid		(ii) High costs of corrosive resistant equipment (iii) Formation of inhibitors
Solvents	(i) Lignin and hemicellulose hydrolysis (ii) Capability to dissolve high loadings of various types of biomass (iii) Mild processing conditions as low temperature	(i) Partial hemicellulose degradation (ii) Acid catalyst needed to make processes efficient with high lignin content material (iii) Toxic compound generation
Steam	(i) Cost effective (ii) Lignin transformation and hemicellulose solubilization (iii) High yield of glucose and hemicellulose in two-step process	(i) Partial hemicellulose degradation (ii) Acid catalyst needed to make process efficient with high lignin content material (iii) Toxic compound generation
AFEX (Ammonia Fiber Explosion)	(i) High effectiveness for herbaceous material and low lignin content biomass (ii) Cellulose becomes more accessible (iii) Causes inactivity between lignin and enzymes (iv) Low inhibitors formation	(i) Recycling of ammonia needed (ii) Less effective process with increase lignin content (iii) Alters lignin structure (iv) High cost of ammonia
ARP (Ammonia Recycle Precolation)	(i) Remove majority of lignin (ii) High cellulose content after pretreatment (iii) Herbaceous materials are most influencial	(i) High energy costs and liquid loading

2.2.2.2 Hydrolysis

Hydrolysis process will break down the hydrogen bonds in hemicellulose and cellulose fraction into their sugar components as pentose and hexose. Hydrolysis of cellulose is more difficult than for starches because cellulose is in a crystalline from with hydrogen bonding. The main hydrolysis process could be performed by dilute acid, concentrated acid and enzymatically.

1) Acid hydrolysis

The prominent advantage of the acid hydrolysis as the acids could penetrate lignin without any preliminary biomass pretreatment to do breaking down the cellulose and hemicellulose polymers to from individual sugar molecules. For instance, serveral types of acids in form of concentrated or diluted could be applied such as sulfurous, sulfuric, hydrochloric, hydrofluoric, phosphoric, nitric and formic acid. Sulfuric and hydrochloric acids are most common used catalysts for hydrolysis of lignocellulosic biomass (Lenihan et al., 2010). A comparison between concentrated- and diluted-acid hydrolysis methods shown in **Table 2-6**.

Table 2-6: Comparison between concentrated- and dilute-acid hydrolysis methods

Hydrolysis methods	Advantages	Disadvantages
Concentrated acid hydrolysis	- Operated at low temperature - High sugar yield	- High acid consumption - Equipment corrosion - High energy consumption for acid recovery - Longer reaction time (e.g. 2-6 h)
Dilute acid hydrolysis	- Low acid consumption - Short residence time	- Operated at high temperature - Low sugar yield - Equipment corrosion - Formation of undesirable by products

2) Dilute acid hydrolysis

As the chemical hydrolysis methods, dilute acid hydrolysis is probably most regular applied. It is can be used either as a pretreatment preceding enzymatic hydrolysis or as the actual method of hydrolyzing lignocellulose to the sugars. The outstanding advantage of the dilute acid hydrolysis is the low acid demanded as 2-5%. This process proceeds at high temperatures to achieve acceptable rates of cellulose conversion. The high temperature raises up the rate of hemicellulose sugars decomposition causing toxic compounds formation such as furfural and 5-hydroxymethy-furfural (HMF). These compounds inhibit yeast cells and the subsequent fermentation stage that causing low ethanol production rate (Maarten et al., 2009). In addition, these compounds lead to reduce fermentable sugars (Maarten et al., 2009). So, high temperature could destroy the equipment corrosion.

3) Concentrated acid hydrolysis

Concentrated acid hydrolysis process are reported to produce higher sugar yield (e.g. 90% of theoretical glucose yield) and consequently higher ethanol yield, compared to diluted acid hydrolysis process. Moreover, the concentrated acid hydrolysis process can operate at low temperature as 40°C which is advantage if compared to dilute acid hydrolysis process. However, the concentration of acid is quite high in this method around 30-70% and dilution and heating of the concentrated acid during the hydrolysis process make it severly corrosive. Therefore, the cost of process is too high due to the process requires special non-metallic constructions such as ceramic or carbon-brick lining and the acid recovery is an energy-demanding process. In addition, when sulfuric acid is used, the neutralization process produces bulk of gypsum. Thus, the environmental impact strongly limits the application of hydrochloric acid.

4) Enzyme hydrolysis

Enzymatic hydrolysis of cellulose is conducted by cellulase enzymes that are highly specific. The products of hydrolysis are regularly reducing sugars including glucose. There are two technology as enzymatic and direct microbial conversion methods. The chemical pretreatment of the cellulose biomass is necessary

prior enzymatic hydrolysis. Enzymatic hydrolysis processe is considered because enzymes catalyze only specific reactions. Not similarlity with acid hydrolysis, there are on side reactions or by products and the hydrolysis could be run at yields approaching 100% of theoretical. Shielding of the cellulosic surface by lignin, crystallinity, and the inaccessibility of the cellulose to the enzymes are possible obstacle to enzymatic attack. For increasing efficiency of enzyme hydrolysis, it is necessary to obtain accessible to the molecules to be hydrolyzed. This required some kind of pretreatment process to remove hemicellulose and break down the crystalline structure of the cellulose. Including removal of the lignin expose the cellulose and hemicellulose molecules.

The comparison between acid and enzymatic hydrolysis shown in **Table 2-7**.

Table 2-7: Comparison between acid and enzymatic hydrolysis (Taherzadeh and Karimi, 2007)

Comparing variable	Acid hydrolysis	Enzyme hydrolysis
Hydrolysis	High temperature (100-240°C)	Mild temperature condition (40-50°C)
Yields	High sugars recovery is not possible in dilute acid hydrolysis	High yields of sugars
Inhibitors	Yes inhibitor formation	No inhibitor formation
Product inhibition during hydrolysis	No	Yes
Cost of catalyst	Low	High
Time of hydrolysis	Short time periods	Long time periods

2.2.3 Literature review of Butanol production

2.2.3.1 Pretreatment of cellulosic biomass

Eliana et al. (2014) studied the effects of the primary treatment process on enzymatic hydrolysis and ethanol fermentability of the elephant grass's cellulosic

fraction. Elephant grass (*Pennisetum purpureum*) is a lignocellulosic grass in tropical zone that hold the great potential in ethanol production. Chemical and physico-chemical pretreatments like alkaline delignification, diluted acid hydrolysis, steam explosion, alkaline peroxide and aqueous ammonia soaking, they involved the effects on the hydrolysis and the fermentability of the cellulosic fraction. As the result, the alkaline pretreatment with NaOH gave the most concentration of reducing sugars. The optimal condition for pretreatment exposed as 120˚C for 1 hour with 2 wt.% NaOH and ratio of solid to liquid as 1:20 (wt.) gave the highest ethanol yield as 26.1 g/L (141.5 mg ethanol/ g dry biomass, 95% of theoretical yield).

Morandim-Giannetti et al. (2013) studied the delignification process on Napier grass by using calcium oxide (CaO) and hydrogen peroxide (H_2O_2). The best condition was presented as 9.00% CaO for a period 2.73 hours offered the result as 74.99% delignification and 66.58% cellulose. The best conditions for the bleaching process were presented as pH 12 and hydrogen peroxide at concentration 4.2% at 40˚C for 6 hours that was consequent 90.98% delignification and 99.21% cellulose.

Pirasao (2013) studied the potential of bioethanol production from *Pinnisetum purpureum* cv. Pakchong1 grass. The results showed that a 60 minutes of immersing time in 3% (v/v) concentration of the NaOH solution at 90˚C, gave the highest yield of lignin degradation to 26.99$\pm$0.52% with maximum cellulose concentration of 73.65$\pm$0.03%. The pretreated Napier Pakchong1 grass was investigated for an optimized hydrolysis condition of diluted-acid and commercial enzyme utilization. The results illustrated that diluted-acid hydrolysis yields were significantly lower process cost than enzymatic hydrolysis with similar production efficiency. The optimal condition was 4.10% (w/v) solution concentrate of H_2SO_4 at 118˚C process temperature and 180 minutes reaction time with 31.00 g/L of reducing sugar. Initial reducing sugar was fermented by separate hydrolysis and fermentation (SHF) process that produced bioethanol at 2.08 g/L hydrolyzed solutions which equivalent to 1.62 L/ton of fresh Napier Pakchong1 grass.

Takata et al. (2013) studied Napier grass in the objective for monosaccharides production by hydrothermal process and phosphoric acid. To treat the Napier grass with 3 wt% phosphoric acid at 160˚C for 15 minutes. The xylose product

attained 10.3 wt% which accordant to 72.0% of the xylan. To conduct the combined process by applying treatment of 85 wt% phosphoric acid at 60˚C for 1 hour and tracing by hydrothermal treatment 3 wt.% phosphoric acid. The primary treatment with intense phosphoric acid contributed mostly xylan was hydrolyzed to xylose, and the crystalline cellulose was transformed to amorphous form. The hydrolysis process cellulose to glucose was vitally improved according hydrothermal process with 3 wt.% phosphoric acids at 200°C for 8 minutes. As the result, 77.2% yield of xylose and 50.0% yield of glucose were obtained from a combined process.

Wongwatanapaiboon et al. (2012) studied the potential production of cellulosic ethanol from grasses in Thailand. Grass samples were pretreated with alkaline peroxide then processed by enzymatic hydrolysis to examine the enzymatic saccharification. The total reducing sugars in grasses ranging from 500-600 mg/g grasses (70-80% yield) were obtained. The feedstock from 11 grass species were used for producing ethanol by simultaneous sacchairfication and cofermentation (SSCF). Hydrolysis process was worked by cellulase and xylanase enzymes whereas yeasts as Saccharomyces cerevisiae and Pichia stipitis were adopted for cofermentation at 35°C for 7 days. The results illustrated that Sri Lanka ecotype vetiver grass contributed the highest yield of ethanol as 1.14 g/L or 0.14 g/g substrate equivalent to 32.72% of the theoretical values.

Nlewem, et al. (2010) studied comparison the pretreatment methods considering the lignin residual that influence on the enzymatic hydrolysis of switchgrass. To study three different pretreatment methods for switchgrass as the condition aqueous sodium hydroxide (0.5-10% w/v, 90˚C, 1 hour), dilute H_2SO_4 (0.5-5.0% v/v, 121˚C, 1 hour) and hot water (100˚C, 1 hour). The result indicated that pre-treated switchgrass with 0.5% w/v sodium hydroxide produced more concentration of glucose than sulfuric acid and hot water pre-treated sample. To study SEM on the pre-treated samples that showed the good pore formation in the NaOH pre-treated samples and a few or without physical changing on the acid and hot water pre-treated samples. The pre-treated samples were shown a significant reduction of lignin content by using NaOH and only slightly reduction in lignin content for the other pretreatment methods.

2.2.3.2 Butanol production

Kiyoshi, et al. (2015) studied butanol production from alkali-pretreated rice straw by co-culture of *Clostridium thermocellum* and *Clostridium saccharoperbutylacetonicum.* The co-culture of cellulolytic *Clostridium thermocellum* NBRC 103400 and butanol-producing *Clostridium saccharoperbutylacetonicum strain* N1-4 produced 5.5 g/L of butanol from 40 g/L of delignified rice straw pretreated with 1% (w/v) NaOH. Cellulose adding (100 U/g biomass) in a co-culture system significantly raised up butanol yield to 6.9 g/L by using 40 g/L of delignified rice straw. To compare the control, increasing of butanol yield was accounted to the expanding of exoglucanase activity on lignocellulose degradation in experimental samples. The results illustrated that the co-culture system in conjunction with expanding of exoglucanase activity contributed the result in cost-effective butanol production from delignified rice straw.

Gao and Rehmann (2014a) studied acetone butanol ethanol (ABE) fermentation from enzymatic hydrolysate of NaOH-pretreated corncobs. Corncob pretreatment was operated on the condition 0.5 mol/L NaOH at 121˚C for 30 min. and followed by enzymatic hydrolysis (cellic CTec 2 at 50˚C for 72 h). Reducing sugars production as 917 g/kg was pretreated. To use water for diluted the enzymatic hydrolysates of the NaOH-pretreated corncobs that derived sugar concentration 60 g/L prior fermentation by *Clostridium saccharobutylicim* DSM 13864. A solvent production 19.44 g/L with 12.27 g/L butanol was derived from 52.22 g/L sugars resulting ABE yield of 350 g/kg and production rate as 0.54 g/L/h.

Gao et al. (2014b) studied butanol production from alkali-pretreatment switchgrass (*Panicum virgatum)* and phragmites (*Phragmites australis).* Two species of grasses were pretreated with 1% (w/v) NaOH, s ratio of olid and liquid 1:10 at 121˚C for 30 minutes and aimed to enzymatic hydrolysis (cellulase at 50˚C for 72 h). Total reducing sugar yields for switchgrass and phragmites were 365 and 385 g/kg raw biomass respectively. Fermentation of the hydrolysates resulted in the entire yields of ABE shown as 146 and 150 g/kg (per kg dry plant material) with a theoretical maximum of 189 and 208 g/kg respectively.

Nanda et al. (2014) studied butanol and ethanol production from lignocellulosic feedstock. This study examined the influence of varied doses of H_2SO_4 (0-2.5%) in three lignocellulosic feedstock materials such pinewood, timothy grass and wheat straw at temperature 121°C for 1 hour. Feedstock pretreatment was purposed to enzymatic hydrolysis by utilizing cellulase, β-glucosidase and xylanase at 45°C for 72 hours. The biomass hydrolysates comprised of glucose and xylose as monomeric sugars were fermented using *Saccharaomyces cerevisiae* and *Clostridium beijerinckii* for ethanol and butanol production. The evaluation of comparison the ethanol and butanol concentrations, residual sugars as byproducts such as acetone, acetate and butyrate from biomass hydrolysates were proceeded. Pinewood provided high ethanol levels as 24.1 g/L followed by wheat straw 23.3 g/L and timothy grass 22.6 g/L. ABE fermentation using *C. beijerinckii* B-592 and the butanol concentrations were 11.2, 11.9 and 9.3 g/L from 50, 100 and 150 g/L of glucose substrate respectively. The butanol levels defined by the biomass hydrolysates were 11.6, 11.2 and 10.8 g/L for pinewood, wheat straw and timothy grass respectively.

Cheng et al. (2012) studied the high yield of bio-butanol production by solvent-producing bacteria microflora. In this study, high butanol-producing anaerobes were isolated from H_2-producing sludge that taken from a sewage treatment plant. Based on denaturing gradient gel electrophoresis (DGGE) analysis and 16s rDNA comparison, four strains from butanol-producing microflora were identified as *Clostridium saccharaperbutylacetonicum, Clostridium butylicum, Clostridium beijerinckii* and *Clostridum acetobutylicim.* The influence of glucoses, $FeSO_4.7H_2O$ and concentration of yeast extract on the butanol production by the mixture cultures were examined on batch process. The intermediate composition for bio-butanol production was optimized by using the Box-Behnken design and response surface methodology (RSM). The highest butanol production rate (0.25±0.02 g/L.h) and concentration 12.4 g/L were obtained under the condition as glucose concentration 60 g/L, $FeSO_4.7H_2O$ 0.516 g/L and yeast extract concentration 5.13 g/L. To add 6.0 g/L butyric acid significantly raised up the butanol titer to 17.51±0.49 g/L. Furthermore, combining butyric acid addition and pressurized fermentation strategies prominent enhanced butanol fermentation

performance, providing a maximum butanol concentration, productivity and yield of 21.1 g/L, 1.25 g/L/h and 0.8 mol butanol/mol glucose respectively.

Kanchanatawee, S. (2012) studied the acetone-butanol-ethanol production from cassava by fermentation process. The major objective of this study was to demonstrate the feasibility of using cassava materials as carbon sources supplemented with a brewer's yeast extract as a nitrogen source for acetone, butanol and ethanol fermentation by *Clostridium* TISTR 1462 in batch culture. The results illustrated that *C. acetobutylicum* TISTR 1462 enabled to produce solvents efficiently from cassava materials. The batch experiment without controlled pH of cassava starch resulted as 14.33 g/L of total solvents compare with 15.39 g/L of total solvents when glucose was used. Furthermore, the result indicated that enzymatic pretreatment of the gelatinized cassava starch before the fermentation did not improve solvent production as compared with direct fermentation of the gelatinized starch. The lower solvent production as 19.48% was observed when cassava materials was hydrolyzed with acid before the fermentation. In the experiment with controlled pH during solventogenic phase, the highest total solvents production 20.08 g/L was obtained with a controlled pH of 5.5. At controlled pH of 6.0 or higher, the fermentation produced mostly organic acids with a small amount of solvents. The approximate cassava starch concentration presented 20-80 g/L, the highest total solvents production 14.33 g/L was obtained at 60 g/L initial cassava starch concentration. The fermentation performance using primary cassava concentration lower than 30 g/L was acidogenic rather than solventogenic.

Raganati et al. (2012) studied butanol production from lignocellulosic-based hexoses (glucose and mannose) and pentose (arabinose and xylose) by fermentation with *Clostridium acetobutylicum.* The primary concentration of each sugar was defined at 60 g/L. The results showed that glucose was confirmed as the sugar characterized by the best performance. The fermentation performances of the other sugars were decreased by the order mannonse, arabinose and xylose. The performance of fermentation was poor when using xylose due to the residual acid concentration was very high. In addition, the conversion into solvents was very strongly enhanced by the presence of $CaCO_3$ in the fermentation medium.

Thirmal and Dahman (2011) studied biobutanol production from wheat straw. Three different physical and chemical pretreatment methods for the wheat straws were observed water, acidic and alkaline pretreatment. As the entire cases, physical pretreatment sample as 1 mm resize of the straws was used before each pretreatment. The results illustrated that 13.91 g/L glucose concentration was produced from saccharification with only in the physical pretreatment (no chemical pretreatment). This represented 5-20% lower sugar emitt in saccharification when compared to other pretreatment processes. Saccharification with acid pretreatment attained the high sugar concentration as values were 18.77 g/L glucose and 12.19 g/L xylose. The second study produced butanol from simultaneous saccharification and fermentation (SSF) using wheat straw hydrolysate and *Clostridium beijerinckii* BA101. The results indicated that neither butyric acid nor butanol production was originated approximate 24 hours. Butanol production from solely physical pretreatment or with water pretreatment prior to SSF provided several advantages that optimum butanol yield 10%. These processes produced low butanol concentration and needed low biomass concentration.

Liu et al. (2010b) studied the butanol production by *Clostridium bejerinckii* ATCC 55025 from the wheat bran (by-product of the wheat milling industry). Hydrolysate of wheat bran proceeded to pretreat with dilute sulfuric acid that the bran was used as a substrate to produce ABE. The wheat bran hydrolysate was holding 53.1 g/L total reducing sugars that include 21.3 g/L of glucose, 17.4 g/L of xylose and 10.6 g/L of arabinose. *C. bejerinckii* ATCC 55025 could utilize hexose and pentose simultaneously in the hydrolysate to produce ABE. After 72 hours of fermentation, the total ABE in the system derived 11.8 g/L whereas acetone, butanol and ethanol were 2.2, 8.8 and 0.8 g/L, respectively. The fermentation resulted in an ABE yield as 0.32 and productivity as 0.16 g/L/h.

Qureshi et al. (2010a) studied the production of butanol from corn stover and switchgrass hydrolysates by using *Clostridium beijerinckii* P260. Glucose was used as substrate in the controlled experiment which resulted in the production of 21.06 g/L total ABE. Fermentation of untreated corn stover hydrolysate (CSH) had no ABE production. However, base on water dilution (two fold) and wheat straw hydrolysate

(WSH, ratio 1:1), 16.00 and 18.04 g/L ABE was produced, respectively. This study resulted in ABE productivity in 0.17-0.21 g/L/h. CSH was defined as inhibitors, it was removed by treating the hydrolysate with $Ca(OH)_2$ (overliming). The culture could produce 26.27 g/L ABE after inhibitor removal. Without treated switchgrass hydrolysate (SGH) was poor fermented and the culture did not produce more than 1.48 g/L ABE which was grown to 14.61 g/L.

Qureshi et al. (2010b) studied the production of butanol (a biofuel) from barley straw hydrolysate. The results illustrated that the fermentation of dilute sulfuric acid barley straw hydrolysate (BSH) by *Clostridium beijerinckii* P260 provided the production of 7.09 g/L ABE, yield as 0.33, and productivity as 0.10 g/L/h. This value of ABE was further less than that observed in a control experiment as 21.06 g/L where initial glucose concentration 60 g/L was used as a substrate. The experiment under controlling, an ABE yield and productivity were 0.41 and 0.31 g/L/h, respectively. The comparison advised that BSH was toxicity to the culture. To decrease the effect of toxicity condition, BSH was necessary to treat with lime ($Ca(OH)_2$) followed by fermentation. The treated BSH resulted in accomplishment of fermentation and ABE concentration as 26.64 g/L was achieved. So, this fermentation derived an ABE yield of 0.43 and productivity of 0.39 g/L/h.

Mookploy (2009) studied the optimization of butanol production from rice straw hydrolysate by *Clostridium acetobutylicum* JCM 1419. Pretreatment of rice straw with sodium hydroxide resulted in the high level of delignification, xylan and glucan solubilization than sulfuric acid and hydrogen peroxide pretreatment. After that, rice straw was hydrolysed with 1% (v/v) sulfuric acid solution under high temperature and pressure (121˚C, 15 psi). The medium was optimized by using the Central Composite Design (CCD). The best medium for butanol production were composed of 70.27 g/L of glucose, 7.03 g/L of $(NH_4)_2SO_4$, and 2.56 g/L of yeast extract. Effects of fermentation condition, including temperature, initial pH and incubation time on butanol production in anaerobic batch culture were studied. *C. acetobutylicum* JCM 1419 produced the most butanol production 2.64 g/L in anaerobic batch culture at 30˚C and an initial pH of 6.0 during 96 hours of culture.

Zhang et al. (2009) studied the continuous acetone-butanol-ethanol production by corn stalk immobilized cells. Corn stalk was used for supporting to immobilize *Clostridium beijerinckii* ATCC 55025 for ABE production. The maximum total solvent concentration of 8.99 g/L was attained at dilution rate of 0.2 h^{-1}. Increasing the dilution rate between 0.2 and 1.0 h^{-1} resulted in increasing of solvent productivity, and the highest solvent productivity was obtained at 5.06 g/L/h with a dilution rate of 1 h^{-1}. The maximum solvent yield from glucose as 0.32 g/g was observed at 0.25 h^{-1}.

Qureshi et al. (2008) studied the removal of fermentation inhibitors from alkaline peroxide pretreated and enzymatically hydrolyzed wheat straw for butanol production by *Clostridium beijerinckii* P260 in batch reactor. Pretreatment of wheat straw was hydrolyzed using cellulolytic and xylanolytic enzymes. The hydrolysate was used to produce butanol. The culture produced acetone-butanol-ethanol lower than 2.59 g/L from alkaline peroxide wheat straw hydrolysate (APWSH) that without treatment process to reduce salt concentration. Butanol fermentation was attained after salt as inhibitor was removed from hydrolysate by electrodialysis. The ABE production and productivity were 22.17 g/L and 0.55 g/L/h, respectively. As the result, the concentration of NaCl over 2 g/L enabled to cease the cell growth and ABE fermentation.

CHAPTER 3

PART I: Determination of the optimum conditions for hydrothermal conditioning and mechanical dehydration

3.1 Introduction

Energy consumption in Thailand has been increased continuously since the year 2011 due to the expansion of domestic demands. Thailand's Alternative Energy Development Plan: AEDP 2015 has been launched with the target of using renewable and alternative energy to replace up to 30 percent of final energy consumption (in form of electricity, heat and bio-fuel) by 2036. This plan partly focuses on the utilization of energy crop, in which 680 MW of electricity is expected to be produced from biogas production using energy crop as the raw material (Energy Policy and Planning Office, 2017). Grass is one of the most important energy crops for Thailand because it is a perennial plant and can grow in every region of the country. Compared to other grass species, Napier Pak Chong1 grass has a higher production yield (up to 75 ton/ha-yr and carbohydrate content 36-38% as dry basis (Negawo et al., 2017; Rengsirikul et al., 2013). These characteristics make Napier Pak Chong1 grass be suitable as a feedstock for biogas production and combustion. However, as grasses are lignocellulosic biomass, they are rather recalcitrant to anaerobic fermentation (Bruni et al., 2010). Low methane yields at long retention time have been observed from anaerobic biodegradation of grasses (Richter et al., 2011; Richter et al., 2009). In addition, long term of mono-digestion of grass may result in the decrease of biogas production due to the effect of trace element deficiency (Thamsiriroj et al., 2012). Moreover, system failure due to the floating of grasses could cause the blockage in the gas pipe (Thamsiriroj & Murphy, 2010). Most of biogas plants using grasses as the feedstock in Germany are co-digestion of grass silage with manure to stabilize the process and maintain biogas production. As the source for renewable energy production, grass can also be used as a substrate for combustion. However, there are many problems due to its high element concentrations which would cause ash slagging (K, Mg), corrosion (Cl, S) and emissions (Cl, S, N) (Jenkins et al., 1998; Obernberger et al., 2006).

The better method of utilizing Napier Pak Chong1 grass could be the integrated generation of solid fuel and biogas from biomass (IFBB) (Wachendorf et al., 2009), which has been developed for increasing efficiency of conversion and methane production yield. In IFBB method, grass is separated into two parts, i.e. press fluid and press cake (**Figure 1-1**). Hydrothermal conditioning, which is the process of grass soaking and heated under continuous stirring for cell wall maceration, and mechanical dehydration process are done in order to transfer the elements and organic compounds into the press fluid for efficient anaerobic digestion. Several works have been conducted using this hydrothermal conditioning and mechanical dehydration process with semi-natural grassland (Wachendorf et al., 2009); green cut material from landscape (Hensgen et al., 2011) and sward maturity (Richter et al., 2011). The process was reported to be able to efficiently transfer minerals and organic compounds required for biogas production to the press fluid. The press fluid of semi-natural grassland was found to contain high crude protein and had the methane yield (0.40-0.43 Nm^3/kg VS after 13 day) two times higher than the whole crop grassland silage (0.22 Nm^3/kg VS after 27 day) (Richter et al., 2009). Similarly, Reulein et al. (2007) observed the high value of methane yields 0.500 Nm^3/kg VS from the press fluids of whole crop silages of maize and wheat. The press cake obtained after the mechanical dehydration process is a high solid fibrous fraction (cellulose, hemicellulose and lignin). This solid is a high-quality fuel as it contains low element concentrations, e.g. potassium, magnesium and chloride (Bühle et al., 2012) which would produce less amounts of air pollution after combustion.

Efficiency of the dehydration process of a biomass depends on several factors, e.g. solid: liquid ratio, temperature, incubation time, mechanical pressing, detergent and harvesting time (Wachendorf et al., 2008; Jia et al., 2013; King et al., 2012; Kuila et al., 2011). This study aimed to utilise Napier Pak Chong1 grass to produce renewable energies using IFBB process. Therefore, the objective of this work was to (1) optimize the hydrothermal conditioning, i.e. harvesting time (day), ratio of solid to water (by weight), soaking time (min) and temperature (°C) and (2) determine the methane yield of the obtained press fluid.

3.2 Materials and methods

3.2.1 Napier Pak Chong 1 grass

Napier Pak Chong1 grass was collected from Chiang Mai Fresh Milk farm, Lamphun, Thailand. After harvested and delivered to the laboratory, the grass was chopped by a hammer mill (Nimut Engineering Company, Thailand) to the average size of 2 cm (**Figure 3-1**). Grass sample were analysed characteristics and stored at 4°C before each use.

Figure 3-1: Hammer mill

3.2.2 Design of experiment

The 2-level full factorial design of experiment with center points and the Central Composite Design (CCD) were employed to obtain the optimum Napier Pak Chong1 grass pressing condition (**Table 3-1**). For the full factorial experiments, the chopped Napier Pak Chong1 grass samples (harvesting time 30 and 60 d) were hydrothermally conditioned by mixing with water (solid: water = 1:3 and 1:5 weight by volume) at different temperatures (37 and 80°C) and different soaking times (10 and 240 minutes) in a 100 L stainless tank. Then the conditioned Napier Pak Chong1 samples were

gravitationally separated from water. Subsequent mechanical dehydration of the Napier Pak Chong1 samples were conducted using screw press (Arkarnsin machinery company, Thailand) (**Figure 3-2**). Axial points used in the CCD for constructing the response surface to estimate the coefficients of quadratic terms are as follows; harvesting time (15 and 75 d), ratio of grass to water (1:2 and 1:6 weight by volume), soaking time (0 and 355 minutes) and soaking temperature (15.5 and 90°C). All experiments were developed and the results were analyzed using MINITAB version 16. Organic substance in form of mass of total COD (TCOD) was used as the response for optimization as it was the most pertinent parameter relating to biogas production potential. Mass of TCOD was calculated from the sum of mass of TCOD in press fluid and in drained water generated after the hydrothermal process.

3.2.3 Biochemical methane potential (BMP) test

The press fluid obtained from the optimum condition for pressing conditioning was investigated for biogas production potential using the BMP test. The BMP test was conducted according to the German Standard Procedure VDI 4630 (VDI, 2006) using 1000 ml glass bottle GL 45 (Schott Duran, Germany) with a working volume of 400 mL. Inoculum was collected from the final part of an anaerobic channel digester treating cow dung of Chiang Mai Fresh Milk farm in Lamphun province, Thailand. The inoculum was diluted to 20 g VS/L with medium solution (**Table 3-2**).

Press fluid and inoculum added in each bottle were 164 and 236 mL, respectively, equivalent to a ratio of press fluid to inoculum of 0.5 (by VS). Also, a bottle with only inoculum and distilled water was prepared and used as the control. All the experiments were done in triplicate including the control experiment. Microcrystalline cellulose was also used as a reference sample for checking the activity of inoculum.The gas production of this reference sample should be at least 80% of 0.74-0.75 $Nm^3/kg\ VS_{added}$. Nitrogen gas was used in flushing the headspace for 3 minutes to ensure the anaerobic condition. Each bottle was sealed with PTFE/silicone septa with PP screw cap and then kept in the temperature controlled room at 35°C. The gas volume was measured indirectly by pressure equipment (Kimo, model MP112) and then converted to that at STP condition

(0°C and 1 atm) . Complete anaerobic digestion was obtained when daily biogas production rate was less than 1% of total volume biogas production.

Figure 3-2: Hydrothermal conditioning and mechanical dehydration process

3.2.4 Analytical method

Total solid, volatile solid and COD were analysed according to standard methods (APHA, AWWA, 2012) . The methane composition was measured using a gas chromatograph (Agilent 7890A) with a thermal conductivity detector (TCD) . The temperature of the injector and detector were 120°C and 150°C, respectively. The carrier gas was He with the flow rate of 10 mL/ min. Methane potential was calculated as Nm^3 CH_4/ kg VS_{added}. The modified Gompertz model (Eq. 3-1) was used to predict the maximum methane yield (Ho & Shihwu, 2010).

Table 3-1: Design of experiment for grass juice

Run order	Harvesting time (d)	Grass to waster ratio (kg: L)	Soaking time (min)	Temperature (°C)	
1	-1 (30)	-1 (1:3)	-1 (10)	-1 (37)	
2	+1 (60)	-1 (1:3)	-1 (10)	-1 (37)	
3	-1 (30)	+1 (1:5)	-1 (10)	-1 (37)	
4	+1 (60)	+1 (1:5)	-1 (10)	-1 (37)	
5	-1 (30)	-1 (1:3)	+1 (240)	-1 (37)	
6	+1 (60)	-1 (1:3)	+1 (240)	-1 (37)	
7	-1 (30)	+1 (1:5)	+1 (240)	-1 (37)	Full
8	+1 (60)	+1 (1:5)	+1 (240)	-1 (37)	Factorial
9	-1 (30)	-1 (1:3)	-1 (10)	+1 (80)	Design
10	+1 (60)	-1 (1:3)	-1 (10)	+1 (80)	
11	-1 (30)	+1 (1:5)	-1 (10)	+1 (80)	
12	+1 (60)	+1 (1:5)	-1 (10)	+1 (80)	
13	-1 (30)	-1 (1:3)	+1 (240)	+1 (80)	
14	+1 (60)	-1 (1:3)	+1 (240)	+1 (80)	
15	-1 (30)	+1 (1:5)	+1 (240)	+1 (80)	
16	+1 (60)	+1 (1:5)	+1 (240)	+1 (80)	
17	0 (45)	0 (1:4)	0 (125)	0 (58.5)	
18	0 (45)	0 (1:4)	0 (125)	0 (58.5)	Center
19	0 (45)	0 (1:4)	0 (125)	0 (58.5)	Points
20	0 (45)	0 (1:4)	0 (125)	0 (58.5)	
21	-α (15)	0 (1:4)	0 (125)	0 (58.5)	
22	α (75)	0 (1:4)	0 (125)	0 (58.5)	
23	0 (45)	-α (1:2)	0 (125)	0 (58.5)	
24	0 (45)	α (1:6)	0 (125)	0 (58.5)	Axial
25	0 (45)	0 (1:4)	-α (0)	0 (58.5)	Points
26	0 (45)	0 (1:4)	α (355)	0 (58.5)	
27	0 (45)	0 (1:4)	0 (125)	-α (15.5)	
28	0 (45)	0 (1:4)	0 (125)	α (90.0)	
29	0 (45)	0 (1:4)	0 (125)	0 (58.5)	Center
30	0 (45)	0 (1:4)	0 (125)	0 (58.5)	Points

Table 3-2: Medium solutions

Chemical	Concentration	Unit
KH_2PO_4	0.27	g/L
$Na_2HPO_4.12H_2O$	1.12	g/L
NH_4Cl	0.53	g/L
$CaCl_2.2H_2O$	0.075	g/L
$MgCl_2.6H_2O$	0.10	g/L
$FeCl_2.4H_2O$	0.02	g/L
$Na_2S.9H_2O$	0.10	g/L
$MnCl_2.4H_2O$	0.50	mg/L
H_3BO_3	0.05	mg/L
$ZnCl_2$	0.05	mg/L
$CuCl_2$	0.03	mg/L
$Na_2MoO_4.2H_2O$	0.01	mg/L
$CoCl_2.6H_2O$	1.00	mg/L
$NiCl_2.6H_2O$	0.10	mg/L
Ns_2SeO_3	0.05	mg/L

$$M = P \times exp\left\{-exp\left[\frac{R_m \times e}{P}(\lambda - t) + 1\right]\right\} \quad [3\text{-}1]$$

Where, M is the cumulative methane yield ($Nm^3/kg\ VS_{added}$), P the maximum methane yield ($Nm^3/kg\ VS_{added}$, R_m the maximum methane production rate ($Nm^3/kg\ VS_{added}/d$), λ the lag phase (days), t the digestion time (days), e the exp(1)=2.718. All parameters (P, R_m, and λ) were estimated by the least square method using Solver function in Microsoft®Office Excel 2013. The sum of the squared errors (SSE) was set to minimize. The error value was the difference between the experimental value and predicted value.

3.3 Results and discussions

3.3.1 Characteristic of Napier Pak Chong1 grass

Characteristics of Napier Pak Chong1 grass are shown in **Table 3-3**. The TS of Napier Pak Chong1 grass was in the range of 14.30-16.44%. This result was similar with those of Lounglawan et al. (2014) who found that the dry matter of King Napier grass was 13.37-18.39%. However, Ansah et al. (2010) reported TS values of four varieties of Napier grass in Ghana in the higher range (48-51%) at harvesting time 60-120 day. Lower TS values of Napier Pak Chong1 grass found in this current study could be attributed to the variety of Napier grass species, planting location and climate and, in particular, the shorter harvesting time. As found in this current study, the 60 d-grass had higher lignin compared to those at shorter harvesting times, which could affect volumes and characteristics of the obtained grass juice.

Table 3-3: Characteristics of Napier Pak Chong1 Grass

Composition	Harvesting time (day)			
	30 (n=1)	45 (n=4)	60 (n=4)	75 (n=2)
Total solid (%)	14.38	13.68±1.70	14.77±0.41	17.47±2.31
Ether extract (% as dry matter)	3.63	3.72±0.54	3.21±0.53	2.48±0.21
Crude fiber (% as dry matter)	33.18	31.95±1.77	34.43±2.44	40.81±2.18
Crude protein (% as dry matter)	9.07	13.13±0.96	11.99±1.91	7.18±3.71
Ash (% as dry matter)	12.04	13.12±1.34	12.19±0.79	10.92±1.34
Nitrogen-free extract, NFE (% as dry matter)	42.08	38.08±2.99	38.17±0.96	38.62±3.08
Cellulose (% as dry matter)	40.44	36.35±0.78	38.41±1.85	44.23±1.32

Table 3-3: Characteristics of Napier Pak Chong1 Grass (continued)

Composition	Harvesting time (day)			
	30 (n=1)	45 (n=4)	60 (n=4)	75 (n=2)
Hemicellulose (% as dry matter)	20.09	22.41±1.24	24.03±0.70	22.35±1.17
Lignin (% as dry matter)	4.04	4.74±0.85	5.38±1.05	6.86±0.25
Potassium (% as dry matter)	1.32	0.38±0.45	0.36±0.28	n.a

3.3.2 Optimization of press fluid

Normally, grasses have high water content up to 80-85%. The preservation methods of grasses, such as silage or drying, for use as a raw material is essential (Xiu & Shahbazi, 2015). For the green biorefinery of biomass, mechanical dehydration with screw press is the primary method used to press grasses to press fluid. To increase maceration of the cell walls a pretreating method of biomass by adding water needs to be applied before press juice separation is conducted by screw press (Arlabosse et al., 2011). Effects of harvesting time (A), grass to water ratio (B), soaking time (C) and temperature (D) on the organic substance (as mass of TCOD) obtained in the press juice were investigated. The experimental data and the regression model of the mass of TCOD (at confidence level of 90%) are shown in **Table 3-4** and **Table 3-5**, respectively. The mathematical equation for the relationship between mass of TCOD and values of studied factors (uncoded values) gained from regression analysis can be shown in Equation 3-2. The boundaries of parameters used for constructing this equation was 15-75 d of harvesting time, 1:2 to 1:6 (weight by volume) of ratio of grass to water, 0-355 minutes of soaking time and 15.5-90°C of soaking temperature. The experimental data showed quadratic correlation between studied factors and the responses. Moreover, interaction effects between experimental variables had also been found.

Table 3-4: Experimental Data of Design of Experiment for the Mass of TCOD of the Press Fluid, In Term of Coded Factor

Run order	Harvesting time (d)	Grass to water ratio (kg: L)	Soaking time (min)	Temperature (°C)	Mass of TCOD (g)	
					Experimental	Predicted
1	-1 (30)	-1 (1:3)	-1 (10)	-1 (37)	122.88	110.24
2	+1 (60)	-1 (1:3)	-1 (10)	-1 (37)	142.33	128.25
3	-1 (30)	+1 (1:5)	-1 (10)	-1 (37)	139.81	145.32
4	+1 (60)	+1 (1:5)	-1 (10)	-1 (37)	160.54	163.32
5	-1 (30)	-1 (1:3)	+1 (240)	-1 (37)	144.42	148.05
6	+1 (60)	-1 (1:3)	+1 (240)	-1 (37)	185.55	166.06
7	-1 (30)	+1 (1:5)	+1 (240)	-1 (37)	173.03	183.13
8	+1 (60)	+1 (1:5)	+1 (240)	-1 (37)	200.41	201.14
9	-1 (30)	-1 (1:3)	-1 (10)	+1 (80)	121.21	121.71
10	+1 (60)	-1 (1:3)	-1 (10)	+1 (80)	141.28	139.72
11	-1 (30)	+1 (1:5)	-1 (10)	+1 (80)	153.06	156.79
12	+1 (60)	+1 (1:5)	-1 (10)	+1 (80)	178.11	174.80
13	-1 (30)	-1 (1:3)	+1 (240)	+1 (80)	129.61	127.90
14	+1 (60)	-1 (1:3)	+1 (240)	+1 (80)	139.45	145.91
15	-1 (30)	+1 (1:5)	+1 (240)	+1 (80)	164.29	162.97
16	+1 (60)	+1 (1:5)	+1 (240)	+1 (80)	171.66	180.98

Table 3-4: Experimental Data of Design of Experiment for the Mass of TCOD of the Press Fluid, In Term of Coded Factor (continued)

Run order	Harvesting time (d)	Grass to water ratio (kg: L)	Soaking time (min)	Temperature (°C)	Mass of TCOD (g)	
					Experimental	Predicted
17	0 (45)	0 (1:4)	0 (125)	0 (58.5)	130.71	134.59
18	0 (45)	0 (1:4)	0 (125)	0 (58.5)	135.63	134.59
19	0 (45)	0 (1:4)	0 (125)	0 (58.5)	126.67	134.59
20	0 (45)	0 (1:4)	0 (125)	0 (58.5)	133.99	134.59
21	-α (15)	0 (1:4)	0 (125)	0 (58.5)	167.29	161.97
22	α (75)	0 (1:4)	0 (125)	0 (58.5)	189.84	197.99
23	0 (45)	-α (1:2)	0 (125)	0 (58.5)	92.26	109.04
24	0 (45)	α (1:6)	0 (125)	0 (58.5)	195.61	179.18
25	0 (45)	0 (1:4)	-α (0)	0 (58.5)	136.60	132.15
26	0 (45)	0 (1:4)	α (355)	0 (58.5)	181.92	166.11
27	0 (45)	0 (1:4)	0 (125)	-α (15.5)	179.14	188.30
28	0 (45)	0 (1:4)	0 (125)	α (90.0)	174.09	162.31
29	0 (45)	0 (1:4)	0 (125)	0 (58.5)	142.60	144.11
30	0 (45)	0 (1:4)	0 (125)	0 (58.5)	125.93	144.11

Table 3-5: Results of Regression Analysis of the Mass of TCOD from the Press Fluid

Model term	Regression coefficient	Standard error coefficient	t-statistic	P-value
Constant	136.07	3.585	37.954	0.000
Block	-5.523	2.089	-2.644	0.016
A (Harvesting time)	9.004	2.143	4.201	0.000
B (Grass to water ratio)	17.536	2.143	8.182	0.000
C (Soaking time)	9.530	2.415	3.946	0.001
D (Temperature)	-2.242	2.286	-0.981	0.338
A^2	9.093	1.964	4.631	0.000
C^2	4.977	2.622	1.898	0.072
D^2	9.911	2.409	4.114	0.001
CD	-7.907	2.625	-3.012	0.007
R^2 = 88.98%	R^2(adj) = 70.65%			

$$Y_{COD} = 172.364 - 3.037\,(Harvested\ time) + 17.536\,(Grass{:}\,water\ ratio) + 0.176\,(Time) - 2.213\,(Temp) + 0.04\,(Harvested\ time)^2 + 0.0004(Time)^2 + 0.021(Temp)^2 - 0.003\,(Time\ x\ Temp) \quad [3\text{-}2]$$

Where: Y_{COD} = mass of TCOD, g

Harvested time = Harvesting time of Napier Pak Chong1 grass, days

Grass: water ratio = Ratio of grass mixed with water, kg: L

Time = Soaking time, min

Temp = Temperature of soaking, °C

As the press fluid from Napier Pak Chong1 grass was intended to be used for biogas production. TCOD was chosen as the response for optimization as it was the most pertinent characteristics for the bioreactor feedstock. The result showed that harvesting time, the ratio of grass to water and soaking time had significant effects on the total mass of TCOD ($P < 0.1$). Optimum conditions obtained from the optimization were harvesting time 75 d, grass: water ratio 1: 6 (kg: L), soaking time 355 min and temperature 15.5°C. Under these conditions, the predicted maximum mass of TCOD was 85.06 g/kg wet weight Napier Pak Chong1 grass. Hensgen et al. (2011) and Wachendorf et al. (2009) found that increase of water temperature in the range of 40-60°C did not increase of mass flow of minerals into the press fluid. Likewise, King et al. (2012) studied the effect of water temperature of hydrothermal conditioning process at 20, 40 and 60°C and reported that concentrations of elements in the press fluid from grass silage obtained at these temperatures were not significantly different. However, Richter et al. (2011) found that higher temperature of hydrothermal conditioning increased mass flows of elements into press fluids and decreased concentrations of elements in press cake when the soaking time and silage grass: water ratio were maintained at 10 min and 1:4 (w/w), respectively. Reasons for different effects of temperature on the quality of press fluid found in these studies are not clear. However, grass species (and structure), characteristics of soaking water and level of grass pretreatment might play some parts on the difference found. In this current study, it was found that the optimum temperature was lower than the normal ambient temperature in Thailand, which was not suitable for actual use. Therefore, the temperature was adjusted to the ambient temperature which was about 25°C. The mass of COD obtained in these adjusted conditions was 56.60 g/kg wet weight Napier Pak Chong1 grass equating to 71.5% of the value predicted by the model (79.17 g/ kg wet weight Napier Pak Chong1 grass). Therefore, this is suitable conditions for producing press fluid from Napier Pak Chong1 grass to press fluid. Kuila et al. (2011) reported that increasing soaking time also increased the reducing sugar production from cashew apple bagasse. The maximum yield of 56.89 (g reducing sugar/100 g dry substrate) was obtained at liquid: solid of 3.26 (mL/g), pH 6.42, incubation time 6.30 h and temperature 52.27°C. Similarly, in this current study, increasing of solid: liquid ratio also resulted in the mass of TCOD and reducing sugar being increased.

3.3.3 Biochemical methane potential (BMP) test

The press fluid from the optimum hydrothermally conditioned grass (harvesting time 75 d, grass: water ratio 1: 6 (kg: L), soaking time 355 min and temperature about 25°C) was investigated for biogas production potential using the BMP test. The average methane yield of press fluid was 0.40±0.05 $Nm^3 CH_4/kg\ VS_{added}$. The average methane content was 68.6%. Relatively, high methane yield could be attributed to the fact that press fluid contained mainly the biodegradable and soluble organic substances. The obtained methane yield is in the same range as those found in the studies of Hensgen et al. (2014), Hensgen et al. (2011), Nayono et al. (2010) and Richter et al. (2009) though different grass species and conditioning conditions before fluid pressing were used. Hensgen et al. (2014) found that methane yields of press fluid from IFBB for twelve European semi-natural grassland varied between 0.31- 0.40 $Nm^3 CH_4/kg\ VS_{added}$. In this work, the ensile sample were sprinkled with 25°C warm tap water and the ratio of biomass to mash water was 1:8. The previous study of Hensgen et al. (2011) reported that different water temperature in hydrothermal conditioning (40 and 60°C) did not affect the methane yields of the press fluid, in which 0.40 – 0.42 $Nm^3 CH_4/kg\ VS_{added}$ were obtained. Richter et al. (2009) also found that methane yields of press fluid from different types of semi-natural grassland conditioned under hydrothermal conditions were ranged 0.30-0.52 $Nm^3 CH_4/kg\ VS_{added}$. This means that the optimum conditioning conditions achieved in this current study is as effective as those reported in previous works. Compared with the whole crop, methane yields of press fluid gained in this current study was significantly higher than that reported from the whole crop silage (0.22 $Nm^3 CH_4/kg\ VS_{added}$) (Richter et al., 2009). Even though, Thamsiriroj & Murphy (2010) reported relatively high methane yield (0.45 $Nm^3 CH_4/kg\ VS_{added}$) from the Irish silage, the organic loading rate used was only 1 kg $VS/m^3.d$ and hydraulic retention time was more than 70 days. As the required digestion time of press fluid was only 15 d (time duration required to reach the maximum biogas production during the BMP test and the pipe clogging problem, normally found when the fibrous whole grass was used as the substrate (Hensgen et al., 2014), is not the issue for press fluid, renewable energy production according to IFBB process is clearly more advantageous.

The cumulative methane data was used to fit with the modified Gompertz model (Equation 3-1) to estimate the microbial kinetic parameters, with an assumption that

biogas production is a function of the methanogens growth in batch digester. The best fit to modified Gompertz equation is compared with the experiment data as illustrated in **Figure 3-3**. The regression coefficient (R^2) was 0.995 demonstrating the suitability of the model for accurate estimation of the anaerobic digestion of press fluid. The methane yield potential (P), the maximum methane production rate (R_m) and lag phase time (λ) were 0.41 Nm^3 CH_4/ kg VS_{added}, 0.05 Nm^3 CH_4/ kg VS_{added}/ d and 4.36 days, respectively. Kacprzak et al. (2012) studied the kinetics of anaerobic digestion of canary grass by using modified Gompertz model. They found that the yield of biogas production and the lag phase were 0.65 Nm^3/ kg VS_{added} and 14.67 d. In addition, Xie et al. (2011) reported specific methane yield and lag phase of co-digestion of pig manure and grass silage ratio at 1:0, 3:1, 1:1 and 1:3 which were equal to 0.28, 0.30, 0.30 and 0.27 Nm^3 CH_4/kg VS_{added} and 29.5, 28.1, 24.6 and 21.3 d, respectively. Furthermore, Prapinagsorn et al. (2017) found that the ratio of grass with cow dung and silage with cow dung at 3:1 by VS gave the maximum methane yield of 0.18 and 0.21 Nm^3 CH_4/kg VS_{added}, respectively. The lag phase of these conditions were 11.9 and 5.9 day, respectively. Compared to microbial kinetic values obtained when grasses were using as either the sole substrate or codigested with animal manures, it is obvious that the grass press juice used in this current study rendered much higher maximum methane yield and shorter lag phase time. This means that, to produce the same amount of biogas, a reactor needed for biogas production from the grass press juice could be less complicated (as the mixing system is not necessary); smaller in size (as it is very likely to accepted higher organic loading rate); easier to start-up (as it needs shorter time for microbial acclimatization) and cheaper to operate (as elements required for microbial degradation are transferred into the press juice in soluble form).

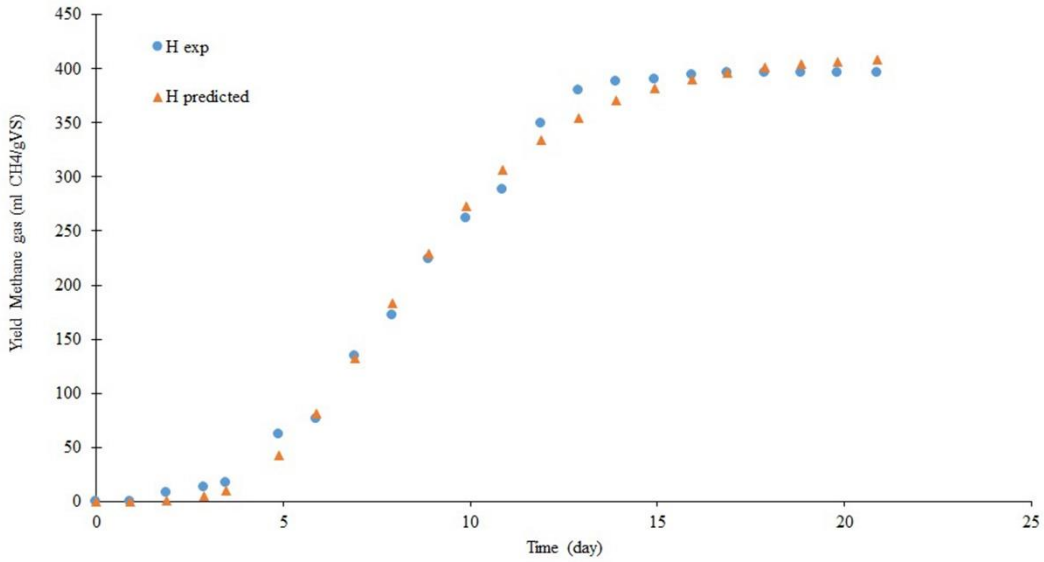


Figure 3-3: Comparison between the Experimental Data and Modified Gompertz Equation Data

3.4 Conclusion

The study revealed that the optimum hydrothermal conditioning conditions for Napier Pak Chong1 grass were as follows; harvesting time 75 d, ratio of grass to water of 1:6 (kg: L), ambient temperature (about 25°C) of the water and the soaking time of 355 min. The mass of COD in the press juice obtained in these conditions was 226.42 g equating to 71.5% of the value predicted by the model (316.68 g). Results from the BMP test showed that methane yield of press fluid was 0.40 $Nm^3 CH_4/kg\ VS_{added}$ with methane content of 68.6%. The microbial kinetic coefficients and biogas yield potential of press fluid were properly fitted with the modified Gompertz equation (adjusted $R^2 = 0.995$). The methane yield potential (P), the maximum methane production rate (R_m) and lag phase time (λ) were 0.41 $Nm^3 CH_4/kg\ VS_{added}$, 0.05 $Nm^3 CH_4/kg\ VS_{added}/d$ and 4.36 days, respectively. Producing biogas from the press fluid was clearly superior to that from the whole grass in a commercial scale.

CHAPTER 4

PART II: Investigation of effects of organic loading rates and effluent recirculation rates on the performance of an anaerobic baffled reactor

4.1 Introduction

Thailand's final energy consumption has been increasing due to continued economic growth. The main sources of energy used in the country's energy production are fossil fuels and crude oil, which are limited and uncertain supply of conventional energy sources. To cope with this problem, the Alternative Energy Development Plan (AEDP) aiming to establish renewable and alternative energy usage up to 30% of final energy production (in form of electricity, heat and bio-fuel) in the year 2036, has been established. Renewable energy production from biogas is one of the renewable forms of energy that the government targets for electricity generation. Currently, the main feedstock for biogas power plant is manure and wastewater from agro-industries. Therefore, the power plant from energy crops is a relatively new issue in Thailand.

Energy crops are considered as important sources for biogas production, considering Thailand being located in a tropical climate. Grass is the most important energy crops because it is a perennial plant and can grow in every region of the country. Compared to other grass species, Napier Pak Chong1 grass has higher production yields, up to 68 ton dried weight/ha.yr (Wijitphan et al., 2009). Cellulose, hemicellulose and lignin of Napier Pak Chong1 grass are 36-48%, 16-25% and 10-19% as dry basis, respectively.These characteristics render Napier Pak Chong1 grass suitable as a feedstock for biogas production (Bedoić et al., 2019; Nimmanterdwong et al., 2017) and combustion. However, as grasses are lignocellulosic biomass (Paul and Dutta, 2018), they are rather recalcitrant to anaerobic fermentation (Zheng et al., 2014). Low biogas (0.50-0.60 m^3/kg VS_{added}) and methane yields (0.192 - 0.275 $Nm^3 CH_4$/kg VS_{added}) at long retention times had been observed from anaerobic biodegradation of grasses (Bedoić et al., 2019; Mattioli et al., 2017; Richter et al., 2011; Richter et al., 2009). Furthermore, long term operation of mono-digestion of grass may result in biogas production being

decreased due to the effect of trace elements (FitzGerald et al., 2019; Wall et al., 2014; Thamsiriroj et al., 2012). Wachendorf et al. (2009) found that the integrated generation of solid fuel and biogas from biomass (IFBB) method could be developed for increasing conversion efficiency and methane yield. In IFBB method, grass is separated into two parts, i.e. press fluid and press cake using hydrothermal conditioning and mechanical dehydration processes. The aim of this process is to transfer the elements and organic compounds into the press fluid. The obtained press fluid is a suitable substrate for anaerobic digestion that can be easily digested and converted to biogas.

The press fluid of semi-natural grassland was found to contain high crude protein and had the methane yield twice as higher (0.40-0.43 Nm^3 CH_4/kg VS_{added} after 13 d) as those of the whole crop grassland silage (0.218 Nm^3/kg VS after 27 d) (Richter et al., 2009). Reulein et al. (2007) observed similar values (0.50 m^3/kg VS_{added}) for the methane yields of the press fluids from whole crop silages of maize and wheat. Likewise, Qiao et al. (2011) found that the supernatant obtained from centrifugation of hydrothermally treated biomass wastes (at 170°C, 1 h) had higher biogas production than without hydrothermal treatment. Biogas production from the press fluid or liquor from biomass eliminates effects of hydrolysis step which is the rate limiting step in anaerobic digestion when the whole crop biomass is used as the feedstock (Hensgen et al., 2014).

In order to get the maximum biogas yield in continuous operation, suitable conditions of digestion such as characteristics of substrate, pH, temperature, organic loading rate (OLR), hydraulic retention time (HRT), feeding scheme, carbon to nitrogen ratio (C/N ratio), alkalinity or buffer and trace elements addition need to be attained. These factors affect gas production, gas production rate, gas composition and stability of anaerobic digestion process. The highest methane yield of 0.66 m^3/kg COD at OLR 1.772 kg COD/m^3.d was reported under continuous mesophilic anaerobic digestion of grass silage liquor with upward-flow packed bed reactor with the effluent recirculation rate of 14 (Q_R/Q) at HRT of 0.65 d (Abu-Dahrieh et al., 2011). While, using residual liquid effluent (brown juice) after protein concentrated from green juice as the substrate in an UASB, 0.20 m^3 CH_4/kg COD (0.31 m^3 CH_4/kg VS) was obtained at OLR of 13.9 kg COD/m^3.d and HRT of 3 d (Martinez et al., 2018). Moreover, trace elements (TEs)

additions are an important key factor for the performance and stability of anaerobic digestion due to demand of microorganisms (Lebuhn et al., 2008). The role of TEs are as co-factors for enzymes involved in the digestion of organic substances for methane gas production (Fermoso et al., 2015; Pobeheim et al., 2011). Many researchers have been interested in increasing methane yield of energy crop and crop residues by trace element supplements (FitzGerald et al., 2019; Wall et al., 2014). Among several studied TEs, Fe, Ni and Co were found to be critical for improving biogas production when lignocellulosic biomass was used as feedstock. Up to 35% increase of biogas production was observed with Fe, Ni and Co additions (Hinken et al., 2008) and improved process stability was observed along with higher biogas production when Ni and Co were supplemented for mono-digestion of maize silage (Pobeheim et al., 2011). Improved methane yields and decreased VFA accumulation at the higher OLR (more than 2.5 kg VS/m^3.d) were also reported from mono-digestion of grass silage with Fe, Ni and Co supplements (FitzGerald et al., 2019).

The anaerobic baffled reactor (ABR) is a one of the most economical but efficient high rate anaerobic reactors. This reactor type has several advantages including simplicity (without requirement of mechanical mixing), low energy consumption and ability in separating acidogens and methanogens longitudinally of the reactor (Zhu et al., 2015). The most important characteristics of ABR are long solid retention time and performance stability under fluctuation of OLRs due to configuration of chamber (Jürgensen et al., 2018). High efficiencies have been reported using ABR in treating several lignocellulosic waste, e.g. vegetable waste (Gulhane et al., 2017), and alkali-decrement wastewater of polyester fabrics (Yang et al., 2018).

Even though, there have been some previous works conducted to determine the suitable conditions for biogas production from grass liquor, most of those experiments have been done using the batch (BMP test) investigating each or a few conditions at a time (Piepenschneider et al., 2016; Hensgen et al., 2011; Richter et al., 2009; Reulein et al., 2007). Moreover, the continuous operation investigation has mostly been done in the completely mixed reactor (Abu-Dahrieh et al., 2011), which is not economical for the liquid substrate. To our knowledge, there have been no works done to systematically

determine several conditions for efficient biogas production from Napier Pak Chong1 grass liquor in the cost-effective ABR. In order to holistically determine the suitable conditions required, this study investigated effects of organic loading rates, feeding scheme, trace element additions and effluent recirculation rates on the performance of ABR for biogas production from press fluid of Napier Pak Chong1 grass.

4.2 Materials and methods

4.2.1 Grass liquor

Grass liquor was obtained from the hydrothermal conditioning and mechanical dehydration process of Napier Pak Chong1 grass harvested from Chiang Mai Fresh Milk farm, Lamphun, Thailand described in Chapter 3. Briefly, the 75 d old grass was chopped by hammer mill (Nimut Engineering company, Thailand) to 2 mm and mixed with water (grass: water = 1: 6 (kg: L)) in a 100 L stainless tank for 355 min at ambient temperature water (approximately 25°C) in the hydrothermal conditioning process. Then the conditioned Napier Pak Chong1 samples were gravitationally separated from water. Subsequent mechanical dehydration of the Napier Pak Chong1 samples was conducted using screw press (Arkarnsin machinery company, Thailand) to obtain grass liquor and grass cake. Grass liquor was stored at 4°C before use. Characteristics of the grass liquor are shown in **Table 4-1**.

4.2.2 Inoculum

In order to obtain the large population of anaerobic microorganisms, especially methanogens, the inoculum was collected from the final part of commercial scale anaerobic channel digester treating cow manure at Chiang Mai Fresh Milk farm, Lamphun, Thailand. Inoculum characteristics are shown in **Table 4-1.**

4.2.3 Anaerobic baffled reactor (ABR)

The laboratory scale ABR was constructed from stainless steel. The reactor was rectangular containing five compartments (**Figure 4-1**) with the total working volume of 37.5 L. The dimensions of reactor were $0.30 \times 1.25 \times 0.10$ m. Each compartment was divided into down flow and up flow sections with volume of 1.88 and 5.62 L, respectively.

Ratio of the width of down flow section to up flow section was 1:3 and the end of the baffle for down flow part in each chamber was 45°C angled. An individual pipe for biogas collection was installed at the top of each compartment. The polyvinylchloride (PVC) gas bags were used for collection of biogas from each compartment. The reactor was operated in the temperature-controlled room at 35±2°C

Table 4-1: Characteristics of grass liquor and inoculum

Parameter	Grass liquor	Inoculum
pH	4.19 – 5.30	7.80
TCOD (mg/L)	18,765 – 30,195	-
FCOD (mg/L)	8,704 – 14,585	-
TS (mg/L)	20,535 – 40,052	-
VS (mg/L)	12,385 – 24,202	-
SS (mg/L)	7,105 – 13,781	59,593
VSS (mg/L)	6,752 – 11,156	48,160
TKN (mg/L)	502 – 1,156	-
TP (mg/L)	112 - 383	-

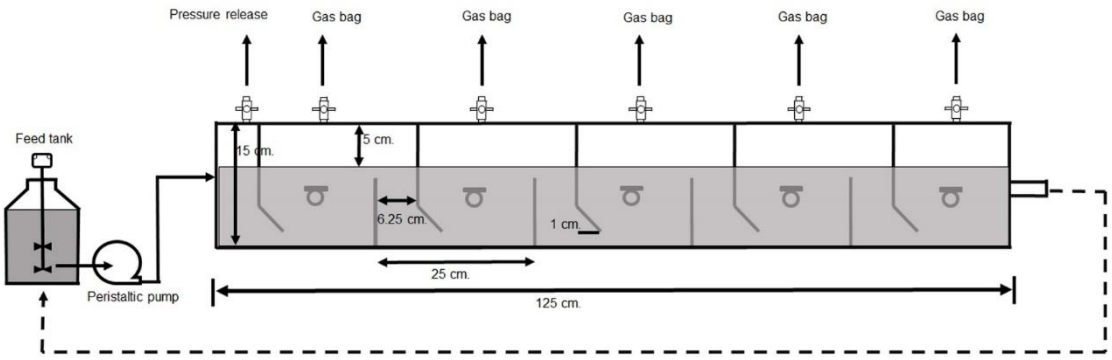
4.2.4 Experimental conditions and set-up

To gain an insightful effects of studied factors, i.e. organic loading rate, feeding scheme, trace element addition and effluent recirculation rate, on biogas production by ABR, the experiments were conducted under several level of these factors using the one-factor-at-a-time method described as follows;

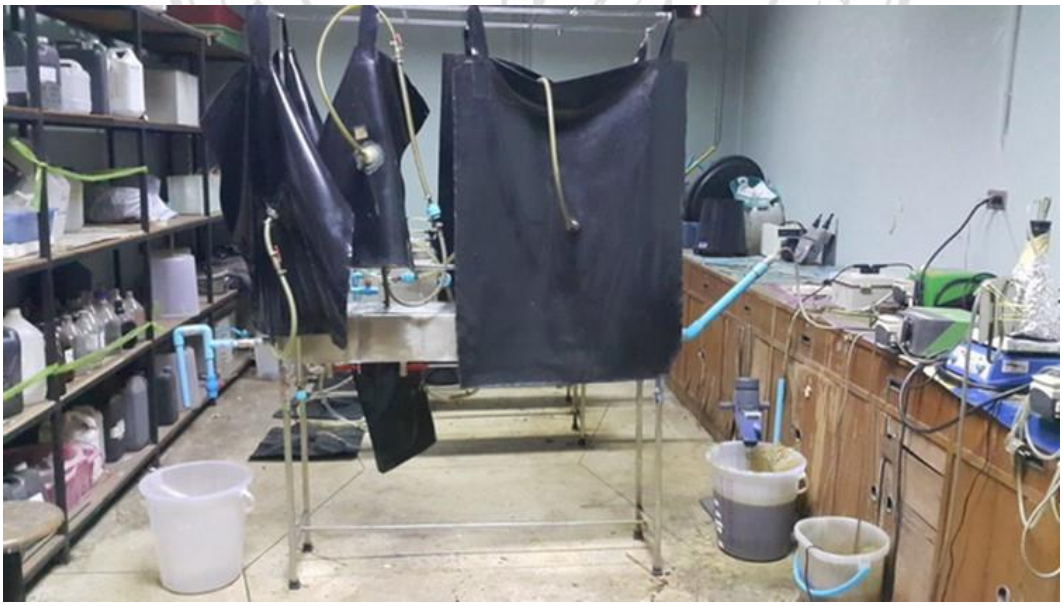
1) Investigation of effects of organic loading rates

The ABR reactor was started up by adding 11 L of inoculum and 26.5 L of water (30% and 70% of working volume, respectively). During start-up period (14 d), the organic loading rate (OLR) was kept constant at 0.5 kg COD/m^3.d and the reactor was fed once a day with the previously prepared grass liquor. During this period, pH of

grass liquor was adjusted to 5.00-5.50 by $NaHCO_3$ (4 mg in 2.3 L of grass liquor) as original pH of grass liquor was low (4.19). After that, in all experiments, there was no alkalinity addition. Studied OLRs were controlled at 1, 2, 4 and 8 kg COD/m^3.d by adjusting feeding flow rates of grass liquor according to the COD concentrations of the obtained grass liquor. All experimental conditions are shown in **Table 4-2**. Each experiment was conducted for the period of at least two times of the reactor HRT.



(a)



(b)

Figure 4-1: The Anaerobic baffled reactor (ABR): a) Dimensions and details of the anaerobic baffled reactor (ABR), b) ABR reactor used in this study

2) Investigation of effects of feeding schemes and trace element additions

To investigate effects of feeding schemes and trace element additions, the ABR was re-started (after reactor failure under OLR of 8 kg COD/m^3.d) under the semi-continuously fed scheme in which the grass liquor was fed 6 times per day at the flow rate of 0.25 L/min starting from OLR 2 and 3 kg COD/m^3.d during day 226-240. After that, it was operated at OLR 4 kg COD/m^3.d on day 241-264 (S1). Grass liquor used in this study contained 24 and 0.06 mg/L of Fe and Ni while Co concentration was below the detection limit. To investigate effects of trace element additions on biogas production efficiency from grass liquor, concentrations of Fe, Ni and Co of grass liquor were adjusted to 40, 0.5 and 10 mg/L, the minimum doses suggested for liquid feedstock (Speece, 1996), using $FeCl_2.4H_2O$, $NiCl_2.6H_2O$ and $CoCl_2.6H_2O$, respectively. On day 265-306, trace elements was added to grass liquor at OLR 4 kg COD/m^3.d (ST1). OLR was then increased to 6 kg COD/m^3.d on day 307-323 (ST2). All experiments in this topic were conducted mainly to assess the requirement of the system for semi-continuous feeding scheme or trace element addition.

3) Investigation of effects of effluent recirculation rates

To investigate effects of effluent recirculation rates, the effluent was mixed with influent at the rate of 25%, 50%, 100% and 200% of the influent flow rate for STR1, STR2, STR3 and STR4, respectively (**Table 4-2**). The effluent recirculation experiments were operated at OLR 4 kg COD/m^3.d, under semi-continuous feeding scheme with trace element additions.

4.2.5 Analytical methods

The influent and effluent were analyzed for pH, Total COD (TCOD), Filtered COD (FCOD), Alkalinity, TS, VS, SS, VSS, TKN and TP, following the Standard Methods (APHA, 2012), while VFA of the effluent were determined by direct titration method (Dilallo and AlbertSon, 1961). At the end of each experiment, a sample was taken from each chamber (at the middle height of the ABR) and was measured for the pH, VFA, SS and VSS. The biogas compositions were analyzed using a gas chromatography (Agilent 7890A) equipped with thermal conductivity detector (TCD) and two columns; molecular

sieves 5A 60/80 and HayeSep Q 80/100. Molecular sieves 5A 60/80 column was used to separate H_2, O_2, N_2, CH_4 and CO gas. HayeSep Q 80/100 column was used to separate CO_2 gas. The temperature of injection, oven and detector were 120°C, 150°C and 250 °C, respectively. The carrier gas was helium gas at flow rate of 10 mL/min. The volume of gas production from each gas bag collected was measured every day with a gas meter (YAZAKI model VY2A, Tecun company, Columbia) coupled with a vacuum pump. The steady-state conditions were considered to achieve when methane yields were less than 15% deviation.

Table 4-2: Experimental conditions

Experiment	Day	Feeding scheme	OLR (kg COD/m^3.d)	Trace element addition	Effluent recirculation rate
Effects of organic loading rates					
Start up	1 - 14	Once a day	0.5	No	No
B1	15 - 109	Once a day	1	No	No
B2	110 - 186	Once a day	2	No	No
B3	187 - 217	Once a day	4	No	No
B4	218 - 225	Once a day	8	No	No
Effects of feeding schemes and trace element additions					
Re-start	226 - 240	Semi-continuous	2 and 3	No	No
S1	241 - 264	Semi-continuous	4	No	No
ST1	265 - 306	Semi-continuous	4	Yes	No
ST2	307 - 323	Semi-continuous	6	Yes	No
Effects of effluent recirculation rates					
Re-start	324 - 343	Semi-continuous	4	Yes	No
STR1	344 - 367	Semi-continuous	4	Yes	0.25

Table 4-2: Experimental conditions (continued)

Experiment	Day	Feeding scheme	OLR (kg COD/m^3.d)	Trace element addition	Effluent recirculation rate
Effects of effluent recirculation rates					
STR2	368 - 391	Semi-continuous	4	Yes	0.50
STR3	392 - 412	Semi-continuous	4	Yes	1.00
STR4	413 - 434	Semi-continuous	4	Yes	2.00

4.2.6 Statistical analysis

All results were analyzed and differences among results from different experiments were tested using either Student's t test or ANOVA at 95% confidence level.

4.3 Results and discussions

4.3.1 Effect of organic loading rates

Results of ABR performances at different OLRs are shown in Table 3. At steady states of all experiments (B1-B4), the effluent pH at OLR 1, 2, 4 and 8 kg COD/m^3.d were 7.56±0.04, 7.52±0.06, 7.34±0.10 and 7.00±0.24, respectively. However, imbalance between the acid and methane formers could be obviously noticed at OLR 8 kg COD/m^3.d (B4) as VFA/Alk ratio in each chamber was in the range of 0.90 - 1.80 and significantly higher (P=0.001) than those detected in other experiments (**Figure 4-2**). Correspondingly, COD removal efficiencies especially in forms of FCOD obtained at B4 (OLR 8 kg COD/m^3.d) were significantly lower (P=0.021) than those gained at other OLRs (B1-B3; **Table 4-3**). These results initially implied that the OLR 8 kg COD/m^3.d was not suitable for biogas production from grass liquor in the studied ABR. Imbalance between acid and methane formers found at this OLR could be a result of increase of influent flow or decrease of HRT which led to the methanogens washout. This claim is supported by the fact that SS removal efficiencies achieved from B4 were minus values and significantly lower than those found from B1 and B2 (P=0.018, **Table 4-3**. Correspondingly, majorities

of microbial mass (64-79% of total mass) were retained in chamber 1-3 in B1-B3, while 58% of total mass was measured in the same chambers for B4. Another explanation for the imbalance between acid and methane formers found at OLR 8 kg COD/m^3.d (B4) could be that as acid formers are kinetically superior to the methanogens (Aris et al., 2017), accumulation of VFA could be expected when too big step of OLR increase was taken (from 4 to 8 kg COD/m^3.d in B3 and B4, respectively). The later experiment (ST2) was conducted to prove this assumption and it was found that even less increase of OLR was undertaken (from 4 kg COD/m^3.d in ST1 to 6 kg COD/m^3.d in ST2 under semi-continuous feeding scheme with trace element additions), sludge washout was still observed (**Table 4-4**). Therefore, deterioration of reactor performance found in this current study at OLR higher than 4 kg COD/m^3.d should be caused mainly by the failure in retaining the microbial sludge at higher OLRs. Sayedin et al. (2018) also found increased biomass washout when OLR was increased from 3.5 to 6 kg COD/m^3.d (HRT decreased from 20 d to 11.7 d) when a hybrid ABR was utilized for treating thin stillage. Increase of OLRs, which resulted in HRT being decreased, was reported to adversely affect COD removal efficiencies and accumulation of VFAs from domestic wastewater using ABRs (Aqaneghad et al., 2017). Similar results were also found when ABRs were used to treat high strength baker's yeast manufacturing wastewater when COD removal efficiencies were decrease from 94.3% to 78% when the HRT decreased from 6 to 2 d (Pirsaheb et al., 2015). Though, Abu-Dahrieh et al. (2011) found that increase of OLRs did not deteriorate COD removal efficiencies when grass silage liquor was used as a feedstock for biogas production by the continuous armfield reactor, the values and range of OLRs (0.851 to 1.77 kg COD/m^3.d) investigated in their work was rather low and narrow.

Table 4-5 shows results reported in previous studies using grass or processed grass liquors as feedstock to produce biogas compared to those found in this current study. The methane yields attained in this study at OLR 1, 2 and 4 kg COD/m^3.d were 0.40±0.03, 0.38±0.05 and 0.40±0.04 Nm^3 CH_4/kg VS_{added}, or 0.27±0.02, 0.29±0.04 and 0.25±0.02 Nm^3 CH_4/kg COD, respectively, which were not significantly different. However, when OLR was increased to 8 kg COD/m^3.d, significantly lower (P=0.000) methane yield (0.10±0.04 Nm^3 CH_4/kg VS_{added}) was gained as the result of sludge washout previously

explained. Methane yields obtained from the BMP test using grass liquor from hydrothermal conditioning and mechanical dehydration were in the range of 0.24-0.43 $Nm^3 CH_4/kg\ VS_{added}$ (Piepenschneider et al., 2016; Hensgen et al., 2011; Richter et al., 2009, **Table 4-5**). Santamaŕia-Fernández et al. (2018) and Martinez et al. (2018) found that methane yields from brown juice in batch and UASB reactor were in the range of 0.31-0.54 $m^3 CH_4$/kg VS (**Table 4-5**). Even though, Abu-Dahrieh et al. (2011) reported relatively higher methane yield 0.52 m^3/kg COD of grass silage liquor using armfield reactor, the experiment was conducted under relative low OLR (1.77 kg COD/m^3.d). As most of previous studies presented in Table 5 were either conducted under the batch experiment or in the continuously mixed reactor, results obtained from this current study indicate that comparable or even better methane yield can be gained from the economical ABR under mesophilic condition using grass liquor as the feedstock. Moreover, the optimum OLR was found at 4 kg COD/m^3.d, which is considerably higher than most reported in the previous studies.

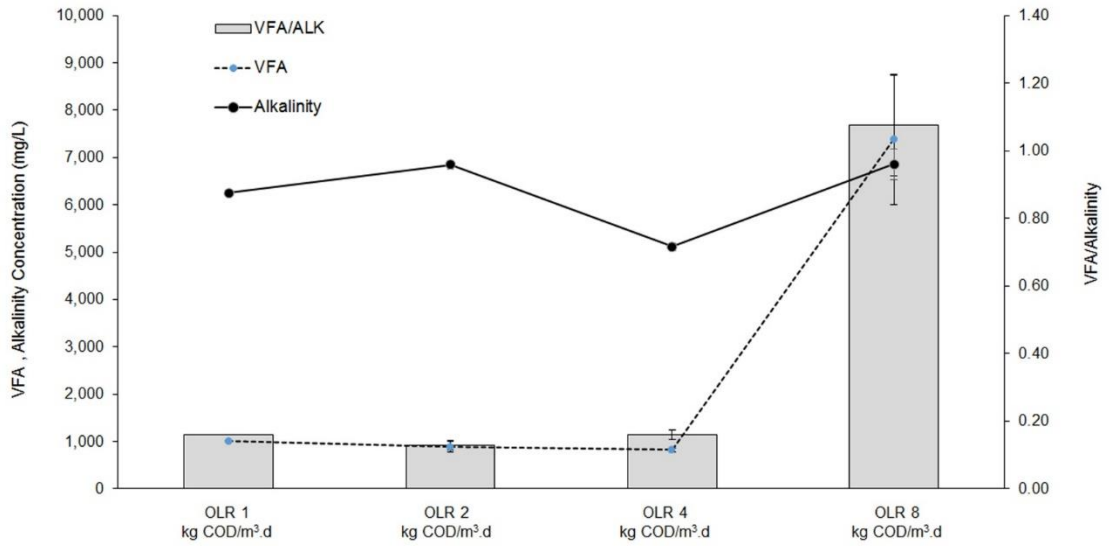


Figure 4-2: The VFA and Alkalinity and VFA/Alk ratio at each chamber of ABR at various organic loading rates

Table 4-3: Performance of ABR at different OLRs under the once a day feeding scheme

Experiments	OLR (kg COD/m^3.d)	VFA (mg/L)	Alkalinity (mg/L)	VFA/Alk	TCOD removal (%)	FCOD removal (%)	SS removal (%)	Methane yield (Nm^3/kg VS_{added})
B1	1	1,011±16^{b}	6,252±48^{a}	0.16±0.00^{b}	88.88±1.83^{a}	95.34±1.01^{a}	90.17±5.52^{a}	0.40±0.03^{a}
B2	2	886±110^{b}	6,845±100^{a}	0.13±0.01^{b}	88.89 ±0.33^{a}	95.00±0.52^{a}	82.62±3.84^{a}	0.38±0.05^{a}
B3	4	818±43^{b}	5,114±76^{b}	0.16±0.01^{b}	76.99±6.96^{a}	96.05±0.30^{a}	57.35±7.25^{a,b}	0.40±0.04^{a}
B4	8	7,386±1,377^{a}	6,860±326^{a}	1.08±0.15^{a}	55.29±10.69^{b}	83.63±4.90^{b}	-10.35±36.05^{b}	0.10±0.04^{b}

Results shown as Mean±SD, Different superscripts in the same column indicate differences ($p < 0.05$)

Table 4-4: Performance of ABR at different conditions under semi-continuous feeding scheme

Experiments	OLR (kg COD/m^3.d)	VFA (mg/L)	Alkalinity (mg/L)	VFA/Alk	TCOD removal (%)	FCOD removal (%)	SS removal (%)	Methane yield (Nm^3/kg VS_{added})
S1	4	591±86^{a}	5,265±44^{a}	0.11±0.01^{a}	92.41±0.54^{a}	96.06±0.11^{a,b}	93.17±1.06^{a}	0.44±0.03^{b}
ST1	4	476±185^{a}	6,700±491^{a}	0.07±0.02^{a}	90.60±0.01^{a}	95.50±0.66^{a,b}	84.87±3.38^{a}	0.43±0.02^{b}
ST2	6	436±49^{a}	5,267±575^{a}	0.08±0.00^{a}	-29.89±56.22^{b}	96.61±0.13^{a}	-81.70±68.43^{b}	0.32±0.02^{c}
STR1	4	922±205^{a}	5,889±645^{a}	0.16±0.05^{a}	86.85±0.00^{a}	97.41±0.00^{a}	87.42±0.00^{a}	0.44±0.02^{b}
STR2	4	552±89^{a}	5,296±156^{a}	0.10±0.01^{a}	81.18±8.54^{a}	94.97±0.33^{a,b}	81.57±1.50^{a}	0.49±0.05^{a}
STR3	4	860±236^{a}	5,983±304^{a}	0.14±0.03^{a}	76.17±5.57^{a}	90.37±3.75^{b,c}	74.26±7.62^{a}	0.30±0.01^{c}
STR4	4	882±548^{a}	4,968±486^{a}	0.18±0.09^{a}	52.34±0.67^{a,b}	87.32±0.83^{c}	32.80±7.00^{a}	0.21±0.02^{d}

Results shown as Mean±SD, Different superscripts in the same column indicate differences ($p < 0.05$)

Table 4-5: Comparison of biogas production from press fluid in previous studies and this study

Feed stock	Fermentation process	Condition	Methane yield		Reference
			$Nm^3/kg\ VS_{added}$	$Nm^3/kg\ COD$	
Grass liquor	Continuous ABR, 35˚C, once daily-feeding	OLR 4 kg COD/m^3.d, HRT 5.7 d	0.40	0.25	This study
Grass liquor	Continuous ABR, 35˚C, semi-continuous feeding	OLR 4 kg COD/m^3.d, HRT 6.2 d	0.44	0.34	This study
Grass liquor	Continuous ABR, 35˚C, semi-continuous feeding	OLR 4 kg COD/m^3.d, HRT 6.2 d, trace element additions	0.43	0.36	This study
Grass liquor	Continuous ABR, 35˚C, semi-continuous feeding	OLR 4 kg COD/m^3.d, HRT 5.3 d, trace element additions, recirculation rate ratio of 0.50	0.49	0.39	This study
Grass silage liquor	Continuous Armfield digester, 35˚C	OLR 1.77 kg COD/m^3.d, HRT 10 d		0.52[b]	Abu-Dahrieh et al. (2011)
Brown juice	Continuous UASB, 37˚C	OLR 13.9 kg COD/m^3.d, HRT 3 d,	0.20[a]	0.31[b]	Martinez et al. (2018)

Table 4-5: Comparison of biogas production from press fluid in previous studies and this study (continued)

Feed stock	Fermentation process	Condition	Methane yield		Reference
			$Nm^3/kg\ VS_{added}$	Nm^3/kg COD	
Grass silage liquor	Continuous Armfield digester, 35°C	OLR 1.77 kg COD/m^3.d, HRT 0.65 d, recirculation rate ratio of 14		0.66[b]	Abu-Dahrieh et al. (2011)
Grass silage liquor	BMP test, 38°C			0.38[b]	Abu-Dahrieh et al. (2011)
Grass silage liquor	BMP test, 37°C		0.24 - 0.29		Piepenschneider et al. (2016)
Press fluid (Green cut material from landscape)	BMP test, 37°C		0.25 - 0.36		Hensgen et al. (2011)
Press fluid from semi-natural grassland	BMP test, 37°C		0.40 - 0.43[a]		Richter et al. (2009)
Brown juice	BMP test, 37°C		0.38 - 0.50[a]		Martinez et al. (2018)

Table 4-5: Comparison of biogas production from press fluid in previous studies and this study (continued)

Feed stock	Fermentation process	Condition	Methane yield		Reference
			$Nm^3/kg\ VS_{added}$	$Nm^3/kg\ COD$	
Brown juice	Continuous UASB, 37˚C	OLR 13.9 kg COD/m^3.d, HRT 3 d,	0.20[a]	0.31[b]	Martinez et al. (2018)
Brown juice	BMP test, 37˚C		0.43 - 0.54[a]		Santamaŕia-Fernández et al. (2018)

Remark: a means data shown in unit $m^3/kg\ VS_{added}$ and b means data shown in unit $m^3/kg\ COD$

4.3.2 Effects of feeding schemes and trace element additions

1) Effects of feeding schemes

Operated under the continuous feeding scheme at OLR of 4 kg COD/m^3.d (S1), improvement of methane yield compared to that gained under the once a day feeding scheme at the same OLR (B3) was not significantly observed (P=0.062). Contrarily, obvious improvement in biogas production (34.2% increase) by continuous process from animal wastes compared to the batch process was reported by Obiukwu and Nwafor (2016). This difference could possibly be attributed to the fact that animal wastes are the high-strength feedstock containing high amounts of suspended solids compared to the grass liquor used in this current study and the continuous process could alleviate the undesirable conditions, e.g. low pH, high VFA, that would happen during the batch process. However, it was found that the semi-continuous feeding scheme used in S1 of this current study rendered higher process stability, in which it provided higher average COD and SS removal efficiencies and lower VFA concentrations in the effluent compared to those obtained from the reactor in B3 (**Table 4-4** and **Table 4-5**). Furthermore, the overall volumetric methane production rate of S1 was found to be considerably higher than that of B3 (**Figure 4-3**), especially in the first chamber. These results implied that ABR operated under semi-continuous feeding scheme (S1) still had more capability to digest more substrates as the microorganisms functioning in chamber 2 to 5 were not yet fully utilized. Advantages of the semi-continuous feeding scheme were also reported by Svensson et al. (2018) who found that the laboratory CSTR reactor treating steam exploded food waste that was fed more frequency (every 2.14 h), methane yield was 20% higher and reactor was more stable than the reactor had less frequency feeding (once a day).

2) Effects of trace element additions

Additions of TEs (ST1) tended to improve the performance of ABR compared to that without TEs addition (S1; **Table 4-4**). Lower average effluent VFA concentrations and VFA/Alk ratios were observed in ST1 compared to those in S1, though the differences were not significant at 95% confidence level. Moreover, higher methane composition in each chamber of ST1 (62, 68, 65, 60 and 62% to 49, 60, 66, 62 and 60% in chamber 1, 2, 3, 4 and 5 of ST1 and S1, respectively) suggested that TEs addition could be important to

reactor performance in long term operation, especially under high OLRs. Thamsiriroj et al. (2012) had revealed a risk of reactor failure in long term operation owing to TEs deficiency when grass was used as a substrate. Positive effects of trace element additions when lignocellulosic biomass was used as the biogas reactor feedstock have been reported. Upon trace element additions, decrease or stabilization of VFA concentrations at high OLR was observed in the maize silage (Abdoun and Weiland, 2009) and brewer's spent grains digester (Bougrier et al., 2018). Restorations of biological stability and increase of methane yield at high OLRs was detected for grass silage (FitzGerald et al., 2019) and food waste digestions (Voelklein et al., 2017).

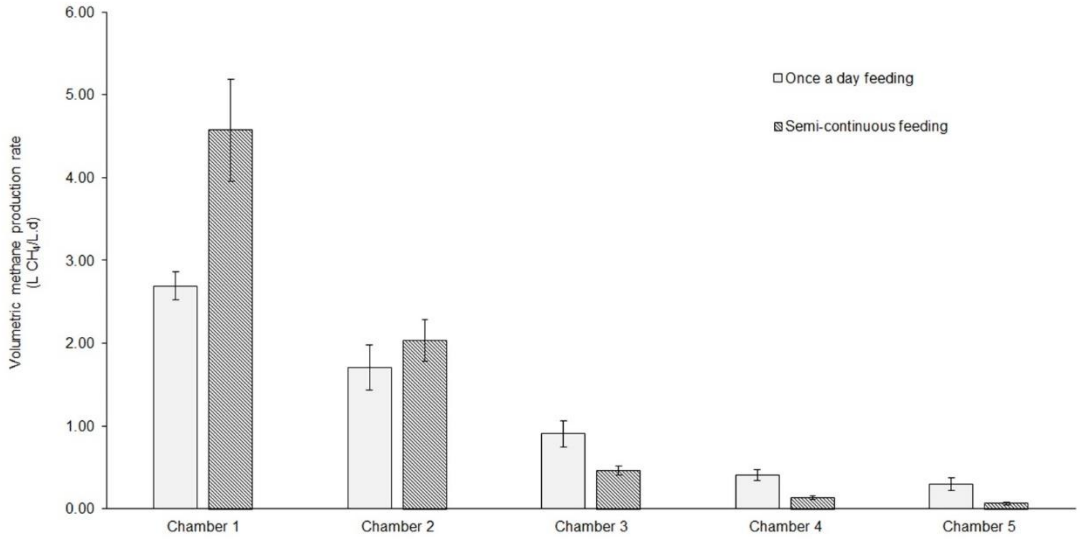


Figure 4-3: Volumetric methane production rate at each chamber under semi-continuous feeding scheme (S1) and once a day feeding scheme (B3)

In this current study, trace element additions helped to maintain VFA at low concentrations when OLR was increased from 4 kg COD/m^3.d in ST1 (476±184 mg/L as CH_3COOH) to 6 kg COD/m^3.d in ST2 (436±49 mg/L as CH_3COOH; **Table 4-4**). However, higher flow rate used in ST2 disturbed the sludge retaining capability of the studied ABR leading to sludge washout and deteriorated SS and COD removal efficiencies (**Table 4-4**). Hence, from the results obtained in this study, it could be concluded that the optimum conditions for biogas production from grass liquor by the

ABR were under OLR of 4 kg COD/m^3.d and semi-continuous feeding scheme with trace element additions.

4.3.3 Effects of effluent recirculation rates

There were no noticeable trends of effects of recirculation rates, i.e. 0.25, 0.50, 1.00 and 2.00 (STR1-4), on VFA and alkalinity concentrations. However, ratios of VFA and alkalinity of STR1-4 were 0.10 - 0.17 which could maintain stability or buffering capacity of the digester. Effluent recirculations have been reported to increase process buffering, and reduce the cost of chemical additions for pH adjustment (Saritpongteeraka and Chaiprapat, 2008; Kennedy and Barriault, 2005). COD removal efficiencies tended to be decreased with increase of recirculation rates especially the FCOD (Table 4). At higher recirculation rates (STR3-4), volumetric methane production rates were obviously decreased (**Figure 4-4**), presumably by the consequent increase of mixing intensity leading to sludge washout. This claim was partly supported by the fact that the average SS removal efficiencies of STR4 were obviously lower than those of STR1-2. Higher effluent recirculation was reported to increase mixing intensity resulting in the plug flow characteristics of ABR reactor being changed to completely mixed process (Sarathai et al., 2010; Kennedy and Barriault, 2005).

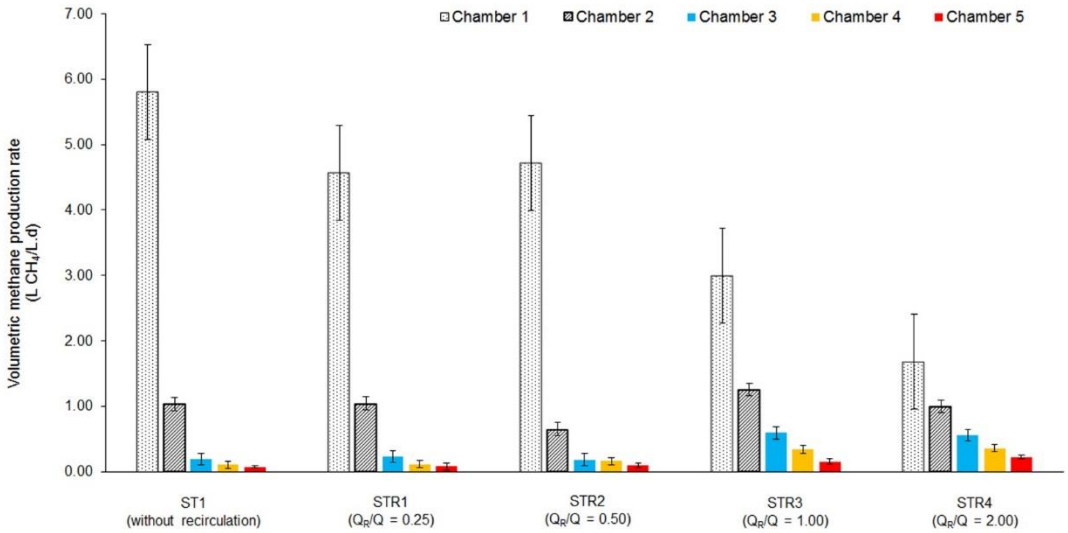


Figure 4-4: Volumetric methane production rate at each chamber of the reactor of ST1 (without effluent recirculation) and STR1-STR4 (with effluent recirculations)

The higher methane yield (0.49 ± 0.05 $Nm^3 CH_4/kg\ VS_{added}$) was obtained at the recirculation rate of 0.5, while the lowest value (0.21 ± 0.02 $Nm^3/kg\ VS_{added}$) was found at the recirculation rate of 2.0. Interestingly, the highest methane yield was found when the most amount of biomass (in form of VSS) was detected in the studied ABR (amounts of biomass in the reactor at steady-states were 1,039, 1,222, 729 and 801 g at recirculation rates of 0.25, 0.50, 1.0 and 2.0, respectively). Results found in this current work implied that advantages of effluent recirculation could be attained when grass liquor was used as the feedstock for the ABR as long as the recirculation rate did not become too excessive to damage the capability of the reactor in retaining microorganisms.

It was also found that, under the same OLR, methane yield of STR2 (operated at recirculation rate of 0.50) was significantly higher ($P=0.000$) than that of ST1 (without recirculation). From the literatures, the optimum recirculation rate depends on types of wastewater and operating conditions for ABRs. Improvement of ABR performance by effluent recirculation has been reported when different feedstock is used; e.g. vegetable market waste (Gulhane et al., 2016), aircraft de-icing fluid (Kennedy and Barriault, 2005), PVA-containing wastewater (Rongrong et al., 2011).

4.4 Conclusion

Effects of different operating conditions on biogas production from Napier Pak Chong1 grass liquor using ABR were investigated. Sludge washout and lower methane yields were observed when ABR was operated under high OLR. ABR operated with semi-continuous feeding scheme showed better performance than that with once a day feeding scheme. Additions of TEs helped the ABR to have higher methane compositions and were expected to maintain stability of the reactor in long term operation. The highest methane yield was detected at effluent recirculation rate of 0.5. At higher effluent recirculation rates, methane yields and COD removal efficiencies were significantly lowered.

CHAPTER 5

PART III: Determination of the suitable bacterial strain and investigation of effects of initial sugar concentrations and pH values on the efficiency of biobutanol production

5.1 Introduction

Over the past decades, the price of gasoline in Thailand has highly been fluctuated due to a decrease of fossil fuels and continuous increase of imported petroleum. To solve this problem, Thailand's government has increased the production of alternative biofuel from biomass, i.e. bio-ethanol, bio-diesel and compressed biomethane which are currently commercial biofuels. However, the use of these biofuels still has some limitations, e.g. moisture content in bio-ethanol is problematic for pipe transporting. In recent years, there have been interests in biobutanol as an alternative biofuel (Vivek et al., 2019; Wu et al., 2019; Amiri et al., 2018; Nanda et al., 2017). Biobutanol has many advantages properties over ethanol, such as higher energy content (Wackett, 2008) and lower volatility, which makes it safer to use. It is also less corrosive and can be distributed through existing petrol pipeline. Moreover, biobutanol can be utilised directly or blended with gasoline or diesel. Mixtures of butanol and gasoline (at any ratios) are superior to those of ethanol and gasoline for using in automobile engine or vehicles without any needs of modification, thanks to its energy density and octane number which are the same as those of gasoline (Luo et al., 2017).

Napier Pak Chong1 grass is one of the most important energy crops in Thailand because it is a perennial plant and can grow in every region of the country. Compared to other grass species, Napier Pak Chong1 grass has significantly higher production yields, up to 68 ton dried weight/ha.yr (Wijitphan et al., 2009). Cellulose, hemicellulose and lignin of Napier Pak Chong1 grass are 36-48%, 16-25% and 10-23% as dry basis, respectively (Tsai et al., 2018; He et al., 2017b; Cardona et al., 2016; Wen et al., 2015; Eliana et al., 2014; Yasuda et al., 2014). These characteristics render Napier Pak Chong1

grass suitable material for renewable energy productions, such as biofuel (butanol and ethanol), bio-based chemical production and biogas. Generally, the refinery of lignocellulosic biomass requires the hydrolysis of cellulose with enzyme and conversion of sugars to usable products by microorganisms (Lui et al., 2017; Procentese et al., 2017; Nieves et al., 2015). However, lignin, which is a polymer forming a complex network cross-linking the cellulose and hemicellulose together, can cause difficulties for enzyme in degrading cellulose and hemicellulose to monomeric sugars. Thus, pretreatment is required to increase the enzymatic digestibility by removing of lignin contents and break down of structural linkages to reduce the crystallinity and surface area of cellulose (Tsai et al., 2018; Phitsuwan et al., 2016).

There are many pretreatment methods, e.g. physical method (milling, grinding, extrusion), chemical method (acid, alkaline, ozone, solvent), physiochemical method (steam explosion, ammonia explosion, carbon dioxide explosion) and biological method (microorganism). Among all, alkaline pretreatment is effective on agricultural residues because it is relative inexpensive, less corrosive and requires less energy (Kim et al., 2015). There have been reports that alkaline pretreatment has greater effects on dissolving lignin than that on cellulose or hemicellulose (Tsai et al., 2018; van der Pol et al., 2015). Alkaline pretreatment with sodium hydroxide has been widely used for agricultural residues due to its effectiveness in removing lignin and suitability for industrial application. Phitsuwan et al. (2016) and Cardona et al. (2016) found that among the chemicals used for alkaline pretreatment of Napier grass, i.e. NaOH, $Ca(OH)_2$, NH_3 and alkaline H_2O_2, NaOH pretreatment resulted in the highest lignin removal at 84-94%. In addition, alkaline pretreatment can dissolve lignin rather than cellulose and hemicellulose (He et al., 2017b), therefore, it generated higher amounts of remained solid fraction than acid pretreatment (Tsai et al., 2018).

Biobutanol has been produced via biological acetone-butanol-ethanol (ABE) fermentation using clostridium strain (Amiri and Karimi, 2018). The ABE fermentation is an attractive process for bioconversion of lignocellulosic biomass as clostridia are able to ferment glucose and pentose derived from hemicellulose and cellulose, respectively. Various lignocellulosic biomasses can be used for the feedstock for butanol production

by *Clostridium* sp., such as Napier grass stem (He et al., 2017b), switchgrass (Gao et al., 2014; Qureshi et al., 2010a), sweet sorghum stem juice (Sirisantimethakom et al., 2018), corn cob (Boonsombuti et al., 2016; Gao and Rehmann, 2014), corn stover (Ding et al., 2016), palm kernel cake (Shukor et al., 2014) and barley straw (Qureshi et al., 2010b). There have been studies related to butanol production from several agricultural residues and new strains of *Clostridium* sp. (Shanmugam et al., 2018). However, research on the production of biobutanol from Napier Pak Chong1 grass is still lacking. In the previous study (Chapter 3), utilization of Napier Pak Chong 1 grass according to the integrated generation of solid fuel and biogas from biomass (IFBB) method has been conducted. The main concept was to separate grass into two parts, i.e. press fluid and press cake using hydrothermal conditioning and mechanical dehydration processes (Chapter 3). The press fluid was used as the substrate for biogas production by anaerobic baffled reactor (Chapter 4). Instead of being used as the solid fuel, the press cake containing high cellulose content (41.69% as dry basis) has great potential to be used as a substrate to produce biobutanol, which is a very promising method for investigating alternative sustainable utilization of Napier Pak Chong 1 grass to produce renewable energy.

This work aimed to determine optimum conditions, i.e. pH and sugar concentration, for biobutanol production from Napier Pak Chong1 press cake. The press cake was converted into butanol by NaOH pretreatment, enzymatic hydrolysis, and butanol fermentation using *Clostridium beijerinckii* TISTR 1461.

5.2 Materials and methods

5.2.1 Grass cake

Grass cake or press cake (PC) was obtained from the hydrothermal conditioning and mechanical dehydration process of Napier Pak Chong1 grass harvested from Chiang Mai Fresh Milk farm, Lamphun, Thailand as described in Chapter 3. Briefly, the 75 d old grass was chopped by hammer mill (Nimut Engineering company, Thailand) to 2 mm. and mixed with water (grass: water = 1: 6 kg: L) in a 100 L stainless tank for 355 min at ambient temperature water (approximately 25°C) in the hydrothermal conditioning process. Then the conditioned Napier Pak Chong1 samples were gravitationally separated

from water. Subsequent mechanical dehydration of the Napier Pak Chong1 samples was conducted using screw press (Arkarnsin machinery company, Thailand) to obtain grass juice and grass cake. Grass juice was used as the feedstock for biogas production in anaerobic baffled reactor (Chapter 4). Grass press cake was dried in the hot-air oven (Memmert, Germany) at 90°C for 1 day. The dried press cake was grounded to small size and sieved through 100 mesh and stored in a sealed plastic bag at room temperature until used in the alkaline pretreatment. Compositions of cellulose, hemicellulose and lignin of dried grass cake were 41.68±4.76%, 20.76±3.90% and 14.07±3.84%, respectively.

5.2.2 Alkaline pretreatment

Sodium hydroxide was used in the alkaline pretreatment method to remove lignin content of press cake. Dried press cake (60 g) was soaked in 600 mL of 3% (w/w) NaOH (solid to liquid ratio of 1:10). The slurry was mixed and boiled at 90°C for 1 h. After that, pretreated grass cake was washed with tap water to adjust the pH to neutral. Then, the pretreated grass cake was dried in the hot-air oven (Memmert, Germany) at 80°C for 2 d or until the constant weight was obtained (**Figure 5-1**). The pretreated press cake sample was analyzed for cellulose, hemicellulose and lignin contents and stored in zipper plastic bag at room temperature until used for the enzymatic hydrolysis.

5.2.3 Enzymatic hydrolysis

Enzymatic hydrolysis of NaOH-treated press cake was done using the commercial cellulase (iKnowZyMe AC cellulase; Reach Biotechnology, Thailand). Pretreated press cake (10 g) was mixed with 100 mL of 0.1 M sodium-citrate buffer (pH 4.8) in 250 mL Erlenmeyer flasks. The enzyme was added to the mixture solution at 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mL, corresponding to loading of 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 mL/g substrate, respectively. Then, the mixture was incubated at 50°C and 150 rpm (GYROMAXTM737, USA) for 3 d. The hydrolysate was centrifuged at 4°C and 9,000 rpm (Universal 320R, Germany) for 10 min (**Figure 5-2**). Supernatant was analyzed for reducing sugars and monosaccharide concentrations by dinitrosalicylic acid (DNS) method (Miller, 1959) and high performance liquid chromatography (HPLC) technique. The suitable enzymatic loading was used for butanol fermentation studies.

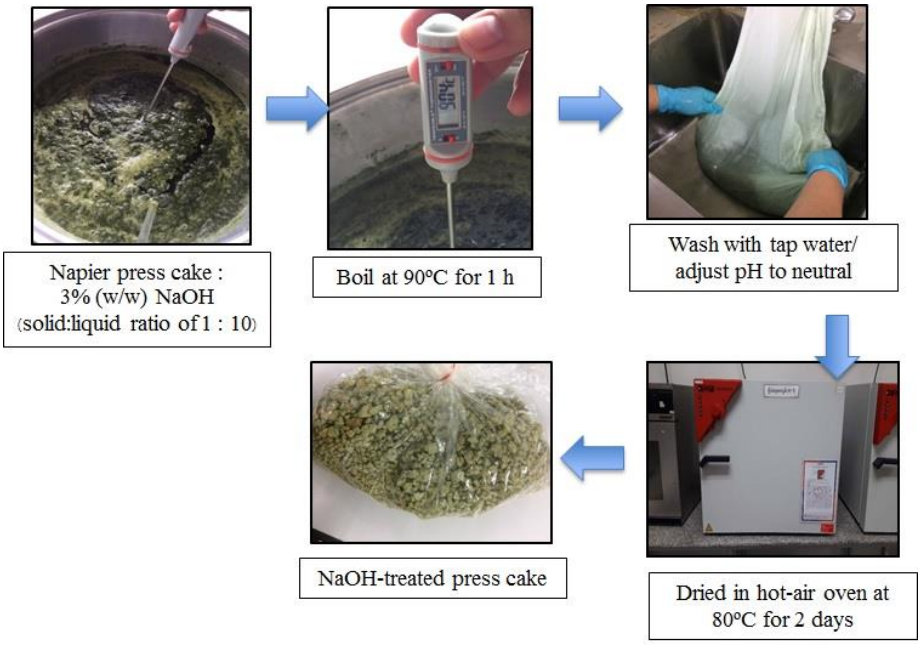


Figure 5-1: Alkaline pretreatment of press cake

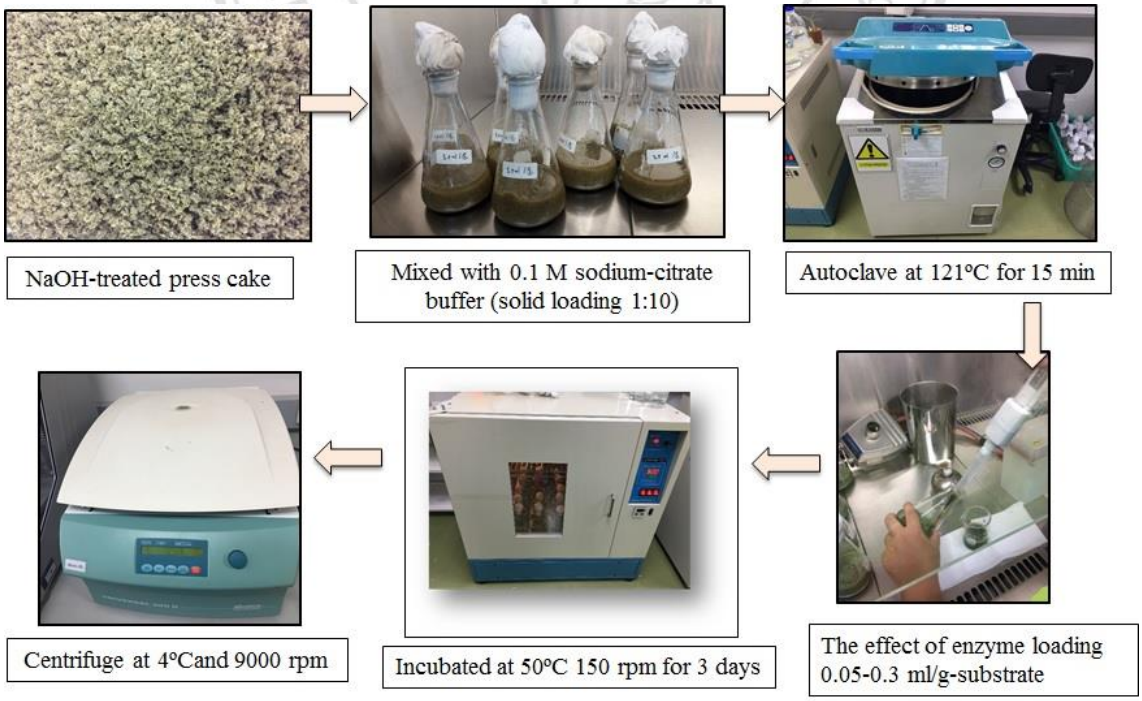


Figure 5-2: Enzymatic hydrolysis of pretreated press cake

5.2.4 Microorganism and culture activation

Clostridium beijerinckii TISTR 1461 and *Clostridium acetobutylicum* TISTR 1462 were purchased from the Thailand Institute of Scientific and Technological Research (TISTR). The commercial *Clostridium beijerinckii* JCM 1390 and *Clostridium acetobutylicum* JCM 1419 were purchased from the Japan Collection of Microorganisms (JCM). The commercial *Clostridium beijerinckii* DSM 791 was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ). All clostridia were freeze-dried cultures. The cultures were inoculated in sterilized cook meat medium (CMM, Sigma-Aldrich) and incubated in anaerobic jar (Merck™, Germany) at 37°C for 2 days. Then, the cultures were inoculated on reinforced clostridia medium (RCM, BD Difco™) agar plate and incubated in anaerobic jar at 37°C for 2 days (**Figure 5-3**). The stock cultures were maintained in 20% (v/v) glycerol at -20°C.

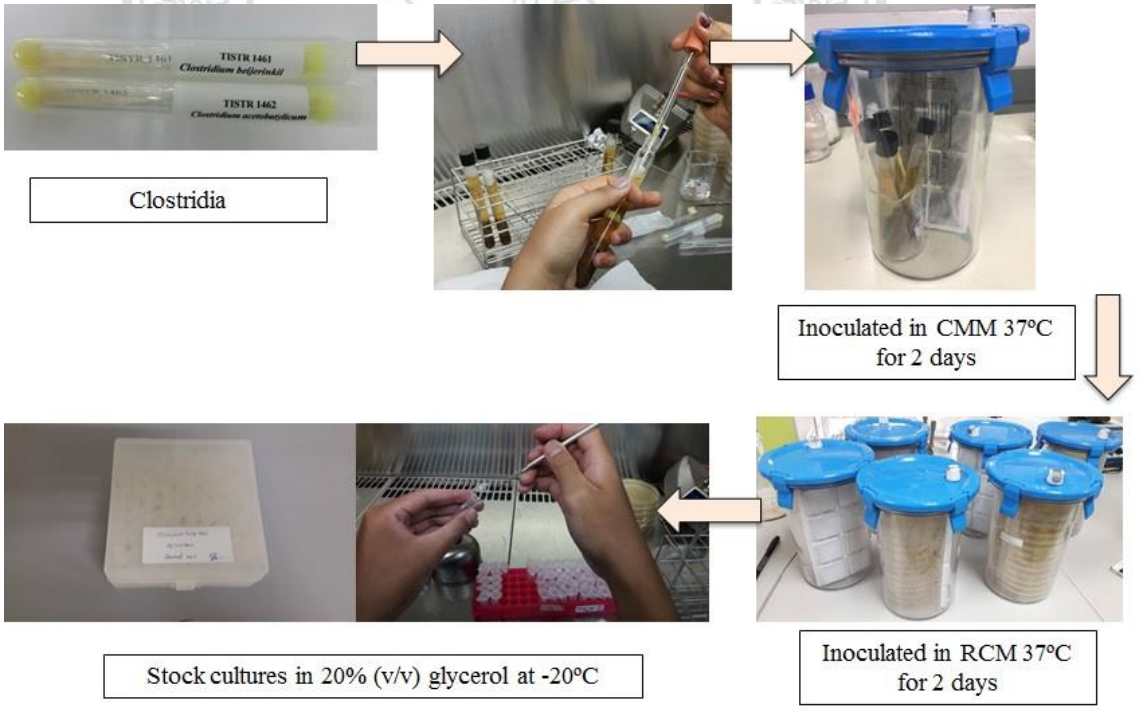


Figure 5-3: Culture activation

5.2.5 Strain selection

The stock cultures were inoculated in reinforced clostridia medium (RCM, BD Difco™) and incubated in anaerobic jar (Merck™, Germany) at 37°C for 2 days. Then, the quantities of growth pattern were determined at optical density of 660 nm. Efficiencies of sugar utilization in butanol fermentation of actively growing cultures (optical density of 1.0 at 660 nm) were then compared. Sugar substrates contained 60 g/L of glucose, xylose, arabinose, each of which was used as the sole substrate and also mixture of glucose, xylose, and arabinose with the selected strain which optical density of 1.0 at 660 nm. Then, selecting strain that provided the best sugar utilization for butanol production (**Figure 5-4 and Figure 5-5**).

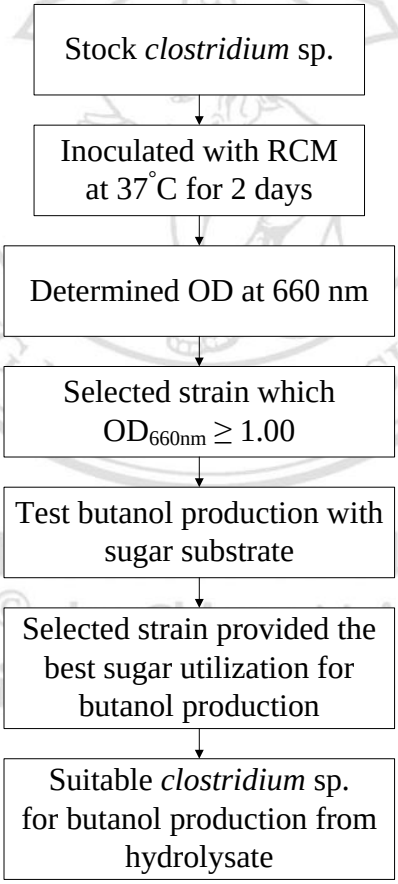


Figure 5-4: Process of selected *clostridium* sp.

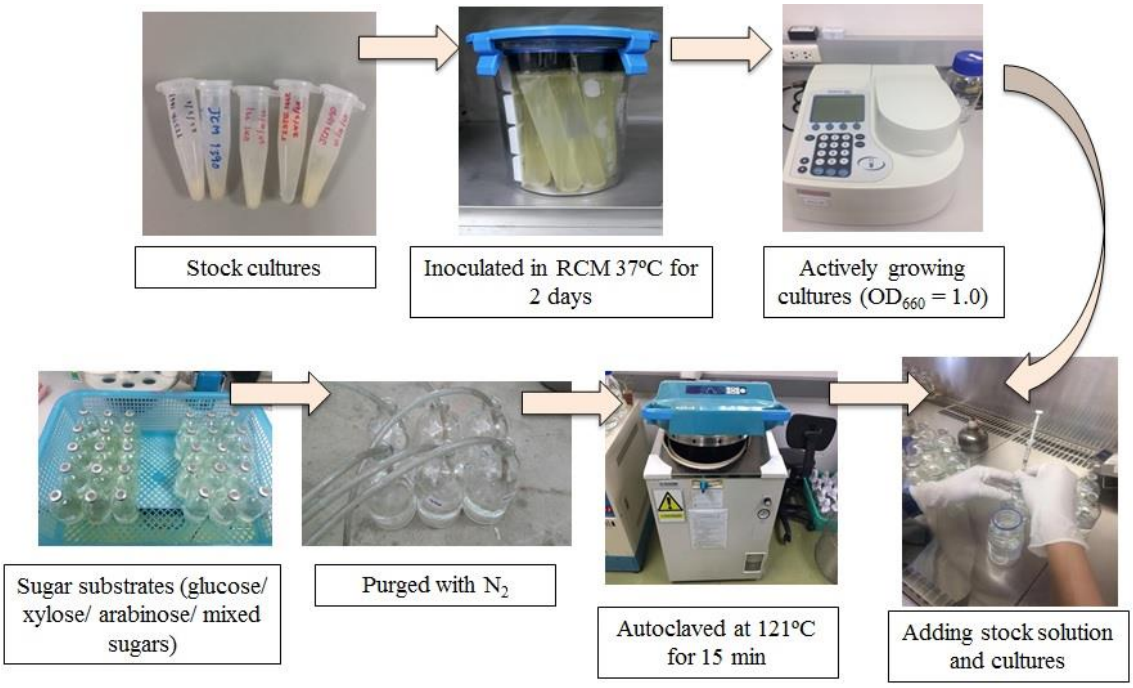


Figure 5-5: Strain selection

5.2.6 Biobutanol production

1) Culture preparation

The stock *Clostridium beijerinckii* TISTR 1461, the most efficient culture in producing butanol gained previously, was inoculated in reinforced clostridia medium (RCM, BD Difco™) and incubated in anaerobic jar (Merck™, Germany) at 37°C for 2 days. After that, 6 mL of preculture was added to 54 mL of sterilized (autoclaved at 121°C for 15 min) P2 medium (30 g/L glucose and 1 g/L yeast extract) in screw-cap tube and the growth was conducted at 37°C for 12-15 h.

2) Experimental design

The 2- level full factorial design with center points and the Central Composite Design (CCD) of experiment were employed to obtain the optimum initial pH and sugar concentration (g/L) for the butanol production from enzymatic hydrolysate of

NaOH-treated press cake. Butanol yield was specified as the response for optimization. The coded and actual values of design of the experiments are shown in **Table 5-1**.

Table 5-1: Experimental design for butanol production

Run	pH	Sugar concentration (mg/L)
1	-1 (5.50)	-1 (40.0)
2	+1 (6.50)	-1 (40.0)
3	-1 (5.50)	+1 (60.0)
4	+1 (6.50)	+1 (60.0)
5	-α (5.29)	0 (50.0)
6	α (6.71)	0 (50.0)
7	0 (6.00)	-α (35.0)
8	0 (6.00)	α (64.0)
9	0 (6.00)	0 (50.0)
10	0 (6.00)	0 (50.0)
11	0 (6.00)	0 (50.0)
12	0 (6.00)	0 (50.0)
13	0 (6.00)	0 (50.0)

Butanol yields from all experiments were fitted into a second-order quadratic model, as shown in Eq. 5-1:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_2 X_2^2 + \beta_{12} X_1 X_2 \quad (5\text{-}1)$$

Where Y is the predicted response, X_1 represents the initial pH, X_2 represents the initial reducing sugar (g/L), β_0 represents the constant coefficient and β_1, β_2 represents the linear coefficient and β_{11}, β_{12} represents the quadratic coefficient. The statistical and mathematical analyses of CCD and optimization variables were evaluated using MINITAB version 15.

3) Butanol fermentation

The fermentation process was conducted in 100 mL culture vessel with 50 mL working volume. The substrate was purged with nitrogen gas for 5 min to develop the anaerobic condition. After that, the substrate was autoclaved at 121°C for 15 min and each of sterilized stock solutions A, B and C (**Table 5-2**) at 0.5 mL was added. Stock solution A and B was autoclaved at 121°C for 15 min. Stock solution B was filter sterilized with 0.2 μm cellulose acetate membrane. Then, inoculations with 0.4 mL of actively growing culture (optical density of 1.0 at 660 nm) and 0.4 mL of 0.2 M cysteine solution (as anoxic solution) were done. Fermentation was incubated at 37°C for 192 h (**Figure 5-6**). Samples were taken every 24 h and analyzed for ABE (acetone, butanol and ethanol), acetic, butyric acid and reducing sugar concentrations.

Table 5-2: Stock solutions

Stock solution	**Concentration (g/L)**
A: Buffer solution	
KH_2PO_4	5
K_2HPO_4	5
Ammonium acetate	22
B: Mineral solution	
$MgSO_4.7H_2O$	2
$MnSO_4.H_2O$	0.1
$FeSO_4.7H_2O$	0.1
NaCl	0.1
C: Vitamin solution	
Para-aminobenzoic acid	0.01
Thiamine	0.01
Biotin	0.001

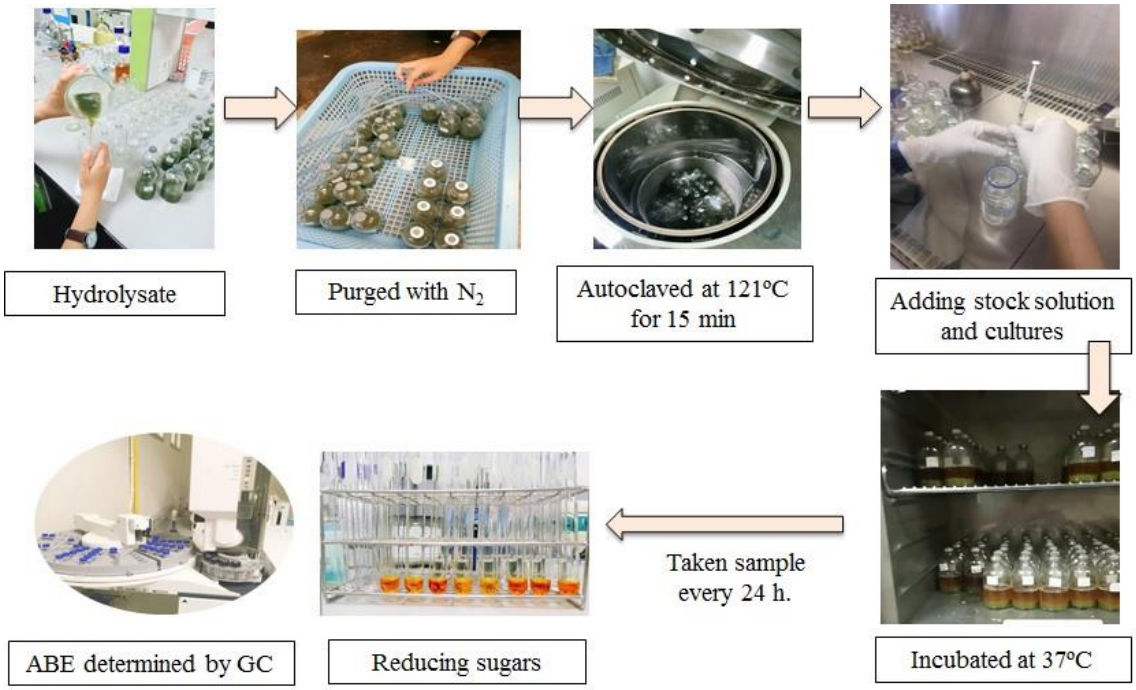


Figure 5-6: biobutanol production of hydrolysate press cake

5.2.7 Analytical methods

Samples of enzymatic hydrolysate and fermentation were centrifuged at 8,000 rpm for 15 min to removed insoluble particles. Reducing sugar in supernatant of enzymatic hydrolysate and fermentation were analyzed by dinitrosalicylic acid (DNS) method (Miller, 1959). Monosaccharide concentration of enzymatic hydrolysate was analyzed by HPLC (Bio-Rad) equipped with an Aminex HPX 87H column (300 x 7.8 mm; Bio-Rad, USA) and a refractive index detector (RID-10A). The column was operated at 40°C with 5 mM H_2SO_4 as an eluent at a flow rate of 0.60 mL/min (Qureshi et al., 2015; Boonchuay et al., 2016). ABE, acetic and butyric acid in fermentation were determined by a gas chromatography (Agilent 7890A) equipped with flame ionization detector (FID) and capillary column (DB-FFAP, 30 m x 250 μm x 0.25 μm) using helium as carrier gas. The injection volume was 1 μL. For acetone, butanol and ethanol analyzing, the oven temperature was maintained at 60°C for 4 min and then programmed with the increment 10°C/min to 200°C for 2 min and increased to 240°C for 3 min. The temperature of the

injector and detector was 150°C and 250°C, respectively. For acetic and butyric acid analyzing, the centrifuged fermentation was adjusted pH to 2 with phosphoric acid, and settled the particles for 2 h. The supernatant was filtered through a syringe filter PVDF with a pore size 0.2 μm. The oven temperature was maintained at 95°C for 2 min and then programmed with the increment 10°C/min to 140°C and increased 40°C/min to 200°C for 5 min. The temperatures of the injector and detector were 150°C and 240°C, respectively. Butanol yields are calculated as the butanol produced divided by the amount of fermentable sugar utilized and expressed as g/g reducing sugar utilized.

5.2.8 Statistical analysis

All results were analyzed using MINITAB version 15 at 95% confidence level.

5.3 Results and discussions

5.3.1 Pretreatment of grass cake

Changes of press cake compositions and solid recoveries before and after alkaline pretreatment are shown in **Table 5-3** and **Figure 5-7**. After pretreatment, cellulose compositions in pretreated press cake was greatly increased due to the loss of hemicellulose and lignin compositions. As high as 77.25±16.56% and 77.02±3.20% of lignin and hemicellulose were removed whereas removal of cellulose was only 27.57±10.06%. The alkaline pretreated *Miscanthus floridulus* grass and Napier grass (*Pennisetum purpureum*) stem were found to contain much less lignin contents confirmed by the scanning electron microscope (SEM) images (Yeh et al., 2018) and increase of porosity and surface area, properties required for enzymatic hydrolysis (Phitsuwan et al., 2016). Similar lignin removal efficiencies were reported under comparable feedstock and pretreatment conditions. Lignin removal of 86.10% was gained when pretreating Napier Pak Chong1 grass by 3% (w/v) NaOH at 121°C for 60 min (Pensri et al., 2016). Using Napier grass stem as the feedstock with 2% (w/v) NaOH and autoclaved at 121°C for 60 min for pretreatment, 84.10% of lignin reduction was reported (Phitsuwan et al., 2016). At higher temperature (100°C for 2 h) and amount of alkaline to biomass (13.3:1 w/w) compared to those used in this current work (90°C for 1 h and 10:1 w/w), up to 94% of

lignin in King grass was reduced (Cardona et al., 2016). At relatively higher NaOH concentration (10% at the solid to liquid ratio of 1:20 (w/v) at 90°C for 1 hour), however, only 63% lignin removal from Napier grass was found (Tsai et al., 2018). Lower lignin reduction found in Tsai et al. (2018)'s work could be attributed by higher lignin content (25.00±0.30%) of Napier grass. Compared to those found in other studies, the efficiency of lignin removal depends on concentration of alkaline solution, incubation temperature, duration time of the process and characteristics of the pretreated biomass. The solid recovery of pretreated press cake obtained in this current study was 41.32±2.84%. This figure is similar to 46.0% solid remaining found by Minmunin et al. (2015) when Elephant grass was pretreated at 10% NaOH and incubation temperature of 70°C for 2 h.

Table 5-3: Compositions of press cake before and after pretreatment

Composition	Raw press cake	Pretreated press cake
Cellulose (% dry basis)	41.68±4.76	73.08±1.13
Hemicellulose (% dry basis)	20.76±3.97	11.54±0.87
Lignin (% dry basis)	14.03±3.84	7.73±2.56
Solid recovery (%)	100	41.32±2.84

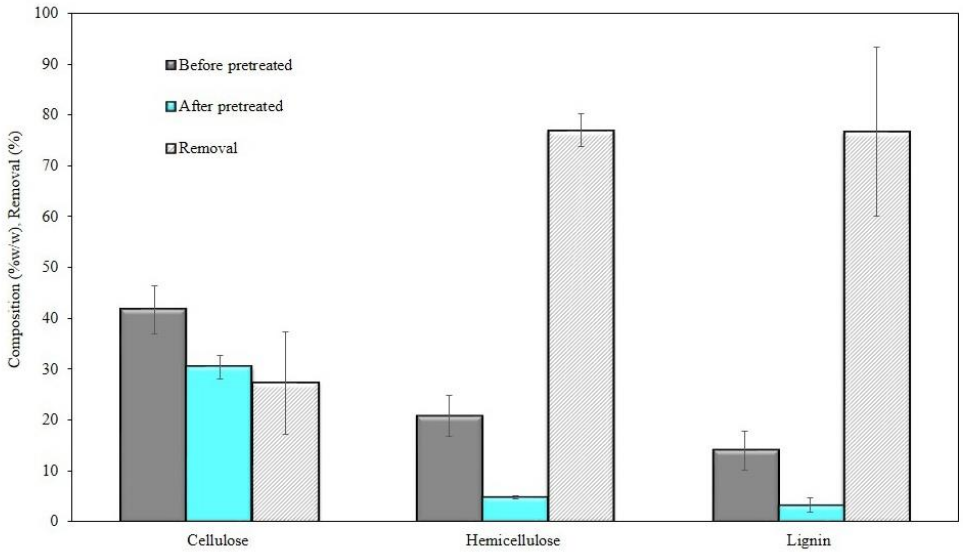


Figure 5-7: Comparison of cellulose, hemicellulose and lignin before and after NaOH-pretreatment

5.3.2 Enzymatic hydrolysis

Effects of enzyme loading volumes on reducing sugar productions are shown in **Table 5-4** and **Figure 5-8**. Total reducing sugars were found to be positively varied with enzyme loading volumes. The produced reducing sugars were stabilized at the enzyme loading volume of 0.20 mL/g with the reducing sugar concentration of 62.75±2.26 g/L as concentrations of total reducing sugar at enzyme loading volume of 0.20-0.30 mL/g pretreated biomass were not significantly different (P=0.081). The major products in the hydrolysate were glucose, xylose and arabinose (**Figure 5-9**). Concentrations of glucose, the main substrate for biobutanol production, obtained at enzyme loading volumes of 0.20 and 0.25 mL/g pretreated biomass were 34.51±1.43 and 35.96±4.98 g/L which were not significantly different (P=0.677). The suitable enzyme loading volume, therefore, was at 0.20 mL/g pretreated biomass, which provided reducing sugar yield of 627±23 mg/g pretreated biomass or 259 ± 9 mg/g dried press cake. Yields of glucose and total reducing sugars have been found to depend on several factors, i.e. composition and structure of pretreated biomass, type of enzyme, enzyme loading volume, condition of hydrolysis. Cardona et al. (2016) reported lower reducing sugar yield (268.8 mg/g pretreated biomass) from the enzymatic hydrolysis (using Accellerase 1500) of NaOH-pretreated grass compared to that found in this current study. Moreover, glucose concentrations obtained in this current work (34.51 – 35.96 g/L) were considerably higher than 18.5 g/L found in the hydrolysate of 2% (w/v) NaOH-pretreated stem Napier grass using combined Cellic CTec2 cellulase and Cellic HTec2 xylanase (Phitsuwan, et al., 2016). Compared to those found in this current work, slightly higher glucose yield (740 g/g treated biomass) was gained by Liong et al. (2012) using alkali-pretreated grass, possibly thanks to the use of higher solid to liquid ratio (1 g: 100 mL of distilled water), while less of glucose concentration (7.4 g/L) was produced. At higher enzyme loading volume (2 mL/g substrate, ten times higher than that used in this current work), 768 mg/g pretreated biomass and 522 mg/g pretreated biomass for reducing sugar and glucose yields were observed (Pensri et al., 2016). Tsai et al. (2018) reported relatively high glucose (51.6 g/L) and xylose (13.5 g/L) concentrations from the hydrolysis of alkaline-pretreated Napier grass. High glucose yield (681 mg/g pretreated biomass) was also obtained from

the hydrolysis of NaOH-pretreated Thai mission grass by dilute acid with cellulase from Trichoderma reesei ATCC 26921 (Prasertwasu et al., 2014).

Table 5-4: Effect of enzyme loading volume on the sugar fermentation at 72 h of fermentation

Enzyme loading volume (mL/g pretreated biomass)	Total reducing sugar (g/L)	Total reducing sugar yield	
		mg/g pretreated biomass	mg/g raw biomass
0.05	24.91±3.60	249±36	103±15
0.10	37.06±7.29	371±73	153±30
0.15	51.11±7.47	511±75	211±31
0.20	62.75±2.26	627±23	259±9
0.25	71.68±7.49	717±75	296±31
0.30	72.63±6.71	726±67	300±28

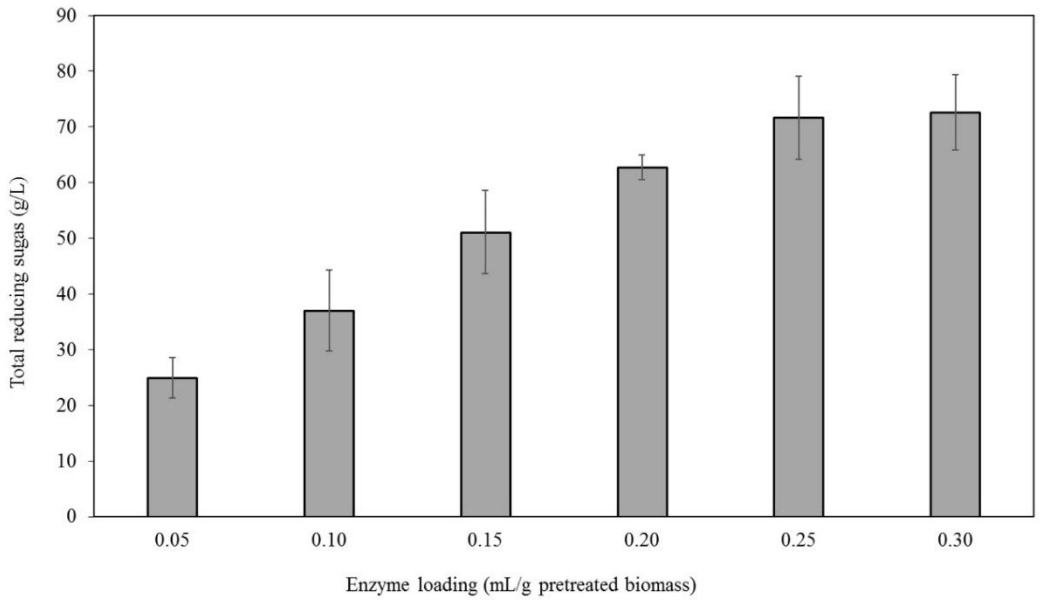


Figure 5-8: Total reducing sugar of hydrolysate at various of enzyme loading volume

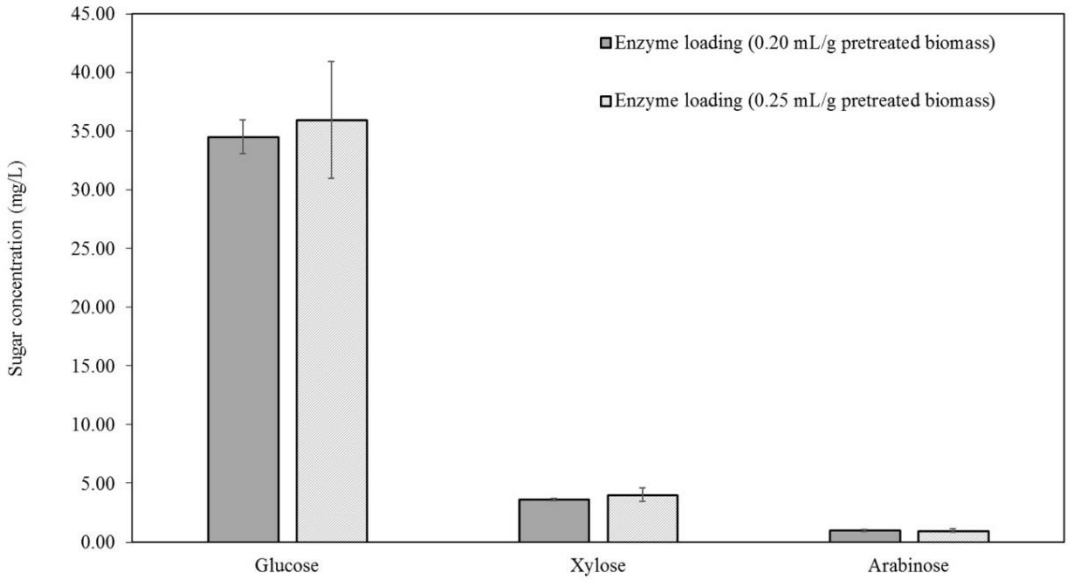


Figure 5-9: Glucose, xylose and arabinose concentrations of hydrolysates at enzyme loading volume 0.20 and 0.25 mL/g pretreated biomass

5.3.3 Selection of Clostridia

Results of gram staining of inoculated stock *Clostridium* species are shown in **Figure 5- 10**. Clostridia cells were rod shaped and the most amounts of cells of *C. beijerinckii* TISTR 1461 were detected, while *C. beijerinckii* DSM 791 was found at the least amounts. After the reactivation process of stock cultures, *C. beijerinckii* TISTR 1461 and *C. acetobutylicum* JCM 1419 were the most actively growing with the OD of 1.276 and 1.291, respectively, while *C. beijerinckii* JCM 1390 and *C. beijerinckii* DSM 791 were the least actively growing with the OD of 0.639 and 0.786, respectively. Therefore *C. beijerinckii* TISTR 1461, *C. acetobutylicum* JCM 1419 and *C. acetobutylicum* TISTR 1462 were selected for sugar utilizing test in butanol fermentation to attain the suitable culture for butanol production.

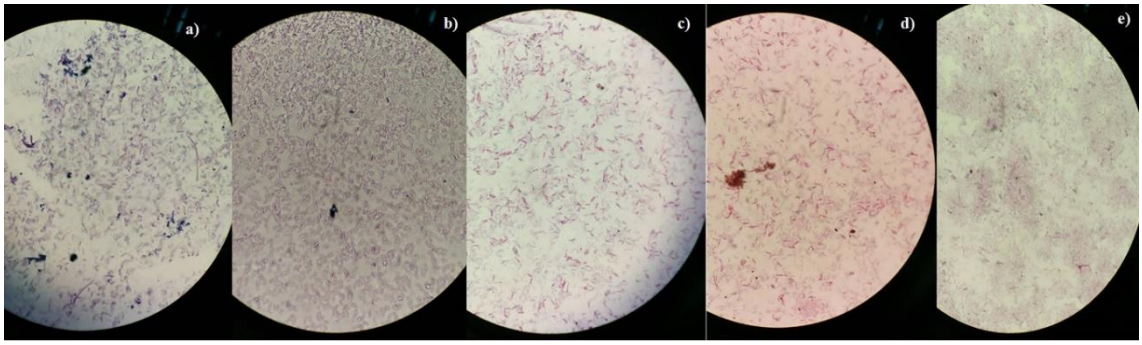


Figure 5-10: Gram staining of clostridia a) *Clostridium beijerinckii* TISTR 1461, b) *Clostridium acetobutylicum* TISTR 1462, c) *Clostridium beijerinckii* JCM 1390, d) *Clostridium beijerinckii* DSM 791, e) *Clostridium acetobutylicum* JCM 1419

As detected in the hydrolysate of NaOH-pretreated press cake; glucose, xylose, arabinose individually (60 g/L each) as the sole substrate and mixture of sugars (glucose, xylose, and arabinose) were used for butanol production test. Reductions of studied sugars at different times (**Figure 5-11**) show that *C. beijerinckii* TISTR 1461 provided the best sugar utilization rate for butanol production compared to other cultures. At 120 h of fermentation (**Table 5-5**), *C. beijerinckii* TISTR 1461 could utilize up to 66.4% of glucose for butanol production, while less than 50% of glucose was utilized by *C. acetobutylicum* JCM 1419 and *C. acetobutylicum* TISTR 1462. Less amounts of *C. acetobutylicum* TISTR 1462, presenting by only 0.870 of OD could partly explain the inferiority of this culture in transforming sugars compared to *C. beijerinckii* TISTR 1461. There have been reports of positive effects of inoculum size on butanol production (Shukor et al. (2014), Ranjan et al. (2013) and Razak et al. (2013)). Generally, optimum inoculum size was 10% (v/v) for the butanol production from glucose by *C. acetobutylicum* NRRL B527 (Mane and Deshmukh, 2013). As glucose was the main hydrolysis composition of sugars found in this work, preference of *C. acetobutylicum* JCM 1419 to arabinose made this culture unsuitable for butanol production. Transformation of glucose into ABE by *C. beijerinckii* TISTR 1461 is presented in **Figure 5-12**. Maximum butanol production at 120 h was 5.10 g/L corresponding to butanol yield of 0.127 $g/g_{glucose\ utilized}$. Similar butanol yields from glucose were observed using different cultures, e.g. *C. Cellulovorans* (0.134 $g/g_{glucose\ utilized}$) and *C. pasteurianum*

GL11 (0.167 $g/g_{glucose\ utilized}$) (Ou et al., 2017; Xin et al., 2016). Judged by its growth and performance in converting sugars into butanol, *C. beijerinckii* TISTR 1461 was found to be the most suitable strain for biobutanol production in this study.

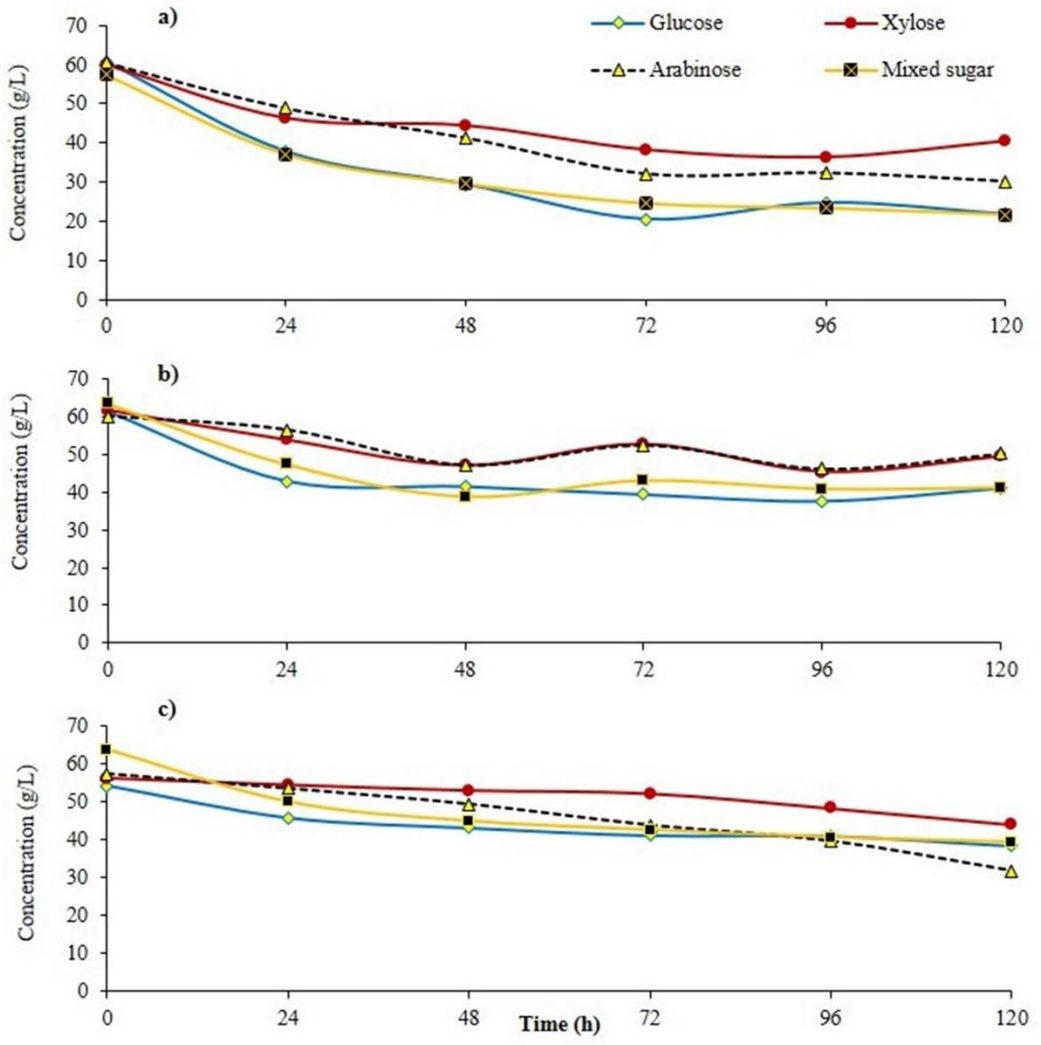


Figure 5-11: Reduction of sugars by (a) *C. beijerinckii* TISTR 1461, (b) *C. acetobutylicum* TISTR 1462, and (c) *C. acetobutylicum* JCM 1419

Table 5-5: Sugar utilizing of *C. beijerinckii* TISTR 1461, *C. acetobutylicum* TISTR 1462 and *C. acetobutylicum* JCM 1419

Sugar utilizing (%)	*C. beijerinckii* TISTR 1461	*C. acetobutylicum* TISTR 1462	*C. acetobutylicum* JCM 1419
Glucose	66.40±5.14	37.22±1.99	32.72±2.97
Xylose	45.43±0.68	26.14±3.36	31.16±4.70
Arabinose	44.69±1.60	24.37±4.40	44.61±6.47
Mixed sugars	62.07±4.21	35.70±1.64	38.10±1.83

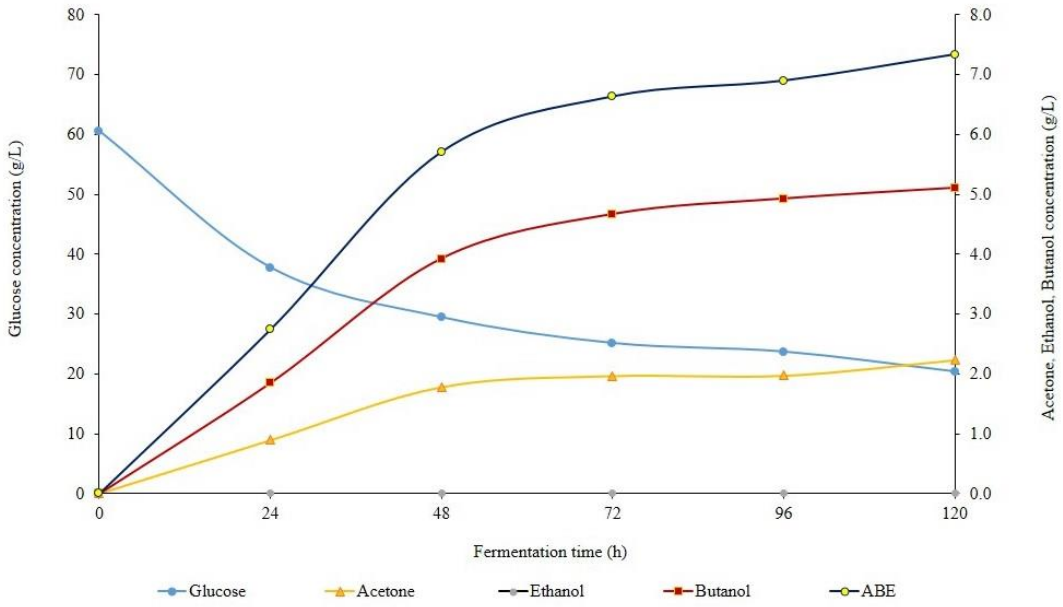


Figure 5-12: Transformation of glucose into ABE by *C. beijerinckii* TISTR 1461

5.3.4 Butanol production

Butanol productions from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 and statistical analysis of the results are shown in **Table 5-6** and **Table 5-7**. Butanol yields and butanol productions were in the range of $0.091–0.183$ g/$g_{\text{reducing sugar utilized}}$ and 3.28–4.40 g/L, respectively (**Table 5-6**). Both pH and sugar concentration had significant linear and quadratic effects on butanol yield (at 95% significant level). Wang and Blaschek (2011) also found that sugar concentration and initial pH were the most

significant factors affecting biobutanol production by *Clostridium brijerinckii* NCIMB 8052 from oil palm decanter cake hydrolysate. Apart from glucose concentration and initial pH, the ratio of inoculum was found to significantly affect butanol production from oil palm decanter cake hydrolysate by *Clostridium acetobutylicum* ATCC 824 (Razak et al., 2013), which was in accordance with the roles of inoculum size (%) and initial pH in butanol production from palm kernel cake by *Clostridium saccharoperbutylacetonicum* N1-4 that was found to be greater than effects of temperature incubation (Shukor et al., 2014). From the multiple regression analysis of the experimental data, yields of butanol production (Y) can be calculated using Eq. 5-2, which combines all significant independent variables in terms of uncoded (real) values. This equation can be appropriately used to predict the butanol yields under different pH levels and sugar concentrations as the coefficient of determination (R^2, **Table 5-7**) is acceptably high (92.19%) and the P value of lack of fit is adequately low (0.245, **Table 5-8**).

Table 5-6: The results of butanol production

Run	pH	Sugar concentration (mg/L)	Butanol (g/L)	Butanol yield ($g/g_{reducing\ utilized}$)
1	-1 (5.50)	-1 (40.0)	3.62	0.145
2	+1 (6.50)	-1 (40.0)	4.37	0.155
3	-1 (5.50)	+1 (60.0)	3.39	0.115
4	+1 (6.50)	+1 (60.0)	3.28	0.091
5	-α (5.29)	0 (50.0)	3.47	0.094
6	α (6.71)	0 (50.0)	4.13	0.118
7	0 (6.00)	-α (35.0)	4.28	0.154
8	0 (6.00)	α (64.0)	3.57	0.110
9	0 (6.00)	0 (50.0)	4.40	0.170
10	0 (6.00)	0 (50.0)	4.08	0.163
11	0 (6.00)	0 (50.0)	4.39	0.161
12	0 (6.00)	0 (50.0)	3.61	0.160
13	0 (6.00)	0 (50.0)	4.32	0.183

Table 5-7: Regression analysis of butanol production from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461

Term	Standard error coefficient	*T* value	*F* value
Constant	0.714	-6.760	0.000
A-pH	0.221	6.992	0.000
B-Sugar concentration	0.008	3.064	0.018
A^2	0.017	-6.808	0.000
B^2	0.000	-3.771	0.007
AB	0.001	-1.541	0.167
R-squared = 92.19%			
Adjust R-squared = 86.61%			

Table 5-8: Analysis of variance for butanol production from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461

Source	DF	Seq SS (Sequential sum of squares)	F value	P value
Regression	4	0.009991	17.11	0.001
Linear	2	0.003166	22.40	0.001
A-pH	1	0.000050	39.78	0.000
B-Sugar concentration	1	0.003116	9.24	0.016
Square	2	0.006825	23.38	0.000
A^2	1	0.005054	39.55	0.000
B^2	1	0.001771	12.13	0.008
Residual Error	8	0.001168		
Lack-of-Fit	4	0.000791	2.10	0.245
Pure Error	4	0.000377		
Total	12	0.011159		

$$Y = -4.31 + 1.39pH + 0.01Sugar\ concentration - 0.12(pH)^2 - 0.00016(Sugar\ concentration)^2 \quad (5\text{-}2)$$

Where: Y = Butanol yield, $g/g_{reducing\ sugar\ utilized}$

pH = Initial pH of hydrolysate

Sugar concentration = Initial reducing sugar concentration of hydrolysate, g/L

The boundaries of parameters used for constructing this equation was 5.29-6.71 of pH and 35.0-64.0 g/L of sugar concentrations. Combined effects of initial pH levels and initial reducing sugar concentrations on butanol yields are graphically shown in **Figure 5-13**. Yields of butanol were increased with increase of initial pH values and reducing sugar concentrations of hydrolysate.

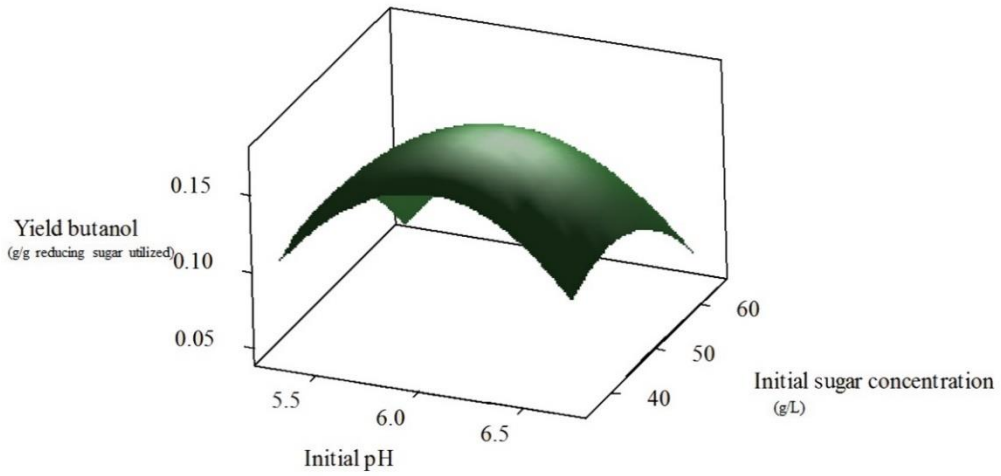


Figure 5-13: Response surface plot of butanol yield as a result of initial pH and initial reducing sugar concentration of hydrolysate of NaOH-treated press cake

According to the regression model, the optimized pH and reducing sugar concentration for butanol production from hydrolysate of NaOH-treated press cake by *C. beijerinckii* TISTR 1461 were 6.08 and 43 g/L, respectively. The maximum butanol yield estimated at these conditions at 74% of composite desirability was 0.17 $g/g_{reducing\ sugar\ utilized}$. The validating experiments conducted at the obtained optimized conditions (**Figure 5-14**) gave the butanol yield of 0.14±0.00 $g/g_{reducing\ sugar\ utilized}$. This butanol yield

was accounted to 77.33% of the estimated butanol yield suggesting that the response surface methodology approach was reasonably effective. Under unsuitable initial pH (4.2), Sanguanchaipaiwong and Leksawasdi (2018) found that butanol yield of pineapple waste juice by *C.beijerinckii* TISTR 1461 was only 0.08 $g/g_{reducing\ sugar\ utilized}$ while butanol yield of glucose (60 g/L) was 0.182 $g/g_{glucose\ utilized}$. **Table 5-9** shows results reported in previous studies using Napier grass, glucose, and other substrates to butanol yield compared to this current study. Using Napier grass as the substrate for butanol production, He et al. (2017b) found that butanol yield attained by semi-simultaneous saccharification fermentation using *C.acetobutylicum* ATCC 824 was 0.22 $g/g_{glucose\text{-}xylose\ utilized}$. This value was not substantially different from that gained at the optimum condition found in this current study considering that butanol yield was presented per gram of reducing sugar in this current work instead of gram of glucose and xylose used in the study of He et al. (2017a). Comparably, butanol yield from sweet potato by *C.acetobutylicum* was 0.18 $g/g\text{-}sugar_{total}$ (He et al., 2017b), while butanol production from alkali pretreated switchgrass and phragmites were 0.23 and 0.20 $g/g_{glucose\text{-}xylose\ utilized}$, respectively (Gao et al., 2014). Bellido et al. (2015) also found that butanol yield of pretreated sugar beet pulp by *C. beijerinckii* DSM 6422 was 0.22 $g/g_{sugar\ utilized}$. On the other hand, Wu et al. (2019) found that co-culture of *Clostridium beijerinckii* F-6 and *Saccharomyces cerivisiae* could enhance butanol yield. Adding *S. cervisiae* at 0.02 g/L after 12 h fermentation of *C.beijerinckii* F-6, butanol yield was 270% increased (0.27 g/g) from that gained using the monoculture of *C. beijerinckii* F-6 fermentation (0.10 g/g). Accordingly, it can be perceived that different butanol yields compared to what found in this current study have been reported under different fermentation conditions such as type of microorganism (Jonglertjunya et al., 2014), pH (Khamaiseh et al., 2013), temperature (Khamaiseh et al., 2013), nutrient supplementation (Khamaiseh et al., 2014) and fermentation technique (Srinorakutara et al., 2018).

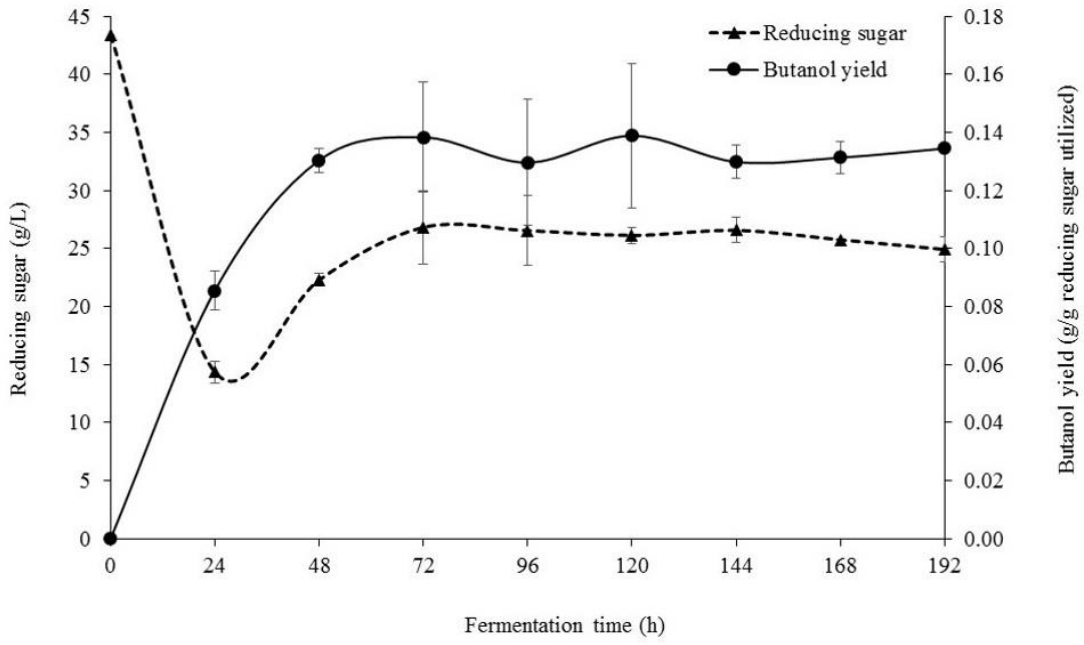


Figure 5-14: Butanol yieldS at the optimized conditionS

5.4 Conclusion

Performances of different *Clostridium* strains and effects of fermentation factors on biobutanol yields from Napier Pak Chong 1 press cake were investigated. *Clostridium beijerinckii* TISTR 1461 had the highest glucose utilization for butanol production. The optimization by Full Factorial design and CCD found that initial pH and sugar concentration had significant effects on butanol yields from enzymatic-hydrolysed of NaOH-pretreated press cake by *Clostridium beijerinckii* TISTR 1461. At optimum conditions, the maximum butanol yield from hydrolysate of NaOH-pretreated press cake was 0.135 g/g reducing sugar utilized. Net energy production analysis revealed that transformation of Napier Pak Chong 1 grass into biogas and biobutanol according to the method proposed in this work had a great potential to be the most sustainable utilization method for renewable energy production.

Table 5-9: Comparison of butanol yield from Napier grass and other substrate in previous studies and this study

Substrate	*Clostridium* sp.	Butanol yield		Reference
		$g/g_{reducing\ sugar\ utilized}$	$g/g_{glucose\text{-}xylose\ utilized}$	
Napier Pak Chong1 press cake	*C.beijerinckii* TISTR 1461	0.014		This study
Napier grass	*C. acetobutylicum* ATCC 824		0.22	He et al. (2017b)
Sweet potato	*C. acetobutylicum*		0.18[a]	He et al. (2017a)
Switchgrass	*C. saccharabutylicum* DSM 13864		0.23	Gao et al. (2014)
Phragmites	*C. saccharabutylicum* DSM 13864		0.22	Gao et al. (2014)
Sugar beet pulp	*C.beijerinckii* DSM 6422		0.22	Bellido et al. (2015)
Glucose	*C.beijerinckii* F-6 and *Saccharomyces cerivisiae*		0.27[b]	Wu et al. (2019)

Remark: a means $g/g_{sugar\ total\ utilized}$,

b means $g/g_{glucose\ utilized}$

CHAPTER 6

Comparisons of energy productions for Napier Pak Chong1 utilisation scenarios

6.1 Integrated utilization of Napier Pak Chong1 grass to produce renewable energy using modified IFBB process

This study aimed to utilise Napier PakChong1 grass to produce renewable energies (in form of biogas and biobutanol) using the modified IFBB process. Material flow diagram of Napier Pak Chong1 utilisation is shown in **Figure 6-1**.

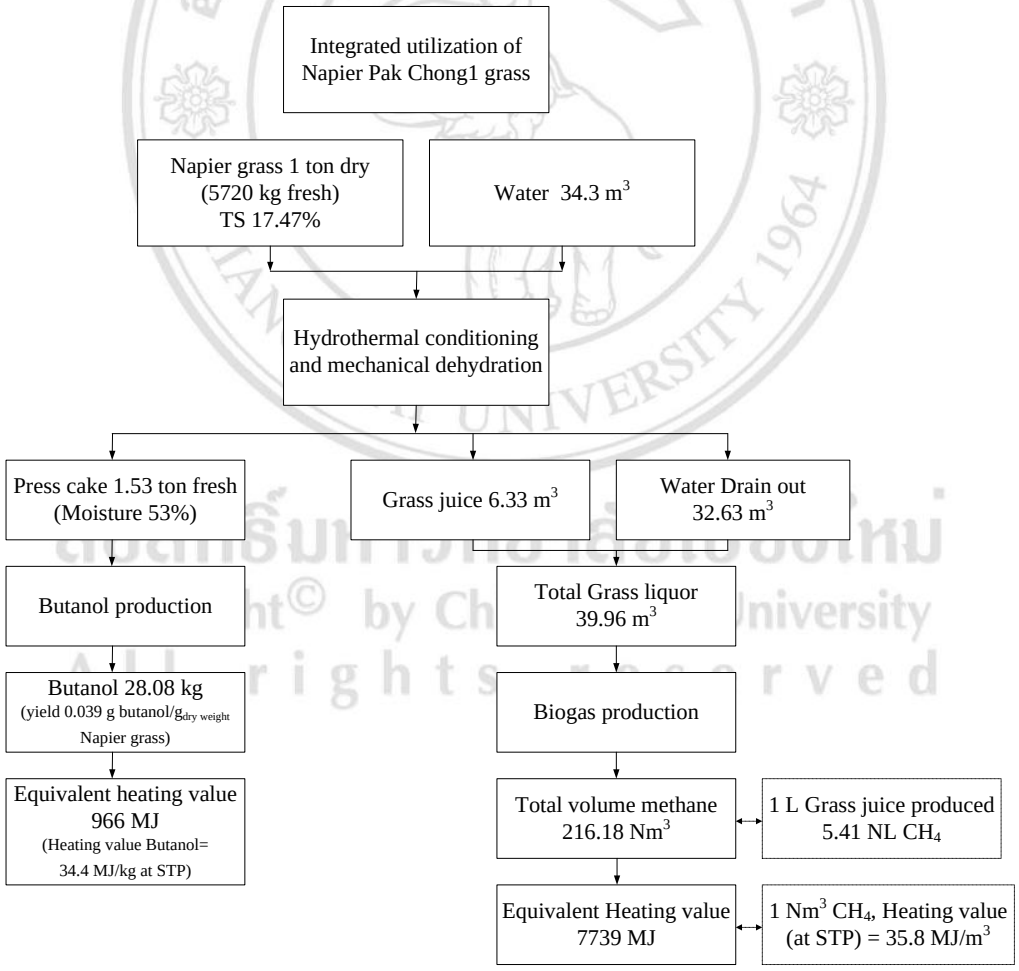


Figure 6-1: Material flow diagram of Napier Pak Chong1 utilisation in this study (Scenario A)

According to **Figure 6-1**, 1 ton of the dry Napier Pak Chong1 grass produces 216 Nm^3 and 28 kg of biogas and biobutanol, which are equivalent to heating values of 7739 and 966 MJ, respectively.

6.2 Comparisons of Napier Pak Chong1 grass utilization scenarios

Comparisons among different Napier Pak Chong 1 utilization scenarios; i. e. conversion to biogas and butanol (A), biogas and solid fuel (B), biogas (C), butanol (D) and ethanol (E), were made based on the produced energy presented in terms of heating values (**Figure 6-1 to 6-5**). Utilisation of Napier Pak Chong1 grass to produce biogas and solid fuel using the original IFBB (Scenario B) is shown in **Figure 6-2**. The green solid fuel is claimed to obtained in this scenario as it contains low element concentrations which would produce less amounts of air pollution after combustion. However, the process for producing solid fuel requires energy for moisture reduction (from 53% to 10%, the moisture content suitable for combustion). This requirement makes the generated heating value of Scenario B less than that of Scenario A. Energy productions in forms of heating values attained from different scenarios are in order as C>A>B>D>E. Though, scenario C is found to generate higher energy production, the produced digestate still needs to be properly managed. This digestate could not be directly combusted as it contains large amounts of water and a great amount of energy would be needed to lower the moisture content of remaining solids to be used as the biofuel. If the biobutanol yield from the grass press cake in scenario A could be improved by optimizing other pertinent factors, e. g. type of microorganism, temperature, nutrient supplementation and fermentation technique, the merit of this scenario could be increased. Considering that butanol has many advantages over other forms of biofuel, i.e. biogas, ethanol and biofuel, Scenario A could be the most promising way for Napier Pak Chong 1 utilisation if all aspects of related processes, especially the biobutanol production, are meticulously optimized.

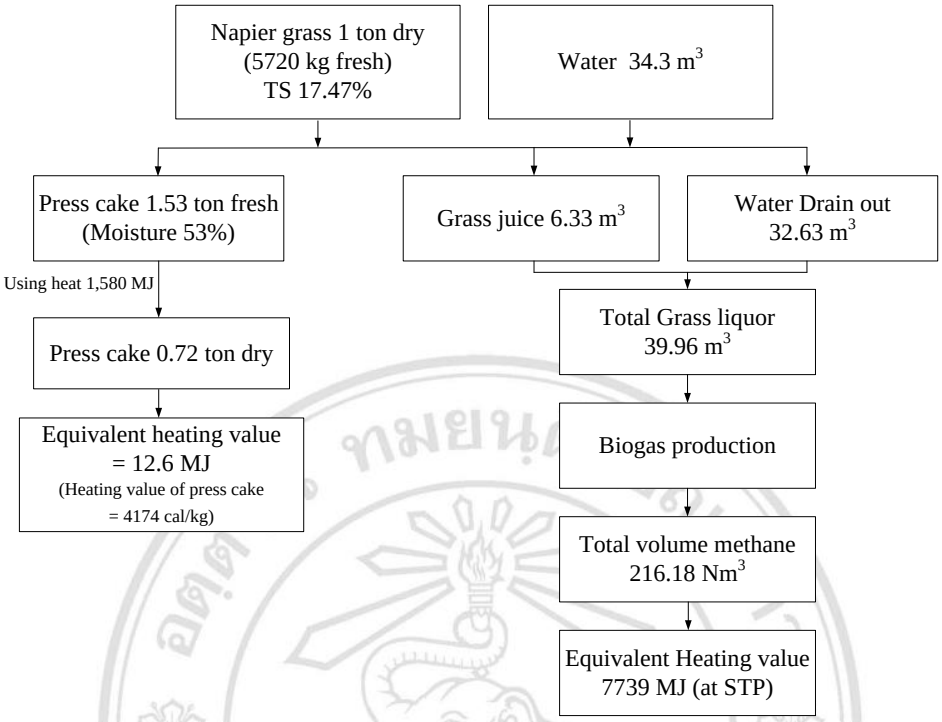


Figure 6-2: Material flow diagram of Napier Pak Chong1 utilisation in Scenario B

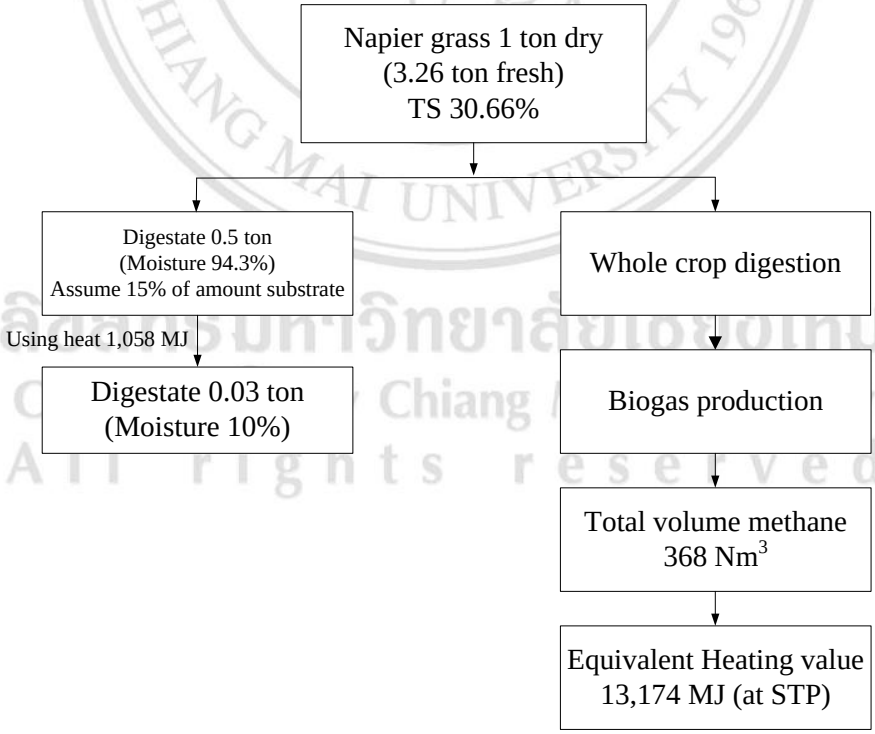


Figure 6-3: Material flow diagram of Napier Pak Chong1 utilisation in Scenario C (calculated based on data presented in Nizami et al., 2012)

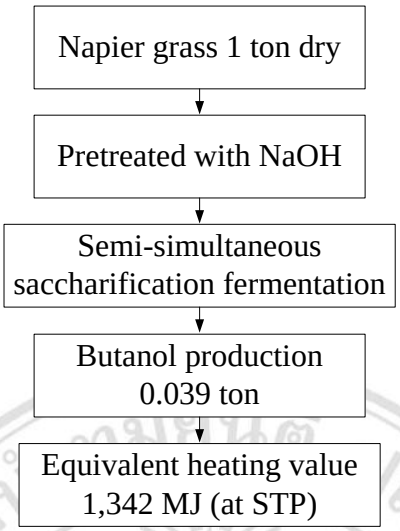


Figure 6-4: Material flow diagram of Napier Pak Chong1 utilisation in Scenario D (calculated based on data presented in He et al., 2017)

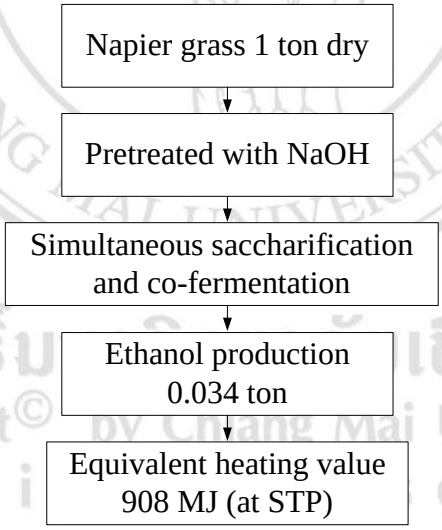


Figure 6-5: Material flow diagram of Napier Pak Chong1 utilisation in Scenario E (calculated based on data presented in Liu et al., 2017)

CHAPTER 7

Conclusion

7.1 Conclusion

This study aimed to utilise Napier PakChong1 grass to produce renewable energies (in form of biogas and biobutanol) using the modified IFBB process which shown as in which are divided into three parts. The three parts of the experiment are concluded as follow;

7.1.1 Part I: Determination of the optimum conditions for hydrothermal conditioning and mechanical dehydration

The optimum hydrothermal conditioning conditions for Napier PakChong1 grass were as follows; harvesting time 75 d, ratio of grass to water of 1: 6 (weight: volume), ambient temperature (about 25°C) of the water and the soaking time of 355 min. The mass of COD in the press juice obtained in these conditions was 226.42 g equating to 71.5% of the value predicted by the model (316.68 g). Results from the BMP test showed that methane yield of press fluid was 0.4 Nm^3CH_4/kg VS with methane content of 68.6%. The microbial kinetic coefficients and biogas yield potential of press fluid were properly fitted with the modified Gompertz equation (adjusted $R^2 = 0.995$). The methane yield potential (P), the maximum methane production rate (R_m) and lag phase time (λ) were 0.41 Nm^3CH_4/kg VS, 0.05 Nm^3CH_4/kg VS /d and 4.36 days, respectively. Producing biogas from the press fluid was clearly superior to that from the whole grass in a commercial scale.

7.1.2 Part II: Investigation of effects of organic loading rates, feeding scheme, trace element addition and effluent recirculation rates on the performance of an anaerobic baffled reactor (ABR)

Effects of different operating conditions on biogas production from Napier Pak Chong1 grass liquor using ABR were investigated. Sludge washout and lower methane yields were observed when ABR was operated under high OLR. ABR operated with semi-

continuous feeding scheme showed better performance than that with once a day feeding scheme. Additions of TEs helped the ABR to have higher methane compositions and were expected to maintain stability of the reactor in long term operation. The highest methane yield was detected at effluent recirculation rate of 0.5. At higher effluent recirculation rates, methane yields and COD removal efficiencies were significantly lowered.

7.1.3 Part III: Determination of the suitable bacterial strain and investigation of effects of initial sugar concentrations and pH values on the efficiency of biobutanol production

Performances of different *Clostridium* strains and effects of fermentation factors on biobutanol yields from Napier Pak Chong 1 press cake were investigated. *Clostridium beijerinckii* TISTR 1461 had the highest glucose utilization for butanol production. The optimization by Full Factorial design and CCD found that initial pH and sugar concentration had significant effects on butanol yields from enzymatic-hydrolysed of NaOH-pretreated press cake by *Clostridium beijerinckii* TISTR 1461. At optimum conditions, the maximum butanol yield from hydrolysate of NaOH-pretreated press cake was 0.135 g/g reducing sugar utilized. Net energy production analysis revealed that transformation of Napier Pak Chong 1 grass into biogas and biobutanol according to the method proposed in this work had a great potential to be the most sustainable utilization method for renewable energy production.

7.2 Suggestions for the future work

Comparisons among different Napier Pak Chong 1 utilization scenarios; i.e. conversion to biogas and butanol (A), biogas and solid fuel (B), biogas (C), butanol (D) and ethanol (E), were made based on the produced net energy presented in terms of the heating value. Net energy productions in forms of heating values attained from different scenarios are in order as C>A>B>D>E. Though, scenario C was found to generate higher energy production, large amounts of digestate containing relatively low concentrations of suspended solids are problematic to use and still need to be managed. If the biobutanol yield from the grass press cake in scenario A could be improved by optimizing other

pertinent factors, e.g. nutrient supplementation and fermentation technique, the merit of this scenario could be much greater.

Loss of press cake solids (up to 58.68%) during the pretreatment process for butanol production could be considered as the waste of resource. It is interesting to investigate for improved pretreatment processes for press cake to reduce loss of raw substrate and to increase biobutanol production, e.g. by using mild alkaline solution pretreatment. Alternative utilisations for grass juice are also promising further studies, e.g. the high-value concentrated protein production by lactic acid fermentation of grass juice. Moreover, the more efficient machine for mechanical dehydration process to produce higher quality grass juice and press cake is one of the very important topics for study.

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LIST OF PUBLICATIONS

1. Suaisom, P., Pholchan, P., Che Man, H., Aggarangsi, P. (2019). Optimization of hydrothermal conditioning conditions for *Pennisetum purpureum x Pennisetum americanum* (Napier Pakchong1 grass) to produce the press fluid for biogas production. *Pertanika Journal of Science and Technology*, 27, 109-122.

2. Pitchaya Suaisom[a,b], Patiroop Pholchan[a,c] and Pruk Aggarangsi[b]. "Holistic determination of suitable conditions for biogas production from *Pennisetum purpureum x Pennisetum americanum* liquor in Anaerobic Baffled Reactor" Submitted to Journal of Environmental Management, Accepted.

APPENDICES

A-1 Data of Grass juice from full factorial design and Central Composite design

Experiment	Weight of grass (kg)	water (L)	Volume of grass juice (L)	Weight of press cake (kg)
1	4	12	3.12	1.30
2	4	12	2.55	1.60
3	4	20	2.40	1.20
4	4	20	2.90	1.55
5	4	12	2.45	1.10
6	4	12	2.83	1.50
7	4	20	3.25	1.10
8	4	20	2.90	1.60
9	4	12	1.80	1.00
10	4	12	2.55	1.80
11	4	20	2.15	1.00
12	4	20	2.10	1.40
13	4	12	2.15	1.00
14	4	12	2.10	1.30
15	4	20	1.90	0.90
16	4	20	2.82	1.30
17	4	16	2.40	1.20
18	4	16	2.60	1.50
19	4	16	2.70	1.20
20	4	16	2.50	1.30
21	4	16	2.50	1.50
22	4	16	3.05	1.70
23	4	8	2.49	1.40
24	4	24	3.00	1.30
25	4	16	3.40	1.60
26	4	16	2.90	1.50
27	4	16	3.70	1.60
28	4	16	2.29	1.20
29	4	16	2.40	1.30
30	4	16	2.43	1.20

A-2 Characteristics of grass juice from full factorial design and Central Composite design

Experiment	COD_t (mg/L)	TKN (mg/L)	TS (mg/L)	VS (mg/L)	WSC (mg/L)	Reducing sugar (mg/L)
1	11,805	426	12,180	8,380	2,264	2,094
2	8,185	414	11,020	6,910	4,191	4,517
3	8,901	289	8,880	6,260	1,283	1,209
4	9,499	346	10,090	6,790	2,644	2,937
5	12,728	435	14,700	10,560	2,152	2,156
6	11,042	488	13,000	8,520	3,461	3,521
7	10,467	350	9,900	7,080	1,634	1,851
8	7,516	346	9,150	6,090	2,186	2,495
9	10,774	402	13,880	9,620	1,899	1,974
10	12,338	536	14,720	9,100	3,253	3,211
11	7,646	250	8,400	5,980	1,388	1,323
12	7,166	278	8,830	5,730	2,028	1,846
13	12,777	462	15,240	10,720	2,824	2,994
14	14,071	663	15,760	9,810	2,796	2,707
15	8,793	271	8,600	6,000	2,637	2,790
16	9,297	423	10,450	6,780	2,095	1,772
17	8,729	374	10,430	6,690	1,410	1,546
18	9,862	413	11,160	7,190	1,507	1,603
19	9,226	469	11,650	8,200	1,054	991
20	9,704	535	13,033	9,467	1,645	1,714
21	9,476	513	11,700	7,800	2,923	2,863
22	11,698	215	13,620	10,280	5,806	5,556
23	11,017	438	12,500	8,420	3,904	4,354
24	8,089	310	8,820	5,920	2,756	3,024
25	12,046	460	14,400	9,800	3,177	3,692
26	11,922	472	13,167	9,367	3,860	4,370
27	11,058	413	12,800	8,660	2,796	3,007
28	12,773	403	13,520	9,240	4,130	4,048
29	10,381	469	12,167	8,167	2,162	2,181
30	9,676	551	12,533	8,700	1,404	1,223

A-3 Characteristics of grass cake from full factorial design and Central Composite design

Experiment	TKN (mg/kg)	TS (mg/kg)	VS (mg/kg)	Moisture (%)
1	2,694	412,658	385,696	59
2	3,641	298,760	286,434	70
3	2,305	307,808	288,630	69
4	4,654	380,105	365,841	62
5	3,277	419,432	394,773	58
6	2,796	275,032	264,000	72
7	2,305	260,897	244,167	74
8	3,189	295,553	283,567	70
9	3,941	459,213	430,073	54
10	3,651	269,535	256,698	73
11	2,567	346,830	325,648	65
12	2,762	222,073	212,269	78
13	3,622	424,095	399,714	58
14	4,318	360,388	347,442	64
15	2,979	444,141	417,980	56
16	3,594	365,074	352,647	63
17	3,040	398,301	380,392	60
18	3,798	335,775	319,358	66
19	2,466	339,548	323,544	66
20	3,823	335,598	319,590	66
21	3,607	2,61493	245,522	74
22	1,622	604,167	586,042	40
23	3,451	291,961	272,353	71
24	3,512	308,857	287,714	69
25	3,190	270,000	251,714	73
26	4,088	493,514	468,378	51
27	2,845	335,714	315,738	66
28	3,810	390,172	373,621	61
29	2,730	239,722	227,037	76
30	3,490	409,795	391,393	59

A-4 Characteristics of dried grass cake from full factorial design and Central Composite design

Experiment	TS (mg/kg)	VS (mg/kg)	Heating value (cal/g)
1	978,594	917,191	4,088.01
2	961,574	916,296	3,824.96
3	990,543	934,457	4,271.92
4	964,078	921,748	3,945.07
5	973,784	917,387	4,387.13
6	987,778	944,444	4,096.79
7	922,310	866,924	4,370.70
8	998,532	957,431	4,031.65
9	975,933	915,189	4,051.97
10	983,905	934,476	4,021.78
11	994,241	939,494	4,113.11
12	993,100	953,400	4,153.70
13	983,317	926,487	4,422.00
14	1,022,700	983,400	4,224.33
15	989,130	935,024	4,263.61
16	975,738	945,328	4,153.38
17	988,165	939,266	4,082.08
18	996,140	943,041	4,048.02
19	1,005,285	960,434	4,302.85
20	1,018,823	971,879	4,222.84
21	1,011,856	953,735	4,177.57
22	1,003,358	966,917	4,029.10
23	999,091	940,000	4,196.54
24	1,015,928	960,587	3,948.13
25	1,028,444	960,444	3,992.98
26	1,063,243	1,010,000	3,984.43
27	1,019,625	958,950	4,011.38
28	1,053,305	1,003,940	4,140.31
29	1,018,769	970,000	4,050.80
30	1,021,158	976,575	4,162.57

A-5 Data of BMP test of grass liquor

Time (d)	cumulative methane yield (m^3/kg VS_{added})
0.00	0.00
0.90	0.00
1.88	8.18
2.88	13.41
3.46	17.36
4.89	62.19
5.88	75.95
6.92	134.04
7.92	171.70
8.88	223.75
9.90	261.82
10.88	288.50
11.90	349.45
12.90	380.05
13.89	388.35
14.92	389.94
15.92	394.20
16.87	396.07
17.89	396.32
18.86	396.20
19.82	396.26
20.88	395.87

A-6 Characteristics of grass juice influent for ABR at OLR 1.0 kg COD/m^3.d

Date	OLR (kg COD/m^3.d)	pH	Temp ($^{\circ}$C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
23/4/2558	1.76	5.54	18.5	14,215	4,189	15,900	10,850	7,615	6,525		
27/4/2558	1.06	5.00	19.0	13,102	3,422	15,410	10,670	7,750	6,575		
30/4/2558	1.43	4.74	19.0	17,756	7,197	26,830	16,910	8,200	6,850		
4/5/2558	1.14	4.80	20.0	16,970	11,360	23,460	14,100	7,210	6,070		
7/5/2558	1.08	4.80	20.5	16,142	10,168	24,580	14,330	8,075	6,805	1,324	192
11/5/2558	1.11	4.87	22.0	16,553	11,813	23,880	14,200	9,160	7,700		
14/5/2558	1.07	4.86	22.0	15,870	8,908	23,070	13,900	6,190	5,065		
18/5/2558	0.95	5.14	24.0	14,183	6,099	15,120	9,410	5,620	4,545	893	138
21/5/2558	0.91	5.16	24.5	13,533	5,034	17,750	11,470	7,590	6,525		
25/5/2558	0.85	5.55	24.7	12,598	6,876	17,350	10,240	6,625	5,885		
28/5/2558	1.31	5.50	18.0	19,543	8,104	18,170	11,430	7,415	6,315		
1/6/2558	1.26	5.87	14.3	18,676	6,972	17,470	10,620	7,895	6,655	1,000	112
4/6/2558	1.27	4.73	18.6	18,855	10,436	23,600	14,150	6,315	6,050		
8/6/2558	1.21	4.75	20.0	18,063	9,056	24,090	13,630	7,270	6,160		

A-7 Characteristics of grass juice effluent from ABR at OLR 1.0 kg COD/m^3.d

Date	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
23/4/2558	7.20	37.1	1,758	265	4,705	1,722	1,340	1,092		
27/4/2558	7.20	35.4	426	262	4,018	1,113	211	109		
30/4/2558	7.38	35.8	759	443	5,132	1,793	375	293		
4/5/2558	7.84	34.5	1,037	539	6,945	1,875	579	401		
7/5/2558	7.72	34.0	1,820	836	8,525	2,735	1,084	850	691	71
11/5/2558	7.79	34.0	1,847	1,242	9,532	2,445	784	633		
14/5/2558	7.70	34.5	3,484	1,859	10,258	2,810	848	702		
18/5/2558	7.70	31.8	3,786	1,989	11,595	3,882	1,919	1,399	1,166	56
21/5/2558	7.81	35.0	2,502	1,216	11,530	3,700	1,271	982		
25/5/2558	7.58	35.8	2,325	1,133	10,062	2,245	1,350	1,025		
28/5/2558	7.50	35.0	2,289	1,273	9,975	3,118	633	512		
1/6/2558	7.50	35.0	1,836	737	8,948	2,290	467	319	903	47
4/6/2558	7.60	33.6	2,341	1,014	9,885	2,760	867	758		
8/6/2558	7.74	34.8	2,177	901	10,070	2,950	1,282	919		

A-8 Alkalinity and VFA of grass juice effluent from ABR at OLR 1.0 kg COD/m^3.d

Date	Alkalinity (mg/L)	VFA (mg/L)	VFA/ALK
23/4/2558	2,884	170	0.06
27/4/2558	2,829	318	0.11
30/4/2558	3,377	503	0.15
4/5/2558	4,597	654	0.14
7/5/2558	4,959	608	0.12
11/5/2558	6,235	1,059	0.17
14/5/2558	6,380	1,027	0.16
18/5/2558	6,966	1,693	0.24
19/5/2558	7,114	1,657	0.23
21/5/2558	6,820	1,411	0.21
25/5/2558	6,832	1,294	0.19
28/5/2558	6,388	1,131	0.18
1/6/2558	6,218	1,022	0.16
4/6/2558	6,286	1,000	0.16
8/6/2558	6,476	417	0.06

A-9 Volume of biogas of each chamber at OLR 1.0 kg COD/m^3.d

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
22/4/2558	11.40	7.60	1.00	0.40	0.40	20.80
23/4/2558	10.80	4.40	0.40	2.60	2.40	20.60
24/4/2558	8.20	8.40	0.20	0.80	0.60	18.20
25/4/2558	11.00	7.40	0.00	0.40	0.60	19.40
26/4/2558	10.80	7.40	0.20	1.40	1.40	21.20
27/4/2558	9.00	8.60	0.40	0.40	1.20	19.60
28/4/2558	10.60	8.80	0.40	1.00	0.80	21.60
29/4/2558	10.60	7.60	0.80	1.60	1.40	22.00
30/4/2558	11.00	10.00	0.20	0.40	0.20	21.80
1/5/2558	11.40	11.40	2.80	1.40	0.40	27.40
2/5/2558	10.60	10.00	3.00	1.20	0.20	25.00
3/5/2558	10.60	10.80	4.20	0.60	0.40	26.60
4/5/2558	11.80	10.00	5.00	0.40	0.20	27.40
5/5/2558	7.40	2.20	8.60	2.20	0.40	20.80
6/5/2558	9.60	7.40	4.60	1.40	0.60	23.60
7/5/2558	12.80	1.60	3.20	2.20	1.40	21.20
8/5/2558	15.40	9.60	5.40	3.40	1.80	35.60
9/5/2558	15.60	11.40	5.40	2.20	2.00	36.60
10/5/2558	15.90	8.60	3.70	1.70	2.00	31.90
11/5/2558	17.50	10.00	5.70	2.20	1.80	37.20
12/5/2558	14.40	8.80	3.00	1.60	0.80	28.60
13/5/2558	12.80	8.00	3.00	1.40	2.10	27.30
14/5/2558	14.60	8.40	4.80	1.40	0.80	30.00
15/5/2558	14.00	7.60	3.40	1.00	1.00	27.00
16/5/2558	13.60	8.00	3.40	2.20	2.20	29.40
17/5/2558	11.00	6.80	3.00	1.50	1.30	23.60

A-9 Volume of biogas of each chamber at OLR 1.0 kg COD/m^3.d (continue)

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
18/5/2558	13.00	6.20	1.80	1.00	0.80	22.80
19/5/2558	12.40	7.00	1.20	1.60	1.00	23.20
20/5/2558	13.20	7.20	2.20	1.20	1.20	25.00
21/5/2558	14.00	7.60	3.40	1.60	1.40	28.00
22/5/2558	13.60	6.80	1.60	1.20	1.20	24.40
23/5/2558	13.20	5.80	3.00	1.20	1.20	24.40
24/5/2558	15.40	8.00	0.20	1.00	2.00	26.60
25/5/2558	16.60	2.60	1.40	0.80	0.60	22.00
26/5/2558	12.00	3.20	1.60	1.00	0.60	18.40
27/5/2558	13.00	3.80	1.60	1.00	0.60	20.00
28/5/2558	13.20	3.20	1.20	1.20	0.80	19.60
29/5/2558	16.20	4.80	1.60	1.00	1.00	24.60
30/5/2558	14.10	4.00	1.80	1.00	2.40	23.30
31/5/2558	16.20	3.70	2.10	1.20	1.80	25.00
1/6/2558	16.10	3.70	1.80	1.00	1.80	24.40
2/6/2558	15.00	4.20	2.40	1.00	1.00	23.60
3/6/2558	16.60	2.00	0.80	0.60	0.60	20.60
4/6/2558	15.80	2.64	0.80	0.60	0.40	20.24
5/6/2558	10.40	7.80	2.20	1.00	1.60	23.00
6/6/2558	12.20	5.60	2.40	1.00	1.20	22.40
7/6/2558	16.60	6.80	2.20	1.60	1.00	28.20
8/6/2558	18.60	2.60	0.80	0.40	1.40	23.80

A-10 Composition of CH_4 at OLR 1.0 kg COD/m^3.d

Date	CH_4 Composition (%)				
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5
23/4/2558	58.8	63.5	63.5	45.6	15.6
27/4/2558	57.3	67.3	64.4	51.8	31.3
30/4/2558	53.9	68.1	64.6	53.8	28.6
7/5/2558	55.5	61.3	71.2	70.7	44.6
12/5/2558	61.4	72.7	72.0	69.5	46.0
14/5/2558	62.2	71.7	66.8	65.4	49.4
18/5/2558	67.0	73.5	71.3	66.1	42.1
20/5/2558	65.9	71.3	70.1	61.7	50.5
25/5/2558	68.4	71.5	63.2	64.3	63.6
28/5/2558	63.1	65.5	64.2	51.1	50.8
2/6/2558	66.7	65.0	63.3	48.7	40.0
4/6/2558	64.2	64.9	62.9	51.3	38.8
8/6/2558	68.3	68.6	60.1	46.4	35.6

A-11 Characteristics of grass juice influent for ABR at OLR 2.0 kg COD/m^3.d

Date	OLR (kg COD/m^3.d)	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
15/6/2558	2.50	6.07	19.6	18,612	8,236	31,420	15,130	10,295	8,825	1,620	234
18/6/2558	4.11	5.68	20.0	30,569	16,120	30,880	16,910	12,670	10,195		
22/6/2558	4.02	4.85	21.2	29,919	13,847	29,050	14,470	10,760	8,775		
25/6/2558	3.32	4.09	19.7	24,703	14,431	26,320	14,690	7,595	6,120		
29/6/2558	3.00	3.92	11.4	22,296	10,814	27,690	16,460	9,955	8,335	1,039	183
2/7/2558	1.97	5.37	11.7	20,919	8,757	31,350	16,600	13,905	11,020		
6/7/2558	2.05	5.45	11.1	21,758	7,778	32,550	18,180	15,040	12,055		
9/7/2558	1.86	3.99	10.0	13,862	7,128	19,950	11,220	6,285	5,135		
13/7/2558	2.11	3.19	6.9	15,716	7,702	20,620	11,690	7,330	6,155	724	116
16/7/2558	2.53	3.95	9.5	18,804	8,972	19,260	10,770	6,870	5,510		
20/7/2558	1.82	4.31	10.0	22,619	9,743	20,660	12,190	8,070	6,475		
23/7/2558	1.46	4.22	9.7	18,160	6,063	21,250	12,440	9,075	7,505		
27/7/2558	2.25	3.88	8.9	27,925	15,581	27,550	16,950	10,980	8,220	675	222
3/8/2558	2.66	4.22	10.0	33,028	25,088	30,380	18,047	12,640	10,035		
6/8/2558	1.67	4.26	10.0	27,053	15,288	28,820	15,720	10,350	8,435		
10/8/2558	1.87	4.08	10.0	30,293	8,363	28,170	17,170	10,635	8,450	686	172
13/8/2558	1.65	4.45	10.0	22,725	8,786	27,300	14,900	11,190	8,900		
17/8/2558	1.82	4.50	11.2	22,584	10,040	29,150	16,970	12,400	10,160		
20/8/2558	1.81	4.41	14.5	22,383	9,649	29,650	18,135	15,163	12,153		
24/8/2558	1.59	4.50	14.7	17,892	9,011	27,060	15,825	13,170	11,215	686	191

A-12 Characteristics of grass juice effluent for ABR at OLR 2.0 kg COD/m^3.d

Date	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
15/6/2558	7.77	37.2	8,889	2,092	18,340	8,800	8,033	6,700	1,375	75
18/6/2558	7.89	37.0	10,074	3,442	20,170	8,137	5,873	3,993		
22/6/2558	7.68	36.2	8,886	1,976	20,575	6,570	7,195	5,005		
25/6/2558	7.85	36.5	6,252	2,263	19,845	5,920	3,580	2,333		
29/6/2558	7.68	36.1	6,080	1,964	18,430	5,690	5,360	3,745	1,080	86
2/7/2558	7.78	35.0	6,890	1,349	18,485	5,820	6,128	4,325		
6/7/2558	7.79	34.8	3,536	1,111	16,595	4,535	3,940	2,748		
9/7/2558	7.86	34.8	3,845	1,158	18,550	5,105	3,767	2,728		
13/7/2558	7.66	36.5	5,739	1,530	16,990	5,195	4,840	3,295	1,192	57
16/7/2558	7.70	37.0	5,810	1,232	15,260	4,500	4,112	2,822		
20/7/2558	7.42	29.6	8,191	1,659	16,490	5,760	5,915	4,427		
23/7/2558	7.69	35.1	3,017	1,441	13,010	2,800	2,437	1,672		
27/7/2558	7.60	34.5	6,197	1,141	15,280	5,845	5,267	2,855	870	74
3/8/2558	7.78	34.6	4,574	2,027	13,873	4,180	2,565	1,745		
6/8/2558	7.71	34.0	3,805	1,465	15,005	3,200	2,421	1,729		
10/8/2558	7.60	34.8	3,201	1,162	13,920	3,630	2,206	1,360	629	54
13/8/2558	7.69	35.0	2,096	864	13,350	2,780	1,725	1,079		
17/8/2558	7.50	35.0	2,561	1,046	13,780	2,770	2,492	1,707		
20/8/2558	7.63	35.2	2,435	1,199	14,000	3,660	2,224	1,652		
24/8/2558	7.71	37.8	2,238	895	13,710	3,680	2,294	1,620	629	81

A-13 Alkalinity and VFA of grass juice effluent for ABR at OLR 2.0 kg COD/m^3.d

Date	Alkalinity (mg/L)	VFA (mg/L)	VFA/ALK
11/6/2558	6,738	2,589	0.38
15/6/2558	6,430	2,536	0.39
18/6/2558	8,570	3,888	0.45
22/6/2558	9,730	2,077	0.21
25/6/2558	8,877	2,290	0.26
29/6/2558	7,534	2,088	0.28
2/7/2558	7,299	1,389	0.19
6/7/2558	8,240	1,128	0.14
9/7/2558	8,560	1,180	0.14
13/7/2558	7,926	1,652	0.21
16/7/2558	7,246	1,078	0.15
20/7/2558	6,097	1,200	0.20
23/7/2558	5,958	1,267	0.21
27/7/2558	6,042	1,328	0.22
3/8/2558	6,123	1,276	0.21
6/8/2558	5,975	836	0.14
10/8/2558	6,522	698	0.11
13/8/2558	6,664	777	0.12
17/8/2558	6,774	809	0.12
20/8/2558	6,916	964	0.14
24/8/2558	6,870	892	0.13

A-14 Volume of biogas at OLR 2.0 kg COD/m^3.d

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
31/7/2558	25.80	13.00	1.60	2.60	1.20	44.20
1/8/2558	26.40	16.20	1.20	2.40	1.20	47.40
2/8/2558	26.20	19.00	2.00	2.80	1.20	51.20
3/8/2558	26.20	18.40	2.20	2.40	1.00	50.20
4/8/2558	27.00	17.00	4.60	0.60	1.20	50.40
5/8/2558	27.40	13.00	6.20	2.20	0.80	49.60
6/8/2558	26.20	10.20	3.20	1.60	1.00	42.20
7/8/2558	27.20	10.40	3.40	2.40	1.40	44.80
8/8/2558	29.00	9.60	5.00	0.60	1.40	45.60
9/8/2558	27.40	10.00	3.20	1.80	1.00	43.40
10/8/2558	29.00	5.80	2.00	1.60	0.80	39.20
11/8/2558	28.00	13.60	2.60	1.80	1.20	47.20
12/8/2558	26.00	7.80	1.80	1.40	1.40	38.40
13/8/2558	31.80	5.40	3.60	2.60	1.60	45.00
14/8/2558	32.40	5.80	1.00	1.00	1.00	41.20
15/8/2558	28.00	6.60	4.40	1.00	1.00	41.00
16/8/2558	28.00	4.40	2.00	1.20	0.80	36.40
17/8/2558	27.00	5.20	2.80	1.20	0.80	37.00
18/8/2558	31.20	5.20	2.00	1.20	0.60	40.20
19/8/2558	22.80	9.80	2.00	0.80	0.80	36.20
20/8/2558	23.60	12.40	3.00	1.40	0.80	41.20
21/8/2558	24.60	14.40	6.60	2.00	1.00	48.60
22/8/2558	24.40	13.20	6.20	2.20	1.20	47.20
23/8/2558	21.00	17.20	10.80	3.80	2.00	54.80
24/8/2558	29.20	12.00	6.50	3.90	1.00	52.60

A-15 CH_4 composition at OLR 2.0 kg COD/m^3.d

Date	CH_4 Composition (%)				
	Chamber1	Chamber2	Chamber3	Chamber4	Chamber5
11/6/2558	67.7	61.7	59.2	51.4	42.4
15/6/2558	66.9	76.4	79.6	78.3	63.7
18/6/2558	64.6	76.4	72.0	64.7	66.4
22/6/2558	69.8	78.7	82.8	83.1	82.0
25/6/2558	62.6	69.7	81.7	81.7	82.1
29/6/2558	54.6	63.0	78.1	77.5	64.9
2/7/2558	48.3	71.7	80.6	81.1	82.2
6/7/2558	61.4	77.9	79.1	79.6	77.8
9/7/2558	68.5	77.4	78.5	77.7	77.2
16/7/2558	64.1	68.7	59.8	60.6	66.4
20/7/2558	41.1	58.1	53.2	69.5	68.3
23/7/2558	48.0	63.6	65.8	69.2	53.6
27/7/2558	60.6	67.1	61.3	66.6	44.5
30/7/2558	62.2	64.6	57.4	66.1	61.8
3/8/2558	65.8	64.2	62.2	67.4	63.5
6/8/2558	65.2	68.8	64.9	65.7	63.2
13/8/2558	55.4	59.1	58.8	58.1	59.0
20/8/2558	53.0	60.9	58.8	58.5	57.2
24/8/2558	65.2	63.1	63.1	63.4	60.4

A-16 Characteristics of grass juice influent for ABR at OLR 4.0 kg COD/m^3.d

Date	OLR (kgCOD/m^3.d)	pH	Temp (^{o}C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
27/8/2558	3.79	4.47	17.3	21,353	8,307	28,700	17,625	12,522	10,450		
31/8/2558	4.83	4.59	16.3	27,231	11,500	27,760	17,160	13,640	11,450		
3/9/2558	5.00	4.57	11.6	28,193	8,750	29,360	17,200	14,585	12,355		
7/9/2558	4.86	4.87	13.0	27,408	16,243	26,860	15,510	10,845	8,685		
10/9/2558	5.17	4.52	13.5	29,609	15,839	27,060	14,310	13,870	11,295		
14/9/2558	4.09	5.04	14.8	23,425	10,669	21,970	12,870	12,630	10,445	502	139
17/9/2558	3.82	4.08	20.7	21,877	13,209	26,670	14,700	8,865	7,245		
21/9/2558	3.48	4.89	13.4	19,930	10,310	22,990	13,680	10,985	9,315		
24/9/2558	5.60	4.20	14.0	32,021	17,066	36,100	20,370	8,740	6,955	1,156	383

A-17 Characteristics of grass juice effluent for ABR at OLR 4.0 kg COD/m^3.d

Date	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
27/8/2558	7.48	34.6	4,715	1,471	16,640	5,910	3,795	2,845		
31/8/2558	7.35	35.2	3,857	1,328	15,085	4,815	2,931	2,137		
3/9/2558	7.56	35.2	6,879	1,483	16,990	5,560	7,005	5,185		
7/9/2558	7.43	34.7	3,259	1,403	13,975	4,150	2,061	1,472		
10/9/2558	7.43	35.2	5,527	1,583	15,390	3,650	4,147	3,310		
14/9/2558	7.49	35.8	3,234	1,193	13,280	3,660	2,450	1,734	435	73
17/9/2558	7.34	34.8	3,958	818	13,030	4,060	3,326	2,348		
21/9/2558	7.38	34.1	5,566	832	14,610	4,900	5,247	3,732		
24/9/2558	7.00	34.9	7,635	3,024	19,030	7,620	5,633	4,063	1,098	255

A-18 Alkalinity and VFA of grass juice effluent for ABR at OLR 4.0 kg COD/m^3.d

Date	Alkalinity (mg/L)	VFA (mg/L)	VFA/ALK
27/8/2558	6,870	1,554	0.23
31/8/2558	7,137	732	0.10
3/9/2558	6,561	799	0.12
7/9/2558	6,902	933	0.14
10/9/2558	6,615	855	0.13
14/9/2558	6,027	693	0.11
17/9/2558	5,167	788	0.15
21/9/2558	5,060	849	0.17
24/9/2558	5,246	2,698	0.51

A-19 Volume of biogas at OLR 4.0 kg COD/m^3.d

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
29/8/2558	32.20	23.60	11.00	6.00	4.00	76.80
30/8/2558	28.20	12.80	8.40	6.40	6.40	62.20
31/8/2558	27.20	22.40	8.20	4.60	3.20	65.60
1/9/2558	40.00	28.40	14.60	7.80	4.00	94.80
2/9/2558	34.00	24.00	11.60	6.40	4.20	80.20
3/9/2558	34.00	25.00	14.00	7.20	3.80	84.00
4/9/2558	34.00	23.40	12.60	6.20	3.60	79.80
5/9/2558	36.70	26.60	15.30	6.90	2.60	88.10
6/9/2558	33.40	27.60	12.60	4.80	3.10	81.50
7/9/2558	39.10	26.60	14.00	5.60	2.80	88.10
8/9/2558	36.20	24.20	13.20	6.20	2.60	82.40
9/9/2558	34.40	29.20	10.80	5.60	4.40	84.40
10/9/2558	34.20	28.40	14.20	6.60	3.60	87.00
11/9/2558	32.60	28.00	12.00	6.00	4.20	82.80
12/9/2558	34.80	21.80	9.20	4.40	3.70	73.90
13/9/2558	34.30	22.20	9.20	4.20	3.10	73.00
14/9/2558	33.50	24.00	11.50	4.90	3.60	77.50
15/9/2558	36.10	26.20	14.70	5.00	3.00	85.00
16/9/2558	34.40	19.20	11.80	5.00	3.00	73.40
17/9/2558	33.60	19.60	9.40	4.80	4.80	72.20
18/9/2558	34.00	19.00	9.60	4.60	4.50	71.70
19/9/2558	34.90	21.70	12.80	5.80	4.60	79.80
20/9/2558	32.60	27.10	12.00	5.50	3.80	81.00
21/9/2558	31.40	25.40	11.40	4.60	2.60	75.40
22/9/2558	30.80	21.00	14.80	6.60	2.80	76.00

A-20 CH_4 composition at OLR 4.0 kg COD/m^3.d

Date	CH_4 Composition Chamber (%)				
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5
27/8/2558	64.1	66.2	66.5	65.9	62.0
31/8/2558	68.8	70.8	71.2	67.9	68.4
3/9/2558	74.1	73.3	73.0	71.4	68.1
7/9/2558	76.8	76.8	76.9	75.3	71.6
18/9/2558	61.0	56.8	56.2	56.3	59.3
21/9/2558	60.7	57.9	57.4	57.3	60.8

A-21 Characteristics of grass juice influent for ABR at OLR 8.0 kg COD/m^3.d

Date	OLR (kgCOD/m^3.d)	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
28/9/2558	11.09	4.20	13.5	34,372	15,637	40,435	24,275	13,160	10,910		
1/10/2558	8.39	4.17	14.0	26,019	13,534	39,670	24,150	13,310	11,125		

A-22 Characteristics of grass juice effluent for ABR at OLR 8.0 kg COD/m^3.d

Date	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
28/9/2558	6.90	35.0	17,965	6,819	27,790	13,040	11,168	8,248		
1/10/2558	7.28	34.8	9,665	3,359	30,500	14,755	18,080	13,540		

A-23 Alkalinity and VFA of grass juice effluent for ABR at OLR 8.0 kg COD/m^3.d

Date	Alkalinity (mg/L)	VFA (mg/L)	VFA/ALK
28/9/2558	6,629	6,412	0.97
1/10/2558	7,090	8,360	1.18

A-24 Volume of biogas at OLR 8.0 kg COD/m^3.d

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
25/9/2558	13.20	19.00	22.00	11.00	5.00	70.20
26/9/2558	9.80	11.00	18.00	10.00	12.40	61.20
27/9/2558	13.40	11.40	12.00	19.40	16.00	72.20
28/9/2558	10.40	6.20	10.40	17.20	9.60	53.80
29/9/2558	14.20	9.40	10.80	22.00	1.20	57.60
30/9/2558	10.60	8.80	3.60	20.80	14.40	58.20
1/10/2558	12.80	12.00	12.60	14.10	16.80	68.30
2/10/2558	3.20	2.60	4.20	7.80	9.20	27.00

A-25 CH_4 composition at OLR 8.0 kg COD/m^3.d

Date	CH_4 Composition Chamber (%)				
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5
25/9/2558	28.2	35.5	68.5	59.3	62.0
28/9/2558	5.2	20.6	45.5	71.7	69.8
1/10/2558	5.2	7.1	16.8	54.6	54.8
2/10/2558	5.9	12.4	26.5	51.7	66.0

A-26 Characteristics of grass juice influent for ABR at OLR 4.0 kg COD/m^3.d (semi-continuous feeding scheme)

Date	OLR (kgCOD/m^3.d)	pH	Temp (^{o}C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
7/12/2558	3.90	5.00	15.0	24,168	7,316	27,830	15,950	13,890	11,625		
10/12/2558	2.48	4.60	14.7	15,400	5,160	21,390	12,700	10,315	8,785	608	330
14/12/2558	2.71	4.30	13.8	16,784	5,030	21,420	12,720	9,125	7,735		
17/12/2558	3.23	4.20	13.0	20,045	6,579	25,510	16,490	9,460	7,780		
21/12/2558	4.36	4.12	12.7	27,047	12,295	30,000	19,330	11,700	9,595	633	377
24/12/2558	3.44	4.12	13.0	21,302	12,656	29,090	18,650	12,700	10,930		
28/12/2558	3.24	4.12	13.0	20,067	5,533	25,600	16,520	14,675	12,585		
31/12/2558	3.25	4.03	14.3	20,154	9,098	25,230	14,830	9,175	7,750	568	382

A-27 Characteristics of grass juice effluent for ABR at OLR 4.0 kg COD/m^3.d (semi-continuous feeding scheme)

Date	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
7/12/2558	8.40	33.8	3,090	1,320	15,425	3,710	1,649	1,286		
10/12/2558	8.35	34.0	2,000	1,290	13,500	2,650	1,098	769	913	139
14/12/2558	8.33	33.6	2,441	1,323	11,980	1,985	726	534		
17/12/2558	8.47	34.0	2,173	1,059	10,950	2,410	772	463		
21/12/2558	8.41	35.0	1,974	874	10,573	2,170	829	610	369	141
24/12/2558	8.35	34.0	1,697	857	11,195	2,423	963	652		
28/12/2558	8.30	34.0	1,446	775	11,335	2,290	892	682		
31/12/2558	8.04	34.0	3,850	1,295	13,250	3,115	2,592	1,664	381	290

A-28 Alkalinity and VFA of grass juice effluent for ABR at OLR 4.0 kg COD/m^3.d (semi-continuous feeding scheme)

Date	Alkalinity (mg/L)	VFA (mg/L)	VFA/ALK
7/12/2558	8,044	732	0.09
10/12/2558	6,737	843	0.13
14/12/2558	5,928	585	0.10
17/12/2558	5,321	548	0.10
21/12/2558	4,778	495	0.10
24/12/2558	5,234	530	0.10
28/12/2558	5,296	652	0.12
31/12/2558	4,832	1,512	0.31

A-29 CH_4 composition at OLR 4.0 kg COD/m^3.d (semi-continuous feeding scheme)

Date	CH4 Composition (%)				
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5
7/12/2558	56.3	69.7	66.7	63.3	56.9
11/12/2558	52.2	63.6	67.0	64.7	55.0
14/12/2558	52.8	62.4	67.0	65.8	56.2
17/12/2558	51.4	61.2	63.7	60.7	52.3
21/12/2558	53.2	63.5	70.7	62.5	51.9
28/12/2558	49.1	60.0	65.8	62.2	59.7
29/12/2558	50.9	62.0	64.0	58.8	60.9

A-30 Volume of biogas at OLR 4.0 kg COD/m^3.d (semi-continuous feeding scheme)

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
7/12/2558	31.40	11.60	4.00	1.60	0.60	49.20
8/12/2558	32.80	10.60	2.00	1.20	1.00	47.60
9/12/2558	34.00	8.60	3.00	1.40	1.00	48.00
10/12/2558	37.60	12.30	5.30	2.40	1.50	59.10
11/12/2558	41.60	14.60	6.40	0.40	0.60	63.60
12/12/2558	45.80	14.00	6.30	1.70	0.60	68.40
13/12/2558	44.80	12.80	4.80	2.00	1.60	66.00
14/12/2558	50.00	13.80	3.20	1.80	1.10	69.90
15/12/2558	51.40	15.40	4.40	1.80	0.60	73.60
16/12/2558	54.80	15.20	2.00	1.80	1.00	74.80
17/12/2558	54.20	16.40	4.60	1.93	0.77	77.90
18/12/2558	55.20	18.60	3.20	1.60	1.00	79.60
19/12/2558	66.30	18.80	3.60	1.40	0.80	90.90
20/12/2558	65.50	22.20	5.00	2.80	0.50	96.00
21/12/2558	65.60	22.60	4.20	1.40	0.70	94.50
22/12/2558	68.40	20.20	4.60	1.40	0.70	95.30
23/12/2558	70.80	23.00	4.40	1.40	0.60	100.20
24/12/2558	61.30	20.90	4.40	1.40	1.00	89.00
25/12/2558	72.10	26.10	5.30	2.00	0.80	106.30
26/12/2558	68.40	24.70	4.70	1.60	0.80	100.20
27/12/2558	57.60	25.10	5.10	1.70	1.10	90.60
28/12/2558	61.60	31.40	6.80	1.60	1.00	102.40
29/12/2558	39.10	16.70	4.40	1.80	1.00	63.00
30/12/2558	39.00	20.30	18.30	9.20	3.20	90.00
31/12/2558	40.20	20.80	14.20	8.50	4.40	88.10

A-31 Characteristics of grass juice influent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and trace element addition

Date	OLR (kg COD/m^3.d)	pH	Temp ($^{\circ}$C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
14/1/2559	4.38	4.00	9.0	27,144	13,689	29,870	18,330	10,960	8,790		
18/1/2559	3.64	4.00	9.0	22,557	9,722	24,996	15,640	9,070	7,425		
21/1/2559	2.65	4.02	8.7	16,453	8,420	27,750	17,250	14,760	8,210	842	275
25/1/2559	3.82	4.31	11.1	23,688	10,826	26,080	15,190	7,945	6,560		
28/1/2559	4.00	4.02	7.7	24,781	13,284	28,510	16,630	10,795	9,100	989	260
1/2/2559	4.58	4.08	15.4	28,396	13,999	31,190	17,910	11,480	9,580		
4/2/2559	3.12	4.06	8.2	19,328	11,016	23,080	14,190	7,330	6,170		
8/2/2559	3.86	4.23	10.9	23,932	11,354	25,360	16,180	8,135	6,945	864	167
11/2/2559	3.20	4.08	10.1	19,859	6,748	27,010	17,360	9,190	7,710		
15/2/2559	3.71	4.19	9.7	23,000	8,820	27,990	17,700	7,525	6,410		
18/2/2559	3.41	4.28	10.6	21,167	13,077	31,560	19,440	9,190	7,625	1,049	214
22/2/2559	3.98	4.07	13.6	24,651	12,534	31,240	19,350	9,190	7,855		

A-32 Characteristics of grass juice effluent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and trace element addition

Date	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
14/1/2559	7.54	34.3	4,630	1,623	14,860	4,360	3,146	2,264		
18/1/2559	7.70	34.0	2,960	1,266	12,866	3,068	2,204	1,662		
21/1/2559	7.59	34.5	2,082	1,191	14,100	2,740	1,548	1,044	374	141
25/1/2559	7.86	33.3	6,155	897	14,040	3,900	3,443	2,510		
28/1/2559	8.33	35.1	5,224	947	12,502	2,105	1,048	737	622	74
1/2/2559	8.26	34.2	2,442	1,130	13,458	1,688	1,900	1,416		
4/2/2559	7.78	34.8	1,632	1,123	13,170	2,880	1,190	899		
8/2/2559	8.11	34.7	1,897	1,263	12,155	2,295	798	561	634	43
11/2/2559	8.13	34.7	1,913	814	11,420	1,925	897	635		
15/2/2559	8.17	33.0	2,658	766	11,510	2,175	876	683		
18/2/2559	8.28	34.0	1,987	1,051	14,095	3,755	1,171	866	803	39
22/2/2559	8.22	33.4	2,318	996	13,230	3,030	1,610	1,213		

A-33 Alkalinity and VFA of grass juice effluent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and trace element addition

Date	Alkalinity (mg/L)	VFA (mg/L)	VFA/ALK
14/1/2559	6,017	1,892	0.31
18/1/2559	6,302	612	0.10
21/1/2559	4,893	989	0.20
25/1/2559	5,859	504	0.09
28/1/2559	5,940	381	0.06
1/2/2559	6,032	581	0.10
4/2/2559	6,266	510	0.08
8/2/2559	6,256	520	0.08
11/2/2559	5,507	395	0.07
15/2/2559	5,599	407	0.07
18/2/2559	6,352	345	0.05
22/2/2559	7,047	606	0.09

A-34 CH_4 composition at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and trace element addition

Date	CH_4 Composition (%)				
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5
21/1/2559	54.7	66.0	60.5	58.8	58.5
25/1/2559	58.4	61.8	56.5	54.3	56.1
28/1/2559	57.6	61.5	53.5	54.3	50.5
1/2/2559	61.3	61.9	58.2	56.2	57.0
11/2/2559	55.0	56.8	55.4	55.0	51.9
15/2/2559	56.1	57.0	57.0	54.0	54.0
18/2/2559	56.0	60.0	58.0	55.0	56.4
19/2/2559	51.7	63.8	59.5	56.0	55.0
20/2/2559	70.2	72.1	70.5	62.0	66.8
21/2/2559	64.0	70.0	66.0	65.0	70.0
22/2/2559	67.8	73.0	71.2	61.4	55.7

A-35 Volume of biogas at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and trace element addition

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
11/2/2559	73.00	6.20	1.40	1.40	0.80	82.80
12/2/2559	76.20	7.20	2.00	1.40	1.00	87.80
13/2/2559	77.60	7.40	1.50	1.70	1.00	89.20
14/2/2559	84.30	8.40	2.20	1.30	1.10	97.30
15/2/2559	83.20	10.80	2.00	1.30	1.00	98.30
16/2/2559	73.60	12.00	2.40	1.60	1.00	90.60
17/2/2559	73.80	12.20	2.20	2.00	1.20	91.40
18/2/2559	73.60	11.00	2.00	1.60	1.00	89.20
19/2/2559	73.60	11.00	2.00	1.60	1.00	89.20
20/2/2559	73.80	9.80	2.00	1.60	1.00	88.20
21/2/2559	68.80	13.00	2.25	0.75	0.75	85.55
22/2/2559	71.90	14.35	3.00	0.75	0.75	90.75

A-36 Characteristics of grass juice influent for ABR at OLR 6.0 kg COD/m^3.d under semi-continuous feeding scheme and trace element addition

Date	OLR (kg COD/m^3.d)	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
14/3/2559	4.90	4.28	10.0	15,179	6,563	26,700	16,990	12,640	10,615		
17/3/2559	6.52	4.10	10.0	20,216	5,292	25,990	17,050	12,195	10,350		
21/3/2559	5.24	4.10	10.5	16,230	8,187	26,990	17,300	11,595	9,815	845	116
24/3/2559	8.08	4.09	11.1	25,035	11,601	37,490	22,940	15,425	12,815		
28/3/2559	6.73	4.42	10.5	20,854	7,680	35,280	21,750	14,820	12,135		
30/3/2559	4.22	4.50	13.0	17,444	4,466	32,260	20,220	14,030	11,545	1,064	124
31/3/2559	5.14	4.51	12.2	21,255	4,522	31,380	19,680	15,215	12,490	1,064	251

A-37 Characteristics of grass juice effluent for ABR at OLR 6.0 kg COD/m^3.d under semi-continuous feeding scheme and trace element addition

Date	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
14/3/2559	7.67	35.0	5,051	1,265	15,115	5,115	3,552	2,480		
17/3/2559	8.13	33.8	8,624	1,007	16,845	6,715	7,132	5,178		
21/3/2559	7.40	38.9	8,794	814	14,370	4,790	4,985	3,625	838	51
24/3/2559	7.36	34.7	22,564	826	29,675	17,905	20,565	15,880		
28/3/2559	7.50	34.0	35,377	725	46,960	29,360	34,100	25,730		
30/3/2559	8.20	33.7	7,278	932	19,580	7,490	8,225	5,720	930	54
31/3/2559	8.16	33.1	4,541	1,148	17,690	5,800	4,587	3,270	887	46

A-38 Alkalinity and VFA of grass juice effluent for ABR at OLR 6.0 kg COD/m^3.d under semi-continuous feeding scheme and trace element addition

Date	Alkalinity (mg/L)	VFA (mg/L)	VFA/ALK
14/3/2559	5,086	1,181	0.23
17/3/2559	5,294	764	0.14
21/3/2559	5,204	621	0.12
24/3/2559	4,860	402	0.08
28/3/2559	5,673	471	0.08
30/3/2559	5,938	741	0.12

A-39 CH_4 composition at OLR 6.0 kg COD/m^3.d under semi-continuous feeding scheme and trace element addition

Date	CH_4 Composition (%)				
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5
14/3/2559	60.6	63.0	63.8	63.4	60.7
17/3/2559	59.3	65.7	62.2	66.7	58.0
21/3/2559	55.1	62.4	64.3	58.2	61.8
24/3/2559	51.1	63.1	65.1	58.2	51.0
28/3/2559	55.2	64.2	65.1	58.2	56.0
30/3/2559	51.0	61.5	69.0	53.2	61.0

A-40 Volume of biogas at OLR 6.0 kg COD/m^3.d under semi-continuous feeding scheme and trace element addition

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
13/3/2559	62.10	11.20	34.50	4.40	2.10	114.30
14/3/2559	59.30	32.80	20.00	7.40	2.60	122.10
15/3/2559	59.30	32.80	20.00	7.40	2.60	122.10
16/3/2559	72.20	33.00	18.00	7.00	2.00	132.20
17/3/2559	70.80	30.00	11.80	7.00	2.00	121.60
18/3/2559	79.00	28.20	11.40	7.60	2.00	128.20
19/3/2559	61.50	37.90	15.80	3.50	6.50	125.20
20/3/2559	45.80	60.60	15.30	4.70	2.00	128.40
21/3/2559	92.20	26.00	20.60	8.60	2.80	150.20
22/3/2559	71.40	38.80	15.20	4.60	1.20	131.20
23/3/2559	84.00	31.20	14.80	7.00	1.80	138.80
24/3/2559	78.20	32.60	16.80	6.00	1.80	135.40
25/3/2559	91.40	36.60	17.20	3.80	2.60	151.60
26/3/2559	86.00	37.20	23.40	8.40	4.00	159.00
27/3/2559	92.30	34.90	23.00	7.10	2.00	159.30
28/3/2559	94.90	38.80	12.60	18.40	3.40	168.10
29/3/2559	98.00	32.80	18.20	6.00	2.80	157.80

A-41 Characteristics of grass juice influent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 0.25 with trace element addition

Date	OLR (kg COD/m^3.d)	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
4/8/2559	3.98	5.18	15.3	16,844	8,544	25,330	10,570	7,050	5,710	1,108	364
8/8/2559	3.33	5.17	19.0	14,063	6,641	25,650	12,840	6,500	5,155		
11/8/2559	3.06	5.68	20.9	12,924	4,816	21,000	11,690	7,020	5,680		
15/8/2559	2.45	5.36	17.3	10,361	4,694	19,800	10,480	6,660	5,390	777	942
18/8/2559	3.02	4.97	19.1	12,768	6,867	21,700	11,380	6,250	5,155		
22/8/2559	3.64	4.86	17.7	15,405	7,140	20,840	10,270	4,680	3,890		
25/8/2559	3.43	4.80	16.5	14,504	5,537	20,000	9,950	4,820	4,090	662	743

A-42 Characteristics of grass juice effluent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 0.25 with trace element addition

Date	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
4/8/2559	8.34	34.0	1,809	868	14,820	2,885	881	604	959	72
8/8/2559	8.26	34.0	2,876	736	15,343	3,352	2,826	2,051		
11/8/2559	7.88	33.7	4,391	333	17,460	5,170	5,575	3,940		
15/8/2559	7.52	33.8	2,696	864	15,060	4,220	3,563	2,630	1,029	189
18/8/2559	8.06	34.0	1,679	853	12,190	2,220	786	580		
22/8/2559	7.82	34.0	18,097	346	24,220	12,360	13,600	11,465		
25/8/2559	8.26	34.0	6,783	425	17,100	6,500	7,305	5,595	888	491

A-43 Alkalinity and VFA of grass juice effluent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 0.25 with trace element addition

Date	Alkalinity (mg/l)	VFA (mg/l)	VFA/ALK
4/8/2559	7,769	824	0.11
8/8/2559	7,871	673	0.09
11/8/2559	8,075	627	0.08
15/8/2559	7,605	775	0.10
18/8/2559	5,726	590	0.10
22/8/2559	6,345	777	0.12
25/8/2559	5,433	1,067	0.20

A-44 CH_4 composition at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 0.25 with trace element addition

Date	CH4 Composition (%)				
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5
4/8/2559	63.6	71.4	68.3	57.5	50.5
8/8/2559	56.5	64.0	66.6	63.6	50.5
11/8/2559	67.3	72.2	67.3	55.9	50.5
15/8/2559	61.9	70.5	65.7	55.9	58.4
18/8/2559	64.9	69.4	65.3	55.9	53.5
22/8/2559	67.3	72.3	62.2	59.6	50.4
24/8/2559	65.7	70.2	63.3	58.5	50.5
25/8/2559	66.6	71.3	65.6	60.0	50.5

A-45 Volume of biogas at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 0.25 with trace element addition

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
2/8/2559	64.20	11.60	2.20	1.20	1.00	80.20
3/8/2559	64.00	13.20	4.20	1.00	0.80	83.20
4/8/2559	64.80	15.40	4.40	1.00	0.80	86.40
5/8/2559	59.90	12.50	3.40	1.60	0.80	78.20
6/8/2559	60.20	15.60	2.00	2.20	0.80	80.80
7/8/2559	58.20	16.80	4.40	1.60	0.60	81.60
8/8/2559	59.20	18.00	4.40	2.00	0.80	84.40
9/8/2559	43.60	16.80	5.00	1.60	0.80	67.80
10/8/2559	64.40	15.60	4.20	2.00	0.80	87.00
11/8/2559	56.60	17.20	6.00	3.80	0.80	84.40
12/8/2559	40.90	12.20	3.40	2.20	3.10	61.80
13/8/2559	43.40	12.10	2.00	1.10	1.50	60.10
14/8/2559	41.40	13.00	4.80	2.30	1.30	62.80
15/8/2559	46.00	7.60	3.40	1.00	1.00	59.00
16/8/2559	42.40	14.20	5.00	1.00	0.80	63.40
17/8/2559	41.20	14.00	4.40	1.00	1.20	61.80
18/8/2559	46.40	10.60	4.60	1.00	1.00	63.60
19/8/2559	52.80	10.60	2.80	1.40	1.20	68.80
20/8/2559	46.80	14.30	3.60	2.40	2.40	69.50
21/8/2559	56.10	7.30	2.70	0.70	0.60	67.40
22/8/2559	50.40	13.00	4.60	1.00	0.60	69.60
23/8/2559	52.20	10.00	1.20	1.40	0.60	65.40
24/8/2559	51.40	10.20	2.20	1.80	0.80	66.40
25/8/2559	51.00	11.00	1.80	1.40	1.60	66.80

A-46 Characteristics of grass juice influent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 0.50 with trace element addition

Date	OLR (kgCOD/m^3.d)	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
29/8/2559	5.24	5.68	18.6	17,722	7,848	21,030	10,460	5,257	4,443		
1/9/2559	4.69	5.99	19.7	15,854	7,416	18,620	9,880	5,313	4,347		
5/9/2559	3.84	6.19	19.8	12,980	4,508	16,545	7,975	4,530	3,817	658	189
8/9/2559	2.64	6.04	19.0	8,925	4,060	17,120	8,740	4,775	4,022		
12/9/2559	3.14	6.18	19.7	10,605	5,793	15,425	7,085	4,840	4,075		
15/9/2559	2.30	6.18	18.9	8,166	6,055	15,295	7,360	4,002	3,322	644	150

A-47 Characteristics of grass juice effluent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 0.50 with trace element addition

Date	pH	Temp (°C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
29/8/2559	8.16	34.1	4,724	790	13,320	3,050	2,911	2,169		
1/9/2559	7.82	34.2	9,367	727	19,225	8,185	8,795	6,708		
5/9/2559	8.08	34.7	3,194	409	15,250	4,950	3,983	3,050	723	132
8/9/2559	8.23	35.0	870	486	10,187	2,243	537	408		
12/9/2559	8.11	34.0	2,636	509	10,830	2,315	2,767	1,910		
15/9/2559	8.16	34.0	1,044	430	9,270	1,200	780	540	602	101

A-48Alkalinity and VFA of grass juice effluent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 0.50 with trace element addition

Date	Alkalinity (mg/L)	VFA (mg/L)	VFA/ALK
29/8/2559	5,668	700	0.12
1/9/2559	5,691	571	0.10
5/9/2559	5,405	511	0.09
8/9/2559	5,049	508	0.10
12/9/2559	5,406	615	0.11
15/9/2559	5,186	489	0.09

A-49 CH_4 composition at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 0.50 with trace element addition

Date	CH_4 Composition (%)				
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5
29/8/2559	66.7	71.8	63.3	61.0	54.6
1/9/2559	66.8	70.7	64.2	57.0	58.4
5/9/2559	66.0	72.2	65.6	64.0	58.5
8/9/2559	67.8	71.9	65.4	63.2	58.2
12/9/2559	71.4	71.6	65.0	62.7	58.2
15/9/2559	69.9	67.9	61.4	62.6	55.8
19/9/2559	71.2	69.3	63.1	57.4	55.8

A-50 Volume of biogas at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 0.50 with trace element addition

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
26/8/2559	50.40	10.60	2.40	1.00	1.00	65.40
27/8/2559	48.00	10.40	5.40	4.10	0.50	68.40
28/8/2559	47.00	8.70	3.50	1.00	0.80	61.00
29/8/2559	55.40	13.60	3.80	1.00	1.00	74.80
30/8/2559	45.20	9.20	2.80	1.60	1.20	60.00
31/8/2559	43.00	7.00	2.40	3.00	2.00	57.40
1/9/2559	52.80	13.40	7.80	1.00	1.00	76.00
2/9/2559	41.20	12.30	3.90	3.50	2.90	63.80
3/9/2559	40.50	11.10	3.20	2.20	1.30	58.30
4/9/2559	43.80	9.30	2.50	1.90	1.40	58.90
5/9/2559	46.20	10.00	4.00	2.00	1.50	63.70
6/9/2559	42.20	9.80	3.40	2.00	3.40	60.80
7/9/2559	50.60	10.20	3.00	2.00	1.60	67.40
8/9/2559	46.60	9.40	3.00	1.60	1.00	61.60
9/9/2559	46.00	8.00	2.60	1.40	1.00	59.00
10/9/2559	43.60	7.20	2.40	1.50	0.90	55.60
11/9/2559	42.30	7.70	3.20	1.30	1.00	55.50
12/9/2559	50.00	5.40	2.60	1.30	1.00	60.30
13/9/2559	48.60	5.20	1.80	1.40	1.00	58.00
14/9/2559	48.60	7.20	1.40	2.20	1.20	60.60
15/9/2559	54.20	5.60	2.40	2.00	1.00	65.20
16/9/2559	60.80	6.40	2.80	1.00	1.00	72.00
17/9/2559	40.00	11.60	0.80	4.00	2.20	58.60
18/9/2559	45.20	7.00	3.20	1.40	1.20	58.00

A-51 Characteristics of grass juice influent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 1.00 with trace element addition

Date	OLR (kg COD/m^3.d)	pH	Temp (^{o}C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
19/9/2559	3.10	7.32	26.8	8,250	2,492	14,870	7,110	5,083	4,203		
22/9/2559	2.94	7.14	20.3	7,808	2,568	15,440	7,060	5,137	4,187		
26/9/2559	2.32	7.00	20.0	6,173	2,900	14,335	6,220	4,363	3,557	802	122
29/9/2559	2.47	7.00	20.0	6,553	2,526	15,045	6,745	4,097	3,377		
3/10/2559	2.44	7.23	22.4	6,489	2,482	16,778	8,133	4,465	3,780		
6/10/2559	3.98	7.27	24.0	10,586	3,688	17,525	8,138	4,555	3,895	942	89

A-52 Characteristics of grass juice influent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 1.00 with trace element addition

Date	pH	Temp (^{o}C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
19/9/2559	7.93	34.7	1,141	562	9,577	2,173	883	582		
22/9/2559	7.99	34.0	20,914	522	22,720	12,710	14,650	12,145		
26/9/2559	8.00	34.2	2,013	744	10,825	2,733	1,736	1,329	751	102
29/9/2559	8.10	34.2	1,752	774	10,230	2,277	949	728		
3/10/2559	8.12	34.2	1,802	797	11,520	2,833	1,390	1,008		
6/10/2559	8.12	34.2	2,106	739	11,700	2,839	927	727	655	34

A-53 Alkalinity and VFA of grass juice influent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 1.00 with trace element addition

Date	Alkalinity (mg/L)	VFA (mg/L)	VFA/ALK
19/9/2559	5,403	737	0.14
22/9/2559	5,475	639	0.12
26/9/2559	5,609	771	0.14
29/9/2559	5,346	870	0.16
3/10/2559	6,198	1,027	0.17
6/10/2559	5,768	693	0.12

A-54 CH_4 composition at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 1.00 with trace element addition

Date	CH_4 Composition (%)				
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5
22/9/2559	66.0	69.1	64.7	65.0	55.8
26/9/2559	61.4	71.7	68.4	68.6	53.2
29/9/2559	61.6	69.6	65.2	69.7	52.7
3/10/2559	61.6	71.7	66.7	69.1	58.5
6/10/2559	67.2	69.7	66.2	67.4	52.8
9/10/2559	65.2	72.8	70.0	70.8	50.0
10/10/2559	61.3	69.1	67.6	66.8	53.8

A-55 Volume of biogas at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 1.00 with trace element addition

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
19/9/2559	35.70	9.30	3.30	2.00	1.00	51.30
20/9/2559	32.00	13.40	4.00	3.00	0.80	53.20
21/9/2559	36.60	14.00	7.60	5.00	0.60	63.80
22/9/2559	36.40	11.80	5.60	2.50	0.80	57.10
23/9/2559	39.40	12.80	5.80	2.60	2.20	62.80
24/9/2559	31.30	12.10	5.30	3.30	2.60	54.60
25/9/2559	24.30	11.50	4.60	2.80	1.30	44.50
26/9/2559	30.40	13.00	4.80	2.50	1.00	51.70
27/9/2559	30.40	11.00	5.20	2.60	1.60	50.80
28/9/2559	33.00	13.00	6.20	3.20	1.60	57.00
29/9/2559	29.70	10.30	5.20	2.50	1.20	48.90
30/9/2559	27.50	9.40	5.60	3.20	2.20	47.90
1/10/2559	29.20	10.40	7.20	4.00	1.70	52.50
2/10/2559	29.40	10.50	6.10	4.10	2.60	52.70
3/10/2559	32.60	13.20	7.60	3.50	1.80	58.70
4/10/2559	33.40	12.60	5.80	2.60	2.20	56.60
5/10/2559	32.20	13.40	6.80	4.00	1.60	58.00
6/10/2559	34.70	13.90	6.30	3.00	1.60	59.50
7/10/2559	34.20	11.60	6.00	3.40	2.60	57.80
8/10/2559	38.00	14.00	7.00	4.20	1.60	64.80
9/10/2559	35.30	13.20	6.60	5.20	3.10	63.40

A-56 Characteristics of grass juice influent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 2.00 with trace element addition

Date	OLR (kg COD/m^3.d)	pH	Temp (^{o}C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
10/10/2559	11.93	7.56	28.0	21,136	3,883	21,069	10,492	12,510	10,010		
13/10/2559	5.29	7.19	25.4	9,370	3,667	17,913	7,420	4,527	3,560		
17/10/2559	5.35	7.22	24.8	9,482	4,089	18,933	7,653	5,230	4,170	1,044	133
20/10/2559	2.80	7.28	27.0	4,958	2,329	15,530	6,020	3,613	2,958		
24/10/2559	3.81	7.28	27.0	6,744	1,745	14,003	6,060	4,070	3,211		
27/10/2559	3.34	7.37	28.1	5,917	1,894	13,235	5,350	3,142	2,462	699	133
31/10/2559	2.97	7.19	26.3	5,260	1,254	13,233	5,827	2,725	2,155		

A-57 Characteristics of grass juice effluent for ABR at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 2.00 with trace element addition

Date	pH	Temp (^{o}C)	TCOD (mg/L)	FCOD (mg/L)	TS (mg/L)	VS (mg/L)	SS (mg/L)	VSS (mg/L)	TKN (mg/L)	TP (mg/L)
10/10/2559	7.88	34.3	18,870	652	22,200	10,920	14,205	10,760		
13/10/2559	7.93	34.0	3,455	1,163	13,560	3,525	2,196	1,492		
17/10/2559	7.89	33.7	3,888	1,235	14,640	3,253	3,800	2,915	1,008	282
20/10/2559	7.14	33.6	3,540	650	14,298	3,820	3,685	2,763		
24/10/2559	7.18	34.0	2,885	817	11,865	3,085	2,586	1,884		
27/10/2559	7.43	34.0	2,792	785	10,932	2,472	1,956	1,288	566	121
31/10/2559	7.89	34.0	2,532	636	11,190	3,040	1,966	1,402		

A-58 Alkalinity and VFA of grass juice effluent for ABR at OLR 4.0 kg COD/m3.d under semi-continuous feeding scheme and effluent recirculation rate of 2.00 with trace element addition

Date	Alkalinity (mg/L)	VFA (mg/L)	VFA/ALK
10/10/2559	6,431	638	0.10
13/10/2559	6,819	1,153	0.17
17/10/2559	6,744	1,018	0.15
20/10/2559	6,473	792	0.12
24/10/2559	5,721	699	0.12
27/10/2559	5,312	1,269	0.24
31/10/2559	4,625	494	0.11

A-59 CH_4 composition at OLR 4.0 kg COD/m3.d under semi-continuous feeding scheme and effluent recirculation rate of 2.00 with trace element addition

Date	CH_4 Composition (%)				
	CH_4	CO_2	O_2	BAL	Total
13/10/2559	68.3	71.5	67.4	67.2	58.0
17/10/2559	71.1	75.4	71.9	72.2	66.0
20/10/2559	66.6	70.9	69.2	70.4	62.2
24/10/2559	69.1	74.0	70.9	69.8	67.0
27/10/2559	63.0	71.3	63.2	69.3	55.4
31/10/2559	69.2	72.3	66.9	62.4	59.4

A-60 Volume of Biogas at OLR 4.0 kg COD/m^3.d under semi-continuous feeding scheme and effluent recirculation rate of 2.00 with trace element addition

Date	Volume of biogas (L/d)					
	Chamber 1	Chamber 2	Chamber 3	Chamber 4	Chamber 5	Total
10/10/2559	37.40	14.60	7.00	3.00	2.60	64.60
11/10/2559	30.80	13.20	6.40	3.20	3.40	57.00
12/10/2559	28.60	15.00	9.00	4.40	3.40	60.40
13/10/2559	28.00	15.00	8.00	7.00	3.00	61.00
14/10/2559	26.40	16.60	8.00	8.60	3.40	63.00
15/10/2559	26.70	13.20	7.20	8.80	4.40	60.30
16/10/2559	27.70	12.60	6.50	5.90	2.70	55.40
17/10/2559	27.60	15.00	8.00	6.40	2.50	59.50
18/10/2559	30.00	15.60	7.40	4.60	2.80	60.40
19/10/2559	30.20	14.40	8.60	7.00	2.80	63.00
20/10/2559	23.00	11.80	6.60	6.00	2.50	49.90
21/10/2559	22.00	12.20	7.00	5.00	0.80	47.00
22/10/2559	15.30	10.20	6.10	4.20	3.50	39.30
23/10/2559	17.20	9.90	5.00	3.60	3.10	38.80
24/10/2559	17.50	9.70	5.70	4.00	3.40	40.30
25/10/2559	21.80	12.80	7.40	3.20	3.00	48.20
26/10/2559	19.00	10.00	5.00	3.00	2.00	39.00
27/10/2559	19.00	9.80	5.60	3.00	2.80	40.20
28/10/2559	16.20	9.80	6.60	5.00	3.20	40.80
29/10/2559	18.30	9.50	6.70	4.90	2.80	42.20
30/10/2559	13.40	9.90	6.00	4.20	2.80	36.30
31/10/2559	22.40	9.00	5.60	4.00	2.50	43.50

A-61 Data of growth curve of *C.beijerinckii* TISTR 1461, *C.acetobutylicum* TISTR 1462 and *C. acetobutylicum* JCM 1419

Time (h)	OD (660 nm)		
	C.beijerinckii TISTR 1461	*C.acetobutylicum* TISTR 1462	*C. acetobutylicum* JCM 1419
0	0.056	0.061	0.044
6	0.872	0.720	0.102
12	1.434	1.550	0.479
18	1.289	1.517	1.207
24	1.336	1.416	1.658
30	1.317	1.238	1.619
36	1.235	1.223	1.400
42	1.202	1.241	1.547
48	1.138	1.234	1.552
54	1.131	1.268	1.426
60	1.047	1.157	1.523

A-62 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 1 (pH 5.50 and sugar concentration 40 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	40.58	0.00	0.00	0.00	0.00	0.00
24	21.60	0.76	1.39	0.00	1.07	0.08
48	26.97	1.20	2.63	0.12	0.72	0.06
72	19.16	1.29	2.91	0.13	0.76	0.14
96	19.82	1.15	2.44	0.12	0.71	0.17
120	19.47	0.93	2.79	0.13	0.75	0.18
144	18.68	1.02	2.92	0.14	0.62	0.13
168	17.48	1.45	3.31	0.14	1.03	0.59
192	15.53	1.56	3.62	0.15	1.28	0.55

A-63 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 2 (pH 6.50 and sugar concentration 60 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	61.41	0.00	0.00	0.00	0.00	0.00
24	38.64	0.67	0.89	0.06	3.35	0.15
48	39.11	1.22	1.67	0.12	2.35	0.16
72	34.97	1.51	2.03	0.15	2.13	0.14
96	37.76	1.56	2.07	0.16	1.73	0.25
120	35.25	1.20	2.12	0.11	0.69	0.10
144	34.30	1.68	2.52	0.16	1.86	0.17
168	35.65	1.75	2.87	0.20	2.07	0.46
192	25.14	1.51	3.28	0.25	2.37	0.94

A-64 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 3 (pH 5.50 and sugar concentration 60 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	61.41	0.00	0.00	0.00	0.00	0.00
24	46.68	0.17	0.13	0.00	1.86	0.20
48	45.76	0.58	0.78	0.00	1.95	0.09
72	47.44	1.15	1.42	0.09	1.35	0.27
96	40.60	1.04	1.61	0.08	0.95	0.15
120	38.44	1.16	2.12	0.16	2.00	0.16
144	31.27	2.05	4.22	0.23	0.47	0.05
168	31.32	2.03	4.21	0.24	0.50	0.05
192	31.83	1.71	3.39	0.15	1.46	0.37

A-65 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 4 (pH 6.50 and sugar concentration 40 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	40.58	0.00	0.00	0.00	0.00	0.00
24	19.33	0.82	1.42	0.00	0.00	0.26
48	26.09	1.46	2.93	0.13	2.21	0.17
72	17.63	1.70	3.28	0.16	2.01	0.23
96	15.36	1.71	3.10	0.18	2.19	0.19
120	16.64	1.86	3.34	0.18	1.73	0.40
144	12.89	2.16	4.07	0.21	1.82	0.34
168	13.54	2.09	4.35	0.21	1.59	0.33
192	12.41	2.05	4.37	0.22	1.51	0.46

A-66 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 5 (pH 6.00 and sugar concentration 50 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	49.91	0.00	0.00	0.00	0.00	0.00
24	39.91	0.33	0.53	0.00	1.74	0.36
48	35.33	0.71	1.13	0.06	1.37	0.16
72	34.93	0.98	2.19	0.10	1.45	0.34
96	31.16	1.11	2.63	0.12	1.58	0.21
120	29.38	1.40	3.14	0.14	1.17	0.06
144	26.04	1.89	4.04	0.19	0.85	0.08
168	24.16	2.09	4.45	0.23	0.80	0.07
192	24.02	2.01	4.40	0.20	0.59	0.05

A-67 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 6 (pH 6.00 and sugar concentration 50 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	49.91	0.00	0.00	0.00	0.00	0.00
24	41.34	0.33	0.60	0.00	1.47	0.37
48	37.52	0.68	1.25	0.05	1.22	0.13
72	36.29	0.86	1.92	0.08	1.20	0.23
96	31.74	1.16	2.77	0.11	1.14	0.14
120	29.28	0.16	3.58	0.16	1.09	0.07
144	25.11	1.95	3.99	0.18	0.76	0.07
168	25.81	1.88	3.60	0.20	0.84	0.09
192	27.24	1.61	3.43	0.16	1.28	0.13

A-68 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 7 (pH 6.00 and sugar concentration 50 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	49.91	0.00	0.00	0.00	0.00	0.00
24	40.55	0.41	0.77	0.00	1.64	0.25
48	37.60	0.86	1.80	0.09	1.19	0.11
72	34.70	0.97	2.21	0.10	1.25	0.22
96	30.63	1.22	2.78	0.11	1.30	0.17
120	30.47	1.80	3.73	0.19	0.86	0.12
144	25.02	1.14	3.05	0.13	1.38	0.40
168	23.33	1.82	4.01	0.22	0.98	0.13
192	24.85	1.93	4.08	0.21	1.17	0.11

A-69 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 8 (pH 5.29 and sugar concentration 50 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	51.27	0.00	0.00	0.00	0.00	0.00
24	26.65	0.77	1.48	0.08	0.97	0.10
48	24.29	1.20	2.84	0.15	1.08	0.07
72	22.21	1.50	3.88	0.17	0.34	0.07
96	17.93	1.74	4.38	0.18	0.00	0.00
120	17.76	1.54	3.75	0.16	0.29	0.06
144	18.51	1.53	3.48	0.14	0.46	0.09
168	15.26	1.69	3.81	0.14	0.00	0.38
192	14.60	1.52	3.47	0.13	2.26	0.89

A-70 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 9 (pH 6.71 and sugar concentration 50 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	51.27	0.00	0.00	0.00	0.00	0.00
24	26.50	0.49	0.93	0.00	1.76	0.20
48	21.31	1.17	2.95	0.13	0.49	0.11
72	17.95	1.58	3.80	0.21	0.90	0.12
96	14.94	1.87	4.50	0.23	0.88	0.14
120	12.81	1.40	3.47	0.21	0.91	0.19
144	13.01	1.62	3.97	0.21	1.00	0.18
168	14.37	1.65	3.91	0.20	0.98	0.14
192	16.30	1.58	4.13	0.19	2.57	0.22

A-71 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 10 (pH 6.00 and sugar concentration 35 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	36.41	0.00	0.00	0.00	0.00	0.00
24	12.54	0.73	1.97	0.08	0.94	0.12
48	12.81	1.23	3.73	0.15	1.41	0.26
72	8.94	1.58	4.62	0.21	1.41	0.61
96	11.78	1.78	5.21	0.23	0.90	0.35
120	6.98	1.55	4.45	0.17	0.00	0.28
144	9.40	1.35	4.22	0.19	0.94	0.37
168	9.28	1.50	4.38	0.19	0.77	0.25
192	8.74	1.54	4.28	0.16	1.77	0.24

A-72 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 11 (pH 6.00 and sugar concentration 64 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	64.67	0.00	0.00	0.00	0.00	0.00
24	42.90	0.21	0.28	0.00	1.81	0.31
48	41.10	0.75	1.58	0.09	1.69	0.17
72	40.10	0.65	1.35	0.00	1.80	0.34
96	34.53	0.92	2.29	0.00	1.32	0.25
120	32.07	1.15	3.15	0.16	1.20	0.18
144	25.30	1.48	3.52	0.20	0.89	0.17
168	29.51	1.65	3.33	0.20	1.04	0.17
192	32.14	1.44	3.57	0.14	3.52	0.39

A-73 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 12 (pH 6.00 and sugar concentration 50 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	49.91	0.00	0.00	0.00	0.00	0.00
24	39.87	0.28	0.40	0.00	1.73	0.33
48	35.89	0.81	1.45	0.06	1.28	0.15
72	34.38	0.93	2.02	0.08	1.38	0.30
96	31.83	1.00	2.61	0.11	1.47	0.20
120	31.07	1.39	3.30	0.15	1.12	0.06
144	25.02	1.99	4.18	0.19	0.82	0.08
168	23.06	2.07	4.32	0.22	0.87	0.12
192	22.68	2.04	4.39	0.21	0.66	0.09

A-74 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 13 (pH 6.00 and sugar concentration 50 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	49.91	0.00	0.00	0.00	0.00	0.00
24	40.91	0.34	0.61	0.04	1.67	0.33
48	38.76	0.69	1.27	0.05	1.39	0.21
72	36.85	0.87	1.92	0.09	1.33	0.33
96	32.02	1.19	2.81	0.13	1.28	0.19
120	27.63	1.57	3.35	0.15	1.10	0.07
144	24.70	2.04	4.02	0.22	0.77	0.07
168	25.74	2.05	4.08	0.20	0.85	0.09
192	27.27	1.67	3.61	0.16	1.34	0.14

A-75 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of Run order 14 (pH 6.00 and sugar concentration 50 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	49.91	0.00	0.00	0.00	0.00	0.00
24	40.83	0.38	0.66	0.00	1.71	0.27
48	36.69	0.87	1.82	0.09	1.26	0.14
72	37.24	0.97	2.19	0.10	1.29	0.24
96	28.64	1.26	2.84	0.12	1.34	0.19
120	26.33	1.94	4.13	0.21	0.91	0.06
144	27.88	1.14	3.07	0.14	1.36	0.42
168	23.54	1.82	4.08	0.22	0.92	0.13
192	26.36	1.96	4.32	0.22	1.11	0.11

A-76 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of optimization1 (pH 6.07 and sugar concentration 43 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	43.43	0.00	0.00	0.00	0.00	0.00
24	28.24	0.42	1.18	0.06	1.45	0.10
48	20.60	0.83	2.99	0.16	0.97	0.07
72	19.72	1.01	3.51	0.20	0.91	0.12
96	20.24	1.77	3.32	0.13	2.46	0.14
120	18.05	1.27	4.26	0.23	1.06	0.27
144	17.38	0.92	3.27	0.19	0.87	0.18
168	17.60	1.18	3.53	0.19	0.82	0.23
192	17.45	1.15	3.55	0.16	1.31	0.36

A-77 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of optimization2 (pH 6.07 and sugar concentration 43 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	43.43	0.00	0.00	0.00	0.00	0.00
24	28.88	0.41	1.28	0.06	1.34	0.10
48	21.00	0.75	2.83	0.16	1.01	0.08
72	16.61	1.15	4.04	0.24	1.00	0.21
96	15.94	0.80	2.88	0.17	0.99	0.20
120	16.74	0.95	3.31	0.20	0.96	0.23
144	17.57	1.00	3.31	0.19	0.90	0.20
168	17.73	0.93	3.39	0.18	0.70	0.15
192	19.58	0.97	3.18	0.17	0.90	0.23

A-78 The results of butanol fermentation from hydrolysate of NaOH-treated press cake by *C.beijerinckii* TISTR 1461 of optimization3 (pH 6.07 and sugar concentration 43 g/L)

Time (h)	Concentration (g/L)					
	Reducing sugar	Acetone	Butanol	Ethanol	Acetic acid	Butyric acid
0	43.43	0.00	0.00	0.00	0.00	0.00
24	30.16	0.43	1.21	0.06	1.58	0.18
48	21.72	0.88	2.92	0.15	0.94	0.07
72	13.41	1.00	3.50	0.17	0.99	0.14
96	14.41	1.17	4.09	0.21	0.88	0.15
120	17.03	1.27	3.31	0.21	0.88	0.17
144	15.57	1.18	3.80	0.20	0.79	0.13
168	17.64	1.00	3.24	0.16	0.91	0.19
192	18.43	1.16	3.35	0.14	1.41	0.25

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