OPTIMIZING METHANATION OF CASSAVA PULP
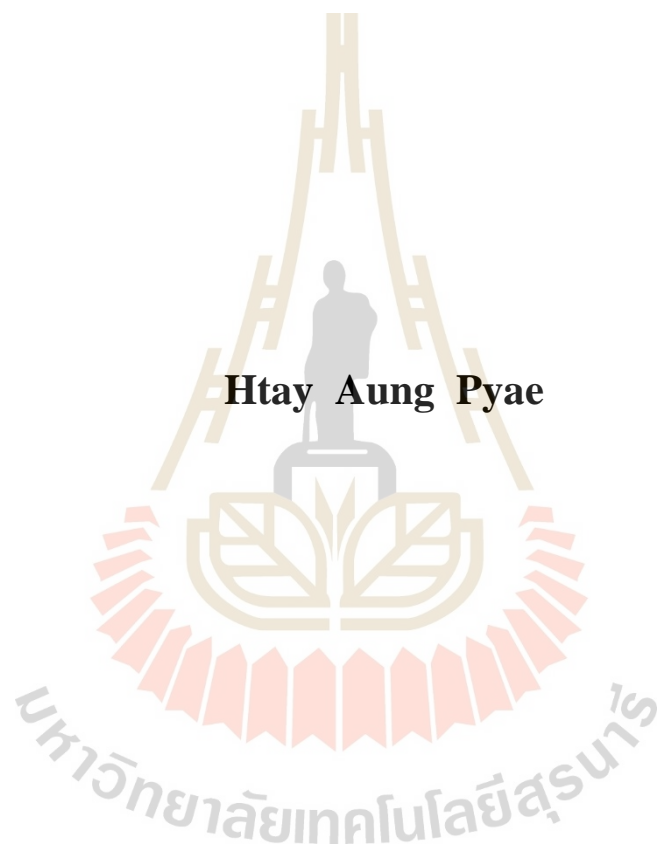
DIGESTION BY ZERO VALENT IRON

Htay Aung Pyae

A Thesis Submitted in Partial Fulfillment of the Requirements for the

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Suranaree University of Technology

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สภาวะที่เหมาะสมในการผลิตมีเทนจากกากมันสำปะหลังด้วยเหล็กประจุศูนย์

นายเต อ่อง พเย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรดุษฎีบัณฑิต
สาขาวิชาวิศวกรรมสิ่งแวดล้อม
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OPTIMIZING METHANATION OF CASSAVA PULP DIGESTION BY ZERO VALENT IRON

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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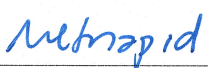_____
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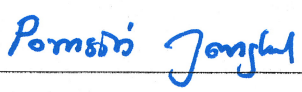
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เต อ่อง พเย : สภาวะที่เหมาะสมในการผลิตมีเทนจากกากมันสำปะหลังด้วยเหล็กประจุ
ศูนย์ (OPTIMIZING METHANATION OF CASSAVA PULP DIGESTION BY ZERO
VALENT IRON) อาจารย์ที่ปรึกษา : อาจารย์ ดร.ฉัตรเพชร ยศพล, 235 หน้า.

งานวิจัยนี้ก็มีจุดมุ่งหมายเพื่อปรับปรุงการเกิดมีเทนในการหมักกากมันสำปะหลังด้วยการ
เติมเหล็กประจุศูนย์อันเป็นสารรีดิวซ์ในการหมัก ในเบื้องต้นนั้นได้มีการทดลองบำบัดขั้นต้น
สำหรับน้ำเสียจากอุตสาหกรรมแป้งมันสำปะหลังผสมกับกากมันสำปะหลังที่สัดส่วนของกากมัน
สำปะหลังต่างกัน 3 ค่า (3% 5% และ 10%) การบำบัดขั้นต้นได้แก่การเติม H2SO4 การเติม NaOH
และการให้ความร้อนที่ 150 องศาเซลเซียส เพื่อหาสภาวะที่เหมาะสมที่สุด ซึ่งพบว่าที่สภาพความ
เป็นกรดสูงและสภาพความเป็นด่างสูงนั้นมีการย่อยสลายเซลลูโลสได้มากที่สุด แต่สภาพความเป็น
ด่างนั้นจะเหมาะสมกว่า ส่วนการเปรียบเทียบชนิดของด่างที่ใช้ในการบำบัดขั้นต้นนั้น พบว่าการใช้
KOH NaOH และ Ca(OH)2 นั้นให้ก๊าซชีวภาพเพิ่มขึ้น 100% 86% และ 103.7% ตามลำดับ และพบ
ว่าการใช้ด่าง Ca(OH)2 ที่พีเอช 10 นั้นเป็นสภาวะที่เหมาะสมที่สุดในการทดลองในขั้นต่อไปใน
การเติมเหล็กประจุศูนย์ช่วยในการหมัก จากนั้นได้มีการศึกษาลักษณะของเหล็กประจุศูนย์ 3 แบบ
คือ R12 M100 และผงตะไบ ใน 4 วิธีการ ซึ่งพบว่า R12 และ M100 มีออกซิเดชั่นสเตต 0 มากกว่า
ผงตะไบ โดยที่ R12 มีความเข้มข้นสูงสุดที่ 96.86% และได้มีการใช้ SEM เพื่อศึกษาลักษณะของผง
เหล็ก รอยแตก รูปทรง รูปร่าง และโครงสร้างอีกด้วย

จากนั้นได้นำน้ำเสียที่ได้เตรียมไว้และเหล็กที่ประจุศูนย์ตามที่ได้ศึกษาลักษณะแล้วมา
ทดลองหมักที่ความเข้มข้นของกากมันสำปะหลังที่แตกต่างกันด้วยระบบ CSTR ที่ภาระสารอินทรีย์
3.25 gVSS/L/day ซึ่งพบว่าได้มีเทนเพิ่มขึ้นถึง 75% และได้ก๊าซชีวภาพเพิ่มขึ้นถึง 35% เมื่อเทียบกับ
การหมักในสภาวะควบคุมด้วยการเติมผงตะไบเหล็ก 20 g/L และพบว่าการเติม M100 1 g/L และ
R12 8 g/L นั้นให้มีเทนเพิ่มขึ้นถึง 93% และให้ก๊าซชีวภาพเพิ่มขึ้นถึง 67% เมื่อเทียบกับการหมักใน
สภาวะควบคุมที่ไม่ได้เติมเหล็กประจุศูนย์ การเติม M100 ขนาดไมโครเมตรในการหมักนั้นมีการ
กระตุ้นการเกิดปฏิกิริยาในทันทีแต่ในท้ายที่สุดแล้วระบบจะล้มเหลวเป็นส่วนมาก ส่วนการเติม
R12 ขนาดไมโครเมตรในการหมักแบบ CSTR นั้นเหมาะสมที่สุดในแง่ของการให้มีเทน การ
ควบคุมการทำงาน (เนื่องจากมีการแตกตัวช้าและพร้อมสำหรับการนำไปใช้โดยจุลินทรีย์ในการ
หมักแบบไร้อากาศได้ดี) และการแยกนำเหล็กประจุศูนย์กลับมาใช้ใหม่ การเติมเหล็กประจุศูนย์ยัง
ช่วยยืดเวลาของความเป็นบัฟเฟอร์ และลดการเติมสารบัฟเฟอร์ในการควบคุมพีเอชอย่างเช่น
NaHCO3 ส่วนในการทดลองหมักแบบ ABR (Anaerobic Baffled Reactor) นั้นพบว่าให้มีเทน
สูงขึ้น 62% เมื่อเทียบกับการหมักแบบ CSTR ในสภาวะเดียวกัน ซึ่งถึงแม้ว่าปริมาณมีเทนที่ได้ใน
การหมักแบบ ABR นั้นไม่แตกต่างจากการหมักแบบ CSTR เท่าใดนัก แต่การหมักนั้นมีความ

สม่ำเสมอในแต่ละห้องย่อยของ ABR ซึ่งบ่งบอกถึงการทำให้ระบบมีเสถียรภาพที่ดี และพบว่าได้
มีเทนเพิ่มขึ้น 5.17% 6.03% และ 8.62% ในการหมักด้วยการเติมผงตะไบ M100 และ R12
ตามลำดับ และยังพบว่าการกวนผสมที่ดีและการปรับปรุงระบบ ABR สำหรับการเติมเหล็กประจุ
ศูนย์ในการหมักนั้นจะช่วยเพิ่มมีเทนมากขึ้นเนื่องจากการเกิดปฏิกิริยาจลนศาสตร์ระหว่างเหล็ก
ประจุศูนย์และสารอาหารนั้นมีความเป็นเนื้อเดียวกันมากขึ้น

สาขาวิชา <u>วิศวกรรมสิ่งแวดล้อม</u>

ปีการศึกษา 2562

ลายมือชื่อนักศึกษา <u>HTAY AUNG PYAE</u>

ลายมือชื่ออาจารย์ที่ปรึกษา <u>ว่าสนิทธิ์</u>

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HTAY AUNG PYAE : OPTIMIZING METHANATION OF CASSAVA PULP DIGESTION BY ZERO VALENT IRON. THESIS ADVISOR : CHATPET YOSSAPOL, Ph.D., 235 PP.

CASSAVA PULP/ ZERO VALENT IRON/ METHANE/ ANAEROBIC DIGESTION

This study aims to improve methnation of cassava pulp digestion by readily available reducing agent, Zero Valent Iron (ZVI) in cassava pulp digestion as common feedstock material. Initially, the mixture of cassava pulp and its wastewater were pre-treated in three different solid contents (i.e. 3%, 5%, and 10%) with concentrated sulfuric acid (H_2SO_4) – Sodium Hydroxide alkaline (NaOH) in 150 °C to examine best digestion setting. The extreme acid and alkali attack resulted in highest solid degradability, but alkaline pre-treatment offered more satisfactory conditions for anaerobic digestion. Among different alkaline pre-treatments (i.e. potassium hydroxide (KOH), sodium hydroxide (NaOH), and calcium hydroxide $Ca(OH)_2$ in different pH ranges, pre-treatment by $Ca(OH)_2$ at pH 10 was found ideal and compatible to subsequent ZVI addition in the digester. Biogas production increased up to 100%, 86%, and 103.7% using KOH, $Ca(OH)_2$ and NaOH respectively. Again, selected ZVI sources (i.e. R12, M100, and Scrap Iron) were characterized in four differential methods. R12 and M100 possessed identical oxidation state (0) more than scrap iron with atomic concentration up to 96.86% in R12 materials. Scanning Electron Microscope (SEM) images disclosed conceivable fragmentation and fractured size of selected irons by its surface morphological pattern, shape and structure. With the abovementioned established cassava substrates setting and ZVI characteristics, ZVI supplementation in different concentrations into lab-scale

Continuous Stirred Tank Reactor (CSTRs), under the optimum organic loading rate (OLR) of 3.25 g VSS L-1 day-1, bio-methane enhanced up to 75% with 35% additional gas yield than that of the control reactor when scrap iron was added up to 20 g/l. Under the optimum dosage for 1 g/l and 8 g/l for M100 and R12, up to 93% more bio-methane enhancement with 67% added biogas volume than ZVI free control reactor. The presence of finer micro-particle M100 ZVI in AD system had instantaneous and stimulatory impact by its readily reactivity with aqueous substrate, but it led digestion failure even with few margins of excess. The micro-particle size R12 supplementation in CSTRs demonstrated the ease of better digestion performance, process control and recoverable properties. The presence of iron cut the frequency of re-buffering, and thus reduced buffer chemical ($NaHCO_3$) consumption for pH control and provided with a longer buffer resistance period. Methane gas was enhanced up to 62% with the integration ideal dosage obtained from CSTRs operation into Anaerobic Baffled Reactor (ABR). Despite this increment is not a significance figure than that of the result of CSTR operation, ZVI addition proved moderating uniform digestion in all the compartments to maintain reactor in healthy status. In term of volumetric gas yield as well, gas volume increased 5.17%, 6.03%, and 8.62% for scrap iron, M100 and R12 respectively. There is a strong evidence that mixing arrangement or modification to conventional ABR system is recommended to enhance bio-methane by the effect of ZVI. So that, the kinetics among ZVI and substrate keep in homogeneity.

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ACKNOWLEDGEMENTS

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SYMBOLS AND ABBREVIATIONS

ABR   =   Anaerobic Baffled Reactor

ACL   =   Anaerobic Covered Lagoon

AD   =   Anaerobic Digestion/Digester

AFF   =   Anerobic Fixed Film

BL   =   Beamline

BMP   =   Bio-Methane Potential

CNG   =   Compressed Natural Gas

COD   =   Chemical Oxygen Demand

CSTR   =   Continuous Stirred Tank Reactor

DEDE   =   Department of alternative Energy Development and Efficiency

EGSB   =   Expanded Granular Sludge Bed

EPPO   =   Energy Policy and Planning Office

HRT   =   Hydraulic Retention Time

LNG   =   Liquified Natural Gas

MLSS   =   Mixed Liquor Suspended Solid

MLVSS   =   Mixed Liquor Volatile Suspended Solid

OLR   =   Organic Loading Rate

PFR   =   Plug Flow Reactor

SEM   =   Scanning Electron Microscopy

SRT   =   Solid Retention Time

TA   =   Total Alkalinity

SYMBOLS AND ABBREVIATIONS (Continued)

TDS   =   Total Dissolved Solid

TOC   =   Total Organic Carbon

TS   =   Total Solid

TTSA   =   Thailand Tapioca Starch Association

UASB   =   Up-flow Anaerobic Sludge Bed

VFA   =   Volatile Fatty Acid

VS   =   Volatile Solid

VSS   =   Volatile Suspended Solid

WtE   =   Waste to Energy

XAS   =   X-ray Absorption Spectroscopy

XPS   =   X-ray Photoelectron Spectroscopy

XRD   =   X-ray Diffraction

XRF   =   X-ray Fluorescence

ZVI   =   Zero Valent Iron

CHAPTER I

INTRODUCTION

1.1 Energy: Thailand Context

Most of the energy that drives our economy and society origins from fossil fuel (i.e. coal, crude oil and natural gas) in which manufacturing industry and transportation sectors are major energy consumers. Experts warn that the fossil energy reserve will exhaust within next decades or in a century utmost (Shafiee & Topal, 2009; Ritchie and Roser, 2019). Therefore, every nation attemptswith their highest capacity in shifting their dependency on fossil fuel, and invests more on alternative and renewable energy such as hydropower, solar, wind, geothermal, nuclear, biomass, and tidal. The efforts produce momentum to ensure energy security under control sinceits threats are devastating to the society and being reckoned as a pressing issue more than ever. The development of biogas technology and the concept of Waste to Energy (WtE) as renewable sources attract developers to invest more in renewable energy while by-products and co-products from manufacturing process chains could be altered into valuable source for power generation particularly forthe countries oriented in agriculture and agro-industry.

Thailand confronts all aforementioned challenges. Heavily depending on imported fossil fuel, it is attempting to cut energy dependency from regional exporters by supporting energy efficiency and utilization alternative energy in its utmost capabilities. In all the consecutive cabinets, they set series of strategies and policies

that enable the Country switching into alternative energy source by uplifting renewable energy and potentials available within the Country. With the energy sources available, Thai Government plans to boost energy supply from renewable source from 6.4% in 2008 to 20.3% by the year 2022 (DEDEReport, 2009). As the agriculture sector dominates the state's economy, Thailand can take this advantage to enhance the role of renewable energy by sourcing the energy within its biomass and agricultural wastes from agro-industry. Cassava, rice, sugarcane, corn and palm oil are Thailand's top product and export among several energy and cash crops. The annual cultivation of these energy crops soars as a result of increased market demand, consumption in value added products, and government supports through innovation, subsidies and tariff exemptions. Therefore, the generation of agricultural residues from crops processing is also on the rise creating more opportunity to harness renewable energy from these agricultural residues and by-products. This Chapter highlight, the scenario of cassava pulp as common feedstock for biogas production, current biogas technologies, justification and research objectives.

1.2   Cassava Industry in Thailand

Thailand ranks the first as the world's biggest cassava exporter and the third highest cassava producer, after Nigeria and Brazil. The strength of well-established cassava or tapioca (botanical name - Manihot Esculenta) institution and their concerted efforts in research and development actions encourages Thailand to harvest higher yield per area than that of Nigeria and Brazil. The stable price in trade, application of cassava starch in value added and related products in both food and consumers products, and export market attract cassava farmers to increase plantation more across

Thailand with the exception to some southern provinces. According to Tapioca Starch Network of Thailand, since 2007, cassava cultivation was increasing having more than 50% in the Northeast, followed by the Central Plain (33%) and the North (15%) of agricultural land. The plantation areas scatter in 48 provinces equivalent to 7 million rais (Office of Agricultural Economics, 2007). To this day, based on the survey conducted by Thai Tapioca Starch Association (TTSA), the cultivation and plantation continues to grow annually. Despite some decrease in yield, according to 2014/2015 figures, the total plantation across Thailand reached over 8.6 million rai, producing over 33.6 million tons on average yield of 3.86 ton/rai. The table 1.1 under shows the status of cassava production in 2014/2015. TTSA forecasted that the cultivation would increase for the year 2015/2016. And, the figures continue to grow.

Table 1.1 Cassava Plantation across Three Provinces in Thailand

Region/Province	Plant Area (rai)	Production (ton)	Yield (ton/rai)	Potential Renewable Energy Sources	
	2014/2015	2014/2015	2014/2015	Wastewater (m³)	Cassava Pulp (tons)
Northern	1,975,131	7,516,727	3.806	33,923,471	2,499,624
Northeast	4,459,600	17,451,887	3.913	78,761,485	5,803,477
Central	2,263,217	8,641,873	3.818	39,001,327	2,873,781
Total All Region	**8,697,948**	**33,610,487**	**3.864**	**151,686,283**	**11,176,882**

Source:Source: Thai Tapioca Survey Team (TTST)

Cassava starch is produced in starch processing factory upon passing screening processes such as root preparing and washing, rasping, extraction, separation, drying and Packing. Common by-products are cassava chips/pellet, cassava pulp and cassava wastewater. While the cassava chips/pellet undergo further process for animal feeds, the cassava pulp and wastewater are used for major feedstock for biogas and bio

ethanol production. In this Chapter, the significance of cassava pulp for biogas production is highlighted. According to cassava starch production, the process flow shown in figure 1.1, typically for every one ton of cassava starch requires 4.21 tons of cassava tubers which then generate approximately 19 m^3 of cassava wastewater and 1.4 ton of cassava pulp residues.
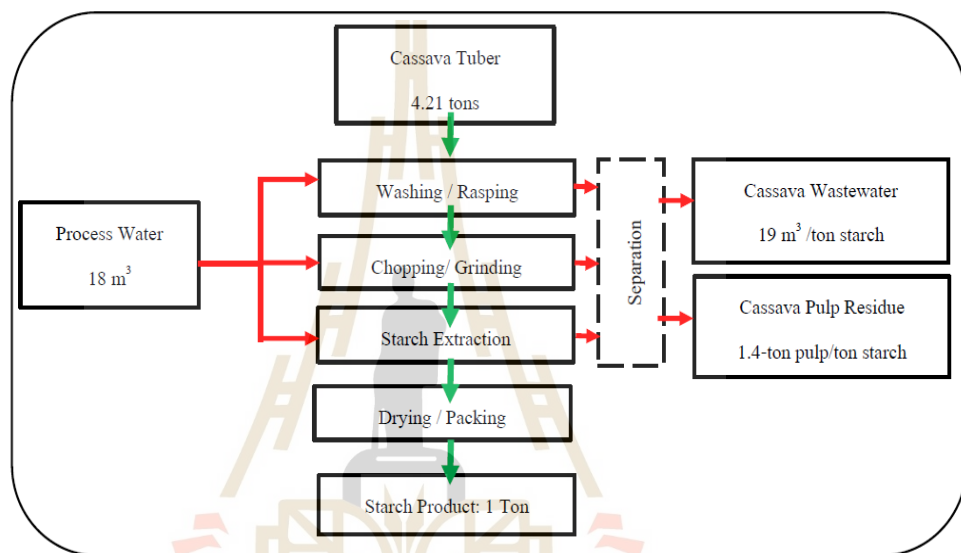
Figure 1.1   Cassava Pulp and Wastewater Production in Typical Cassava Starch Factory (Source: Chavalparit and Ongwandee, 2009)

About 70 large scale cassava starch factories produced 3-4 million tons of processed dry cassava starch annually in Thailand (Warinthorn, 2013).

The cassava pulp generation is ranging between 0.95 - 2.86 tons per one ton of cassava starch output. The figure depends on the choice of manufacturing process, technology and cassava tuber varieties. In total, estimated 6-7 million tons of cassava pulp are being produced each year from cassava starch industry. However, the application of cassava pulp as major substrates remains limited for bio-digester and

being targeted as cheap animal feeds and fertilizers. The low protein values in cassava pulp make it unpopular to use them as animal feeds for growth promoter. On the other hand, as the consequence of the increased cassava production, resulting cassava pulp generation from starch mill surpasses the consumption by livestock industries. Therefore, the surplus bulge of cassava pulp ends as natural fertilizer through composting. This results in both economic lost and environmental pollution. The degradation of cassava pulp in the environment release acids and gasses during fermentation and leaching to soil, water sources and nuisance to the ambient environment. Hence, in order to avert the environmental pollution triggered by conversion into natural fertilizer, utilizing cassava pulp as major biomass for bio-digester is prime option in eliminating these problems. But, the decision to adopt diverse technological options and selection of suitable digester type for utmost biogas production capacity still remains as the challenges.

Cassava industry can reliably apply the concept of Waste to Energy (WtE) for producing energy, biogas (methane: CH_4), by using by-products namely cassava wastewater and pulp in the Anaerobic Digester (AD). This energy recovery process helps reducing the whole cassava factory to rely on electricity supplier by generating power from its own waste for more income from exporting the energy back into national power grid. Higher cassava production means more acquisition of biomass/feedstock for bio-digester and the industry becomes more sustainable in renewable energy production. Therefore, to conserve fossil fuel and efficient utilization of energy, agro-industrial wastes possess higher renewable and sustainable potentials than other renewable sources. Taking the advantage of agricultural

productivity, Thailand's renewable energy goal is more likely to be met through improvement of energy crop production and energy salvaging efforts.

1.3 Cassava Pulp and Biogas Technologies in Thailand

The biogas technology was introduced in Thailand starting from 1960s with small scale digester that used animal manure aiming to produce biogas for cooking purposes. After late 1990s, with the combination of government's support, incentives and development of technological know-how and innovation, the biogas technology and the industry boomed. As of government 2011 statistics, there were more than 1800 biogas plants throughout Thailand (Kamsamrong & Sorapipatana, 2014; Pruk, 2017). Generally, biogas is produced in four common types of biomass such as industrial, municipal, farm and agro-industrial wastes. Sometimes co-digestion among these biomasses is applied for better yield. The production technology is composed of four major processes, namely pre-treatment, biogas conversion, biogas upgrading and biogas utilization. There are 4 major pre-treatment processes (i.e. physical, chemical, biological, and advanced technological) for biomass before biogas conversion. Except biological pre-treatment, in Thailand, the remaining three processes are adopted but the complexity of biomass's physio-chemical properties hinders widespread use of this technology. In biogas conversion technology, reactors are divided into high and slow rate reactors (Suwanasri et al., 2015). High rate reactors are those which include Anaerobic Fixed Film (AFF/AF), Up-flow Anaerobic Sludge Bed (UASB), Expanded Granular Sludge Bed (EGSB), Continuous Stirred Tank Reactor (CSTR), Anaerobic Baffle Reactor (ABR), and Plug Flow Reactor (PFR). Low rate reactors are Anaerobic Covered Lagoons (ACL). All reactors undergo the same fundamental of methanation or biogas

conversion process.But the organic loading rate and configuration of digestion phase have strong influence on the selection of reactors type and design; these factors govern operation cost and economic return as well. UASB and CSTR reactors are highly favoured in Thailand because of more simplicity in operation and less running cost. Out of biogas plants built starting 1995 to present day, UASB and CSTR reactors stands 70% of high rate digester category (Suwanasrie et al., 2015). The following table 1.2 shows the status of technology adoption for biogas production in Thailand.

Table 1.2 Technology Development Status for Biogas Production in Thailand

Pre-treatment		Biogas Conversion Technology		Post Treatment	
Methods/ Processes	**Status**	**Methods/ Processes**	**Status**	**Technology**	**Status**
		High Rate			
Mechanical	Partially	AFF/AF	Fully developed	Hydrogenation	Not Developed
Thermal	Partially	USAB	Fully developed	Electro-methanogenesis	Not Developed
Chemical	Partially	EGSB	Partially Developed	Scrubbing	Fully Developed
Enzyme	Not Developed	CSTR	Partially Developed		
		ABF	Partially Developed		
		PFR	Partially Developed		
		Slow Rate			
		Cover Lagoon	Fully developed		

(Soruce: Warinthorn Songkasiri 2013)

1.4 Cassava Pulp and Current Biogas Yields: Overview

Being one of the major by-product of cassava starch industry, cassava pulp contains large amount of carbohydrate (see table 1.3). This amply available carbon source is strongly favourable for biogas production. On sampling, cassava pulp has a chemical oxygen demand (COD) of 1,251 g/kg, dry and volatile solids (VS) of 98% (Paepatung et al., 2009). When cassava pulp as major substrates for biogas production is subjected for digestion in anaerobic

reactor, the bioconversion processes take place under complex set of bacteria that degrade the substrates in symbiotic environment. There are four stages in biogas conversion (i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis) (Alvarez et al., 2000). The rate of conversion depends on substrate properties and characteristics. Cassava pulp has the following physiochemical properties described in table 1.3 (Pires et al., 2019). The rate of methanation and gas yield evolved depends on factors such as pH, temperature, organic loading, nutrients, carbon/nitrogen (C/N) ratios, substrate particle size and inorganic minerals. Inhibition occurs as the result of changes in these conditions as well. By Bio-Methane Potential test (BMP), cassava pulp produces ultimate yield of 0.37 l/g VS added (Liu et al., 2013)). This figure represents maximum specific methane production rate of 36.57 ml/day and 76% biodegradability. On a commercial scale, a typical cassava starch factory, having 200 TPD capacity generates 280 ton/day of cassava pulp with 20% TS and 95% VS, produces about 16,000 m^3/day of methane gas fromthe operation in ABR reactor (Pires et al., 2019). Likewise, the project called Korat Waste-to-Energy, the facility processes 750 TPD, produces 75,000 m^3/day of bio-methane (Cohen, 2004). Similarly, Chok Chai Starch, one of the leading cassava starch manufacturers, produces methane 30,000 Nm^3/day capacity(Geoff, 2015).

Regardless of any reactor types adopted, it was found out that biogas has methane composition, which is major energy calorificvalue between 40 – 65 %. Therefore, gas upgrading or purification process which is under the post treatment of biogas production plays a crucial role to enrich energy value of biogas. Therefore, the technology to produce biogas containing high methane content needs to be innovated to eliminate heavy use of chemicals and the burden of removing inert and toxic gases such as carbon dioxide (CO_2) and hydrogen Sulphide (H_2S).

Table 1.3 Physiochemical Properties of Cassava Pulp

Parameter	Units	Content
Moisture	%	81.60
VS	% dry mass	98.07
COD	g/kg dry	1251
Carbon	% dry basis	44.68
Hydrogen	% dry basis	6.31
Nitrogen	% dry basis	1.85
Oxygen	% dry basis	46
Sulphur	% dry basis	0.13
Cellulose	% dry basis	12.56
Lignin	% dry basis	1.86
TKN	g/kg dry	2.76
NH_3-N	g/kg dry	0.58
Starch	% dry basis	50

(Source: Paepatung et al., 2009)

1.5 Problems Statement and Justification

Biogas evolved from different substrates produces fluctuated results in terms of quantity and quality which is considered as energy gas, Methane CH_4. The available options for digestion system, environmental variables and mode of operation make it rather complicated to achieve ideal gas yield between the determinants of cost and benefit standpoint within given resource inputs. Therefore, the technology for biogas extraction from different sources needs constant investigation and research ondigestion settings and conditions when even a minor modification in the manufacturing processes is made.

According to Alternative Energy Development Plan (AEDP), there are about 500 biogas plant installed as of 2018 data and Energy Policy and Planning Office's (EPPO) 2013 figures, about 60 cassava feed biogas plants are in operation across

Thailand, generating 385.82 million m^3 annually. But the industry encounters varying in gas yield due to the fact that the absence of dedicated biogas technology for cassava substrate with adaptable and innovative research and development on biogas fermentation process when raw materials in starch extraction and cassava verities have been modified in the milling processes (Chavalparit & Ongwandee, 2009). Depending on the choice of substrates for biogas production, it needs to apply specific technical knowhow on reaction kinetic, management on the micro-organisms, and digester variables for achieving optimal yield.

With nutritional fact of cassava pulp and its physio-chemical properties for anaerobic digestion, cassava pulp had been proven an eligible substrate for biogas plant (Sriroth et al, 2000). However, digestion failure still persists due to the lack of nutrient supplementations and chemical stimulators to maintain balance growth of methane forming bacteria and substrate utilization in the digester. Although researchers have been conducting on technical parameters and variables for optimum biogas yield using cassava pulp, few emphasizes on the research areas focusing onthe role of catalyst for enriching methane composition regarding cassava residues (Demirel and Scherer (2011), and Schattauer et al. (2011)). This study highlights essential roles of chemical reductant, particularly to Zero Valent Iron (ZVI), to optimize digestion performance for greater yield and biogas quality. Therefore, the finding might fill the research gaps for the requirements of specific research for applicability of catalysing agent in biogas process that focuses on cassava pulp for anaerobic fermentation for ensuring stable biochemical reaction throughout digestion processes.

1.6   Research Aims and Objectives

This research is aimed at 1) uncovering ideal presence of catalytic agent, Zero Valent Iron (ZVI) in term of either powder or granule in cassava pulp fed anaerobic digester after feedstock material are chemically pre-treated and 2) optimizing methanation by applying ZVI in mono-digestion of agro-industrial by products. In order to accomplish these research aims; followings were undertaken as specific objectives.

i)   To determine optimum solid content of feedstock material (cassava pulp) for selected chemical pre-treatment

ii)   To determine compromised chemicals (acid − alkaline − thermal) pre-treatment configuration setting by considering practicality and feasibility for commercial application.

iii) To discover ultimate anaerobic digestion settings through pre-tests in batch reactors.

iv)   To characterize and examine different ZVI materials for optimum elemental iron with ZVI properties in differential investigation methods (XAS, XPS, XRD, XRF, and SEM).

v)   To discover ideal presence of ZVI materials by means of addition in different concentrations in Continuous Stirred Tank Reactor (CSTR).

vi)   To evaluate significance of ZVI addition to anaerobic digestion processes in CSTR, anaerobic digester, and examine gases content and yield by gas chromatography.

vii)   To evaluate significance of ZVI addition to anaerobic digestion processes in Anaerobic Baffled Reactor (ABR) system, and examine gases content and yield by gas chromatography.

1.7   Research Questions

This study was inspired by the following key research questions:

(1)   What is the relative significant of zero valent iron in cassava feed digester?

(2)   How much bio-methane volume can be promoted by mean of ZVI supplementation against standardized control reactors?

(3)   Is it feasible to use collective addition of both Zero Valent Iron (ZVI) in biogas process?

(4)   What challenges could the finding face in commercial application?

1.8   Research Hypothesis

The primary assumption of this study is that if Zero Valent Iron supplemented cassava pulp feed anaerobic bio-digesters will produce more methane, either by producing more biogas or by producing biogas with a higher methane percentage. The subsequent hypothesis also aims at if optimized collective ZVI supplementation formula could be integrated into pilot level.

This hypothesis will be investigated through series of sampling and analysis throughout research period until desirable results discovered. Type of analysis and strategy to achieve the research goals are stated in-depth in Chapter 2.

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CHAPTER 2

RESERACH SCOPE DEFINITION AND FRAMEWORK

2.1   Introduction

This Chapter highlights research activities undertaken to answer the research questions and hypothesis. The pertinent literatures reviews, materials and methods, justification, objectives, results and discussion, and the conclusion of each particular tasks is presented in the relevant Chapters.

2.2   Research Scope Definition and Framework

Presented in figure 2.1, research framework describes the processes bymeans ofhow research hypothesis and questions have been answered to achieve research goals. It is divided into 3 parts. Part 1 includes pre-treatment to major feedstock materials (i.e. cassava pulp and wastewater) for examining optimal biogas yield, whereas part 2 and part 3 include characterizing major catalyst, Zero Valent Iron (ZVI), and optimizing methanation of cassava pulp digestion by ZVI in 2 different bioreactors. The detailed structure of this thesis is shown in the table 2.1 under. The pertinent information regarding publications are further presented.

Biogas technology includes interdisciplinary fields and mechanisms ranging from biotechnology for biomass application to advanced engineering for high process efficiency. Nevertheless, by ensuring strictly anaerobic environment, biogas technology could be divided into 3 stages (i.e. pre-treatment, digestion, and post-treatment). The summary of important biogas related researches could be classified as in the figure

2.2in which the scope of this study is underlined on the left and the boundary of biogas related research on the right onion chart.

Table 2.1 Structure of Thesis

THESIS STRUCTURE		Publication Status
CHAPTER 1		
Objectives	Literature Review and Background	Review Paper [Published]
Research Framework		
CHAPTER 3		
Objectives	Pre-test in Batch System - Pre-treatment (Acid-Alkaline-Thermal) to Cassava Pulp's Physiochemical Characteristic for Ideal Anaerobic Digestion Settings	Proceedings [Published]
Research Framework	**PART I**	
CHAPTER 4		
Objectives	Pre-test in Batch System - Comparative Alkaline Pre-Treatment to Cassava Pulp for Optimal Biogas Yield	Research Paper [Published]
Research Framework	**PART I**	
CHAPTER 5		
Objectives	Verification and Characterization Selected ZVI source for ultimate Reducing Potential in Anaerobic Digestion Process	Research Paper [Published]
Research Framework	**PART II**	
CHAPTER 6		
Objectives	Optimizing Bio-methanation Potential of Cassava Pulp Digestion by Scrap Iron in CSTRs	Review Paper [Published]
Research Framework	**PART II**	
CHAPTER 7		
Objectives	Inhibition Threshold for Micro-Particle Presences in Cassava Pulp Digestion in CSTRs	Review Paper [Published]
Research Framework	**PART II**	

Table 2.1 Structure of Thesis (Continued)

THESIS STRUCTURE		Publication Status
CHAPTER 8		
Objectives	Optimizing Bio-Methanation of Cassava Pulp Digestion in CSTRs by Differential Size ZVI Supplementation	**Submittal [Pending]**
Research Framework	**PART II**	
CHAPTER 9		
Objectives	Integration Ideal ZVI Concentration in ABR system-Pilot Scale Study	Proceedings [Published]
Research Framework	**PART II**	
CHAPTER 10		
Objectives	Conclusion-Recommendations-Future Work-Research Novelty	
Research Framework	**PART III**	
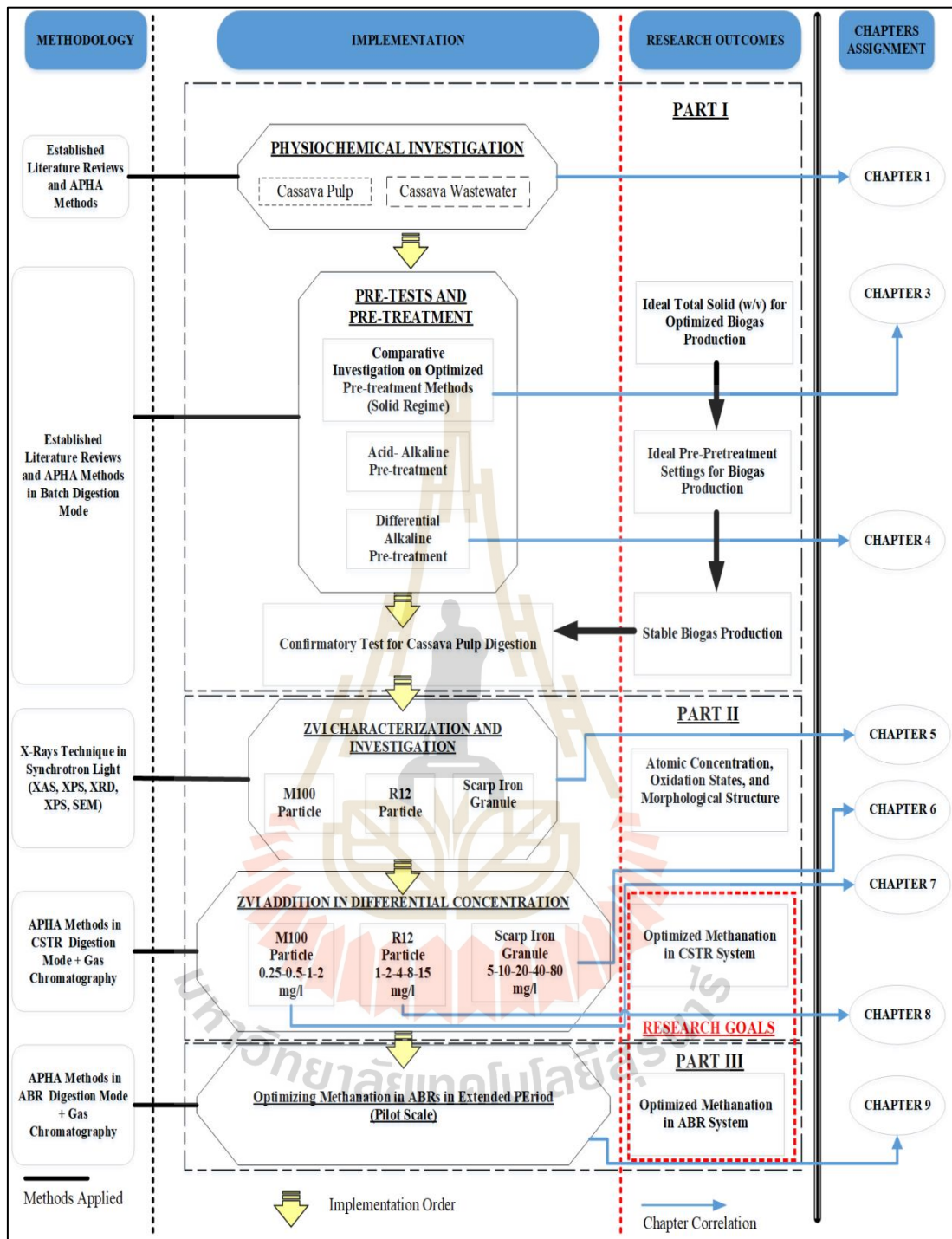
Figure 2.1 Research Framework
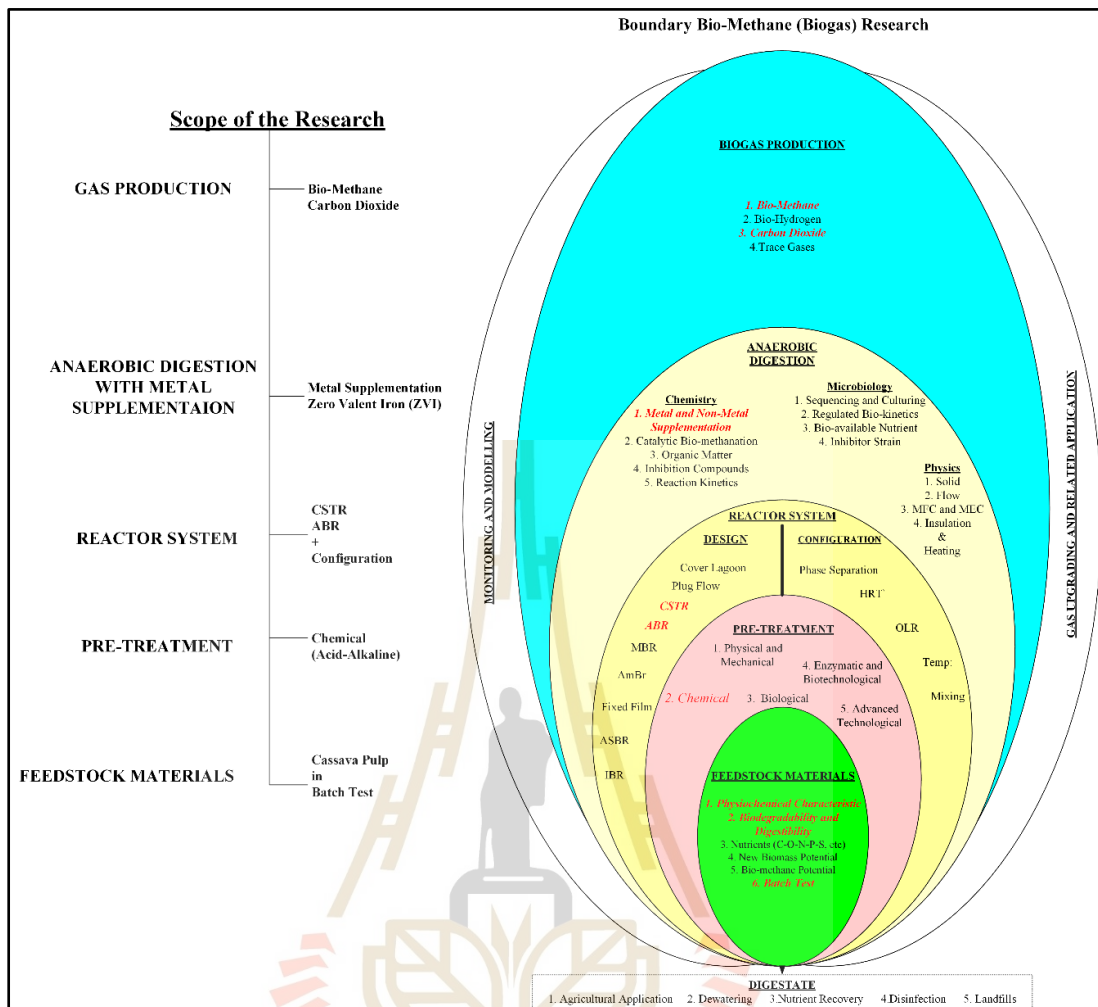
Figure 2.2 Research Scope Definition

(Adopted from Biogas Research Centre (BRC) Report 2014:2.)

2.3   Implications

This study intends to investigate the positive impact of zero valent iron which are being widely used for environmental remediation technology by its reducing properties to oxidize harmful chemical compounds in water, and as a stimulator for microorganism symbiosis. By using these combined advantages into cassava pulp feed digester, biogas is strongly believed to be promoted both quantity and quality. Finally, the research is expected to be forefront research on zero valent iron application in cassava pulp feed fermenters and the research

findings will become breakthrough technological finding for ongoing cassava biogas plant and alternative choice for biogas plant developer in future.

CHAPTER 3

EFFECT OF ACID-ALKALINE AND THERMAL PRE-TREATMENT TO CASSAVA PULP FEED IN OPTIMIZATION OF BIOGAS YIELD IN BATCH REACTORS

3.1   Graphical Abstract
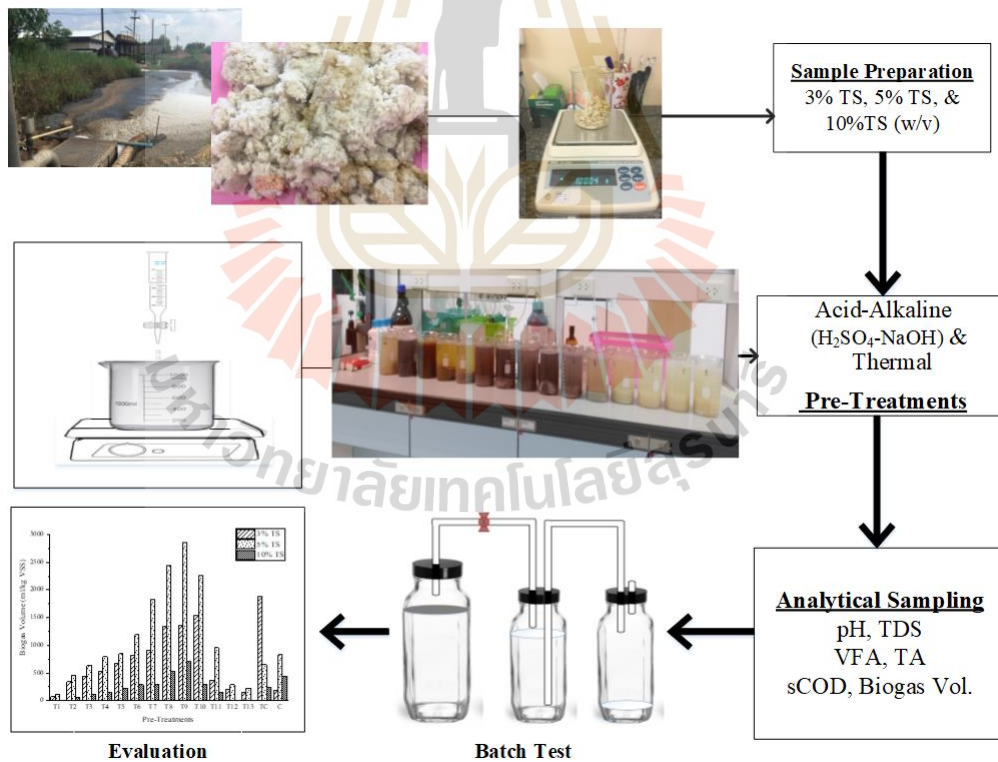
3.2 Highlights

-   Acid-alkaline pre-treatment accelerates solubility of the carbon source attached to the lignocellulosic structure of cassava pulp and helps adjusting pH back to the desired pH.

-   Thermal and extreme acid-alkaline pre-treated samples resulted in highest lignocellulose solubility but upholds less possible bacteria diversity.

-   Extreme pH pre-treated sample sets (T1, T2, T11, T12, and T13) generated up to 60% more biomass transformation potential.

-   The experiments revealed that sCOD and TDS could be more enhanced by either acid-alkaline and thermal pre-treatment than control substrate.

-   5% TS pre-treatment resulted in best digestion performance than its counterparts.

3.3 Abstract

Anaerobic digestion technology for biomass offers reliable and renewable energy, however, the question on resource consumption and energy output remains as a challenge to adopt it. This study used acid-alkaline and thermal pre-treatment to modify the cassava pulp substrate as feedstock material to enhance biogas yield. Concentrated 36N sulfuric acid (H_2SO_4) and 20 M sodium hydroxide (NaOH) were chosen as acid-alkaline pre-treatment to adjust required pH for the substrates, and 45 min. at 200°C for thermal pre-treatment for the objective of better solid degradability. Extreme pH adjusted substrates such as T1, T2, T12 and T13 required both acid and alkaline in high volume, and inhibition occurred from both acid and alkaline radicals

resulting in retarded fermentation and digestion failure. The results showed soluble
Chemical Oxygen Demand (sCOD) obtained from decomposition of lignocellulosic
structure of fresh cassava pulp by combined thermal-chemical pre-treatmentwas
found highest in T1 and T12 at which sCOD were 92 g/L and 95 g/l respectively.
Though sCOD could be enhanced by acid-alkaline pre-treatment, it led to inhibition
driven by radicals of acid and alkaline, and food overloading to microorganism. Three
different mixing ratios, i.e. 3%, 5%, and 10% (w/v) were compared against the one
without pre-treated samples, and found 5% Total Solid (TS) proved most suitable
after subjected to acid-alkaline pre-treatment and produced biogas yield at 2845 ml/kg
VSS in batch digestion for 21 days. The optimum pre-treatment to cassava pulp
offered up to 6 times of more biogas volume than the one without pre-treated samples
saving resource consumption and optimized yield.

3.4 Introduction

Biomass in general composed of cellulose, hemicellulose, and lignin which in
combination are known as lignocellulosic substances (Zhao et al., 2012; Leu and Zhu,
2013). Each individual biomass possesses distinct lignocellulosic compositional
properties making them difficult to break down during AD processes (Taherzade&
Karimi, 2008; Anwar et al., 2014). Although cellulose of common biomass is soluble
in water and hemicellulose with lignin chains are insoluble but they are sensitive to
thermal and chemicals subjected to suitable pre-treatments (Wang et al., 2012). They
tend to dissolve into enriched nutrient content and more carbon source of the
feedstock material. The selection of pre-treatment methods depends on type of
biomass which have unparalleled characteristics of organic compositional structure to

obtain higher organic solubility subjected to dedicated pre-treatment for better substrate degradability (Carrère et al., 2010; Châu, 2014). The advantages of pre-treatment to biomass prior to initiate Anaerobic Digestion (AD) process improves biodegradability of cellulosic material of biomass producing fermentable sugars, amino acids and volatile fatty acids as the products of hydrolysis stage (Zheng et al., 2014). Pre-treatment offers more food source from specific loading for hydrolytic bacteria and subsequently all microorganisms within AD system as a whole for more biogas yield (Wellinger et al., 2013; Taherzade& Karimi, 2008). Out of several pre-treatment options available, while thermal, chemical, and ultrasonic method were proved effective, time saving, and economically feasible, whereas the biological and enzymatic methods remain unattractive because of complexity and slow rate (Leu and Zhu, 2013; Seidl & Goulart, 2016; Chen et al., 2017). The selection of pre-treatment methods relies on the properties of individual biomass and applicability.

In recent years, researchers had been focusing on diverse ranges of biomass, specifically from forest, agricultural residues, herbaceous grass to municipal waste (Zheng et al., 2014; Behera et al, 2014). Though cassava tuber and its residues are proven for biogas productionthrough bioconversion, the research pertinent to pre-treatment of cassava pulp for more biogas volume under specific resource input remains elucidated. Several authors reported application of pre-treatment methods for cassava waste to produce diverse ranges of product from bio-ethanol to hydrogen. Phowan and Danvirutai (2014) reported that hydrogen yield of 342 ml H_2 g^{-1} COD $_{reduced}$ and hydrogen production rate of 3381 ml H_2 L^{-1} d^{-1} was achieved by using dilute sulfuric acid pre-treatment up to 5% within reaction time 30 mins and temperature at 121˚C. Similarly, for ethanol fuel, a study conducted by Srinorakurata

and kaewvimol et al., (2007), by using combination of dilute sulfuric acid of 0.2-5.0 M and enzyme type of α-amylase at the temperature of 60-120 ˚C for 30 min, maximum ethanol production was found 3.62% (w/v) in 10L fermenter at 24 hrs. Also, Kosugi and Kondo et al., (2009) conducted pre-treatment of cassava pulp to produce ethanol by using hydrothermal and enzymatic hydrolysis. In the studies, the researchers used H_2SO_4 having concentration 0.1 or 2.0 %, K7 strain, and heated at 120 to 180 ˚C for 1h, proving ethanol could be produced up to 40 ml/kg cassava pulp within 7 days of fermentation.

This study highlights optimization of biogas volume from cassava pulp feed in batch rectors by concentrated acid-alkaline and thermal pre-treatment. Cassava pulp were first subjected to strong acid, sulfuric acid (36N - H_2SO_4), strong sodium hydroxide alkaline solution (20M - NaOH) and thermal hydrolysis to boiling point prior to execute anaerobic digestion in batch reactors in term of different pH ranges and 3 different solid contents, i.e. 3% TS, 5% TS and 10% TS. The effect of acid-alkaline and thermal pre-treatment results in biogas forming potential were compared against the control sets. The objective of this work is to find out compromised pre-treatment option among acid, alkaline and thermal pre-treatments, and ideal solid regime (TS%) for optimum biogas production.

3.5. Experiments

3.5.1 Feedstock material and Inoculum

Fresh cassava pulp was collected from Korat Starch Factory located in Nakhon Ratchasima province in north-eastern Thailand. Qualitative surveying was made prior to collect in determining cassava tuber variety from local farmers, and

found four major species, namely CMC 76, KU 50, Rayong 60, and Rayong 90 are cultivated commercially in this province (Watana et al., 2006; Piyachomkwan & Tanticharoen, 2011). Upon proximate analysis undertaken on each feedstock biomass sample collection, fresh cassava pulp was found having moisture contents between 55-70% on wet basis, 60-65% of starch and 15-18% of fibre respectively (AOAC, 1990). Wastewater from the starch mill was chosen for hydrolysis and stored under 4˚C in cold storage until used. Hydrolysing for 15 mins was carried out prior to pre-treatments to ensure homogeneity of the bulky cassava pulp and wastewater. Sodium bicarbonate ($NaHCO_3$) was added for buffer control to ensure pH during AD process remained between 6.8-7.5.

Inoculum was obtained from parent biogas plant, maintained at 35 ˚C, and utilized within 12 hours of collection period. Inoculum properties having pH value about 7.5-8.0. Laboratory samplings for physicochemical properties were carried out prior to starting anaerobic digestion in batch reactors (figure 3.1).

3.5.2 Acid-Alkaline and Thermal Pre-treatment

Feedstock material - cassava substrates were made in 3% TS, 5% TS and 10 % TS (w/v) by mixing solid cassava pulp with 1000 ml mill effluent wastewater. Concentrated sulfuric acid (H_2SO_4) of 36 N and sodium hydroxide (NaOH) of 20 M were used to adjust desired pH ranging 1-13 denoting pre-treatments from T1 to T13 shown in the figures. Since the boiling point of the substrates which is the mixtures of fibrous cassava pulp and mill effluent starts at 150 ˚C, and for practical approach in biogas plant, temperature and reaction time for acid-alkaline and thermal pre-treatment were set at 200 ˚C for 45 min on hot plate, and left for cooling and further acid-alkaline attack for 6 hrs. Finally, hydrolysed samples were

neutralized back to pH 7.5 for anaerobic digestion in batch process. Therefore, the whole pre-treatment mechanism includes forward and backward neutralization to reach desired pH set by concentrated H_2SO_4 and NaOH solution. Two control sample sets, one with neither acid-alkaline nor thermal pre-treated (C1) and with only thermally pre-treated (TC2) were compared against all acid-alkaline pre-treated sample sets (T1-T13). The investigation on the chemical consumption for acid-alkaline pre-treatments experiment on 3% TS was retained as a reference. Then, the chemical consumption for remaining 5% TS and 10% TS was evaluated from the result of 3% TS. Microscopy images were taken by Zeiss Microscope Primo Star under 400X zoom lens.

3.5.3 Biogas Potential Assay and Analytical samplings

To obtain optimum gas yield from the batch reactors of each set, batch reactors were set up in polypropylene bottles of 650 ml content shown in the figure (3.1). The head space was largely eliminated by adding 300ml of prepared substrate and 300 ml of inoculum. Batch reactors in three different solid contents, i.e. 3% TS, 5% TS and 10% TS were set up separately and compared. The Food to Microbial ratio (F/M) was set at 1:1 and anaerobic digestion was maintained under 33 ˚C (+/- 2) in thermo-control room. Digestion period (HRT=SRT) was set 3 weeks (21 days). Biogas was collected in water displacement system and the total biogas yield was measured at the end of digestion period.
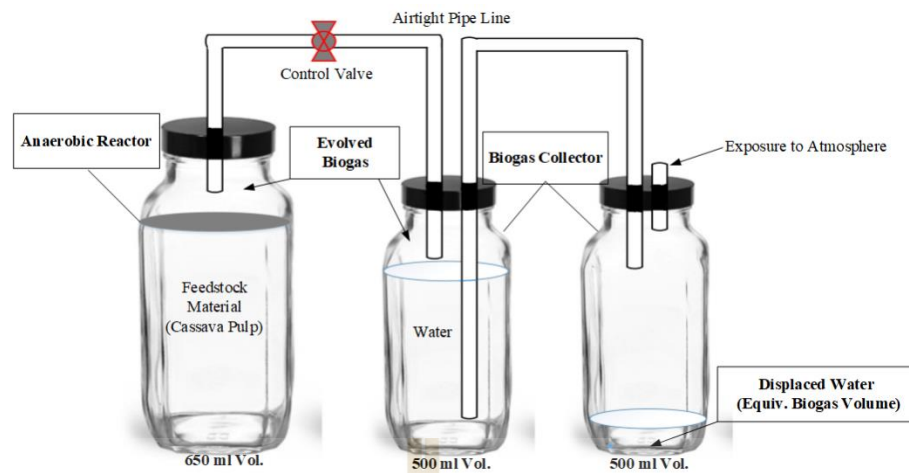
Figure 3.1 Schematic Diagram of Batch Mode Anaerobic Digestion Assay

The results of pre-treatments during hydrolysis were analysed using APHA standard methods (1995) in several key parameters, such as total dissolved solid (TDS), soluble Chemical Oxygen Demand (sCOD), Total Alkalinity (TA) and Volatile Fatty Acids (VFAs). To avoid possible interference, the variances between each sample during laboratory investigation of measured parameters were eliminated by triplicating each individual sample set. For all parameter of the experiments involved in this research, the t-test has been constructed to determine whether the difference between the average of triplicated numerical result of measured samples and the control equals. The descriptive statistics and t tests were generated in origin 2018 statistical software package. The hypothesis is as follows:

H_0:   there is no difference in term of numerical values between specific parameter and control set.

H_a: there is a difference in term of numerical values between of specific parameter and control set.

At the same time, comparison among measured samples are not likely equal to that of the control sample. Therefore, computed P-value is always less than 0.05, and the null hypothesis can be rejected.

3.6   Results and Discussion

3.6.1   Chemical Demand and Effect of Pre-treatments of on Microbial Diversity

The effect of acid-alkaline pre-treatment contributes not only to accelerate solubility of the carbon source attached to the lignocellulosic structure of cassava pulp but also to adjust back to the desired pH upon addition of the relevant acid or base. The stronger the acid or base, the more rapid the changes in pH during hydrolysis. Thermal treatment at 200 ˚C did not significantly change pH of the substrate (a mixture of cassava pulp and mill effluent) from its benchmark pH 4.3. The addition of concentrated H_2SO_4 and NaOH catalysed decomposition of lignocellulosic structure of cassava pulp. The amount of acid and alkali required is reported in the Figure 3.2.

The justification of using concentrated acid and base solution in this work is to control overall w/v of substrate in hydrolysis and rapid chemical attack to the lignin attached to lignocellulose of cassava pulp. Thereby, uniform substrate volume was maintained for digestion volume and solid regime. Based on 3% TS pre-treatment experiment, in extremely acid or alkaline treated samples (i.e. T1, T2, T12 and T13) corresponding pH 1, pH 2, pH 12, and pH 13 respectively, required higher volumes of both 36N H_2SO_4 and 20M NaOH. Alkaline demand reached up to 15.9 mL and 17.5 mL, respectively, in T1 and T13, and acid consumed up to 7.2 mL in T1 and 6.5 mL in T13. Overall, all sets of substrates required NaOH addition to adjust to

the designated pH range during hydrolysis and to neutralize back to pH 7.5, the optimum pH to trigger anaerobic digestion (Clark & Speece, 1971; Stronach et al., 1986). The accumulated acid and base pre-treatment could result in acid-base reaction and acid-base radical formation within aqueous substrate. The chemical demands in 5% TS and 10% TS required additional 10% and 30% respectively accordingly to those of 3% TS pre-treatment experiments. As consequence, the impact of chemical toxicity on microbial diversity due to acid and base radical was apparently subtle. In comparison among low and high pH adjusted samples, with the exception of T12 and T13 those samples which had been pre-treated in low pH (T1 -T4) required high volume of both acids for obtaining dedicated pH and alkaline to adjust back to neutral pH 7 (Figure 3.2). Therefore, acid pre-treatment is objectionable since even in 3%TS pre-treatment mode, the state of chemical consumption was observed very high, resulting in more possible inhibition risks in AD process.
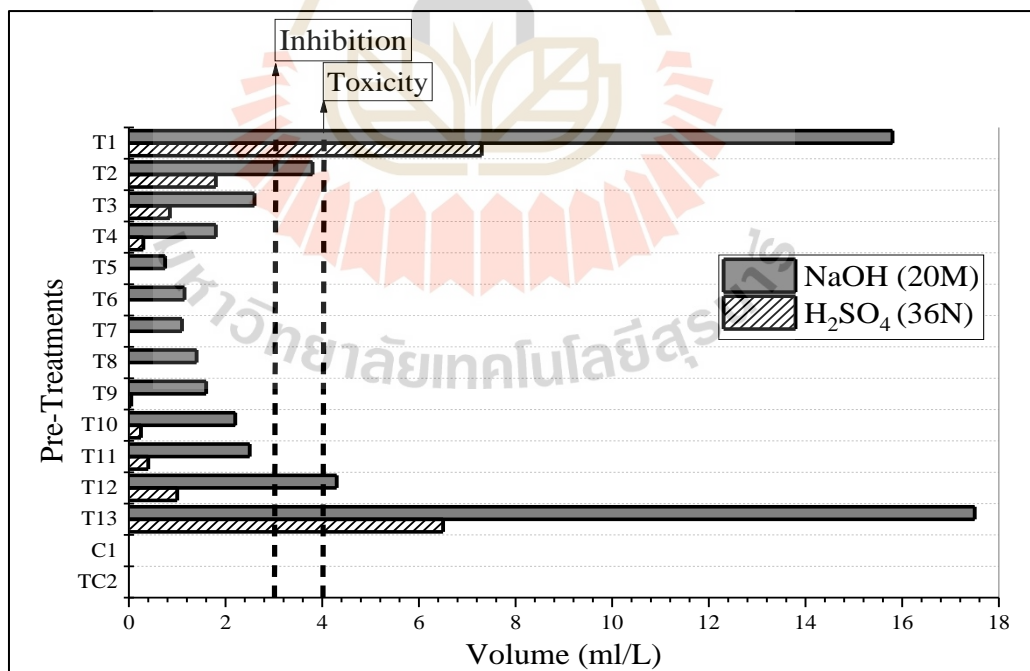
Figure 3.2 Chemicals Consumption for each Pre-treatment in 3% TS

NaOH in which Na^+ radical evolved as an inorganic salt during chemical reaction with carbohydrate from cassava pulp, they serve as a nutrient source for microorganisms in the AD process (Mrafkova et al., 2003; Ye et al., 2008). While Na^+ content in the range of 100-200 mg L^{-1} had stimulatory effects, inhibitory effect starts in the excess of more than 3,500 mg L^{-1}, and toxicity above 5,500 mg L^{-1} respectively (Ye et al., 2008). The experiment also revealed that the extreme pH treated substrates demanded substantial volumes of 36N H_2SO_4 (up to 7 ml L^{-1}). Sulphate SO_4^{2-} radical formation could result inferior digestion performance in comparison to those substrates treated with neutral ranges. This inhibition to microorganism could occur by acid and base radical, such as sodium (Na^+) and sulphate (SO_4^{2-}) ions occurs during anaerobic digestion (Choi & Rim, 1991; Chen et al., 2008, Chen et al., 2014). The effect of excess chemical toxicity is eliminated in thermal pre-treatment. However, at high temperature (above 65 ℃) and long pre-treatment duration, extermination effect takes place to diverse micro-organism which responsible for the preceding anaerobic process (Wiegel ,1992; Lowe et al., 1993).

Extreme acid-alkaline pre-treatment (T1, T2, T12, and T12) also disturbs the biodiversity of anaerobic microbes, and even intoxicates the acidophile and the alkaliphile of anaerobic extremophile alternately in course of extreme acid and alkaline pre-treatment (i.e. pH< 2 and pH>12) to maintain the ecology of anaerobic community to sustain early AD process (lowe et al., 1993; Najafpour, 2015). Visual investigation under microscope revealed that those samples pre-treated within pH 4 and pH 12 maintain cells survival (Figure 3.3). Nevertheless, thermal and extreme acid-alkaline pre-treated samples, though resulted highest lignocellulose solubility, upholds less possible bacteria diversity than those of sample C and samples treated

within pH4 and pH 12 (T4-T12). This resulted longer time for microbial growth for initiating anaerobic digestion and high food to microbial ratio (F/M) to activate bioconversion process. In addition, with low cell residence state, food over loading is highly possible, leading to digestion failure even in the initial state of anaerobic digestion processes.
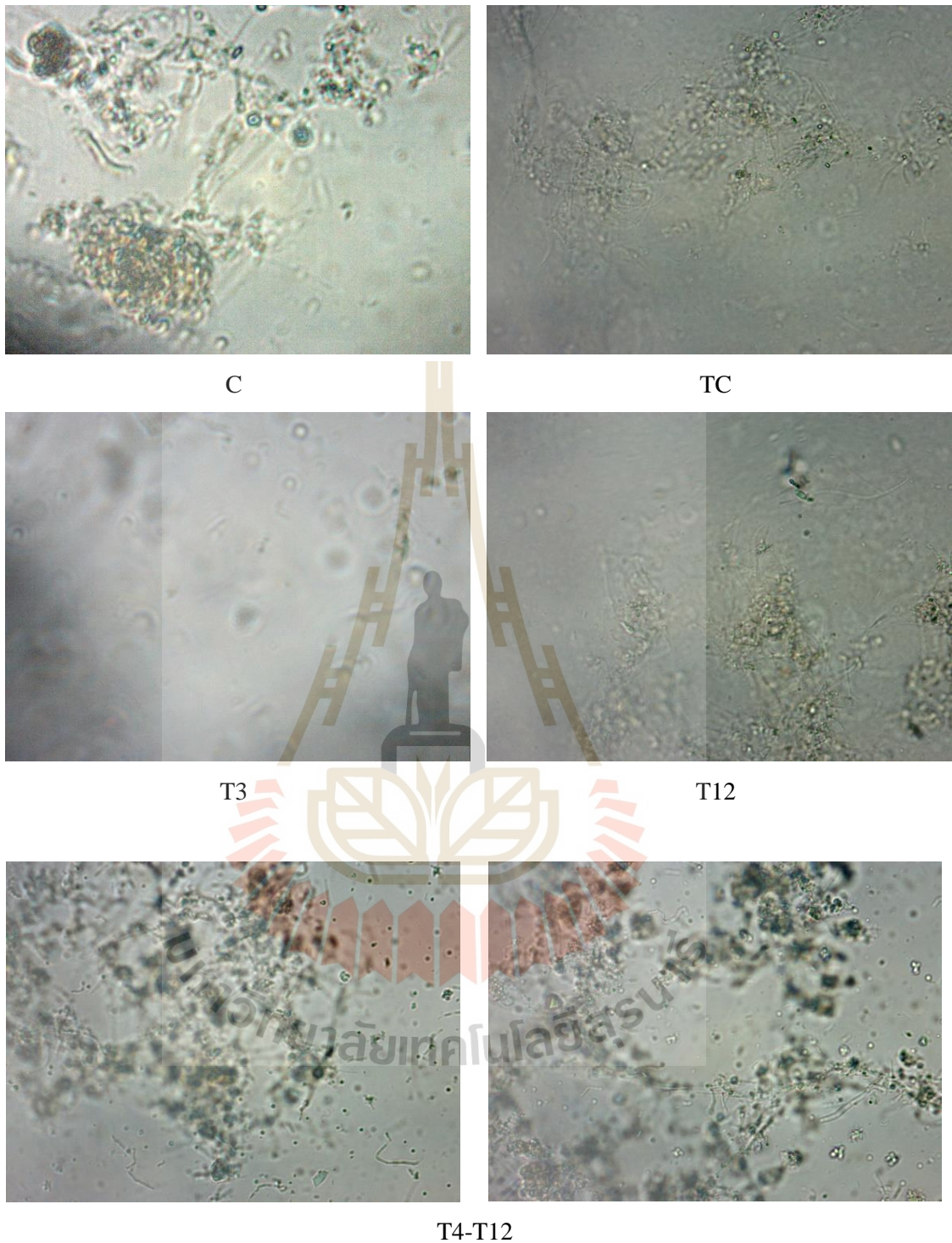
Figure 3.3 Observed Biodiversity of Microscopic Cell After Pre-Treatment

3.6.2 Effect of Pre-treatments of on Solid Degradability and Soluble Chemical Oxygen Demand

The result of solid analysis is one of indicators for the outcome of pre-treatments. As the result of the combination of sustained acid-alkaline attack on lignocellulosic material of cassava pulp and mixing action, both soluble carbon chain (cellulose) and insoluble fibre (lignin and hemicellulose) dissolves into aqueous substrate as carbon source for digestion (Sluiter et al., 2010; Evstigneev, 2011). Therefore, the Total Dissolved Solids (TDS) which is the combination of volatile Solid (VS) and volatile Suspended solids (VSS) inferred the consequence of pre-treatments. The experiments uncovered acid-alkaline pre-treatments fuelled solubility of cassava in each case with respect to the extent of chemical consumptions. Hoverer, physical transformation of Total Solids (TS) by thermal pre-treatment, TC was found having ultimate conversion capability in all case (i.e. 3%TS, 5%TS and 10%TS) than those of acid-alkaline pre-treatments. However, in term of efficiency, it decreases from 93% in 3%TS to 83% in 10%TS (Figure 3.4: a-c). The higher the solid content, the more viscosity of the substrate presents by the starchy characteristic of cassava pulp and increased saturation by heat treatment. Nonetheless, up to 4-5 times solubility potential is hopeful by thermal pre-treatment in comparison against referenced control sample. Amin et al., 2017 reviewed out of available edge-cutting pre-treatment technologies for biomass, the authors strongly recommend to adopt thermal pre-treatment for biomass which have highly lignin and hemicellulose content. However, the question over in light of feasibility and practicability remains a concern for application of thermal pre-treatment (Baruah et al., 2018; Yang et al., 2018).
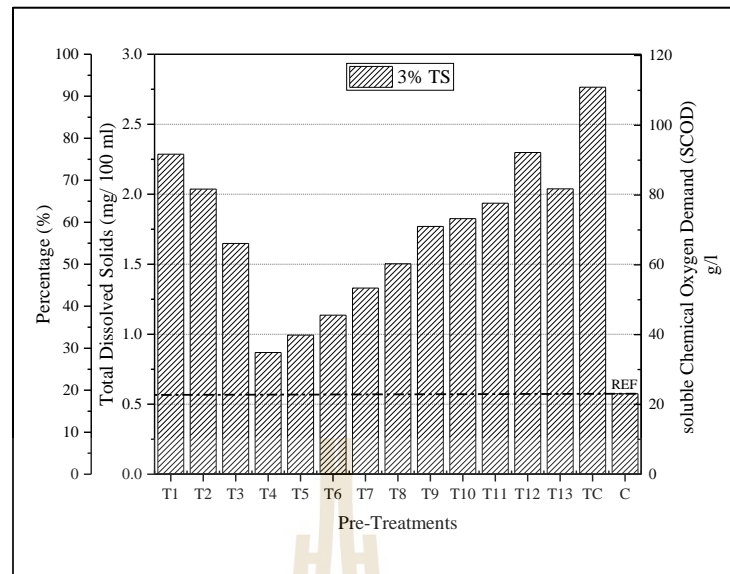
Figure 3.4a Total Dissolved Solids by 3%TS Pre-Treatments

Since initial pH of the samples before pre-treatment were between pH 4.0 and pH 4.5, the effectiveness of acid-alkaline pre-treatment among sample C and T4 were found insignificant. In the case of 3%TS pre-treatment experiment, the results from figure (3.4a) indicate higher TDS was converted as increased in chemical consumption. Extreme pH pre-treated sample sets (T1, T2, T11, T12, and T13) generated up to 60% more transformation potential (2.2 mg/100ml) than control sample (C). Though more TDS formation achieved, these samples have a tendency of triggering inhibition for microorganisms during AD processes. Thus, sample sets from T5 to T9 are more favourable with TDS formation from 35-60 % than control set since these samples lack cross-chemicals contamination stresses of concentrated H_2SO_4 and NaOH. These samples need no acid intervention even after pre-treatment process undertaken since their pH restore to neutral range because of hereditary acidity of cassava wastewater having pH 4.0 – pH 4.5 and cassava pulp having pH 5.0 to pH 5.5 which in combination deters pH level of samples in sustaining in alkaline

range. But in the case of T10, T11, T12 and T13, the acidity of cassava pulp and wastewater exhausted by high alkaline addition.

The development of soluble chemical oxygen demand (sCOD) in parallel to that of Total Dissolved Solids (TDS) as in the figure (3.4a). sCOD is the derivative of total organic carbon of cassava pulp comprised of COD and sCOD among which sCOD is predominantly assessible by microorganism as food source for anaerobic digestion (Orhon & Çokgör, 1997; Liu et al., 2016). Lignin fragment remains as Suspended Solid (SS) which retains further COD. The heat pre-treatment generated highest sCOD of more than 110 g/l sCOD because of thermal solubility of cassava pulp in high temperature (Figure 3.4a). With the exception of substrates treated to T13, the remaining sample sets produced sCOD ranging between 60 to 90 g/l sCOD as increased in chemicals consumption accordingly. It can be concluded that highly acid subjected substrate created higher sCOD in comparison against those alkaline treated samples. In addition, the formation of sCOD varied inversely proportional to the TSS and VSS since complex polymer compounds of cassava pulp were dissolved during hydrolysis. sCOD production increased as increased in total solid contents in the raw substrates (Figure 3.4b and 3.4c). Among the different total solid comparative investigations, it was observed that optimum solubility of cassava was found 5-7.5 % TDS under 10% TS regime (Figure 3.4c) and sCOD formation potential will elevate in accordance to percent increase in TDS and TS.
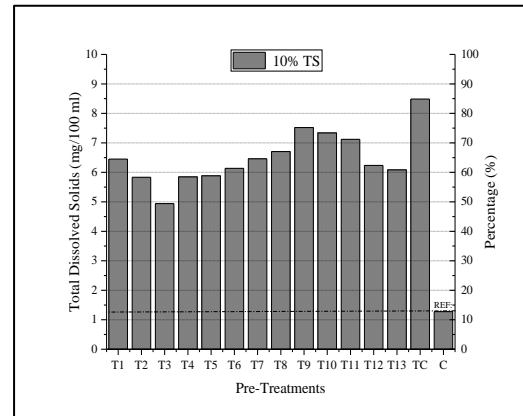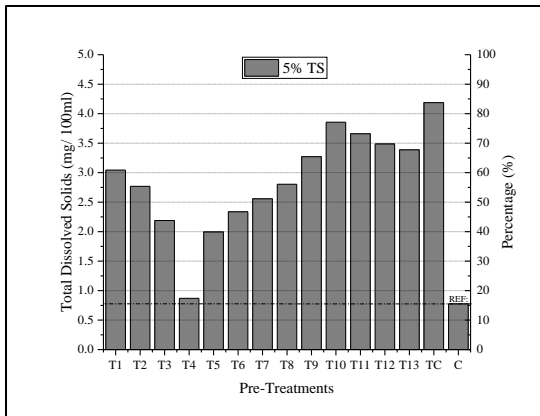
Figure 3.4b Total Dissolved Solids by 5%TS   **Figure 3.4c** Total Dissolved Solids

by 10%TS

Alternately, applicable pre-treatments range T8-T11 in 3% TS case, the sCOD was from 60-80 g/L. From 3%TS figures, the sCOD for 5%TS and 10%TS could be integrated to maximum 166% to 333% sCOD formation potential (i.e. 100 g/l to 200 g/l sCOD). Therefore, total solid contents of more than 5% are not recommended and the excess to this content can result in inhibition effects by over feeding to microorganisms, and consequently disrupting biogas fermentation processes. In addition, since the physicochemical properties of cassava pulp are highly enriched with starchy carbohydrate (Gunorubon, 2012), therefore upon being subjected to thermal pre-treatment, sCOD and TDS in all cases were found significantly high. Nevertheless, the pre-treatment experiments revealed that sCOD and TDS which deemed as common food source anaerobic digestion could be enhanced by either acid-alkaline and thermal pre-treatment than control substrate. This enable saving raw material, cassava pulp, requirement in optimizing biogas production under specific resources.

3.6.3   Effect on Volatile Fatty Acids and Total Alkalinity

The status of Volatile Fatty Acids (VFAs) and Total Alkalinity (TA) upon treatments were neutralized at pH 7 after acid-alkaline and thermal pre-treatments is showed in the figure (3.5). They are the initial values of VFAs and TA prior to execute AD process in batch mode. Although the syntrophic action of hydrolytic, acidogenic or fermentative bacteria of anaerobic groups generate volatile fatty acids as their synthesized products (Bitton, 1994, Gerardi, 2003), this group of bacteria was not active in pre-treatment stage since extreme acidic, alkaline, and thermal treatment led to the cassava feedstock inhospitable environment. The VFAs found in treatments was associated with residual VFA present in fresh cassava pulp and wastewater. Hence, control sample's VFAs remained very high at 1150 mg/l. Acid pre-treated samples has higher VFAs (up to 1500 mg/L) and it depleted to as low as below 100 mg/l as increase pH by alkali addition. This is due to the fact that saponification by adding NaOH that reacts with acids in the substrate resulting in diminishing acidity present in the samples (Goss & Petrucci, 2007). It was also observed that high heat pre-treatment (TC) resulted in VFAs exhaustion.
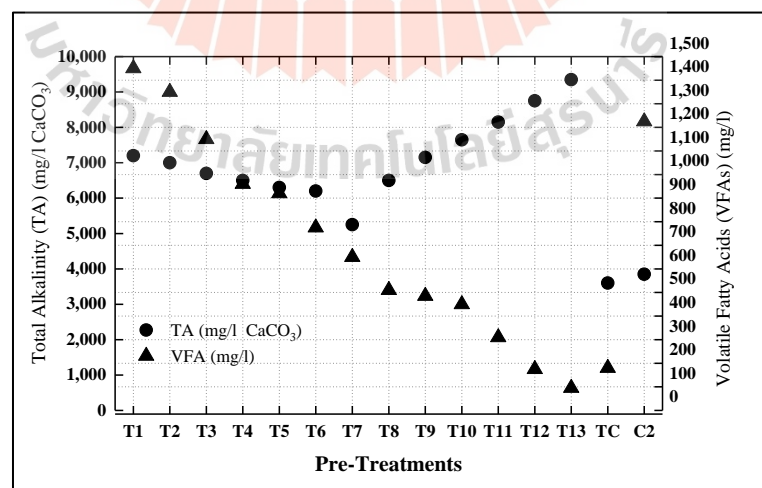
Figure 3.5 VFAs and TA by Pre-Treatments

In contrast, the condition of total alkalinity found elevated in all treatments. Since all the samples were neutralized back to pH 7, with the exception of extreme acid-alkaline pre-treatment, total alkalinity of all samples was quite identical ranging between 6000-7000 mg/l as $CaCO_3$. Although the optimum alkalinity for the success of reactor during anaerobic digestion being $2500 - 5000$ mg/L as $CaCO_3$, the observed alkalinity of pre-treated substrates was significantly higher than optimum range (Graef & Andrews, 1974). However, this excess amount helps in buffering and reserving for pH drop when fermentation progresses during acidogenesis stage. The VFA/TA ratio ranging between $0.1 - 0.4$ which is ideal range for healthy biogas fermentation, (Buswell, & Mueller, 1952), and all pre-treated substrates were to be verified by AD performance. Nevertheless, this acid-alkaline pre-treatment revealed the cut-off treatment ranges that is either objectionable or inhibiting for anaerobic digestion.

3.6.4 Effect of Pre-treatment in Biogas Production by Different Solid Contents

The total biogas yields were calculated at the end of digestion periods of 21 days. Three different solid ratios such as 3% TS, 5% TS and 10% TS were investigated (Figure 3.6). The results indicated that the batch reactors in which substrates were subjected to both extreme acid and alkaline, i.e. T1, T12, and T13 pre-treated batch reactors produced less gas yield or completely failure in some cases more than those were subjected moderately (Figure 3.2 and 3.6). This was due to the fact that they encountered excessive inhibition of chemicals on microorganisms triggered by sodium and sulphate from alkaline and acid. Upon the digestion period ended, their biogas yields were found below 200 ml/kg VSS. The batch reactors in

which substrates were treated from pH 7- pH 10 proved better biogas fermentation and yielded more than 3 folds of those treated with extreme pH. Substrates which had been treated to pH 9 (T9) and pH 10 (T10) have highest biogas yield of 1550 and 2845 ml/kg VSS for 3% TS and 5% TS respectively. Maximum biogas yield for 10% TS was found in T9 which is pH 9 treated samples at 700 ml/kg VSS. The results provided withthe evidence that solid content of 5% provided best performance under acid-alkaline (H_2SO_4 and NaOH), and thermal pre-treated cassava pulp with 4125.5 ml/kg TS biogas yield under digestion period of 21 days in batch reactors.

Taking as a whole, the experiments uncovered that acid-alkaline pre-treatment in 5% TS resulted in better digestion performance than its counterparts. Nevertheless, thermally pre-treatment in 3%TS has as good bio-conversion capability as that of 5% TS acid-alkaline pre-treatment in neutral pH range. Highly solid degradability and sCOD formation is believed to be responsible for it. Since this experiment applied batch mode AD digestion, the condition biochemical conversion and its kinetic during AD process could not be investigated. However, optimum ranges for each solid regime (i.e. T8, T9, and T10) for acid-alkaline pre-treatment for ultimate biogas production was discovered. In comparison against control reactor, these optimum ranges were also proved enhancing biogas yield in a given cassava pulp input. Though, thermal pre-treatment in 3% TS could generate higher biogas yield than that of optimum acid-alkaline pre-treatments, energy consumption for heating remains as deterrent in terms of feasibility and practicability for commercial level.
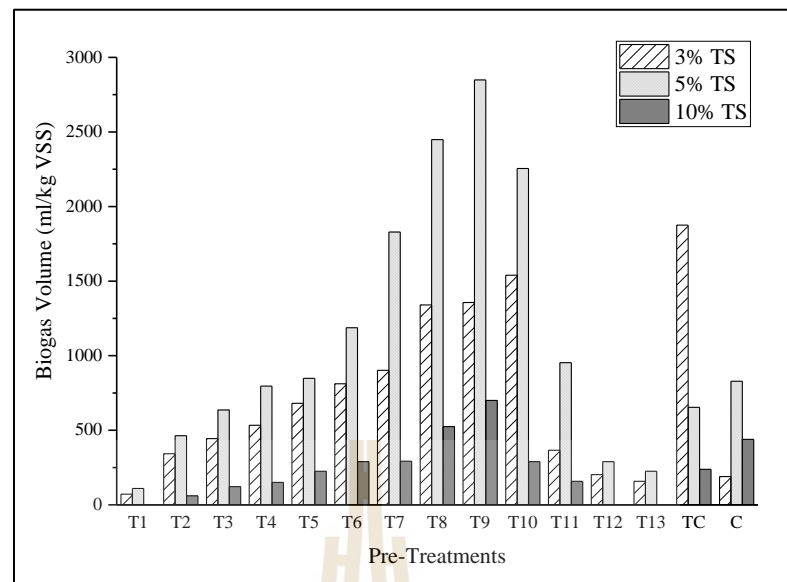
Figure 3.6 Reactor Performance by Pre-treatments in Different Solid Content

3.7   Conclusion

Anaerobic digestion is an effective mean to manage mounting agro-industrial waste in factories, the lack of research and development tailored to individual biomass hinders biogas developers to generate environmentally friendly and reliable bioenergy from cash crop residues. This study proved there is high potential to produce higher biogas yield than conventional practice by using either/both acid-alkaline or thermal pre-treatments prior to execute biogas fermentation. Having highly acidity in cassava pulp and wastewater, after hydrolysis in slightly alkaline pH range, the result provides with the evidence that alkaline pre-treatment is recommended for the objective of optimizing biogas yield by increasing solid degradability of lignocellulosic cassava pulp for high sCOD formation. In addition, even though highly acidic pre-treated substrates produced higher sCOD, SO_4^{2-} and Na^+ toxicity was observed upon undergoing AD process and obtained lesser biogas yield than those substrates treated

to pH 9 (T9) and pH 10 (T10). Therefore, this study uncovered compromised volume of acid-alkaline addition while selecting of H_2SO_4 and NaOH as common chemical pre-treatment to cassava pulp in different solid contents versus the ranges of pH to extract optimum biogas volume. Although plenty of literature had proved acid and alkaline make better disintegration of lignocellulosic properties of biomass, in the case of cassava pulp in the studies, combination of both moderately higher than neutral pH by alkaline-acid pre-treatment, thermal pre-treatments for 45 min at 200°C, and 5% TS produced best outcome from batch mode of AD fermentation.

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CHATPER 4

EFFECT OF DIFFERENT ALKALINE PRE-TREATMENT ON CASSAVA PULP FOR OPTIMUM BIOGAS PRODUCTION

4.1   Graphical Abstract
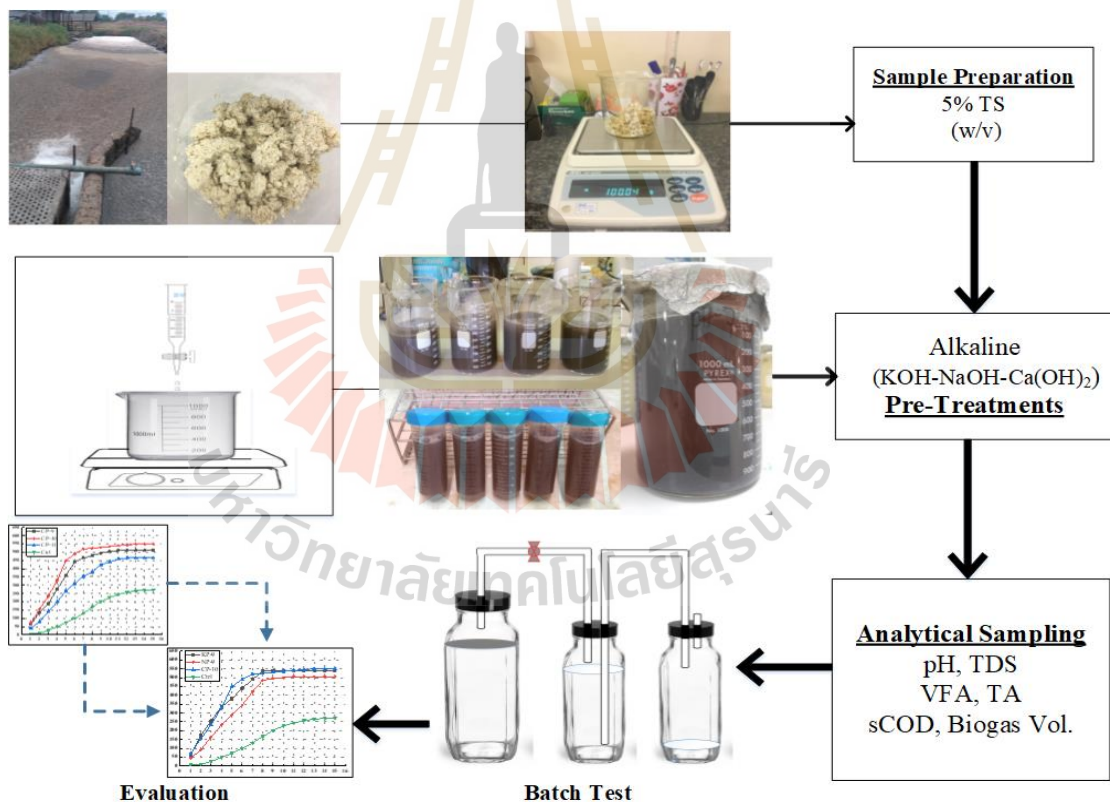
4.2 Highlights

- While NaOH and Ca (OH)$_2$ were found very identical biomass conversion capability, KOH was found having best TDS generation under same condition.

- sCOD productions were increased from 24.5% to 37.6%, from 22.8% to 32.8%, and from 21.4% to 30.7% for KOH, NaOH, and (Ca(OH)$_2$) pre-treatment respectively.

- Highly alkaline pre-treatment is objectionable since high VFA/TA gap against ideal range could result in delayed digestion process, and in extreme case digestion failure is envisaged.

- The total gas yields of all Ca (OH)$_2$ for CP-10 at the end of digestion with highest gas yield in all Ca (OH)$_2$ pre-treatments than its counterparts.

- With alkaline pre-treatment, gas yield could be improved 14% − 103.7 % than untreated substrate.

4.3 Abstract

Produced in vast quantity as one of by-product from cassava starch processing chains, cassava pulp has great potential for energy recovery by harnessing biogas through Anaerobic Digestion (AD). This study aims to enhance biogas production by comparative alkaline pre-treatment investigation in batch mode digestion. 5%TS w/v of cassava pulp mixed with mill effluent were pre-treated with 20 molar potassium hydroxide (KOH), sodium hydroxide (NaOH), and calcium hydroxide (Ca (OH)$_2$) solution for 6 hours contact time. Effects of different alkaline pre-treatment on cassava substrate were assessed in Total Dissolved Solid (TDS), soluble Chemical Oxygen Demand (sCOD), Volatile Fatty Acids (VFA) to Total Alkalinity ratio

(VFA/TA), and gas yield over 15 days digestion period. Daily accumulated biogas yield was taken as final indicator of the effect of different pre-treatment. KOH pre-treatment in pH 11 resulted highest Total Dissolved Solid (TDS) 4.5 mg/ 100 ml (90%), and improved soluble Chemical Oxygen Demand (sCOD) formation up to 37.61% (3.6 g/L) than control substrate. The experiment revealed peak biogas production by KOH pre-treated substrate was found from day 6 - 9 after digestion executed, and achieved 540 ml. The finding proves that different pre-treatment methods applicable to cassava pulp, alkaline pre-treatment could realistically increase biogas production. Biogas production increased up to 100%, 86%, and 103.7% using KOH, Ca(OH)$_2$ and NaOH respectively. However, when future provision to the technology for AD system and design is concerned, the choice of highly reactive alkali could lead to complication in the system. Among different alkaline pre-treatments with pH 9 to pH 10 in 3 different ranges, pre-treatment by Ca(OH)$_2$ at pH 10 was found ideal and compatible to exiting starch manufacturing chains and subsequent waste management.

4.4   Introduction

Climate change and global warming link to detrimental impacts of the massive fossil fuel consumption across the globe. This has to be reconsidered to avoid mounting environmental problems and dwindling resources. Therefore, it is mandatory to tap environmentally friendly energy to lessen dependence on fossil energy (Alfstad, 2008). Renewable energy is one of the substitutions in the energy transformation. In comparison to its counterparts, biomass gains renewed interest because of its inherent advantages in large scale renewability, availability, storage

capability, and adaptable to produce different bioenergy from diverse biomasses (Bain, 2007). Biogas or bioethanol could be produced from agro-industrial by-products generated from production chains. Based on market demand and the dimension of agricultural sector, every country has unique potential to produce biomass as feedstock material for bioenergy production. Cassava or tapioca related foodstuff is one of the top agriculture products of Thailand as well as in the region. Thai Tapioca Starch (TTSA) reported the cassava production reached more than 33.94 million tons in 2016, and the figure is increasing. As of Thailand's Alternative Energy Development Plan (AEDP) 2015 which is to foster cutting greenhouse gases (GHGs) emission and boost carbon credit rating, out of biogas energy potential of 657 MW, it was targeted to increase from current figure of 312.95 MW to 600 MW by 2036 (AEDP 2015 Report). Though the target is achievable, technological challenges remain as major hindrance that the AEDP goal is to be met (Sriroth et al., 2000).

Biogas technology in its generic term is the anaerobic digestion (AD) process in which any digestible biomass is subjected for conversion into biogas from fermentation through symbiotic action of bacteria under strictly anoxic condition along four common stages (i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis) (Price & Cheremisinoff, 1981; Marchaim, 1992). The process of biogas could be divided into three divisions which are pre-digestion, digestion and post-digestion. While pre-digestion phase is related to processing feedstock material and digestion phase is associated with environmental variables and digester configuration, post-digestion is linked to proliferation of raw biogas into highly energy calorific values (Achinas et al., 2017). The advantages of pre-treatment to biomass prior to initiate AD process improve biodegradability of cellulosic material of biomass producing

fermentable sugars, amino acids and volatile fatty acids as the products of hydrolysis stage (Mosier et al., 20056; Zheng et al., 2014). Pre-treatment offers more food source from specific loading for hydrolytic bacteria and subsequently all microorganisms within AD system as a whole for more biogas yield.

Kim & Holtzapple (2005) reported that during fermentable sugar generation under calcium hydroxide pre-treatment for 0.5 g $Ca(OH)_2$ for 4 weeks contact time with up to 55˚ C temperature to corn stover biomass, the overall yield of glucose and xylose were 91.3 and 51.8 at 15 FPU/g cellulose. Similarly, Chang et al., (2001) recommended that $Ca(OH)_2$ of 0.1 g/dry mass produced effective digestibility and better reducing sugar yield and more lignin solubility. Likewise, calcium hydroxide, Sodium hydroxide has been widely used in different biomass pre-treatments. By using NaOH up to 5 w/v, it was reported that total reducing sugar was produced 350mg/g in 20.5% NaOH with 120 ˚ C in bioethanol synthesis by kans grass (Komolwanich et al., 2014). On contrary, pre-treatment with NaOH alone and even with variable concentration, mixing rate and temperature resulted in low delignification levels of only 5-21% and low saccharification yield of 3-8% in bioethanol production studies (Sun & Cheng, 2002). In a comparative investigation among potassium hydroxide (KOH) and calcium hydroxide $(Ca(OH)_2)$ of corn stover for bio-gasification study, under the same bio-methane potential productivity, the dosage of KOH could be reduced up to 4 folds. By using one particular KOH, with different temperature and contact time settings, Sharma et al., (2013) proved that carbohydrate conversion has been achieved 91.8% in switchgrass for sugar generation and subsequent biofuel production. In general, alkaline pre-treatment has been extensively investigated to biomass which are difficult to biodegrade under bioconversion process. But with

regard to the research related to cassava tubers and its related residues was not much investigated as its counterparts.

This study fills this gap highlighting optimization of biogas yield from comparative alkaline pre-treatment in the absence of thermal effect to cassava pulp in batch rectors by three commercially available alkali namely caustic potash (KOH), caustic soda (NaOH), and slaked lime (Ca(OH)$_2$). The results of biogas yield from different alkaline pre-treated substrates were compared against alkali free control sets. Demonstrated in previous chapter (Chapter 3), the acid pre-treatment is objectionable for anaerobic digestion process. This study aims to achieve ultimate biogas yield of alkaline pre-treatment to cassava pulp for biogas plants in cassava industry and promotes the capability of enhancing biogas generation than conventional practice applied in biogas plant in the region.

4.5   Materials and Methods

4.5.1   Substrate, wastewater and Inoculum Characteristics

Fresh cassava pulp as common substrate was collected from Korat Starch Factory located in Nakhon Ratchasima province in north-eastern Thailand. Upon proximate analysis, fresh cassava pulp of all variety was found consisting majority in carbohydrate and crude fibre as in the table 1. Wastewater from the starch mill was chosen for hydrolysis and stored under 4˚C in cold storage until used. Hydrolysing for 15 mins was carried out prior to alkaline pre-treatment to ensure homogeneity of the bulk cassava pulp and wastewater.

Table 4.1 Nutritional Composition of Cassava Pulp (Pinheiro et al., 2018)

Nutritional Content	Composition (%)
Carbohydrate	70-72
Crude Fibre	18-20
Crude Ash	3-5
Crude Fat	2-3
Protein	1-2
Moisture	3-5

Table 4.2 Characteristic of Wastewater and Inoculum

Parameter	Unit	Wastewater	Inoculum
pH	-	4.2 ± 0.2	7.8 ± 0.2
Total Solid (TS)	% (w/v)	2.5 ± 0.5	8.0 ± 0.5
Volatile Solid (VS)	g VS /L	0.8 ± 0.2	2.85 ± 0.2
Total COD	g/L	21.5 ± 5	76.5 ± 5
Soluble sCOD	g/L	6.0 ± 5	27.5 ± 5
VFA *(observed)*	mg/L	180 ± 50	1250 ± 50

Triplicate analysis was carried out to each parameter.

APHA standard methods (1995) were applied to analyse substrate characteristics. Laboratory samplings were done prior to start anaerobic digestion in batch reactors. Characteristic of substrates and inoculum were shown in the Table4.2. Inoculum was taken from covered lagoon type biogas plant operated in parent cassava starch factory, acclimatized at 35 ˚C up to 3 hours, and executed AD process within 6 hours of collection time.

4.5.2 Alkaline Pre-treatment Experiments

Cassava pulp substrates were made in 5% TS (w/v) by mixing solid cassava pulp with 1000 ml effluent wastewater from starch mill. 20 M chemical

solution of each potassium hydroxide (KOH), sodium hydroxide (NaOH), and calcium hydroxide (Ca(OH)$_2$) were prepared for alkaline pre-treatment and used to adjust desired pH ranging 9-11denoting KP, NP and CP for each chemical respectively. To obtain the ultimate contact time, prepared samples were left stirring for alkali attack to lignocellulosic material of cassava pulp for 6 hrs. Finally, hydrolysed samples were neutralized back to pH value of 7.5 for digestion with concentrated hydrochloric acid (HCL). NaHCO$_3$ was added for buffer agent for maintaining digestion pH from 7.0 – 7.2. Control sample which was not subjected to chemical pre-treatment was prepared in its original condition with same hydrolysis contact time for 6 hrs, and was compared against all other comparative alkaline pre-treated sample sets.

4.5.3 Analytical Method, Biogas Potential Assay and Statistical Analysis

The results of pre-treatments during hydrolysis were analysed by using APHA standard methods (1995) in several key parameters, such as total dissolved solid (TDS), soluble Chemical Oxygen Demand (sCOD), total alkalinity (TA), and Volatile Fatty Acids (VFAs). To obtain optimum gas yield from the batch reactors of each set, batch reactors were set up in glass bottle of 650 ml content. The head space was largely eliminated by adding 300ml of prepared substrate and 300 ml of inoculum (Figure 3.1). The inoculum to substrate ratio (F/M) was set at 1 and anaerobic digestion was maintained under 33 ˚C (+/- 2) in thermo-control room. Digestion period (HRT=SRT) was set 15 days. Mixing was conducted in 150 rpm for 2 min/12 hours. Biogas was collected in water displacement system and the total biogas yield was measured on daily basic before mixing to investigate accumulated biogas production rate and end of digestion when food source exhausted. To avoid possible interference, the variances between each sample during laboratory investigation of control parameters were eliminated by triplicating each individual sample set.

For all parameter of the experiments involved in this research, the t-test has been constructed to determine whether the difference between the average of triplicated numerical result of measured samples and the control equals. The descriptive statistics and t tests were generated in origin 2018 statistical software package. The hypothesis is as follows:

H0:   there is no difference in term of numerical values between specific parameter and control set.

Ha: there is a difference in term of numerical values between of specific parameter and control set.

At the same time, comparison among measured samples are not likely equal to that of the control sample. Therefore, computed P-value is always less than 0.05, and the null hypothesis can be rejected.

4.6   Results and Discussion

4.6.1   Effect of Pre-treatment on Biomass Solubility

Complex insoluble organic matters, polymers are converted into soluble organic compounds, monomers with the help of hydrolytic bacteria during hydrolysis (Gunaseelan, 1997; Mudhoo, 2012). In term of solids analysis, among dissolved solid and suspended solid, only dissolved solids is accessible by bacteria for bioconversion processes (Khalid et al., 2011; Montag & Schink, 2016). Therefore, the total dissolved solids (TDS) represents digestible food source generated from Total Solid (TS). As a result of pre-treatment, the higher formation of TDS indicates higher potential of foods source for microorganism (Kumar, & Wyman, 2013). The results from the experiments revealed that KOH pre-treated substrates produce slightly higher TDS yield. KP-11 was found producing highest dissolved soluble solid at 4.5 mg in 100 ml substrate, while NaOH and $Ca(OH)_2$ pre-treated substrates' TDS were ranging between 3.5 to 4.0 mg/ 100ml regardless of pH values. In comparison to control sample (Ctrl), due to the alkaline pre-treatment, TDS could be

enhanced from 200% to 300%. In term of comparative performance among three alkalis, while NaOH and Ca(OH)$_2$ were found very identical biomass conversion capability, KOH was found having best TDS generation under same condition (Figure 4.1).
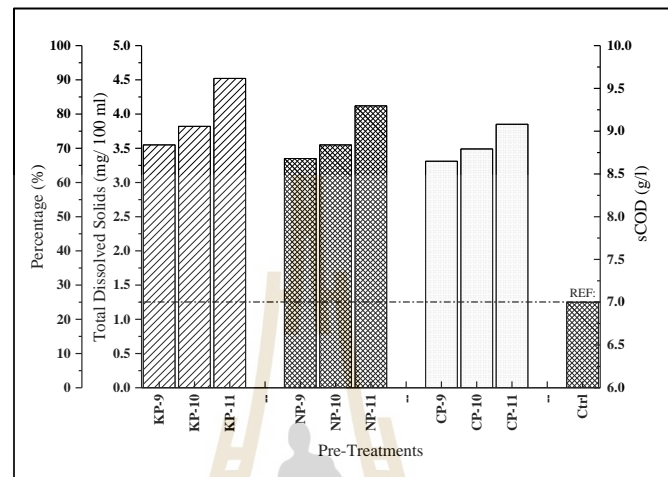
Figure 4.1 Solid Degradability by Effect of 3 Alkaline Pre-treatments in 5%TS

Although higher pH of each alkaline solution resulted in better TDS formation, presumptive experiments indicated that pH value higher than 11 required more alkaline concentration, and this surpasses inhibition threshold for microorganism to execute further biogas conversion process (Price, & Cheremisinoff, 1981; Kumar et al., 2009). Therefore, separation of digestion face and hydrolysis phase for pre-treatment versus remaining AD phases is highly recommended in order to avoid inhibition caused by highly alkaline condition (Koyama et al., 2017). In addition, previous study in Chapter 3 also implied pre-treatment in extreme pH demanded high volume of chemicals (both acid and alkali) for neutralizing back to pH 7, thereby acid-alkali complication occurred leading to the digestion failure.

Alternately, soluble Chemical oxygen demand (sCOD), which is common food source for microorganism during AD process is another indicator for effect of alkaline pre-treatment (Behera et al., 2014; Kim et al., 2016). The original pH of substrate which is the mixture of cassava pulp and mill effluent was found at approximately pH 4.3- pH 4.5. The benchmark sCOD of the substrates which is control (Ctrl) samples was found 7.0 g/l after 5% TS addition to wastewater which as 6.0 g/L sCOD (Table 4.2). Likewise, TDS, the experiment discovered that KOH pre-treated substrates generated more sCOD within the same pH of other alkaline pre-treatment. sCOD formation in substrates were found directly proportional to those of increased alkaline pre-treatment in all cases. In comparison to control set (Ctrl), sCOD productions were increased from 24.5% to 37.6%, from 22.8% to 32.8%, and from 21.4% to 30.7% for KOH, NaOH, and $(Ca(OH)_2)$ pre-treatment respectively. COD in insoluble form is also considered as available food source for microorganism in AD processes, therefore, total Chemical Oxygen Demand (tCOD) in which insoluble organic carbon can also behave as food source for certain type of bacteria to during fermentation or acidogenesis process (Kim et al., 2003). Nevertheless, the possibility of enhancing sCOD by alkaline pre-treatment by this experiment paves the way that food provision is improved for more gas yield within specific feedstock material sources by manageable alkalis present in the substrate and negligible further acids needed in neutralizing back to pH 7 (figure 3.2).

4.5.2 Influence of Pre-treatments on Common Intermediary Products of AD Process

Major intermediary products, Volatile Fatty Acids (VFAs) evolves as a result of hydrolysis by hydrolytic and acidogenic bacteria in course of fermentation

(Wang et al., 1999; Kim et al., 2018). From the results of pre-treatment, higher pH ended up in lower VFAs values. Controlled sample (Ctrl) was found having highest VFAs values at 1100 mg/l since the condition of VFAs present in original alkali exposed cassava substrates has high VFA by active fermentation. Contrary to VFAs, total alkalinity increased as in the increased in pH values because of the alkaline addition, pH 11 of KOH, NaOH and $Ca(OH)_2$ treated substrates were found reaching TA values of 6150, 7000 and 3750 mg/L equivalent to $CaCO_3$. As a result of VFA/TA of the pre-treatment, while Control substrate's VFA/TA was found between 0.05 – 0.23, those substrates which have been treated to NaOH alkaline solution were found having VFA/TA under 0.1. this figure is far too low than optimum VFA/TA 0.44 (Chen et al., 2008). Therebefore, low VFA/TA ratio in respective alkaline pre–treatments could encounter retarded methane synthesis by later phase of AD process (i.e. acetogenesis and methanogenesis).

The ideal range of VFA/TA for AD process 0.15 – 0.3 has been suggested in literature (Li et al., 2014; Li et al., 2018; Masebinu et al., 2018). VFA/TA of some KOH and all NaOH pre-treated substrates were observed below 0.15. And, the remaining KP-9 and all $Ca(OH)_2$ pre-treated substrates were found within the range of 0.15 to 0.3 (Fig. 3). Despite the fact too much higher or lower VFA/TA represents substrates that are more likely to digestion failure, the range within 0.1 – 1.5 falls under manageable level by mean of adopting suitable mode of digestion (Li et al., 2018). Therefore, $Ca(OH)_2$ is more favourable for sustainability for AD processes. Massive VFA exhaustion in KOH and NaOH pre-treated samples in comparison against control set were attributed to molar mass and molecular weight of each chemicals. With $Ca(OH)_2$ has 74.09 g/mol and larger atomic size than those of

KOH and NaOH (i.e. (56.1056 g/mol and 39.997 g/mol), the latter alkalis readily react with acetic acids (CH_3COOH) present in feedstock materials. Furthermore, the application of slake lime or calcium hydroxide ($Ca(OH)_2$) during the cassava starch production particularly in juice extraction and clarification process also contributes the existence of residual $Ca(OH)_2$ in cassava wastewater which in combination save chemical consumption in alkaline pre-treatment (Breuninger et al., 2009). This could also help in avoiding inter-alkali toxicity or excess salinity to AD microorganism (Kaster et al., 2011; Ogata et al., 2016). In this regard, concentrated H_2SO_4 was also avoided for minimal neutralizing in pH 10 and pH 11 alkaline pre-treated substrates since sulphur was applied in starch extraction process (Sriroth et al., 2000). Hence, the complication triggered by residual sulphur in cassava wastewater was eliminated. This study employed batch mode which is totally enclosed chamber, the resultant kinetic of VFA/TA at subsequent AD process could not be investigated. Therefore, for precautionary measure, highly alkaline pre-treatment is objectionable since high VFA/TA gap against ideal range could result delayed digestion process and in extreme case digestion failure is envisaged for high alkalinity present in the substrate. Evaluation could only be made through daily accumulative gas yield and digester performance at the end of the digestion period.

This comparative alkaline pre-treatment of biomass investigation revealed effect of alkaline pre-treatment reduced VFAs formation as increased in pH of respective alkaline solution. The rate of reduction was weak in $Ca(OH)_2$ treated sample while those of KOH and NaOH treated substrates were found more vigorous. However, in comparison against control sample, VFA depletion found stronger in pH increase and according to reactivity series of metal. This study contrasts Fang et. al.,

(2019)'s work in which NaOH prolong pre-treated of rise husk resulted 72.9% increase in TVFAs generation. Likewise, 37% VFA improvement was achieved by similar study by using sugarcane filter cake (Janke et al., 2016). In both cases, VFAs were measured at the mid of AD process of either semi or full continuous reactor, whereas in this study because of batch process, measurement could not be done in the intermediate AD process along digestion period. However, biogas yield improvement in latter discussion is the evidence of VFAs were improved by the effect of comparative alkaline pre-treatment.
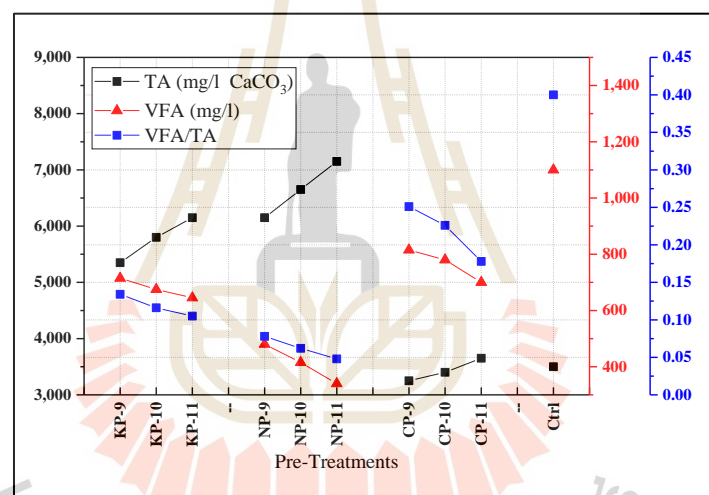
Figure 4.2 Initial Volatile Fatty Acids (VFAs) and Total Alkalinity (TA) in Different Pre-treatments

4.5.6 Effects of Alkaline on Pre-treatment Biogas Yields and Production Rate

Based on AD digestion under equal Food to Microbial ratio (F/M) in batch mode, the results indicated that the performance of reactor in which KOH pre-treated substrates produced, under the same pH range, slightly higher biogas volume

in comparison to all other alkaline pre-treated counterparts. While biogas production was found inactive after day 11 of digestion period, the peak biogas production rate was found from day 2 to day 6 in all KOH pre-treated substrates (Fig. 4a). Maximum accumulated gas yield for KOH pre-treated sample was found for KP-9 at 546 ml and gas yield decreased as increased in pH (Kp-10 and Kp-11). This is due to fact that available food source exhausted for AD microorganism and K^+ salinity as increased in pH. In the case of NaOH Pre-treatment, the highest rate of biogas production occurred at day 7 and day 8 in NaOH pre-treated substrate. The volume of biogas produced in NaOH pre-treated substrates were also found lower as increased in pH. While NP-9 which the substrate pre-treated with NaOH in pH 9 produced 513 ml at the end of digestion period, NP-10 and NP-11 produced 385 ml and 310 ml respectively (Figure 4b). In contrast to KOH and NaOH pre-treatment, the biogas production rate of $Ca(OH)_2$ were found stable throughout digestion period with approximately about 65 ml per day and the total gas yields of all $Ca(OH)_2$ for CP-10 at the end of digestion with the highest gas yield in all $Ca(OH)_2$ pre-treatments than its counterparts (Figure 4c). Food source exhaustion occurred within 7 days after AD process executed. The experiments revealed that due to the alkaline pre-treatment, biogas yield could be increased 35% to 100% in KOH pre-treatment, 14% to 86% in NaOH pre-treatment, and 72% to 103.7% in Ca(OH)2 pre-treatment (Figure 4d). Thomas et. al., (2018) reported 67-227% increase in methane production under co-digestion with cattle manure in lime (Ca(OH)2 pre-treatment. Even in the case of rice straw which has high lignin percentage, pre-treated with Ca(OH)2 to rice straw, more than 30% methane was enhanced in short duration pre-treatment (Gu et al., 2015). Under mono-digestion, biogas production increase up to 101%, 92%, and 70% using

KOH, Ca(OH)$_2$ and NaOH respectively. At least 100% methane improvement was obtained using NaOH for oil palm residues (Nieves et al., 2011). By using KOH pre-treatment, 77.5% more methane yield was possible for wheat straw (Liu et alk., 2015).

(a)

(b)

(c)

(d)
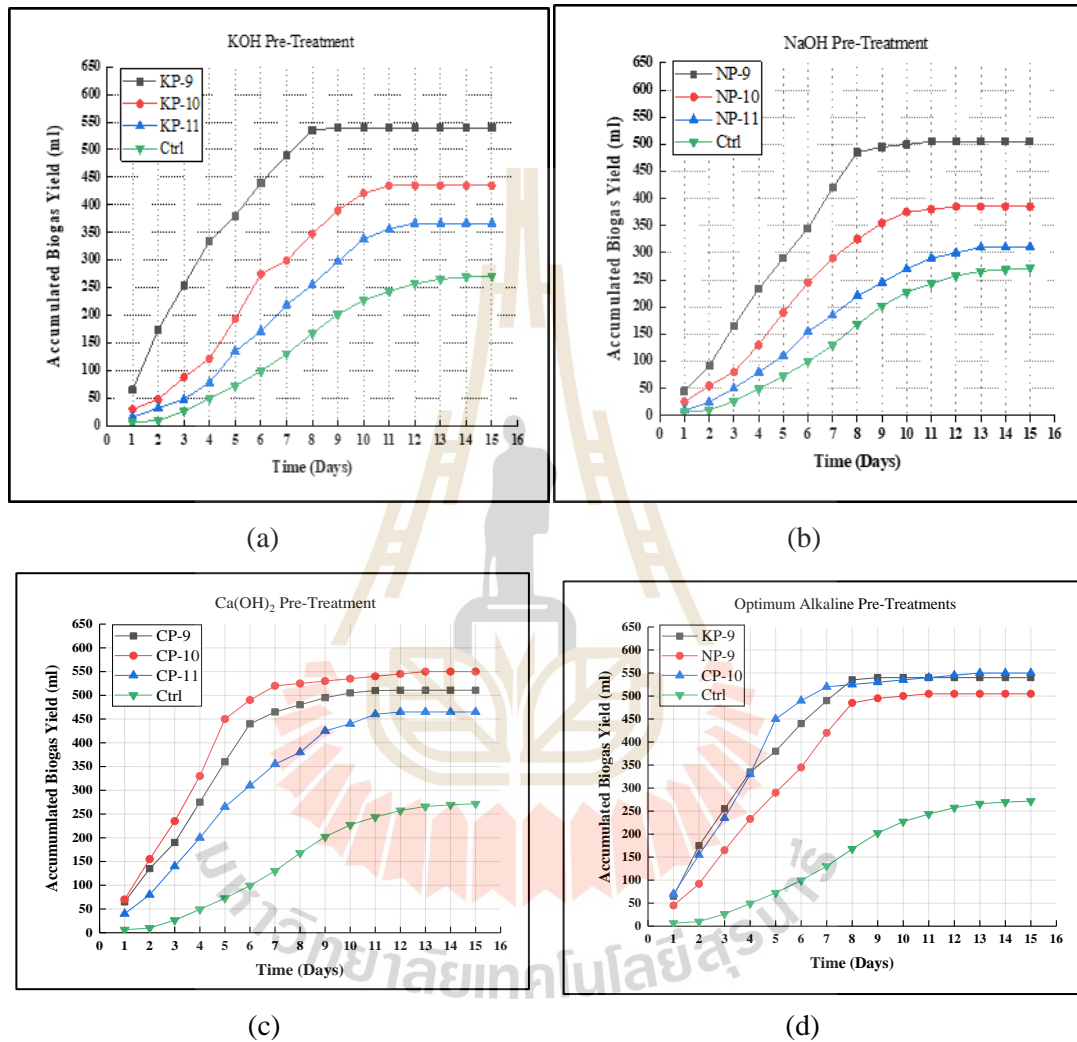
Figure 4.3 Cumulative Biogas Yield of Different Alkaline Pre-treated Substrates

(a: KOH Pre-Treatment b: NaOH Pre-treatment c: Ca(OH)$_2$ Pre-Treatment d: Optimum Biogas Pre-Treatment Comparison)

In this study,out of 3 different alkaline pre-treatments under the same pH range, NaOH performed lowest process enhancement but found almost identical gas volume at each respective optimum pre-treatment range (Figure 4c). Produced highest gas volume in CP-10, however, when the cost of the chemical is concerned, Ca (OH)$_2$ is the most economy to its counterparts. Furthermore, though Ca(OH)$_2$ required more chemical quantity because of its highest molecular weight, its insoluble characteristic was found persistence in feedstock material in combination with residual lime from cassava starch production chains. Slaked lime or Ca(OH)$_2$ pre-treatment ensure prolonged alkaline pre-treatment, and compatible in subsequent waste management process in the industry (Sriroth et al., 2000; Kaster et al., 2011; Ogata et al., 2016). In most literatures, NaOH had been chosen for alkaline pre-treatment (Frigon & Guiot, 2010; Chandra et al., 2012; Montgomery & Bochmann, 2014). Nevertheless, if there is no further modification in the anaerobic digestion by catalytic agents or metal supplementation as nutrient supplementation, KOH and NaOH is more suitable for generating more biogas because of its solid conversion potential under control AD variables. But when advanced processes will be employed into the AD system for future provision, these two chemicals can lead to complication with other supplemented chemicals into the system. Therefore, the choice of chemicals for biogas enhancement should be made based on provision in AD system and reactor configuration.

4.7 Conclusion

With cassava pulp having fine particles sized and highly carbohydrate residues, among several pre-treatment methods available, alkaline pre-treatment

method proved promising for rapid conversion of cassava pulp substrate for optimizing biogas production. In addition, to enhance gas yield, the comparative investigation among three alkaline (KOH, NaOH and $Ca(OH)_2$), KOH was proved best substrate degradability and gas yield within specific pre-treatment range. But $Ca(OH)_2$ offered highest biogas volume at pH-10 treatment. With alkaline pre-treatment, gas yield could be improved 14% − 103.7 % than untreated substrate. In view of resource and chemicals consumption for alkaline solution preparation, KOH and NaOH demand less alkaline quantity during pre-treatment. Therefore, by adopting the results of the study on alkaline pre-treatment to cassava substrate, significant amount of biogas could be generated in cassava processing plants. However, for future provision into AD system, the choice of chemicals could be a concern for the complication within biogas process when reactive alkaline agent is used for biogas enhancement.

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CHAPTER 5

INVESTIGATION AND CHARACTERIZATION OF IRON POWDERS FOR ZERO-VALENT IRON (FE^0) IN SYNCHROTRON RADIATIONS

5.1   Graphical Abstract
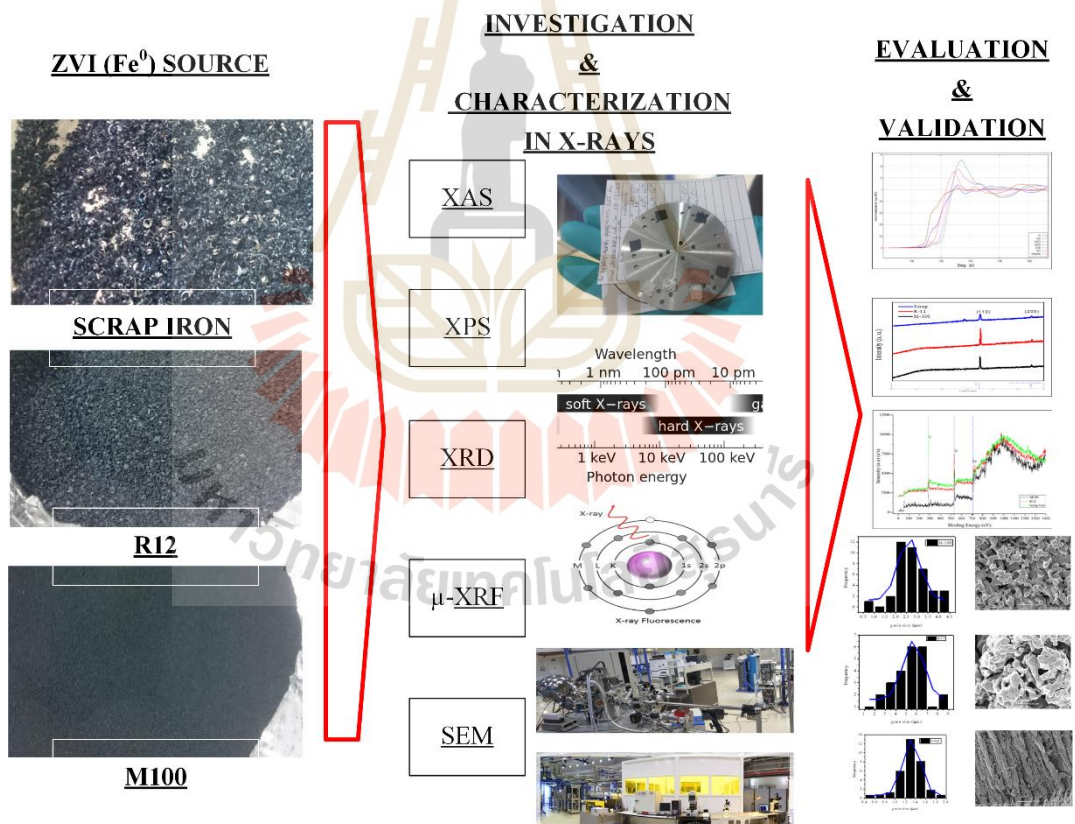
5.2 Highlights

- Four differential X-Ray techniques to characterize the composition of underlying Zero Valent Iron (ZVI) and highlights the importance of engaging multiple investigation methods in sourcing ZVI.

- XAS surface investigation disclosed the oxidation state (Fe^0) in M100 and R12 had identical category (0) despite distinct particle size, and majority of scrap iron's valency stood rather at +1, inferring it was partial or semi oxidized.

- Through argon [Ag] sputtering in XPS experiment, atomic concentration of Fe found in R12 was higher than its counterparts.

- Since crystallite sizes of the M100 and R12 samples were found as 32.24 and 33.28 nm respectively R12 and M100 had corresponding reactive and breakable properties in term of XRD pattern.

- Surface mineralogical study in SEM uncovered M100 and R12 had similar interlinked spherical structure with different gap size and intact particle size, but scrap iron had layered rocked texture.

5.3 Abstract

This study employs four differential synchrotron radiation techniques to characterize the composition of underlying Zero Valent Iron (ZVI) source in three readily commercially available iron particles (code name - M100, R12, and Scrap Iron) and highlights the importance of engaging multiple investigation methods in sourcing ZVI. With ZVI or Fe^0 having reducing properties to convert harmful chemicals to harmless substance, its widespread functional application in the

environmental remediation purposes was on the rise. Consequently, attempts were being made in choosing iron powders as ZVI source in interdisciplinary researches for exploring catalytic chemical reactions of ZVI. XAS-XANES and XPS spectra revealed scrap iron could not be regarded as hopeful ZVI sources since its edges and occurrences were detected entirely in contrast against standard iron foil having noticeable valency zero, and rather resembling to iron oxides. M100 and R12 were found consisting more percentage of zero valence properties than iron foil. Homogeneity and phase identification were further investigated by mean of XRD, and discovered R12 and M100 were comparable to reference iron standards. In addition, μ-XRF uncovered possible cross contaminants existed in the samples. Finally, SEM analysis disclosed distinctive metallic morphology, formation and texture of selected iron particles. This study resolved the controversial assumption that all iron sources consist of credible ZVI source for its catalytic reaction to take place. And contradictory iron oxides reactions could be highly possible on conditions when irons are not taken comparative characterization methods prior to source ZVI for requisite purpose.

5.4 Introduction

Among major abundant mineral reserves on earth, iron embodied about 5 percent, the fourth most abundant element on earth crust (USGS Report, 2008). Being indispensable and cheap metal to modern world, iron plays in crucial role of human society representing about 90% of all refined metal production today. Application of iron in construction, manufacturing, automobile industry is because of its appealing physical properties in strength, toughness and versatility as a result of its phase,

density, melting, and specific capacity (Yellishetty et al., 2010). With the development of physical chemistry in recent decades, like other metal element, iron was subjected for catalytic agents for chemical reactions (Pollack et al., 1963). With the synthesis in laboratory, scientists discovered iron possesses reducing properties (oxidizing agent) as catalyst. Iron in compounds occurs in numerous oxidation states (-2 to +6), among which +2 and +3 are more prevalent in iron oxides when exposed to oxygen (Wilke et al., 2001). In addition, being transitional element and thus highly reactive, the surface of iron particle tends to mostly reduce electron from another atom rather than reducing itself (Wilke et al., 2001; Murr et al; 2017). Earliest application of iron as chemical catalyst dated back to Habar-Bosch Process for ammonia manufacturing from nitrogen and hydrogen by iron complex in which iron speeds up the reaction at higher rate under lower temperature (Travis, 1993; Kozuch & Shaik, 2008; Kandemir et al; 2013). Fischer–Tropsch (F-T) synthesis evolved as another game changing application of iron in converting carbon-based syngas into liquid hydrocarbon (Schulz, 1999; Laan et al; 1999; Dry, M.E., 2002). By mean of catalyst irons in F-T synthesis, the higher waste-gas shift activity was proved realistic, and the more suitability for hydrogen and carbon monoxide exchanged was achieved (Dry, M.E., 2002). Since then, iron's reactive properties became the focal point in the realm of physical chemistry and material science inspiring researchers to the potentials of iron powders as chemical addictive. While commercial grade of different iron powders is readily available in the market, iron particles of different grade and particle size could also be synthesized by dedicated chemical reactions in the laboratory scale.

The discovery on the properties of Zero-Valent Iron's (ZVI, Fe^0) as reducing agent to convert oxidizable substance opens new focal research to iron as catalyst for material science and applied chemistry. Though controversial, since then, the application of ZVI in environmental remediation like contaminated land and groundwater treatment as reductant or sorbent has become research attention nowadays (Joo & Cheng, 2006; Li et al., 2006; Khin et al., 2012). In general, metallic pure iron powder, also refers to ZVI (Fe^0) oxidizes toxic and solubilizes toxic substances into harmless, stable, and insoluble ions and compounds. ZVI's active reducing properties degrades highly toxic organic contaminant and inorganic compounds under natural environment, ending itself into Fe^{2+} to Fe^{3+} (Zhang et al., 2006; Noubactep et al., 2012; Zou et al., 2016). The background kinetic iron converts toxic heavy metals of different oxidation states by direct electron transfer (a.k.a. electron donating capability) (Ansaf et al., 2016). The transformation process takes place through the adsorption or redox mechanism of its metallic oxidation state into less harmful stages (Boparai et al., 2011; Ansaf et al., 2016). The application of ZVI in environmental remediation measures can be classified into (1) stoichiometric ZVI reaction pertinent to dedicated pollutants as Permeable Reactive Barrier (PRB) in contaminated land or groundwater, and (2) electron transfer properties in conjunction with aqueous compounds for redox capabilities (Cundy et al., 2008; Fu et al., 2014; Zou et al., 2016).

In recent years, there have been considerable renewed interests to application of ZVI in enhancing bio-methane. Researchers prove that ZVI could stimulate methanogenic activities through chemically or biologically (Feng et al., 2014; Liu et al., 2015). In particular, hypothesizing aforementioned properties of ZVI, their

application into anaerobic bioreactors could accelerate microbial symbiosis among diverse fermentation bacteria and methane forming archaea (Zhang et al., 2015; Liu et al., 2015). ZVI had been proved enhancing methane production by means of its oxidation/reduction potential to intermediary products evolved during anaerobic digestion (Shi et al., 2011). ZVI was also proved having buffer capacity to regulate pH as a result of hydrogen gas liberated in the course of biogas processes, and consequently produced more methane (Bae & Hanna, 2015; Carpenter et al., 2015). Literature reveals that researchers opted either commercially available iron powders or discarded iron scraps which were considered genuine ZVI or synthesis of ZVI in the laboratory (Chang, 2008; Chekli et al., 2016). Nevertheless, while pure ZVI could be synthesized, the drawback was low in yield and insignificant for sizable application, commercial ZVI product needed extraordinary measures to avoid self-oxidation (Stefaniuk et al., 2016). Especially, when ZVI was to be applied on the condition where ZVI was strictly limited factor to achieve ideal chemical reactions. In the comparative investigation among commercially grade ZVI nanoparticles (cNZVI), iron fillings (bulk iron powder, ZVI) and synthesized ZVI (sNZVI) with no spectroscopy investigation, despite all ZVI source could improve biogas yield, cNZVI resulted best outcomes(Carpenter et al., 2015). There were complex results of NZVI application, this was due to the fact that toxicity of reduced Fe species and disturbance of electron transfers (Yang et al., 2013). In other studies, with no surface investigation by X-Ray spectrum, when three different types of ZVI (iron power, clean scrap and rusty scrap) were added to methane production from waste active sludge (WAS), iron powder performed inferior than the latter sources (Liu et al., 2015; Ibrahim & Abulaziz. 2016). Without specifying characteristic of ZVI, the

addition of iron powders added methane yield up to 43% (Ignace et al., 2016). In contrary, in a study of ZVI's effect on methane production, the researcher used non-rusty scrap and rusty scrap iron to investigate anaerobic reactor, while ZVI enhanced 1.27 times of methane, scrap irons resulted in better methane yields at 2.45 to 2.7 times (Zhen et al., 2015). Applying Energy Dispersive X-ray (EDX) in investigating ZVI source, the author concluded the potential application of ZVI in scrap iron again proved promoting methane formation. However, with XRD investigation to two different commercial grade microscale ZVI powder for biogas studies, methane production could be increased up to 40% (Sreekanth & Sahu, 2015). The discrepancy behind these controversial effects of ZVI is being the selection of ZVI source and ZVI verification technique. Though pure ZVI was consistent to its characteristic and properties, it was important to use differential methods in investigation of ZVI.

The development in advanced electromagnetic and radiographic technologies enabled research in material science with new scientific breakthroughs like never before. Apparently, with the help of spectroscopy, the surface properties of matter could be investigated to more thorough understanding to the ranges of material with their electronic properties and structural heterogeneity. However, each electromagnetic radiation technique had specific function and capability to detect structural configurations and constituents (Jenkins, 1995). Under the detection of X-ray spectrums for materials' surface analysis, while X-ray photoelectron spectroscopy (XPS) assisted in investigating a broad range of elements in their quantitative and chemical states information of the material, X-ray Absorption Spectroscopy (XAS) could comprehensively detect individual element's metal oxidation states, site symmetry, coordination number, covalency, etc (Watts & Wolstenholme, 2003;

Bokhoven & Lamberti, 2016). Similarly, in micro-beam energy dispersive X-ray fluorescence (μ-XRF), it was designed to determine elemental composition and chemistry of material, whereas X-ray Diffraction (XRD) was applied for inspecting characterization, identification and quantitative study of material (Majumdar et al., 2012; Speakman, S2014). Thanks to these techniques, researchers could speculate the properties of the substances. However, each process provided with their respective unique implications with pros and cons. Therefore, it was imperative to use multiple techniques to validate the surface composition and oxidation prior to application.

The study aims at investigating and characterizing in three different ZVI sources prior to application in anaerobic digestion to validate whether selected ZVIs be pertaining reliable zero valency and their relative composition to ensure high bio-methane production capability, oxidation-reduction potential, and acid buffer properties. In this work, 3 different ZVIs were chosen (i.e. scrap iron, R12, and M100), and they were subjected to 4 differential X-Ray radiation methods in Synchrotron and one electron microscopy for characterization. The importance of comparative investigation methods in verifying ZVI had been stressed by means of individual assessment and their limitation. Finally, the study intends to highlight the importance of investigation methods in choosing ZVI application for ensuring ideal chemical reaction.

5.5 *Experimental Procedure*

5.5.1 Materials and Preparation of Iron Particles

The Zero Valent Iron (ZVI) investigation for this study was applied to two commercially available iron powders, M 100 and R12 of Swedish based metal

powders manufacturer Höganäs AB Inc., and discarded iron scrap from mechanical workshop of Suranaree University of Technology. Properties of selected ZVI sources are summarized in the table (1). Prior to comparative X-ray investigations, while the surface rust of scrap iron was eliminated by octane immersion for 24 hours, rinsed thoroughly in DI water, then dried under sunlight, powder samples were subjected under heat treatment (150 ˚C) for 24 hours in oven to shed pre-oxidized layer and avoid possible further surface oxidation by ambient air and humidity. Characterization was performed at NANOTEC- SLRI, Thailand.

Table 5.1 Physical Properties of Selected Iron (Source: Höganäs, AB)

Samples	Apparent Density (g/cm^2)	Specific Surface (BET) (m^2/kg)	Particle Sizes
M100	2.4	130	< 10 µm
R12	1.4	225	< 100 µm
Scrap Iron	1.1	265	∝1~5mm

5.5.2 Surface Analyses and Techniques

5.5.2.1 X-Ray Absorption Spectroscopy (XAS)

Electron activated by bending magnet in high voltage (1.0 ~ 1.2 GeV) beam, the B.L 5.2. of SUT-NANOTEC SLRI supports energy range between 1240-12100 eV with two major modes (i.e. Transmission and Florescence) (Kidkhunthod, 2017). The existence of the structures around target element could be verified either Near Edge Structure (XANES) or Fine Structure (XAFS) which is major contrast, and depends upon the surface location of subjected sample. Since this study focuses metal oxidation and covalency of elemental iron, XANES mode with ion chamber approach was applied. The beamline is capable with energy resolution up

to 2 x 10^{-4}/light energy. The spectra of Fe foil, pure iron, occurs at 7112 eV. Then, samples were compared with standard iron spectra of Fe^0 (Fe), Fe^{2+} (FeO), Fe^{3+} (Fe_3O_4). Strict calibration measures were followed to prevent precision errors, biases and invalidity. Samples were treated with in situ nitrogen (N_2) gas flashing arrangement lest surface oxidation incidence prior positioning into sample chamber. Instead of adhesive coloured tape, clear plastic sachet was used for samples when mounting to have x-ray attenuation and absorption in check. 6 scans were attempted to reduce spectra noises and ensure consistency between each scanning. In order to retrieve scanning data, beamline edge energy thresholds were set from ~20ev before and ~150eV above the Fe edge 7110 eV. Retrieved spectra data were further presided by Demeter XAS data processing and analysis software package.

5.5.2.2 X-ray Photoelectron Spectroscopy (XPS)

Following the same procedures of XAS, X-ray photoelectron spectroscopy (XPS) spectra for this study was conducted in SUT-NANOTEC SLRI, but different beamline (B.L 5.1). By the excitation of electron in relatively shallow core level by mean of K_α surface analysis system, the XPS phenomenon itself is flexible in detecting diverse material with its functional groups, chemical compositions and state of elements, while survey scan consists of numerous sharp peaks that emerged across binding energy range (i.e. 0~1400 eV) with respect to intensity (c/s), whereas narrow scan accentuates specific element in which it arises within the whole spectrum. Common notation exhibit in XPS are in electron orbitals (e.g. $1s_{1/2}$, $2s_{1/2}$, $2p_{1/2}$, $2p_{3/2}$, etc.), and different electron spins located across the spectrum. Argon (Ar) gas sputtering was applied to reduce surface oxidation preceding to implant on sample platter. Thorough coarse navigation was examined through optical view window for

adequate XPS point analysis. During the investigation, all samples were first taken for 2 surrey scans (0~1400 eV) to identify possible clusters, after validation for 6 narrow scans dedicating to Fe related binding energy range (705~720 eV) and spin-orbit splitting of peaks (i.e. 2s, $2p_{1/2}$ and $2p_{3/2}$) was measured. Best ZVI location source was chosen by automatic optical detector. The observed Fe peaks were further substantiated against standard XPS database and analysed using PHI multiPak XPS data processing application with a combination of Gaussian and Lorentzian components.

5.5.2.3 X-Ray Diffraction (XRD)

Beamline 6 (BL-6) of SUT-NANOTEC SLRI, X-ray Diffraction (XRD) was used to investigate the size and the shape of the unit cell of any compound. It delivered an unfocused monochromatic X-Ray in 1.25 − 10 keV photon energy in adjustable energy intensity (Klysubun et al., 2017). Diffraction patterns gave information on size and shape of unit cell from peak positions and information on electron density inside the unit cell, namely where the atoms were located from peak intensities. It was performed by using monochromatic Cu K-α radiation (λ=1.54056 Å), operated at 40 kV and 40 mA. Iron samples were scanned from 10 °C to 70 °C in the diffraction angle, 2θ with a step-size of 0.01°. The crystallite sizes were estimated by using Debye Scherrer's equation:

$$D_{crys} = \frac{k\lambda}{\beta cos\theta} \tag{6.1}$$

where,

D_{crys} =   crystallite size (nm),

K =   Scherrer constant (about 0.89 for Fe_3O_4 and Fe_2O_3),

λ =   wavelength of Cu-K-α (λ=1.54056 Å),

θ = peak angle (deg.), and

β = width of the XRD peak at half height (radian).

5.5.2.4 Micro Beam Energy Dispersive X-ray Florescence (μ-XRF)

Beamline 6b of SUT-NANOTEC SLRI, Energy dispersive micro-XRF technique was used to analyse the qualitative and quantitative composition of solid or liquid samples. It could be used to measure virtually every element from K to U in the periodic table in concentrations ranging from a few ppm to nearly 100 percent (Adams, 2010). Measurement of wavelength of the X-ray photons emitted by the sample element allowed the identification of the elements. It couldpermit to obtain images with element distributions on the surface of the iron samples. Powder samples were pressed into pellet (1 cm diameter) by applying pressure of 5 ton for 5 minutes using a hydraulic press. The analysed element was counted in the absorption K edge energy range of 0-15 keV in order to calibrate the energy scale. The measured spectra were fitted using fit line profiles for powder and scrap samples. To get the reliable data, the instrument was calibrated using the expected elemental concentrations from the standards versus the calculated elemental concentration. The best fit of standards on the regression line indicated that the analysed concentrations were at or near the given concentrations.

5.5.2.5 Scanning Electron Microscopy (SEM)

The surface morphology and microstructural properties of iron samples were observed by using scanning electron microscopy (SEM) at department of physics, Mandalay University, Myanmar. The average grain size of M-100, R-12 and scrap samples were examined from the observed SEM images. It can create the

maps of element distribution on the surface of samples. The device used the electron

beam to produce a magnified image of iron sample. In the device, the electron gun

produced a high intensity electron beam, which passed through a series of

electromagnetic lenses to be focused and scanned across the sample. The incident

electron beam causes secondary electrons to be emitted from the iron sample. Then

the image was reconstructed from the signals backscattered from the surface of the

sample and magnified on the screen. The resolution of the SEM could approach from a

few nano-metre (nm) to micro-metre (μm) and it could operate at magnifications that

are easily adjusted from about 10X-300,000X. The objective of SEM investigation was

to validate commercial grade selected iron powders' surface shape, crystallinity and

possible disintegration during chemical reaction taken place in determined application.

5.6 **Results and Discussion**

5.6.1 **X-Ray Absorption Spectroscopy (XAS) Analysis**

Since XAS comprises the combination of XANES (X-ray Absorption

Near Edge Structure) and EXAFS (Extended X-ray Absorption Fine Structure), while

XANES (pre-edge of the spectra) infers for metal oxidation and co-valances, whereas

EXAFS (post-edge) is associated with ligands, bond angle etc. (Rehr & Ankudinov,

2005). Fe XANES spectra in the figure (1) reveals respective spectrum of 7

comparative irons. Among which, M100, R12 and Scrap Iron were ZVI hypothesized

samples, and they were evaluated against remaining 4 standardized Fe (Fe Foil), FeO,

Fe_2O_3, Fe_3O_4 having oxidation states 0, +2, +3, and +2/+3 respectively (Crocombette

et al., 1995; Ravel et al., 2002). The elemental Iron (Fe) having valency zero which

the relative energy occurs at 7112 eV was the principal sample against which the

concerned samples (M100, R12 and Scrap Iron) were differentiated whether they owed their zero valent properties. The remaining iron oxides testified the reference to the condition of different iron oxides' oxidation states and spectra patterns. The XAS results (figure: 1a) indicates that the edge of M100 and R12 arose noticeably from left to the standardized iron foil (Fe) giving evidence that the samples (M100 and R12) are consistent proof of being in zero oxidation state.

In contrast, the spectra of scrap iron which occurred more isolated and shifting sparsely right to standardized iron foil (Fe) and far secluded to M100 and R12. Since the edge of Scrap Iron emerged between standard iron elemental (Fe) and Iron II oxide (FeO), it is the evidence that scrap iron's metal oxidation state (valency) is distinct to those of the M 100 and R12. This is due to the fact that scrap iron which is the discarded iron fragments derived from cast, wrought and rolled iron bar or plate from machinery workshop which intrinsically had been under prolong exposure to the air and contained other metal from manufacturing processes. Consequently, its surface environment was entirely oxidized. In contrast, among M100 and R12 which are genuinely specified as elemental iron powder, the spectra of R12 was discovered closer to standard Fe spectra, meaning R12 iron powder is highly in consistence with higher ZVI source. The pre-edge and edge spectra stacked plot (figure:1b) provides with further confirmation that while the characteristic of elemental irons (i.e. Fe (Foil), M100, and R12) conformed to identical approach at 7112 eV, iron oxides (i.e. FeO, Fe_2O_3, and Fe_3O_4) were observed near 7120 eV respectively. And, among which Scrap Iron's peak appeared between 7112 eV and 7120 eV. However, XANES in XAS method in this stage could not further validate quantitatively composition of metal elements.

(a) Normalized Pre-edge and Edge
edge

(b) Stacked normalized pre-edge and
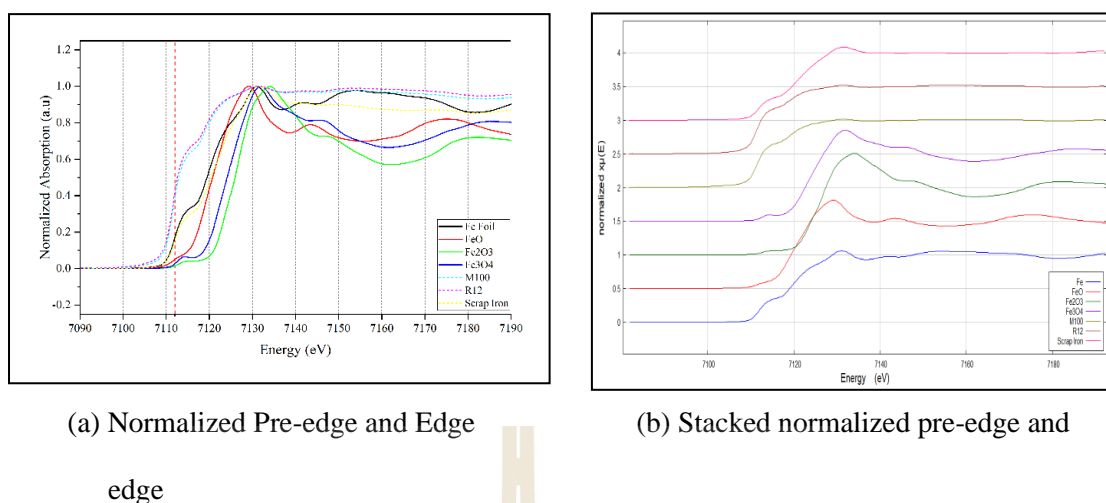
Figure 5.1 XANES Spectra of Different Iron Sources

The localized spatial edges' discrepancies within Fe spectra and those of R12, M100 and Scrap iron could possibly have triggered by their distinct phases (texture, size, density, and BET) and morphology of the samples. While the standardized Fe spectra was obtained from elemental iron foil, M100 and R12 spectrums were of Micro- and Nano-particles. Nevertheless, with spectra shift in obvious eccentricity to elemental iron (Fe foil) and the peaks conformation below known oxidation state, the relative oxidation states of M100, R12 and Scrap Iron could be enumerated by contradiction to valency states of Fe, FeO, Fe_2O_3, and Fe_3O_4. By Gaussian curve fitting to all energy peaks and linear regression to x-ray absorption, the peaks of all hypothesized samples progressed below known iron oxides (Fe (II), Fe (III), and Fe (II/III)) spectrum; this ascertains that all three selected samples, with the exception of scrap iron having inferior to Fe foil in term of valency 0, M100 approached proximity to zero valence oxidation state, then followed by R12. This further substantiates that M100 and R12 hold highest zero valent credibility. Since the target samples were

elemental iron (Fe) participating single iron element and with exclusion and comparison among different compounds, k,R,q space driven by EXFAS analysis and extended spectra constituency was ignored. Other potential metal impurities were further inspected by other differential methods.
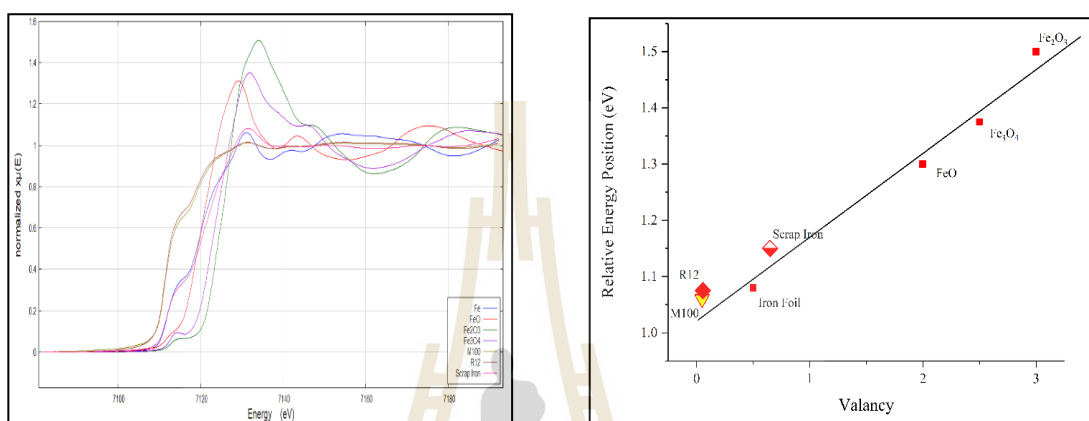
(a) Irons XANES peak fitting in

energy excitation

(b) Relative Valency of Irons

Figure 5.2 Localized Peak Formation During Energy Excitation

5.6.2 X-ray Photoelectron Spectroscopy (XPS) Analysis

The advantages of XPS over XAS being capability in survey scanning (0~1400 eV) in detecting multiple atomic species and their relative quantitative concentration in the sample described in a single spectrum (Ebel et al., 1988). Vice versa, this is also major drawback of incapability to identify specific diverse oxidation states of the metals in XPS (Grieken & Markowicz, 2001; Groot & Kotani, 2008). Therefore, the separate narrow scanning which configured binding energy range is obligatory requisite in characterizing individual elements. XPS Survey Scanning in the figure (3) reveals that satellite sharp peaks were discovered in all three samples

(M100, R12, Scrap Iron) representing the existence of Carbon (C), Oxygen (O) and iron (Fe) across different orbital edge.
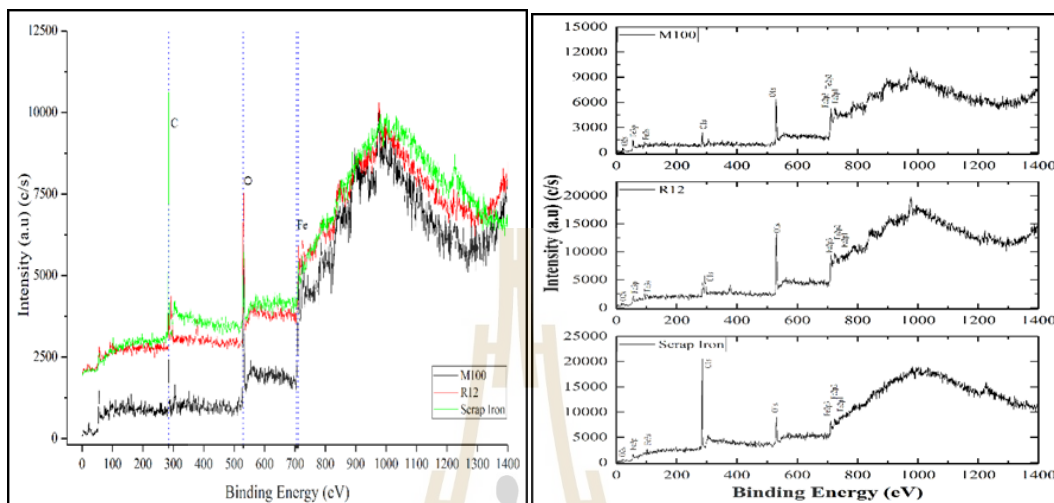
Figure 5.3 XPS Survey Scanning Spectrum of Irons

Apparent atomic Carbon and Oxygen were identified in s-shell at binding energy 285 ± 5 eV, 530 ± 5 eV, and 706 530 ± respectively. Numerical results generated from synchrotron light indicated that R12 had highest atomic Fe concentration with minimum atomic oxygen (Table 2). Therefore, sample R12 could be considered to have highest iron (Fe) source. This result is being controversial to those of XAS in which M100 was previously proved convincing zero valent iron source. However, the justification being while XAS focuses oxidation states of elements, XPS, on the other hand, emphasizes quantitative composition of iron in both element and compounds by function of sharp peaks establishment. Therefore, the XPS spectra reveals different elements along wider energy range by means of peaks developed by respective energy intensity. Followed by M100 after R12's highest iron

(Fe) composition, and finally scrap iron turned out resulting in lowest atomic iron and highest carbon and oxygen to its counterparts since generic scrap iron derived from smelting chains consisting higher carbon because of commercial grades and fabrication processes. The size of the particle and the texture of the samples could also be the factor for these differences. Since the XPS surface technique analyse 2~5 nm probing depth of the sample surface, which is extremely shallow depth, with this, instantaneous oxidations could take place when subjected to local environmental exposure and the rate varies as increased in particle sizes, apparent density, and relative humidity. Therefore, it is advisable that other corresponding methods should be used in validating and characterizing core-shell structure and composition. Unlike XAS, in which hypothesized samples were compared against other standardized specimen (i.e. Fe Foil, FeO, Fe_2O_3, and Fe_3O_4), in XPS investigation, samples were evaluated alongside with XPS database (NIST, 2018).

Table 5.2 Atomic Concentration of Selected Irons

Samples	Atomic Concentration (%)			
	Fe	**O**	**C**	**Others**
M100	93.01	4.85	0.66	0.48
R12	96.86	1.26	0.92	0.96
Scrap Iron	73.22	17.51	6.36	2.91

Narrow scanning in accordance to associated binding energy for focused element opens another measure in characterizing iron and its compounds. Theoretically, the peak of Zero Valent Iron (ZVI) in photoelectron spectra analysis could not be pinpointed on the generic iron spectrum since ZVI peak being tiny arc and barely unnoticeable unless samples are Ar^+ sputtered (Grosvenor, et al., 2004;

Fondell et al., 2018). Based on the XPS narrow scanning with Argon (Ar^+) sputtering in this the study, overall Fe oxide peaks were easily distinguishable and significantly shifting at higher binding energy of the metal iron (figure 3). While metal iron (Fe) peak develops between 706 ~ 708 eV, in which zero valent iron positioned on the edge (figure 4b). Then, irons oxides (Fe_xO_x) in their particular orbital spinning orientation progressed after 710 eV in the Fe XPS spectrum. Since, oxides of iron peaks generally shifted to higher energy level than metal state, from the experiment, it was discovered that Fe metal peak of M100 and R12 were found beneath that of Scrap iron and followed within the reference energy boundaries (figure 4a). When spectra are compared in detailed against standard Ar+ sputtered Fe_2O_3 spectrum (figure 4b), all of the iron sample could possibly be oxidized to Fe II and Fe III, among which M100 and Scrap Iron were found partially being oxidized because those spectra trend were found identical to Fe II and Fe III satellite zones.
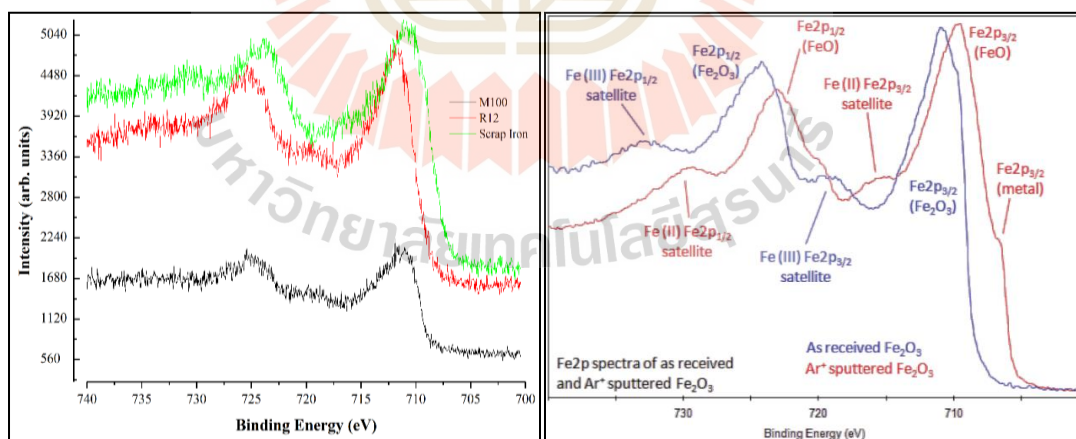
(a) Customized Scanning Spectrum of Irons   (b) Standard Iron Spectra orbital state.

(source: www.xpssimplified.com)

Figure 5.4 XPS Narrow Scanning on Irons vs. Standard XPS Fe Spectra

Besides, acquiring higher Fe atomic concentration and the trend of XPS spectrum of M100 and R12 were comparatively in parallel, this adds further supportive evidence that scrap iron has more oxidized characteristics. This implies that M100 and R 12 hold more oxidizing potentials. Even though, exact valance could not be interpreted from XPS spectrum, it can provide with the possible oxidation states by reaction response when exposed to the chemicals. The recognizable rise of scrap iron's spectra after the Fe peak proved that scrap iron is either partially or completely oxidized in surface layer during purifying processes, while the R12 and M100's spectrum remains in lower intensity as increased in binding energy. This is further proved by atomic concentration of each sample shown in the table (2). The presence of other minute metal concentration could also trigger these variances. Hence, the characterization of ZVI for selected irons was further elucidated by with XRD and μ-XRF.

5.6.3 X-Ray Diffraction (XRD) Analysis

The XRD spectra of iron-powder and scrap were indicated as shown in the figure (5). The upper side of XRD profile represents the observed profile while the lower side indicates the standard (reference) of Fe JCPDS (Joint Committee on Powder Diffraction Standards) library (P. D,1977). Upon Compared standard data with the diffracted peaks to observed XRD pattern, the peaks were indexed. M100
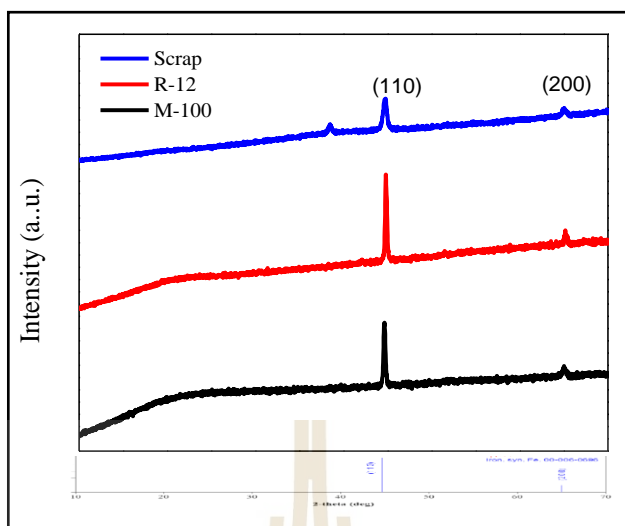
Figure 5.5 XRD pattern of Selected Iron Powder and Scrap Iron

and R12 were named for Nano and Micro size iron samples. On the iron-powder XRD patterns, there were only two identified peaks (110) and (200) at $2\theta=44.61°$, $65.16°$. The micron size iron sample (R12) had more intensive peaks than nano-metre scale M100 sample. Three peaks were clearly observed on the iron-scrap XRD pattern but (110) and (200) peaks at $2\theta=44.68°$, $64.93°$ were well matched with those of the standard (reference) Fe JCPDS library file. The other peak at $2\theta=30°$ could not be identified. The diffraction line at $2\theta=44.61°$ was the strongest in intensity among the collected M100 and R12 XRD lines. To calculate the crystallite size, the strongest diffraction line had been applied. Crystallite sizes of the M100 and R12 samples were obtained as 32.24 and 33.28 nm. The smallest crystallite size is iron-scrap sample and it is obtained as 16.38 nm. Hence, it could be interpreted that the R12 and M100 have corresponding properties in term of XRD pattern, whereas scrap iron's XRD consists

additional isolated peak, representing the latter sample has more impurities, atypical size and characteristics.

5.6.4 Micro Beam Energy Dispersive X-ray Florescence (μ-XRF) Analysis

Raw μ-XRF spectra were fitted using data analysis tool named PyMCA for all samples. The black line showed the experimental data. The red solid line showed the best fit of the experimental data. The green line was continuum line model where background was subtracted from the experimental data. The pattern of peaks observed in the spectrum is directly related to the elements present in the iron sample. Figure 8 (a-c) shows the presence of one or more metals including Sn, Mn other than Fe in XRF spectra. The fitted KL3 lines of Sn, Mn and Fe on each spectrum were 5900, 6490 and 7058 eV respectively. The atomic Fe concentration percentage of 67, 72 % were recorded for M100, R12 iron powder samples after using iron 70 % occupied material as a standard reference sample. Therefore, by correlating to the result of XPS, it could be presumed both M100 and R12 possesses highest Fe concentration. Detected Mn and Fe peak in all samples' spectra implies possible contamination involves in the samples. The 0.144 and 0.148 of the full width at half maximum (FWHM) estimated on Mn and Fe peaks of spectrum and fit areas were 3.845×10^3 and 1.118×10^6 for R-12 and 5.8035×10^2 and 1.048×10^6 for M-100 at KL3 lines. Therefore, comparative X-ray investigation is strongly suggested to examine whether the presence of Sn and Mn element is either from original source or cross-contamination along preceding samples investigated in the same beam line.
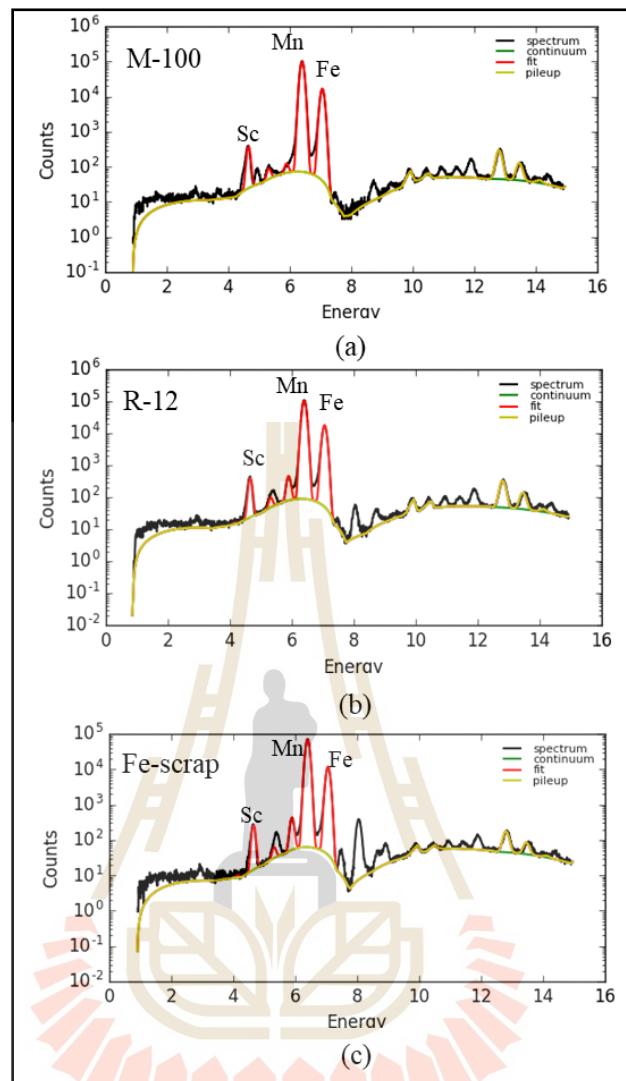
Figure 5.6 Fit Peak Results for Iron Powders and Scrap Iron

5.6.5　Scanning Electron Microscope (SEM) Analysis

The photomicrographs of iron powders and scrap iron samples were shown in the figure 7 (a-c). Distribution and orientation of grains and compact layers in iron sample were morphologically investigated using JSM-5610 Scanning Electron Microscope (SEM) with accelerating voltage 15 kV and 850 times magnification. In all samples, same bar code system of 20 µm was used. The surface morphology of

iron powders was seemed to be sponge shape and the grain sizes were apparently large in micron size R12 samples. In nano size M100 sample, the agglomerated M100 iron particles were noticeably much smaller than R12 sample. But the grain distribution was uniform in both powder irons (R12 and M100) (Figure 8). The shapes like rock layers were found in scrap iron sample. SEM findings provides with more morphological information of iron available in commercials. Furthermore, SEM image revealed potential disintegration when reactive chemical attached to samples' surface. Under the same magnification range, it has been observed than M100 iron particle is more likely to dissolve and decompose when exposed to other matter. Following M100, R12 and Scrap iron found having harder and sturdy structural and crystal integrity.

(a) M 100   (b) R 12   (c) Scrap Iron
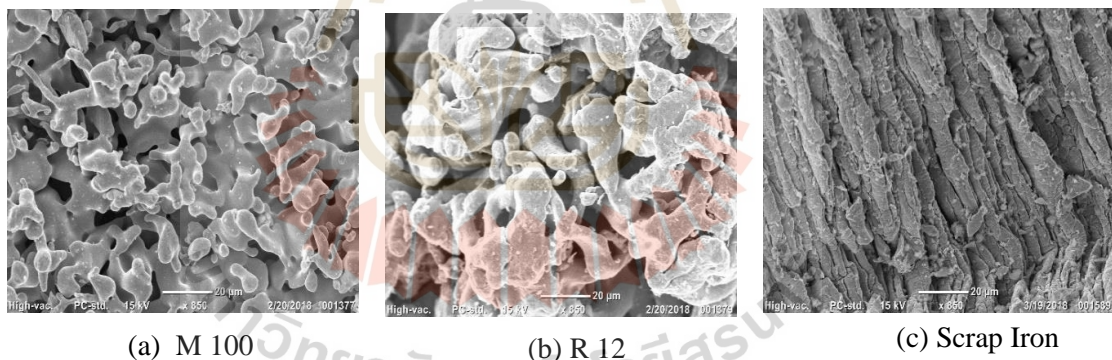
Figure 5.7 SEM Images of Fe Powders and Scrap Iron

The grain sizes and gap distribution of M100, R12 and scrap samples by electronic SEM histogram revealed the average spaced-grain size observed in receptor plate under electronic recognition. The distance between grains and its distribution were presented in the form of a number-frequency histogram. As shown in the figure 8 (a), the gap and whole particle size of M100 sample were in the range 0.5–4.5 µm.

R12 sample had minimum size of 1 µm and maximum size of 9 µm space between particles (figure. 8 (b)). The distribution curve peaks at 5.5 µm in gap size at R12. This represents majority space between fractured surfaced are mostly 5.5 µm. likewise, in the figure 8 (c), compact iron layers with different sizes in the range 0.4 – 2.0 µm were found. In R12 sample, the presence of large space between particle may be attributed to their high surface energy and high surface tension of ultrafine micro particles. The intact particle size can be varying according to localized observation under the microscopic view finder. Exact particle size shall be made by compromised results of specialized particles size analyser and physical analysis under sieve analysis in micron to nanometre scale openings.
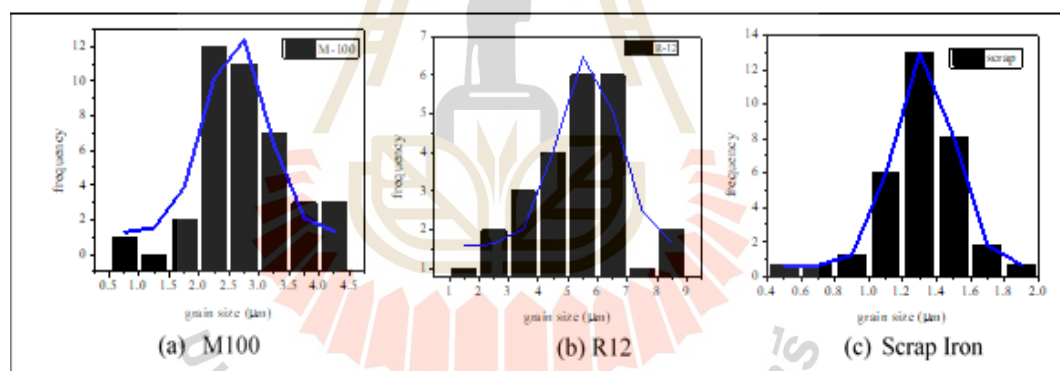
Figure 5.8 Size Distribution Histogram of Iron Powders and Iron

5.7   Conclusion

Since widespread functional applications of zero valent iron (ZVI) as catalyst in the environmental remediation purposes, investigation and characterization is crucial lest detrimental iron reactions are eliminated, and validation is preferred if iron powder is genuinely in ultimate zero valent iron composition. In this regard,

undergoing 4 differential methods to three selected iron sources (i.e. M100, R12 and Scrap Iron) for validating credential ZVI source. Qualitative and quantitative characterization revealed that scrap iron was found consisting insignificant ZVI matter and apparently more swayed against ZVI criteria than its counterparts (R12 and M100). However, there could be a drawback from R12, nano particle ZVI since it could lead to more instantaneous chemical reaction than micro size grain M100 and Scrap Iron under exposure to any liquid. It can be readily oxidized itself prior to application in intended purposes. This limitation could likely be exacerbated when nanoparticles are in contact with reactive chemical solution. Detrimental results could be envisaged due to improper handling and incorrect ZVI sourcing. Therefore, it is crucial to validate ZVI before designated reaction to be taken place. Possible paradoxical chemical outcome is also likely to be envisaged when scrap iron is undergoing application as ZVI function. By SEM investigation, the grain size distribution and particle density of selected samples was found apparently distinctive as per visual and categorial description, meanwhile, with synchrotron lights, M100 and R12 were analysed having comparable results and evidences as metallic zero valent iron. Thus, this two samples proved fit for enabling certain ZVI reactions for further ZVI function. However, special precaution is advisable when choosing M100 because of readily oxidizable potential (reducing or catalytic properties) by ambient exposure forming subsequent iron oxides within short period of product lifespan.

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CHAPTER 6

COMPARATIVE STUDY ON THE PERFORMANCE OF

IRON-AMENDED CASSAVA PULP FEED

METHANATION IN CONTINUOUS STIRRED TANK

REACORTS (CSTRs)

6.1   Graphical Abstract
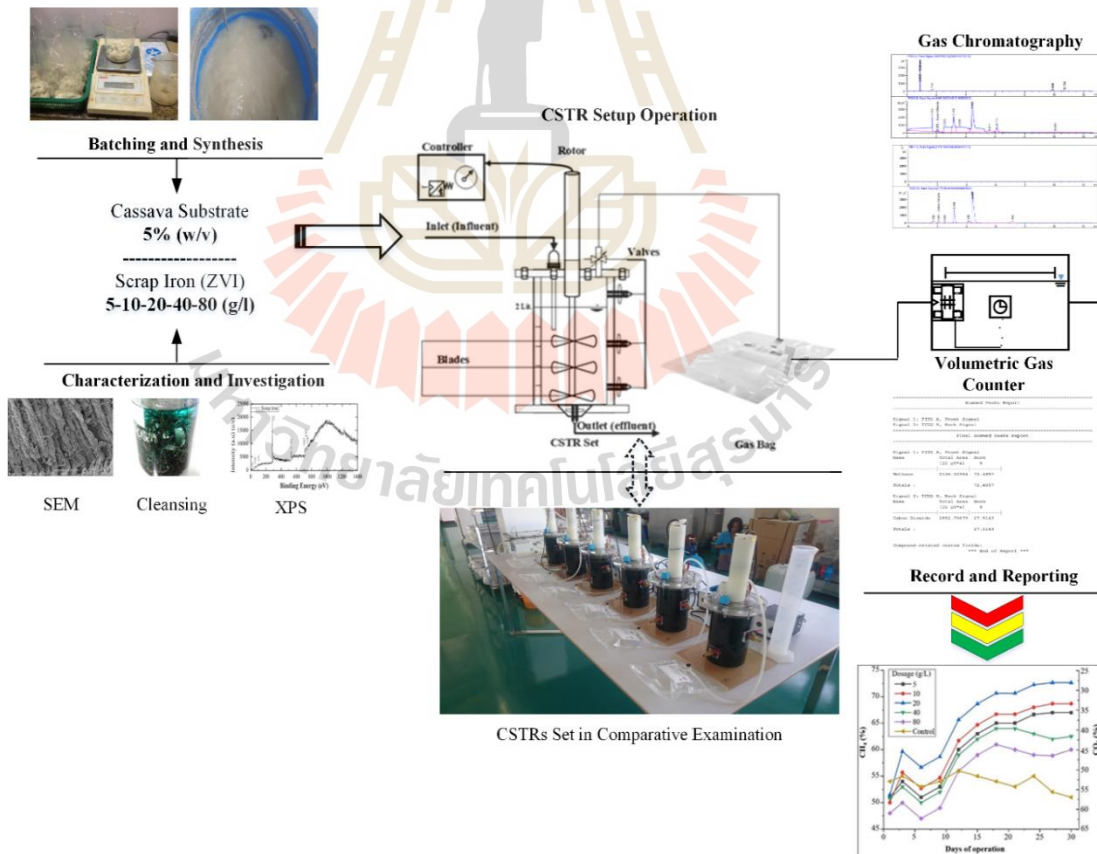
6.2 Highlights

- Using scrap iron at various levels in CSTRs showed improved digestion performance and gas yield compared to iron free control reactors.

- The combination of iron amendment and mixing arrangement helped to retard rapid pH changes within active digestion by avoiding sludge and substrate isolation for ensuring sustainability of methanation process.

- VFA/TA ratio interacts directly proportional to iron concentration present in reactors.

- Stimulatory effect of iron metal was found between 5 to 20 g/L, among which optimal dosage was 20 g/L with more than 95% sCOD removal with daily tCOD addition.

- The net increase in energy calorific value of biogas were 25%, 18% and 16% improvement by 20, 10, 5 g/L by iron addition respectively.

6.3 Abstract

In recent years, the advances in the biogas related technology and plant design open new possibilities in biogas production from various biomasses other than activated waste sludge from a waste water treatment plant. Although, the anaerobic digestion (AD) process offers sustainable and environmentally friendly energy to curb heavily reliance on fossil fuels to a certain extent, the low methane (CH_4) content of biogas remains as a challenge. Using discarded scrap iron and its ultimate properties of zero valent iron (ZVI) potential, this Chapter attempts to enhance the biomethane content of biogas in terms of quality and quantity. The characteristics of scrap iron were verified using XPS and SEM. The optimum iron level was observed at 20 g/L

where COD removal reached 95%. This represents a 20% enhancement more than that of the control reactor with 75% removal. Bio-methane production could further be improved using iron with superior characteristics (particularly zero valent iron, ZVI). Nevertheless, by means of the current iron amendments in cassava pulp feed CSTRs with an optimum organic loading rate (OLR) of 3.25 g VSS L^{-1} day^{-1}, methane was enriched from 50% to 75% with 10% to 35% additional gas yield more than that of the control reactor. VFA/TA levels are a critical control factor. Inhibition starts when iron addition exceeds 20 g/L. The outcome of iron addition is seen immediately, making the process easier to control with better stability during digestion. The presence of iron cut the frequency of re-buffering and thus reduced chemical consumption for pH control and provided for a longer buffer resistance period. Iron amendment during anaerobic digestion of cassava pulp was shown to promote higher levels of bio-methane production.

6.4   Introduction

In curbing global carbon emissions and heavily reliance on fossil fuels, renewable energy plays pivotal role in global energy transformation to ease dependence on dwindling oil and gas reserves and reduce climate change. In this regard, efforts are being made to foster competitiveness, sustainability and energy security of bioenergy. Among several alternative biomasses, agriculturally derived biomass has a distinct potential to produce bioenergy (García et al., 2014). The increased demand for food manufacturing generates proportional agro-industrial by-products from production chains, which becomes ample feedstock for bioenergy generating processes (Forsell & Selosse, 2012). As an agricultural nation, Thailand, to

a great extent, has an energy profile based on resource imports to cover rising consumption. Around 60% of the country's energy is imported in different mineral forms and through the electrical grid system (IRENA Outlook Thailand 2017). Therefore, the country has set a renewable energy development agenda to promote and support energy security. AEDP 2015-2036 targeted to generate up to 716 MW of power by 2021 and 1283 MW by 2036 from biomass residues or energy crops (Achawangkul, 2017). This target could be achieved by means of biogas plant expansion and optimizing biogas related technologies applied in existing biogas plants.

Biogas manufacturing process is a well-established technology. Carbonaceous biomasses are subjected to digestion under a strictly anoxic environment (Ostrem, 2004). Anaerobic microorganisms are responsible for converting organic feedstocks into biogas through four sequential steps (i.e. hydrolysis, acidification, acetogenesis and methanogenesis) (Verma, 2002; Alverez, 2003; Ostrem, 2004; Weiland, 2010). Anaerobic digestion produces biogas which is a combination of methane (CH_4) 55%~65%, carbon dioxide (CO_2) 40% ~55%, and some trace gases at levels of less than 1% (Karki, 2005; Vintila et al., 2012; Sun et al., 2015). Bio-methane gas represents the energy value of biogas and thus, the higher the methane content, the better the quality of biofuel for energy production (Karki, 2005; Sun et al., 2015). The substrates' physicochemical properties and process design of reactors are the fundamental considerations of biogas quality and quantity (Angelidaki et al., 1999; Salomon & Lora, 2009). Thereby, microbiological, chemical and substrate characteristic are key factors for the success of an anaerobic digestion (AD) producing

biomethane (Wilkie, 2005; Yu et al., 2014). These factors must be under process control/monitoring. Unlike co-digestion, in which two or more feedstock materials are dispensed into the AD system, mono-digestion requires either catalytic or nutritional supplementation to maintain balanced growth of the diverse bacteria contributing to each bio conversion process (Hinken et al., 2008). Literatures gave evidence that the micro-nutrient (i.e., Fe, Co, Ni, Se, Mo, and W) requirements is essential to ensure process stability in anaerobic reactors (Möller & Müller, 2012; Choong et al., 2016; Güiza et al., 2016).

Out of aforementioned metal nutrient, in recent years, the importance of iron for methane-forming bacteria has drawn new research interest (Feng et al., 2014). In particular, using elemental iron or zero valent iron (ZVI), researchers demonstrated that iron reduces CO_2 into CH_4 by means of electron donation from elemental iron (Carpenter et al., 2015; Joo et al., 2018). The presence of iron in the reactors, acetic acid and acetate, which are the key intermediary products of acidogenesis promote CH_4 formation (Feng et al., 2014). The reaction kinetic pathway of iron in the process is according to Eq. (1-4). Iron in the biogas process reduces itself, forming more H^+ in an aqueous substrate to convert CO_2 to CH_4. This is known as hydrogenotrophic methanation (Eq. (1 & 2)). Additionally, through the acetoclastic pathway of Eq. (3 & 4), ZVI has been shown to stimulate syntrophic action, enabling a bioconversion process to form more biomethane from acetate and acetic acid (Feng et al., 2014; Carpenter et al., 2015; Joo et al., 2018).

$$CO_2 + 4Fe^0 + 8H^+ \rightarrow CH_4 + 4Fe^{2+} + 2H_2O \qquad (6.1)$$

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + H_2 + 2OH^- \qquad (6.2)$$

$$CH_3COO^- + H+ \rightarrow CH_4 + CO_2 \qquad (6.3)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad (6.4)$$
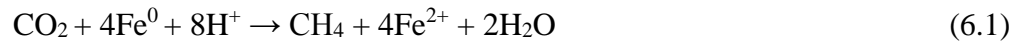
There have been several studies with contradicting results. In Ibrahim and Abdulaziz's (2016) batch studies concluded that the presence of scrap iron increased methane production by up to 61% in waste activated sludge. Likewise, Liu et. al. (2015) found that either clean or rusty scrap iron were found more effective than iron powder in methane enhancement. This study paved the way for introducing iron powder for increased biomethane production in the methanogenic phase and consequently harnessing more energy gas and reducing upstream process burdens. 17% increase in methane production was achieved when waste scrap iron metal was added to a waste up-flow anaerobic blanket reactor. This also yielded an additional 21% COD removal (Zang et al., 2011). The studies highlighted that iron decreased the oxidation/reduction potential (ORP) and helped in buffer control. In contrast, Yanget al., (2013) observed that nano-particulate ZVI and ferrous iron could disrupt certain types of bacterial cell membranes and caused inactivation in unaerated conditions. Yang et al., (2013) reported application of extremely minute ZVI particles (< 100nm) led to inhibition on methanogenesis. The author suggested iron powder with a larger grain size increased methane production. Using 0.2 mm grain size iron, Feng et al., (2014) demonstrated that up to 43.5% methane production was possible in a waste

sludge from UASB reactor. Ignace et al., (2016) used iron powder to increase methane yield to 43.6% in sewage sludge. The aforementioned experiments and literature reports were conducted in either mesophilic or thermophilic environments with waste activated sludges, and their outcomes are controversial. However, studies of iron amendments in organic biomass fermentations at ambient temperatures is yet to be elucidated.

This study emphasizes the performance of anaerobic digestion in Continuous Stirred Tank Reactors (CSTRs) with iron used as a catalyst to enrich the methane content. The objective is to examine the improvement of biomethane conversion using iron amendment, reducing CO_2 to CH_4 under temperature swings and controlled settings in an optimizing methane fermentation of cassava pulp and wastewater to develop a practicable option in existing biogas plant. Since a higher CO_2 content affects the quality of biogas, the finding may help in cutting CO_2 scrubbing costs by mean of enhanced methane. With the evidence found at the lab scale, this study aims to foster biogas process that could be improved by simple catalytic iron amendment in bio-reactors.

6.5   Materials and methods

6.5.1   Substrate characteristic and synthesis

Cassava pulp, which is a common feedstock for fermentation, was used as a substrate in this study. It was collected in batches from Korat Starch Factory in Nakhon Ratchasima City, Thailand. The physicochemical characteristics of the fresh cassava pulp and wastewater sample were analysed upon collection after which it was stored at the temperature below 4 ℃. Then, a cassava substrate (5 TS % w/v) was

synthesized for the entire experiment in 50-liter polypropylene barrels, and its characteristics were periodically compared against fresh wastewater from the factory. The justification for applying 5% TS solid regime is referred to Chapter 3. The characteristics of fresh and synthesized wastewater are shown in the Table 6.1.

Table 6.1 Substrate Characteristics

Parameter (Units)	Fresh Cassava Wastewater	Synthesized Cassava Wastewater
pH	4.5 ± 0.2	$4.2 \sim 4.5$
Total Suspended Solids (TSS - g/l)	$2 \sim 3$	5 ± 0.5
Volatile Suspended Solids (VSS – g/l)	$2.55 \sim 2.8$	3.25 ± 0.25
Total Chemical Oxygen Demand (tCOD – mg/l)	$20,000 \sim 25,000$	$10,000 \sim 12,500$
Soluble Chemical Oxygen Demand (sCOD – mg/l)	$5,000 \sim 8,000$	$5,500 \pm 500$
Volatile Fatty Acids (VFAs – mg/l) at pH 4.2 ~ 4.5	$4,000 \sim 6,000$	$4,500 \pm 500$
Volatile Fatty Acids (VFAs – mg/l) at pH 7.0	-	1200 ± 100
NH_3-N (mg/l)	$100 \sim 300$	N.D.
TKN (mg/l)	$700 \sim 1,000$	N.D.

*N.D. = Not Detected

Aeration arrangement was incorporated for 30 mins in every 4 hours by an automatic aerator to make it more resemble to fresh wastewater and sustain the growth of fermentative microbiota in course of hydrolysis and mixing. This study includes two steps (separate fermentation) in which one being hydrolysis of the feedstock material took place in an aerobic tank, and the other, methanation was

performed in anaerobic CSTRs. Seeds (inoculum) were collected from a parent anaerobic covered lagoon prior to executing the methanation process. In order to maximize initial CH_4 content of biogas, the pH was set to 7.5 before iron amendment. Commercial grade anhydrous sodium bicarbonate ($NaHCO_3$) powder was used as weak base buffering agent for controlling active acidification insides reactors upon feed loading.

6.5.2 Digestion settings

Laboratory scale CSTR is schematically shown in the figure 6.1, the anaerobic digestion in this study was divided into two phases. The first phase includes an equalization process to achieve steady state in 6 CSTRs operating in parallel. This was done to determine the hydraulic retention time (HRT) at an optimum Organic Loading Rate (OLR) with respect to the maximum raw biogas yield prior to iron amendment. Six CSTRs were set up, each with a two-litre working volume and an equal food to microbe ratio (F/M) under ambient environmental conditions. The experiments were conducted in an opened indoor site where the ambient temperature ranged between 24 ~ 32 ºC throughout the day during three months of the rainy season (June to August 2018). Stirring was controlled in timer at a rate of 150 rpm for 15 mins of operation in every two hours. Under steady state, a stable organic loading rate (OLR, 3.25 ± 0.25 g VSS L^{-1} day^{-1}) was obtained among the CSTRs with $\pm 10\%$ variance in biogas yield (600 ± 50 ml /day) at a hydraulic retention time (HRT) of 16 days. A stable volatile fatty acids (VFAs) to total alkalinity (TA) ratio (VFA/TA) of 0.5 was maintained for a healthy reactor. Then, reactors were operated for up to 10 days before iron amendment.
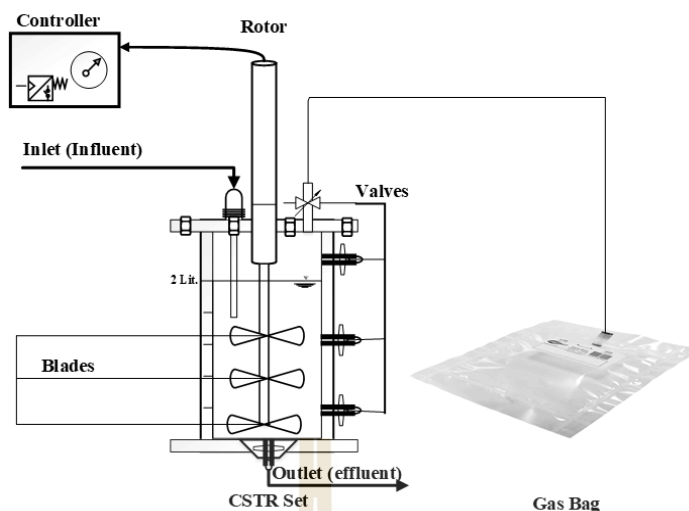
Figure 6.1 Schematic Diagram of AD CSTR System

The second phase, iron amendment at five different levels (5-10-20-40-80 g/L) was executed under the same operational configuration as in the first phase. The performance of the CSTRs with and without iron amendment was monitored for next 30 days. The current study highlights performance of CSTRs after iron treatment (second phase) with regard to process stability and methanation after CSTRs attained steady state to examine effect of iron amended CSTRs for comparative investigation on optimum methanation.

6.5.3 Scrap iron and characterization

Scrap iron from a mechanical workshop at Suranaree University was chosen as catalyst. These fragments of iron are industrial residues of commercial wrought iron plates and bars which resemble scrap iron from an iron works, and they are readily available. The scrap iron was thoroughly rinsed in octane, cleaned and dried with de-ionized water before characterization using X-ray photo-electron

spectroscopy (XPS) at beamline 6.2b of the synchrotron light research institute (SLRI), Thailand and scanning electron microscopy (SEM).
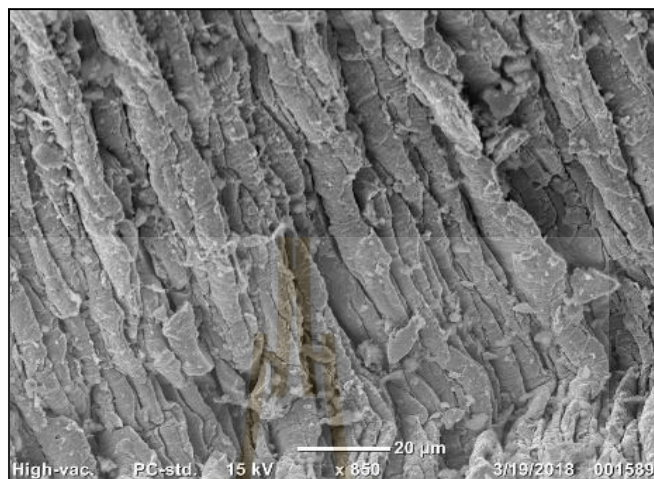
Figure 6.2 SEM Image of Scrap Iron

Varying in grain size from 0.5 – 5 mm, the SEM image of scrap iron in the figure 6.2 reveals a crystalline structure of strip pattern with a partially fragmented surface. Through surface contact with a substrate, this bonding fragmentation acts as a catalyst for bio-degradation during substrate conversion and bioavailable source of iron for microorganism. XPS analysis also revealed the atomic concentration of elements on the iron's surface layer. The respective peak along XPS spectrum of selected scrap iron samples (Figure 6.3) showed that it is composed of 73.22% Fe, 17.51% O, 6.36 % C and 2.91% other inorganic matter.
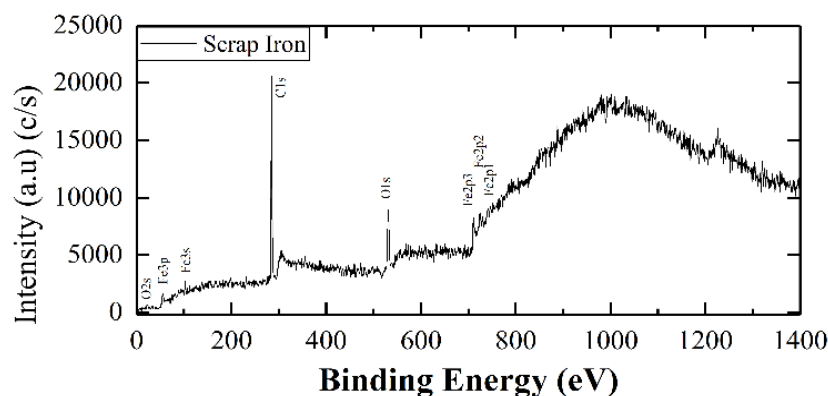
Figure 6.3 XPS Spectra of Scrap Iron

APHA standard methods (2012) were applied for determination of VFAs, residual solids, and chemical oxygen demand (COD). For volatile fatty acids (VFAs) and total alkalinity (TA) profiles, a three-point GLP titration method was employed using a Titroline 7000 automatic SI analytic machine in accordance to ASTM D4274-99 standards. The pH was measured with a HORIBA Scientific pH meter. Analytical samplings were conducted in triplicate for each sample on a daily basis throughout experimental period. Quantitative biogas volume was determined using an equivalent specific water replacement method in a pressure head swing. Biomethane was collected in one-liter sized SKC Tedlar® sample bags, then the relative content of the gas was analysed using an Agilent 7890A GC system. Chromatographic gas demarcation was performed every other day throughout the iron amended methanation period.To avoid possible interference, the variances between each sample during laboratory investigation of measured parameters were eliminated by triplicating each individual sample set. For all parameter of the experiments involved in this research, the t-test has been constructed to determine whether the difference between the average of triplicated numerical result of measured samples and the

control equals. The descriptive statistics and t tests were generated in origin 2018 statistical software package. The hypothesis is as follows:

H_0:   there is no difference in term of numerical values between specific parameter and control set.

H_a: there is a difference in term of numerical values between of specific parameter and control set.

At the same time, comparison among measured samples are not likely equal to that of the control sample. Therefore, computed P-value is always less than 0.05, and the null hypothesis can be rejected.

6.6   Results and discussion

6.6.1   Effect on buffer resistance of substrates

The longer the time it took for pH to drop below the critical pH 6.9 value, the higher the buffer resistance of the substrate is (Cioabla et al., 2012). The figure 6.4 reveals buffer resistance test. The results of the experiment uncovered the substrate varied in a way that was directly proportional to exposure to the iron. Although, the hydrolysed feedstocks with low pH values (4.2~.4.5) could be adjusted to the optimum condition of anaerobic digestion (pH 7.0~7.5), it is impossible to neutralize fresh substrate on daily basis by buffering it to the optimum pH. Dissolved in an acidic substrate, $NaHCO_3$ which is weak base, increased pH to slightly above 7.0. Thus, it exhausted the VFAs and escalated TA rapidly, leading to a saponification effect. This is because $NaHCO_3$ has alkaline properties. Its presence in the buffering process of the system promotes conversion of long chain fatty acids into other organic functional groups leading to further VFA depletion. Subsequently it disrupts the

balance VFA to TA ratio. As a result of adding a buffering agent, VFAs dropped from 4500 ± 500 mg/L at pH 4.5 to 1200 ± 100 mg/L at pH 7.0. While VFAs are the primarily derivative in acetate syntrophy by acetoclastic bacteria, acid buffering of the substrate can impede the acetogenesis process. This potentially causes a nutrient shortage for the subsequent methanogenesis by methanogenic archaea (Manyi-Loh et al., 2013). Using a separate two-step digestion (fermentation and methanation), a hydrolysed organic substrate was used to produce methane in this study. As the result of acidic organic feed loading which possesses high VFAs loading, the TA requires adjustment in line with added VFAs in the reactors (Cioabla et al., 2012). Therefore, because of the added VFAs from OLR and subsequent synthrophic action of AD bacteria, the pH drop occurred upon the next OLR loading, and thus frequency of buffering and $NaHCO_3$ chemical consumption was increased to maintain the optimum pH (7.0 ~7.5). Without iron amendment, the CSTRs required buffering by chemical addition every two days to maintain the optimum pH for an ideal anaerobic digestion.
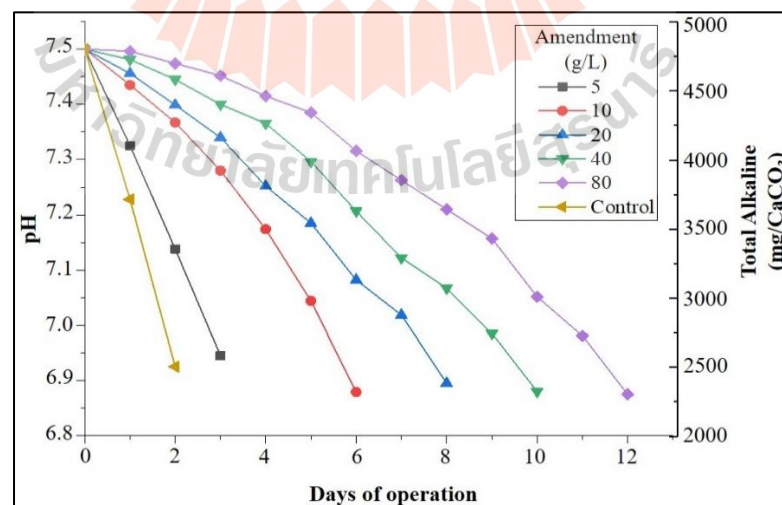
Figure 6.4 Buffer Resistance of Substrates by Iron Amendment at Various Concentrations

The increased iron concentration caused a greater buffering capacity inside the reactors. The 80 g/L level was the strongest, requiring lease frequency for re-buffering only once in 12 days. Higher levels of iron addition resulted in stronger buffering. Reaction kinetics among products of anaerobic digestion process also caused a pH drop. To counter this situation, the mass and specific surface area of scrap iron contributes iron bioavailability to the microbial community and stability of the AD process (Smith & Carliell, 2009). Stirring promotes surface contact between iron surface and substrate to lessen the pH drop and creates a buffering action until the iron is exhausted (Adamson & Gast, 1967). Higher amendment levels provide with more contact surface in the CSTR. Therefore, surface reactions of iron with the liquid media in CSTRs were promoted by intermittent mixing action. The combination of iron amendment and mixing arrangement helped to retard rapid pH changes within active digestion by avoiding sludge and substrate isolation for ensuring sustainability of anaerobic digestion in the CSTRs during the methanation process. It also reduces the frequency of chemical needs for buffering because of substrate homogeneity inside the CSTR reactor. On average, it needed 7-9 g of anhydrous $NaHCO_3$ powder to buffer one litre of either fresh or synthesized cassava wastewater to obtain pH 7.0. By cutting the frequency of re-buffering throughout the digestion period, as shown in the figure 6.4, less sodium bicarbonate ($NaHCO_3$) was used for pH control, and this subsequently fostered process stability, ease of chemical intervention and economic cost for long-term operation.

6.6.2 Effect on VFA/TA ratio

The VFA/TA ratio represents the overall stability of an anaerobic digestion processes under which acid and methane forming microorganisms are held

in balance (Li et al., 2013). Since end products of each step of the anaerobic process are the major source for the subsequent step in the system, it is crucial to maintain a balanced proportion of the various types of anaerobic microorganisms for syntrophy (Venkiteshwaran et al., 2015; Nguyen et al., 2019). A low VFA/TA ratio implies a low pH with increased acidification inhibiting the growth of methane forming archaea. Alternatively, a high VFA/TA may lead to nutrient exhaustion for methanogens and reduce the numbers of fermentative bacteria. An ideal VFA/TA has been suggested in several literature reports as 0.3. VFA/TA ratios lower than 0.15 or higher than 0.5 will result in inhibition (Kim et al., 2006; Appels et al., 2008). Taking the benchmark VFA/TA at approximately 0.5 for all steady state CSTRs prior to iron amendment, the effect of iron on the VFA/TA ratio was investigated. As the consequence of the pH adjustment, the VFAs of fresh substrate were reduced from 4500 ± 500 mg/L at pH $4.0 \sim 4.5$ to 1200 ± 100 mg/L by $NaHCO_3$ saponification (Table 6.1).Results for 30 HRT days of iron supplementation revealed that the presence of iron reduced the VFA/TA ratio in direct proportion to iron concentration instantly upon its addition and sustained the VFA/TA drop as long as iron existed in the reactors (Figure 6.5). Out of the five different levels, 20 g/L iron concentration maintained the ideal VFA/TA ratio. 80 g/L amendment inhibited digestion. Sequentially doubling the iron amendment (5-10-20-40-80 g/L) resulted in decreased VFA/TA ratio of 8%, 14%, 32%, 50% and 70% respectively, of that of the iron free control reactor.
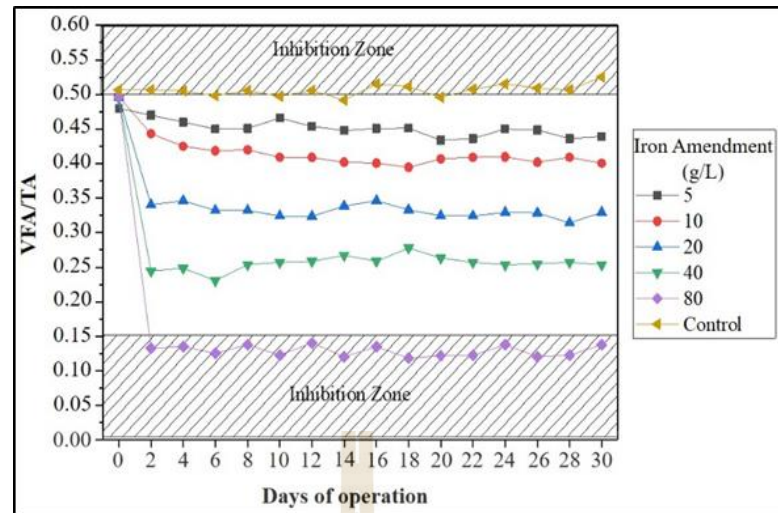
Figure 6.5 VFA/TA Profile During 30 Operation Days

6.6.3   Effect on COD removal and process stability

During operation, the soluble Chemical Oxygen Demand (sCOD) represents the major food source for the diverse anaerobic bacteria in the biogas process, particularly to the early AD processes (Verma, 2002; Mata-Alvarez, 2003). Therefore, the state of reactor is usually reported in terms of COD or solids removal in digestion processes since AD processes were discovered (Grau et al., 1975). The total COD (tCOD) includes indigestible and soluble COD (sCOD), but only sCOD is accessible by microorganisms for bioconversion into biogas. Prior to iron amendment, the optimum OLR was found to be 3.25g VSS L^{-1} day^{-1}, which is equivalent to approximately 3000 mg/l O_2 of sCOD loading. This OLR remained unchangedto examine the comparative performance of iron amended CSTRs against controlled ones. It was discovered that the stimulatory effect of iron metal was at iron levels of 5 to 20 g/L. The optimal amount was found to be 20 g/L, and this resulted in more than 95% sCOD removal with daily tCOD addition. From Figure 6.5, inhibition occurred

when iron amendment exceeded 20 g/L. Levels of 40 and 80 g/L resulted in toxicity to microorganism in the reactors. The level of sCOD removal dropped to as low as 20% at the end of the digestion period from its initial stage of 78% (Figure 6.6). The accumulation in residual sCOD from daily OLR triggered disproportionately high nutrient levels culminating in excessive microbial growth and digestion failure. This is due to iron toxicity from accumulation of oxidized iron (Fe^{2+}) or its conjugates with organic acids (Jackson, 1990).
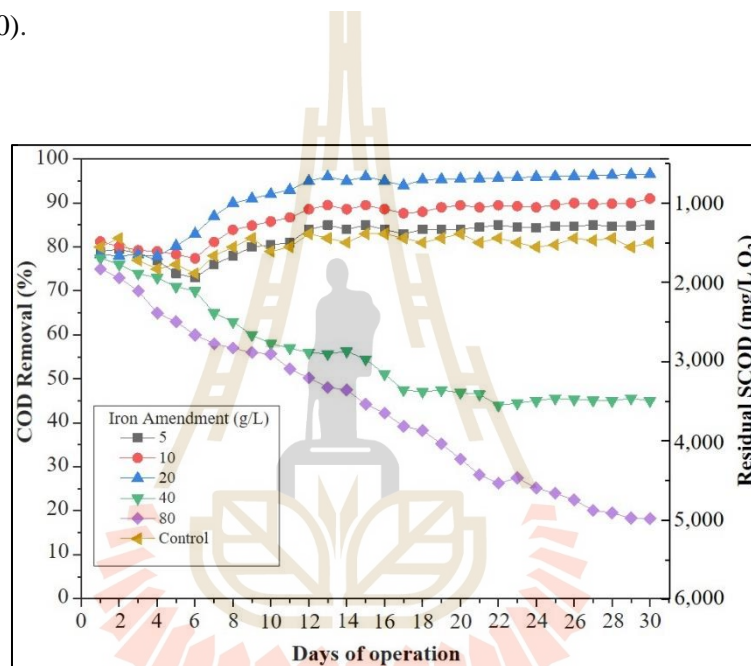
Figure 6.6 Effect of Iron Amendment on Digestion Performance

The sCOD removal rate of the control CSTR varied by around 80% throughout the investigation period. The issue of residual sCOD in a healthy CSTR can be handled by means of daily feed flow (inflow/outflow) from the CSTRs. Despite improved digestion in the CSTRs with 5-10-20 g/L of iron supplementation after 7 days, a diminishing effect was seen in all CSTRs in the first few days (up to day 4). This was either a result of the influence of ambient environmental factors

(prolonged torrential rain) during days 2 and 6 or the instantaneous response of microorganisms to iron amendment. Nevertheless, the impact of iron addition could be differentiated from recovery of stimulatory rates (5-10-20 g/L) and of inhibitory rates (40-80 g/L) against a control CSTR after 6 days of iron amendment (Figure 6.6). The sCOD removal declined with inhibitory levels of iron. This was the consequence of iron toxicity that disrupted the balanced among anaerobic microorganisms and intermediary products in the system. This assumption is further substantiated by the performance of CSTR in gas yield and quality.

6.6.4 Effect on bio-methane enhancement and gas yield

The quality and quantity of biogas enhancement by catalytic action of iron is illustrated in the figure 6.7 (a and b). Neglecting the trace gases (i.e., H_2S and NH_3), the biogas consists of methane (CH_4) and carbon dioxide (CO_2). The CSTRs had initial optimal CH_4 content between 46~54% before iron was introduced. Based on the chromatographic investigation on the biogas produced in CSTRs after iron amendment, 20 g/L level generated highest bio-methane content (up to 73%) as the investigation progressed. The control reactor remained almost unchanged (around 50% methane). The outcome of iron modified methanation was obvious after 10 days of iron exposure (Figure 6.7a). The net increase in caloric energy of biogas was about 25%, 18% and 16% improvement by 20, 10, 5 g/L by iron additionrespectively. The bio-methane improvement happened either as in Eq. (1) or the ideal amendment supported the anaerobic digestion process along with bio-conversion processes. However, higher levels of iron treatment resulted in slightly lower methanation in CSTRs with 40 and 80 g/L. Therefore, the hypothesis of iron modified formethanation was justified by the results obtained. In the experiment of Agani et al. (2016),

methane production was 77.6% using iron powdercompared to 58% in a control reactor. Comparing rusty scrap iron and new iron, Ibrahim and Abdulaziz (2016) reported that 82% methane increase was obtained using 15 g/L rusty iron concentration from a waste sludge anaerobic digestion . Similarly, using waste activated sludge, observed only 30% increase in biogas production using 10 g/L of scrap iron (Liu et al., 2016). In this work, applying cassava pulp organic waste, only 25% more biogas volume was obtained with 20 g/L of scrap iron supplementation. However, 91% increase in methane yield using iron powder was produced compared to a control group in waste activated sludge (Zhang et al., 2015).

(a) Biomethane Enhancement   (b) Daily Biogas Yield
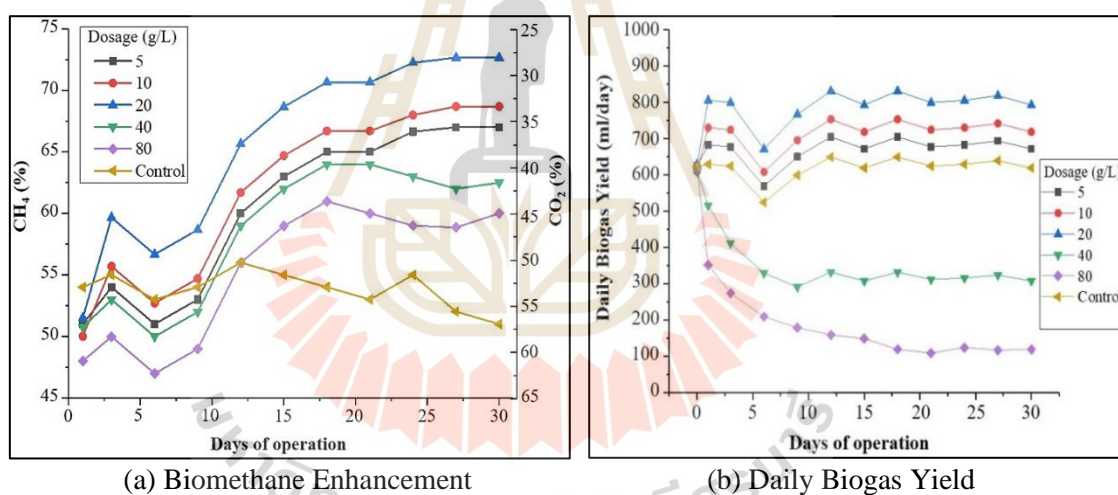
Figure 6.7 Effect of Iron Amendment on Methane Enhancement

In terms of gas volume, under the same OLR of 3.25 ± 0.25 g VSS L^{-1} day^{-1} (500 ml synthesized substrate added), the control CSTR produced about 600 ml/day, while the CSTR with 20g/L iron delivered 800 ml//day, which is about 35% increase. However, supplementation beyond 20 g/L was found problematic despite the 5-10% higher values of bio-methane over the control reactor, the gas yield declined sharply

to less than 200 ml/day after iron amendment (Figure 6.7b). Lower gas production represents the collapse of the anaerobic digestion process as the consequence of excessive iron levels leading to iron toxicity. In the recent study of Wei et al., (2018), a relative increase in methane content of 27% increase was reported when extremely fine iron powder was used for methane production in a primary sludge. This study suggests that using micro and nano sized iron powder may lead to superior biogas quality. This assumption was supported by Carpenter et al., (2013) in which a maximum increase of 28% methane production was possible by using nano-sized iron powders.

6.7   Conclusions

As a part of waste-to-energy technology, anaerobic digestion processes are an alternative to recover green and sustainable energy from agro-industries from its biomass by-products. However, the limitation of biogas is its low bio-methane content and high process costs. These remain major challenges. Using a long HRT (30 days) in this comparative study, it was observed that only amendment of a low-cost iron scrap iron as catalyst resulted in bio-methane with an enhanced caloric content from 16% to 25% and 10% to 35% greater gas volume. Even better bio-methane results can be envisioned when better quality iron source is applied. Additionally, the presence of iron in an anaerobic digestion has been found beneficial in iron amended reactors in comparison to control reactors in this study, regulating the VFA/TA ratio. Thus, the process performance found more stable.

This study validated that iron assists in methanation processes. Furthermore, the benefit of iron amendment in anaerobic digestion can also provide with the

economic benefit of reduced costs for alkaline chemicals for buffering and process stability. Higher methane content reduces the burden of gas purification in post biogas production processes. Hence, iron supplementation promotes not only the anaerobic digestion process for improving methanation but also lower subsequent costs in upstream processes.

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CHAPTER 7

MICRO-PARTICLE ZVI INHIBITION THRESHOLD IN CASSAVA PULP METHANATION

7.1 Graphical Abstract
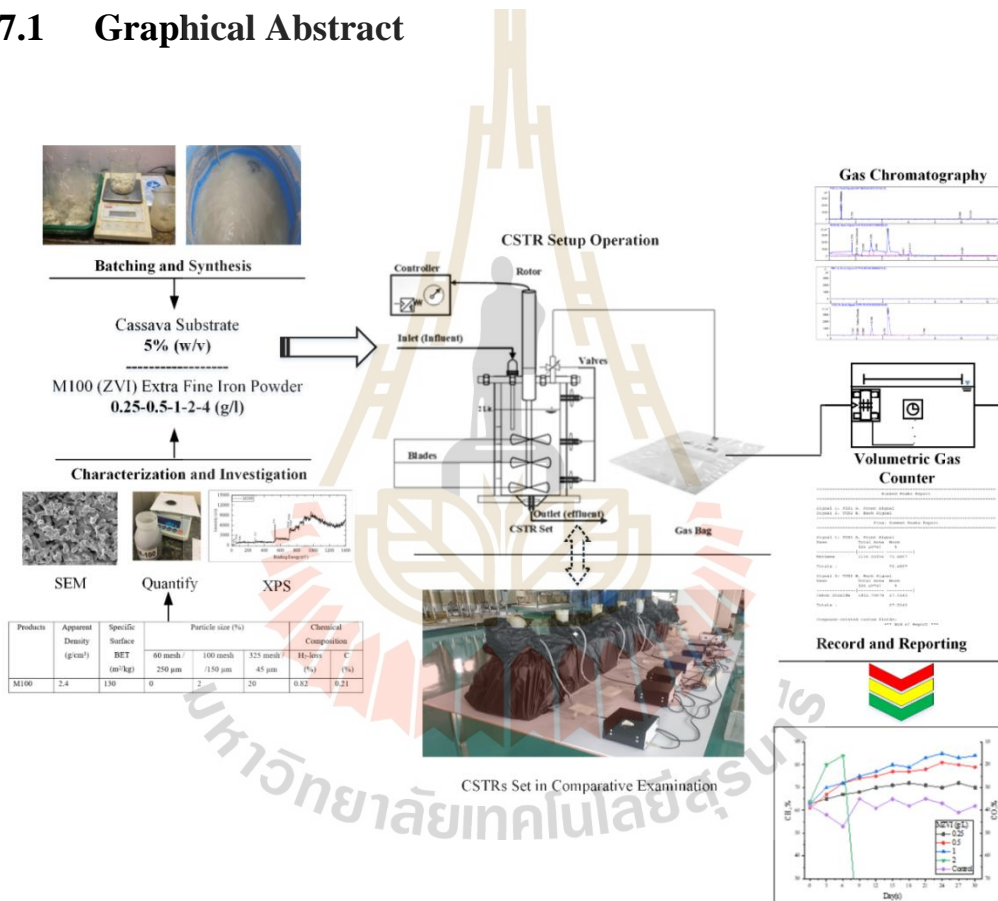
7.2 Highlights

- XPS survey scanning revealed atomic concentration of M100 iron micro particle was found having Fe, O and C in 93.01%, 4.85%, 0.66%, and negligible other inorganic metal 0.48% respectively.

- 1 g/L ZVI micro particle concentration resulted maximized sCOD removal (up

to 95%) generating biogas for 900 ml/day/OLR with highest methane content (85%).

- Inhibition started when iron concentration exceeds 1 g/L ending digestion failure by iron toxicity after 5 days along comparative study for 30 days operation time.

-   Up to 350ml (58 %) gas volume enhanced in 1g/L concentration with 40% methane increase.

-   Excessive presence of MZVI led imminent digestion failure when extra fine microparticle zero valent iron is applied as catalytical agent in biogas process for organic biomass.

7.3   Abstract

One setback of mono-digestion for biogas production is low methane (CH_4) content, the core energy calorific value. Catalyst and nutrient supplementation are the option to improve biogas both in quantity and quality by reducing carbon dioxide (CO_2) back to methane (CH_4). Using CSTRs, this study attempted to enhance bio-methane from cassava pulp and its wastewater by readily available reducing agent, Zero Valent Iron, Fe^0 (ZVI). M100 iron in micro-meter size particle was verified and characterized by synchrotron lights (XPS) whether it is in state of valency 0 for optimum reactional kinetic. Introducing ZVI in 0-0.25-0.5-1-2 g/L dosage, it was discovered that 1 g/L ZVI microparticle concentration resulted maximized sCOD removal (up to 95%) generating biogas for 900 ml/day/OLR with highest methane content (85%). Although the presence of ZVI is stimulatory, inhibition started when iron concentration exceeds 1 g/L ending digestion failure by iron toxicity after 5 days along comparative study

for 30 days HRT. Meanwhile, the performance of CSTRs in remaining doses function stably among which ZVI microparticle in 1 g/L supplemented CSTR processed in ideal anaerobic digestion conditions. This study inspires the possibility of enriching more bio-methane by cutting hefty cost and chemicals consumption in subsequent gas upgrading processes.

7.4   Introduction

In order to avoid climate change, Countries are abandoning fossil fuel that triggers major greenhouse gas emission, particularly coal for electricity generation. It is therebefore, renewable sources become pivotal role to ease dependence on this controversial and environmental polluting option (Staudt, 2011). However, each individual renewable energy source has inherent limitations. Despite the fact that advanced technologies inspire mass production of solar panel and wind farms in cutting initial cost of investment and offers more attractive in unit cost per unit electricity, temporal, seasonal and locational factors being major concerns (Bazilian et al., 2012; IEA Report (2013)). Bioenergy lacks these shortcomings and the technology is more compatible with agriculture-oriented country to produce biofuels from agro-industrial residues and by-products. Out of several alternatives of biofuels, the merit of biogas over its counterparts are economic feasibility, ease of process control, applicability of various organic material and more (Sriram & Shahidehpour, 2005; Das, 2009).

Anaerobic Digestion (AD) for biogas includes four backbone processes (i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis) by syntrophic action of diverse microorganism of each step of biogas process. On average, biogas consists of

40-65% of bio-methane (CH_4), 35-60% of carbon dioxide (CO_2), and some traced gases (NH_3, H_2S, SO_2, etc.). The setback of biogas is low methane content, the energy calorific value for energy production. Most literature emphasizes biogas production from different biomass materials and higher yield through pre-treatment, co-digestion with other organic/inorganic source, and nutrient supplementation (Kim et al., (2010); Ariunbaatar et al., 2014). However, low methane (CH_4) content remains unresolved leading biogas upgrading process at higher cost of chemical consumption and system configurations (Yadvika et la., 2014; Chen et al., 2008).

The application of catalysts draws renewed interest for enhancing bio-methane within AD process. Zero Valent Iron (ZVI) is believed to have oxidation-reducing potential (ORP), and thus serves as an acid buffer. This stimulates the growth of methanogens which produce methane by metabolizing of volatile fatty acids and carbon dioxide (Liu et al., 2015). ZVI improved methane production by 30-40% and methane concentration for 5-13% when activated sewage sludge was subjected to AD processes in batch experiment (Su et al., 2013). In an attempt to study the influence of ZVI in livestock substrate, the presence of ZVI in 20mg/L increased biogas volume and methane content by 1.45 and 1.59 times respectively (Abdelsalam et al., 2016). ZVI was also proved as electron-donor and promoted H_2 consumption by methanogens in enriching methane up to 38.2% (Zhen et al., 2015). The hypothesis of reaction kinetic of ZVI with intermediary products of anaerobic digestion is to improve methane as per following equations (Liang et al., 2000; Feng et al., 2014).

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + H_2 + 2\ OH^- \qquad\qquad (7.1)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad\qquad (7.2)$$

Besides beneficial of ZVI in AD process, Yang et al., (2013) suggested that ZVI exhibits disruption of cell integrity, and inhibits methanogenesis leading methane production by more than 20%. Again, Lee et al., (2008) and Auffan et al., (2008) highlighted that ZVI inhibits the growth of bacteria even at concentration as low as few mg/L since it induces reductive stress and interrupt cell membrane. At the concentration of 1.5 g/L ZVI in pure culture study, shu et al., (2011) reported that ZVI inactivated sulpahte reducing bacteria (SRB) and disrupt cell growth. Higher particle size was recommended and nanoparticle cut off point shall not begreater than 1mM and above, since ZVI stimulates higher reactivity and hydrogen gas more readily in liquid interface (Yang et al., 2013). Therefore, there is mixed reaction over the impact of ZVI on bacterial growth (Lee et al., 2008; Li et al., 2010; Xiu et al., 2010a, 2010b). On top of that, most studies employed activated sewage sludge and livestock manure. Thus, the impact of microparticle ZVI on organic biomass in methane enhancement remains to be elucidated.

In this study, the impact of microparticle ZVI (MZVI) in different concentration to improve bio-methane was investigated by mono-digestion of agro-industrial residues, cassava pulp in CSTRs. The objective of the study is to find out the stimulatory and inhibition threshold of microparticle ZVI in optimizing bio-methane.

7.5   Materials And Methods

7.5.1   ZVI Materials

M100 Microparticle Zero Valent Iron (MZVI) (20µm) was obtained from Höganäs AB, a leading Swedish based metallic powder manufacturer. Physical characteristic of M100 powder is as shown in the table 7.1. Surface investigation by X-ray Photoelectron Spectroscopy (XPS) in synchrotron light in reveals (figure 7.1) the characteristics and atomic concentration of selected iron powder whether retaining iron in elemental stage. The interpretation to the sharp peak of XPS survey scanning being the atomic concentration of M100 microparticle was found having Fe, O and C in 93.01%, 4.85%, 0.66% and negligible other inorganic metal 0.48% respectively.

Table 7.1 Size Characteristic of M100 Iron Powder (Höganäs Ab; Hu 2005)

Products	Apparent Density (g/cm³)	Specific Surface BET (m²/kg)	Particle size (%)			Chemical Composition	
			60 mesh / 250 µm	100 mesh /150 µm	325 mesh / 45 µm	H_2-loss (%)	C (%)
M100	2.4	130	0	2	20	0.82	0.21

Figure 7.1 XPS Survey Scanning of M100 Micro-particle

7.5.2   Substrate and Seeds

To make 5% TS cassava substrate, cassava pulp and wastewater waste were collected from cassava starch mill (Korat Starch Co. Ltd). Synthesized cassava wastewater was prepared in the laboratory throughout experiment period. Periodical physiochemical analysis was made to ensure synthesized wastewater is identical to fresh wastewater from the mill. Table 7.2 shows characteristic of fresh and synthesized wastewater applied in the experiment. Seeds inoculum was collected from parent covered lagoon biogas plant. Anaerobic digestion was executed within 24 hours after collection. Inoculum characteristics were found pH 7.75 ± 0.2, TS 12.15 g/l ($\pm$ 2.50), VS 10.55 g/L ($\pm$ 0.5), and C/N 25.02 ± 3.5.

Table 7.2 Characteristic of Fresh and Synthesized Cassava Wastewater

Parameter (Units)	Fresh Cassava Wastewater	Synthesized Cassava Wastewater
pH	4.5 ± 0.2	4.2 ~ 4.5
Total Suspended Solids (TSS - g/l)	2 ~ 3	5 ± 0.5
Volatile Solids (VS – g/l)	2.55 ~ 2.82	3.25 ± 0.25
Total Chemical Oxygen Demand (TSCD – mg/l)	20,000 ~25,000	10,000 ~ 12,500
Soluble Chemical Oxygen Demand (SCOD – mg/l)	5,000 ~ 8,000	5,500 ± 500
Volatile Fatty Acids (VFAs – mg/l)	4,000 ~ 6,000	4,500 ±500
NH_3-N (mg/l)	100 ~ 300	N.D.
TKN (mg/l)	700 ~1,000	N.D.

7.5.3 Reactor Setup and Analytical Methods

2 litres size Continuous Stirred Tank Reactors (CSTRs) were utilized in this study. Methanation was undertaken in ambient temperature with initial F/M ratio of 1. Over 30 days HRT period, daily organic loading (OLR) was set at 3.25 g VS/L day and mixing rate at 150 rpm @ 15 mins/2 hours. Microparticles were added on daily basic in 0-0.25-0.5-1-2 g/L concentration. Standard methods for examination of water and wastewater (A.P.H.A, 2012) was applied to analyse Volatile Fatty Acids (VFAs), Total Alkalinity (TA), soluble Chemical Oxygen Demand (sCOD), while pH was inspected by Horiba Scientific pH meter. Biogas was collected in 1-liter sized SKC Tedlar® sample bag, then the relative content of the gas was analysed in Agilent 7890A GC system. Gas volume was measured in pressure swing water displacement system. The schematic diagram of reactor set up is shown in figure 7.2.
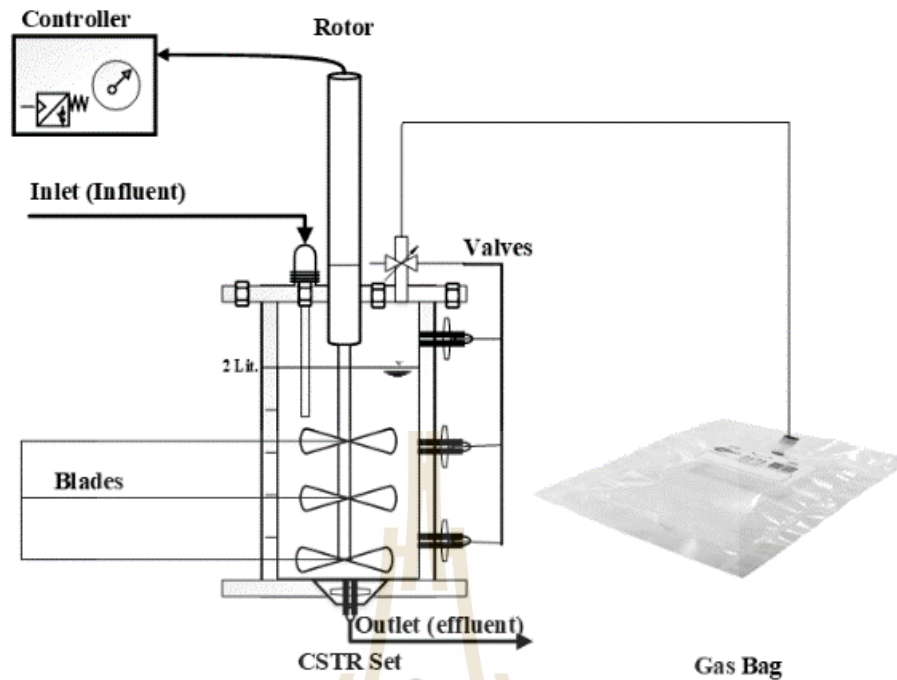
Figure 7.2 Schematic Diagram of CSTRs System

7.6 Result and Discussion

7.6.1 Effect of MZVI on Process Stability

Since the substrates for anaerobic digestion were subjected to fermentation during hydrolysis and acidogenesis, they tend to have acidic properties (low pH) prior to methanogenesis stage. Therefore, perpetual drop in pH encounters alongside digestion processes. It is necessary to preserve pH level in the digester slightly above 7.0 with +0.15 to +0.3 margin. And, the presence of ZVI halves abrupt pH fluctuation upon next organic loading and maintains pH during the course of methanation (Liu and Lowry, 2006). ZVI is responsible for this ZVI oxidation reaction ($Fe^0 + 2H^+ \rightarrow Fe^{2+} + H_2$) in which available protons from fluid substrate build-up pH rise (Carpenter et al., 2015). Although, pH range

between 6.5 to 8.0 is ultimate limit for digestion, the ideal pH ranged is between 6.8 to 7.3 (Ciobla et al., 2012). ZVI supplementation in this study revealed (Figure 7.3) that while pH statuses of ZVI free control reactor dropped along 30 days HRT period than its initial stage, those of ZVI added reactors' pH rose directly proportional to the concentration. Noticeable pH increment was observed after few days of ZVI was introduced. High pH indicates depletion of organic acids (volatile fatty acids, VFAs) resulting food shortage for methane forming methanogens and stress added to fermentative anaerobic microorganism leading imbalanced bio-synergetic processes. Thus, ZVI dose up to 1 g/L satisfied under optimum limit of pH as healthy rector, the excess to that concentration approached to the expanse of inhibition margin.
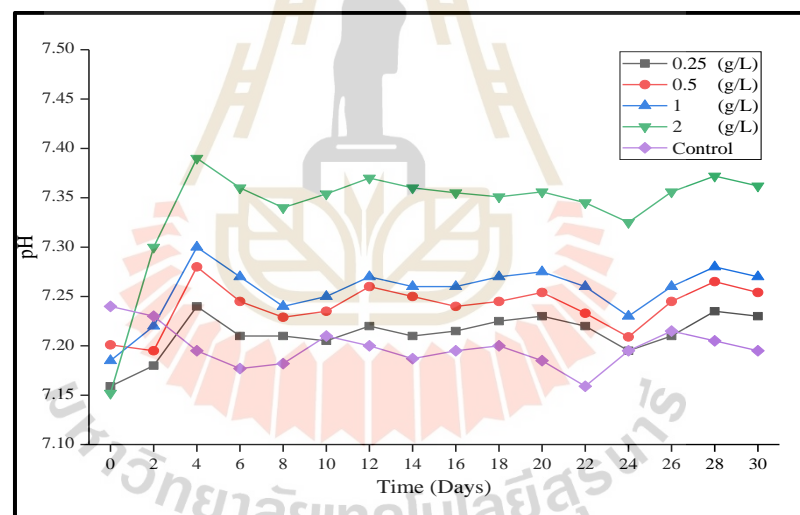
Figure 7.3 pH Changes by effect of M100 ZVI

In carpenter et al., 2015 experiment, pH rose relatively from 6.6 to 7.4 using commercially available and synthesized nanometre size ZVI as increase in concentration (1.25 g/L and 2.5 g/L). The authors concluded that reduced particle size (below 100nm dia.) may trigger more undesirable effect to the system by highly

reactivity properties of ZVI and instantaneous H_2 production. This assumption was testified by Yang et al., 2013's investigation in which average ZVI particle size of 55 ± 11 nm were applied resulting pH surged to 8.0 on 30mM ZVI (1.675 g/L equiv.) added, demonstrating 1mM (0.06 g/L equiv.) and above nanoparticle concentration inhibited methanogenesis. Nevertheless, in this study, the inhibition starting point is expected in excess of micro particle ZVI over 1 g/L concentration.

Volatile Fatty Acids (VFAs) and Total Alkalinity (TA) interpreted in ratio (VFAs/TA) reflects to the condition of the digestion, besides pH. The accumulation of VFAs from daily OLR and products of acidogenesis of anaerobic digestion process drags total alkalinity in the system which is vital for progression of methanogens for methanation, and vice versa, for fermentative bacteria. According to Lossie and Pütz (2008), ideal VFAs/TA had been recommended between 0.2 and 0.3. Process inhibition encounters beyond these margins as a result of either high and low organic loading, and syntrophic action among diverse bacteria (Sinaga et al., 2017). The result from the figure 7.4 of this study revealed that ZVI helped process stability by dissolution itself to break down excess VFAs into acetate from the organic feed loading by feed degradation. As in the figure 7.4, while VFAs/TA of control reactor were almost unchanged approaching upper inhibition thresholds (weak TA) throughout HRT period, VFAs/TA decreased from its initial stage of 0.29 to 0.2 in ZVI concentration up to 1 g/L. However, ZVI dose of 2 g/L resulted VFAs exhaustion leading to another inhibition thresholds (strong TA). In the experiment of Yang et al. (2013), reactors amended with 30mM (1.675 g/L) nanoparticle ZVI led failure by toxicity of nanoscale iron to methanogens. Alternatively, Zhang et al., (2015) demonstrated that ZVI assists accelerating VFA consumptions for acetate production

using larger particle size 0.2 mm diameter for which 2 g/L ZVI concentration performed most rapid VFA/TA drop along 30 days fermentation time. Literature suggested the particle size has strong influence on process stability of the anaerobic digestion while ZVI is employed for reducing agent in enriching biomethane (Carpenter et al., 2015).
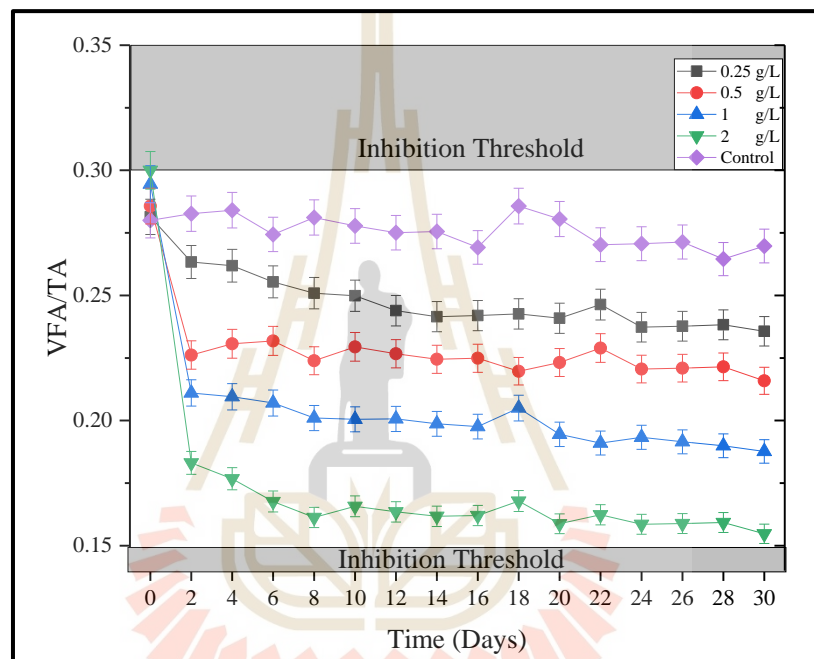
Figure 7.4 VFAs/TA Profile

7.6.2 Effect of MZVI on SCOD removal

Soluble Chemical Oxygen Demand (sCOD) acts as the food source which is accessible by diverse microorganism in anaerobic digestion in early phase of anaerobic digestion process (viz. hydrolysis and acidogenesis) (carpenter et al., 2015). The benchmark sCOD per daily OLR is as in the table 7.2which presents 5500 ± 500 mg/L. The higher removal represents better bioconversion of biomass feedstock into organic acids (volatile fatty acids). Alternatively, it is also the indicator for the

performance of reactor in which microbial activities are synergetically functioning for methanation. Since MZVI introduced, the results from the figure 7.5 indicated that MZVI helped in sCOD removal. Initial removal was found at unanimously at 75%, then the efficiency increased steadily with less residual sCOD than control reactor since MZVI introduced, reaching maximum removal rate for 97% at day 7 in bioreactors. The performance increased as increase in MZVI concentration, establishing 1g/L as ultimate yield point, since sCOD removal rate plunged below 30% when MZVI 2g/L rate was added and consequently more residual sCOD as the experiment proceeded. This satisfied to the studies of Liu et al., 2011 and Zhang et al., 2011b in which COD removal increased dramatically from 60.3% to 90.7% and from 70.2 % to 91.7% respectively.
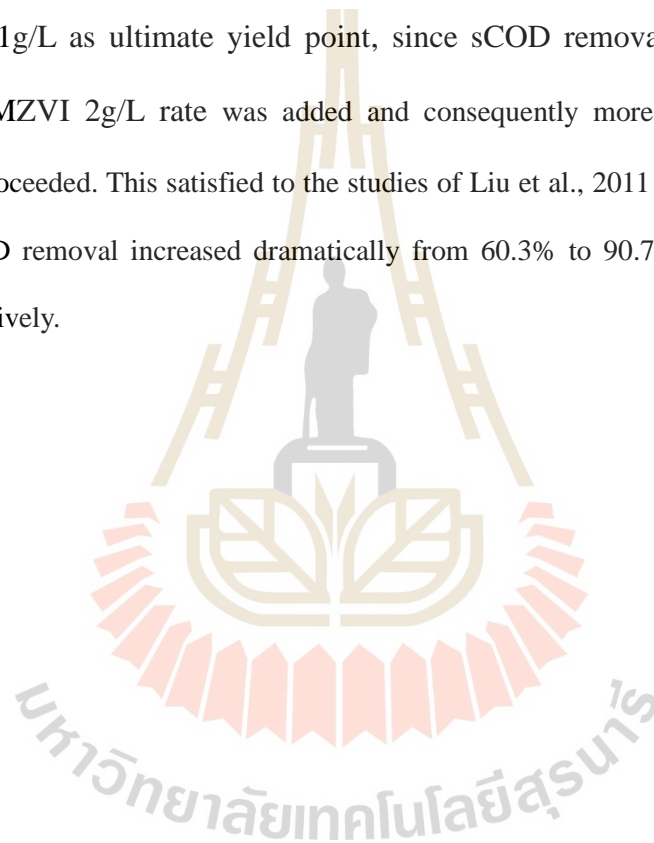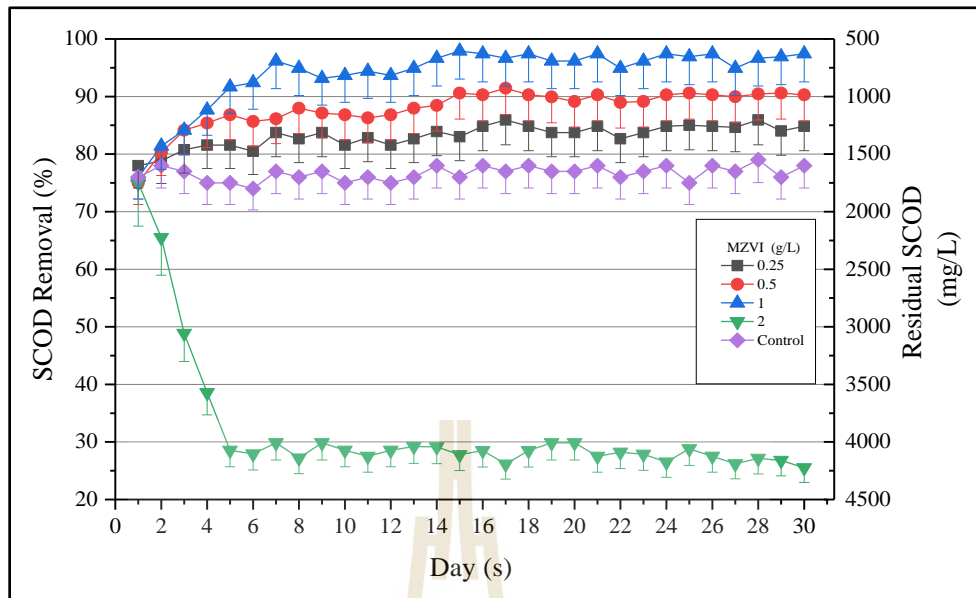
Figure 7.5 sCOD removal Efficiency by M100 ZVI Supplementation

Although, MZVI supports biodegradation of biomass by stimulating microbials' activities, the excess concentration could intoxicate the growth of hydrolysing and fermenting bacteria resulting complication in subsequent acetogenesis and methanogenesis stages of biogas process (Yang et al., 2013). Applying larger granular ZVI powder, Wu et al., 2015 reported that up to 90% COD removal was achieved within 30hrs. Again, using mm. diameter size ZVI granules, Zhang et al., (2015) presented 10 g/L of ZVI concentration was found highest sCOD reduction for VFA formation and most methane enhancement. In a recent study of Jia et al., (2017), 1 g/L of nanoparticle ZVI concentration resulted compromised biogas production and COD removal rate at 76% which is lower than present study. Therefore, as per previous reports of ZVI studies for methanation, it could be presumed that smaller ZVI grain size and surplus presence is objectionable in order to avoid instantaneous release of

zero valent iron (Fe^0) for bioavailability lest inciting biochemical reaction disorders within anaerobic digestion system.

7.6.3 Gas Yield and Quality

In co-relation to sCOD removal, since higher efficiency means greater bioconversion of organic degradability by anaerobic microbial, thus more biogas was produced.Upon introducing MZVI, it was discovered as in thefigure 7.6(a) that MZVI encouraged not only in improving gas yield but also the gas quality within the boundary concentration of up to 1 g/L concentration. Both biogas yield and quality improved immediately after MZVI supplementation within these margins. While, MZVI unamended reactor's gas yield was stable around 600ml/day/OLR, gas volume rose as increase in concentration. Up to 350ml (58 %) gas volume enhanced in 1g/L concentration with 40% methane increase (Figure 7.6(b)). Nevertheless, in the case of 2g/L dose reactor, though highest methanation was achieved in initial few days, digestion failure progressively occurred resulting continuous decreased in gas volume with rapid bio-methane content reduction. This is due to the fact that surplus of MZVI is more than 1g/L inhibited the sustainability of microbial growth resulting total breakdown to the whole the anaerobic system. Following the kinetic of the reaction, $Fe^0 + CO^2 + H_2O \rightarrow FeCO_{3(s)} + H_2$, more H_2 is produced in the system. Once again, the evolved H_2 recombined with CO_2 through the reaction of $2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$ known as hydrogenotrophic pathway subsequently producing more terminal product, biomethane by H_2 utilizing methanogens (Kotsyurbenko et al., 2004) (Yang et al., 2013). Complying to aforementioned mechanism and using 10µm ZVI particle size, methane formation could be increased from 0.0232 to 0.3098 mmol CH_4/mol Fe^0 day (Li et al., (2013)). In the recent study of Wei et al., (2018),

maximum biomethane could be enhanced 12-27% by 1g/l and 4g/L 0.2mm diameter size ZVI powder. Their finding was found inasmuch as to present study but different feedstock material. However, with the same activated sludge, Zhang et al., (2015) stated ZVI accelerated in methanation by 91.5% with shortened lag time for achieving steady state. However, in the case of nanoscale ZVI with 1g/L dose, cumulative biogas production could be increased of only 18.11% with 6.93% methane in the experiment of Jia et al., (2017). As a result of comparative literature so far, there is large discrepancy in both particle size and concentration in optimizing biomethane production potential. The physiochemical characteristic of feedstocks material also influences on ZVI's kinetic in biochemical conversion processes.

(a) Daily Biogas Yield   (b) Bio-Methane Content
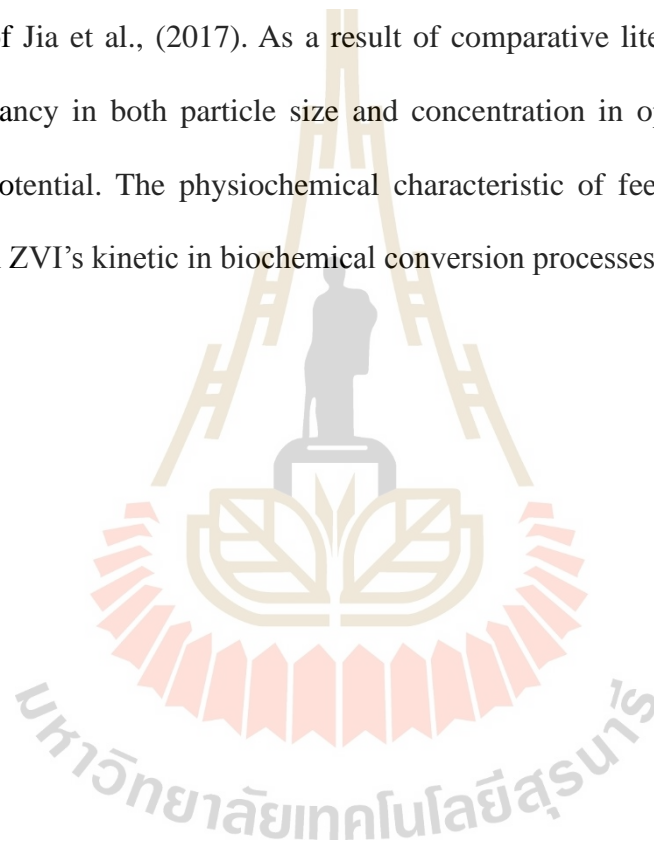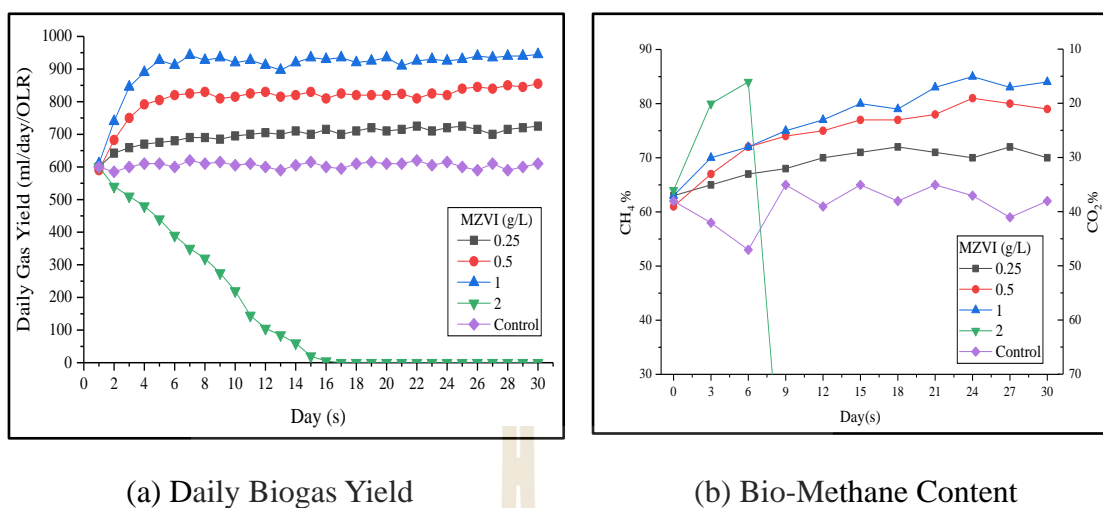
Figure 7.6 Volumetric Gas Yield and Methane Enhancement by M100 ZVI

7.7 Conclusion

The advantages of being abundant and cheap makes iron possible for using as reducing agent in hydrogenotrophic pathway is more favourable in economic sense. This investigation on the impact of microparticle MZVI for optimizing methanation of cassava pulp and wastewater uncovered the inhibition threshold of MZVI which was found 1 g/L concentration. Within this boundary concentrations, MZVI was proved stimulatory in process stability by maintaining reactors and provided with biomethane enhancement. Under the same unit OLR and controls factors in comparative CSTRs, biogas volume and methane content were increased 58% and 40% respectively against control reactor. However, excessive presence MZVI also indicated imminent digestion failure. Hence, precaution should be taken when microparticle zero valent iron is applied as catalytical agent in biogas process for organic biomass.

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CHAPTER 8

OPTIMIZING METHANATION BY DIFFERENTIAL ZERO VALENT IRON (ZVI) PARTICLE SIZE IN CASSAVA PULP FEED IN CONTINUOUS STIRRED TANK REACTORS (CSTRs)

8.1 Graphical Abstract

8.2 Highlights

- Under the optimum dosage for 1 g/l and 8g/l for M100 and R 12, up to 93% bio-methane enhancement has been achieved with 67% added biogas volume.

- The presence of nano-particle M100 ZVI in AD system had instantaneous and stimulatory impact by its readily reactivity with aqueous substrate, but it leads digestion failure even with few margins of excess.

- Instantaneous decline in VFA/TA was observed upon ZVI supplementation, while ZVI free control reactor's VFA/TA remained closer upper inhibition threshold of 0.3 in both nano-particle (M100) and micro-particle (R12) cases.

- Microscale ZVI (R12) amended reactors were more resistance to reaction kinematic stress and longevity for bioavailability.

- ZVI addition in optimal concentration proved stimulatory for methane enhancement and gas yield up to 93 and 67% respectively.

8.3 Abstract

The inherent limitation of biogas from Anaerobic Digestion (AD) process is high in carbon dioxide (CO_2) which deters and remains as a challenge for ultimate applicability of biogas as bio-energy for multi-purposes. The use of metallic powder as catalyst within AD process is promising to reduce both economic and environmental cost of post biogas upgrading technologies available nowadays. Utilizing Zero Valent Iron (ZVI) in differential particle size, M100 (nano-particle) and R12 (micro-particle), in cassava pulp and wastewater as feedstock materials for methanation, this study attempted to optimize methane (CH_4) concentration of biogas in Continuous Stirred Tank Reactors (CSTRs). Synchrotron X-ray radiations and Scanning Electron

Microscopy (SEM) were applied for characterizing the valency, atomic concentration, and particle size. The experiments revealed under the optimum dosage for 1 g/l and 8g/l for M100 and R 12, up to 93% bio-methane enhancement has been achieved with 67% added biogas volume than ZVI free control reactor. The presence of nano-particle M100 ZVI in AD system had instantaneous and stimulatory impact by its readily reactivity with aqueous substrate, but it leads digestion failure even with few margins of excess. The micro-particle size R 12 amended CSTRs demonstrated ease of better digestion performance, process control and recoverable properties by its slow disintegration and bio-availability in AD system.

8.4   Introduction

Amid global population growth, food and energy security has been a challenging issue for the world. As of recent UN's figures, by 2030, world population is expected to surpass 8.6 billion people from current number of 7.8 billion people (UN World Population Prospect: 2017). As the result of increase in population, attempts are being made to meet mounting challenges triggered by rising food consumption and energy demand (GAP Report 2017). On the other hand, the dwindling fossil fuel reserves and fluctuated crude oil price exacerbate the energy issues being confronted in every modern society. Despite the fact that the sustainable and renewable energies (i.e. solar, wind, hydropower and geothermal) assists the world with energy sufficiency to some extent, each technology holds inherent limitation from scarcity in resources from seasonal or temporal variation (Flohn, 1977). These shortcomings are eliminated in bioenergy technology in which the main substrate could either be biomass form agricultural by-products or wastes from numerous sources as long as feedstock materials consisting mainly carbohydrates, fat and protein (Hermiati et al., 2011; Jain et al., 2015). According to USDA 2018 reports, the global grain-starch

export and consumption of top producing countries like United States and China were steadily increasing from 2.5 million tons in 2007/08 fiscal year to 4.8 million tons in 2017/18 (WAP, 2018). Likewise, the corn starch production alone in Thailand increased 3~6 % in 2018 from that of 2017 figure reaching 5.3 million metric tons. Thailand also ranks world 2nd cassava root producer after Nigeria, and 1st producer and exporter for cassava-derived foodstuffs (Piyachomkwan & Tanticharoen, 2011). The population growth results in the application of cassava starch products in diverse products, the production surged from 22 million tons in 2010/11 to 33 million tons in 2016/17 (Nguyen et al., 2007; Pingmuanglek et al., 2017; TTSA, 2018). The increased production generates more wastes and by-products which are ideal resource for bioenergy. Therefore, agro-industrial countries benefit green and reliable energy from its agriculture potential to harness attractive renewable bio-energy.

Bioenergy offers heart and biofuels (i.e. biogas, bioethanol, hydrogen etc.). However, the practicability varies among two determinants of available resource and best technology. While heat energy could be converted directly by incineration in all region, biofuel production is only feasible in areas where biosynthesis is favourable. The generic advantages of biogas over its counterparts being ease of workability, management, environmentally friendly and rate of return per resource input (Nguyen et al., 2007; Jain et al., 2015). By means of Anaerobic Digestion (AD) process, biomass is converted into biogas through series of bioconversion phases in orders (i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis) (Paylostathis & Gaialdo-Gomez, 1991, Geradi, 2003). Microbial communities are responsible for each step of symbiosisstrict in anoxic environment under multiple controlling factors such as pH,

temperature, organic loading, solid regime, carbon to nitrogen ratio (C/N) and so on (Boone et al., 1993; Veeken & Hamelers, 1999; Monnet 2003; Naik et al., 2014). No matter how these factors could be configured, bio-methane content, the sole energy calorific value of biogas regenerated in low quantity remains as an obstacle. Biogas is the combination of methane (CH_4) - 65% max, carbon dioxide (CO_2) - up to 35% and some trace gases (Rasi, 2009; Appels et al., 2011). Although methane gas could be proliferated by several gas upgrading technologies, the technology itself is still in infancy and most of them are economically unviable for commercial purposes (Chen et al., 2008; Amoah et al., 2019). Since recent decades, research pertinent to new and better digestibility of biomass for higher biogas yield has been emphasizing comprehensively. Nevertheless, efforts focusing for better methanation draws few attentions, and it needs to be elucidated.

Researchers believes introducing catalyst into the AD process could promote microbial activities and thus further accelerate biochemical reactions taken place within intermediary products and all biogas process as a whole (Waite, 2002; Mohamed & Nageh, 2015; Yun, 2016; Romero et al., 2016). In this regard, metals (i.e. Fe, Mn, Co, Cu, Se, and Co) supplementation has been renewed interest to enhance methane during biogas process (Demirel & Scherer, 2011; Ma et al., 2015; Choong et al., 2016, Cai et al., 2018). Along with a range of metal catalysts, Zero Valent Iron (Fe^0) or ZVI as reducing agent and dominate electron donor is considered to have high potential in contributing reducing CO_2 to CH_4, and foster beneficial effects for microbial communities particularly to acetogenic and methanogenic phase which is responsible for hydrogenotrophic methanation (Feng et al., 2014; Liu et al., 2015; Ganzoury & Allan, 2015; Carpenter et al., 2015; Hao et al., 2017). In spite of

the low cost and stimulatory, the impact of metallic ZVI's (Fe^0) impact on anaerobic biota and its mechanism remains ambiguous. The principal kinetic pathway of ZVI with intermediary products within anaerobic digestion processes as reducing agent are as per following key reactions (Karri et al., 2005; Hu et al., 2015; Hao et al., 2017),

$$CO_2 + 4Fe^0 + 8H^+ \rightarrow CH_4 + 4Fe^{2+} + 2H_2O \qquad (8.1)$$

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + H_2 + 2OH^- \qquad (8.2)$$

Based on aforementioned reaction kinetics, since ZVI's serves lowering oxidation-reduction potential (ORP), and acid buffer, it is being widely used for the purpose of methane enhancement especially in activated sewage waste sludge and wastewater. ZVI was proved enhancing methane production capability up to 27% from primary sludge of wastewater treatment plant in batch test with varying ZVI concentration with 0.2mm diameter size for 0-20 g/L added (Meng et al., 2013; Carpenter et al., 2015). Demonstrated in laboratory Biochemical Methane Potential (BMP) tests, ZVI as chemical addictive resulted enhanced methane production and de-waterability but without specifying CH_4/CO_2 fraction (Wei et al., 2018). Using same substrate, Zhang et al., (2015) suggested that ZVI helps in VSS removal up to 60% increased through $0 – 5$ g/L ZVI powder added and thus consequently generated increased methane yield by 91.5%. ZVI was also investigated impacting positively for 2.46 -3.53 times increase for growth of methanogens only under 10 g/L dose by 16s rRNA genetic sequencing (Antwi et al., 2017). However, controversial over verifying reliable ZVI sources, forms and dose applied in AD system exists, and few emphasizes effect of ZVI in particular to organic biomass (Liu et al., 2015; Wei et al.,

2018; Cai et al., 2018). Although, highly purified ZVI could be synthesized at laboratory scale, its low output is unfeasible for commercial purpose (Li et al., 2006; Zhang et al., 2006). In addition, though iron powders are readily available in variable particle sizes (μm $-$ mm diameter) and sizable quantity (Hu, 2005), it oxidizes itself upon exposure to humidity, and thus requires cautious handling prior to application. The reactivity and response of ZVI in anaerobic process largely depends on its particle size with respect to substrate characteristics (Li et al., 2006; Mansoori et al., 2008). However, digestion response with respect to the particle size and dose remains uncertain especially to agro-industrial biomass, and prevailing ZVI literatures investigated predominantly in activated waste sludge.

In this study, by using cassava pulp and wastewater, the impacts of two different sizes of ZVI powder (nm and μm) on bio-methane production was evaluated through validating selected ZVI sources and alternative doses. Our previous work, using scrap iron and same feedstock materials, concluded that characterization of ZVI is crucial in verifying valency Zero in iron sources to ensure ultimate ZVI's function, and discovered scrap iron supplementation in CSTRs resulted slightly higher biogas and more resilient to temperature stress. This study intends to investigate the response of anaerobic digestion by two differential ZVI particle size and examine optimum dose for optimum bio-methane enhancement.

8.5   Materials and Methods

8.5.1. Substrate and Inoculum Source

Main substrate (5% TS w/v) for anaerobic digestion were prepared from major by-products of commercial cassava starch mill, cassava pulp and its wastewater. The characteristic of cassava wastewater was analysed and synthesized further for extended use throughout digestion period. Synthesized in large polypropylene barrels, the process included fermentation by cassava wastewater from parent wastewater pond and newly blended cassava pulp mixture, and active fermentation was maintained by intermittent aeration arrangement. Table (8.1) represents the physiochemical characteristic of both fresh and synthesized feedstock substrate. Prior feed loading into the reactor, synthesized substrate was neutralized to pH 7.0 by $NaHCO_3$ as buffer chemical. Seeds (inoculum) were collected from covered lagoon and anaerobic digestion was executed within 24 hours. Inoculum characteristics were found pH 7.75 ± 0.2, TS 12.15 (g/l) ± 2.50, VS 10.55 (g/l) ± 0.5, and C/N 25.02 ± 3.5.

Table 8.1 Substrate Characteristics

Parameter (Units)	Fresh Cassava Wastewater	Synthesized Cassava Wastewater
pH	4.5 ± 0.2	$4.2 \sim 4.5$
Total Suspended Solids (TSS - g/l)	$2 \sim 3$	5 ± 0.5
Volatile Suspended Solids (VSS – g/l)	$2.55 \sim 2.82$	3.25 ± 0.25
Total Chemical Oxygen Demand (TSCD – mg/l)	$20,000 \sim 25,000$	$10,000 \sim 12,500$
Soluble Chemical Oxygen Demand (SCOD – mg/l)	$5,000 \sim 8,000$	$5,500 \pm 500$
Volatile Fatty Acids (VFAs – mg/l)	$4,000 \sim 6,000$	$4,500 \pm 500$
NH_3-N (mg/l)	$100 \sim 300$	N.D.
TKN (mg/l)	$700 \sim 1,000$	N.D.

N.D. = Not Detected

8.5.2 Zero Valent Iron (ZVI)

Powder ZVI - M100 and R12 utilized in this study was obtained from Swedish metal powder manufacturer, Höganäs AB. Table (8.2) indicates typical properties of selected products. From the disparity of particle size distribution, it could be graded that M100 as extremely fine powder (nm) and R12 as coarse powder (µm) (Hu, 2005). Since iron powders are in commercial grade, they were subjected to heat treatment prior to application to eliminate impurities and self-oxidation by atmospheric humidity.

Table 8.2 Physical Characteristics of Selected Iron Powders

Products	Apparent Density (g/cm^3)	Specific Surface BET (m^2/kg)	Particle size (%)			Chemical Composition	
			+60 mesh / 250 µm	+100 mesh /150 µm	-325 mesh / 45 µm	H_2-loss (%)	C (%)
M100	2.4	130	0	2	20	0.82	0.21
R12	1.4	225	46	79	3	1.8	0.02

With metal iron existing in numerous oxidation states, powders were further investigated for valency and atomic concentration by synchrotron radiation lights for X-ray Absorption Spectroscopy (XAS) and X-Ray Photoelectron Spectroscopy (XPS) at beamline 6.1 and 6.2 of synchrotron light research institute, Thailand, and metal surface crystallinity structure and size by Scanning Electron Microscopy (SEM). Peaked in binding energy at 712ev, XPS spectrum of both M100 and R12 verified that they are predominantly consisting elemental iron (Fig. 8.1). The atomic concentration was found majority in Fe with trace of O and C (Table 8.3). Generated from XAS spectra of different iron oxides, M100 and R12 fall on zero as per relative valency of

iron(s) (Fig. 8.2). Finally, surface morphological and crystallographic state of the sample was observed as per SEM images (Fig. 8.3).

Table 8.3 Atomic Concentration of M100 and R12 ZVI

Samples	Atomic Concentration (%)			
	Fe	**O**	**C**	**Others**
M100	93.01	4.85	0.66	0.48
R12	96.86	1.26	0.92	0.96
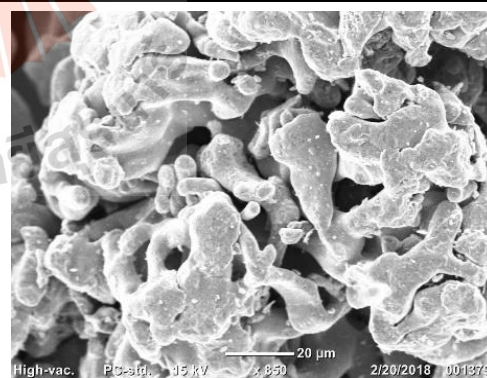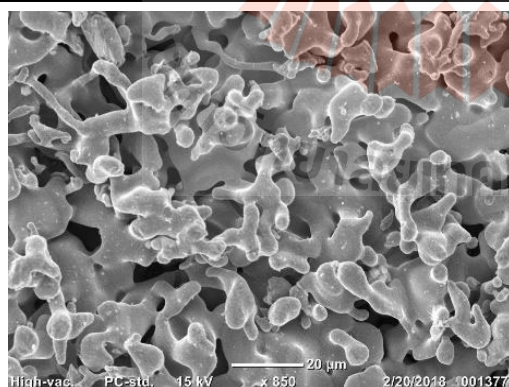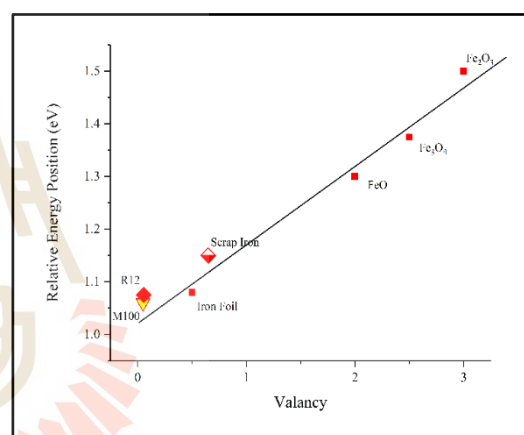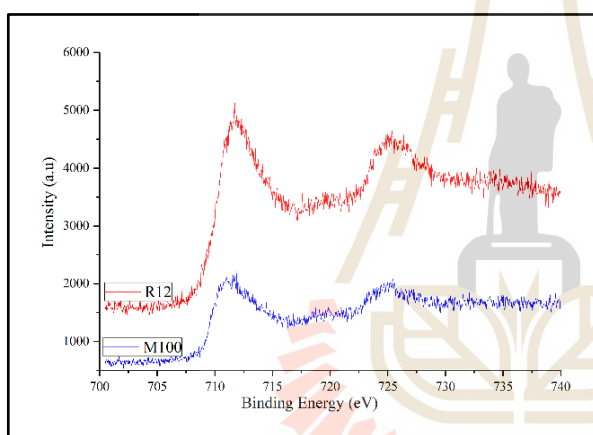
Figure 8.1 XPS Spectrum of R12 and M100

(a)

Figure 8.2 Relative Valency of Irons

(b)

Figure 8.3 SEM Images of a: M100 and b:R12

8.5.3 AD Set up and Configuration

Methanation in this study was conducted in Continuous Stirred Tank Rectors (CSTRs) shown in the figure8.4. Divided into two phases, the first phase includes equalization process in which 6 CSTRs operated in parallel in order to determine Hydraulic Retention Rime (HRT) in optimum Organic Loading Rate (OLR). Reactors were set up in 2-litre sized 6 CSTRs in equally food to microbial ratio (F/M) under ambient environmental condition where ambient temperature ranging between 24 ℃ min: ~ 32 ℃ max: throughout the day. Stirring or mixing was controlled at the rate of 150rpm with 15 mins operation in every 2 hours. Stable organic loading rate (OLR − 3.25 ± 0.25 g VSS L^{-1} day^{-1}) among CSTRS with ± 10% variance biogas yield (600 ± 50 ml/OLR/day) was achieved at hydraulic retention time (HRT) 16 days. Volatile Fatty Acids (VFAs) to Total Alkalinity (TA) ratio (VFA/TA) was maintained below 0.3 as per the limitation for healthy reactor.
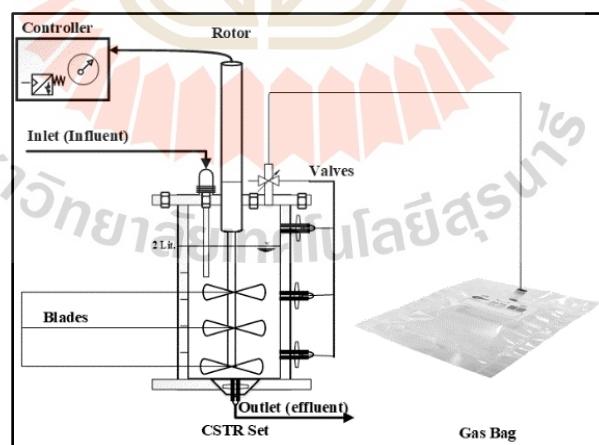
Figure 8.4 Schematic Diagram of CSTR system

Then, in the second phase, ZVI supplementation was proceeded up to next 30 Operation days. Initially, M100 and R12 ZVI powder in 5 different fixed dosages (1-2-4-8-15 g/L) was introduced to assess inhibition threshold of each differential particle size. Based on initial assessment for digestion response of M100 and R12 ZVI particle, further iron concentration adjustment was made. The performance of with and without ZVI input CSTRs were comparatively examined. This study covered the second phase on the response of differential particle size upon ZVI addition with regard to methanation.

8.5.4 Analytical Methods

The APHA standard methods (2005) were applied for soluble Chemical Oxygen Demand (sCOD). For Volatile Fatty Acids (VFAs) and Total Alkalinity (TA) profiles, 3 points GLP titration method was employed by Titroline 7000 automatic SI analytic machine. pH and buffer were measured by HORIBA Scientific® pH meter. Analytical samplings were conducted triplicate for each sample in daily basis throughout experiment period. Quantitative biogas volume was examined in equivalence of specific water replacement method in pressure head swing. Bio-methane was collected in 1-liter sized SKC Tedlar® sample bag, then the relative content of the gas was analysed in Agilent 7890A GC system. Chromatographic gas demarcation was performed in every other day throughout methanation period.To avoid possible interference, the variances between each sample during laboratory investigation of measured parameters were eliminated by triplicating each individual sample set. For all parameter of the experiments involved in this research, the t-test has been constructed to determine whether the difference between the average of triplicated numerical result of measured samples and the control equals. The

descriptive statistics and t tests were generated in origin 2018 statistical software package. The hypothesis is as follows:

H_0:   there is no difference in term of numerical values between specific parameter and control set.

H_a: there is a difference in term of numerical values between of specific parameter and control set.

At the same time, comparison among measured samples are not likely equal to that of the control sample. Therefore, computed P-value is always less than 0.05, and the null hypothesis can be rejected.

8.6   Results and Discussion

8.6.1   Initial Assessment on fixed ZVI Dose for Digestion Response

Since the early studies on the effect of ZVI on bioenergy were mostly related to Waste activated Sludge (WAS), few references relating ideal dose for agricultural biomass is unavailable. By applying ZVI addition in fixed concentration (1-2-4-8-15 g/L) for both M100 and R12, and comparing against ZVI free control reactors, the proximate stimulatory and inhibiting concentration thresholds of each ZVI class for cassava pulp substrate was identified (Fig. 8.5.). After ZVI addition into bioreactors, digestion response in M100 ZVI nanoparticle supplemented sets encountered inhibition when concentration exceeds 2 g/L (Fig. 8.5a). Iron toxicity occurred when the dosing is beyond 2 g/L causing digestion failure in the later days. A compromised new M100 dose (0.25-0.5-1-2 g/L) was established to determine optimum methanation potential with 1.0 g/L ZVI concentration was found ideal concentration (Fig. 8.5a). Identically, using WAS in biochemical methane potential (BMP) assay Zhen et al., (2015) and Jia

et. al., (2017) suggested 1.0 g ZVI/g VSS dose achieved highest methane production. Suanon et. al., (2016) studies also stressed that the effect of nanoscale ZVI addition at 0.75 g/150 g dewatered waste sludge led higher substrate degradation and methane forming. In a separate BMP test of Yang et. al., (2013), at the concentration 30mM (1.68 gm/ L) of Nano Zerovalent Iron (nZVI), methane production was noticeably reduced. It is therefore cassava pulp substrate follows around 1 g/L margin of nano ZVI supplementation for improved digestion and reaction response exhibiting in bioreactors.
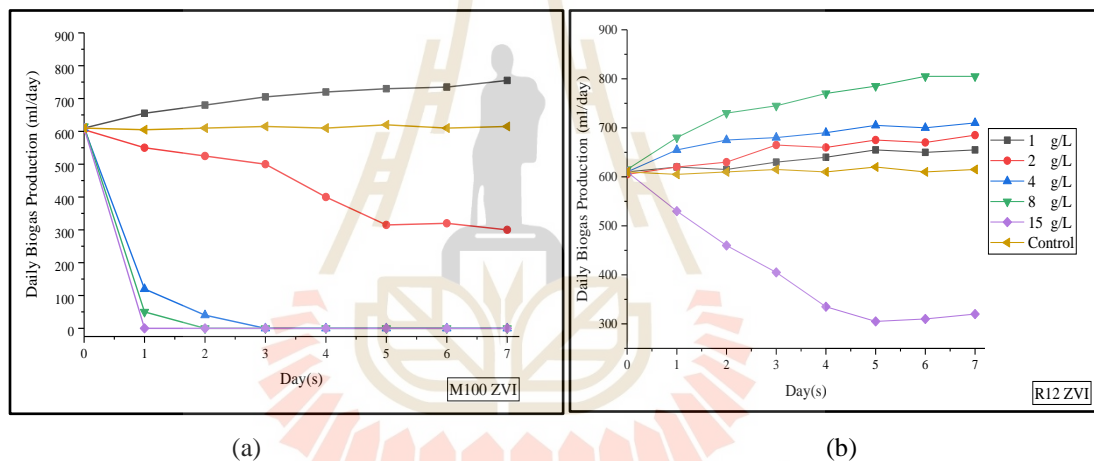
Figure 8.5 Digestion Response of ZVI Addition (a) M100 (b) R12

In contrast to M100 ZVI nanoparticles, the initial digestion response of R12 detected at 8g/L concentration (Fig. 8.5b). While at the lower dose (4g/L), the stimulatory performance of AD was insignificant, the negative impact of R12 arose at 15 g/L dose. Unlike nanoparticles (M100), the inhibition of R12 micro-particle in initial response of digester was found gradual while those of nanoparticles were rapid. Zhao et. al., (2018) revealed at 10 g/L ZVI powder (0.2mm diameter) addition, 30~35%

methane enhancement was achieved than ZVI free reactor. Dosing 10 g/L micro ZVI particle, Liu et. al., (2012) explained powder ZVI helps in COD removal and process stability. The authors further suggested powder ZVI (0.2mm diameter) accelerate acidogenesis and thus subsequent treatment receives beneficial impact. 43.5% of methane productivity was proved by addition 20 g/L in waste activated sludge (Feng et al., 2014). In the case of Ibrahim and Abdulaziz (2016), 15g/L addition led 82% increase in biogas production. This figure contradicts to this study and thus R12 dosage was set at 2-4-8-15 g/L in extended studies.

8.6.2   Extended study to ZVI Impacts during Methanation Process

Applying optimized concentration of (0.25-0.5-1-2 g/L) and (1-2-4-8-15 g/L) for M100 nano-particle and R12 micro-particle respectively, effect of particle size on several key indicators during biogas processes was analysed for extended hydraulic retention time (HRT)

(i) pH Buffer

(ii) VFAs /TA Ratio

(iii) Process Efficiency

8.6.2.1 pH Buffer

Since M100 and R12 ZVI supplementation were not conducted in parallel under same period, relative performance for comparative evaluation is inaccessible. However, applying the compromised stimulatory doses, the result from the separate investigation uncovered the effect of each type ZVI particles on pH in extended digestion period (Fig. 8.6). ZVI had been proved encouraging buffer resistance in AD system (Li et al., 2013; Carpenter et al., 2015; Wu et al., 2015). The current study satisfied with the hypothesis that ZVI assists buffer resistance capacity.

Despite there were slightly different in initial pH (7.15 ~7.25) prior to M100 ZVI addition, pH surged as increase in ZVI concentration and the increment were noticeably most active during the first 4 days, then stabilize in the case of M100 nano-particles (Fig. 8.6a), but steadily increasing in R12 micro-particle (Fig. 8.6b). In Carpenter et al., (2015) studies, intense pH changes happened at the first 3 days of iron amendment. While the rate of pH rise in 2 g/L dose supplementation were most dynamic, the rate in0.25, 0.5 and 1 g/L dosage were found just marginally increase. After day 4, the trend of pH fluctuation in M100 ZVI amended reactors were almost corresponding to that ZVI free (control) reactor, this is due to the fact that nanoparticles ZVI has been completely disintegrated under instant reaction providing with less resistance to stresses impending by other AD variables.

In contrast, pH response in micro-particle ZVI (R12) were gradually increasing after day 2 and maintaining pH below 7.5 at the end of digestion period. All compromised dose of R12 satisfies under optimum pH for healthy digestion is between pH 7.0 and 7.5 (Clark and Speece, 1971). Under this scenario, the frequency of ZVI addition becomes a justification factor for workability and ease of process monitoring in commercial operation. With lower concentration, nanoparticles M100 ZVI required daily addition upon next organic loading. However, in micro-particle R12, though higher dose, it required only single amendment for the whole digestion period. Therefore, R12 ZVI addition saves more chemical consumption for buffer resistance and daily chemical consumption in neutralizing acidic substrates against M100. Excess ZVI can also lead to digestion failure immediately in nanoparticles, volatile fatty acids (VFAs) depletion, balanced growth of microorganisms (Yang et al., 2013; Wu et al., 2015). The slow atomic iron release and surface decomposition in

R12 ZVI lacks those limitations and thus offers the advantage on ease of application and process control over M100.
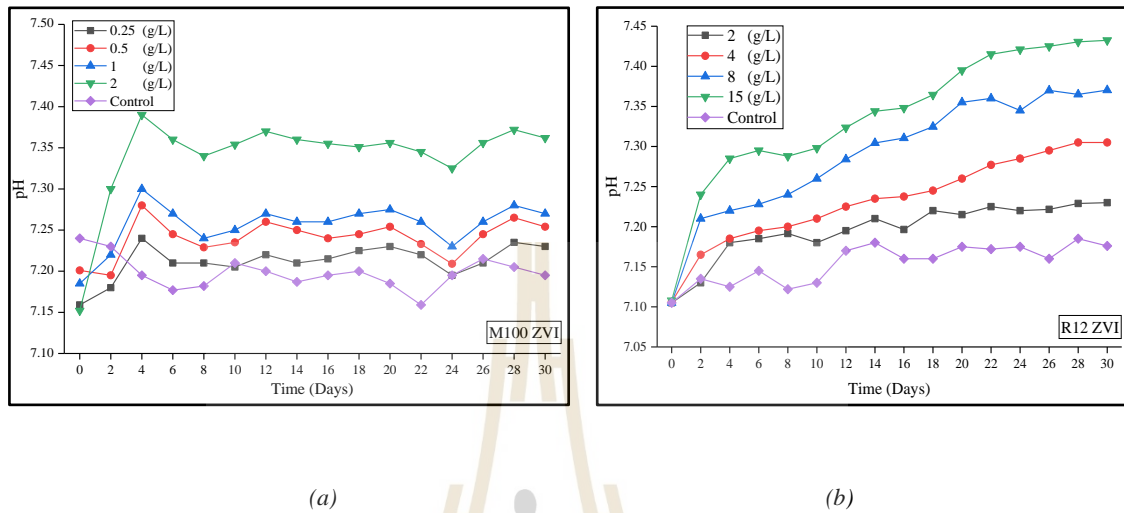
Figure 8.6 Long-term pH Response of ZVI in CSTRs (a) M100 (b) R12

8.6.2.2 VFA/ TA Ratio

The state of Volatile Fatty Acids (VFAs) and Total Alkalinity (TA) in equilibrium is one of the essential criteria in ensuring healthy digester in bioconversion process of biogas (Drosg, 2013; Li et al., 2014). While TA changes is triggered by buffering substrate to neutral pH by bases chemicals, VFAs are an intermediary product formation from decomposition of organic substances by acids formers, acidogenic and acetogenic bacteria communities. The methane forming archaea (methanogens) favours alkaline conditions and they consume VFA and generates terminal products, Bio-methane (CH_4) and Carbon dioxide (CO_2). High VFA formation leads to rapid drop in pH and consequently intoxicates them. Vice versa, high alkalinity condition for acidogenic bacteria diminishes VFA formation leading foods shortage for proper symbiosis to all sort of anaerobic bio communities.

Therefore, VFA to TA ratio (VFA/TA) must be maintained between 0.15 ~ 0.3 for diversification of both acidic and basic favoured microbial communities in equilibrium between pH 6.8~7.5 (Mata-Alvarez, 2002; Khalid et al., 2011; Appels et al., 2011). In spite of saponification by $NaHCO_3$ in synthesized substrate VFAs could be reduced to ideal range below 500 mg/L (Mouneimne et al., 2003; Battimelli et al., 2009), ZVI was also found actively aided in driving down the VFAs mass by mean of a reactive media.

The experiment revealed instantaneous decline in VFA/TA was observed upon ZVI supplementation, while ZVI free control reactor's VFA/TA remained closer upper inhibition threshold of 0.3 in both nano-particle (M100) and micro-particle (R12) cases (Fig. 8.7.). The higher the ZVI concentration is, the lower the VFA/TA ratio was observed leading to another inhibition threshold underneath as per ideal VFA/TA condition. However, concentration of nanoparticle 1-2 g/L abruptly dropped VFA/TA from 0.3 to under 0.2 margin (Fig. 8.7a). This could lead digestion anomaly by immediate release of iron particle for rapid reaction among iron and organic chemical compounds within digester (Yang et al., 2013; Abdelsalam et al., 2017). For nanoparticle concentration of 2 g/l in extended digestion, the VFA/TA was found critically low ending up in VFA exhaustion. Consequently, there is high likely in low gas yield for food source deficiency for methanogens. It is therefore important to maintain the condition of VFA/TA in-between upper and lower inhibition threshold as provision for fluctuation by daily OLR and persistent anaerobic biochemical reactions (Ahring et al., 1995; Duan et al., 2012). Thus, nanoparticle ZVI (M100) dose between 0.5-1 g/L could be considered as optimal. With slow disintegration R12 micro iron particle, VFA/TA responded contrarily to its counterparts. Once injected, VFA/TA

varied just slightly from 0.3 to 0.25. Iron particle deposit found at the bottom of the digester are evidence that dissolution of micro-particle was slower than those of nanoparticle in which it transformed into sludge, changing substrate's colour into pitch back.

In addition, the time taken for noticeable VFA/TA changes took longer than nanoparticle. The investigation uncovered that since R12 micro particle added, VFA/TA variation were active from first 8 days, then stabilized on later days. In term of VFA/TA changes, with the exception to the highest R12 dose (15 g/L), the remaining micro-particle amended CSTRs reactor performed about at the mid of both inhibition thresholds (Fig. 8.7b). This led CSTRs were more resilient to impending stresses derived from variable anaerobic digestion setting (Aquino & Stuckey, 2007; Romero et al., 2016; Choong et al., 2016). While nanoparticle M100 required sustained daily supplementation to maintain ZVI function, the frequency of micro-particle R12 dosing could save by recycling it into the CSTRs till fully disintegrated. As a result, despite nanoparticle demanded in large quantity in initial stage, over 30 days HRT period, the chemical consumption for nanoparticle M100 was higher in each dosage level.
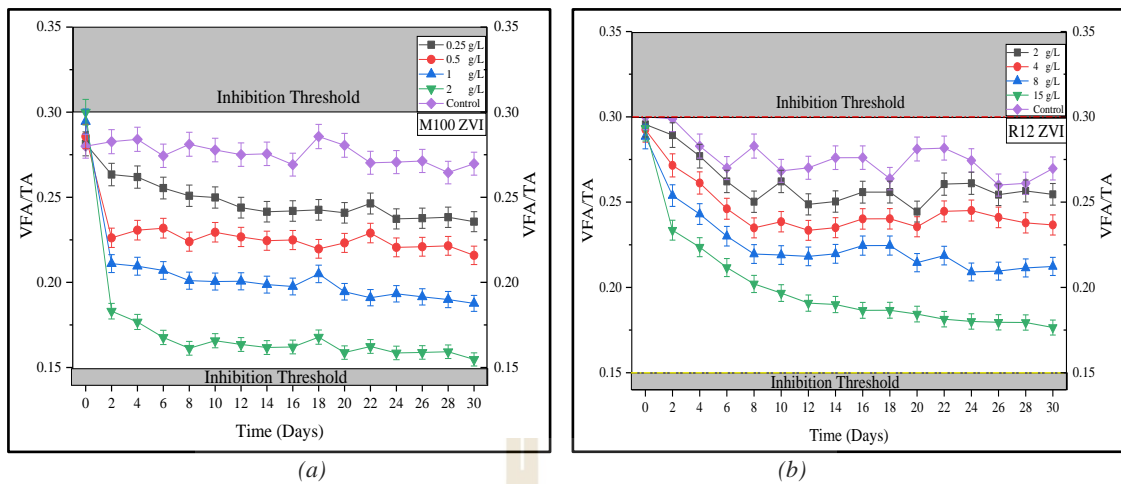
Figure 8.7 VFA/TA Profile of CSTRs (a) M100 (b) R12

8.6.2.3 Process Efficiency

The rate of soluble Chemical Oxygen Demand (sCOD) removal and its residual fraction represents the efficiency of anaerobic digestion process of bio-reactors in converting digestible biomass into bio-methane. Chemical Oxygen Demand (COD) comprises the combination of indigestible or un-biodegradable solid, and digestible or biodegradable soluble solid, among which only soluble solid is accessible by microorganisms as food source as sCOD (Rossle & Pretorius, 2001). With the exception of 2 g/L and 15 g/l in M100 and R12 respectively, the presence of ZVI in both cases promoted active bio-conversion process in long term investigation up to 95 % removal rate with lesser daily residual sCOD upon next loading. While ZVI free control reactor's removal rate was 75%, process efficiency increased with different rate in each ZVI concentration rates (Fig. 8.8). However, the response of finer particle size in M100 ZVI cases were found faster (Fig. 8.8a) than those of R12 ZVI injected CSTRs (Fig. 8.8b). Optimum efficacy in improving sCOD removability of respective concentration of M100 ZVI achieved from just first 4 - 7 days after

introduced, whereas for R12 ZVI, the peak bioconversion rate occurred after day 12 through lower rate. Although finer particle size M100 ZVI provided with shorter duration in increasing reactors' productivity, it led immediate failure by ZVI toxicity when M100 ZVI presence more than 1 g/L in which the reactor irrecoverable state where microbial communities could sustain bio-conversion process. With higher difference in concentration margin between 8 and 15 g/L in R12 coarser ZVI particle, the rate of ZVI inhibition resulted gradual drop in sCOD removal leaving time to recover on time. This alarmed the need for troubleshooting in order to recover active digestion from excessive ZVI introduction. Therefore, in comparison between finer M100 ZVI and coarser R 12 ZVI, the latter has more ease of process control for ensuring optimal conditions under other anaerobic digestion variables (i.e. temperature, mixing rate, OLR, solid content, F/M, etc.).

The relative sCOD removal efficiency with reference to control reactor in this experiment was observed up to 35% increase in optimum conditions when both M100 and R12 ZVI present in CSTRs. In the retrospective studies of similar investigation, COD removal efficiencies increased up to 22% from 54% by finest nano-particle ZVI (nZVI) and 66.2% by grain size iron powder (IP) against control reactor at 44.6 % suggesting larger grain size ZVI for enhancing methane (Suanon et. al., 2017). High level of iron addition along with essential trace elements resulted elevated COD removal rates between 70-80 % process efficiencies when reference reactor's achievement was only 53% (Ortner et. al., 2015). Under the 20g/L ZVI dosage, COD removal declined to 25% within 6 hrs in an effort to enhance anaerobic wastewater treatment (Liu et al., 2012). However, under controlled variables, addition of ZVI according to reactors' real-time performance, COD removal was about at least one

time higher in comparison to that of ZVI free reactor (Liu et al., 2012; Yang et al., 2013). The results from the current study demonstrated that the different in particle size distribution has strong influences on rate of process efficiency and stability.

(a)   *(b)*
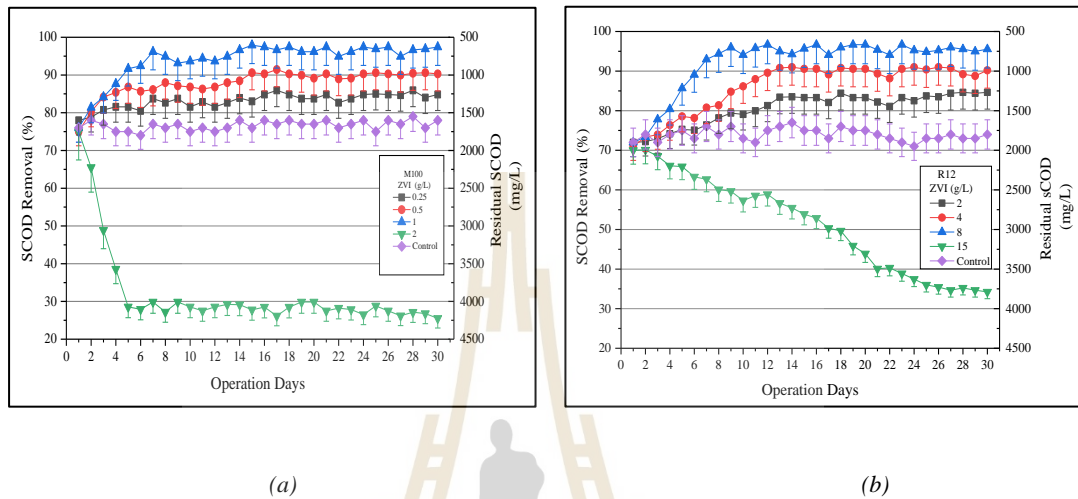
Figure 8.8 sCOD Removal Efficiency and Residual sCOD in CSTRs (a) M100

(b) R12

8.6.2.4 Biogas Yield and Methane Content

According to the investigation throughout 30 operation days after steady state conditions in all parallel CSTRs, upon ZVI introduced, the results (Fig. 8.9: a - c) indicate that the impact on gas volume and methane enhancement of nano-particle size M100 ZVI advanced immediately upon addition, whereas, the trend of micro-particle size R12 ZVI progressed gradually. While the time taken for optimizing bio-methane achieved within first few days in M100 ZVI, in the case of R12, optimum methane improvement occurs on day 15. As a result of M100 ZVI presence, the toxicity occurred when it exceeded 1g/l concentration, it disrupted

overall methanation process ending up into digestion failure. Similarly, in 15 g/l R12 ZVI added reactor, in contrast to that of M100 ZVI, although methane optimization continued till operation period, the daily biogas yield decreases gradually. This is due to the fact that the excessive presence of ZVI unnecessarily elevated pH and buffer within the reactors, then it interrupts proper synthrophy of microorganisms, especially those bacteria responsible for acidogenesis and acetogenesis of anaerobic digestion process (Yang et al., 2013; Suanon et at., 2017). Therefore, the optimum concentration for methane enhancement in cassava pulp methanation of M100 and R12 ZVI was observed at 1 g/l and 8g/l respectively. In comparison to reference control reactor, under unit daily OLR rate, gas yield increased from 600 ± 25 ml to 920 ± 20 ml (67%) in both R 12 and M100 ZVI addition (Fig. 8.9: a and c). Likewise, methane enhancement was found up to 93 % CH_4 concentration from 60 ± 5 % of control reactor (Fig. 8.9: b and d). However, under the flux of mixing mechanism of CSTR set, the impact of particle size in optimizing bio-methane presents as a factor over ease of process control and duration of anaerobic digestion system. Extremely minute M100 ZVI particle unable timely recovery effort because of its promptly reactivity with aqueous substrate once it has been overdosed, while coarser R12 ZVI lacks this limitation and promote bio-availability for methane producing archaea in course of its disintegration along mixing action.

Employing 4 different grades of ZVI nanoparticle (1.120, 0.149, 0.044 and 0.010-mm diameter) in enriching methanogenesis and sulphate reduction in anaerobic sludge, the study suggested the finest particle grade achieved highest rate of methane forming at 0.310 mmol CH_4/mol Fe^0.day and 0.804 mmol SO_4^{2-} reduced/mol Fe^0.day

(Karri et al., 2005). In this investigation, micrometre size R12 ZVI had proved 35% more methane formation than ZVI free reactor. The same increment followed in the case of nano meter size M100 ZVI at 1g/L optimum concentration with identical methane enhancement. Carpenter et al., (2015) reinforced this finding by using commercial ZVI with 150 nm diameter that 28% increase in biogas with 5% increase in more methane than synthesized ZVI in brewery waste water. In comparison to the effect among nano and micro meter size ZVI particles for biogas production, Su et al., (2013) reported nanoparticles ZVI performed superior in methane forming in activated sludge over prolonged digestion with more than 36% (up to 40.4%) enhancement than that of micro ZVI particles. Nevertheless, this finding contrasts to current finding since larger particle size resulted better process control and sustainability of the reactor. Yang et al., (2013) stressed that potential negative impact on methanogenesis with more than 20% inhibition both biogas and methane production in anaerobic digestion when extremely small ZVI particle size (>60 nm) was applied. Liu et al., (2015) demonstrated that granular scrap iron was more effective than ZVI powder (0.2mm diameter) for methanation in waste activated sludge obtaining 10% more CH_4/ kg VS. In the same way, Feng et al., (2014), mentioned larger particle size (up to decimetre level) could have higher possibility in chelation and promote slow released in course of prolonged biochemical processes. Hence, ZVI with larger particle size could be recommended when it is designed to apply as catalytic agent in the bio-reactors for methane enhancement.
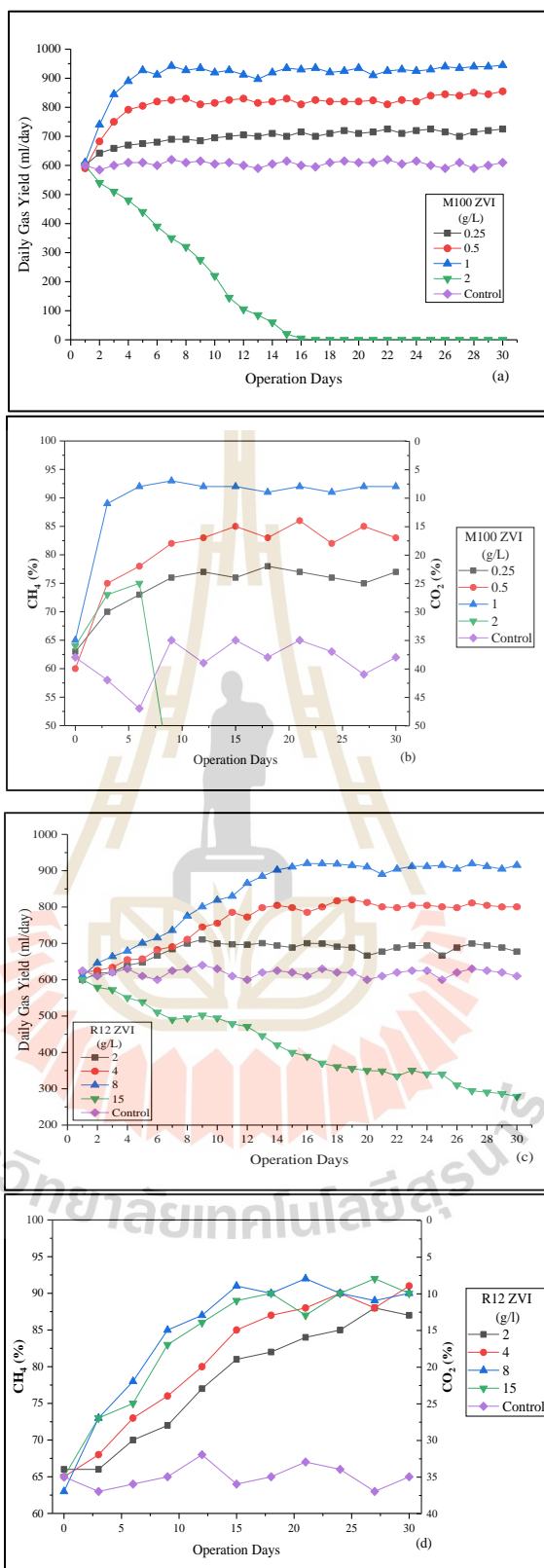
Figure 8.9 Daily Biogas Yield and Methane Content (a-b: M100) (c-d: R12)

The incident of ZVI toxicity of M100 and R12 in this initial assessment indicates how ZVI particle size influences on bioconversion process of both microbial communities and intermediary products. Whiletoxicity took place immediately in nanoparticles (M100 ZVI) injected reactors, those of micro particle (R12 ZVI) were noticed after several days of addition. This is due to the fact that the instantaneous dissolution nanoparticles and its highly reactivity which transforms substrate from grey to pitch black colour and more sludge accumulation, and finally led to digestion failure. Some literatures reported the toxicity mechanism of nano ZVI in diverse anaerobic microbial communities were as the consequence of disruption to the cell membrane integrity, interference with respiration, and damage of DNA or enzymatic proteins activated by release of the metal ions from nanoparticles (Chen et al., 2011; Hajipour et al., 2012; Xie et al., 2017). In comparison between nanoscale and microscale ZVI, Abdelsalam et. al., (2016) highlighted micro scale ZVI treated biota promoted bacterial growth significantly than nanoscale ZVI. Even under anoxic condition, nano ZVI triggered corrosion and surface oxidation by dissolved oxygen, consequently it reduces redox activity (Lee et al., 2008; Lin et al., 2010). Microscale ZVI possesses intrinsic characteristic of surface integrity, higher contact surface and slow atomic exchange (Carpenter et al., 2015; Zhen et al., 2015). Hence, microscale ZVI (R12) amended reactors were more resistance to reaction kinematic stress and longevity for bioavailability. Since R12 ZVI residues were found almost unchanged in both mass and weight on next dose, thus microscale particle also saves dosing frequency by recycling or salvaging the ZVI deposit.

8.7 Conclusion

Catalytic use of metal element and trace element supplementation during anaerobic digestion process will become future technology to reduce the environmental cost and elevated load in biogas upgrading systems in enriching methane concentration of biogas. Nevertheless, metal powder requires characterization and verification to ensure its kinetic reaction with dedicated substrate. Investigated in XAS, XPS and SEM technologies, the application of low-cost elemental iron powder as reducing agent for methanation in this experiment revealed that ZVI addition in optimal concentration proved stimulatory for methane enhancement and gas yield up to 93% and 67% respectively. However, when ease of process control and sustainability are weighed for long-term operation for cassava pulp feed anaerobic bioreactor against differential ZVI particle size, micro-particle size R12 offers superior characteristics than nano-particle size M100.

8.8 References

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CHAPTER 9

INTEGRATION IDEAL ZERO VALENT IRON

CONCENTRATIONS FOR BIOMETHANATION IN ABR

SYSTEM: PILOT STUDY

9.1 Graphical Abstract
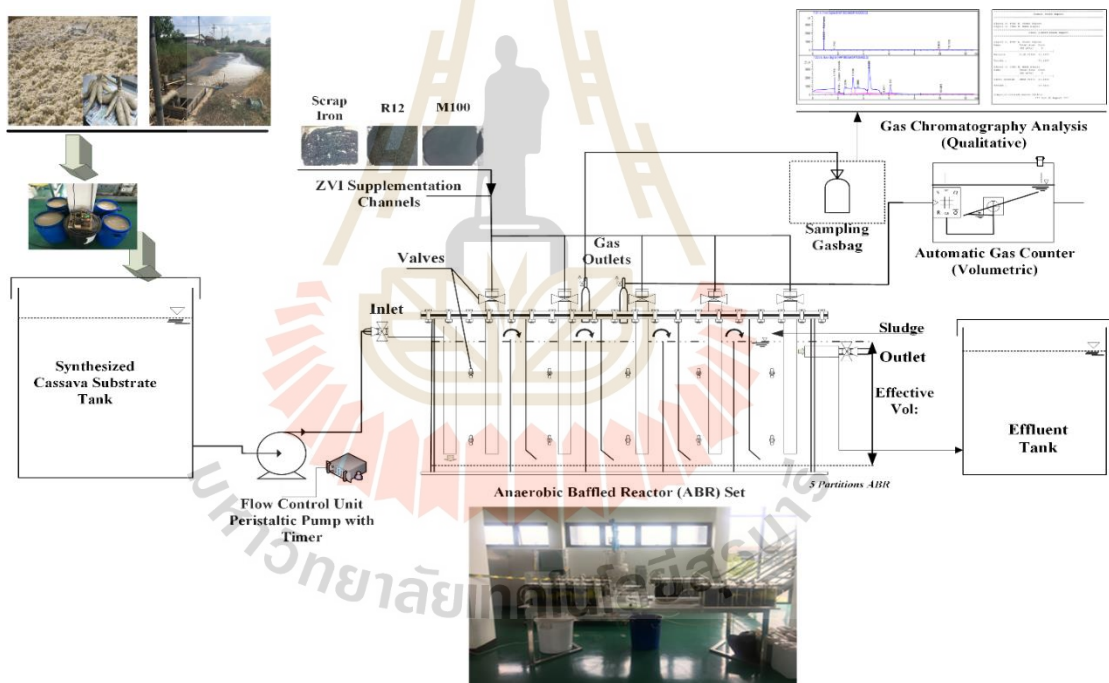
9.2 Highlights

- The production potential in 5% w/v feedstock material of 25 ltrs. effective volume ABR system's ultimate volumetric biogas was found 2.95 l/day in 4.0 kg $COD/m^3/day$ which is 60% increase to minimal OLR rate of 2.0 kg $COD/m^3/day$.

- The static solid accumulation fosters algal bloom that could bring paradoxical and detrimental impact on microorganism communities and food sources availability for dedicated anaerobic bacteria, and archaea inside digester

- ZVI addition encouraged stable digestion performance and cross-feeding of the anaerobic microbe by regulating pH, VFA/TA ratio.

- By ZVI presence, gas volume increased 5.17%, 6.03%, and 8.62% for scrap iron, M100 and R12 respectively.

- R12 consisting highest ZVI concentration, its presence did not generate outstanding results in both volumetric gas yield and methane content

9.3   Abstract

Waste to Energy (WtE) offers minimalistic obstacle with highest feasibility to adopt in comparison to its renewable counterparts in tackling carbon emission and its resultant climate change. Using cassava pulp, a leading by-product generated from cassava starch industry of Thailand, this study emphasized optimizing biogas production in 5 compartmentalized Anaerobic Baffled Reactor (ABR) by means of stepwise inversed variation in Organic Loading Rate (OLR) and Hydraulic Retention Time (HRT). Within 36 days of operation period for steady state, the results uncovered that despite reduce in both total and soluble Chemical Oxygen Demand (tCOD) removal, sCOD removal efficiency recovered up to 90% along with increased OLR. The production potential in 5% w/v feedstock material of 25 ltrs. effective volume ABR system's ultimate volume was found 2.95 l/day in 4.0 kg COD/m^3/day which is 60% increase to minimal OLR rate of 2.0 kg COD/m^3/day. When excess or deficient loading to the prescribed OLRs and HRTs was applied, this novel

experiment identified possible inhibition and failure in following compartments of ABR system by food shortage resulting disruption in anaerobic communities to sustain balanced bio-conversion processes.

Upon Zero Valent Iron (ZVI) supplemented, the presence of iron encourages process stability with iron mass and quantity in ABR rather than concentration of Iron's Valency.Possessing highest ZVI characteristic in order (R12, M100, and Scrap Iron) and integrated their ideal dosage into cassava pulp feed Anaerobic Digestion (AD) system, methane gas was enhanced up to 62% while iron free ABR's methane were ranging between 50%-55%. Despite the increment is not significant than that of the result of Continuous Stirred Tank Reactor (CSTR) operation, ZVI addition proved moderating uniform digestion along the compartments to maintain as healthy reactor. In term of volumetric gas yield as well, gas volume increased 5.17%, 6.03%, and 8.62% for scrap iron, M100 and R12 respectively. There is strong evidence that in order to enhance bio-methane by effect of ZVI to its ultimate potential, mixing arrangement or modification to conventional ABR system is recommended. So that, the reactional kinetic among ZVI and substrate is in homogeneity.

9.4   Introduction

Being one of the pressing issues of the world, ensuring energy security and tapping reliable energy source is imperative factor for the development. In this circumstance, sustainability and green concept exists as an important consideration to counter mounting challenges associated with climate change and environmental risks.

Thanks to advance technologies, energy from sustainable sources such as solar, wind, geothermal and tidal are promising for the eco-system. However, it is still in infancy for adopting aforementioned technologies as mainstream sources because of its readily reliant on seasonal and temporal shortcomings (Stram, 2016: Brett, 2019). Moreover, these technologies are unfeasible for developing countries in which utility cost needs to be set reasonable under the determinant between productivity and per capita income. In this regard, Waste-to-Energy (WtE) offers an option to recuperate energy from diverse wastes particularly in agriculture and agro-industry oriented countries.

Under the WtE technology tree, the inherent advantages of Anaerobic Digestion (AD) technology over its counterparts are economical feasibility, resource availability, ease of process control, and more. Biologically, by means of anaerobic digestion, wastes are converted into bio-energies (i.e. biogas, and bio-ethanol) for electricity generation. As long as wastes consist carbohydrate, fats, and protein, it is adequate for biogas production through biogas processes (Hobson & Wheatley, 1993; Paritosh et al., 2017; Caruso et al., 2019). The kinetic and biochemical theories relating to biogas processes as one of waste treatment options was fully established and uncovered since 1980s (Anderson & Duarte,1980: Chen, 1983: Hobson, & Wheatley,1993).

In recent decades, the combination of global warming triggered by fossil fuel and researchers' extensive research on potential renewable energy led biogas or bio-methane a renewed interest.Strictly controlling in anaerobic environment, a range of digesters are available based on characteristic of wastes, biomass and wastewater with respect to the scale of availability and project size (Grant et al., 2002). While batch

digestion in miniature size is widely applied for Biochemical Methane Potential (BMP) test, for elevated laboratory investigation, either Continuous Stirred Tank Reactor (CSTR) or Anaerobic Baffled Reactor (ABR) system is more prevalent in investigating volumetric biogas potential of specific feedstock material (Mahanta et al., 2002). Then, results from lab-scale are put into operation in more advanced reactor like Up-flow Anaerobic Sludge Bed (UASB) to Expanded Granular Sludge Bed (EGSB) to handle pilot or commercial level continuous operation (Bhatia, 2014).

Nevertheless, fluctuation in gas yield and low methane gas (CH_4) gas content which is core energy calorific value of biogas remains an obstacle to make biogas technology more attractive among renewable alternatives, no matter what system is applied in biogas plant (Mitzlaff, 1988: Noyola et al., 2006). Therefore, anaerobic digestion needs post biogas purification or gas proliferation measures to transform bio-methane competitive and as a substitute for Compressed Nature Gas (CNG) and Liquified Natural Gas (LNG). The background objective of this research is to enrich bio-methane composition of biogas derived from cassava pulp digestion by differential Zero Valent Iron (ZVI). This paper covers evaluating start-up operation and performance of five compartmentalized Anaerobic Baffled Reactor (ABR) system with respect to biochemical kinetic condition within individual compartments and assays prior to reach steady state of readily available agro-industrial residues, cassava or tapioca pulp digestion and its wastewater. Therefore, ABRs are under controlled condition which is acceptable for comparative investigation on the effect of catalyst, ZVI addition. The study aims highlighting the significant of variable Organic Loading Rate (OLR) and its impact of process stability to optimize biogas yield under given reactor design specification. Then, the effect of ZVI presence is to be investigated to

optimize methanation of cassava pulp digestion in the ABR system. The concentrations of ZVI were taken from the finding from optimum dosage of ZVI found in CSTRs operation of Chapter 6, 7, and 8.

9.5   Materials And Methods

9.5.1   ABR System - Set Up and Description

ABR system is built in clear acrylic panel in rectangular box shape containing 5 equal partitions (68 cm x 28 cm x 18.5 cm) shown in the figure (9.1). Each partition accommodates 6000 cm^3 liquid volume (equiv. to 6 litres) over 26.5 cm length, 13.0 cm width, and 17.5 cm height of each individual intact cubicle. Reserved for 1 litre for sludge accumulation (hanging baffles) during operation, therefore the ABR system holds maximum 5 litres of effective volume for stable AD digestion to solid and liquid retention space (standing baffles). Therefore, out of 30 litres working volume, the ABR system accommodates liquid and sludge for 25 litres and 5 litres respectively. Instead of direct circular opening as barriers, the partitions were arranged in an overflow vertical column and one reversed sludge blockage panel with inclined opening. Thus, the reaction kinetic in each compartment is fully baffled as designed, and steady substrate flow is ensured. Schematic diagram of complete ABR system is shown in the figure (9.1). In addition, unlike ordinary ABR system, two layers of valves in each partition for troubleshooting and intervention whennecessary. Polyvinyl Chloride (PVC) pipes are installed on the reactor in this study. The objective of this arrangement is accessibility to each substrate (sampling) and provision for catalyst inlet without disturbing strict anaerobic condition. Substrate influent and effluent tank is connected in 1cm (3/8") silicone pipe in which

flow rate is controlled by peristaltic pump. Two gas outlets in which one channel is connected to automatic gas counter in liquid displacement system and one channel is connected to the gas bag. The reactor was operated under ambient temperature. Laboratory assay points are selected at lower valves of each compartment of ABR (i.e. A_1 - A_5).
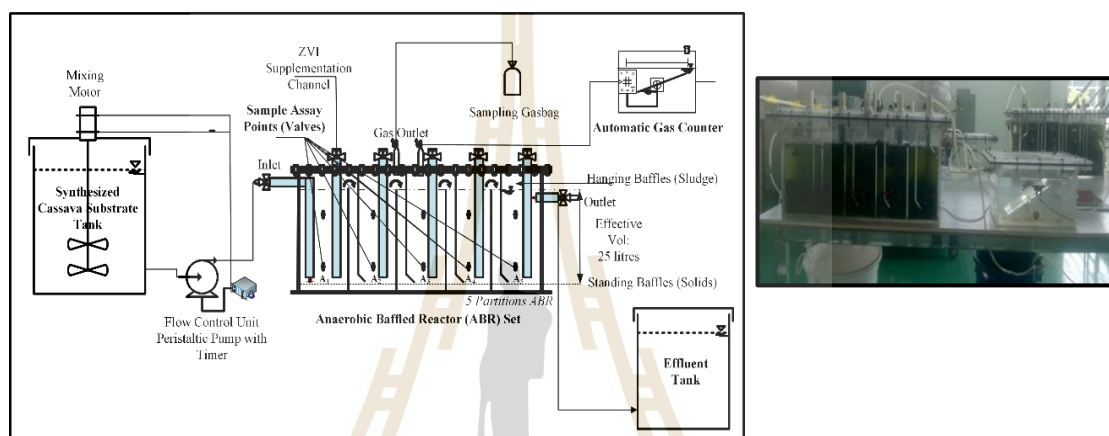
Figure 9.1 ABR System Configuration

9.5.2 Substrate and Inoculum Source

5% TS w/v feedstock for anaerobic digestion were prepared from major by-products of commercial cassava starch mill, cassava pulp and its wastewater. The characteristic of cassava wastewater was analysed and synthesized further for extended use throughout digestion period. Synthesized in large polypropylene barrel, the process included fermentation by cassava wastewater from parent wastewater pond and newly blended cassava pulp mixture, and active fermentation was maintained by intermittent aeration arrangement. Table (9.1) represents the physiochemical characteristic of both fresh and synthesized feedstock substrate. Prior feed loading into the reactor,

synthesized substrate was neutralized to pH 7.0 by NaHCO₃ as buffer chemical when

needed. Seeds (inoculum) were collected from covered lagoon and anaerobic digestion

was executed within 24 hours. Inoculum characteristics were found pH 7.75 ± 0.2, MLSS

121.5 (g/l) $\pm$ 2.50, VSS 105.5 (g/l) $\pm$ 0.5, and C/N 25.02 ± 3.5.

Table 9.1 Substrate Characteristics

Parameter (Units)	Fresh Cassava Wastewater	Synthesized Cassava Wastewater
pH	4.5 ± 0.2	$4.2 \sim 4.5$
Total Suspended Solids (TSS - g/l)	$20 \sim 30$	50 ± 0.5
Volatile Suspended Solids (VSS – g/l)	$25.5 \sim 28.2$	32.5 ± 0.25
Total Chemical Oxygen Demand (TCOD – g/l)	$20 \sim 25$	$10 \sim 13$
Soluble Chemical Oxygen Demand (SCOD – g/L)	$7 \sim 12$	$6 \sim 8$
Volatile Fatty Acids (VFAs – mg/l)	$2,000 \sim 3,000$	$2,000 \pm 500$
NH₃-N (mg/l)	$100 \sim 300$	N.D.
TKN (mg/l)	$700 \sim 1,000$	N.D.

N.D. = Not Detected

9.5.3 Analytical Method and Statistical Analysis

The APHA standard methods (2005) were applied for Chemical Oxygen

Demand (COD) and solid analysis. For Volatile Fatty Acids (VFAs) and Total

Alkalinity (TA) profiles, 3 points GLP titration method was employed by Titroline

7000 automatic SI analytic machine. pH and buffer were measured by HORIBA

Scientific® pH meter. Analytical samplings were conducted in triplicate for each

sample in daily basis throughout experiment period. Evolved biogas was examined in

volumetric automatic counter and gas quality for methane content by Agilent

Technologies 7890A GC System in gasbags and vials. During start-up operation,

samples were taken from each compartment in 4 parallel ABRs. For extended studies,

samples were taken only from influent compartment #1 (C1) and Effluent Compartment #5 (C5) when ZVI was added.To avoid possible interference, the variances between each sample during laboratory investigation of measured parameters were eliminated by triplicating each individual sample set. For all parameter of the experiments involved in this research, the t-test has been constructed to determine whether the difference between the average of triplicated numerical result of measured samples and the control equals. The descriptive statistics and t tests were generated in origin 2018 statistical software package. The hypothesis is as follows:

H_0:   there is no difference in term of numerical values between specific parameter and control set.

H_a: there is a difference in term of numerical values between of specific parameter and control set.

At the same time, comparison among measured samples are not likely equal to that of the control sample. Therefore, computed P-value is always less than 0.05, and the null hypothesis can be rejected.

9.5.4 ABR Initiation and Optimum OLR Adjustment

Prior execution the AD process, Seed sludge were inoculated, acclimatized, and stabilized for 48 hours in order to subtract the residual food source present in collected sample sourced from parent anaerobic covered lagoon. New substrate loading equal to seed volume for which same food to microorganism ratio (F/M = 1) were loaded. Initial loading was kept for 3 days to ensure the lag-log-stationary phases in order for new anaerobic environment within ABR compartments. Steady state for each OLR was considered 5 days. Organic Loading Rate (OLR) were adjusted in three steps wise manner in ascending arrangement based on optimum

sCOD removal in last ABR compartment. For each individual OLR, 5 days duration was taken for steady state and optimum sCOD removal identification for specified ABR. As OLR increased, HRT was reduced in step by step until 24 hours duration to examine optimum volumetric daily biogas yield. The consideration for three different OLR (2.0, 3.0, 4.0 kg COD/m^3/day were based on following equations in which only reactor volume is fixed and the remaining sets are varied under the influence of time and flow rate. By adjusting these variables, steady state operation was obtained for each OLR.

$$
\begin{aligned}
\text{OLR} &= (\text{CODi x Q})/\text{V} \qquad\qquad (9.1)\\
&= \text{CODi x (Q/V)}\\
&= \text{CODi x (1/HRT)}
\end{aligned}
$$

Where; OLR   =   Organic Loading Rate (kg COD/m^3/day or Kg COD/L/day)

CODi   =   Chemical oxygen demand concentration of feed (kg/m^3 or kg/L)

Q   =   Flow of substrates (or feed) in the digester (m^3/day or L/day)

V   =   Volume of reactor (L or m^3)

HRT   =   Hydraulic Retention Time (day) = (V/Q)

9.5.5 ZVI Supplementation

Zero Valent Iron (ZVI) applied in this phase of work is based on the experimental results of CSTR operation from Chapter 6,7, and 8. The ideal concentrations in optimizing methanation of cassava pulp digestion were examined at 20 g/l, 8g/l, and 1 g/l for Scrap Iron, R12, and M100 respectively. The properties of ZVI materials are referred to related Chapters. ZVI addition and removal were carried out on daily basis with respect to substrate inflow volume (per organic loading rate per day).In order to maximize ZVI's ultimate catalytic properties, selected powder and granular

iron were subjected to heat treatment (105 °C) and Argon (Ag) gas flushing to eliminate surface oxidation and impurity by ambient humidity and cross-contamination.

9.6   Results And Dicussion

9.6.1   Part I: Steady State Operation

9.6.1.1 Digestion Performance Within Individual Partition

The effect of increased Organic Loading Rate (OLR) with respect to solid degradability and digestion performance in each assay is stated in thetable 9.2. Having benchmark influent sCOD between 7000-8000 mg/l and the flow was regulated by peristaltic pump in intermittent operation along 24 hours HRT, the velocity of substrate flowing inside the ABR system were observed laminar and sub-critical upon organic loading. Without disturbing steadiness of biomass and anaerobic microscopic communities, gradual settlement occurred once uploading instigated into compartment 1 (A_1) and the substrate overflowed through intermediate partition walls in subsequent compartments. Therefore, total suspended solid accumulation was found highest in first chamber in all 3 different OLR trials. Residual TSS being lowest at last compartment (A_5) was attributed by sustained solid degradability from inoculum and uniform F/M ratio during initial start-up. TSS figures in intermediate cells were a result of evolved sludge agglomeration and flocculation that collided, then settled into succeeding room via aqueous transport channels of cells' wall. Hence, solid concentration declined dramatically in comparison to antecedent assays. Nevertheless, with increase in OLR, solid accumulation was found proportionately accumulated in all scenario. Therefore, digestion performance needs to be evaluated in additional physicochemical factors.

Being early warning for the stability of anaerobic reactor, fluctuation in hydrogen ion concentration [H$^+$] or pH is the important indicator for the performance of AD system. pH level increased as succeeding digestion chamber (Table 9.2). Ideal pH for maintaining anaerobic digestion is between 6.8 – 7.5 (Clark & Speece, 1971). The observed pH in compartment 4 and 5 (A4 & A5)of Trial 1 (2.0 Kg COD/m^3/day OLR) were found beyond optimum range. This infers that though the condition is favourable for methane forming archaea, the community of bacteria that are responsible for active fermentation could not sustain growth and metabolism. Food source depletion is another factor in these zones, and therefore, residual and sCOD plunged and the removal efficiency peaked in comparison to previous digestion rooms (i.e. A3, A2, A1). Even though this situation is highly favourable for water and wastewater treatment purpose, but for generating biogas, it could lead to microbial imbalanced among bio-methane, acetate and volatile acids forming microorganisms (Amani et al., 2010). Thus, increment in food loading was found highly recommended in order that all 5 compartments are undergoing uniform anaerobic digestion for optimum biogas production potential. The context of VFA/TA provided with further evidence that the decrease in VFA level in last room (A5) indicates the upsurge of total alkalinity, and under which circumstance (pH 7.8) acidogenesis could not persists (Moreno et al., 2018). While the ideal VFA/TA for healthy digestion is between 0.15 – 0.4 (Speece, 1996: Lossie & Pütz, 2008), Trial 1 of the study revealed VFA/TA were observed way below to the mid of optimal range.

Table 9.2 Reactors Performance across Each Compartment

Tests	OLR Kg COD /m³/d	Parameters	Assays (24 hrs. HRT)				
			A1	A2	A3	A4	A5
Trial 1	2.00	pH	7.25±0.5	7.36 ±0.5	7.48±0.5	7.59±0.5	7.8±0.5
		VFA/TA	0.21	0.185	0.15	0.11	0.09
		TSS (mg/l)	8.65	5.95	4.45	4.10	3.85
		sCOD (mg/l)	1780	1140	630	270	50
		COD Removal (%)	72.30	81.00	89.35	95.50	99.15
Trial 2	3.00	pH	7.05±0.5	7.08±0.5	7.29±0.5	7.35±0.5	7.45±0.5
		VFA/TA	0.33	0.29	0.24	0.27	0.18
		TSS (mg/l)	15.26	11.35	7.85	5.25	4.15
		sCOD (mg/l)	3220	2520	1730	850	360
		COD Removal (%)	54.25	63.90	75.25	87.85	94.80
Trial 3	4.00	pH	6.87±0.1	7.05±0.1	7.20±0.1	7.27±0.1	7.37±0.1
		VFA/TA	0.44	0.38	0.35	0.31	0.29
		TSS (mg/l)	24.15	20.55	16.8	6.75	4.55
		sCOD (mg/l)	5460	3670	2520	1450	810
		COD Removal (%)	31.65	53.90	68.65	81.95	89.65

Upon OLR increase in stepwise manner up to 4.0 Kg COD/m³/day, pH level within all the ABR system were found satisfying to the ideal pH for healthy anaerobic reactor. However, physicochemical indicators (i.e. pH and VFA/TA) of first partition (A1) was at the threshold of early inhibition state. compromising for the scenario for which all partitions are in actively bioconversion process for biogas production, the

figure could be superseded for optimizing volumetric biogas yield throughout all five compartmentalized ABR units. By static nature and intermittent loading, solid accumulation was found highest in A1 and A2 of respective trial. And, TSS in A3, A4 and A4 of all cases are the residual undegradable solids of lignocellulosic biomass matter of cassava pulp from F/M of ABR start-up.

9.6.1.2 Effect of OLR and HRT on Volumetric Biogas Yield

The Hydraulic Retention Time (HRT) has strong influence on solid degradability and bio-conversion process of any anaerobic digestion process regardless of digester design and configuration. A high HRT allows prolonged exposure of diverse microorganisms to feedstock materials which reside in the system (Dareioti & Kornaros, 2014: Gaby et al., 2017). It is therefore solid removal is accelerated by dynamic microbial communities in course of their log and stationary growth especially during initial stage of digestion phase (Cha & Noike, 1997: Kim et al., 2006). In conformity to this principal, this experiment reveals that high COD (both soluble and total) removal efficiency has been achieved in 96 hrs. HRT. Despite sCOD removal had recovered after extended operation time to 90%, tCOD declined to 60% margin eventually when HRT reduced to 24 hrs. (figure 9.2a). Thus, only sCOD from increased OLR could be contingent as the food source for biogas production under short HRT cycle. The selection of HRT depends on the type of reactor, feedstock materials and the purpose of AD system; it could be modified from few hours in wastewater treatment to months in biofuel production (Nasir et al., 2012: Smith et al., 2012). Typically for ABR system, 20 − 36 hrs. have been adopted. (Nachaiyasit & Stuckey 1995; Li et al., 2013). This study opted 24 hrs. in steady state

condition for comparative investigation along parallel ABRs and as the provision for the effect of catalyst supplementation into the system.
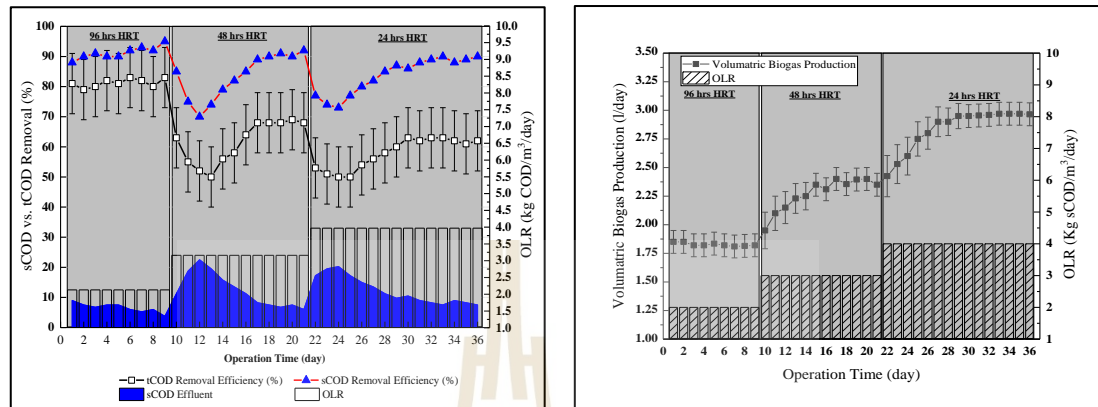
[**a**: Solid Removal (Left) **b**: Volumetric Gas Yield (Right)]

Figure 9.2 ABR Performance under Variable HRT and OLR

Under the steady state operation in 24 hrs. HRT and 4.0 kg COD/m^3/day the relative increment in volumetric biogas production was found 60%(1.05) than that of 2.0 kg COD/m^3/day OLR of given ABR system (Figure 9.2b). Although this figure is not compromised gain from increased OLR, the discrepancies are as the result of shortened HRT and by its reduction in tCOD removal efficiency (Figure 9.2). Nevertheless, the optimum biogas production of cassava pulp digestion by specified ABRs was observed 2.9 l/day. Digestion failure and instability could be predicted by further OLR addition since the performance in first partition of 5 compartmentalized ABR is on the verge of possible inhibition (Trial 3). Alternatetively, trial 1 insinuates, though biogas yield is superior in less resource input, most of the succeeding were found food source exhaustion resulting inefficacy of prescribed ABR system (Table 9.2). In retrospective literatures, 74.1% more methane production had been achieved by modified ABR for co-digestion kitchen waste and organic fraction of municipal solid waste (OFMSW) (Malakahmad et al., 2008). Krishna et al.,

reported ultimate COD removal was possible with more compartment in less HRT. The number of compartments, OLR and HRT variables are important factors for ABR operations. While more compartments are advisable for wastewater treatment, more compartmental arrangement creates hindered bio-conversion for waste to energy purpose (Barber & Stuckey et al., 1999: Jamshidi & Khalesidoost, 2014). With 5 equals compartmentalized ABR in this study, volumetric biogas production has been enhanced from 1.75 to 2.95 $\pm$ 0.2 l/day by inversed OLR and HRT adjustment.

9.6.1.3 Sludge and Solids Occupancy Across ABR Compartments

Unlike CSTR, UASB and Plug Flow system, the distinct features of ABR are being the installed partition walls to ensure baffling capabilities in each particular cell, independent sampling points and localized influent/effluent position. These offers both advantages and disadvantages over its counterparts in waste management system (Barber & Stuckey, 1999). Because of ABR system's compartmentalization option, reactor could be savaged and intervened separately on critical operation condition. However, the partitioned walls themselves become as a divider for free-flow along ABR especially for those fluid in combination with settleable and colloidal solids. Likewise, cassava pulp substrate in 5% w/v TS in this study engages solid withholding in current ABR system. Hence, solid transfer within ABR system is possible by means of dissolved solid transported by flowing through the weirs (partition walls) and overflowing hanging supernatant (sludge) within (Bwapwa, 2010). With more compartment build-ups, more solid is retained in preceding cells.
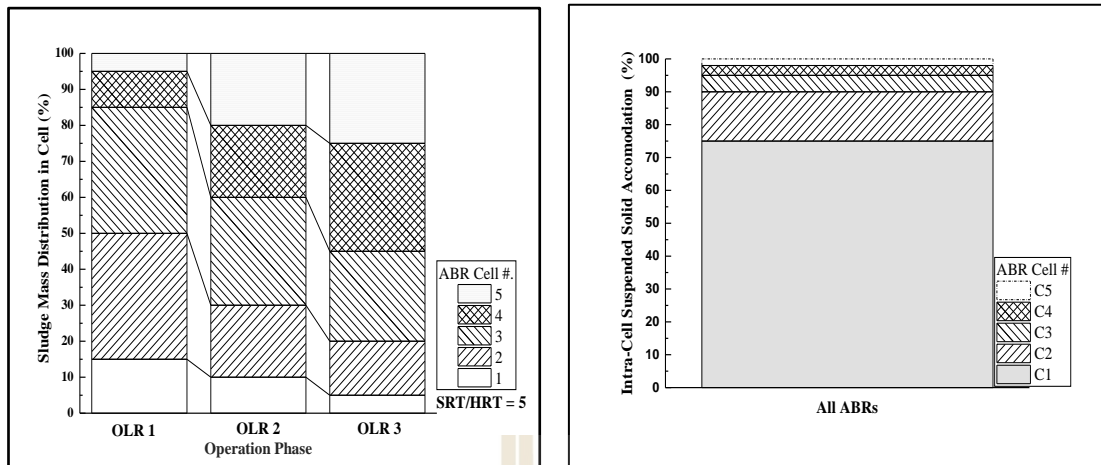
Figure 9.3 Solid Residence in ABR System

Figure 3a. Sludge Mass Distribution on Variable OLR and HRT (Left) Figure 3b. Solids Accumulation within ABR Cells During Operation (Right) Variable in HRT and OLR has significant impact of sludge formation and accumulation in ABR cells. Though conventional ABR does not contain activation and clarification mechanism, longer HRT results in sludge formation by solid degradability by active anaerobic digestion. Figure 9.3a describes sludge mass distribution by Imhoff cone analysis. Under OLR1 operation (96 hrs HRT), most of the sludge mass progresses in intermediary cells (cell no. 2-3-4). Sludge produced in early compartments decreased eventually as increased in OLR (OLR 3) with shortened HRT (24hrs.). The accumulation is as the result of sustained digestion and biodegradation of residual solid from initial F/M. With no considerable solid inflow in followings compartments, sludge accumulation was observed (figure 9.3b). No matter in what trial, more than 70% solid is stockpiled in the first cell (figure 9.3b). Therefore, high SRT/HRT is necessary for solid (digestate) removal to moderate digestion across 5 compartments. Although the efficiency of waste treatment directly proportionately to the number of compartments in ABR system

configuration, however, mass solid accumulation could lead to microbial imbalance and instability of methanation among ABR cells (Ramandeep, 2016; Chinwetkitvanich & Ruchiraset, 2017). In addition, the static solid accumulation fosters algal bloom that could bring paradoxical and detrimental impact on microorganism communities and food sources availability for dedicated anaerobic bacteria and archaea present within digester.

9.6.2   Part II: Integration Zero Valent Iron in ABR (Extended Study)

9.6.2.1 Effect of ZVI Presence in ABR on Methanation

When steady state operation condition is maintained for certain period, based on the presenting results of the figures9.4a and 9.4b, due to ZVI supplementation into ABR system under uniformed 4 kg COD/m^3/day OLR, biogas production rose from 2.9 l/day reference volume in control reactor to 3.15 l/day in R12 supplemented reactors. Empirically, gas volume increased 5.17%, 6.03%, and 8.62% for scrap iron, M100 and R12 respectively. Though, the gas yield increments are not considerable than that of lab scale CSTR experiments, digestion performance were noticed more stable and resilient on condition when temperature fluctuation within the day and alternate weather throughout operation days. With R12 ZVI having highest ZVI's reducing properties for methanation, the mass build-up of iron at the bottom of the ABR housing becomes an obstacle for substrate inlet in each cell since mass iron deposit could block the flow. Though orientation of powdered Zero Valent Iron (R12 and M100) were found adequate at ABR's low datum, in the case of scrap iron for which ideal concentration is 20 g/l which takes up to 100 g per single cell occupying 150 cm^3 (density 1.5gram/cm^3) and impeding free flow of substrate from early compartments. In the absence of mixing arrangement in ABR system, the possibility for intra-cell solids transfer and contact surface among substrate and ZVI's

were set less likely to ensure homogeneity of substrate's physicochemical characteristics.
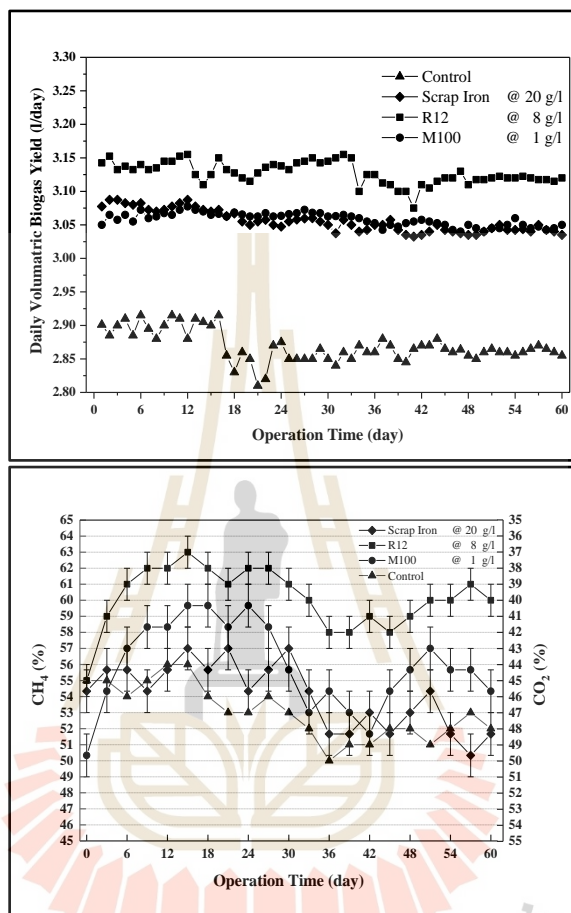
Figure 9.4 Volumetric Daily Gas Yield by ZVI Supplementation into ABR System

Figure 4a. Volumetric Daily Biogas Yield by ZVI (Left)

Figure 4b. Effect of ZVI Supplementation on Methanation Potential in ABR (Right)

The chromatographic demarcation on biogas content by the presence of ZVI in ABR system revealed that the catalytic ZVI assisted biomethane formation improved more slightly than that of no iron amended ABR. By means of highly refined ZVI

(R12), methane could be detected up to 63% during operation (Figure 9.4b). This figure contradicts to the results of CSTR operation for which methane development could be improved up to 85% under same ZVI concentration added. In addition, the significance of ZVI supplementation among scrap iron and M100 extra fine micro particle were discovered identical (55% and 55%), especially at the end of operation period. Regardless of ZVI type, it could be clearly weighed from the experiments' result that the absence of mixing arrangement led ZVI's reaction kinetic with cassava substrate in methanation is confined only to during addition and remained inert because of static interface at the bottom ABR zone. Hence, methanation could not be optimized beyond significant level at 65% in ABR operation. It is highly likely that the low methane formation in this study is presumed to be collective gas evolved across 5 opened unit ABR cells for which biogas was investigated in single gas bag. Zhu et al., (2008) reported that using separately enclosed 4 compartmentalized ABR system, the methane formation where found higher (up to 75% CH_4) than that of preceding compartments. The decrease in methane formation during day 30 and beyond is as the result of temporary ambient temperature fluctuation when night time temperature drops lower than 28°C. As the result, the methane forming archaea which are thermophile in nature could not propagate methanogenesis under impending temperature stress (Yu et al., 2014; Mao et al., 2015). In recent study, employing multiple-compartment ABR for energy production, observed methane formation has been achieved on average 60% along months of operation (Pfluger et al., 2018). Using high strength wastewater in modified ABR system, up to 70% methane could be generated by kitchen waste (Malakahmad et al., 2011). As far as ABR related literatures concerned, it could be summarized that most of the studies targets waste

treatment like COD and solid removal prior to discharge the industrial effluent into natural waterbody nearby. However, studies suggest to have ABR modified when it is aimed for energy production (Barber & Stuckey, 1999; Liu et al., 2010; Ohimain & Izah, 2017). In conformity to these reviews, the result from this study provides with the evidence that the given ABR system needs structural and operational modification in order that the effect of ZVI catalyst is expended and activated to its highest reactional kinetic with feedstock materials.

9.6.2.2 Effect of ZVI on Digestion Performance and Process Stability

The regular investigation on intermediary products of anaerobic digestion during operation period is indispensable and crucial action to ensure whether the reactors are under controlled variables. The pH level of anaerobic digestion is the forefront indicator for stability of digester in which both diverse bacteria are in syntrophy (Kamagata, 2015). While lower pH (<6.5) promotes rapid growth of fermentative and acidogenic bacteria, this condition inhibits methane forming archaea which only survive at pH higher than 6.8 (Clark & Speece, 1971; Amani et al., 2010; Dobre et al., 2014). It is therefore, critical pH margin of 6.8 was maintained in all ABR sets. With higher mass ZVI exists in the compartment, the experiment revealed ZVI proved stronger pH buffer throughout the digestion period (figure 9.5a). Though M100 possess superior ZVI quality than scrap iron, because of its minimalist presence in ABR, pH resistance was found lower than that of scrap iron. The rise of pH within compartment #1 is also associated to the occasional buffering agent (NaHCO$_3$) administration since the synthesized substrates own pH level between 4.0 and 4.5 that continually subside ABR's operational pH level. The significance of ZVI presence in ABR is more apparent in compartment #5 in which pH were elevated up to 7.6 in

highly ZVI enriched iron, while pH level of iron free control reactor was varying 7.3 ±0.5 (figure 9.5b). Taking entry and exist point as point of examination, the pH level along the intermediate compartments (#2, #3, and #4) were deemed within ideal pH for healthy digester.
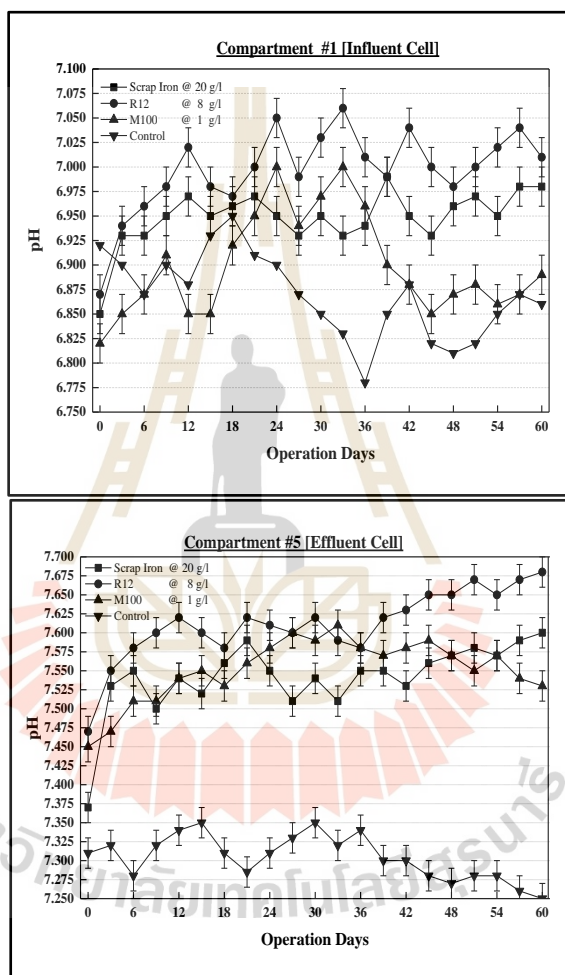

Figure 9.5 pH Changes inside ABR System

Figure 5a. pH Condition in Inlet (Compartment #1) (Left)

Figure 5b. pH Condition in Outlet (Compartment #5) (Right)

The ratio of Volatile Fatty Acids to Total Alkalinity (VFA/TA) represents food source availability to microorganisms. As the result of hydrolysis and acidogenesis, the released VFA was further transformed into acetate, then bio-methane was formed by the synergetic action of methane forming archaea (Zehnder & Mitchell, 1978; Breure & Audel, 1987; Klass, 1984). A well balanced VFA/TA ensures the propagation of anaerobic digestion microorganisms and stable digestion performance. Within the ratio 0.15~0.4 had been widely accepted as ideal ratio for biogas production (Speece, 1996: Lossie & Pütz, 2008). With the inlet channel (compartment #1) received sustained freshly synthesized substrate by continual organic loading, the VFA/TA ratio was found higher than 0.45 and increasing in control ABR (figure 9.6a). By ZVI introduction, the figure was moderate to 0.4 in all iron amended ABRs. The outcome of VFA/TA moderation was further apparent in outlet channel (compartment #5) in which the greater iron mass present, the lesser ratio was dropped down in ABR system (figure 9.6b). While higher VFA/TA ratio represents stronger volatile fatty acids accumulation leading pH drop and high acidity, the opposite of it leads food source exhaustion resulting disruption for the integrity microbial diversity. Since the VFA/TA ratio in compartment #1 and compartment #5 infers that the ratios follow within ideal range, therefore, the ratios of the three intermediate compartments deemed engaging between the initial and final margin (i.e. 0.45 − 0.175). In this regard, ZVI addition encourages stable digestion performance and cross-feeding of the anaerobic microbe (figure 9.6a and 9.6b).
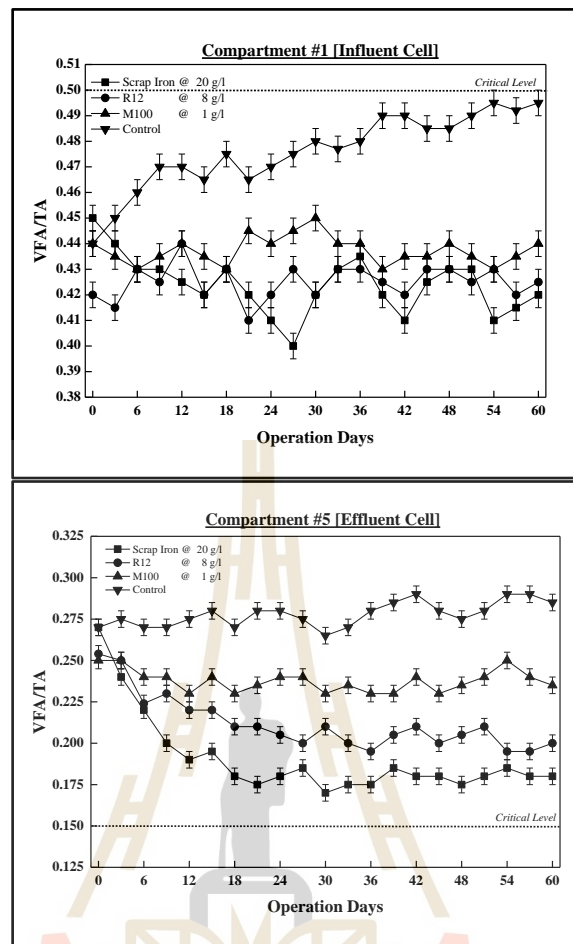
Figure 9.6 VFA/TA Profiles in ABR System

Figure 6a.VFA/TA condition in Inlet (Compartment #1) (Left)

Figure 6b.VFA/TA condition in Outlet (Compartment #5) (Right)

Soluble Chemical Oxygen Demand (sCOD) is one the alternatives when accessing digestion performance. This parameter is rather relevant for initial phase of anaerobic digestion since sCOD derived from the Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC) source which is the major constitute as carbohydrate found in cassava pulp and its wastewater (Clair et al., 2003; Okunade & Adekalu, 2013). Out of TOC present in the feedstock material, only carbon in soluble form is

readily accessible by microorganisms. Hence, sCOD is rationally chosen for process evaluation. Undergoing steady state operation and extended study, compartment #1 of all ABR received sCOD in increasing tendency because of organic loading in timely manner (figure 9.7a). Under this circumstance, sCOD removal could not be executed. Nevertheless, unreliable digestion is progressing under critical conditions at first room of the ABR. Then, the whole digestion was moderated along the ABR channel until effluent point.

Flowing through sequential compartments of ABR system, sCOD as organic fraction for the common food source was breakdown by hydrolytic and acidogenic bacteria. It was further removed in-between cells under satisfactory operational conditions as healthy AD reactor. From the experiments, sCOD was reduced to 500 mg/l in compartment #5 with scrap iron in 20 g/l supplemented ABR. In term of sCOD removal %, more than 90% removing capability has been achieved in all ABR sets. The effect of ZVI presence in ABR were found better sCOD removal than control set (figure 9.7b). However, regardless of ZVI concentration, the quantity of iron input provided with superior sCOD elimination. This is due to the fact that the contact area contributed better sCOD removal than ZVI concentration in the absence of mixing arrangement to foster homogeneity of substrate and activate ferro favoured (i.e. lithotrophs and chemotrophs) bacteria in anaerobic system (Blaut & Gottschalk,1997; Todar, 2011). In addition, HRT and number of cells also have significant influence on removal efficiency of ABR system. For wastewater treatment purpose, literatures affirm that the longer HRT and more compartment provided, the better treatment outcomes could be achieved (Barber & Stuckey, 1999; Reynaud & Buckley, 2015; Ramandeep, 2016). In compliance to aforementioned reviews, under

steady state operation with longer HRT (96hrs), COD and sCOD removal had been examined better than shortened HRT (24hrs) under same compartment scenario. However, this study emphasizes ultimate methanation by utilizing catalytic agent, ZVI into ABR, therefore HRT is shortened. It is highly likely that by introducing mixing arrangement or modifying ABR system, the beneficial effect of ZVI would be obviously observed.
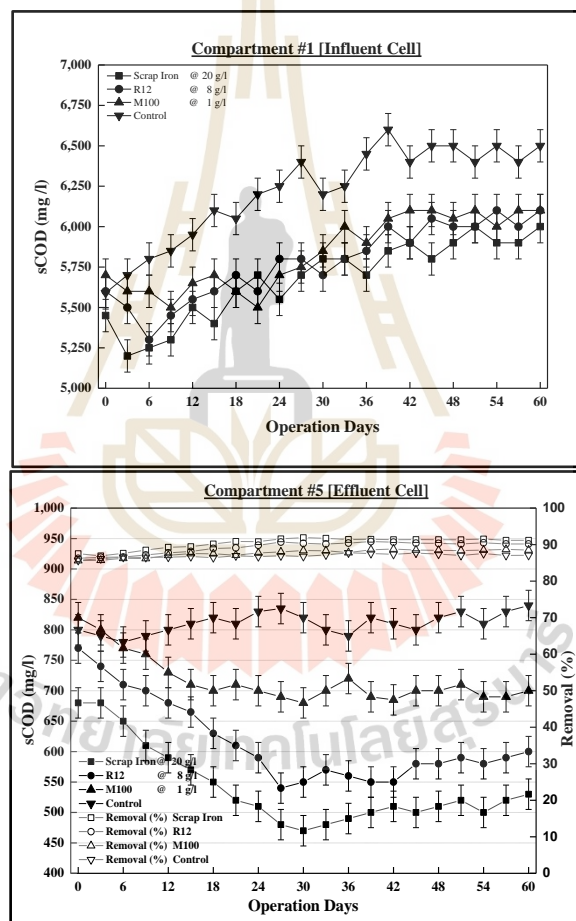
Figure 9.7 sCOD Removal and Efficiency in ABR System

Figure 7a. sCOD condition Inlet (Compartment #1) (Left)

Figure 7b. sCOD condition Outlet (Compartment #5) (Right)

9.7   Conclusion

Phase 1 of this study attempted to optimize biogas yield by ABRs system as part of Waste to Energy (WtE) for energy recovery from by-products of cassava starch factory for reaching steady state to a period. By regulating key variable factors of ABR operation (i.e. OLR and HRT), under steady state within 5 compartments of ABR system, ideal OLR was found 4.0 kg COD/m^3/day in 24 HRT for which the volumetric biogas production was found 2.9 l/day from working in 25 liters ABR volume. Through 3 trials, the experiments revealed possible inhibition and failure point of reference in several crucial parameters for timely intervention operation exceeds when minimum and maximum food loading.

From the results of extended study which is ZVI supplementation (Phase 2), it was discovered that instead of ZVI concentration, the quantity of iron input impacted better digestion performance. R12 consisting highest ZVI concentration, its presence did not generate outstanding results in both volumetric gas yield and methane content. Modification and providing mixing arrangement to given ABR sets are strongly encouraged in order to optimize ZVI's ultimate methanation which has been proved in lab scale CSTR study. Nevertheless, with static and mass ZVI occupancy inside ABR, it encourages stable digestion performance impacted by two major inversed determinants (i.e. HRT and OLR).

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CHAPTER 10

CONCLUSIONS AND RECOMMENDATIONS

10.1 Overview

Within the defined research scope in Chapter 2, this thesis covers the followings 4 separate steps in 3 research segments. They are -

- Pre-treatment to cassava pulp mixture to examine biogas production potential in batch mode.

- Characterization and Investigation for the concentration of Zero Valent Irons (ZVI) in selected powders for ultimate reducing properties and bioavailability in methanation.

- ZVI supplementation in differential doses to examine ideal concentration in optimizing methanation in lab-scale Continuous Stirred Tank Reactors (CSTRs).

- Integration the ideal dose obtained from lab-scale experiments into extended pilot-scale in Anaerobic Baffled Reactor (ABR) system.

Strictly following the research framework, research questions and objectives have been validated.

10.2 General Conclusions

Optimizing methane content of biogas is the primary focus of current research. By mean of cassava pulp digestion with mono-digestion and metal ZVI

supplementation has proved uplifting methane content, major energy calorific value of biogas. While cassava pulp can be amply available under minimalistic limitation, the metal iron is abundant on earth crust. The aspiration of applying these two inexpensive products for anaerobic digestion for methanation, current research topic has been established. Simply, quantitative analysis and sampling methods have been applied for characterizing cassava pulp, selected ZVI materials. Then, necessary trials and pre-pre-treatment options were applied prior ZVIs were subjected to test in CSTR and ABR system whether it could realistically enhance methane content within anaerobic processes. This research revealed ZVI addition in anaerobic digestion enhancing methane content with process stability within the condition when ZVI presence is strictly under stimulatory to anaerobic digestion level. And, their respective ideal concentration has also been uncovered. The role of biogas (biofuel)from agro-industrial by-products as one of the attempts to overcome the challenge of heavily dependency on unreliable and controversial carbon resources of the world has becomes more promising because of current research outcomes.

To sum up part I of the research segment, by using concentrated sulfuric acid (H_2SO_4), Sodium Hydroxide (NaOH), and thermal pre-treatment to cassava pulp, alkaline pre-treatment is more favourable since it saves chemicals during neutralizing back to pH 7 avoiding complication to acid and alkali driven from radicals within cassava substrate. Although thermal pre-treatment lacks detrimental consequences of chemicals, the extreme heat sterilizes all anaerobic microorganisms resulting delayed anaerobic digestion initiation and microbial imbalance. This phenomenon also applies to acid and alkaline pre-treatment in extreme pH level.Alkaline pre-treatment at pH 8-10 resulted highest biogas yield than its counterparts and control sampleAmong 3%,

5%, and 10% (w/v) solid content with comparative acid-alkaline and thermal pre-treated samples, 5% (w/v) generated ideal gas yield in batch digestion mode and save resource consumption. Among different alkaline pre-treatment with pH 9 to pH 10 in 3 different range, pre-treatment by $Ca(OH)_2$ at pH 10 was found ideal and compatible to exiting starch manufacturing chains and subsequent waste management. Though $Ca(OH)_2$ required more chemical quantity because of its highest molecular weight, its insoluble characteristic makes persistence in feedstock material in combination with residual lime from cassava starch production chains. When advanced processes will be employed into the AD system for future provision, these two chemicals (KOH and NaOH) can lead to complication with other supplemented chemicals into the system because of its more highly reactivity than $Ca(OH)_2$.

Employing X-rays technology of synchrotron beamline, irons' valency, atomic concentration, crystallite size, contaminant, and morphological characteristic of selected source (i.e. R12, M100 and Scrap Iron) had been verified and characterized. X-ray Absorption Spectroscopy (XAS) investigation revealed R12 and M100 possess more highly enriched with Valency Zero than Scarp Iron in comparison against iron in different oxidation states.X-ray Photoelectron Spectroscopy spectra revealed R12 has highest Fe atomic concentration with 76.86%.X-Ray Diffraction (XRD) pattern confirmed powder irons (i.e. R12 and M100) has corresponding purity and properties.Micro Beam Energy Dispersive X-ray Florescence (μ-XRF) analysis uncovered the minor existence of Manganese (Mn) and Tin (Sn) within selected iron sources.Scanning Electron Microscopy (SEM) images conclude R12 and M100 proved fit for enabling certain ZVI reactions for further ZVI function but being

readily oxidizable potential (reducing or catalytic properties) by ambient exposure, cleansing measure is highly recommended to shed oxidized surface.

The core of this research being part II and III which is optimizing methanation of cassava pulp digestion by ZVI supplementation. The net increase in methane (CH_4) content of biogas were 25%, 18% and 16% by 20, 10, 5 g/L by iron addition. Inhibition starts when scrap iron addition exceeds 20 g/L. 1 g/L M100 ZVI microparticle concentration resulted maximized SCOD removal (up to 95%) generating biogas for 900 ml/day/OLR with highest methane content (85%). However, the inhibition started when iron concentration exceeds 1 g/L ending digestion failure by iron toxicity. The optimum dosage for R12 ZVI is 8g/l with up to 93% bio-methane enhancement has been achieved with 67% added biogas volume. When ease of process control and sustainability are weighed for long-term operation for cassava pulp feed anaerobic bioreactor against differential ZVI particle size, micro-particle size R12 offers superior characteristics than nano-particle size M100. The application of low-cost elemental iron powder as reducing agent for methanation proved stimulatory for methane enhancement and gas yield if and only if its presence depends on concentration of zero valent iron and disintegration capabilities for bio-available nutrients. However, applying these optimum concentrations of ZVI in ABR system, the results ended up contradictory. ZVI presence in ABR system could only increase, gas volumefor 5.17%, 6.03%, and 8.62% in scrap iron, M100 and R12. These figuresare considerably lower than those of CSTR operation. Nevertheless, ZVI addition proved moderating uniform digestion along the compartments to maintain as healthy reactor. The loophole of inferior in methanation performance in ABR system is the need to introduce mixing arrangement or modifying conventional ABR system.

Static solid accumulation of feedstock material fosters algal bloom that could bring paradoxical and detrimental impact on microorganism communities and food sources availability.

10.3   Recommendations for Future Works

This study emphasizes enhancing bio-methane from the perspective of strictly physiochemical reaction among catalytic agent, Zero Valent Iron and Cassava Pulp within anaerobic digestion system. Since key indicators or intermediary products of biochemical conversion had been analysed throughout the study, following future study is strongly encouraged to understand more on the effect of ZVI in methanation.

i.   Metagenomic analysis and comparison should be conducted before and after ZVI addition to examine the effect of ZVI on individual microorganism species.

ii. Trial study for integration of ideal ZVI concentration should be made in advanced anaerobic reactors providing either mixing or stage separation mechanism.

iii. Effect of micro-nutrient supplementation to AD digestion along with ZVI in catalysing agent role is encouraged like mono feedstock material digestion in this study.

iv. With the exception of chemical pre-treatment, alternative pre-treatment options should be investigated for maximizing solid degradability and methanation by ZVI supplementation.

v.   Heating, insulation, and sunlight exposure system should be provided in no matter what reactor type is applied since while temperature fluctuation impairs

microbial activity; sunlight encourages algal booming affecting food source availability for microbe.

vi. Economic analysis over application of catalyst in cassava pulp methanation along with overall inventory of resources input and turnover.

10.4 Novelty of This Research

Scholarly contribution in answering research hypothesis include

- Forefront research for application of ZVI for optimizing methanation by agro-industrial residues, anticipating all essential criteria under mono-digestion realm.

- Low solid regime is mandatory when cassava pulp is subjected to AD process and optimizing methanation by catalyst supplementation.

- Importance of applying differential methods in characterizing ZVI materials to ensure ultimate reducing potential.

- Ideal concentrations for selected ZVI materials for optimizing methanation of cassava pulp digestion.

- The crucial role of reactor system when ZVI supplementation is applied for methane enhancement.
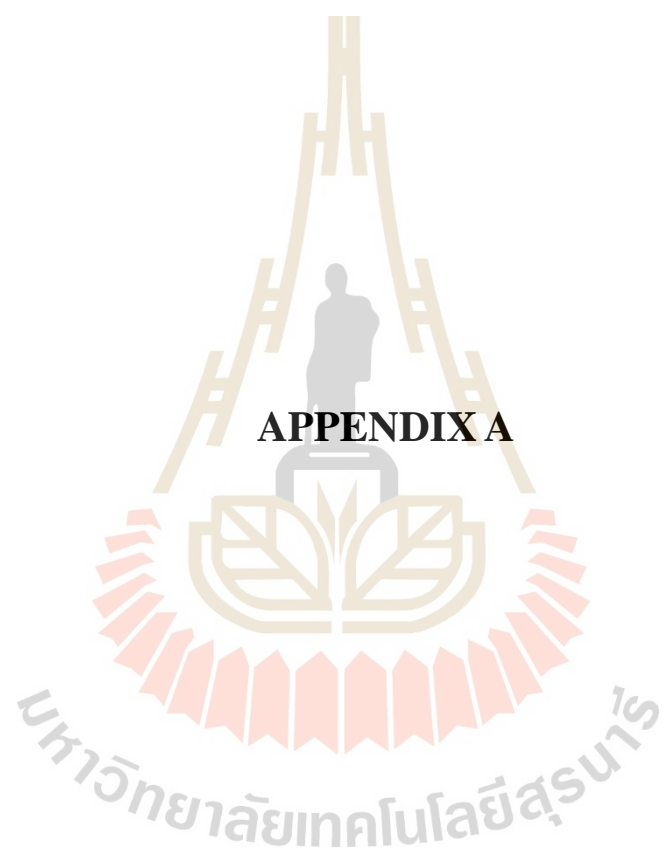
APPENDIX A

List of Publications

International Publications

Pyae, H., Boontian, N., Yingchon, U., & Piasai, C. (2018). Effect of Acid-Alkaline and Thermal Pre-treatment to Cassava Pulp Feed for Batch Reactors in Optimization of Bio-Methane Yield. **Applied Environmental Research**, 40(2), 51-60.

Aye, W., Aye H., Yossapol, C.,&Pyae, H. (2019). Effect of Alkaline Pre-Treatment on Cassava Pulp for Optimum Biogas Production. **International Journal of Recent Technology and Engineering (IJRTE)**, ISSN:2277-3878, Volume-8, Isssue-2, July 2019.

Pyae, H., Aye, W., & Yossapol, C. (2019). Investigation and Characterization of Iron Powders for Zero-Valent Iron (Fe0) in Synchrotron Radiations. **International Journal of Engineering and Advanced Technology (IJEAT)**, ISSN:2249-8958, Volume-8 Issue-3, February 2019.

Pyae, H., Aye, W., Yossapol, C., & Dararatana, S. (2019). Comparative study on the performance of iron-amended cassava pulp feed bio-methanation in CSTRs. **Engineering and Applied Science Research**, 46(3), 219-226.

Pyae., H, Aye, W., Yossapol., & Dararatana, S (2019). Micro-Particle ZVI Inhibition Thresholdin Cassava Pulp Bio-Methanation. **EnvironmentAsia**. Special Issue 12 2019. ISSN 1906-1714; Online ISSN: 2586-8861.

International Conference Proceedings

Pyae, H., Boontian, N., Yingchon, U., & Piasai, C. (2018). Biogas Production from Cassava Pulp: Review on Current Condition and Future Perspective. **Environmental Technology Conference; Environmental Engineering Association of Thailand**, January 2016, Bangkok Thailand.

Pyae, H., Yossapol, C., & Dararatana, S. (2019). Optimizing Volumetric Biogas Yield by Variable Organic Loading Rate and Hydraulic Retention Time in Five Compartmentalized Anaerobic Baffled Reactor (ABR) System. 1st**International Conference on Environment and Sustainable Development**. Yangon, Myanmar.

Poster Publication

Pyae, H., Boontian, N., Yingchon, U., & Piasai, C., (2016). Methanogenic Archaea: A Review on Microbial Communities in Anaerobic Digesters. 5th**International Conference on Environmental Engineering**, Science and Management. May 11-13, 2016. Bangkok, Thailand.

BIOGRAPHY

On 2009, Htay Aung Pyae completed Bachelor of Civil Engineeringfrom Hmawbi Technological University, Myanmar. Then, he pursued Master of Civil Engineering from 2010-2012 form Yangon Technological University, Myanmar. He conducted the research entitled "Evaluation of Proposed Drainage System of Bago" as partial fulfilment for the requirement for the degree. Worked as Civil Engineer at Aerodrome Standard and Safety Division, Department of Civil Aviation, and Environmental Consultant at Ramboll-Environ Global Inc. for some years, he started his Doctoral Studies in Environmental Engineering at Suranaree University of Technology (SUT), Thailand, under the scholarship of SUT Ph.D. ASEAN Scholarship for ASEAN and graduate scholarship of Thailand Institute of Scientific and Technological Research (TISTR) program. By financial support of these two institutes in combination, he completed his Ph.D. research in 2020.