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Biomethanation Potential and Enhancement of Acacia Leaves Waste Via Pretreatment and Co-Digestion Strategy

Supachai Hirunsupachote[1], Pratin Kullavanijaya[2], Orathai Chavalparit[3,4*]

[1] Faculty of Science and Technology, Rajamangala University of Technology Phra Nakhon, Bangkok, 18000, Thailand

[2] Excellent Center of Waste Utilization and Management, Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok, 10150, Thailand

[3] Department of Environmental Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok, 10330, Thailand

[4] Research Unit of Environmental Management and Sustainable Industry, Faculty of Engineering, Chulalongkorn University, Bangkok, 10330, Thailand

* Corresponding author's email: orathai.c@chula.ac.th

ABSTRACT

Acacia leaves waste biomass (AcLW) is an attractive feedstock for biomethane production by its generation amounts practically. This study evaluated the methane productivity of AcLW and its enhancement via alkaline pretreatment and co-digestion strategy. The effect of pretreatment conditions and process configuration on methane yields were investigated. The results showed that raw AcLW digestion in the single-stage process generated about 41.32 m^3-CH_4/kg VS_{added}, which increased significantly by 1.94–2.51 times to be 80.05–103.85 m^3-CH_4/kg VS_{added} for alkaline and 93.31–182.26 m^3-CH_4/kg VS_{added} for alkali-thermal pre-treated samples. The increase of NaOH concentration, soaking time and thermal supplementation affected methane productivity directly, while co-digestion with pulp bio-sludge at identical solid conditions promoted about 3.38 times or 162.7 m^3-CH_4/kg VS_{added} compared to raw AcLW digestion. A profitable operation of two separated stages combining leaching bed acidification and CSTR was also depicted with 152.1 m^3-CH_4/kg VS_{added}.The maximum gases productivity of AcLW digestion was promoted with alkaline-thermal pre-treated biomass for 3.60–4.41 times increase with 67.02–75.59% of total solids reduction. This finding demonstrated the biomethanation potential of AcLW and its enhancement after pretreatment and co-digestion significantly, which increased its possibility as a biogas feedstock.

Keywords: alkaline thermal pretreatment, leaves biomass, methane yield, paper waste, pretreatment

INTRODUCTION

Acacia is increasingly considered a potent economic tree for the pulp and paper industry. The available species of Acacia in Thailand are *Acacia mangium*, *Acacia crassicarpa*, and *Acacia aulacocarpa*. This fast-growing tree has been promoted widely to use substitutionally the Eucalyptus as a wood pulp source for the paper processing industry, which is a large industry demanding about 1.26 million tons of short fiber pulp production (TPPIA, 2019). Acacia has several advantages over other paper making trees, i.e., providing high biomass production yield in the three-year of age than Eucalyptus, higher fibre productivity (Inail et al., 2019), shorter cultivation period which each cycle needs only four years (Sruamsiri, 2001), and flexibility to grow in various pH of soil even in an acidic condition (Bowen and Benison, 2009). Paper processing starts with wood making (Moinul et al., 2019). Acacia twig can use as fuel instead of wood material but leaves biomass is still abundant as waste in the production areas and needs further operation to manage appropriately. This problem remains and will be increasing in the future because of the increase in paper production from Acacia wood cultivation.

Acacia is lignocellulosic biomass consisting of cellulose, hemicellulose, lignin, and biomolecules such as protein, carbohydrates, and fats, which can potentially be converted to methane in anaerobic digestion (AD) and used as fuel for heat or electricity generation (Chaiyapong and Chavalparit, 2016). AD of lignocellulosic biomass, including AcLW, does not proceed quickly by its complex digestible fraction and lignin. In general, the AD process relies closely on two main groups but significantly diversified microorganisms in converting organic material into biogas through four digestion steps called hydrolysis, acidogenesis, acetogenesis, and methanogenesis, under an oxygen-free environment (Liew et al., 2011). In the case of AcLW, hydrolysis is retarding and limiting to the overall biomass degradation. The amorphous heteropolymer network associated with cellulose microfibrils and lignin had been reported as resistant to predicting microbial attack before degradation (Agbor et al., 2011). Several efforts are attempted to enhance biogas and methane production degradation using various strategies, i.e., chemical pretreatment, co-substrate digestion, and the separation stages. Likewise, another biomass, to regulate the degradation rate of AcLW biomass, pretreatment is beneficial in increasing more to access surface structure and digestion easier for microbes. It had been reported that alkaline pretreatment was a suitable choice that could break a complex structure effectively with a reasonable cost of operation compared to the others (Zheng et al., 2014).

Alkaline pretreatment provokes many benefits in enhanced gas production by lignin destabilization (Kullavanijaya and Chavalparit, 2020). Barlianti et al. (2015) reported that the pretreatment of oil palm fruit brunch and fronds by soaking in 1.0% to 10.0% of sodium hydroxide (NaOH) solution could decrease lignin content from 47.3% to 37.8%, respectively. At the same time, about 83.7% to 87.3% of lignin found in sugarcane bagasse was removed after an alkali-thermal process with 1.5% to 2.0% NaOH (Wunna et al., 2017). A similar finding was found for Chaiyapong and Chavalparit (2016), who concluded that Acacia leaves waste digestion increased about 2.0 times after alkaline soaking than the non-pretreated leaves. Despite the regulation of biomass solubilization, the choice of the pretreatment method also plays a vital role in the efficient operation of an anaerobic digester. Sambusiti et al. (2013) concluded that more methane gas production was promoted by 25.0% higher in between alkaline pretreated and non-pretreated sorghum forage, but more digester stability was also observed. In practice, the benefit of this option is to enhance biogas production and reduce the waste storage condition in the paper processing industrial area. Therefore, co-substrate digestion could also enhance gas production as well as waste minimization. Not only pretreatment but it was also found that optimization of process digestion benefits biomass digestion. The two separated stage process was used in many several studies. An increase of biogas from lignocellulosic biomass was reported for various digestion of grass silage, sugar beet, willow leaves, rice straw, and biogas production was 160-390 L/kg VS_{added} (Lehtomaki and Bjornson, 2006; Crine et al., 2008; Zhang and Zhang, 1999). This method also is a selection. This study evaluated AcLW digestion for methane production potential in batch fed conditions to obtain a broader projection in AcLW biomass utilization. Three methods to enhance biogas and methane productivity, including alkaline soaking pretreatment, co-substrate digestion and process configuration or phasing, were investigated for raw and pretreated AcLW biomass. Moreover, the economic consideration was also studied for some energy usage scenarios to guide a proper decision-making condition for further application in the paper industry.

MATERIALS AND METHODS

AcLW biomass

Acacia leaves biomass (AcLW), both raw and pretreated samples, were digested to determine its methane productivity in this study. The selected AcLW was a widespread species, a hybrid of *Acacia mangium* and *Acacia auriculiformis*, as the characteristics are shown in Figure 1. Acacia leaves were harvested from the plantation field located in the Northeast of Thailand. After the harvesting, leaves biomass was shredded mechanically to the size of around 0.5 cm to 1.0 cm (Figure 1 b). The shredded AcLW was packed in a vacuum bag and then kept in the refrigerator at a controlled temperature of 4°C before the pretreatment experiment. For composition analysis, shredded AcLW was dried at 50°C and ground for further analysis of its chemical components such as total solids (TS), total volatile solids (VS),

Figure 1. AcLW biomass samples; (a) Acacia leave, (b) shredded AcLW, and (c) alkaline pretreated AcLW

cellulose, hemicellulose and lignin. It was found that ACLW contained about 47.0% of TS that volatilized the possibility of 44.7%. The lignocellulosic composition of AcLW were 18.8±0.2%, 14.3±0.0%, and 17.4±0.118.8% for cellulose, hemicellulose, and lignin.

For the co-substrate digestion experiment, a waste bio-sludge (WBS) from the pulp and paper factory was selected. This slurry sludge was collected from a sedimentation unit of the activated sludge, a standard treatment used in the paper factory. The sludge contained a COD of 0.6 g/g compared to 0.3 g/g in AcLW, which is considered an additional substrate that may promote more gas production of AcLW digestion and consequently increase the production potential of waste biomass in the exact origin. In practice, fresh shredded AcLW was dried in an oven at 60°C until a stable weight was obtained, then the dried sample was ground and used for further analysis. For inoculum, it was collected from a mesophilic anaerobic digester from the treatment plant of the beverage industry in the central. The mixed inoculum was applied for CSTR and two-stage digestion. This cow manure was collected from a livestock farm located nearby the Acacia cultivation area in the Northeast. It was pre-digested before using to eliminate the effect of the remaining substrate. In Table 1, the essential characteristics of AcLW, WBS, inoculum, and cow manure used in the study are summarized.

Alkaline and alkaline-thermal pretreatment

Acacia leave biomass was pretreated by soaking in alkaline solution (NaOH) at various concentrations and times. For biochemical methane potential (BMP) assessment, the selected concentrations and soaking time from previous the central composite design (CCD) (Reference) and applied to this experiment. The NaOH concentration was varied for 0%, 1.5%, and 3.0%, respectively. The soaking time was also varied for 0, 15, and 30 mins. The alkaline pretreatment was conducted for four conditions by varying the NaOH concentration and soaking time for 1.5–3.0% and 24–48 hours. For thermal pretreatment, the six conditions were selected for comparison with and without pretreated alkaline conditions. AcLW was steamed in an autoclave at 121^{o}C (15 psi) for heating. The others were adding alkaline pretreatment with thermal pretreatment to reduce the soaking time. AcLW was soaked in NaOH 1.5% and 3.0% and steamed for 15 and 30 minutes. The maximum promotion of gases was considered the selected condition for further investigation in

Table 1. Solid and chemical characteristics

Parameter	Feedstock			
	AcLW	WBS	Inoculum	Cow manure
Total solids (%)	47.0±0.1	2.0±0.0	6.7±0.0	19.4±1.1
Volatile solids (%)	44.7±0.1	1.3±0.0	5.9±0.0	16.6±2.2
Ash (%)	2.3±0.0	0.7±0.0	0.6±0.0	2.8±2.2
Cellulose (%TS)	18.8±0.1	n.d.	n.d.	n.d.
Hemicellulose (%TS)	14.3±0.2	n.d.	n.d.	n.d.
Lignin (%TS)	17.4±0.6	n.d.	n.d.	n.d.

Note: AcLW – Acacia leaves waste, WBS – waste bio-sludge, n.d. – not determine.

expanded scale digestion in CSTR and two-stage digestion. For alkali-pretreatment, pretreated biomass by soaking in 3.0% of NaOH for 48 hours with and without heat supplementation was applied for single-stage CSTR and a two-stage reactor operation considering the maximum methane production and operation cost.

EXPERIMENTAL DESIGNED CONDITIONS

Batch BMP assay

Experiments were herein divided into three parts. The effects of pretreatment conditions on methane production were investigated using batch BMP assay. The selected treatment conditions were applied to prepare AcLW for further digestion in the single-stage CSTR and a two-stage digester combining leached bed process and CSTR for acidification and methanation. The methane production performance and yield were compared. A batch BMP assay was conducted to preliminarily study the effect of pretreatment conditions on the biomethanation of AcLW samples. In practice, BMP digestion was carried out in 125 ml serum vials with a liquid working volume of 100 ml. The substrate and inoculum were added to the bottle in the ratio of 60:40 (v/v). The amount of initial substrate fraction was 2.5% of VS. The inoculum with a concentration of 25 mg/l was added to each bottle. NaHCO3 at concentration pf 3.0 g/L was also added to provide buffering capacity. The pH was adjusted to 6.8–7.2. The vials were then flushed with nitrogen gas, capped with butyl rubber stoppers and aluminium crimps. The vials were kept at the ambient condition where the temperature ranged between 30–35°C, intermittently mixed for 15 min hourly by shaker at a velocity of 70 RPM. The produced biogas and their composition were routinely analyzed. Each tested condition was conducted in triplicate, and the controlled substrate and inoculum vials without substrate addition were also operated as the referred condition.

Completely stirred tank reactors

For the lab-scale reactor, the single-stage completely stirred tank reactors (CSTR) with a working volume of 5.0 L were operated to evaluate the methane production potential of the AcLW samples. Each CSTR was configured roughly into four parts: the base part, digestion tank, mechanical mixing device, and gas production measuring device. These CSTR were acrylic made in a cylindrical shape with 16.0 cm and 30.0 cm for an internal diameter and height. Two sampling ports were provided at 10 cm and 25 cm. The mixing device was two-level paddles connected to the motor by the direct shaft. The mixing operation was intermittently for 15-mins hourly. A gas counter device using the water replacement method was attached to each CSTR. Samples were regularly withdrawal for analysis. The experiments were divided into four reactors with different AcLW biomass samples: raw AcLW for CSTR1, alkaline pretreated AcLW biomass (soaked at 3.0%-NaOH for 48 hrs) for CSTR2, AcLW co-digested with WBS at the ratio of 1:1 (VS basis) for CSTR3, and pretreated AcLW (soaked in 3.0%-NaOH for 48 h) co-digested with WBS with ratio 1:1 for CSTR4. The substrate and inoculum ratio used in the reactor was 60:40. In each reactor, substrate fraction was 3.0 L with an initial inoculum of 2.0% of VS, and the inoculum fraction was 2.0 L with an initial 2.5% of VS. The fermentation was conducted at room temperature in mesophilic ranged about 28.0–32.5 °C.

Two separated stages digester

The two stages digester was operated to investigate the effect of process configuration on methane production from AcLW. This reactor operated for two sets of experiments. The two-stage reactor consisted of ABR (Anaerobic leach bed reactor) for acidification and CSTR for methane production. The leach bed reactor was a cylindrical tank with the ratio of height and diameter was 5.76:1.0 (80:11 cm) or working volume of 5.0 L. Reactor was loaded with about 1,000 g of leaves biomass, which was mixed with inoculum in proportion to each test condition. The high porosity spherical plastic balls (3.3±0.2 cm) were mixed with the biomass for clogging preventing. At the bottom of the tank, an acrylic drilled plate was installed for filling and preventing a surplus loss of inoculum and wash-out of biomass through the effluent each ABR connected to pump for feeding and recycling intermittently. A gas counter using the water replacing was also installed to measure the daily gas production in each tank. All experiments were conducted at ambient temperature, which was 28.3°C±3.2. The leached effluent was kept in the storage tank before being recirculated at the top of the reactor. The circulation rate was 4.0 L/day. The acidification was operated for 80 days. This acidic leachate was then fermented in the methane tank using a CSTR reactor with an identical character as in 2.3.2. The cow manure was used as an inoculum to enhance biogas and increase the use of the alternative material in the paper industry area. This two-stage was operated for two experiments; the first experiment was the fermentation of non-pretreated AcLW and pretreated AcLW by soaking in 3.0% of NaOH with thermal addition for steaming at 121°C for 30 mins.

Sample and data analysis

TS, VS, and COD were analyzed according to the standard (AOAC, 1990). Cellulose, hemicellulose and lignin were analyzed following Van Soest's method (Van Soest et al., 1991), while AOAC standard method (AOAC, 2000) was also applied for protein, carbohydrate and fat composition. Biogas was measured routinely by the water displacement method, and methane composition was analyzed using gas chromatography equipped with a thermal detector. The maximum methane yield and methane production rate were simulated by the Gompertz equation using the Solver function of Excel (Nielfa and Euverink, 2015; Ghatak and Mahanta, 2014). The equation is shown below:

$$P = P_m \cdot \exp(-\exp(R_m \cdot e / P_m \cdot (\lambda - t) + 1))$$

where: P – observe methane production (L/kg VS_{added}), P_m – maximum methane production (L/kg VS_{added}), R_m – Methane production rate (L/kg VS_{added}·d), λ – lag phase (day), e – mathematic constant = 2.718.

For reactor operation, the overall performance and stability were monitored, indicating throughout chemical parameters such as pH, SCOD, total volatile fatty acids (TVFA), and alkalinity (Alk) followed the standard procedures for analysis: TS (#2540) and COD (#5220) (APHA, 1998), and titration method of Anderson and Yang (1992) for Alk and TVA analysis. pH was measured by a pH meter. In comparison, an economic consideration when applying the pretreatment process were also analyzed. The operation cost of pretreatment from a chemical used of NaOH soaking and heating for steaming was estimated. The amount of NaOH and energy used from electricity in each pretreatment method were compared to enhanced gas production for each treatment condition at an equal volatile solid addition. The return value was calculated using generated biogas substituted crude oil fuel or electricity per 1 kg VS of feed biomass. For the two-stage reactor, the leaching time circulation and reactor cost were neglected. A statistical analysis of ANOVA (one-way) using SPSS Statistics 17.0 was applied to the results of significant meaning analysis at 95%-confidential. The difference in average operational performance and stability by the influence of each inoculation was determined significantly. Moreover, the surface area of the pretreated samples was analyzed using a scanning electron microscope (SEM) at 1000 times of magnifying power.

RESULTS AND DISCUSSION

Characteristics of raw and pretreated AcLW

The AcLW biomass represents typical lignocellulosic biomass which chemical characteristics of raw AcLW and pretreated ones are shown in Table 2. The result found that the pretreatment changed the chemical characteristics of AcLW. The result implied that the alkaline soaking and heat could transform the hard to easy digestible content. The non-fibre content in AcLW was increased, and cellulose and lignin were decreased significantly when the concentration of NaOH and soaking time was increased experimentally. In the last condition, soaking in 3.0% of NaOH with steaming for 30 min changed the chemical characteristics. The non-fibre content, which was quickly degradable content, was increased from 49.5% to 70.6%. On the other hand, cellulose and lignin, which had complex degradable content, decreased from 18.8 to 14.7% and 17.4 to 7.9%, respectively. The hemicellulose decreased from 14.30% to 7.2%. However, in the same method of pretreatment (alkaline pretreatment or alkali-thermal pretreatment), the concentration of NaOH, soaking time decreased hemicellulose content slightly. The considerable change of lignocellulosic (cellulose, hemicellulose and lignin) components were promoted when the alkali pretreatment was applied, particularly with thermal supplementation that changed the chemical characteristics of AcLW significantly.

It was worth noting that after treatment the increased methane production was expected. Several studies found the positive effect of pretreatment on the raw material characteristic. For example, the cellulose chain was shortening via alkaline hydrolysis (Mozdyniewicz et al., 2016).

Table 2. Characteristics of raw AcLW and pretreated AcLW with different pretreatment conditions

Code	Treatment	TS (%)	VS (%TS)	Non-fiber (%)	Cel[a] (%)	Hem[a] (%)	Lig[a] (%)
A	None (raw AcLW)[b]	47.0±0.1	85.8±0.1	49.5±0.5	18.8±0.1	14.30±0.17	17.4±0.6
B	Soaked in NaOH 1.5%, 24h	40.6±0.1	86.2±0.3	58.1±0.0	18.3±0.1	8.6±0.1	15.0±0.0
C	Soaked in NaOH 1.5%, 48h	38.4±0.0	84.4±0.3	59.6±0.0	18.1±0.0	9.2±0.0	13.1±0.0
D	Soaked in NaOH 3.0%, 24h	36.1±0.0	81.9±0.2	60.9±0.1	17.8±0.0	8.2±0.0	13.1±0.0
E	Soaked in NaOH 3.0%, 48h	47.3±0.2	92.7±0.2	61.0±0.2	17.2±0.2	9.2±0.0	12.6±0.1
F	Steamed 15 min	43.4±0.1	90.8±0.2	60.6±0.0	17.0±0.1	10.2±0.1	12.2±0.0
G	Steamed 30 min	42.2±0.0	93.7±0.1	61.7±0.1	16.6±0.1	9.7±0.1	12.0±0.1
H	Steamed 15 min in NaOH 1.5%	43.2±0.1	95.6±0.1	66.9±0.1	15.9±0.1	7.4±0.1	9.9±0.0
I	Steamed 30 min in NaOH 1.5%	42.4±0.1	91.1±0.0	67.2±0.1	15.7±0.1	8.3±0.1	8.9±0.0
J	Steamed 15 min in NaOH 3.0%	45.2±0.1	93.6±0.2	68.1±0.1	15.5±0.1	8.2±0.1	8.2±0.0
K	Steamed 30 min in NaOH 3.0%	40.0±0.1	94.6±0.1	70.6±0.1	14.6±0.0	7.2±0.1	7.9±0.1

Note: [a] Cel – cellulose, Hem – hemicellulose, Lig – lignin. [b] Raw AcLW or non-pretreated biomass. [c] Soaking in NaOH solution at different concentrations (%) and time (hour, hr). [d] Heat by steaming with/without soaking in NaOH at different concentrations (%) and time (min).

In addition, alkaline pretreatment had a significant effect on lignin but a minor effect on hemicellulose solubilization (Swatloski et al., 2002). The steam explosion had a considerable impact on hemicellulose (Zheng et al., 2009). Some studies found that co-pretreatment such as alkaline plus thermal pretreatment can enhance more methane production. The fibre was hydrolyzed, solubilized, fractionated, and separated before enzymatic hydrolysis (Saha, 2003).

Similar to chemical characteristics, the surface of each treated AcLW and raw ones were analyzed. It was found that after pretreatment, the surface characteristics were changed relatively. Figure 2 shows the SEM micrographs of non-pretreated AcLW (Figure 2a) and pretreated AcLW (Figure 2b) of nine treatments conditions. The results found that the material surface was changed after pretreatment. It was rougher than non-pretreated AcLW. The higher porosity from pretreated material can be found. Mosier et al. (2005) found that the alkaline and thermal pretreatment increased the accessible surface area. In addition, these results confronted the work of Jabasingh and Nachiyar (2011). They found the increasing external surface of narrow-leaf cattail from the alkali concentration and temperature. Another is the word from Rungmee and Sangwichien (2013), who stated the intensity of alkaline concentration similarly influenced the surface roughness of bagasse.

Effect of pretreatment on biomethanation

Figure 3 shows the cumulative methane production of AcLW and pretreated AcLW with different treated conditions. It was found that the significantly increased methane production was promoted maximally after alkali-thermal pretreatment of AcLW. For alkali-pretreatment, the gas production increased rapidly in the beginning and reached steady on the 21st day. The methane accumulation was changed. The methane can be generated longer than from non-pretreated AcLW and reached its steady-state of production on the day 33rd of operation.

Furthermore, the methane productivity was increased significantly with time and treatment conditions and promoted maximally on the 60th day. The increased concentration of NaOH solution and soaking time increased the methane production appropriately, but a significant change was obtained when supplied heat treatment. Figure 3(b) showed the methane accumulation from thermal pretreatment. The methane accumulation can be expanded for more than 60 days. Thus, the maximum methane was predicted by the Gompertz model. The predicted methane was shown as the line in Figure 3. From the Gompertz model, the growth methane accumulation can be found in 3 groups. The methane from single thermal pretreatment was steady on the 40th day. When the NaOH 1.5% was applied with thermal pretreatment, the methane was steady on the 45th day. Moreover, when the NaOH 3.0% was applied with thermal pretreatment, the methane was steady on the 57th day. Moreover, the maximum methane from co-pretreatment was different from single pretreatment significantly. The maximum methane, methane rate production and lag phase from the Gompertz model was shown in Table 3.

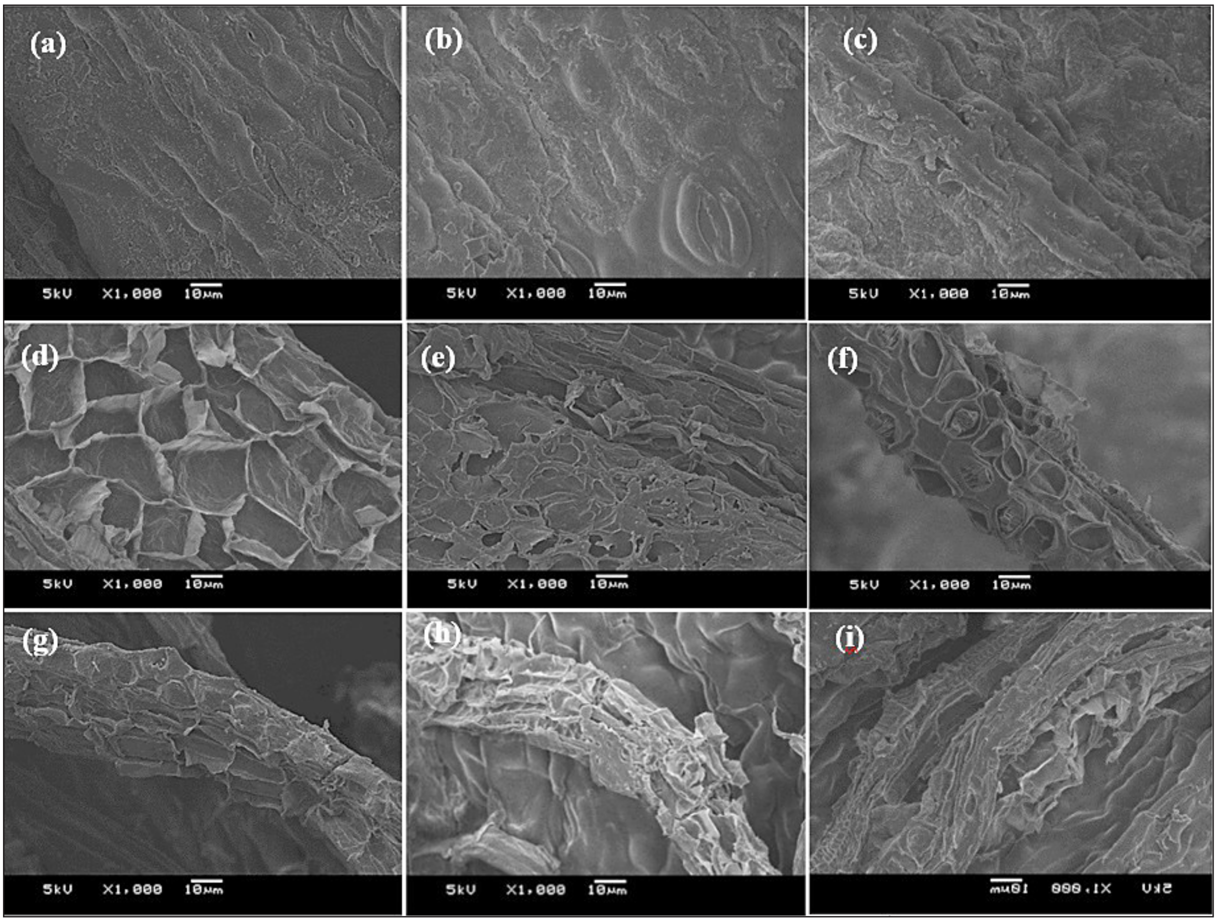

Figure 2. SEM micrographs (at 5kv, x1000) of AcLW samples: (a) raw AcLW, (b) soaked in 1.5% of NaOH for 48 hrs, (c) soaked in 3.0% of NaOH for 48 hrs,(d) steamed at 121°C (15 psi) for 15 min, (e) steamed at 121°C (15 psi) for 30 min, (f) soaked in 1.5% of NaOH and steamed at 121°C (15 psi) for 15 min, (g) soaked in 1.5% of NaOH and steamed at 121°C (15 psi) for 30 min, (h) soaked in 3.0% of NaOH and steamed at 121°C (15 psi) for 15min, (i) soaked in 3.0% of NaOH and steamed at 121°C (15 psi) for 30 min.

Table 3. Kinetic parameters of AcLW and pretreated AcLW digestion simulated by Gompertz equation
Note: Analyzing with ANOVA single factor test and two factors without replication test the accumulation methane in each method had significantly difference at $p < 0.005$

Code	R_m (L/kg VS_{added}.d)	Lag phase (day)	P_{max} (L/kg VS_{added})	Day to 60% production of CH^4	Steady (80%) at day	SCOD (g/g)	Solid reduction (%)
A	2.04	0	41.32	16	36	0.30	30.72
B	4.51	0	80.05	12	24	1.78	51.99
C	4.48	1.89	85.59	14	24	1.99	55.01
D	4.06	4.77	88.48	18	21	2.20	56.02
E	4.79	3.1	103.85	16	26	2.40	59.03
F	4.36	0	93.31	17	36	2.30	59.81
G	5.03	0	103.06	17	36	2.42	59.24
H	6.47	0	148.67	15	28	3.70	67.02
I	7.62	0.64	163.17	15	28	4.03	71.36
J	5.73	4.23	167.73	24	36	4.04	72.25
K	7.15	4.9	182.26	21	34	4.50	75.59

The methane production rate from non-pretreatment AcLW was 2.0 L/kg VS_{added}.d, while it increased by 2.2-2.5 times to 4.1-5.0 L/kg VS_{added}.d after alkaline pretreatment. The supplementation of heat by steaming was also increased the methane production rate to 5.7-7.2 L/

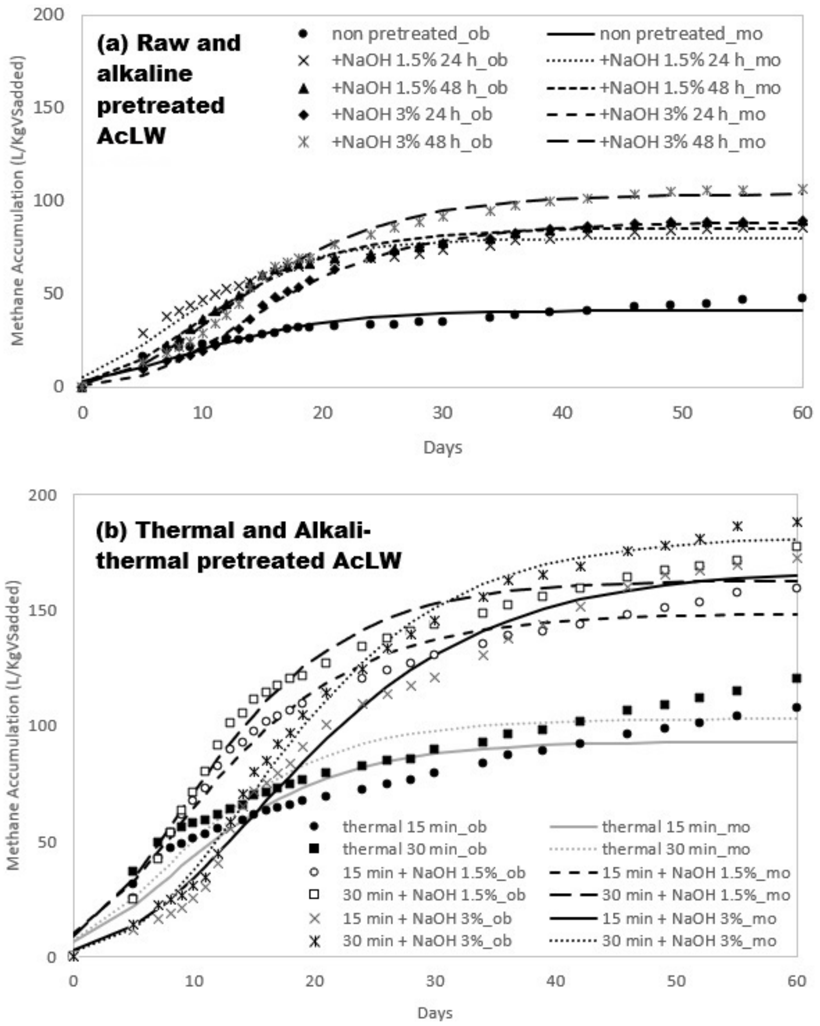

Figure 3. Methane production potential of AcLW with different pretreatment conditions: (a) Raw and alkaline pretreated AcLW, (b) Thermal and alkali-thermal pretreated AcLW

kg VS_{added}.d. The maximum methane yield from alkaline-pretreatment was 103.9 L/kg VS_{added}. It was also found that soaking time was a minor factor influencing methane yield, which directly enhanced the gas productivity more than NaOH concentration increased. Methane production of alkaline pretreated AcLW in 1.5% of NaOH for 24 and 48 hours were 80.1 and 85.6 m^3/kg VS_{added}. The methane production was only about 6.9% promoted while increasing the NaOH concentration increased by 10.5%. However, if the NaOH concentration and soaking time increased, the methane production could significantly increase. From the result, soaking AcLW in 3.0% of NaOH solution for 48 hours increased about 21.3% of methane production compared to a soaked in NaOH of 1.5% for 48 h sample. A similar finding in the positive effect of alkaline soaking, Zheng et al. (2009) reported a suitable time for corn stover soaked in NaOH was three days.

When the steam pretreatment was applied without alkaline pretreatment, the AcLW sample generated maximally methane production around 93.3-103.1 m^3-CH_4/kg VS_{added}. The increase of steaming time also increased productivity. A similar finding was stated by Bali et al. (2014), who studied the effect of the alkali-thermal pretreatment period and found that the incubation time affected sugar yield directly. They concluded that soaking time and temperature were important factors in enhancing the accessibility of cellulose enzymes. At the same time, the low temperature was for longer residence time dissolved a major portion of hemicellulose and exhibited higher cellulose accessibility than high temperature. From the result, increasing incubation of steaming to 15.0 minutes increased the methane production by 10.5%. When comparing an alkaline-pretreatment and thermal pretreatment, the increased methane production did not differ significantly.

However, using alkaline pretreatment with a thermal supplement, the maximum methane yield increased 43.16%-76.47% to be 148.67-182.26 L/kg VS_{added}. Another advantage of thermal pretreatment was pretreated time. Using thermal took only 15-30 minutes, while alkaline soaking needed 24-48 hours to take place at the same productivity. The incubation time was a minor factor. From the result, the increased incubation time of 15.0 min with NaOH 1.5 and 3.0% increased the methane production by 9.8 and 8.7%, respectively. However, using the maximum NaOH concentration and incubation time also promoted the maximum methane production or increased 4.4 times from non-pretreated AcLW.

Considering SCOD and total solids reduction, it was well correlated to methane yield and methane production rate. The SCOD and solids reduction from alkali-thermal treatments were higher than only alkaline or thermal pretreatment significantly. This result was confronted in the last part. The decreased challenging digestible content such as cellulose and lignin and increased quickly digestible content increase methane production. Not only methane production but also methane production rate was increased. Alkaline-pretreatment can improve hemicellulose and lignin solubility and sugars content which is degraded more efficiently and rapidly after alkaline- and autohydrolysis treatment (Swatloski et al., 2002; Agbor et al., 2011). At the same time, Wang et al. (2010) reported similarly that solid, sugar and lignin content were reduced when coastal Bermuda grass was pretreated with NaOH at 121°C. The thermal pretreatment almost changed the hydrolysis step. It could improve the hydrolysed substance and the hydrolysis yield (Hendriks et al., 2009). In this consequence, the faster hydrolysis rate can also increase biogas and methane production.

Methane production enhancement via co-digestion and phase-separation strategy

To investigate the effect of digestion conditions, herein phasing and co-digestion, on methane production enhancement, batch experiments were carried out in CSTR and two-stage reactor. The result of each digestion is summarized in Table 4 and Figure 4. The obtained results depicted that the biogas and methane production from the various digestion were differently promoted. A single CSTR reactor for alkaline-pretreated AcLW increased methane production from 48.0-116.8 L/kg VS_{added} or about 2.44 times, while vial scale digestion was higher than non-pretreated AcLW 2.51 times. Not only the methane production but also methane production rate and SCOD removal increased after biomass pretreatment. The methane composition from alkaline-pretreated AcLW increased from non-pretreated one by 9.5% from 49.0% to 58.5%, while SCOD removal was increased from 69.2% to 81.2%. It was worth noting that a similar result was observed both in batch BMP and single-state CSTR digestion. The methane production was increased when applied pretreatment to AcLW biomass.

In addition, two-stage digestion was evaluated for methane enhancement. The result found that the leaching bed tank for acidification coupling stirred tank for methanation promoted methane of 111.9 m^3/kg VS_{added} from raw AcLW digestion. This production increased by 2.3 times compared to non-pretreated AcLW digestion in a single-stage process. However, compared to the

Table 4. An averaged performance of raw and pretreated AcLW digestion with different conditions

Conditions	pH	Temp. (°C)	Alk[a] (mg/L))	VFA[b] (mg/L)	$SCOD_{rem}$ (%)	Biogas (L/kg VS_{added})	CH_4 (%)	CH_4 (L/kg VS_{added})
One-stage reactor								
Non-pretreatment	7.1-7.40	29.4-31.9	870-2,505	75-225	69.2±10.2	98.0±7.2	49.0±5.3	48.0±3.2
Alkaline-pretreatment	6.8-7.6	28.6-32.0	840-2,640	42-245	81.2±9.60	199.6±9.0	58.5±6.2	116.8±9.5
Two-stage reactors								
Non-pretreatment	7.2-7.3	30.2-32.1	2,150-3,175[c]	855-1,650[c]	73.3±5.3[c]	152.1±12.1	73.6±1.8[c]	111.9±8.2
Alkali-thermal pretreated	7.2-7.4	30.1-31.7	2,175-3,500[c]	875-1,700[c]	73.4±9.0[c]	272.4±19.6	76.7±2.2[c]	208.9±15.2
Co-digestion								
AcLW: WBS (1:1)	7.0-7.6	28.1-31.4	1,000-3,690	90-2,310	82.5±9.1	261.1±18.0	62.3±6.6	162.7±17.2
Pretreated AcLW: WBS (1:1)	7.0-7.7	28.3-31.6	1,100-3,760	110-2,685	84.2±7.7	402.9±25.9	62.6±2.6	252.2±20.56

Note: a – Alk in the unit of mg/L as $CaCO_3$/l, b – VFA in the unit of mg/l as CH_3COOH, c – averaged value of methane tank.

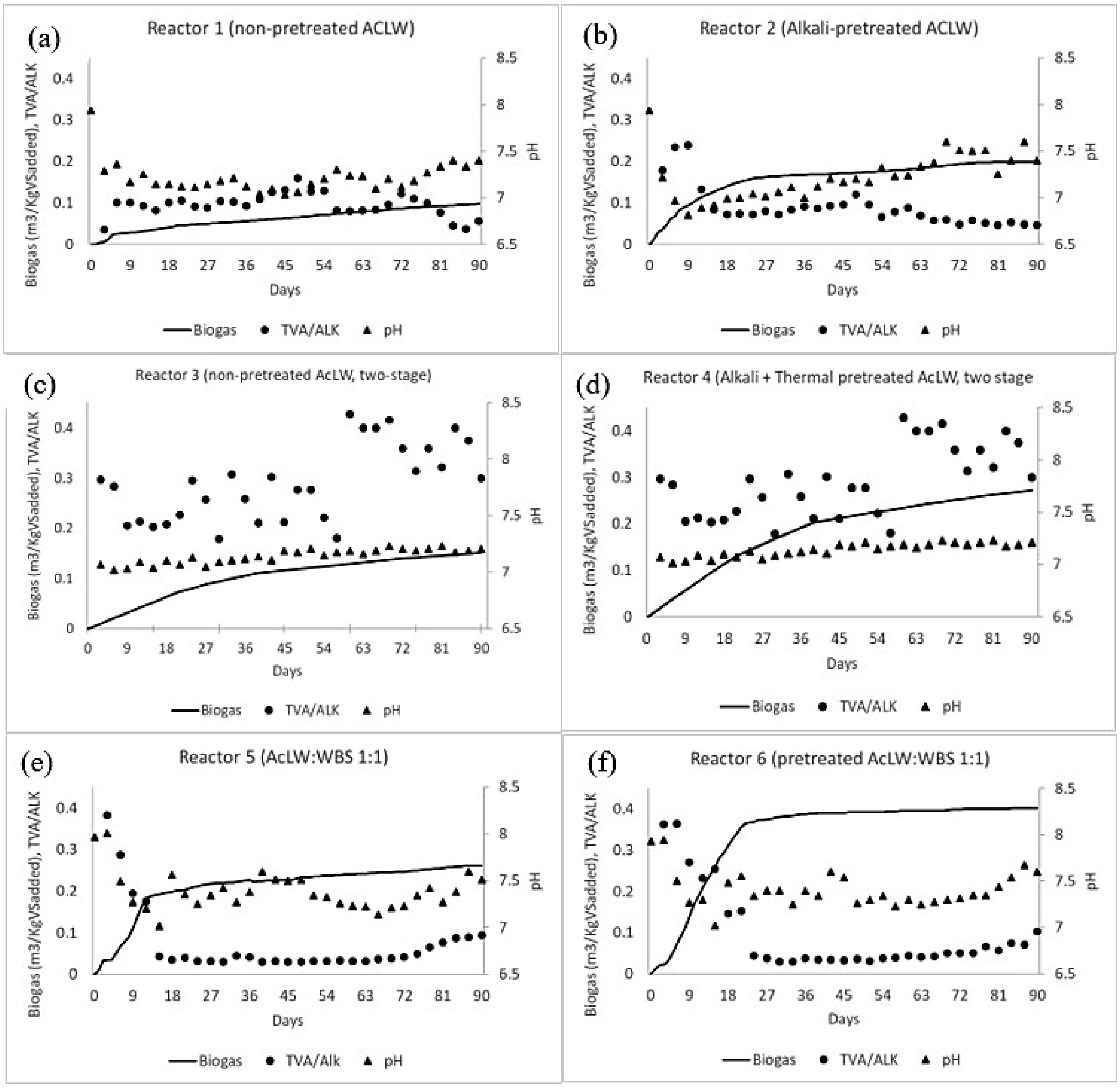

Figure 4. The performance of an aerobic digester (a) non-pretreated AcLW, single stage (b) Alkali-pretreated AcLW, single stage (c) non-pretreated AcLW, two-stage (d) Alkali + Thermal pretreated AcLW, two stage (e) co-digestion AcLW:WBS 1:1, single stage (f) co-digestion pretreated AcLW:WBS 1:1, single stage

same two-stage digester, methane production of alkali-thermal pretreated biomass soaked in 3.0% of NaOH and steamed for 30 min at 121°C increased about 1.86 times or from 111.9 to 208.9 L/kg VS_{added}. The methane composition in produced biogas was increased significantly to 73.6–76.7% compared to non-pretreated and alkali-thermal pretreated AcLW.

The positive effect of phasing was depicted significantly in this study. Compared to a non-pretreated AcLW fermentation in a single-stage CSTR, the methane production was increased by 4.4 times. However, compared to the previous part, pretreatment with 3.0% of NaOH and steamed for 30 min from vial batch experiment also increased the methane productivity by 4.4 times compared to non-pretreated AcLW digestion. Thus, the methane production from pretreated AcLW between the single batch digestion was similar to the two-stage reactor of aw AcLW with a bit differently. For this reason, the use of two separated stage reactors which acidification step as a pretreatment process, was applied correctly. Previously, several studies reported the benefit of a two-stage process. For instance, Lehtomaki and Bjornsson (2006) applied a leach bed reactor to generate the acid from grass silage, sugar beet, and willows leave, while Zhang and Zhang, 1999 used a leach bed reactor for acid-forming from rice straw. This method was suitable for acid-forming from lignocellulosic biomass. The leached water was recirculated. The moisture was increased, and the organic substance could be cumulatively contacted with microorganisms. Thus, it can improve the performance of acid-forming microorganisms in the reactor. From the result, pH and temperature in a single batch and two-stage reactor have not differed significantly. In the acid tank, the acid was monitored and leached until it was stable. Hence, VFA had reached the maximum in the acid-forming condition before fermenting in the methane reactor.

From Table 4, VFA reached a maximum of 1,650-1,700 mg/l CH_3COOH, 7.5 times higher than single batch digestion. Moreover, the range of alkalinity in the two-stage reactor was more comprehensive than in the batch reactor. The lignocellulosic biomass consisted of some complex digestible contents, and it was recirculated and digested more than in a single CSTR. The two-stage application with lignocellulosic biomass can enhance methane and biogas but feed the OLR more than the single batch reactor (0.42 g VS/L.d). For a single-stage reactor, the fed OLR was only 0.13-0.16 g VS/L.d, while in two stages, the feedstock was provided more than the previous only 22.9%. It was more than a single stage for methane composition because the acid-forming microorganism was separated for methane-forming bacteria. Then, methanogens use acetic and hydrogen and carbon dioxides to generate methane as hydrogenotrophic and acetoclastic methanogenesis, respectively (Bassani et al., 2015). For the application of co-digestion in the pulp and paper industry, not only AcLW but WBS from wastewater treatment plants from pulp processing was considered. Waste bio-sludge addition to the same solid condition could enhance biogas production 2.7 times. Moreover, when the AcLW was pretreated with alkaline- and co-digested with WBS, the generated methane was 252.2 $m^3/kg\ VS_{added}$. The methane value increases by 55.6%. In addition, the methane composition increased from single raw material digestion by 13.3%. Table 4 shows the result of the application using AcLW and WBS to generate the biogas on a larger scale. The anaerobic system removed SCOD for 82.5-84.2% and generated biogas and methane with high potential than the natural substrate.

A previous study found that the AcLW co-digested with Napier grass at an equally solid basis could increase biogas production 2.5 times, the methane composition increased from 49.0% to 58.5%, and SCOD removal increased from 69.2% to 80.6% (Chaiyapong and Chavalparit, 2016). Because AcLW is high lignin content biomass, it inhibited an anaerobic digestion process (Liew et al., 2011). Thus, biogas and methane production from AcLW had not high value compared with other substrates such as WBS. After co-digestion, the methane production increased from single substrate digestion 3.4 times, methane composition increased from 49% to 62.3%, and SCOD removal efficiency increased from 69.2 to 82.5% and other digesters. Using the additives' study of Kumar et al. (2013) could help maintain favourable conditions for rapid gas production in the reactor and promote acetogenesis and methanogenesis. Also, non-pretreated AcLW can increase the methane yield from WBS. Moreover, when the AcLW was pretreated, the methane could increase from the pretreatment process. It increased from before pretreatment to 55.56%. The co-substrate between AcLW and WBS is a suitable option for waste management because WBS is waste in the pulp industry. Hence, biogas fermentation between AcLW and WBS can be applied on a larger scale. The reason was to increase the gas quantity from other lignocellulosic wastes in the pulp and paper industry and reduce the industry's waste. Suppose AcLW and WBS can be used as mixed substrates in a biogas plant. It can minimize waste volume and add value to waste in the pulp and paper industry. In this part, the suggested application used two-stage and co-digestion. However, using two-stage was suitable for non-pretreatment conditions. For co-digestion, WBS was presented to co-digest with pretreated AcLW because it can reduce the waste of both AcLW and WBS. In addition, it can generate biogas and methane in high potential biogas and methane. Moreover, the cost of AcLW pretreatment was considerable. This finding demonstrated the biomethanation potential of AcLW and the possibility of enhancing its production via pretreatment and process condition.

Cost-benefit analysis of AcLW biomethanation

Biogas can be used as fuel for steam power generation for bleaching and the chemical recovery processes in the paper industry (Liew et al., 2011). Otherwise, biogas can use substitutionally crude oil fuel to generate electricity used in the industry or sell to grids. The best conditions for the alkali, thermal and alkali thermal pretreatment were selected to compare the cost. The fuel oil and electricity are income—the return of equal biogas electricity and fuel oil from all options, as shown in Figure 5. The cost of pretreatment was considered from alkaline substances and electricity price. The return of biogas as fuel oil was higher than expenses but for electricity generation, but the thermal pretreatment was not still worthy in any case. However, in practice, the cost of the pretreatment process on a larger scale can be reduced because a ton of NaOH would be cheaper, and it can be used more than once. In this case, the cost of 99% of NaOH was 1.5 USD/kg and soaked AcLW 1 kg in a liter of NaOH solution only one time. Moreover, the use of electricity per pretreated raw material in the larger scale of pretreatment would be decreased from the specification of the incubator. In this case, the AcLW was pretreated in steam autoclave for only a kilogram. In this practice, the highest return was from pretreated AcLW biomass with soaking n 3.0% of NaOH for 48 hours regardless of equal electricity or fuel oil without loss. The return value was 135 USD per ton of AcLW during non-pretreated AcLW returned only 92.33 USD. However, when fuel oil demand in the industries was high, it was worth it because of the price of fuel oil. Furthermore, thermal pretreatment can provide more biogas, methane and income consequently. However, the pretreatment time was also an expenditure, which had to be considered. Only alkaline pretreatment takes about 2-3 days, while thermal pretreatment was only 30 minutes.

For using two stages reactor, the capital was energy and operation cost. Although using two steps instead of the single-stage reactor, it was preferred to only non-pretreated AcLW. However, the operation was more complicated and took more operation time. In this experiment, the fermentation time in ABR was 80 days and in CSTR 90 days, while using the batch CSTR reactor was only 90 days. In addition, the expense for reactor and maintenance cost will be double from the single-stage reactor. The returned money from using a two-stage reactor was not different from a single-stage reactor because the expenditure depends on energy cost, and the biogas production from pretreated AcLW between the single and two-stage reactor did not differ significantly. Using co-digestion to enhance biogas production, the income from biogas per ton AcLW increase 5.44 times for electricity and 5.29 times for fuel oil. After pretreated AcLW soaking in NaOH of 3.0% for 48 hours, the revenue from biogas increased by 53.57%. This method is very worthy. The incomes increased, but there was no cost for the WBS. The WBS is the waste in the paper industry and is typically used as fertilizer. Using AcLW and WBS, the waste from the paper industry can be decreased.

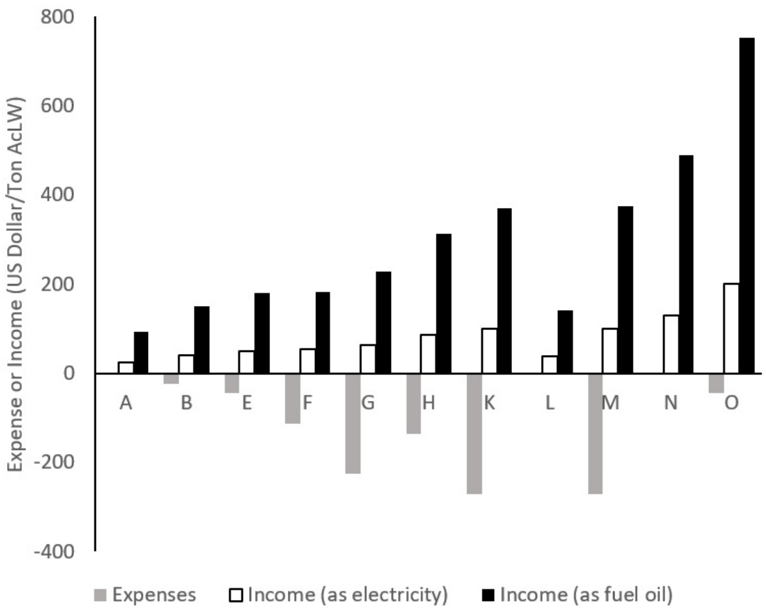

Figure 5. Cost-benefit analysis of AcLW biomethanation

CONCLUSIONS

AcLW is much-generated waste biomass from pulp and paper processing. The value creation of AcLW in anaerobic digestion and its enhancement via different strategies were demonstrated in the study. The limited production of raw AcLW digestion was found to promote raw AcLW digestion. This methane production potential increased significantly after the alkaline pretreatment and co-digestion strategy. The increase of alkaline concentration and soaking time also increased methane productivity, while WBS benefited co-digestion conditions that generated higher methane production. The thermal supplementation during alkaline conditions was the maximized condition of methane production; the increase of gases yield was maximized. It was found that methane production yield was increased when applied pretreatment and other strategies, but the cost of operation also increased. The alkaline pretreatment can return cost benefits whether it is used as electricity or fuel oil. The energy cost-benefit of alkali-thermal pretreatment was not suitable for using biogas for electricity due to low efficiency and energy's price. This finding demonstrated the benefit of pretreatment on methane productivity of AcLW's enhancement, the significantly increased was found the contest benefit simultaneously to be considered.

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