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豬糞尿水初次消化液與稻稈、竹材之厭氧共消化

Anaerobic Co-digestion of Predigested Swine Wastewater Effluent with Rice Straw and Bamboo Waste

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本論文係黃獻宏君（學號 R09631046）在國立臺灣大學生物機電工程學系、所完成之碩士學位論文，於民國 110 年 08 月 16 日承下列考試委員審查通過及口試及格，特此證明

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中文摘要

隨著台灣的養豬戶慢慢轉向大規模養殖，為滿足所需的廢水排放標準，針對養豬場廢水處理系統的需求將越來越高。台灣常見的廢水處理方式是由畜產試驗所（Taiwan Livestock Research Institute, TLRI）開發的三段式處理系統，其中包括 1）固液分離、2）厭氧消化以及 3）好氧處理，依次進行。然而，由於好氧處理的耗能性質，因此提出了另一種省略好氧池的處理系統：可用第二階段厭氧反應槽代替好氧池，第一階段厭氧反應槽的污水可與農業廢棄物如稻草（RS）和林業廢棄物如竹子廢棄物（BW）共同消化。第二階段厭氧共消化代替好氧池的優點是，除了可處理更多的廢棄物，同時又可減少厭氧池的能量消耗，又可產生更多的再生能源。

在本研究，豬糞尿初次消化液（Swine Wastewater Effluent, SWE）是由實驗室規模的第一階段連續攪拌式反應槽（Continuously Stirred Tank Reactor, CSTR）中所收集，該 CSTR 在 37°C 和 HRT 10 天內消化了 TS 為 8%的新鮮豬糞尿（Fresh Swine Manure, FSM）。第二階段的厭氧 CSTR 在 37°C 下分批進行。SWE 的量固定為 3,000 mL，RS+BW 的量固定為 200 g。本研究的目的是比較不同的 RS：BW 混合比例對厭氧共消化之差異，共進行了 100：0（R100）、80：20（R80）、60：40（R60）、40：60（R40）、20：80（R20）和 0：100（R0）6 個批次的試驗。每一批次都是兩重複（Duplicate），包括參考點的空白試驗。

由於 RS 具有較佳的生物降解性，因此 RS 比率越高，固體去除率越高，單位 VS 的甲烷產量就越高。實驗結果顯示最低和最高的總固形物去除率（s-RTS）分別為 R0 批次的 8.09%和 R100 批次的 49.84%。最低和最高的揮發性固形物去除率（s-RVS）在批次 R0 和批次 R100 中分別為 12.17%和 57.37%。單位甲烷產量

（Methane Yield）在 R0 中最低，為 0.036 L CH_4/g VS added，R80 中最高，為 0.171 L CH_4/g VS added，而 R100 的單位甲烷產量僅略低於 R80 為 0.169 L CH_4/g VS added。就去除的 VS 而言，所有批次的單位甲烷產量平均為 0.311 L CH_4/g VS destroyed，它也可以被視為 VS-甲烷轉化率。至於 BW 的生物降解性比 RS 低的原因，過去的研究曾指出纖維素和木質素之和與厭氧生物降解性呈負相關，而本研究所呈現的結果與前人的研究一致。

關鍵詞：厭氧共消化、甲烷、稻稈、竹材、木質纖維素

Abstract

As Taiwan’s swine farmers are moving away from small scale farming to bigger scale farming, more swine farms will need proper wastewater management to meet the required wastewater discharge standard. The common state-of-the-art wastewater treatment in Taiwan is three-stage treatment system developed by Taiwan Livestock Research Institute (TLRI), which includes 1) solid-liquid separation, 2) anaerobic digestion, and 3) aerobic treatment, in sequence. However, since the aerobic treatment is energy intensive, another alternative treatment system without one is proposed: the aerobic tank could be substituted with second-stage anaerobic reactor which co-digest effluent from first-stage anaerobic reactor as the seeding bacteria with agricultural waste, such as rice straw (RS), and forestry waste, such as bamboo waste (BW). The advantage of second-stage anaerobic co-digestion instead of aerobic treatment is that it can treat additional waste, which otherwise would go untreated, while at the same time produce more green bio-energy and save cost. This research explored this idea by experimenting with anaerobic co-digestion in the second-stage anaerobic reactor, using first-stage anaerobic effluent as the seeding bacteria.

In this research, swine wastewater effluent (SWE) was collected from simulated, lab scale, first-stage semi-continuous anaerobic CSTRs that digested TS 8% of fresh swine manure (FSM) at 37 ± 1 ^{o}C and HRT 10 days. The second-stage anaerobic CSTRs were operated in batch at 37 ± 1 ^{o}C. The amount of SWE was fixed with 3000 mL : 200 g ratio compared to biomass mixture, which comprises of RS and BW. The aim of this research is to investigate the differences among 6 different RS:BW mixing ratios: 100:0 (R100), 80:20 (R80), 60:40 (R60), 40:60 (R40), 20:80 (R20), and 0:100 (R0). Each was done in duplicate, including blanks for the reference point.

Since RS has better biodegradability, the higher the RS ratio, the higher the solids removal efficiency, and the higher the methane yield per gram of VS added. Lowest and highest s-RTS is 8.09% in batch R0 and 49.84% in batch R100, respectively. Lowest and highest s-RVS is 12.17% in batch R0 and 57.37% in batch R100, respectively. The methane yield is lowest in R0 at 0.036 L/g VS added, and the highest in R80 at 0.171 L/g VS added, although R100 methane yield was only slightly below at 0.169 L/g VS added. In terms of VS destroyed, all of the batches averaged at methane yield of 0.311 L/g VS destroyed. It could also be interpreted as VS-methane conversion rate.

As for the reason of lower biodegradability in BW as compared to RS, lignocellulosic composition analysis of them was done. Three other studies were also incorporated in the lignocellulosic composition comparison. The result was in line with previous studies' conclusions that the lignin content is negatively correlated with anaerobic biodegradability. Thus, higher lignin content would yield lower biogas and methane yield.

Keywords: Anaerobic Co-digestion, Methane, Rice Straw, Bamboo, Lignocellulose

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List of Abbreviations

Abbreviation	Full Name
ADL	Acid Detergent Lignin
BMP	Biochemical Methane Potential
BB-LS	Mixture of 200g Bamboo with Low S/I Ratio
BB-HS	Mixture of 200g Bamboo with High S/I Ratio
BR-LS	Mixture of 100g Bamboo and 100g Rice Straw with High S/I Ratio
BW	Bamboo Waste
BOD	Biochemical Oxygen Demand
C/N Ratio	Carbon-to-Nitrogen Ratio
CNS	Chinese National Standard
COA	Council of Agriculture
COD	Chemical Oxygen Demand
CSTR	Continuously-Stirred Tank Reactor
FSM	Fresh Swine Manure
HRT	Hydraulic Retention Time
RTS	Removal Efficiency of Total Solids
s-RTS	Co-substrates' Removal Efficiency of Total Solids
t-RTS	Total Mixture's Removal Efficiency of Total Solids
RVS	Removal Efficiency of Volatile Solids
s-RVS	Co-substrates' Removal Efficiency of Volatile Solids
t-RVS	Total Mixture's Removal Efficiency of Volatile Solids
RS	Rice Straw
S/I Ratio	Substrate-to-Inoculum Ratio
SWE	Swine Wastewater Effluent
SS	Suspended Solids
TLRI	Taiwan Livestock Research Institute
TS	Total Solids
VS	Volatile Solids

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Chapter 1 Introduction

As Taiwan is having less and less number of swine farm while having the total swine stocks at around 550 million heads since years ago, it implies that there is an ongoing trend towards large scale swine farms, which, by definition, hold more than a thousand swine in a single farm (COA, 2022). While it might be more advantageous in economic term for the farmers to own a larger scale farm, the concentration of many animals in a small area would raise the issue of wastewater concentration and the challenge of its proper treatment.

In Taiwan, swine wastewater typically undergoes a three-stage treatment system developed by Taiwan Livestock Research Institute (TLRI), which includes 1) solid-liquid separation, 2) anaerobic digestion, and 3) aerobic treatment, in sequence (Sheen et al., 1994). Anaerobic digestion is just one among many alternatives to treat agricultural wastewater, but one of the advantages of anaerobic digestion is that while the organic waste is stabilized, energy is also produced in the form of methane-containing biogas (McCarty, 1964), thus shooting two birds with one stone.

In the usual three-stage treatment system, the effluent from anaerobic digestion goes to the aerobic pond for further treatment, then eventually discharged into water body. Despite it is being the common practice, aerobic treatment is energy intensive, and wastewater discharge cost the swine farmers a fortune.

Here, it is proposed that the aerobic pond could be substituted by a second-stage anaerobic treatment. The second-stage anaerobic digester can co-digest the effluent from the first-stage anaerobic digester together with other dry wastes, such as rice straw and bamboo waste. In general, the volume of wastewater discharge would be reduced as some of the wastewater would be absorbed by the dry wastes. To push the idea even further, in an ideal case, the wastewater would be absorbed totally by the dry wastes such that zero-discharge could be achieved. The dry wastes also act as extra carbon-source to rebalance the C/N ratio that would have been depleted in the first stage anaerobic digester.

Such system can have several advantages: it could save cost for the farmers by 1) reducing energy consumption of aerobic treatment and 2) reducing wastewater discharge volume. In addition, it could 3) treat additional amount of waste, which would otherwise be untreated, and 4) have additional renewable green bio-energy in the form of methane-containing biogas. This research explored this idea of anaerobic co-digestion using predigested swine wastewater effluent with rice straw and bamboo waste.

There have been many indications that anaerobic co-digestion exhibited synergistic effect and increased digester performance compared to mono-digestion (Wang et al., 2012; Zhao et al., 2014). Having more than one substrate might be advantageous due to balanced nutrient composition, synergistic effect of the microorganisms, increased buffering capacity, and diluted toxic compounds (Mata-Alvarez et al., 2014; Sukhesh et al., 2019). Anaerobic mono-digestion presents some drawbacks linked to substrate characteristics (Mata-Alvarez et al., 2014). One such drawback is the seasonality and limited local availability of one single substrate utilized in anaerobic mono-digestion facility. The drawback is the substrate stream would not be steady, while an anaerobic facility requires a steady stream of substrate feeding for stable and optimum performance. Suppose there are other substrates available locally, anaerobic co-digestion can be considered as an option for the facility.

The objective of this research is to investigate how different composition of co-substrates mixtures affect the performance of anaerobic co-digestion of predigested swine wastewater effluent (SWE) with rice straw (RS) and bamboo waste (BW).

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Chapter 2 Literature Review

2.1 Anaerobic Digestion

Anaerobic digestion is the degradation of complex organic materials, conducted by microorganisms in the absence of oxygen, which eventually will be broken down in the form of methane and carbon dioxide (Adekunle and Okolie, 2015). It is a treatment method that can be used to treat both solid and liquid waste, while producing combustible biogas that can be used as an alternative renewable energy. There are four major stages of biochemical conversion that could explain the process of anaerobic digestion: hydrolysis, acidogenesis, acetogenesis, and methanogenesis.

Hydrolysis involves the enzyme-mediated transformation of insoluble organic materials and higher molecular mass compounds into soluble organic materials, i.e., compounds suitable for the use as source of energy and cell carbon such as monosaccharides, amino acids and other simple organic compounds. The monomers produced in the hydrolytic phase are taken up by different facultative and obligatory anaerobic bacteria and are degraded further into shorter chain organic acids such as butyric acids, propionic acids, acetic acids, alcohols, and hydrogen. This is acidogenesis. In acetogenesis, intermediate products which cannot be directly converted to methane by methanogenic bacteria are converted into methanogenic substrates; volatile fatty acids (VFAs) and alcohols are oxidized into methanogenic substrates like acetate, hydrogen and carbon dioxide. Finally, in the methanogenic phase, the production of methane and carbon dioxide from the methanogenic substrates is carried out by methanogenic bacterial under strict anaerobic conditions (Adekunle and Okolie, 2015). There are two major pathways of methane formation, acetic acid cleavage which accounts for about 70% of the methane formation and CO_2 reduction which the rest of the methane is formed. Figure

2-1 visualizes the complex biochemical conversions in anaerobic digestion processes in a diagram (Gujer and Zehnder, 1983; McCarty, 1964; McCarty and Smith, 1986).

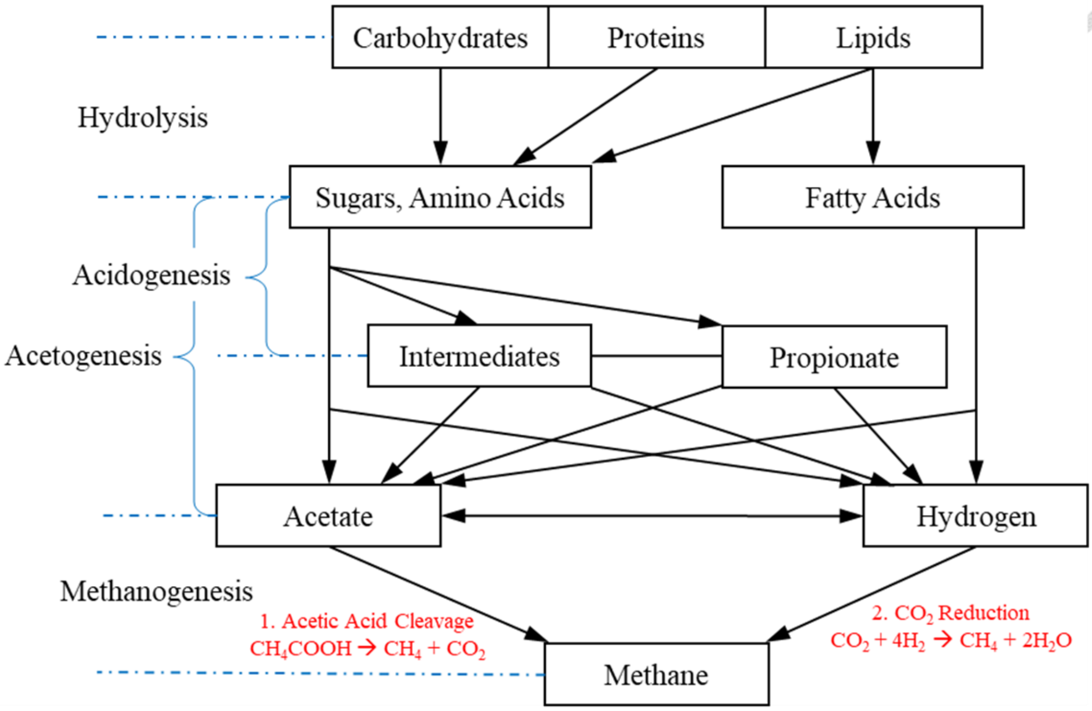

Figure 2-1 Four stages biochemical conversion of anaerobic digestion process and two major mechanisms of methane formation, namely, acetic acid cleavage and carbon dioxide reduction (Gujer and Zehnder, 1983; McCarty, 1964; McCarty and Smith, 1986)

The anaerobic process is in many ways ideal for waste treatment as it has several advantages compared to aerobic treatment, such as the possibility of a high degree waste stabilization, low production of waste biological sludge, low nutrient requirements, no oxygen requirements, and it has methane as the useful end product (McCarty, 1964). Of course, there are several disadvantages too. The slow growth rate of the methanogens implies longer periods of time are required for start-up process and limits the rate at which the process can adjust to changing loads, temperatures, or other environmental conditions (McCarty, 1964).

While the energy input is relatively higher, thermophilic conditions (55 °C) could promote faster methanogens' growth rates compared to mesophilic system (37 °C) (Khan et al., 2016). For dilute waste, the energy output from methane production might not be able to offset the energy input required for the maintaining the high temperature. However, for a more concentrated waste with BOD higher than 10,000 mg/L, the advantages of anaerobic digestion win over the disadvantages (McCarty, 1964).

2.1.1 Anaerobic Co-Digestion

Anaerobic mono-digestion, i.e. digestion with a single substrate, has drawbacks linked to its substrate’s characteristic. For instance, animal manures have high ammonia concentration that might inhibit methanogens, yet crop wastes are seasonal and lack of nitrogen (Mata-Alvarez et al., 2014). Anaerobic co-digestion is an anaerobic process in which two or more substrates are being digested in the same reactor at the same time. By mixing more than one substrates together, drawbacks associated with one single substrate can be mitigated (Siddique and Wahid, 2018).

As Shen et al. (2015) suggested that there are some more additional benefits of anaerobic co-digestion such as higher methane yield, more efficient digester volume utilization, and reduced biosolids production. With the right type and composition of substrates, anaerobic co-digestion can exhibit synergistic effect (Mata-Alvarez et al., 2014), which can either boost specific methane yield or increase biogas production kinetics (Xie et al., 2016). However, if the co-substrates’ properties and composition are not right, antagonistic effects may also occur in co-digestion (Silvestre et al., 2014).

The operational parameters that affect co-digestion are not different than those of mono-digestion: temperature, mixing, hydraulic retention time, and loading rate. However, the complexity lies in how to choose and mix the appropriate co-substrates in a way that the digester has balanced C/N ratio, organic loading, macro- and micro-nutrients, and low amount of inhibitors such as long chain fatty acids and free ammonia (Siddique and Wahid, 2018; Xie et al., 2016).

2.2 Agricultural Waste

2.2.1 Animal Waste

Animal waste contains mostly feces and urine excreted predominantly by cows, swine, and chickens. Typically, however, it could also include bedding materials, wasted feed, drinking or flush water, hair, feather, and soil (Girotto and Cossu, 2017). Based on data presented in Table 2-1 combined together with data on livestock population, Girotto and Cossu (2017) estimated the amount of manure produced all around the world was 55 billion tons in one year.

Table 2-1 Daily manure production per 454 kg animal unit (Girotto and Cossu, 2017)

Livestock Type	**Estimated Manure Production (kg/day)**
Beef	26
Dairy	36
Swine	28
Chicken (layers)	27
Chicken (broilers)	36
Turkey	20

The traditional disposal method of animal waste is just applying it to agricultural land for fertilizer. However, the nitrogen and phosphorus from the animal waste can negatively impact air and water quality when applied excessively. The excess nitrogen and phosphorus can be washed into nearby water stream and causes eutrophication, which eventually lead to a decrease in aquatic life. Excess nitrogen can also escape to the air through its gaseous form nitrous oxides which is a potent greenhouse gas (EPA, 2021). There have been some reports of human pathogens associated with animal manure (Jiang et al., 2015).

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Therefore, there must be a more sustainable animal waste treatment practices. Using black soldier fly larvae for manure treatment has gain some popularity recently (Liu et al., 2022). Of course, energy recovery of animal waste through anaerobic treatment is also widespread.

2.2.1.1 Swine Industry in Taiwan

Figure 2-2 is showing the recent trend of swine farming in Taiwan. According to COA (2022), the number of swine in total is declining, from 7,164 thousand heads in 2001 to 5,471 thousand heads in 2021. The number of swine farm, however, seems to be declining faster from 13,753 farms in 2001 to only 6,308 farms in 2021. This implies that despite there is a decrease in swine population, the number of swine per farm has an upward trend, which is shown by the number: 521 heads/farm in 2001 and up to 867 heads/farm in 2021 (Figure 2-3). The data clearly indicates that small scale farms are disappearing and being replaced by larger scale farms. By the end of November 2021, 71.7% (3,920,528 heads) of the swine were grown in 25.5% (1,577) of the farms (COA, 2022).

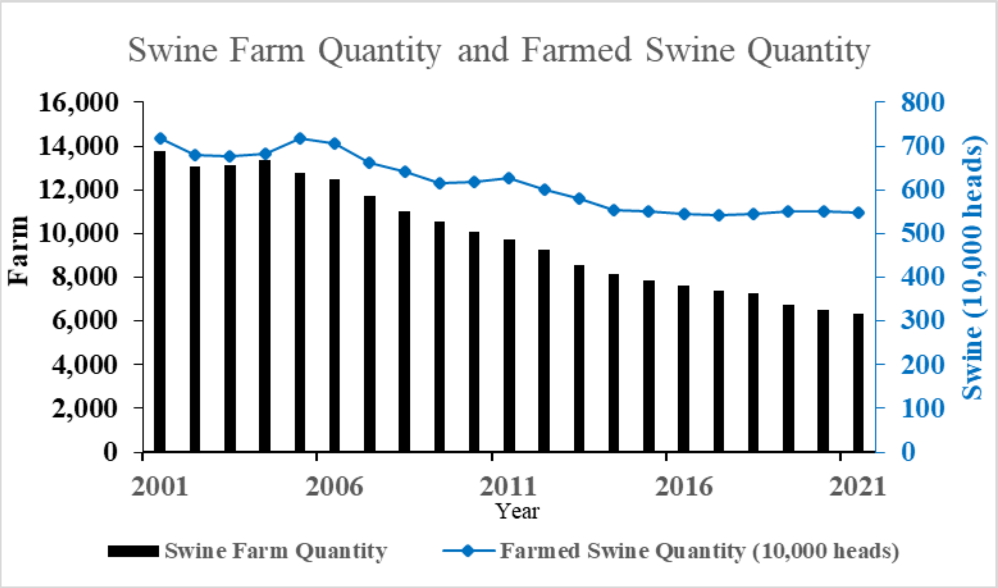

Figure 2-2 Number of swine and swine farm in Taiwan from 2001 – 2021 (COA, 2022)

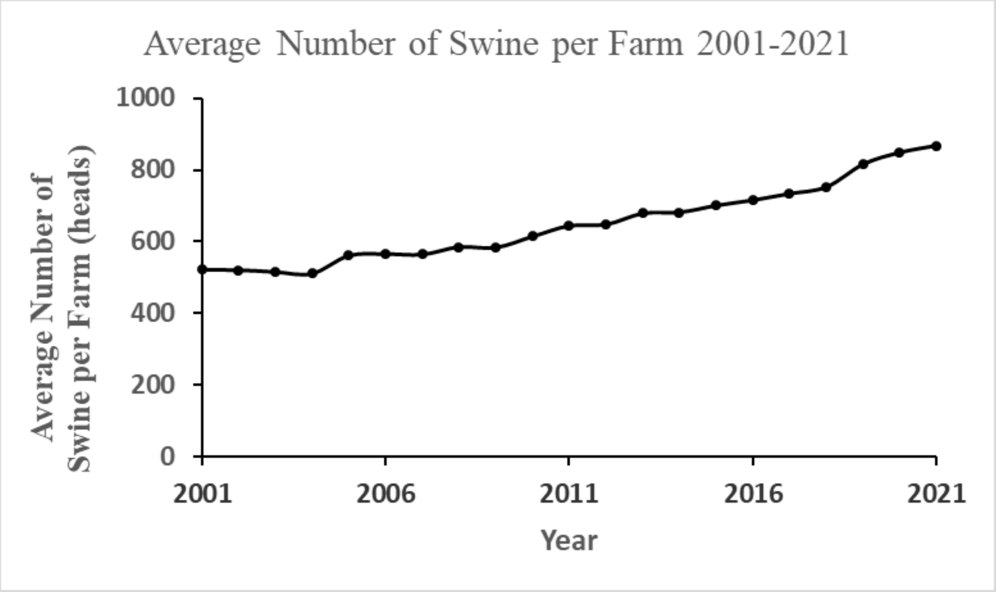

Figure 2-3 Average number of swine per farm in Taiwan from 2001 – 2021 (COA, 2022)

2.2.1.2 Swine Wastewater Production, Characteristics, and Treatment

Swine manure production and characteristics vary from farm to farm, depending on the feed intake, the age, and the breed of the swine. Table 2-2 shows the swine manure production and characteristics estimation based on above mentioned variables. According to COA (2022), Taiwan had swine under 30 kg, between 30-60 kg, and above 60 kg about 1.4 million heads each, not including breeding boar, breeding sow, gilt, and piglet which totaled about 1.4 million heads as well. Thus, with Table 2-1 or Table 2-2 one can estimate the amount of swine manure produced in Taiwan and it would not be less than tens of thousands of tons every day.

With Table 2-2, a typical swine farm should be able to estimate the characteristics of its wastewater depending on how much water the farmer uses to flush and dilute the waste. The final effluent discharge should still meet the standard for discharge shown in Table 2-3.

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Table 2-2 Average amount of swine manure production and characteristics (Koh et al., 2010)

	Hog's Weight, kg	**30**	**50**	**100**
Manure Production	**Feed Intake, g/day**	1327	1678	2661
	Manure Weight, g/day	513	816	981
	Urine Volume, mL/day	1180	2577	2974
Manure Characteristics	**Manure COD, g/kg**	961	969	1093
	Manure BOD, g/kg	251	158	208
	Urine COD, g/L	19	12	17
	Urine BOD, g/L	9	5	9
	Urine SS, g/L	28	18	27

Table 2-3 Standards for discharged livestock wastewater in Taiwan (MOJ, 2019)

Standards for Discharged Livestock Wastewater		
Chemical Oxygen Demand (COD, ppm)	Biochemical Oxygen Demand (BOD, ppm)	Suspended Solids (SS, ppm)
600	80	150

Usually in Taiwan, farmers use the three-stage treatment system developed by TLRI, which includes 1) solid-liquid separation, 2) anaerobic digestion, and 3) aerobic treatment, in sequence, for the treatment of the swine wastewater to meet the standards of wastewater discharge (Sheen et al., 1994). However, in the three-stage treatment system, the aerobic treatment in third stage consumes up to 80% of the whole system's total electricity usage. Since consuming electricity is not considered as green, Taiwan has promoted the reuse of anaerobic effluent (digestate) for agricultural application (Kuo, 2020).

2.2.2 Biomass Waste

2.2.2.1 Rice Straw

Taiwan produces around 1.7 million tons of rice annually (COA, 2021). Such production contributed to around 8% of Taiwan’s agricultural GDP in 2017, nominally NT$38 billion (AFA, 2018). Assuming that 1 ton of rice straw is produced as a byproduct for every ton of rice, annual rice straw production of Taiwan is 1.7 million tons (Chiu, 2009). Such amount of rice straw, if managed incorrectly, could probe some problem. Field burning – both open and close – of rice straw is a common practice to dispose the waste quickly and prepare the field for next crop (Chang et al., 2012). However, uncontrolled combustion in open field burning emits harmful air pollutants, such as CO, CO_2, CH_4, NO_X, SO_2. Some of them like CH_4 and NO_X are greenhouse gases which contribute to global warming (Gadde et al., 2009). Chang et al. (2012) estimated that Taiwan open-burned 27% rice straw during the second harvest season in 2009.

Instead of open-burning it, there are better alternatives of using rice straw: used as a material for bioethanol production, composted as a fertilizer, used as biochar to improve soil productivity, used for energy generation by direct burning, or lastly used for biogas production (Bindu and Manan, 2018). Other than reducing air pollution, Nguyen et al. (2016) showed that the use of rice straw for biogas production could also generate a positive net energy output of 3400-3700 MJ per ton of straw. By co-digesting it with other synergistic material under optimal operating condition (C/N ratio, moisture, etc), optimal methane yield might be achieved (Wang et al., 2013).

2.2.2.2 Bamboo Waste

In Taiwan, bamboo has two main purposes either as raw material for manufacturing, or as edible food. For example, Long-shoot Bamboo (*Bambusa dolichoclada*) and several other species are used for manufacturing furniture, artworks, and chopsticks, while Green Bamboo (*Bambusa oldhamii*) and several other species are used for edible bamboo shoots (Chen, 2017; Wang and Chen, 2017). Assume that for every hectare, 20 tons of bamboo waste would be generated every year (Hung, 2018a), since Taiwan has 175,000 hectares of bamboo forest area (Wang and Chen, 2017), then the amount of bamboo waste generation would potentially be 3.5 million tons every year.

For high quality of Green Bamboo shoots, the big old bamboos need to be cut down during winter and disposed as waste (Chen, 2017). Back in 1980s, these bamboo waste was the raw material for paper industry and oyster cage for oyster farmers (Forestry Bureau, 2019; Hung, 2018a). However, gradually less and less waste was processed. In the end, bamboo farmers started to just open-burning it in the forest, causing air pollution.

Fortunately, there are new innovations being tested to productively consume bamboo waste. For example, it could be dried then turned into biochar either for fertilizer or deodorant (Hung, 2018a). Forestry Bureau (2019), together with other organizations and industry players, utilized the bamboo as a mix of wood pellets for biomass fuel. Hung (2018b) reported gasification as an alternative that could generate 80 kWh of electricity for every 300 kg of bamboo waste. Chen (2017) investigated anaerobic co-digestion of bamboo waste with swine manure for another possible option of bamboo waste treatment.

2.2.3 Application of Anaerobic Co-digestion

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Judging by the increase in number of publications, anaerobic co-digestion has gained popularity among the academics for the past decade (Siddique and Wahid, 2018). It certainly reflects the interest and trend of environment sustainability in our society.

Most anaerobic co-digestion researches used manures as the main substrate (Mata-Alvarez et al., 2014). Typically, animal manures contain high buffer capacity, low carbon and high nitrogen, where ammonia concentrations become too high and inhibit methanogens. Therefore, C-rich wastes are usually used as the co-substrates to increase the C/N ratio to optimum range and thereafter produce satisfactory amount of biogas. Vegetable wastes, glycerol, and cheese whey were only three examples of C-rich wastes among many co-substrates investigated by researchers (Astals et al., 2012; Lin, 2016; Rico et al., 2015).

Lignocellulosic biomass have also been gaining much attention as candidate co-substrates because they do not compete directly with food or feed production. Chen (2017) co-digested swine manure with bamboo waste and achieved highest methane production rate of 2.96 L/L/day. Wang et al. (2012) optimized the feeding composition and C/N ratio for improving methane yield of dairy manure, chicken manure, and wheat straw. Ye et al. (2013) also showed that co-digesting rice straw with kitchen waste and swine manure could exhibit synergistic effect, hence, improving biogas production.

The investigation of anaerobic co-digestion with anaerobic digestate as the main substrate is very few. However, Kuo (2020) showed that poly-lactic acid can be a good carbon source for the predigested swine manure in a co-digestion operation. The co-digestion performed the best when poly-lactic acid was pretreated with sodium hydroxide in thermophilic temperature, showing methane production rate of 1.38 L/L/day, which is 41% higher compared to the untreated poly-lactic acid. In terms of yield, poly-lactic acid pretreated with sodium hydroxide could produce methane 139 mL/g VS added.

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Chapter 3 Materials and Methods

3.1 Research Process and Structure

Figure 3-1 shows the flow chart of the research process and structure.

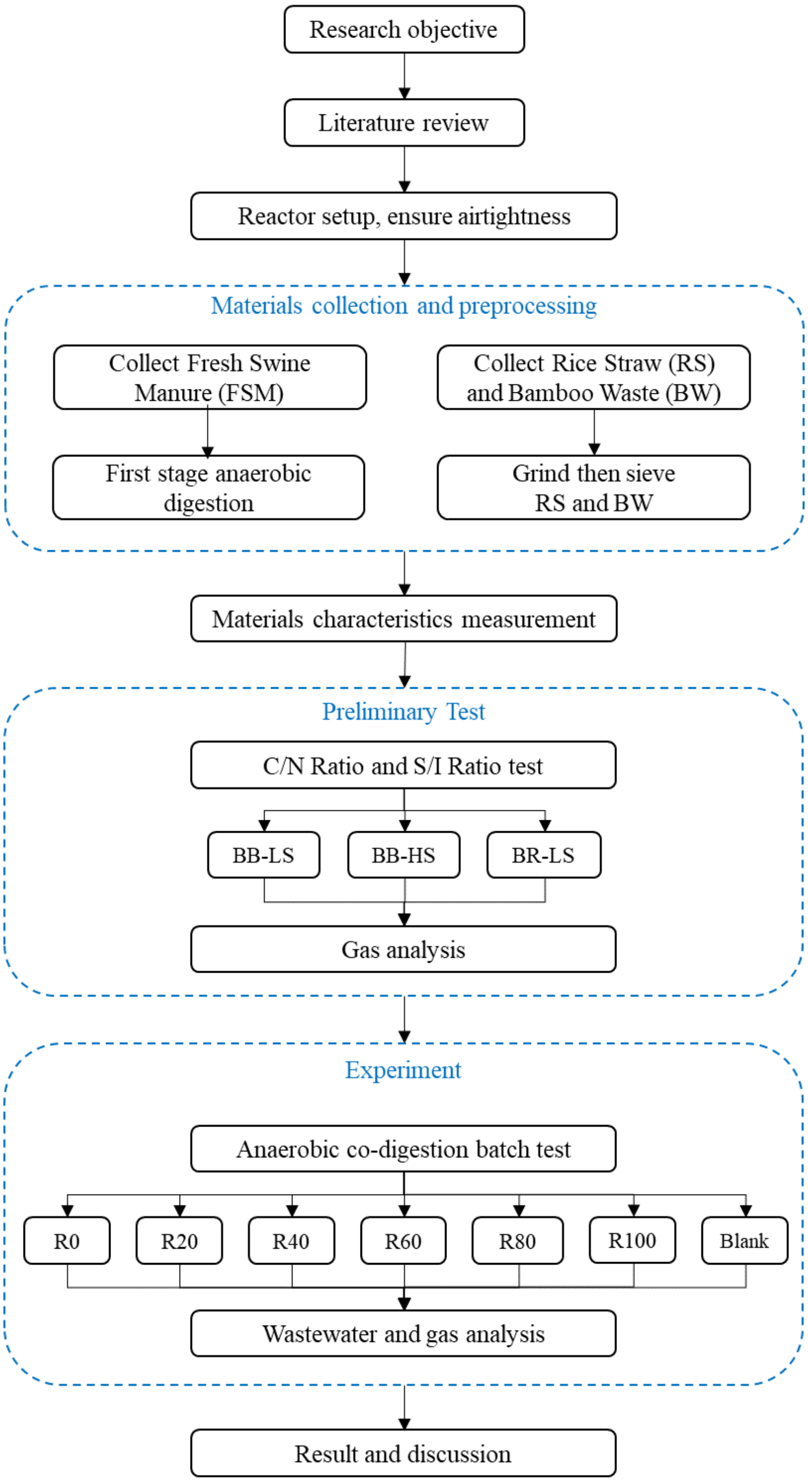

Figure 3-1 The flow chart of research process and structure

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3.1.1 Experimental Design

Before the experiment was designed, a preliminary test was done. The preliminary test investigated two different S/I ratios and two different composition of biomass mixture. Three batches with different waste mixture was prepared (Table 3-1).

BB-LS served as the base for comparison, containing only 200 g of BW mixed with 3000 mL of SWE. This mixture was estimated to have C/N ratio of 21.54, which is still in the optimal C/N ratio range for anaerobic digestion between 20-35 (Khalid et al., 2011). The S/I ratio was 1.44, which was in the acceptable range for Bamboo digestion according to Chen (2017). He showed Bamboo digestion with S/I ratio 0.88, 1.70, and 3.91 had methane yield of 0.117, 0.099, and 0.124, respectively.

BB-HS also consisted of 200 g of BW and 3000 mL of diluted SWE. The SWE was diluted to lower the VS of inoculum, which means the mixture would have higher S/I and C/N ratio. To increase S/I ratio, initially, instead of diluting the whole mixture, increasing the BW amount was a choice, but the mixture TS would get too high and almost in solid-state, which made it unable to be digested in the lab’s liquid digester. This mixture was estimated to have C/N ratio of 28.19, which is in the optimal C/N ratio range for anaerobic digestion. The S/I ratio was 2.82, which was in the acceptable range for Bamboo digestion.

BR-LS replaced 100 g of the BW with 100 g of RS, with the SWE undiluted. The resulting S/I ratio and C/N ratio were 1.50 and 20.28, respectively, which were practically similar with BB-LS.

Table 3-1 Batch name in Preliminary Test and the corresponding S/I ratio and C/N ratio

Batch Name	**SWE (mL) : Water (mL) : BW (g) : RS (g)**	**S/I Ratio (VS Basis)**	**Estimated C/N Ratio***
BB-LS	3000 : 0 : 200 : 0	1.44	21.54
BB-HS	1500 : 1500 : 200 : 0	2.82	28.19
BR-LS	3000 : 0 : 100 : 100	1.50	20.28

*Estimation was based on Table 3-4

Based on the result of Preliminary Test, in the experiment, the effect of change in RS:BW ratio was investigated. The amount of SWE and biomass waste (containing RS and/or BW) would be held constant. For each batch reactor, 3 L of SWE was mixed with 200 g of biomass waste. This specific SWE : biomass waste ratio was chosen based on the Preliminary Test result, which the lower S/I ratio showed better result (see Chapter 4). With this mixture, the S/I ratio and C/N ratio were about 1.5 and 20, respectively.

After mixing the SWE and biomass waste homogeneously, assuming total volume is 3.2 L, the 3 L went into batch CSTR and the rest of it was kept as sample for further wastewater analysis. The thing that was altered between batches was the ratio within the biomass waste, i.e., the RS:BW ratio (Table 3-2). A blank batch containing only 2.8125 L of SWE was also run along with R0, R20, R40, R60, R80, and R100, all were run in duplicate tanks.

Table 3-2 Experimental design batch name with the corresponding RS and BW mixture

Batch Name	**RS:BW Ratio**	**RS Weight (g)**	**BW Weight (g)**
R0	0:100	0	200
R20	20:80	40	160
R40	40:60	80	120
R60	60:40	120	80
R80	80:20	160	40
R100	100:0	200	0

3.2 Materials

3.2.1 SWE as Seeding Bacteria

Fresh Swine Manure (FSM) was collected from Zaoqiao Farm, Zaoqiao Township, Miaoli County, Taiwan. FSM was diluted then put into collection bottles inside below freezing temperature storage. About one week prior to usage, frozen FSM was thawed in 4°C refrigerator. Then, FSM was further diluted to TS concentration of 8% and fed into 5 L anaerobic continuously-stirred tank reactor (CSTR), with working volume of 3 L, HRT 10 days, and operated under stable mesophilic temperature of 37°C. This is to mimic the first stage anaerobic treatment to produce SWE. The SWE was then used as the seeding bacteria in this experiment. The characteristics of both FSM and SWE are shown in Table 3-3.

3.2.2 Rice Straw and Bamboo Waste

Both RS and BW were collected from Taoyuan District Agricultural Improvement Station. The BW was the waste of Green Bamboo. During harvesting, RS and BW had been cut into around 2 cm size. Before usage, physical pretreatment was done to reduce size, RS and BW were ground further smaller with grinder and screened through sifter

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mesh no. 25, of which the hole size is 0.710 mm (Figure 3-2). The characteristics of RS and BW are shown in Table 3-3.

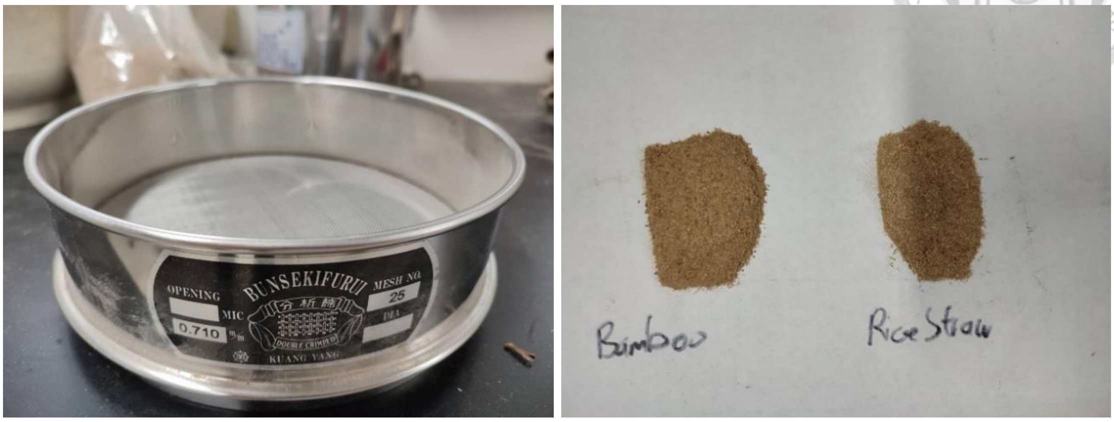

Figure 3-2 Mesh no. 25 (left); BW and RS sample after screened through mesh no. 25 (right)

Table 3-3 The characteristics of the seeding bacteria and co-substrates

Characteristics	**Fresh Swine Manure (FSM)**	**Swine Wastewater Effluent (SWE)**	**Rice Straw (RS)**	**Bamboo Waste (BW)**
TS (%)	8.12 ± 0.05	5.90 ± 0.05	93.78 ± 0.24	94.22 ± 0.25
VS (%)	5.52 ± 0.03	3.71 ± 0.05	84.86 ± 0.21	89.80 ± 0.39
VS (% of TS)	67.93 ± 0.29	62.92 ± 0.29	90.49 ± 0.21	95.30 ±0.16
pH	7.10	7.56	-	-

After being treated in an anaerobic digester, swine manure won’t be fully degraded. In the digestion process, the carbon in the swine manure will be degraded by the bacteria, decreasing the C/N ratio of the swine manure. Eventually, before the carbon being all used up, the C/N ratio would already be too low, which means ammonia inhibition would occur and the environment would not be optimal anymore for the bacteria to grow (Tanimu et al., 2014).

The idea of adding additional waste such as BW and RS in the SWE is to increase the C/N ratio of the mixture to an optimal range. BW and RS served as extra carbon source in this case, and, likewise, the SWE served as a nitrogen source. Since C/N ratio is one important factor in determining waste mixture, the data in Table 3-4 is used to estimate the C/N ratio of the mixtures in this experiment.

Table 3-4 C/N ratio of the seeding bacteria and co-substrates

Parameters	FSM	SWE*	RS	BW
TS (%)**	8.12	5.90	93.78	94.22
TOC (% of TS)	41.9	n.d.	41.30	53.68
TKN (% of TS)	2.62	n.d.	0.81	0.90
TOC (g)	102.07	71.45	77.46	101.15
TKN (g)	6.38	6.32	1.52	1.70
C/N Ratio	15.99	11.31	50.99	59.64
Reference	Chen (2017)	Estimated	Chou (1997)	Chen (2017)

* Estimated that TOC of FSM is destroyed 30%, because VS is destroyed roughly about 30% (Table 3-3), and TKN is destroyed 1%, because nitrogen is utilized 30 times slower in anaerobic digestion (Yadvika et al., 2004).

** Only TS value is from this research

3.2.3 Reactor

Every reactor used in this experiment were set-up as identically as possible. The reactor volume is 5 L and working volume was 3 L. As shown in Figure 3-3, warm water from the water bath, where the temperature was being regulated, flowed through the reactor's warming jacket to maintain the temperature inside the reactor at 37 ± 1 ^{o}C (Figure 3-4).

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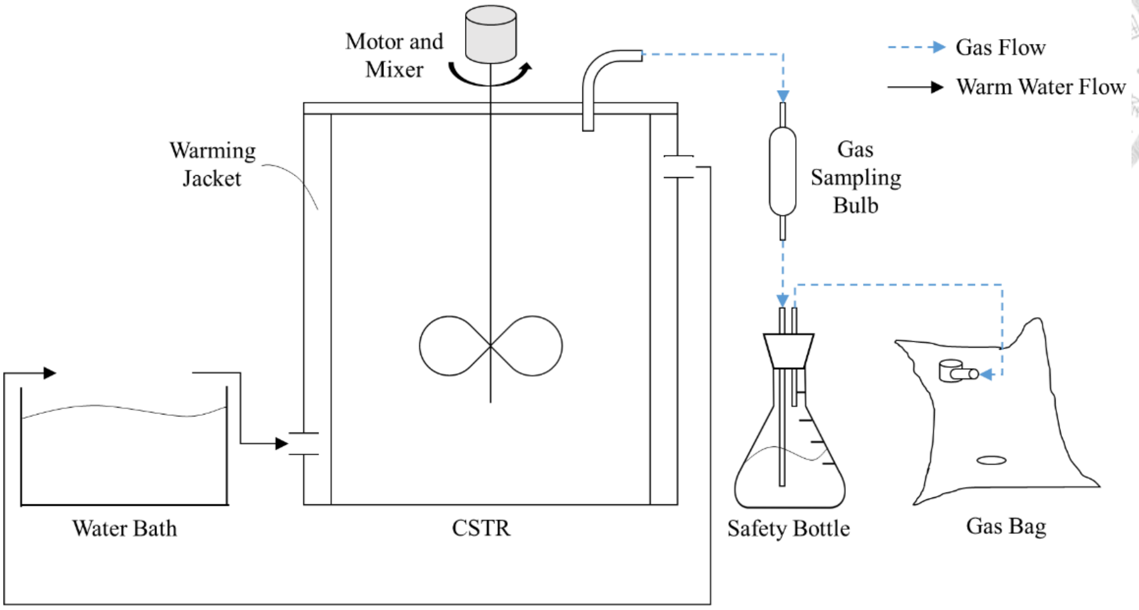

Figure 3-3 The set-up diagram of continuously-stirred tank reactor (CSTR) used in this experiment for anaerobic batch co-digestion

Figure 3-4 Water bath’s front view with its thermostat (left) and top view with the water pumps (right)

Since continuously-stirred tank reactors were used, every tank has one motor to drive the mixer, controlled and rotated by speed control unit at about 60 rpm continuously (Figure 3-5, 3-6). The biogas produced by the microorganisms would flow through the sampling bulb for gas sampling to measure its content, then safety bottle which contain only water to re-ensure reactor's air-tightness, and finally to the gas bag where the biogas was collected.

Figure 3-5 One of the reactor that was used for batch operation (left) and motor that was used to drive the mixer (right)

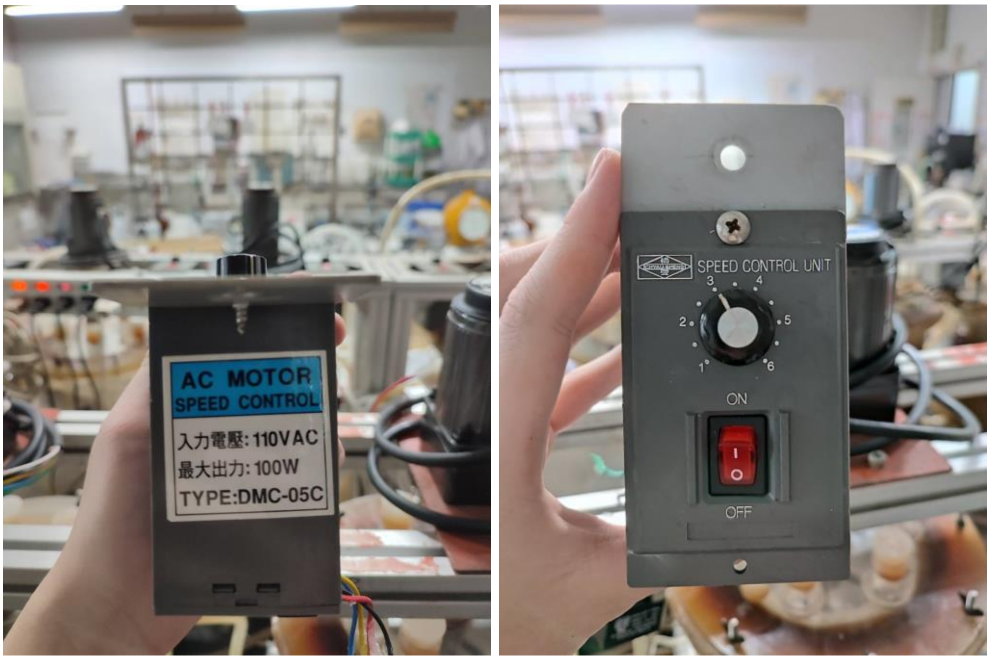

Figure 3-6 Speed control unit's front view with its specification (left) and top view (right)

3.3 Analytical Methods

3.3.1 Wastewater Analysis

Initial condition (influent) and final condition (effluent) of the anaerobic batch co-digestion would be sampled for pH, Total Solids (TS), and Volatile Solids (VS) analysis based on Standard Methods (APHA, 1992). The detailed methods for each analysis are shown in Table 3-5.

Table 3-5 Standard Method for wastewater analysis (APHA, 1992)

Analysis	Standard Method
TS	2540C
VS	2540G

For pH measurement, pH meter PHB-9901 from AI-ON Industrial Corp., USA was used. Before measurement, calibration using standard buffer pH 4 and pH 7 was required.

3.3.2 Gas Analysis

For methane content analysis, gas sample was taken by syringe from the gas sampling bulb. The gas sample would be analyzed using gas chromatography (8700T, China Chromatography Co., Ltd, Taiwan), as shown in Figure 3-7, with Thermal Conductivity Detector (TCD) and Porapak Q Column (Supelco, Inc., MO, USA, 6ft × 1/8 in) for its methane and carbon dioxide content. Helium was used as the carrier gas with flow rate 30 mL/min. The temperature of TCD, injection port, and column is 120℃, 110℃, and 75℃, respectively. Using ChemStation (35900E, Agilent, Wilmington, USA), the area under curve of sampled gas could be obtained, and then comparison with the area under curve of standard methane (99.99%) and standard carbon dioxide (99.99%), which came from Yuan Rong Industrial Gases Co., Ltd., Taiwan, would result in both methane

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and carbon dioxide content in the gas sample. Wet test gas meter (W-NK-0.5, Shinagawa Co., Tokyo, Japan), as shown in Figure 3-7, was used for measurement of gas volume produced that was collected from the gas bag (CAT. NO. 232-01, SKC Inc., USA). Measurement of gas production volume and gas content analysis were done every day during the operation of the batch to obtain gas production rate (GPR) and methane production rate (MPR). Finally, further analysis of the gas production volume with the wastewater characteristics, biogas yield could be calculated.

Figure 3-7 GC (left) and wet test gas meter (right) for gas analysis

3.3.3 Chemical Composition

RS and BW were oven-dried, ground, and screened through 40-60 mesh before the analysis of the chemical composition. Thereafter, samples were subjected to the following standard tests: moisture content (TAPPI T 258 om-06), solvent extractive (CNS 4713), holocellulose (CNS 3085), α-cellulose (CNS 10865), Klason lignin (CNS 14907), and ash content (CNS 3084) (BSMI, 2021).

Chapter 4 Results and Discussion

4.1 Preliminary Test

This experiment investigated three different batches: BB-LS, BB-HS, and BR-LS. Comparing BB-LS with BB-HS is comparing how two batches with the same mixture composition performed with two different S/I ratio. BB-LS represents the lower S/I ratio 1.5, while BB-HS represents the higher S/I ratio 2.8. Comparing BB-LS with BR-LS is comparing two batches with roughly similar S/I ratio performed with two different substrates mixture composition. BR-LS contains half BW and half RS in the co-substrates. The gas production result of BB-LS, BB-HS, and BR-LS can be seen in Table 4-1.

Table 4-1 Preliminary Test Result

Batch Name	**Total Gas Production (L)**	**Total Methane Production (L)**	**Cumulative Methane Content (%)**	**Methane Yield (L/g VS added)**
BB-LC	13.346	6.225	46.64	0.039
BB-HC	9.834	4.252	43.23	0.027
BR-LC	23.908	12.090	50.57	0.078

Comparing BB-LS and BB-HS, the lower S/I ratio BB-LS produced more biogas and methane as compared to BB-HS. The cumulative methane content of BB-LS (46.64%), albeit arguably not high enough, is still higher than that of BB-HS (43.24%). As shown in Figure 4-1, the peak of methane content is higher for BB-LS (62.74%) as compared to BB-HS (57.56%). As shown in Figure 4-2, the peak of daily gas production is higher for BB-LS at 2.17 L/day as compared to BB-HS at 1.27 L/day. The methane yield of BB-LS 0.039 L/g VS added is also higher as compared to BB-HS 0.027 L/g VS added. Therefore, it was assumed that mixture with C/N ratio and S/I ratio around 20 and 1.5, respectively, was better than that of 28 and 2.8.

Comparing BB-LS and BR-LS, the latter which had its mixture half of the BW replaced by RS produced more biogas and methane. The cumulative methane content of BB-LS (46.64%) is lower than that of BR-LS (50.57%). As shown in Figure 4-1, the peak of methane content is lower for BB-LS (62.74%) as compared to BR-LS (67.96%). As shown in Figure 4-2, the peak of daily gas production is lower for BB-LS at 2.17 L/day as compared to BB-HS at 4.38 L/day. The methane yield of BB-LS 0.039 L/g VS added is also lower than BR-LS 0.078 L/g VS added.

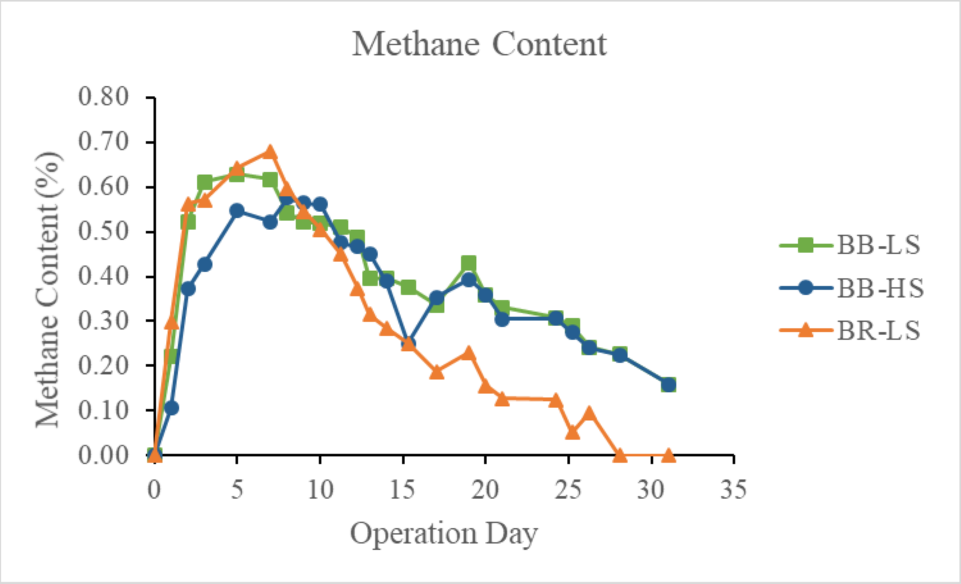

Figure 4-1 Methane Content of three batches in Preliminary Test

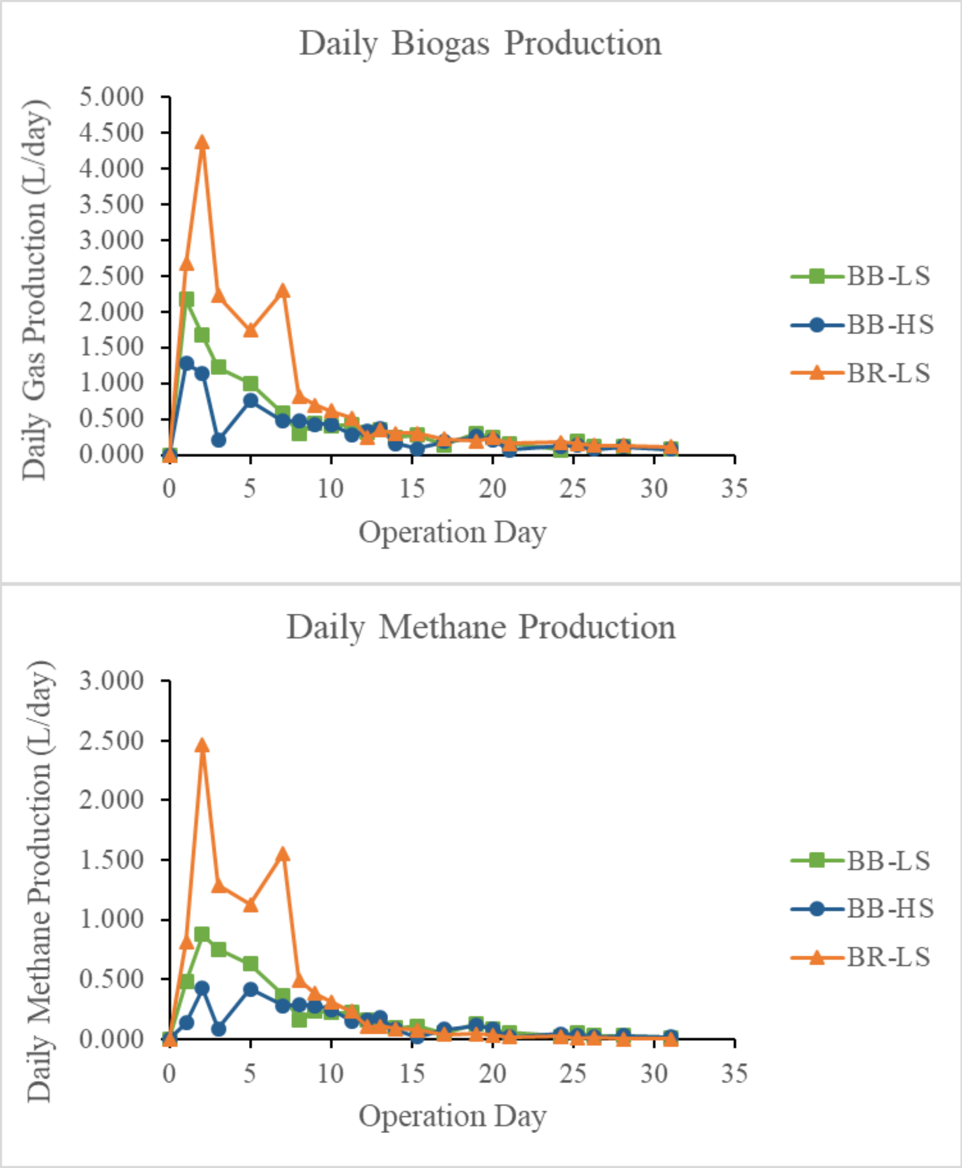

Figure 4-2 Daily biogas production (top) and daily methane production (bottom) of three batches in Preliminary Test

The difference of the methane production between BB-HS and BB-LS is about 50%, using BB-HS as the base. It is the difference between S/I ratio of 1.5 and 2.8. Meanwhile, the difference of the methane production between BB-LS and BR-LS is about 100%, using BB-LS as the base. It is the difference between co-substrates of 200 g all BW and half BW half RS, which the RS composition can still be increased and more dramatic change in methane production can be expected. These differences can be observed visually by the accumulated biogas and methane production graph in Figure 4-3. Therefore, in this case, the change in co-substrates composition affects the methane production more than the change of S/I ratio. This is also why the S/I ratio 1.5 was chosen as the controlled variable in the experiment. In the later sections, more analysis in changing the co-substrates composition will be discussed further in a more detailed manner.

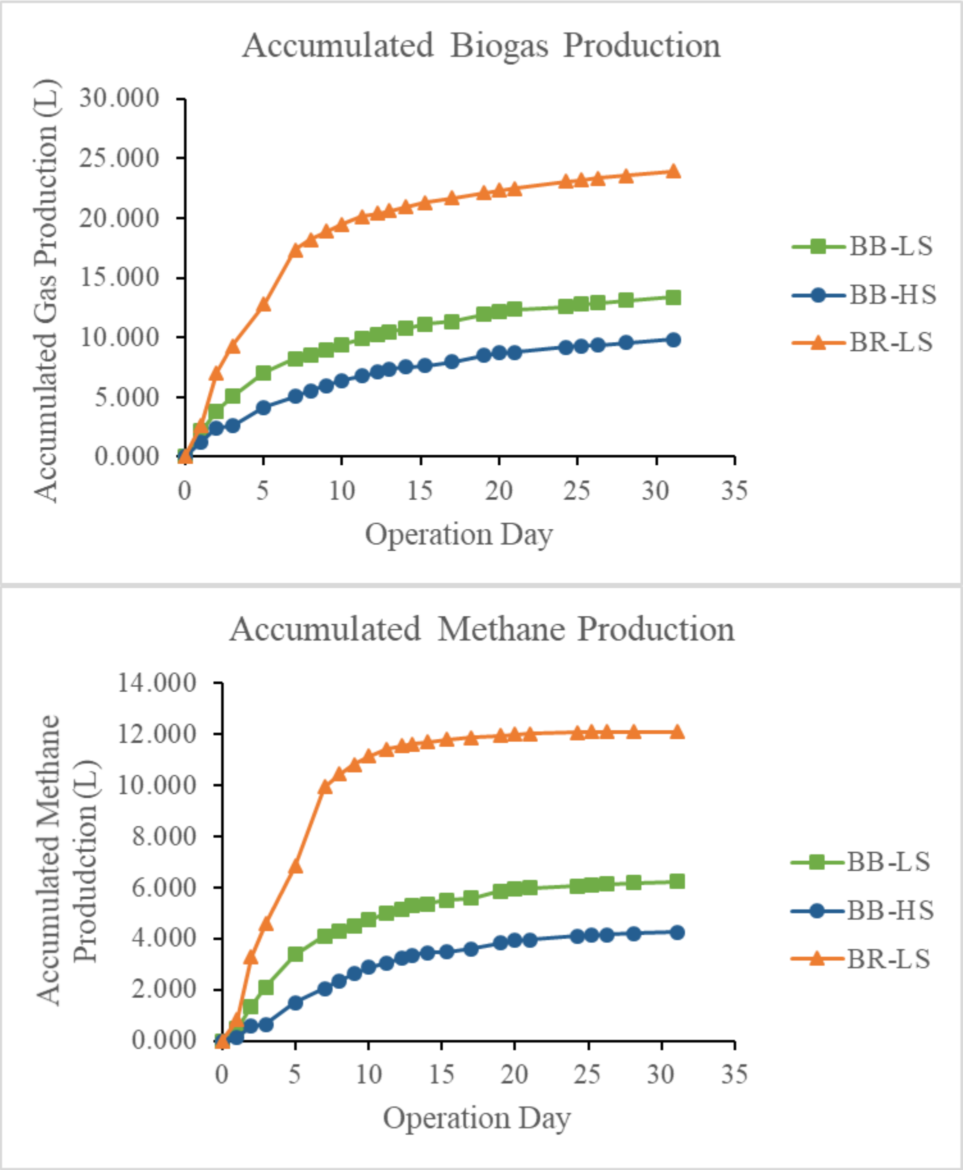

Figure 4-3 Accumulated biogas production (top) and accumulated methane production (bottom) of three batches in Preliminary Test

4.2 Wastewater Analysis

The pH, TS, and VS of both influent and effluent of across all batches are shown in Table 4-2. The influents pHi were all about 7, which is an ideal environment for anaerobic microorganisms. The effluent pHe were increased a little bit at still an ideal environment, which means neither acid nor ammonia inhibition happened.

The TSi and VSi (of influents) were also supposed to be approximately the same across all batches, since the S/I ratio was controlled and RS has similar characteristics as BW. However, as shown in Table 4-2, the TSi were in the range of 11.20% - 11.60% with the exception of R100 10.77%, while VSi were in the range of 8.67% - 8.81% with the exception of R100 8.14%. This stability of TSi and VSi across all batches was caused by similar characteristics in RS and BW (Table 3-3).

Table 4-2 The wastewater quality (pH, TS, and VS) analysis of both influent and effluent

Batch	**Influent**			**Effluent**		
	pHi	**TSi (%)**	**VSi (%)**	**pHe**	**TSe (%)**	**VSe (%)**
R0	7.15	11.20	8.74	7.22	10.08	7.70
R20	7.05	11.35	8.76	7.15	9.58	7.14
R40	7.10	11.34	8.73	7.17	9.25	6.75
R60	7.21	11.60	8.81	7.22	9.00	6.30
R80	7.18	11.55	8.72	7.23	8.55	5.90
R100	7.25	10.77	8.14	7.31	7.43	4.97
Blank	7.09	6.26	3.77	7.20	5.55	3.34

The TSe and VSe (of effluents), however, decreased as the RS composition in the co-substrates increased. The lowest TSe and VSe were in batch R100 at 7.43% and 4.97%, respectively. The highest TSe and VSe were in batch R0 at 10.08% and 7.70%, respectively. Constant TSi and lower TSe means higher removal efficiency of TS (RTS). Likewise, constant VSi and lower VSe, means higher removal efficiency of VS (RVS) (Table 4-3). Therefore, the more RS in the co-substrates' composition, the lower the TSe and VSe, the more TS and VS were removed.

Two possible methods were used to calculate the removal efficiency of TS (RTS) and VS (RVS). The first method accounted for the total mixture (seeding bacteria and co-substrates) as a whole for the calculation, denoted by the prefix t-. The second method only accounted for the co-substrates part for the calculation, denoted by the prefix s-. Equation (4.1) and (4.2) shows how the calculations were done using method 1 and 2, respectively. By knowing the absolute amount of TS and VS in influent and effluent of Mixtures and Blanks derived from Table 4-2, RTS and RVS can be calculated.

$$t = \frac{M_d}{M_i} = \frac{M_i - M_e}{M_i} \tag{4.1}$$

$$s = \frac{S_d}{S_i} = \frac{S_i - S_e}{S_i} = \frac{(M_i - B_i) - (M_e - B_e)}{(M_i - B_i)} \tag{4.2}$$

where: t = Removal Efficiency of Solids calculated using method 1

s = Removal Efficiency of Solids calculated using method 2

M = Total Mixture's Solids, g

S = Substrate's Solids, g

B = Blank's Solids, g

subscript d denotes the amount of solids destroyed or removed, e denotes effluent, and i denotes influent.

Both equations were applied for the calculation of t-RTS, t-RVS, s-RTS, and s-RVS (Table 4-3). For any batch, the s-RVS is higher than the t-RVS. This means that the VS of the co-substrates was destroyed more efficiently than the seeding bacteria SWE.

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This phenomenon is consistent across all batches. The same observation can also be made for TS, yet only R0 that has higher t-RTS than s-RTS. The low RTS of BW suggests the possibility of BW having a low anaerobic biodegradability.

Comparing across different RS:BW composition, there is an apparent trend that the higher RS composition in the co-substrates, the higher the removal efficiency (RTS and RVS), whatever method used for calculation (Figure 4-4). This trend observation implies, lower anaerobic biodegradability of BW compared to RS. Neither synergistic nor antagonistic effects – in terms of solids removal – are apparent. Further discussion about the cause in later part of this chapter.

Table 4-3 TS and VS removal efficiency of different batches

Batch	**t-RTS (%)**	**t-RVS (%)**	**s-RTS (%)**	**s-RVS (%)**
R0	9.95	11.98	8.09	12.17
R20	15.59	18.47	19.95	23.40
R40	18.43	22.66	25.79	30.55
R60	22.44	28.50	34.09	39.60
R80	26.03	32.43	41.56	46.32
R100	31.04	38.96	49.84	57.37
Blank	11.35	11.43	-	-

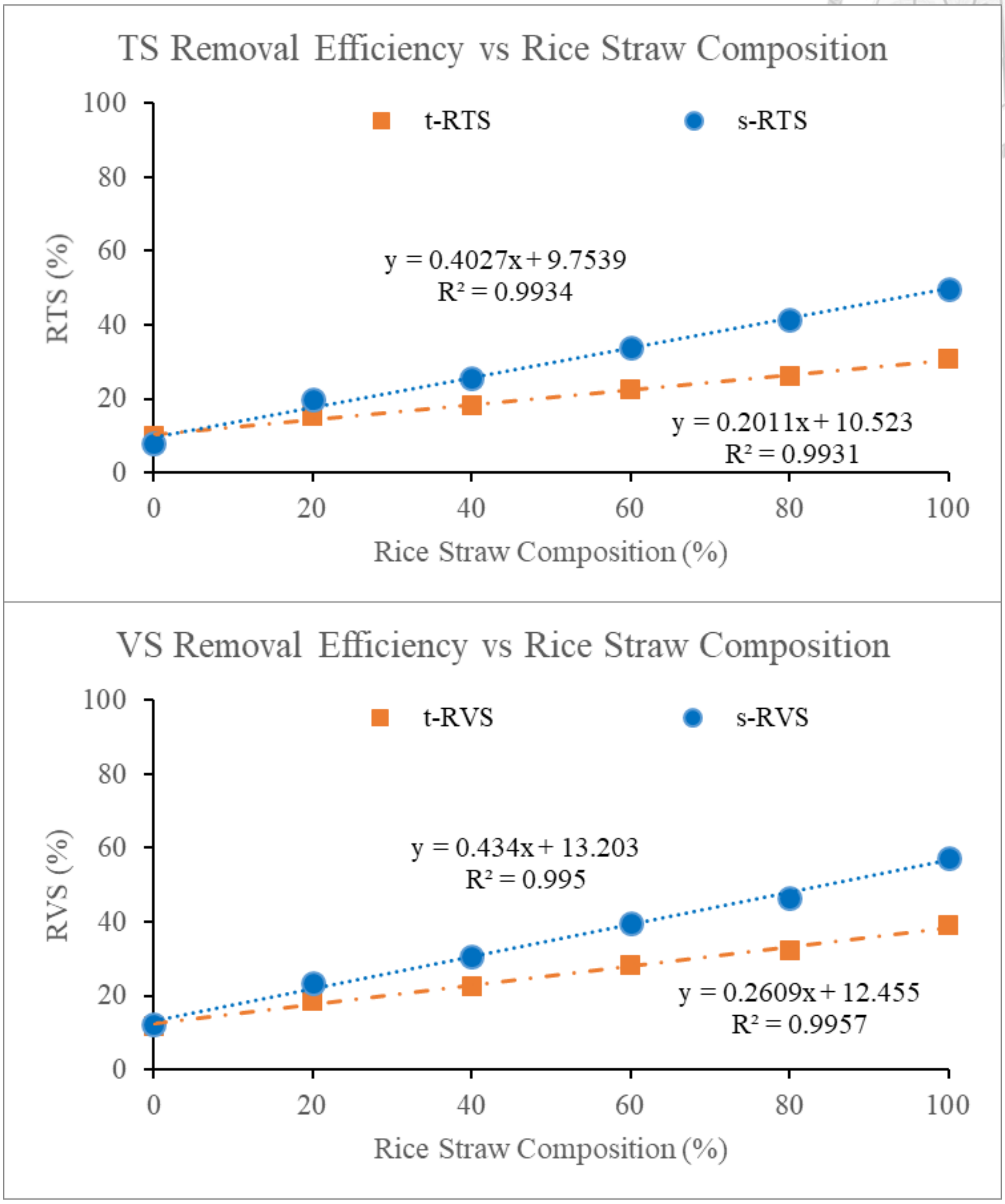

Figure 4-4 The trend of removal efficiency – RTS (top) and RVS (bottom) – as compared to the changes in RS:BW composition in the co-substrate

Each of the regression lines of t-RTS, t-RVS, s-RTS, and s-RVS has a coefficient of determination $R^2 > 0.99$. Intercept of the regression line is the model prediction of removal efficiency when RS composition at 0% (R0). The slope means how much incremental efficiency that could be reached for every 1 percent (or 2 grams) of BW replaced with RS. As shown in Table 4-4, all of the slopes are positive. The method 2 calculation (prefix s-) shows steeper slope compared to method 1.

Table 4-4 The intercepts and slopes of removal efficiencies’ regression lines

	Intercept (%)	**Slope**	**Coefficient of Determination**
t-RTS	10.52	0.20	0.99
t-RVS	12.46	0.26	1.00
s-RTS	9.75	0.40	0.99
s-RVS	13.20	0.43	1.00

4.3 Total Biogas Production and Methane Content

The total biogas and methane production generally went up as RS composition also went up as shown in Table 4-5.

Table 4-5 Cumulative biogas and methane production of different batches

Batch	**Cumulative Biogas Production (L)**	**Cumulative Methane Production (L)**	**Methane / Biogas ratio (%)**
R0	13.346	6.225	46.64
R20	21.814	11.926	54.67
R40	30.568	17.282	56.54
R60	27.434	14.863	54.18
R80	48.429	27.313	56.40
R100	47.711	24.958	52.31
Blank	3.335	0.501	15.02

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R0 has the lowest cumulative biogas production (13.346 L) and cumulative methane production (6.225 L) among all of the batches. In contrast, R80 has the highest cumulative biogas production (48.429 L) and cumulative methane production (27.313 L). R100 trails behind R80 with cumulative biogas production of 47.711 L and cumulative methane production of 24.958 L. The day to day cumulative biogas and methane production curve are produced and shown in Figure 4-5.

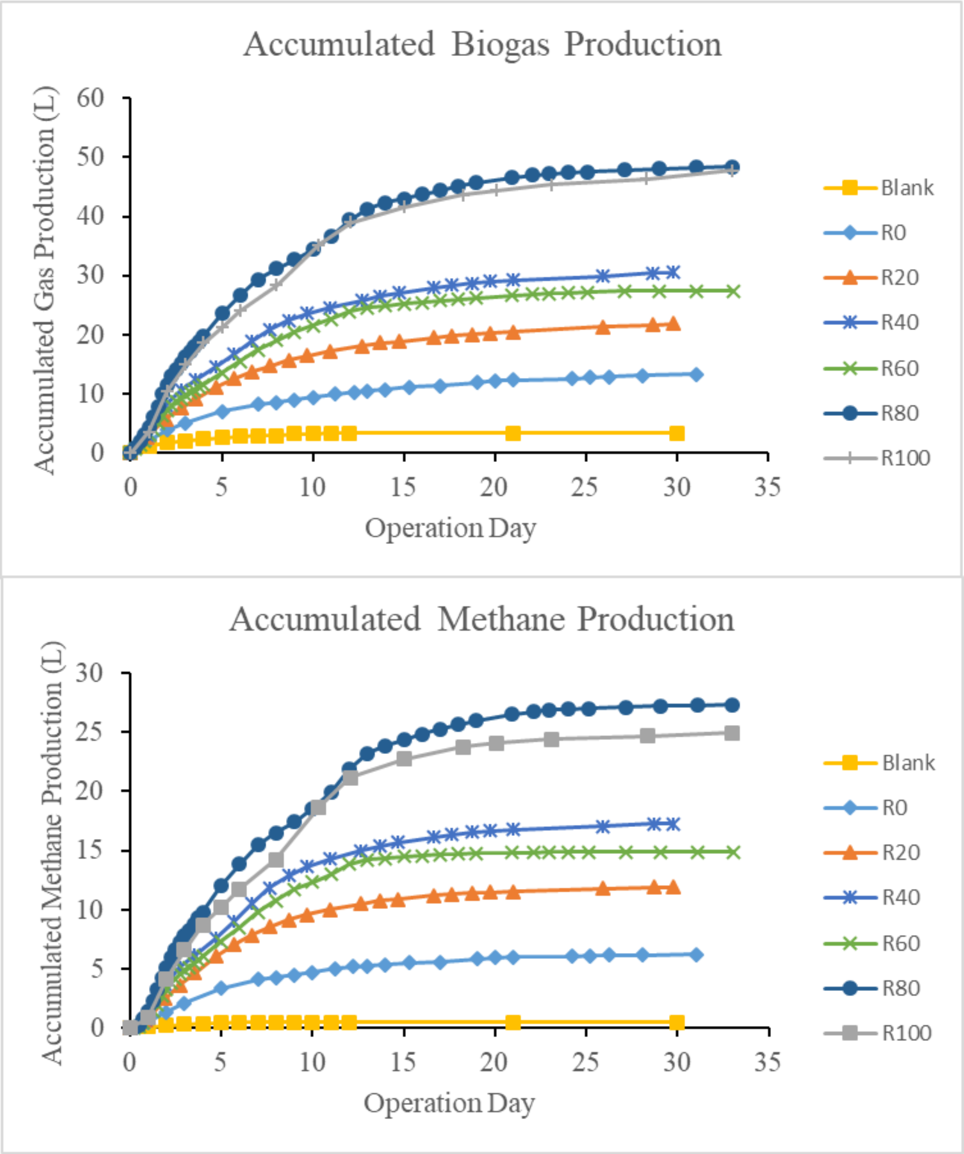

Figure 4-5 Cumulative biogas (top) and methane (bottom) production across all batches

Based on the methane content in the daily biogas produced, all of the batches were healthy, indicated by more than 50% methane content in the biogas produced starting on day 2, which then peaked and slowly dropped due to no more substrate inflow (Figure 4-6). However, inspected cumulatively, Table 4-5 shows that R0's cumulative methane content is lower than 50%, which further support the idea that BW might have a low anaerobic biodegradability.

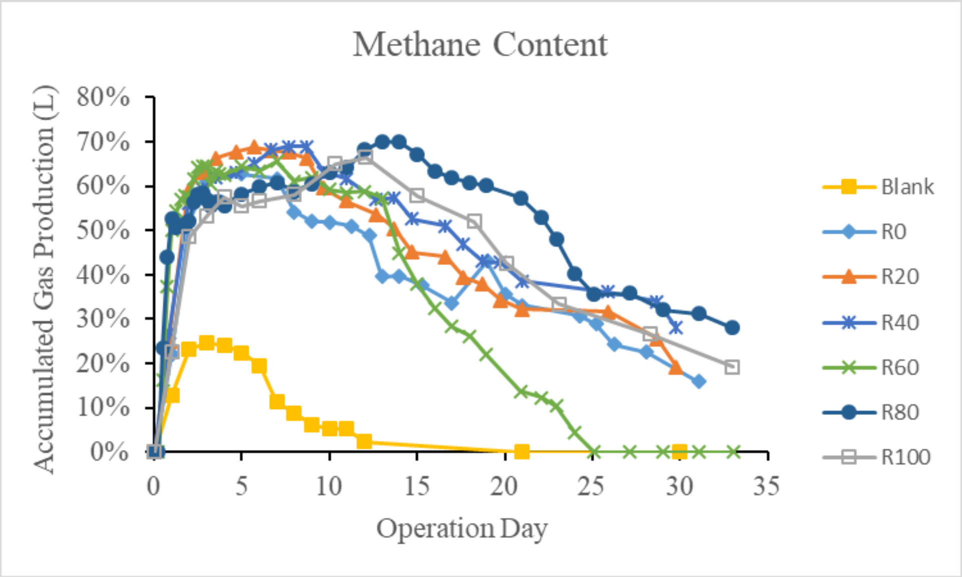

Figure 4-6 The daily methane content of all batches

Every batch's methane content peaked at some particular day (Table 4-6) before it slowly dropped. The lowest methane content peak was observed in, again, R0 at day 5 (62.74%), while the highest was observed in R80 at day 13 (70.53%). It seems that the methane content peak day comes later when RS composition is increased. This might be caused by the longer time that hydrolysis took place before more digestible content could be devoured by the methanogens.

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Table 4-6 Methane content at the day it peaked across all batches

Batch	Peak Day	Methane Content at Peak Day (%)
R0	5	62.74
R20	6	68.72
R40	8	68.96
R60	7	65.51
R80	13	70.53
R100	12	66.48

4.4 Biogas and Methane Yield

However, when comparing both biogas and methane production, it is more useful to discuss in terms of the yield per VS added and/or yield per VS destroyed, which calculations are shown in Equation (4.3) and (4.4), respectively.

$$Y_i = \frac{P - B}{VS_i} \tag{4.3}$$

$$Y_d = \frac{P - B}{VS_d} = \frac{P - B}{VS_i - VS_e} \tag{4.4}$$

where: Y_i = Biogas (or Methane) Yield per VS added, L/g VS added

Y_d = Biogas (or Methane) Yield per VS destroyed, L/g VS destroyed

P = Batch Cumulative Production of Biogas (or Methane), L

B = Blank Cumulative Production of Biogas (or Methane), L

VS_i = Amount of substrate’s VS influent (added), g

VS_e = Amount of substrate’s VS effluent, g

VS_d = Amount of substrate’s VS destroyed, g

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By using Equation (4.3) with P, B, and VS_i obtained from Table 4-2 and Table 4-5, biogas yield and methane yield per gram of substrate's VS added can be calculated. Figure 4-7 displays the calculation results in diagram. Neither synergistic nor antagonistic effects – in terms of yield per VS added – are apparent.

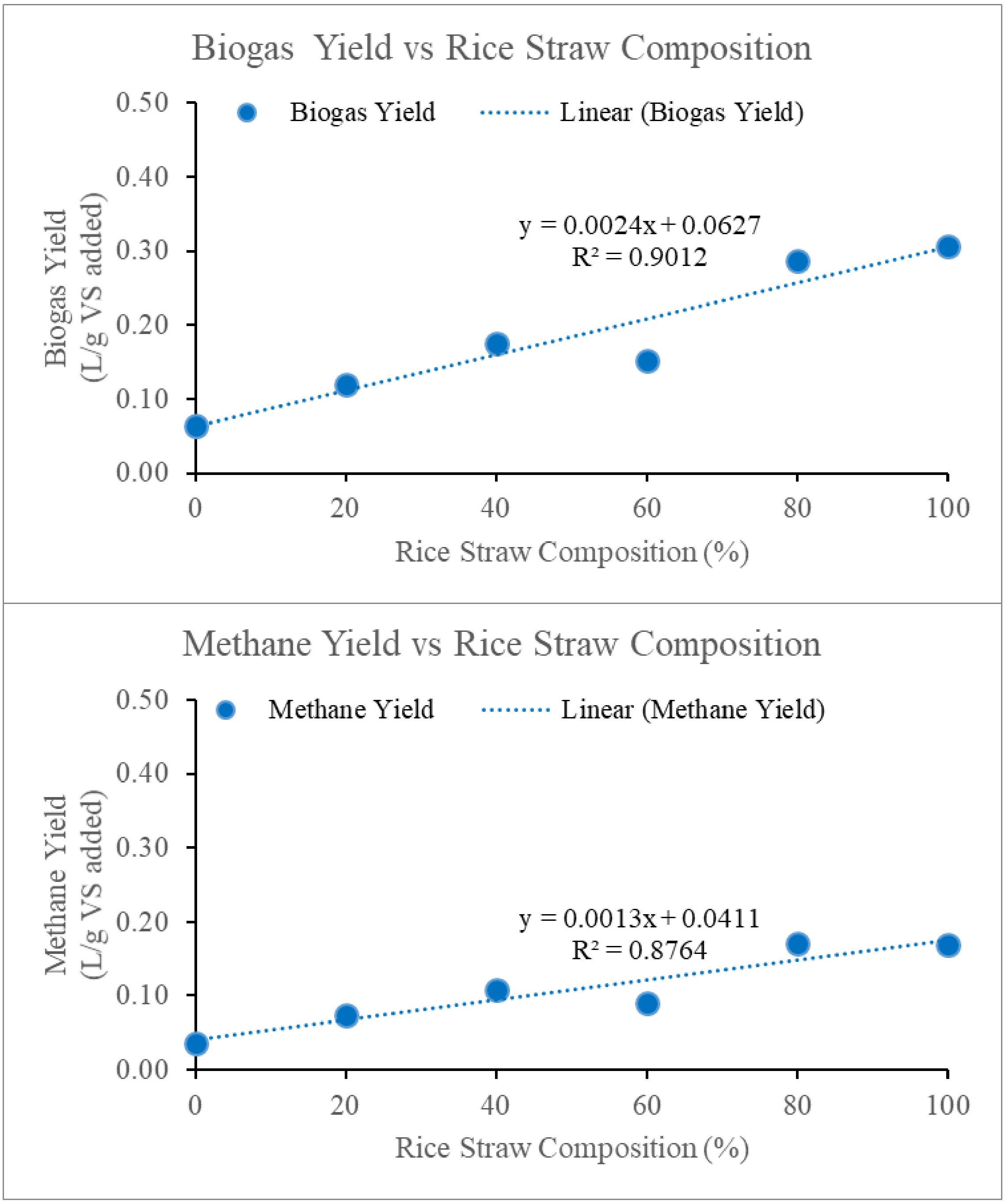

Figure 4-7 Yield of biogas (top) and methane (bottom) per gram of VS added across different RS composition

The lowest biogas yield was R0 at 0.063 L/g VS added, and the highest being R100 at 0.307 L/g VS added. It is apparent that as the RS composition was increased, the biogas yield also tended to increase. The biogas yield can also be described by the following regression equation ($R^2 = 0.90$):

$$Y_B = 0.0024x + 0.0627 \qquad (4.5)$$

where: Y_B = biogas yield per VS added, L/g VS added

x = the percentage of RS composition in this particular experiment

The lowest methane yield was R0 at 0.036 L/g VS added, and the highest being R80 at 0.171 L/g VS added. Methane yield of R100 was slightly below R80 at 0.169 L/g VS added. The trend of methane yield is the same as biogas yield, tended to increase as the RS composition increased. Similar to biogas yield, methane yield can also be described by the following regression equation ($R^2 = 0.88$):

$$Y_M = 0.0013x + 0.0411 \qquad (4.6)$$

where: Y_M = methane yield per VS added, L/g VS added

x = the percentage of RS composition in this particular experiment

By using Equation (4.4) with P, B, VS_i, and VS_e obtained from Table 4-2 and Table 4-5, biogas and methane yield per gram of substrate's VS destroyed can be calculated. Figure 4-8 displays the calculation results in diagram. Neither synergistic nor antagonistic effects – in terms of yield per VS destroyed – are apparent.

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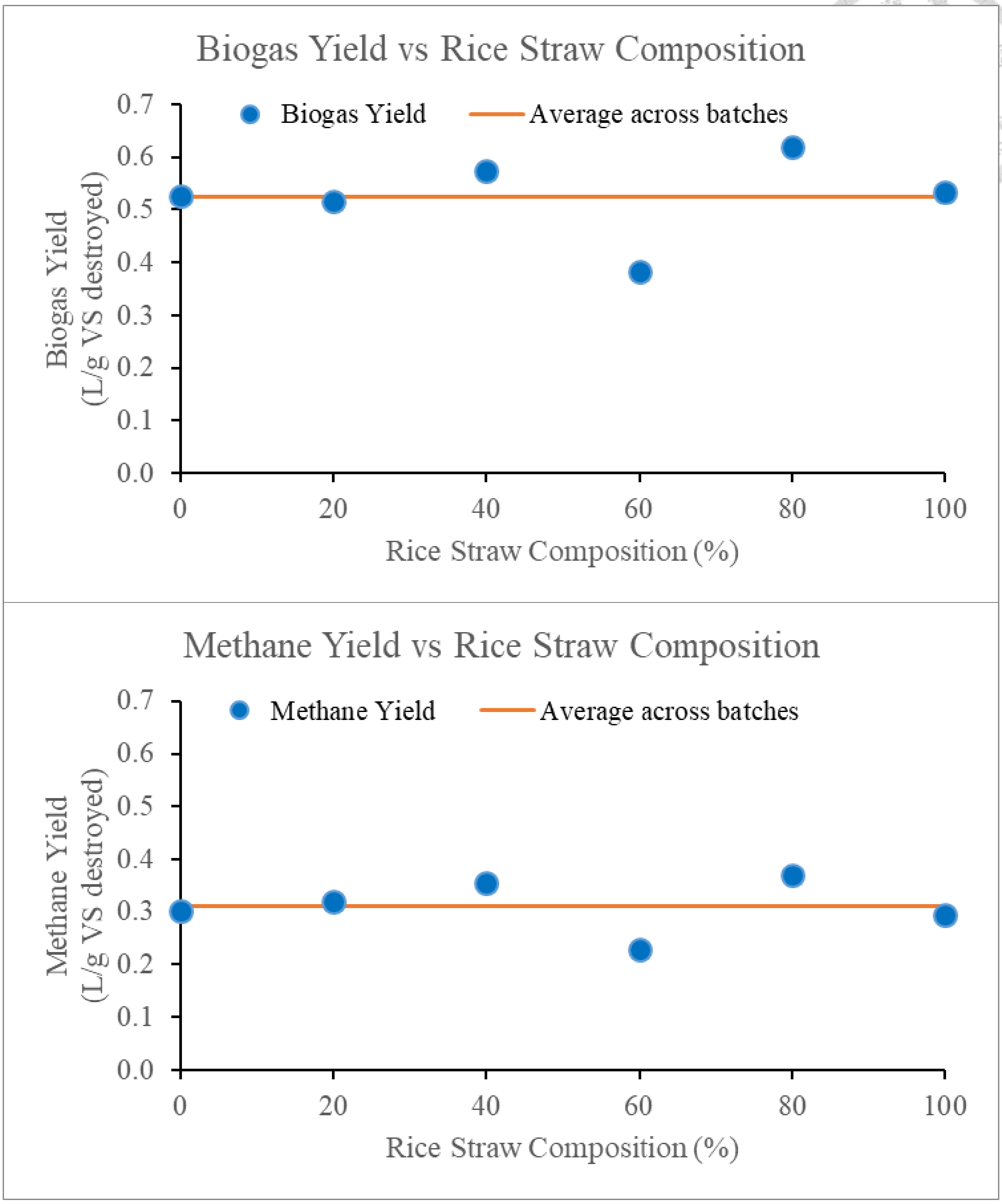

Figure 4-8 Yield of biogas (top) and methane (bottom) per gram of VS destroyed across different RS composition

In terms of VS destruction, there is no apparent trend between the biogas/methane yield and RS composition. The lowest biogas yield was R60 at 0.382 L/g VS destroyed and the highest was R80 at 0.620 L/g VS destroyed with average of 0.526 L/g VS destroyed. The lowest methane yield was R60 at 0.228 L/g VS destroyed and the highest was R80 at 0.369 L/g VS destroyed with an average of 0.311 L/g VS destroyed.

The reason why the yield in terms of per gram VS destroyed is seemingly constant is that the methane can be produced only by breaking down organic matter and reducing organic content, and VS is used as organic content indicator. Thus, what this yield is representing is just the conversion of organic content (VS) to biogas or methane. As the RS composition increased, the increase in biogas/methane production (Figure 4-5, 4-7) did not come from the increase in yield per VS destroyed, rather it came from an increase in the amount of a more biodegradable VS (Figure 4-4), while the VS-biogas and VS-methane conversion rate itself did not change or stayed about constant at 0.526 and 0.311 L/g VS destroyed, respectively (Figure 4-8).

4.5 Effect of Biochemical Composition on Methane Yield and Biodegradability

4.5.1 Lignin

While VS is commonly regarded as a proxy to organic content, it solely cannot tell the story of biodegradability. The biochemical and physicochemical composition might be able to comprehensively explain the biodegradability and biogas yield. For example, each of carbohydrate, protein, and lipid has different theoretical methane yield (Angelidaki and Sanders, 2004; Symons and Buswell, 1933). Substrates with a high lignocellulosic fraction (cell wall) was found to be recalcitrant (Labatut et al., 2011).

Numerous researches have been done for evaluating the statistical correlation between lignocellulosic composition with biodegradability and/or methane yield (BMP). While there are disagreements in the effect of cellulose and hemicellulose, all of them were consistent in their observation about the negative correlation of lignin content and biodegradability. For example, Chandler and Jewell (1980) gathered biodegradability data from various lignocellulosic materials. They developed a negative linear relationship between lignin content and biodegradability, which is shown below:

$$B = 0.83 - 0.028 \times L_{\%VS} \tag{4.7}$$

where: $B =$ biodegradable fraction

$L_{\%VS} =$ lignin content, % of VS

$\%VS =$ volatile solids, % of TS

This relationship between lignin content and biodegradability were further revalidated again and again by recent studies (Buffiere et al., 2006; Dandikas et al., 2015; Monlau et al., 2012; Pabón-Pereira et al., 2020; Thomsen et al., 2014; Triolo et al., 2011). This is because lignin acts as physical barrier surrounding the carbohydrates and it does not degrade anaerobically (Angelidaki and Sanders, 2004; Thomsen et al., 2014). For example, Thomsen et al. (2014) developed a sound statistical model for predicting methane yield by the lignin content from a combined datasets with 64 data points. His final model can be succinctly presented as:

$$Y_M = 347 - 7.85L_{\%TS} + 63D \tag{4.8}$$

where: $D = 1$ when it is Klason lignin, 0 if it is ADL

Since RS and BW are both lignocellulosic biomass, the lignocellulosic composition was analyzed. The results are shown in Table 4-7 together with other studies' results for comparison.

Table 4-7 Lignocellulosic composition, methane yield, and laboratory practices of this study and three other studies

Biomass	BW 1[a]	RS 1[b]	RS 2	RS 3	RS 4
Cellulose (% of TS)	45.45	44.26	26.20	33.40	33.90
Hemicellulose (% of TS)	21.10	13.75	18.80	28.20	20.40
Lignin (% of TS)	32.38	24.24	27.00	7.40	11.30
Residual (% of TS)	1.07	17.75	28.00	31.00	34.40
Methane Yield (L/g VS added)	0.036	0.169	0.217	0.360	0.327
Operation Days	30	30	40	NA	50
Reactor Volume (mL)	5000	5000	600	2000	1000
Working Volume (mL)	3000	3000	400	1500	450
S/I ratio	1.5	1.5	1.0	2.4	1.3[c]
Temperature (°C)	37	37	35	35	55
Mixing Speed (rpm)	60	60	No	120	No
Other Conditions	-	-	Macroelement, buffer solution	C/N ratio 25	-
Lignin type	ADL	ADL	Klason	ADL	Klason
Source	This Study	This Study	Monlau et al. (2012)	He et al. (2008)	Thomsen et al. (2014)

[a] R0, [b] R100, [c] estimated, assuming seeding bacteria VS was 4%.

Comparing RS 1 and BW 1 in this study, the lignin composition is indeed higher for BW. This might serve as an explanation of BW's lower biodegradability and methane yield. Furthermore, by comparing RS 1 from this study with RS 2, RS 3, and RS 4 from other studies similar negative correlation between lignin and methane yield is also shown, with the exception that RS 2 had higher lignin but also higher methane yield compared to

RS 1. This is a result of different lignin type. As Klason consist of acid detergent lignin (ADL) and acid soluble lignin, ADL number is smaller, thus any statistical model must take into account this difference.

A little caveat, there are differences in laboratory practice between the two studies in determining methane yield (the BMP assays). The reactor working volume in this study is 7.5 times bigger than what Monlau et al. (2012) used. In addition, Monlau et al. (2012) controlled the nutrients using macroelement, oligoelement, and buffer solution, whereas the other studies didn’t. Other example would be that Thomsen et al. (2014) controlled the operating temperature at thermophilic, whereas the others used mesophilic temperature. The mixing condition also differ from one study to another. Up until today, every model assumes these nuances as noise.

Having lignin as a less biodegradable fraction itself reduced the methane yield as a whole. In addition, it also affects the biodegradable carbon portion, thus biodegradable C/N ratio, thus further complicate calculation. Miscalculated biodegradable C/N ratio might result in sub optimal digester performance. According to Richard (1996), Van Soest developed log-linear relationship between lignin content and biodegradability that fits better a larger database compared to Equation (4.7). Such relationship can be used to calculate the biodegradable carbon content:

$$C_{biodegradable} = (C_{all})\left(\frac{LF_{\%}}{100}\right)(1-0.0541(L_{\%LF})^{0.76}) + (C_{all})(1-\frac{LF_{\%}}{100}) \qquad (4.9)$$

where: C = carbon content

$LF_{\%}$ = lignocellulosic fraction, % of TS

$L_{\%LF}$ = lignin content, % of lignocellulosic fraction

Adjusting for this, the degradable fraction of carbon is only 75.18% and 82.84% of total carbon for RS and BW, respectively. This means that the C/N ratios for all 6 batches were indeed suboptimal at around 18. This might be one of the reason that RS 1 yielded less methane as compared to RS 2, RS 3, and RS 4.

4.5.2 Cellulose

Pabón-Pereira et al. (2020) developed a similar linear relationship model with Equation (4.7) developed by Chandler and Jewell (1980), except this model used sum of lignin and cellulose instead of just lignin content.

$$B = 0.86 - 0.92 \times (L_{\%VS} + CL_{\%VS}) \tag{4.10}$$

where: $CL_{\%VS} =$ cellulose content, % of VS

Even though currently no consensus on the effect of cellulose is present, there has been several studies agreed that cellulose also correlates with biodegradability negatively. More precisely, the sum of cellulose and lignin showed negative correlation with biodegradability (Buffiere et al., 2006; Pabón-Pereira et al., 2020; Triolo et al., 2011), which mathematical form is shown in Equation (4.10). In light of this, Table 4-7 data is recreated in the form of a graph and shown in Figure 4-9. However, in theory, cellulose as a biodegradable substrate should not correlate negatively with either biodegradability and methane production.

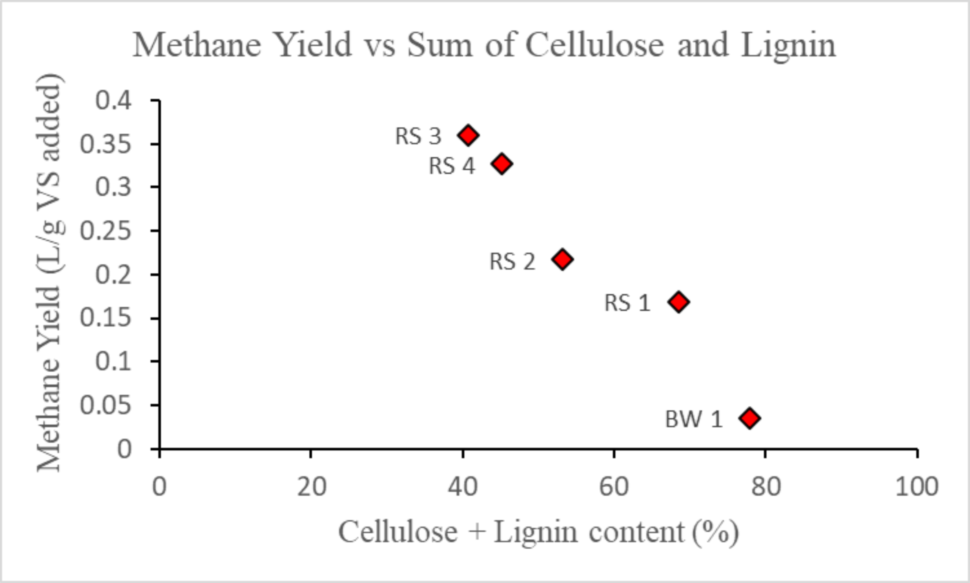

Figure 4-9 Negative correlation between methane yield and sum of cellulose and lignin

4.6 Daily Biogas and Methane Production

All of the daily methane production peaked the highest at the second day. The gas production also peaked the highest at the second day, except for R0 and R20 which peaked the highest at the first day.

As shown in Figure 4-10, in this low RS group, daily gas production and daily methane production peaked the highest at the first day and second day, respectively, then both went down almost every day until the end of operation.

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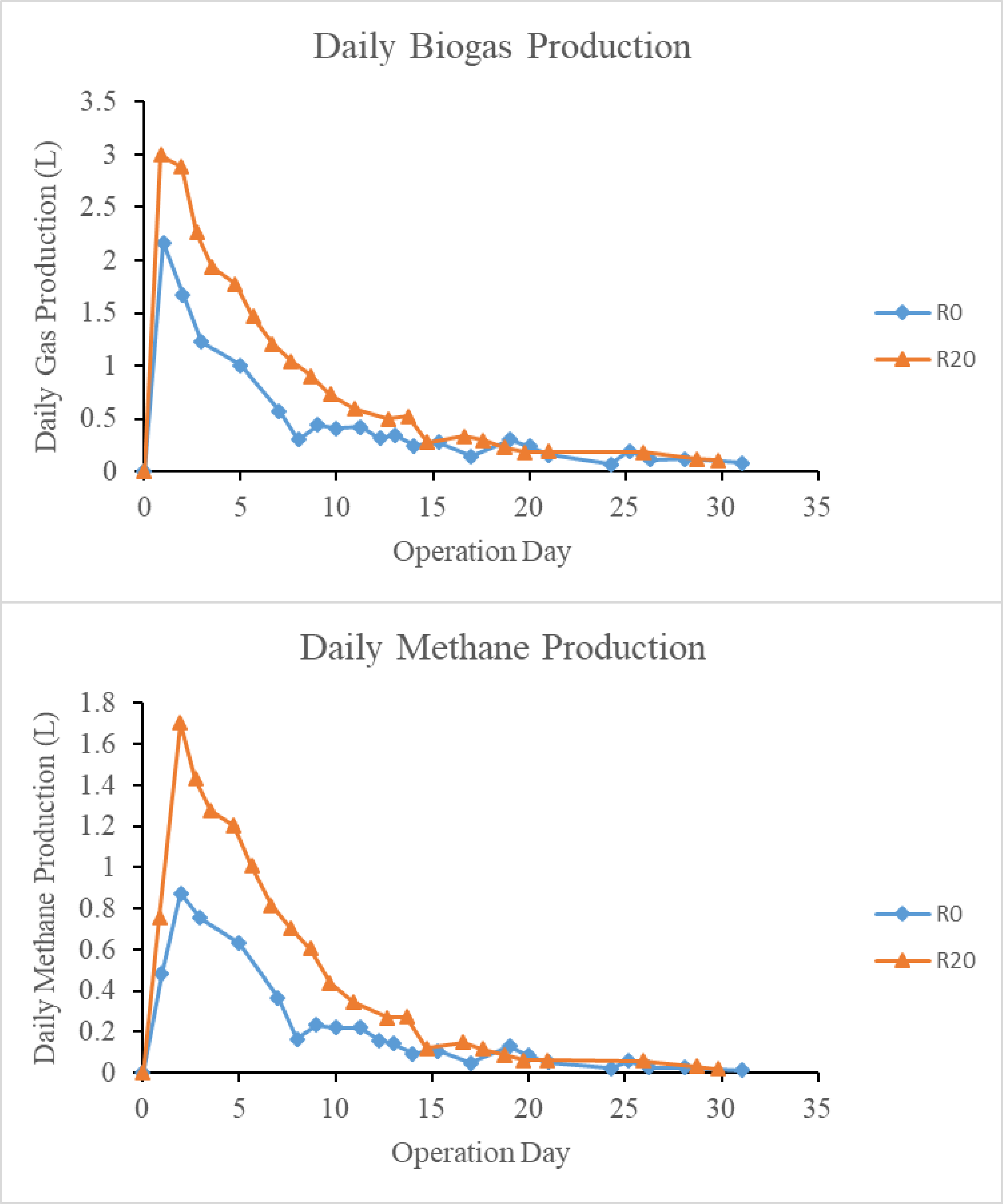

Figure 4-10 Daily biogas production (top) and daily methane production (bottom) of low RS group: R0 and R20

As shown in Figure 4-11, in this mid RS group, both daily gas production and daily methane production peaked the highest at the second day, then both went down but then made a small peak again around day 7. This phenomenon can be observed more clearly in the bottom figure showing daily methane production of R40.

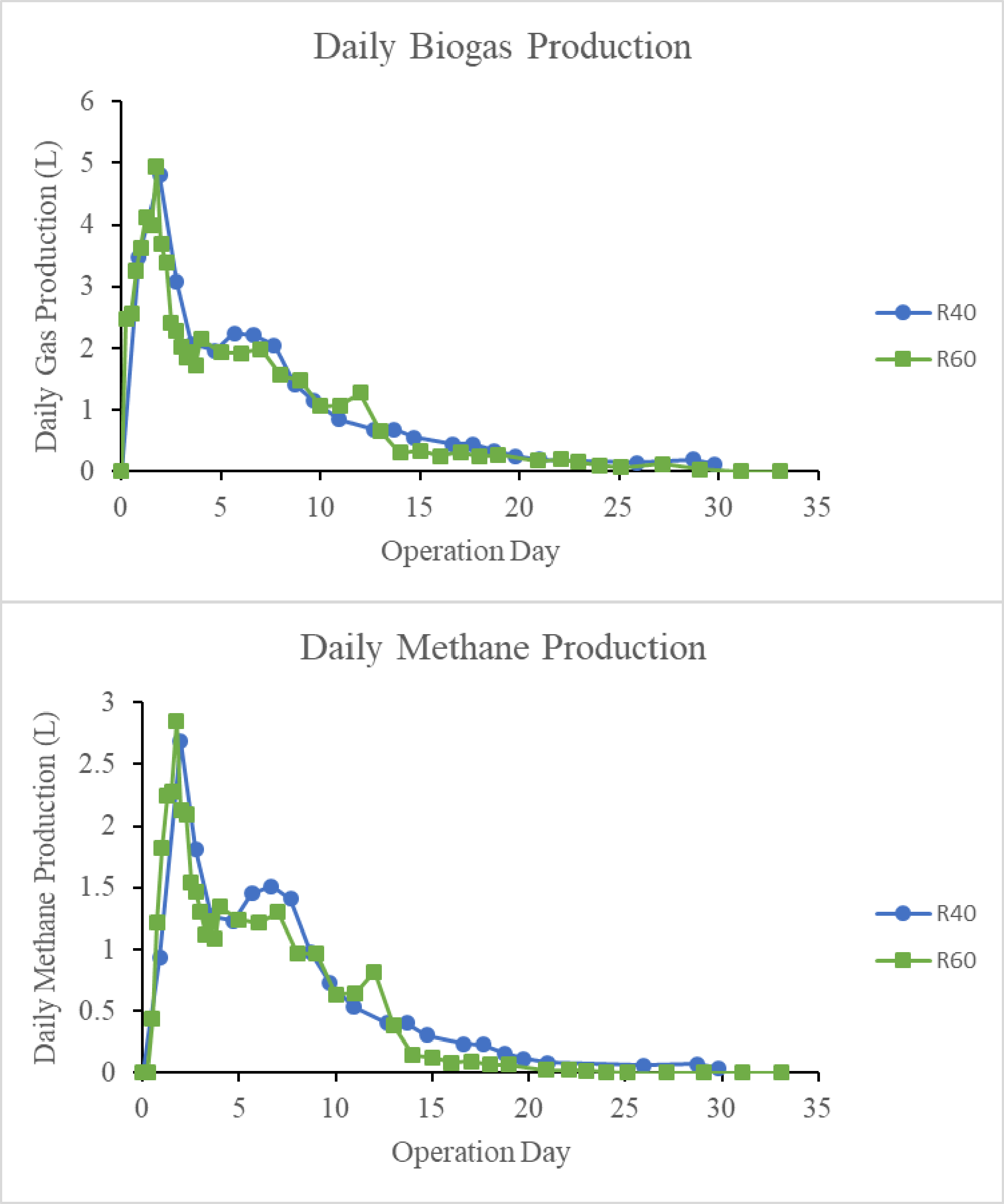

Figure 4-11 Daily biogas production (top) and daily methane production (bottom) of mid RS group: R40 and R60

As shown in Figure 4-12, in this high RS group, both daily gas production and daily methane production peaked the highest at the second day, then both went down but then made a small peak again. This second peak was at day 12 for R80 and day 10 for

R100. The second peaks are a lot more visible compared to mid RS group, and they also peaked later compared to the mid RS group.

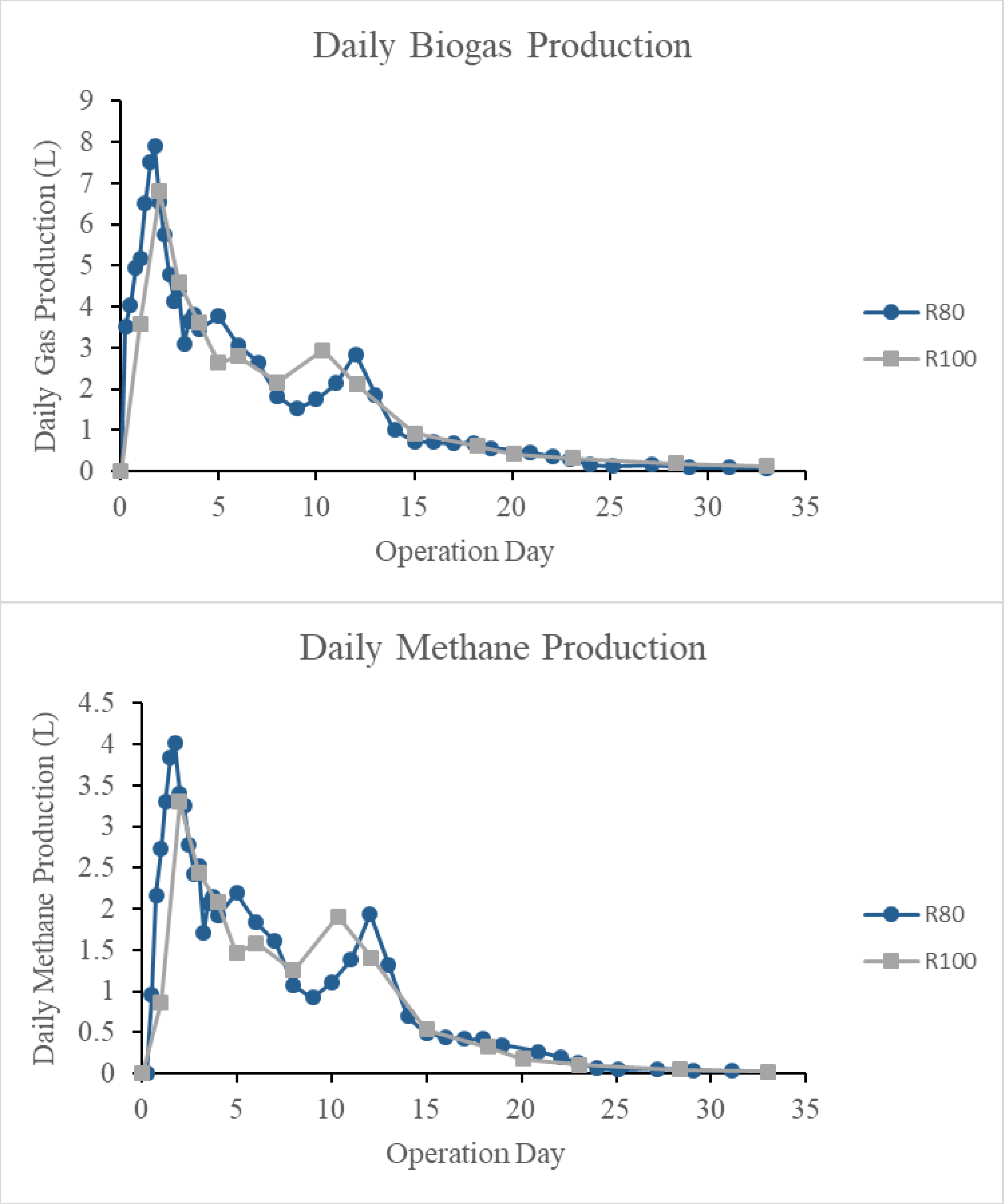

Figure 4-12 Daily biogas production (top) and daily methane production (bottom) of high RS group: R80 and R100

A possible explanation for this phenomenon is that as RS composition is higher, the degradable cellulose in RS compose a larger part of the total degradable fraction of the co-substrates. Cellulose is known to have a relatively lower methane production rate and slower methane production peak (Choi et al., 2020). For example, Choi et al. (2020) showed that cellulose methane production peaked at roughly day 15, while xylan and starch peaked faster at day 10. The appearance of second peaks in higher RS composition might be an indicator that RS contains higher amount of biodegradable cellulose as compared to BW.

Chapter 5 Conclusions and Future Works

5.1 Conclusions

Higher RS ratio in the biomass mixture would result in higher TS and VS removal. Lowest and highest s-RTS is 8.09% in batch R0 and 49.84% in batch R100, respectively. Lowest and highest s-RVS is 12.17% in batch R0 and 57.37% in batch R100, respectively. Subsequently, every gram of VS removed was converted to 0.526 L of biogas or 0.311 L of methane. Therefore, higher RS ratio in the biomass mixture would result in higher biogas and methane yield per gram of VS added. The methane yield is lowest in R0 at 0.036 L/g VS added, and the highest in R80 at 0.171 L/g VS added, although R100 methane yield was only slightly below at 0.169 L/g VS added. In terms of both solids removal and methane yield, neither synergistic nor antagonistic effects were apparent between RS and BW, since they both are lignocellulosic materials which possess similar characteristics.

The disparity between biodegradability of RS and BW could be explained by the lignocellulosic composition. Firstly, BW contains higher lignocellulosic fraction, which was found to be recalcitrant. Secondly, BW contains higher sum of cellulose and lignin, which was found to be negatively correlated with the anaerobic biodegradability. Therefore, BW contained less biodegradable fractions, thus yielded lower methane.

5.2 Future Works

The anaerobic co-digestion of RS and BW with SWE could be examined further. Assuming liquid digester in operation, S/I ratio could be increased if the FSM and SWE are more dilute. Semi-continuous operation could be considered, since many large-scale digester operates semi-continuously. Semi-continuous, instead of continuous, mixing could also be considered to reduce energy usage and probably increase methane

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production. Another way to increase S/I ratio is just increasing more biomass in the mixture and doing a solid-state anaerobic digestion. In this case, it is one step closer to the ideal case of zero wastewater discharge. In addition, a wide range of biomass and organic wastes could be considered as substitutional or even additional co-substrates, depending on the local condition and availability.

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