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Anaerobic biodegradability of organic solid substrates by steam explosion or co-digestion

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TABLE OF CONTENTS

ABSTRACT	1
ACKNOWLEDGEMENT	2
NOMENCLATURE.....	3
LIST OF FIGURES.....	4
LIST OF TABLES	4
I. INTRODUCTION	5
1. Background	5
1.1 Organic waste generation and anaerobic digestion.....	5
1.2. Organic substrates for anaerobic digestion	7
1.3. Strategies to enhance the anaerobic digestion of organic solid substrates.....	8
2. Objective	9
II. MATERIALS AND METHODS	11
1. Inoculum collection.....	11
2. Substrate preparation.....	11
2.1. Substrate collection and storage.....	11
2.2. Substrate and inoculum characteristics	12
3. Steam explosion pretreatment	12
4. Co-digestion of substrates	13
5. Biochemical methane potential (BMP) tests.....	13
6. Equations for parameters calculation and evaluation.....	14
III. RESULTS AND DISCUSSION	17
1. Biogas and methane yield of untreated solid organic substrates.....	17
2. Effect of steam explosion on the biomethane yield	18
3. Effect of co-digestion on the biomethane yield	22
4. Inhibition of the BMP tests	23
4.1. Effect of air content on the biomethane potential	23
4.2. Precision of the replicates	24
IV. CONCLUSION.....	27
V. REFERENCE	29

ABSTRACT

Organic solid waste is such a dominant source which accounts for nearly 50% of the total disposal solid waste. Besides, the fossil fuels, as a main source of energy production, are running out while the global energy demand is growing rapidly. The requirement for an alternative sustainable energy source is crucial to meet this demand, while minimizing greenhouse gas emissions. Anaerobic digestion for biogas recovery from organic solid waste has become an attractive technology to solve those problems with high economic and environmental benefits. In this study, several organic solid substrates including concentrated domestic sludge, industrial sludge from slaughterhouse, spent coffee grounds, microalgae and aquatic weeds were selected to evaluate their biodegradability by steam explosion or co-digestion. Biochemical methane potential tests were conducted in batch assays to verify the biomethane production of each substrate in each strategy. The results showed that industrial sludge from slaughterhouse produced highest methane yield at 745 mL/gVS without any pre-treatment because its component consists of high proportion of lipids. The lowest methane yield was obtained for aquatic weeds mainly due to its high lignocellulosic content in the cell wall. These results supported for the assumption that lipid-rich materials may have higher methane potential in comparison with lignocellulosic materials. Steam explosion at 180°C for 30 minutes did not show a substantial improvement in the methane yield but the process promoted the methane production rate of domestic sewage sludge and aquatic weeds so that reduced the lag phase time, which further shortened the hydraulic retention time of the tests. Some inhibition processes were assessed including acceptable air content inside the vessels and the precision of the triplicates. Those assessments were important in eliminating some inhibited conditions during the digestion process as well as validating the result of biochemical methane potential test.

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Linh Nguyen Thi Thuy
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NOMENCLATURE

BMP	Biochemical methane potential
CDS	Concentrated domestic sludge
SCG	Spent coffee grounds
MA	Microalgae
IS	Industrial sludge
AW	Aquatic weeds
COD	Chemical Oxygen Demand
C:N ratio	Carbon nitrogen ratio
WWTP	Wastewater treatment plant
DWWTP	Domestic wastewater treatment plant
LCFA	Long-chain fatty acid
TS	Total solid
VS	Volatile solid

LIST OF FIGURES

Figure 1: Anaerobic digestion process	6
Figure 2: Scheme of pre-treatment with steam explosion.....	12
Figure 3: Cumulative methane yield and methane production rate of untreated (blue) and pre-treated (orange) concentrated domestic sludge	20
Figure 4: Cumulative methane yield and methane production rate of untreated (blue) and pre-treated (orange) spent coffee grounds	20
Figure 5: Cumulative methane yield and methane production rate of untreated (blue) and pre-treated (orange) microalgae.....	20
Figure 6: Cumulative methane yield and methane production rate of untreated (blue) and pre-treated (orange) industrial sludge.....	21
Figure 7: Cumulative methane yield and methane production rate of untreated (blue) and pre-treated (orange) aquatic weeds.....	21
Figure 9: Air content inside the vessels in BMP test of untreated substrates	24
Figure 10: The methane production rate of pre-treated substrates.....	24
Figure 11: The precision in the triplicates of untreated concentrated domestic sludge and pre-treated spent coffee grounds.....	25

LIST OF TABLES

Table 1: Total solids (TS) and volatile solids (VS) characterization substrates and inocula ..	12
Table 2: Substrate composition and approximate C:N ratio	13
Table 3: Cumulative biogas yield and specific methane yield of untreated substrates.....	18
Table 4: Theoretical methane potential and biodegradability of untreated substrates.....	18
Table 5: Specific methane yield of untreated and pre-treated substrates	19
Table 6: Estimation of maximum methane production rate (R_{max}) and lag phase (λ).....	21

I. INTRODUCTION

1. Background

1.1. Organic waste generation and anaerobic digestion

Solid waste management has become an important issue corresponding with the growing of modernization and urbanization. According to the report “What a waste, a global review of solid waste management” published by the World Bank in 2012, the world cities generate about 1.3 billion tons of solid waste per year. This number is expected to increase to 2.2 billion tons by 2025. In the total disposal solid waste, organic waste accounted for nearly 50% [1]. At the same time, the global energy demand is constantly rising while the fossil fuels, as a main source (made up 70% of the growth in global energy demand [2]), is running out. Accordingly, the governments and managers are making a huge effort to manage these sources with the focus of sustainable development.

Anaerobic digestion from solid organic waste for energy recovery has become in the last decade an attractive renewable energy pathway in the world due to its high economic and environmental benefits. This technology can provide energy for heat, power, and transportation instead of utilizing fossil fuels, therefore reducing the greenhouse gases release into the atmosphere. Besides, the residues after the process can be used as a source of organic fertilizer for growing crops. With the increase of positive policies for renewable energy generation, developed countries has invested more for developing anaerobic digestion plants. For example, Europe has become the world leader for biogas electricity production since its total electricity capacity reach 10.4 GW, as compared to the global electricity of 15GW. In the developing countries including Vietnam, biogas is produced in small, domestic-scale for cooking or heating, while the substrates for this energy are enormous due to large quantity of by-products from agriculture [3]. Therefore, the potential for biogas production from solid organic waste is significant to develop a green, low carbon economy all over the world as well as eliminate the pollution for the environment.

Anaerobic digestion is known as a natural process in which micro-organisms break down complex organic compounds and convert them to biogas in the absence of oxygen. The process of anaerobic digestion involves four main steps that are hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The hydrolysis is responsible for converting complex organic compounds into simpler molecules under the action of the hydrolytic anaerobic bacteria. The products after

hydrolysis process are sugars, amino acids, and fatty acids. These molecules then are degraded by acidogenic anaerobic bacteria into organic acids, alcohol, hydrogen, carbon dioxide, volatile fatty acids and some by-products like ammonia and hydro sulfide. After that, volatile fatty acids and alcohols are converted into acetic acid under the action acetogenic bacteria. Finally, methanogenic bacteria convert acetic acid and hydrogen to methane and carbon dioxide. A small percentage of carbon dioxide and alcohol in the previous stage can be transferred into methane by hydrogenotrophic methanogens and methylotrophic methanogens, respectively. [4] [5] [6]. The summary of the process can be seen on **Figure 1**.

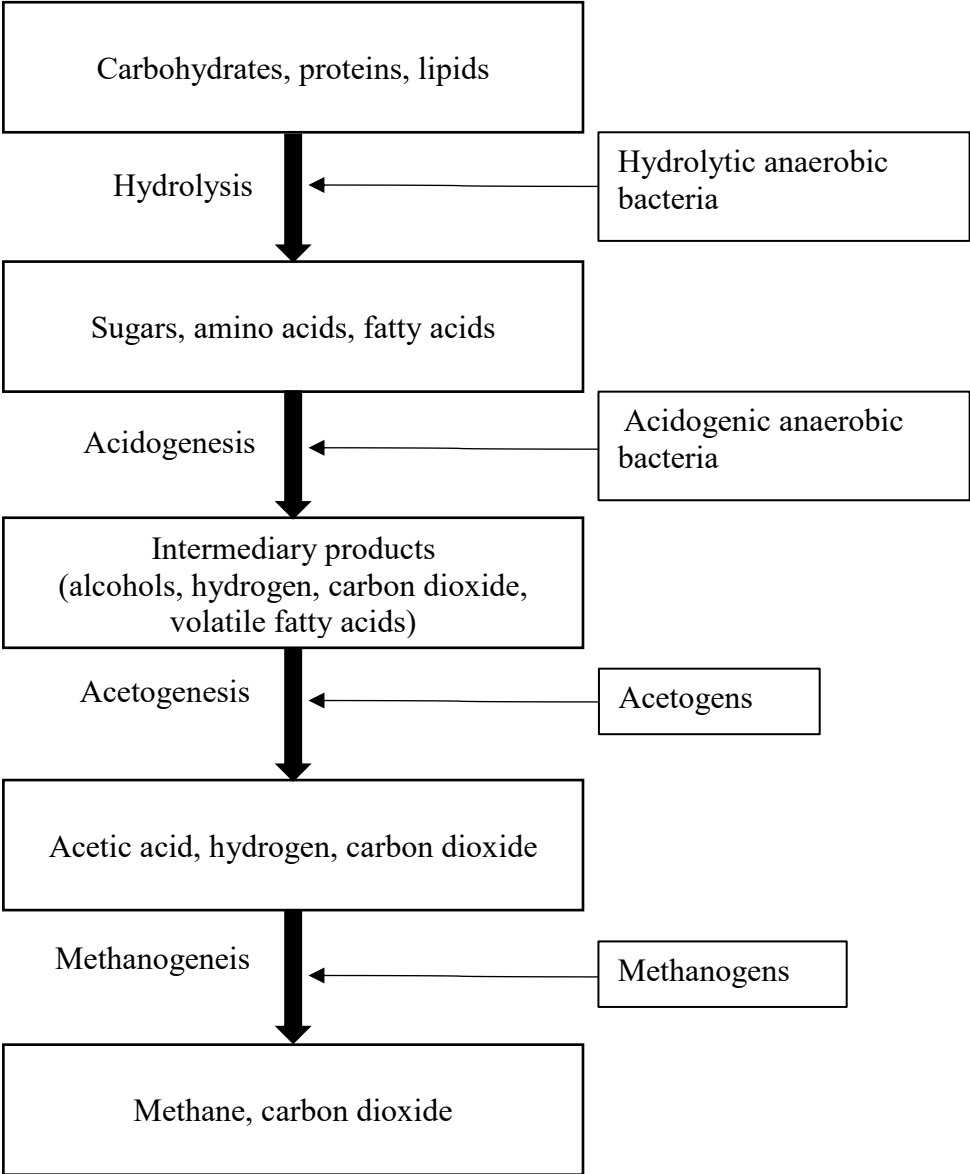


Figure 1: Anaerobic digestion process

1.2. Organic substrates for anaerobic digestion

Many different types of organic materials can be used as substrates in anaerobic digestion. Any substrate which is composed of carbohydrates, lipids and proteins are the desirable feedstock for the process. Theoretically, compounds with high proportion of lipids lead to higher methane potential compared to the ones has high content of proteins and carbohydrates. However, the hydrolysis of lipids takes a longer time compared to other compounds, which in turn prolong the hydraulic retention time of the methane production [7].

Microalgae is one of the promising feedstocks for biofuel production in recent years because many microalgal strains have ability to accumulate large quantity of lipids [8]. Besides, the cultivation of microalgae is beneficial compared to higher plants because of their capacity to grow in barren areas like in desert or coastal land, saline water and waste water, as well as their ability to uptake CO₂ which in turn reduce the greenhouse gas emission [9] [10]. Microalgae is a unicellular and autotrophic organism in which they can absorb sunlight, water, and atmospheric CO₂ to synthesis their biomass. Generally, the microalgal biomass comprises of about 7-23% lipids, 6-71% proteins, and 5-64% carbohydrates. The percentage of those components may vary depending on algal strains and cultivation conditions [10]. Some articles estimated that the structure of microalgae cell wall consists of 25-35% cellulose, 15-25% hemicellulose, 35% pectin and 5-10% glycoprotein [11]. Those components create a rigid protective cell wall that cause a high resistance to microbial attack which therefore limit their availability for anaerobic digestion. *Chlorella sp.* and *Scenedesmus sp.* are two freshwater algal strains that have hard cell walls, and therefore are difficult to disrupt [9] [12]. Some other studies pointed out that the cell wall of *Chlorella sp.* and *Scenedesmus sp.* comprises of a matrix of cellulose and hemicellulose which is made from glucose, mannose and galactose. Those organic macro-molecules with the presence of sporopollenin-like biopolymer contribute a robust cell wall which lead to the less biodegradability of these substrates [13] [14]. Thermal pretreatment is known as one of the most effective and widely applied methods in solubilizing the microalgal cell wall and may increase the methane yield up to 78% [15].

Besides microalgae, **spent coffee grounds** is also a potential substrate for biofuel generation. Spent coffee grounds (SCG) is the insoluble residues after several processes including roasting process, pressurizing with hot water, and spray-drying or freeze-drying to make the soluble or instant coffee [16]. Carbohydrates are the main components of SCG when it makes up for 79% of dry materials. It is followed by 17% proteins, and 4% lipids [17]. Similar with microalgae's cell wall components, cellulose and hemicellulose are prevalent components of SCG. Previous studies estimated that SCG do not need any pretreatment for anaerobic digestion, probably

because it faced with some previous processing stages during the production of instant coffee [18]. However, some other studies showed the improvement of SCG's biogas production by applying pretreatment methods [19] [20].

Sewage sludge is known as the by-product disposed after wastewater treatment processes. It is an effective source for producing biogas because of its high nutrient contents as a worthy food for anaerobic bacteria. A typical sewage sludge is the mixing of primary sludge produced during pre-settling and biological excess sludge produced from the activated sludge system. **Domestic sewage sludge** originated from DWWTP normally comprises high percentage of carbohydrates (58.63% starch and 20.15% lignocelluloses of the total component). While starch is partially soluble and bioavailable, lignocelluloses are more complex and insoluble. [21]

Apart from domestic sewage sludge, **industrial sludge** can also be a feedstock for anaerobic digestion. For example, in slaughter house industry, the wastewater contains higher values of total nitrogen, total phosphorous, total COD and suspended solid in comparison with domestic wastewater which may reflect a higher biogas potential in slaughterhouse wastewater sludge [22]. Lipids (characterized as fats, oils and greases) are the main content of industrial sludge, however, its degradation products – long-chain fatty acids (LCFA) may accumulate and inhibit the methanogenesis which then prolong the lag phase of methane production [23].

Anaerobic digestion is also effective in treating some **aquatic weeds** which have been excessively grown and caused some severe environmental problems to the waterbodies like foul order, water stagnation, fish interference, etc. [24]. Previous studies have pointed out that the methane yield from several aquatic weeds range from 38 to 361 ml/gVS_{added}. Although proportion of organic matter is high, many of weeds have low degradability due to the accumulation of lignocelluloses. In the submerged macrophyte *Potamogeton maackianus*, some organic compositions had been measured and showed that cellulose accounted for the largest ratio of 36.2%, followed by 20.7% lignin, 11.4% hemicellulose and other non-organic contents [25]. Those lignocelluloses are recalcitrant because they are made of phenyl propane units which are combined with other molecules by alkali-stable ether bonds to form lignin/phenolics-carbohydrate complexes.

1.3. Strategies to enhance the anaerobic digestion of organic solid substrates

A promising strategy is to introduce a pre-treatment step prior to the digester to hydrolyze the solid structure. To optimize the biogas potential in the substrates and minimize the lag phase in the biogas production time, several pretreatment methods can be applied, in which thermal

hydrolysis and co-digestion are the most applicable strategies for solid wastes. Thermal hydrolysis by steam explosion is one of the most environmental friendly and widely used process. This method does not use chemicals nor produce pollutants while enhances the solubility of the substrate significantly with a low treatment cost and shorten hydraulic retention time [26]. This is a favorable solution for hydrolyzing lignocellulosic materials. Apart from steam explosion, co-digestion is another effectual method from the point of view of diluting some potential inhibitors, balancing the nutrient ratio, and widening the bacterial strains for a better biodegradability. Normally, substrates with high carbon content (high C:N ratio) can co-digest with the ones rich in nitrogen content (low C:N ratio) to get the optimal C:N ratio from 1:20 to 1:30 [27]. However, in some cases, the co-digestion may be lead to the antagonism for the co-substrates when it promotes some inhibitory factors such as ammonia toxicity, high volatile acid concentration, pH inhibition, among others. [21]

2. Objective

The objective of this work was to examine the biodegradability of five different solid organic substrates and evaluate the effect of steam explosion and co-digestion on the potential methane production and kinetics.

II. MATERIALS AND METHODS

1. Inoculum collection

The inoculum is the anaerobic sludge collected from the anaerobic digester treating mixed sludge in the domestic wastewater treatment plant (DWWTP) in Valladolid (Spain). The inoculum was put in the room at the mesophilic temperature ranging from 33.4°C to 40.9°C for about 4 hours before used.

2. Substrate preparation

2.1. Substrate collection and storage

Sewage sludge from domestic wastewater treatment plant (DWWTP)

The sewage sludge was collected from DWWTP of Valladolid in 09/05/2018. After that, the sludge was centrifuged at 10 000 rpm for 10 minutes, and later mixed with the same sludge that was centrifuged at 10 000 rpm in 5 minutes. The concentrated domestic sludge was stored in the fridge at 4°C before used.

Industrial sludge from slaughterhouse wastewater treatment plant (WWTP)

The industrial sludge was received from a WWTP of a slaughterhouse in Sevilla, in 04/03/2018. This sludge had as higher water content compared with the domestic sludge, and therefore, it was less concentrated. The industrial sludge was also stored with the domestic one in the fridge at 4°C before used.

Spent coffee grounds

The substrate was collected from a coffee processing facility (Seda Outspan Iberia, L.S.) located in Palencia (Spain). It was stored in a dry plastic bottle at ambient temperature before used.

Microalgae

A mixture of *Chlorella sp.* and *Scenedesmus sp.* were collected from a pond treating wastewater in Almería (Spain). They were stored in the freezer at -20°C until use.

Aquatic weeds

The aquatic weed used in this experiment is *Potamogeton maackianus* that was harvested in the Southern Basin of Lake Biwa, Shiga, Japan in October 2017. Before used, it was stored in the freezer at -20°C. Before BMP test, the substrate was defrosted and then fragmented to about 1-2 cm long using a blender.

2.2. Substrate and inoculum characteristics

Total solids (TS) and volatile solids (VS) of substrates and inocula were determined following standard methods. All the results are shown in **Table 1**.

Table 1: Total solids (TS) and volatile solids (VS) characterization of substrates and inocula

Material	TS (g/kg)	VS (g/kg)	VS/TS ratio (%)
Concentrated domestic sludge (untreated)	172.25	124.78	72.4
Concentrated domestic sludge (pre-treated)	26.43	18.96	71.7
Spent coffee grounds (untreated)	450.88	448.35	99.4
Spent coffee grounds (pre-treated)	42.39	41.71	98.4
Microalgae (untreated)	164.31	150.9	91.8
Microalgae (pre-treated)	30.41	27.93	91.8
Industrial sludge (untreated)	134.63	112.38	83.5
Industrial sludge (pre-treated)	32.93	27.76	84.3
Aquatic weeds (untreated)	117.02	90.69	77.5
Aquatic weeds (pre-treated)	13.13	10.53	80.2
Inoculum (for pre-treatment experiment)	19.9	10.57	53.1
Inoculum (for co-digestion experiment)	20.8	15.2	62.5

3. Steam explosion pre-treatment

The process was performed in the pilot-plant facility at the University of Valladolid (Spain).

Picture 2 presents the scheme of pretreatment with steam explosion.

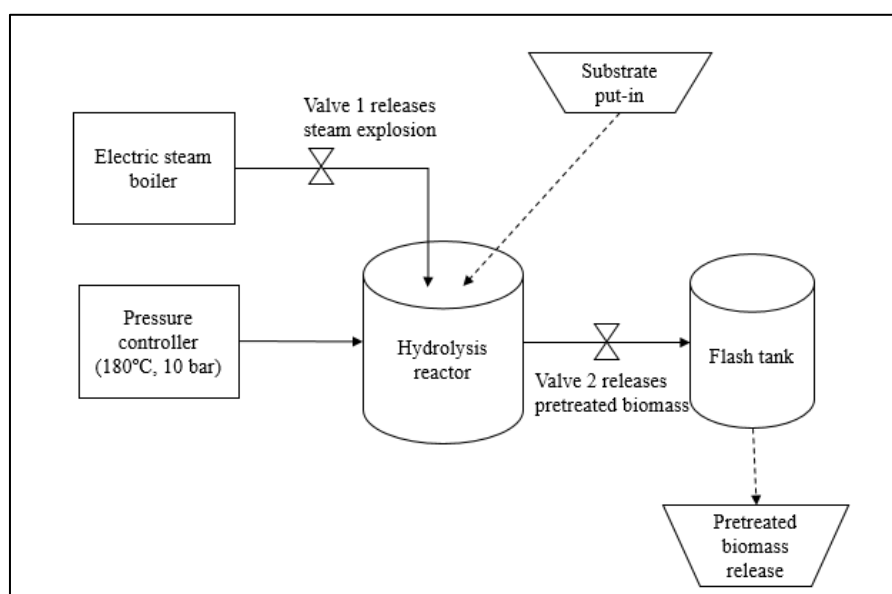


Figure 2: Scheme of pre-treatment with steam explosion

During the pre-treatment, each substrate was put into the hydrolysis reactor together with a litter of distilled water. Valve 1 was opened, allowing steam from the boiler to enter the reactor to heat the substrate at the desired temperature of 180°C (corresponding pressure of 10 bar). After 30 minutes of heating time, valve 1 was closed and the biomass is rapidly depressurized and released into the flash tank by opening the valve 2. The resulting steam exploded substrate was collected and stored at 4°C for further analysis and BMP tests.

4. Co-digestion of substrates

The substrates were co-digested based on their composition. In general, the substrates that have low C:N ratio were mixed with those that have high C:N ratio [28]. The composition and the approximate C:N ratio of the substrates are shown in **Table 2**.

Table 2: Substrate composition and approximate C:N ratio

Substrates	Lipids (%)	Protein (%)	Carbohydrates (%)	C:N ratio	Reference
Concentrated domestic sludge	8.3	6.45	78.78	High	[27] [29]
Spent coffee ground	4	17	79	High	[17]
Microalgae	-	76.35	7.15	Low	[30]
Industrial sludge	23.6	74	2.4	Low	[31]
Aquatic weed	1.91	22.97	45.64	High	[32]

Based on **Table 2**, six combinations of substrates were chosen for co-digestion including the combination of concentrated domestic sludge with four others (spent coffee grounds, microalgae, industrial sludge, aquatic weeds), and the combination of microalgae with two others has high C:N ratio (spent coffee grounds and aquatic weeds). The mixing ratio between substrates is fixed at 1:1. The biogas production from co-digestion was determined by means of the biochemical methane potential test further described in 5.

5. Biochemical methane potential (BMP) tests

The anaerobic digestion was performed in biochemical methane potential tests.

The assays were performed for each substrate in triplicate using 120-ml-closed-vessel (for pretreatment experiment) or 160-ml-closed-vessel (for co-digestion experiment) in which 50% was used as working volume, and the remaining was for gas headspace. A substrate-inoculum-ratio of $0.5\text{gVS}_{\text{substrate}}/\text{gVS}_{\text{inoculum}}$ was fixed as a basis to determine the amount of substrate and inoculum added to the vessels. A blank (control) test without substrate was included to verify the endogenous methane production from inoculum. Certain amount of sodium bicarbonate was

added into the vessels as buffering agent. After adding all the ingredients, the vessels were closed with gastight butyl rubbers and sealed with aluminum crimps. In addition, the air was removed from the reactor vessels by purging the headspace with helium in 3 minutes. All samples were incubated under mesophilic condition in a thermostatic room (the temperature ranges from 33.4°C to 40.9°C) with a continuously constant mixing for the guarantee homogeneous conditions. The experiments were performed at the hydraulic retention time of 33 days (pre-treatment experiment) and 28 days (co-digestion experiment).

The gas pressure was measured everyday using the gauge pressure transducer. Besides, the biogas composition was quantified in a gas chromatography device (Varian CP 3800) equipped with a thermal conductivity detector (TCD). The results were expressed as specific biogas yield (mL/gVS) and specific methane yield (mL/gVS) at standard pressure and temperature (0°C, 1atm).

6. Equations for parameters calculation and evaluation

The maximum methane production rate and the lag-phase were determined by using modified Gompertz equation *Eq. 1*:

$$P(t) = P_{max} * exp \left\{ - exp \left[\frac{R_{max} * e}{P_{max}} * (\lambda - t) + 1 \right] \right\} \quad Eq. 1$$

where **P(t)** is the cumulative specific methane yield (mL/gVS); **P_{max}** is the maximum methane production potential (mL/gVS); **R_{max}** is the maximum methane production rate (mL/gVS/day); **t** is time (day); **λ** is the lag phase (days); and **e** is the Euler's number (**e**=2.718282). [28]

The theoretical methane yield was determined by using the equation *Eq. 2*:

$$B_u = X * x + Y * y + Z * z \quad (\text{mL/gVS}) \quad Eq. 2$$

where **x**, **y**, **z** are the theoretical specific methane yield of lipids, proteins and carbohydrates, respectively (**x** = 1014 mL/gVS, **y**=851 mL/gVS, **z**=415mL/gVS); **X**, **Y**, **Z** are the percentage of lipids, proteins and carbohydrates in the substrates, respectively. [7]

The biodegradability can be calculated as the equation *Eq. 3*:

$$f_D = \frac{B_o}{B_u} \quad \text{Eq. 3}$$

where f_D is the substrate biodegradable fraction; B_o is experimental specific methane yield (mL/gVS) determined from the BMP assays; B_u is the theoretical methane potential (mL/gVS) that is evaluated by *Eq. 2* [33].

III. RESULTS AND DISCUSSION

1. Biogas and methane yield of untreated solid organic substrates

Table 3 presents the cumulative biogas yield and specific methane yield of the raw substrates. It was apparent that industrial sludge had the highest biogas yield and highest methane content at 1059 mL/gVS and 70.3%, respectively. In opposite, the biogas yield of aquatic weeds was the lowest (353 mL/gVS) - only a third biogas produced by industrial sludge. The biogas yield of microalgae was the half compared to industrial sludge (548 mL/gVS). The cumulative biogas yield from concentrated domestic sludge and spent coffee grounds were quite high: 693 mL/gVS and 754 mL/gVS, respectively.

The difference in biogas yield could be explained by the composition of the substrates. Industrial sludge comes from a WWTP of a slaughterhouse, so that it may contain a high proportion of lipids and proteins rather than carbohydrates. As it was pointed out in [26], lipid-rich substrates had higher biogas yield. On the other hand, aquatic weeds and microalgae had high content of non-degradable carbohydrates, so that their biodegradability was much lower than others.

In **Table 4**, the theoretical methane potential and the biodegradability of five substrates were evaluated based on *Eq. 2* and *Eq. 3*, respectively. The results support the assumption that although aquatic weeds and microalgae had substantial methane potential, their biodegradability were very low. Their biodegradable fractions were only at 42.65% and 49.16%, respectively. Conversely, concentrated domestic sludge was supposed to have the highest biodegradability at 91.89% based on this calculation. It was followed by spent coffee grounds (85.55%) and industrial sludge (84.75%). Although the biodegradability of industrial sludge was lower than that in concentrated domestic sludge, its theoretical methane potential may reach 879 mL/gVS, nearly double the value in concentrated domestic sludge (466 mL/gVS).

Table 3: Cumulative biogas yield and specific methane yield of untreated substrates

Substrates	Cumulative biogas yield (mL/gVS)	Specific methane yield (mL/gVS)	Methane content (%)
Concentrated domestic sludge (CDS)	693	428	61.8
Spent coffee grounds (SCG)	754	439	58.2
Microalgae (MA)	548	334	60.9
Industrial sludge (IS)	1059	745	70.3
Aquatic weeds (AW)	353	172	48.8

Table 4: Theoretical methane potential and biodegradability of untreated substrates

Substrates	Theoretical methane potential (mL/gVS)	Experimental methane yield (mL/gVS)	Biodegradability (%)
Concentrated domestic sludge (CDS)	466	428	91.89
Spent coffee grounds (SCG)	513	439	85.55
Microalgae (MA)	679	334	49.16
Industrial sludge (IS)	879	745	84.75
Aquatic weeds (AW)	404	172	42.65

2. Effect of steam explosion on the biomethane yield

Table 5 summarizes the specific methane yield of untreated and pretreated substrates at 180°C for 30 minutes. It is important to take into account that no optimization of operation conditions was performed in this study, and that the results could vary for other temperature-time conditions.

Figures 3-7 show the cumulative methane yield and the methane production rate of the five substrates studied. Under steam explosion, spent coffee grounds and microalgae had no influence on the methane yield compared to those that are untreated. The increase of methane yield for domestic and industrial sludge were trivial at 2.4 % and 7.2%. Aquatic weeds, however, had the highest improvement of methane yield after steam explosion at 16.2% but this figure was not significant. Regarding the kinetics (methane production rate, graphs in the right side), it is evident that steam explosion influenced the methane production rate for two substrates: concentrated domestic sludge and aquatic weeds, and also for microalgae and

industrial sludge, in a smaller extent. The highest methane production rate of pre-treated domestic sludge was at day 16th while that of untreated sludge was at day 28th. In pretreated aquatic weeds, the methane production rate reached the peak on day 7th, while untreated aquatic weeds reached the highest rate on day 20th.

Table 6 presents the kinetics results in terms of maximum methane production rate (R_{max}) and lag phase (λ), estimated by using modified Gompertz model (*Eq. 1*). Regarding maximum methane production rate, all substrates except for spent coffee grounds had higher R_{max} after pretreated. The highest R_{max} was observed in industrial sludge for both untreated and pre-treated samples (43.5 mL/gVS/day and 81.2 mL/gVS/day, respectively). In contrast, the lowest value was for aquatic weeds (8.6 mL/gVS/day for untreated weeds and 22.3 mL/gVS/day for pretreated). The obtained kinetic values were parallel with the experimental results for methane potential: industrial sludge produced highest biomethane while aquatic weeds had the lowest biomethane yield. In terms of lag phase, pretreated domestic sludge and aquatic weeds had the most improvement in lag phase compared with untreated ones. Their lag phase time decreased by approximate two times (from 20.3 days to 11.2 days for concentrated domestic sludge, and from 6.3 days to 2.8 days for aquatic weeds). Other pre-treated substrates had trivial enhancement in lag phase, even the lag phase of pre-treated industrial sludge was longer than that of untreated sludge (It was 11.5 days at untreated sludge while that at pretreated sludge was 13.9 days).

Table 5: Specific methane yield of untreated and pre-treated substrates

Substrates	Specific methane yield (mL/gVS)		CH4 increase (%)
	Untreated	Pre-treated	
Concentrated domestic sludge (CDS)	428	438	2.4
Spent coffee grounds (SCG)	439	438	-0.3
Microalgae (MA)	334	313	-6.4
Industrial sludge (IS)	745	799	7.2
Aquatic weeds (AW)	172	200	16.2

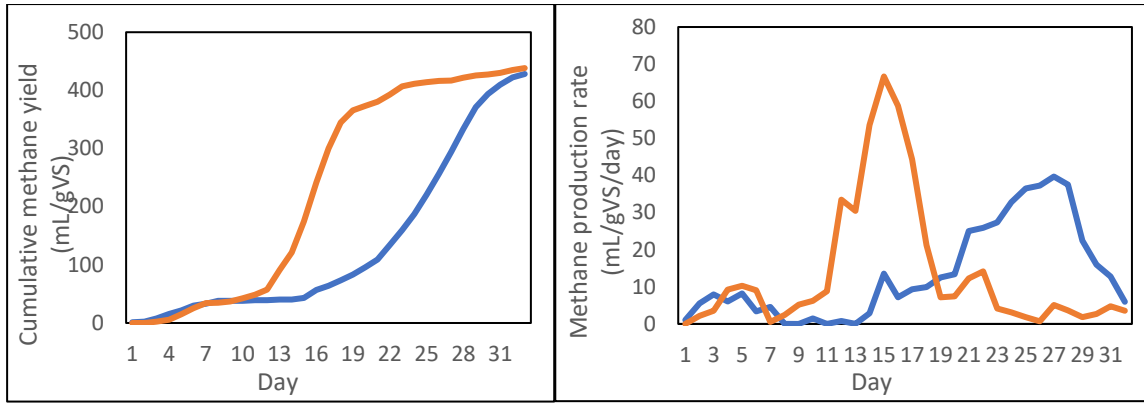


Figure 3: Cumulative methane yield and methane production rate of untreated (blue) and pre-treated (orange) concentrated domestic sludge

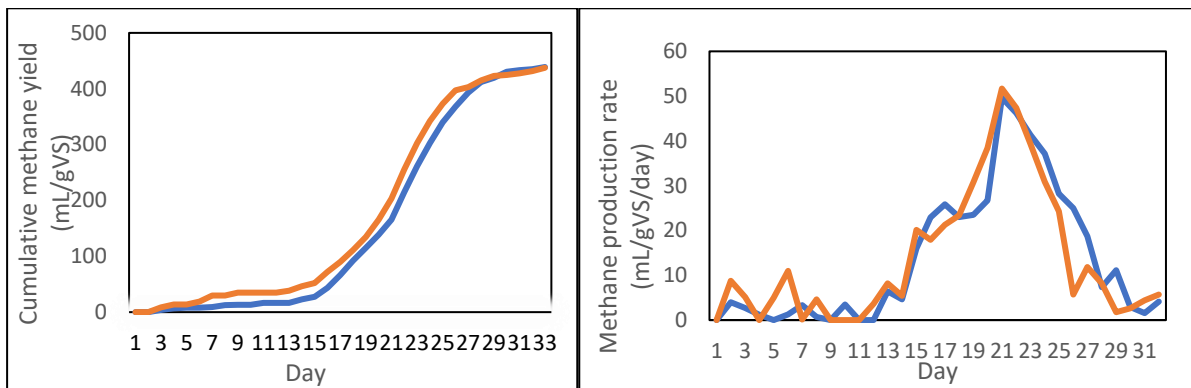


Figure 4: Cumulative methane yield and methane production rate of untreated (blue) and pre-treated (orange) spent coffee grounds

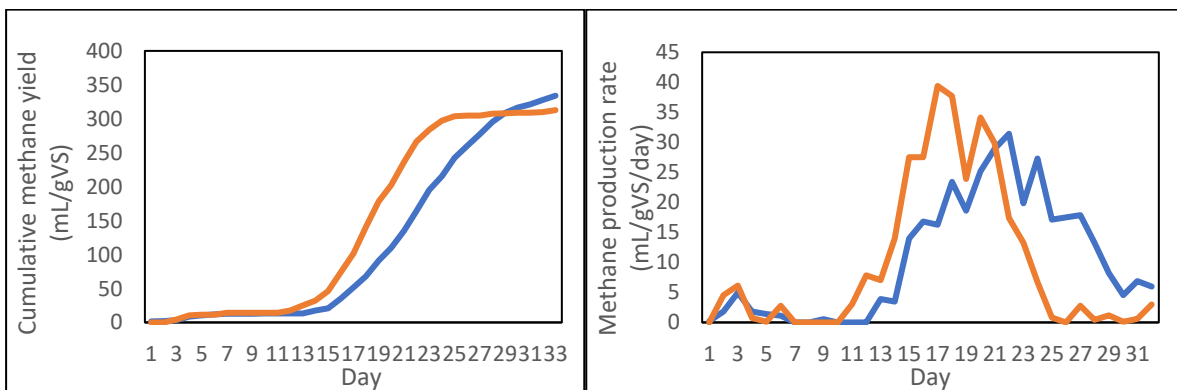


Figure 5: Cumulative methane yield and methane production rate of untreated (blue) and pre-treated (orange) microalgae

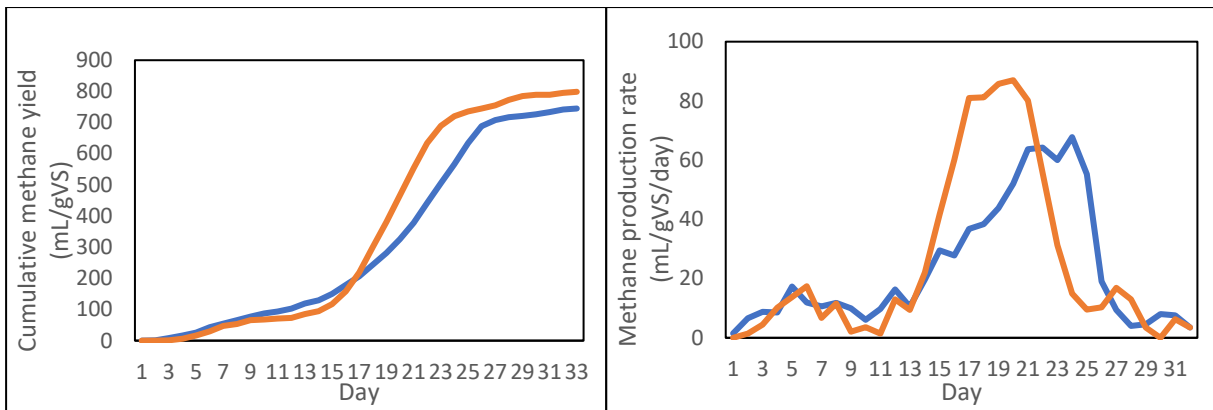


Figure 6: Cumulative methane yield and methane production rate of untreated (blue) and pre-treated (orange) industrial sludge

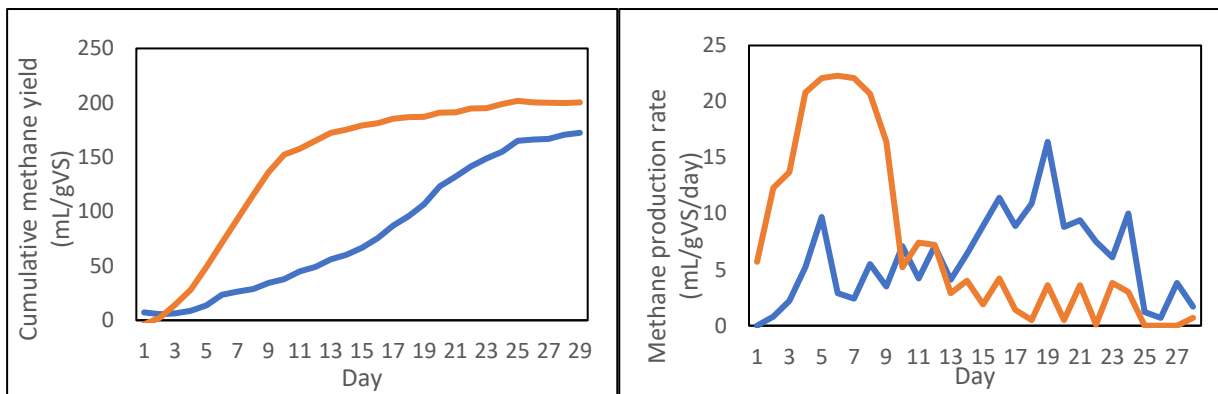


Figure 7: Cumulative methane yield and methane production rate of untreated (blue) and pre-treated (orange) aquatic weeds

Table 6: Estimation of maximum methane production rate (R_{max}) and lag phase (λ)

Substrates	R_{max} (mL/gVS/day)	λ (days)
Concentrated domestic sludge (untreated)	38.1	20.8
Concentrated domestic sludge (pre-treated)	51.6	11.2
Spent coffee grounds (untreated)	38.2	16.0
Spent coffee grounds (pre-treated)	36.3	14.7
Microalgae (untreated)	25.7	15.5
Microalgae (pre-treated)	37.2	14.0
Industrial sludge (untreated)	43.5	11.5
Industrial sludge (pre-treated)	81.2	13.9
Aquatic weeds (untreated)	8.6	6.3
Aquatic weeds (pre-treated)	22.3	2.8

3. Effect of co-digestion on the biomethane yield

Figure 8 presents the specific methane yield of co-substrates compared with mono-substrates. It is evident that the biomethane production of co-substrates varies greatly. Concentrated domestic sludge co-digested with aquatic weeds showed the lowest methane yield at 137 mL/gVS in comparison with other co-substrates. The highest methane potential was obtained for the co-digestion between domestic sludge and microalgae (360 mL/gVS). It was followed by the mixture of concentrated domestic and industrial sludge when its methane potential reached 274 mL/gVS, which doubled the methane yield of co-substrates domestic sludge and aquatic weeds. Three other co-substrates including domestic sludge and spent coffee grounds, microalgae and spent coffee grounds as well as microalgae and aquatic weeds, the methane yield was in range of 141 mL/gVS to 191 mL/gVS.

It seems that most of co-digested samples were antagonistic because their methane production was lower than that at both mono-substrates before co-digested. In cases of co-digestion between concentrated domestic sludge with spent coffee grounds, concentrated domestic sludge with aquatic weeds, and spent coffee grounds with aquatic weeds, the evidence of antagonism may be due to the high lignocellulosic content in the mixed substrates, so that their combination cannot produce high biomethane yield unless applying several pre-treatment methods to solubilize those molecules. Regarding co-substrates between domestic and industrial sludge, the methane potential was not as high as expected probably because of the accumulation of volatile fatty acids originated from industrial sludge – this intermediary product needs long time to decompose. However, the methane yield of the co-substrate was still in range of 270 – 500 mL/gVS found in the study in [34]. The co-digestion between domestic sludge and microalgae is seemed to be synergistic since the co-substrates produced the highest methane yield as compared with the methane yield of mono-substrates. It could be due to the contribution of additional nutrients, enzymes or other amendment that mono substrates by itself may lack. It then resulted in an enhancement of substrate biodegradability which further increased methane yield.

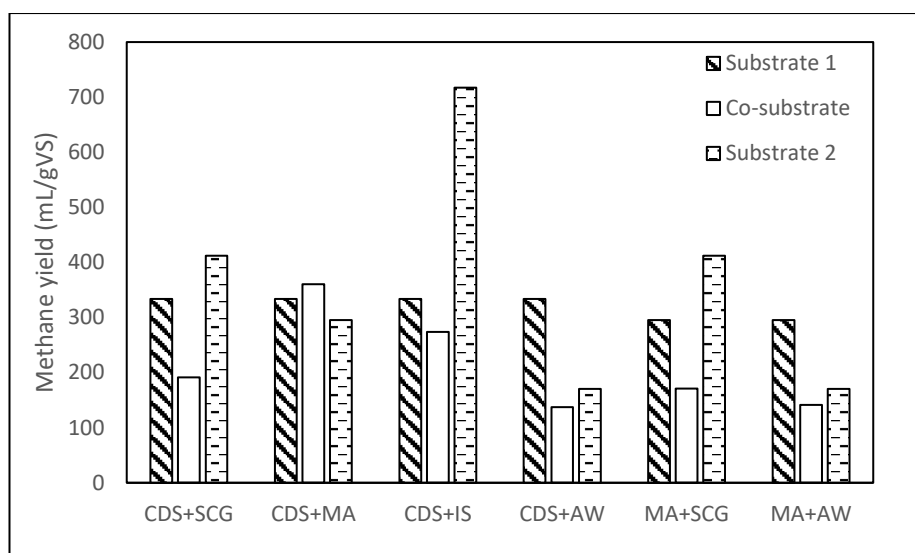


Figure 8: Specific methane yield of co-substrates and mono substrates

4. Inhibition of the BMP tests

The experimentally determined methane production may be underestimated when some uncontrolled conditions contributed during the conduction of the BMP tests. In this study, two factors were discussed: the air content inside the vessels and the precision of the triplicates.

4.1. Effect of air content on the biomethane potential

The air content inside the vessels may affect the digestion of anaerobic bacteria. Although the process of anaerobic digestion occurred in the absence of oxygen, a small amount of air content (mainly nitrogen and oxygen) still existed in the vessels which were detected by the gas chromatography device. The acceptable air content in the vessels were not estimated in previous studies. In this experiment, the air content was likely to be an inhibitory factor affecting the BMP result. **Figure 9** and **Figure 10** illustrate the air content inside the vessels and the corresponding methane production rate of pre-treated substrates in the BMP test. It is evident that during the first 2 weeks, the air content inside the vessels of four substrates (including domestic and industrial sludge, spent coffee grounds, and microalgae) were significant at more than 10%. At the same time, those methane production rates were trivial when they only produce methane at the rate smaller than 20 mL/gVS/day. After the air composition went down to around 5%, the methane production rate started to increase and reached the peak some days after. In the AW, the air content was as low as 5% at the beginning which result in the high production rate during the first 10 days. It was supposed that the high level of air content had inhibited the methane production during the first two weeks of the BMP test. At that condition,

the substrates will be degraded in the presence of oxygen and the products in such case was CO_2 instead of CH_4 .

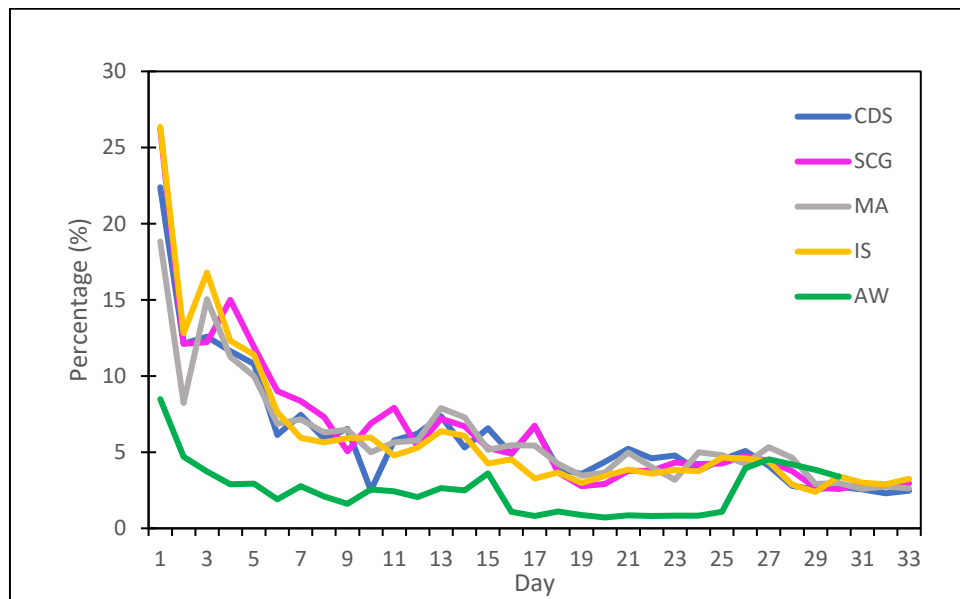


Figure 9: Air content inside the vessels in BMP test of untreated substrates

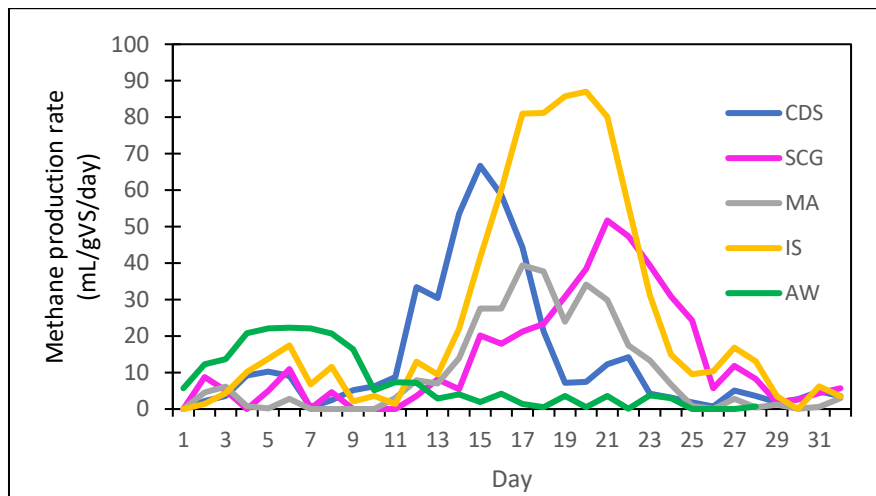


Figure 10: The methane production rate of pre-treated substrates

4.2. Precision of the replicates

The replicates for each substrate should be at least three to guarantee the reproducibility of the assays. However, during the period of the BMP test, if one sample in the triplicates had an abnormal result compared with two others, this outlier should be rejected. For example, **Figure 11** represents two different situations in the triplicates. The triplicate of untreated concentrated domestic sludge seems to be acceptable when three samples produced similar level of methane yield. But in case of pre-treated spent coffee grounds, one sample in the triplicate did not follow

the methane production trend with two others, and therefore, this single outlier should be rejected for validation of BMP test.

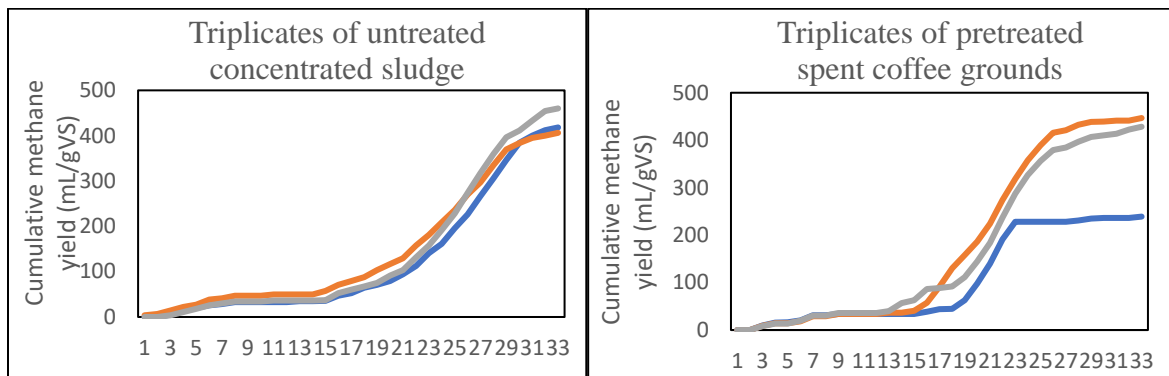


Figure 11: The precision in the triplicates of untreated concentrated domestic sludge and pre-treated spent coffee grounds

IV. CONCLUSION

The study evaluated the biomethane yield from anaerobic digestion of five different organic substrates that were greatly different in composition. It was obtained that industrial sludge produced the highest methane yield of 745 mL/gVS probably due to its high lipid content. Conversely, the methane production of aquatic weeds was the lowest (172 mL/gVS) mainly because lignocellulosic components accumulated in its cell wall which are recalcitrant and difficult to disrupt. After steam explosion pre-treatment at 180°C for 30 minutes, no significant improvement was observed in the methane yield of the substrates. In some cases (such as microalgae and spent coffee grounds), the pre-treatment had no improvement. However, the pre-treatment generally enhanced the methane production rate of the substrates, resulted in the shorter lag phase of the digestion which further lowered the hydraulic retention time. Further optimization of the pre-treatment conditions should be conducted for each substrate. A co-digestion evaluation was also performed. For most of the co-substrates, the effect was antagonistic, except for the case of co-digestion between concentrated domestic sludge and microalgae, in which the co-substrates enhanced the biomethane yield. Some inhibition processes were assessed in the experiment and showed that air content remaining inside the vessels may inhibit the anaerobic digestion at the beginning of the process if their concentration exceed 5% of the total gas volume. Besides, the precision of triplicates in BMP test was important in validating the BMP results. To sum up, the potential of biogas production from organic solids waste is clear and further study in testing several strategies to optimize the biogas yield would be essential in the application of this technology in practise.

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