UNIVERSITI PUTRA MALAYSIA

ENHANCEMENT OF BIOGAS PRODUCTION IN ANAEROBIC CO-DIGESTION OF SUBCRITICAL WATER PRE-TREATED WITH PINEAPPLE WASTE AND COW DUNG

ADILA FAZLIYANA BINTI AILI HAMZAH

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By

ADILA FAZLIYANA BINTI AILI HAMZAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

February 2022

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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February 2022

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The co-digestion of lignocellulosic waste with manure has been seen to improve the C/N ratio required by the systems to produce higher methane yield compared to mono digestion. Despite the success of co-digestion systems, the complex structure of pineapple waste slows down the digestion process. Subcritical Water (SCW) pre-treatment was introduced to improve the digestibility of pineapple waste. This study aims to evaluate suitable organic loading and mixing ratio of pineapple waste and cow dung for co-digestion process. Then, the SCW pre-treatment conditions of pineapple waste such as temperature, time and water to solid ratio were optimized using Response Surface Methodology (RSM). The impact of untreated and pre-treated pineapple waste on the physicochemical and morphology properties, methane production, its kinetic, and performance in an up-scaled anaerobic digester were further assessed at optimal conditions. Based on volatile solid (VS) contents, five different cow dung to pineapple waste ratios were assessed. The highest biogas and methane yield were obtained at ratio 1:3 with 179.08 mL/g VS and 142.89 mL CH_4/g VS. The co-digestion improved the C/N ratio, total ammonia nitrogen (TAN), VS removal, and pH. The kinetic study using the modified Gompertz model showed that the co-digestion process had shortened the lag phase, and the highest biogas production rate was observed at 12.80 mL/g VS. day. For optimization process, the model fitting showed the coefficient of determination (R^2) value of 0.9825. The RSM model predicted a maximum biogas yield of 144.17 mL/g VS for pre-treatment conditions at the water to solid ratio of 7.5:1, the temperature at 160°C and the reaction time of 15 minutes. The optimal condition of SCW pre-treatment was at 128.51°C, water to solid ratio of 5.67:1, and reaction time of 5 minutes. The compositional analysis found that the pre-treated pineapple waste reduced lignin, hemicellulose, and cellulose contents. Pre-treated samples yielded 102.42 mL/g VS, which was 23% higher than untreated. Analysis using Fourier Transform Infrared Spectroscopy (FTIR) and Thermogravimetric Analysis (TGA) verified the presence of cellulosic material in the pre-treated pineapple waste. X-ray

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Diffraction (XRD) analysis indicated changes in the crystallinity index. Scanning Electron Microscopy (SEM) confirmed structural modification of pre-treated pineapple waste and made it accessible for microbial attack. A higher methane yield of 85.05 mL CH_4/g VS was observed in pre-treated pineapple waste with a 44% increment. The co-digestion of optimized SCW pre-treated pineapple waste and cow dung was upscaled using 6.4 L lab-scale anaerobic digester. The cumulative biogas produced was 2100 mL which yielded 17.13 mL/g VS. The methane volume was 440 mL with a 36 to 86% methane content. The kinetic study of upscale anaerobic digester showed that the lag phase had shortened, and the highest biogas production rate was observed at 17.53 mL/g VS. day. This suggests that the co-digestion process using SCW pre-treated sample could increase methane production, enhance process efficiency and significantly reduce the digestion time. The co-digestion of SCW pre-treated pineapple waste with cow dung improves biogas production and produces a higher methane yield in comparison to untreated samples. Enhancing biogas production from SCW pre-treated pineapple waste with cow dung appears to be a promising approach for bioenergy recovery.

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PENINGKATAN PENGELUARAN BIOGAS DALAM PENCERNAAN ANAEROBIK BERSAMA BAGI PRA-RAWATAN AIR SUBKRITIKAL BERSAMA SISA NANAS DAN NAJIS LEMBU

Oleh

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Pencernaan bersama sisa lignoselulosa dengan najis telah meningkatkan nisbah C/N yang diperlukan oleh sistem untuk menghasilkan hasil metana yang lebih tinggi berbanding dengan pencernaan tunggal. Di sebalik kejayaan sistem pencernaan bersama, struktur kompleks sisa nanas melambatkan proses pencernaan. Pra-rawatan air subkritikal (SCW) telah diperkenalkan untuk meningkatkan kebolehcernaan sisa nanas. Kajian ini bertujuan untuk menilai muatan organik dan nisbah pencampuran yang sesuai bagi sisa nanas dan najis lembu dalam pencernaan anaerobik bersama. Kemudian, keadaan pra-rawatan SCW bagi sisa nanas seperti suhu, masa dan nisbah air kepada pepejal dioptimumkan menggunakan kaedah permukaan tindak balas (RSM). Kesan sisa nanas yang tidak dirawat dan pra-rawatan ke atas sifat fizikokimia dan morfologi, pengeluaran metana, kinetik, dan prestasi dalam pencerna anaerobik berskala tinggi juga dinilai pada keadaan optimum. Berdasarkan kandungan pepejal meruap (VS), lima nisbah najis lembu kepada sisa nanas yang berbeza telah dinilai. Hasil biogas dan metana tertinggi diperoleh pada nisbah 1:3 dengan 179.08 mL/g VS dan 142.89 mL CH_4/g VS. Pencernaan bersama meningkatkan nisbah C/N, jumlah nitrogen ammonia (TAN), penyingkiran VS dan pH. Kajian kinetik menggunakan model Gompertz yang diubah suai menunjukkan bahawa proses pencernaan bersama telah memendekkan fasa lag, dan kadar pengeluaran biogas tertinggi diperhatikan pada 12.80 mL/g VS.hari. Untuk proses pengoptimuman, model menunjukkan nilai pekali penentuan (R^2) sebanyak 0.9825. Model RSM meramalkan hasil biogas maksimum pada 144.17 mL/g VS untuk keadaan pra-rawatan pada nisbah air kepada pepejal 7.5:1, suhu pada 160°C dan masa tindak balas 15 minit. Keadaan optimum ialah pada 128.51 °C, nisbah air kepada pepejal 5.67:1 dan masa tindak balas 5 minit. Analisis komposisi mendapati kandungan lignin, hemiselulosa dan selulosa pada sisa nanas yang telah dirawat telah berkurang. Sampel pra-rawatan menghasilkan 102.42 mL/g VS biogas, iaitu 23 % lebih tinggi daripada tidak dirawat. Analisis menggunakan Spektroskopi Inframerah Transformasi Fourier

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(FTIR) dan Analisis Termogravimetrik (TGA) mengesahkan kehadiran bahan selulosa dalam sisa nanas pra-rawatan. Analisis Pembelauan Sinar-X (XRD) menunjukkan bahawa terdapat perubahan dalam indeks kehabluran. Imbasan Mikroskop Elektron (SEM) mengesahkan pengubahsuaian struktur sisa nanas pra-rawatan dan menjadikannya boleh diakses untuk serangan mikrob. Hasil metana yang lebih tinggi sebanyak 85.05 mL CH_4/g VS diperhatikan dalam sisa nanas pra-rawatan dengan peningkatan sebanyak 44%. Sisa nanas pra-rawatan yang optimum dicernakan bersama najis lembu telah ditingkatkan menggunakan pencerna anaerobik skala makmal 6.4 L. Biogas kumulatif yang dihasilkan ialah 2100 mL yang menghasilkan 17.13 mL/g VS. Isipadu metana ialah 440 mL dengan kandungan metana 36 hingga 86%. Kajian kinetik untuk pencerna anaerobik skala tinggi menunjukkan bahawa fasa lag telah dipendekkan, dan kadar pengeluaran biogas tertinggi diperhatikan pada 17.53 mL/g VS. hari. Hal ini menunjukkan bahawa proses pencernaan bersama boleh meningkatkan pengeluaran metana, meningkatkan kecekapan proses dan mengurangkan masa pencernaan dengan ketara. Pencernaan bersama sisa nanas pra-rawatan SCW dengan najis lembu meningkatkan pengeluaran biogas dan menghasilkan hasil metana yang lebih tinggi dibandingkan dengan sampel tidak di rawat. Peningkatan pengeluaran biogas daripada sisa nanas pra-rawatan SCW dengan najis lembu dilihat sebagai pendekatan yang menjanjikan untuk pemulihan biotenaga.

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May Allah reward your kindness with the best of rewards. Thank you all.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by Members of the Supervisory Committee

This is to confirm that:

- the research and the writing of this thesis were done under our supervision;
- supervisory responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2015-2016) are adhered to.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CCD	Central Composite Design
CH_4	Methane Gas
CI	Crystallinity Index
CO_2	Carbon Dioxide
C/N	Carbon to Nitrogen
DTG	Derivative Thermogravimetric
FTIR	Fourier Transform Infrared
g	Gram
GC	Gas Chromatography
GHG	Greenhouse Gases
H_2	Hydrogen Gas
HCL	Hydrochloric Acid
HMF	Hydroxymethylfurfural
H_2SO_4	Sulphuric Acid
L	Litre
mL	Millilitre
NaOH	Sodium Hydroxide
OLR	Organic Loading Rate
R^2	Correlation Coefficient
RMK	*Rancangan Malaysia Ke*
RSM	Response Surface Methodology
SCW	Subcritical Water
SDG	Sustainable Development Goal

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SEM	Selective Electron Microscopy
TAN	Total Ammonia Nitrogen
TAPPI	Technical Association of The Pulp and Paper Industry
TCD	Thermal Conductivity Detector
TGA	Thermogravimetric Analysis
TS	Total Solid
UPM	Universiti Putra Malaysia
VFA	Volatile Fatty Acid
VS	Volatile Solid
XRD	X-Ray Diffraction
2D	Two Dimensional
3D	Three Dimensional
λ	Lag Phase
º C	Degree Celsius

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CHAPTER 1

INTRODUCTION

1.1 Background

The concern for sustainable waste management for agricultural and food processing waste has increased recently. These wastes often end up in landfills, where a substantial amount of waste is transformed into greenhouse gases (GHG) and methane. The particular reason for the situation is that these wastes are easily degraded than other wastes. In line with United Nations' Sustainable Development Goal (SDG), with good food waste management practice, responsible production and consumption by linking the concept of food loss and waste management could be accomplished to reduce the release of toxic matters to the environment (Lemaire and Limbourg, 2019; Rodić and Wilson, 2017). Also, one of the core objectives of Malaysia's National Agrofood policy 2021-2025 highlighted the need to improve agricultural practices and a sustainable food system (MAFI, 2021).

One of the strategies under this core is to reduce food loss and wastage along the food chain by improving efficient resources usage. In recent years, researchers have been focused on the conversion of agricultural and food waste to valuable products based on the waste-to-wealth concept to reduce the downsides of inappropriate waste management practices (Hamzah et al., 2016; Rico et al., 2020; Sindhu et al., 2019). The derivation of energy from animal by-products such as livestock, poultry and other agricultural waste can be seen as a sustainable waste management method. One of the themes introduced in the 12th Malaysia Plan 2021-2025 (RMK12) is pursuing advancing sustainability (RMK-12, 2021). This theme focuses on embracing the circular economy and accelerating the adoption of integrated resources management. This plan encourages the public and private sectors to implement and integrate the SDGs values in their decision-making. Apart from ensuring supply safety, the circular economy will reduce dependency on non-renewable energy, waste production, pollution and GHG emissions.

Bioenergy production based on biogas has been increasing in Malaysia for several years. In Malaysia, solid waste generation had increased from 3856 tonnes per day to 4967 tonnes per day in 2020, and more than 80% of this waste is subjected to landfill (Lim et al., 2021). A biochemical process, using this waste as a feedstock source in biogas plants, appears to be one of the dynamic methods to improve the application and management levels of this waste, especially in country parts (Cigolotti, 2012). Biogas production through an anaerobic digester plant would be convenient on on-site food production zones, predominantly countryside areas with limited energy source connections (McPhail et al., 2012).

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Pineapple has been listed as one of ten premium fruits by the Malaysian government with USD153 million production value (Abu Bakar et al., 2021). As one of the major pineapple producers in Southeast Asia and the increasing demand for pineapples, Malaysia produced more than 100,000 tonnes of pineapple wastes (Ahmad Zamri et al., 2020). High moisture and sugar content in pineapple waste increases the spoilage rate due to microbes, thus contributing to environmental problems. Large capital is needed to dispose of the cumulative amount of waste in landfills (Rico et al., 2020). The conventional techniques for managing this waste include open burning and landfilling, which contribute to air pollution (Yahayu et al., 2017) as well as huge capital expenditure to discard these wastes, and land contributes to greenhouse gas emissions (Banerjee et al. 2018).

Globally, it was estimated that there were approximately 1.5 billion stocks of cow population in 2019, and 683,501 stocks were estimated in Malaysia (FAO, 2021). Rising demand for dairy products in Malaysia in recent years due to increased livestock manure volume generation. Also, out of million tonnes of livestock production in Malaysia, cows had the highest manure, contributing to 5.45 million tonnes a year (Abdeshahian et al., 2016). The improper waste management techniques of cow dung have contributed to odour problems, GHG emission, airborne ammonia, and the release of harmful pathogens (Gopinathan et al., 2018; Gupta et al., 2016; Tufaner and Avşar, 2016). Cow dung will be toxic to the environment and causes nutrient imbalance and contamination if not appropriately managed. They comprise a high nitrogen and phosphorus concentration and traces of harmful elements such as growth hormones, antibiotics, and heavy metals.

1.2 Problem Statement

Bioenergy is renewable, releases fewer toxic mixtures through burning, reduces carbon dioxide emissions, and absorbs the most produced carbon dioxide compared to fossil fuels (Voloshin et al., 2016). Due to the increase of total population in Malaysia, the energy demand has increased about 21%, mainly from industry, transportation, agriculture, and housing and commercial sector (Abdeshahian et al., 2016). On account of the limited availability of fossil fuel, an easily accessible, cheap, and environment-friendly renewable energy source is vital. Methane-rich biogas is produced from various feedstocks, consisting of primarily 50–75% methane, 19–34% carbon dioxide, and less than 1% hydrogen through the anaerobic digestion process (Elina, 2016; Jehan et al., 2017). Pineapple waste and cow dung have been seen as potential feedstocks for biogas production (Deng et al., 2012; Haryanto et al., 2018; Kafle and Chen, 2016; Kumar et al., 2017; Nga and Trang, 2015).

The main challenges in anaerobic digestion especially on mono digestion using sole substrates are the high carbon to nitrogen (C/N) ratio and slow hydrolysis of the substrates. Mono digestion manure often hinders

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anaerobic digestion due to high nitrogen content that could increase ammonia in the systems (Li et al., 2015). To eliminate the effect of nitrogen deficiency in pineapple waste, cow dung is seen as one of the alternative co-substrates to balance the C/N ratio required by the systems. The co-digestion process increases the buffer capacity, reduces the toxic effect, improves the pH and C/N ratio, and enhances biodegradability (Akyol et al. 2016; Li et al. 2018; Shen et al. 2019). The balanced C/N ratio promotes a synergistic effect on the systems. It also creates a nutrient imbalance for the microorganisms in the anaerobic digestion process. However, an appropriate mixing ratio is needed to ensure the C/N ratio of the substrate combination is in the optimal range as suggested by several pieces of literature, whereby the optimal C/N ratio recommended by literature was in the range of 15 to 30 (Hagos et al., 2017; Lee et al., 2019; Xie et al., 2011).

Though cow dung is proved to be reasonable to be used as a single substrate due to the degradability and availability of bacteria needed in the fermentation phase, it is also rich in nutrients, micronutrients, and other trace elements that boost bacterial growth (Achinas et al., 2018; Sidik et al., 2013). Despite this, low biogas production often results from mono digestion of cow dung due to low carbon content and lignin complexes (Achinas et al., 2018; Akyol et al., 2016). Methanogens might inhibit the anaerobic digestion process due to inappropriate C/N ratio and high nitrogen content (Aksay et al., 2018; Zhang et al., 2013). Adding lignocellulosic waste as co-substrates to manure could help balance the C/N ratio due to high carbon and sugar content. High carbon content in lignocellulosic waste balances the high nitrogen content in manure. It is critical to co-digest manure with an appropriate substrate for nutrient balance and lower the process limitation (Achinas et al., 2018). Pineapple waste is known to contain 11.74% glucose, 9.70% fructose, 2.93% xylose, 2.05% sucrose, and 24.04% reducing sugars (Zain et al., 2021). Such composition makes them suitable as feedstocks for biogas production. Several studies have been conducted using pineapple waste as sole substrates (mono digestion) for anaerobic digestion, resulting in a more prolonged lag phase required to produce significant biogas volume (Dahunsi, 2019; Kumar et al., 2017; Muenmee and Prasertboonyai, 2021). Co-digestion of pineapple waste and cow dung would balance the C/N ratio for better biogas production. In addition, co-digestion for cow dung would be beneficial for the circular economy. Obtaining the substrates for biogas production is made easier by the abundance of pineapple waste from the pineapple plantation and cow dung from the feedlot.

Integrating plantation and livestock production created more sustainable and productive agricultural systems for smallholder farming families (Garrity et al., 2010). This type of integrated production could benefit the co-digestion process due to feedstock availability. The Eco-Yap Agriculture and Livestock Farm is an emerging farm in Malaysia that produces pineapple and dairy cows (Lee, 2019). To date, cow dung was used as the compost for pineapple production at the same farm. However, pineapple waste from the farm is still underutilized. Since the farm was also operated as 'Farm Stay' to boost agrotourism (Han, 2020), the potential of utilizing cow dung

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and pineapple waste as co-digestion substrates for on-site biogas production could be another sustainable approach since the biogas produced can generate electricity. The logistical issues associated with transporting the waste could be resolved. Therefore, co-digestion of pineapple waste and cow dung can be viewed as a viable waste management method that could be proposed in a variety of Malaysian integrated farms.

Mono digestion often observes lower biogas yield, and even with anaerobic co-digestion, the biogas yield is not satisfying due to the complexity of the structure. High resistance of lignocellulose to biological hydrolysis is associated with the presence of refractory lignin, its linkage to the strong bond between cellulose and hemicelluloses, and the presence of crystalline cellulose (Paudel et al., 2017). The primary purpose of the pre-treatment for lignocellulose feedstock is to disrupt lignin structure; consequently, cellulose and hemicellulose become accessible for microbial attack. Subcritical Water (SCW) pre-treatment promotes structure-breaking and substrate availability after pre-treatment (Wang et al. 2018).

SCW pre-treatment technology is gaining prominence in biogas production enhancement. SCW pre-treatment involves pre-treatment using water is very sustainable and environmentally friendly since it does not require acid recycling, is non-corrosive, non-toxic, and inflammable (Antwi et al. 2019; Saha et al. 2013). Water enters the lignocellulosic complex to hydrate cellulose, solubilize a significant amount of hemicellulose and partially remove lignin (Ahmad et al., 2018). The effectiveness of the SCW depends on the process parameters such as temperature, reaction time, and water to solid ratio. These parameters are the aspects of dominant importance concerning pre-treatment efficiency and subsequently on the anaerobic digestion. Too high temperature and longer reaction time could contribute to the release of toxic inhibitors compounds. Too low temperature and short reaction time might not be able to treat the substrates effectively. Thus, the proper study should be conducted to identify the appropriate SCW parameter set.

In this study, the application of co-digestion could elevate the potential biogas production from pineapple waste and cow dung and recommend fully utilizing this waste as renewable energy sources. To date, limited study on biogas production investigation focuses on the proper mixing ratio to meet suitable C/N ratio (15 to 30) of pineapple waste and cow dung (Hagos et al., 2017; Lee et al., 2019; Xie et al., 2011). On the other hand, even though anaerobic co-digestion, pineapple waste still could not be utilized fully due to the complex substrates. The complex structure of pineapple consists of lignin structure embedding the cellulose and hemicellulose that resisting microbial attack and subsequent hydrolysis. Pre-treatment could enhance the biogas production by structural modification and make the cellulose accessible for hydrolysis. Application of pre-treatment to pineapple waste prior to co-digestion with cow dung has been seen as one of the solutions

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to the problem. However, it is vital to carefully conduct the pre-treatment at its optimal parameter to avoid the formation of toxic compounds (furfural and hydroxymethylfurfural (HMF)), which can significantly inhibit methanogenesis. A limited study has been conducted on SCW pre-treatment as biogas enhancement method available for pineapple waste especially on the physicochemical, structural and morphological modification of pineapple waste. That information is crucial to evaluate the suitability of SCW pre-treatment in biogas production. It is also essential to assess the ability of the co-digestion method and SCW pre-treatment on an upscale size. The ability of the process to operate in a large-scale anaerobic digester will prove its feasibility for industrial scale. Thus, this study focuses on investigating the proper mixing ratio and the application of SCW pre-treatment to improve methane yield from co-digestion of pineapple waste and cow dung.

1.3 Aim and Objectives

The aim of this study is to investigate the enhancement of biogas production in anaerobic co-digestion of pineapple waste and cow dung by SCW pre-treatment. The specific objectives are as follows:

i. To study the suitable organic loading and mixing ratio of pineapple waste and cow dung for methane production by anaerobic co-digestion in a batch experiment.

ii. To determine the optimum SCW pre-treatment conditions of pineapple waste such as temperature, time and water to solid ratio using Response Surface Methodology (RSM) on biogas production by anaerobic co-digestion.

iii. To assess and characterize the impact of untreated and pre-treated pineapple waste on the physicochemical and morphology properties, methane production, its kinetic and performance in an up-scaled anaerobic digester at optimal conditions.

1.4 Scope of Study

The pineapple wastes (Josephine) were collected from Pasar Borong Selangor, Malaysia. Cow dung was collected from a cow farm at Universiti Putra Malaysia. Inoculum prepared from anaerobic digestion sludge. The characterization of Total Solid (TS), Volatile Solid (VS), pH, ash, moisture content, Carbon ©, Nitrogen (N), Total Ammonia Nitrogen (TAN), and C/N ratio were analyzed prior to the digestion process. The anaerobic mono and co-digestion digestion were performed at 100 mL working volume at mesophilic temperature (37 ±1ºC) at startup pH of 7. The organic loading tested at 14 gVS/L to 30 gVS/L. The mixing ratios VS basis were tested at 1:1,1:2, 1:3, 2:1, 3:1 (Cow dung: Pineapple waste). The gas compositional

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analysis every ten days. In the pre-treatment, only SCW pre-treatment was used. The optimization of SCW pre-treated pineapple waste with cow dung using RSM developed from Design-expert software using the same condition as preliminary study and mixing ratio at 1:3 VS basis using 30 gVS/L organic loading. The optimal SCW pre-treated pineapple waste and untreated were compared on its methane yield, anaerobic process parameters, compositional analysis (lignin cellulose and hemicellulose), Fourier Transform Infrared (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), and Thermogravimetric Analysis (TGA) analysis. The anaerobic co-digestion of SCW pre-treated pineapple waste and cow dung were done in 6.4 L anaerobic digester at 5.4L working volume. Kinetic study throughout the research was validated using modified Gompertz model equation.

1.5 Overview of Thesis

Chapter 1 covers the research background and the objectives of the thesis. Chapter 2 presents a current literature review on pineapple waste and cow dung, and research contributions made towards biogas production. In addition, fundamental of anaerobic digestion process and pre-treatment methods are also discussed. Chapter 3 explains an overall experimental method of anaerobic co-digestion and SCW pre-treatment that includes a detailed experimental set-up and design as well as characterization techniques. Chapter 4 further presents the results and discussions of the study. In this chapter, the mono and anaerobic co-digestion of pineapple waste and cow dung at different organic loading mixing ratios based on VS content were discussed. The SCW pre-treatment using RSM, fit summary and validation of the model, analysis of variance (ANOVA) and model fitting, the interactive effect of the process variable were explained. Optimal set of SCW pre-treatment parameters are generated from RSM and the optimal set of SCW pre-treatments were validated experimentally. The methane yield of untreated and optimized SCW pre-treated pineapple waste were compared. The effect of SCW pre-treated on the characterization, composition, structural modification, and crystallinity index were compared. The last section discussed the performance of anaerobic co-digestion of SCW pre-treated pineapple waste and cow dung in an upscale anaerobic digester. Finally, Chapter 5 presents the conclusion and recommendations for future research.

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