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# Anaerobic digestion of urea pretreated water hyacinth removed from Lake Abaya; bio-methane potential, system stability, and substance conversion

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Water hyacinth (*Eichhornia crassipes*) is characterized as an aquatic plant that can grow very rapidly and freely float over water, and covers the top surface of water. It prevents the penetration of sunlight and reduces nutrients and oxygen from water bodies, and can adversely affect the aquatic ecosystem; however, it can be used in the biogas production as the sole feedstock for anaerobic digestion due to high contents of carbohydrates and biodegradable matter. Thus, this study was aimed to remove water hyacinth from Lake Abaya as a renewable energy resource for biogas production through anaerobic digestion. A lab-scale anaerobic batch reactor was applied to assess its biogas and methane generation potential. The results showed that the total solids of 91.9% at dry basis and volatile solids of 84.82% of TS. The biogas and methane production potential of 474.92 mL g<sup>-1</sup> VS and 213.92 mL g<sup>-1</sup> VS was recorded during 45 days HRT at 37 ± 1 °C. The daily methane production discloses accelerated increment starting from day 8 up to the peak point on day 12 (2170 mL d<sup>-1</sup>). The biochemical conversion of substances in water hyacinth to biogas was recorded as follows: total volatile solids (96.1%), cellulose (92.5%), hemicellulose (88.2%), and chemical oxygen demand (99.3%). Based on results, *E. crassipes* can be the sole feedstock for the anaerobic reactor to produce biomethane, while the effluents can be applied as organic fertilizers.

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## 1. Introduction

Green energy is one of the significant factors to global wealth. The continuous use of fossil fuels as the main energy source has led to worldwide weather change, environmental degradation, and human health problems.<sup>1</sup> The exploration of alternative energy sources is associated with several urgent issues, including energy independence, fluctuation of petroleum fuel costs, the threat of climate change, and the foreseen depletion of non-renewable fuel sources.<sup>2</sup> These resources are draining very fast in most areas where societies have no access to alternative renewable energy sources. There are renewable sources, including wood, plants, dung, falling water, geothermal sources, solar, wind, and wave energy. But each has its own economic, health, and environmental costs, benefits, and risk factors that interact strongly with other governmental and global priorities. Choices must be made, but in the certain knowledge that choosing an energy strategy inevitably means

choosing an environmental strategy.<sup>3</sup> Green energy is often supposed to be the next great technology that will one day replace mankind's dependency on fossil fuels.

Now a day's serious socio-economic and environmental problems are the encroachment of invasive plants, such as water hyacinths, in lakes, ponds, and rivers. Water hyacinth (WH) is an aquatic plant that can grow very rapidly and free-floating over the water's surface. This is due to the leaves and stem containing air-filled sacs, which help them to stay afloat on water. The invasive potential of this plant has created massive environmental problems in many areas. It has shown an extremely high growth rate and the coverage of the whole body of water by forming a thick compact carpet. This thick carpet prevents the penetration of sunlight underwater, and thus the oxygen level in water decreases<sup>4</sup> and numerous problems, including damage of aquatic plants and animals, irrigation problems preventing water transportation, and a breeding place for mosquitoes leading to rise in the mosquito population.<sup>5</sup>

Ethiopia is one of the countries facing the existence of WH in its water bodies.<sup>6</sup> The invasion of water hyacinth on the water bodies in Ethiopia was noticed in 2012 on Lake Tana. Since then, the plant has shown tremendous expansion on Lake Tana.

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Due to its quick expansion, it is becoming a serious challenge for society to get rid of it, which is now not only in Lake Tana, but also in Lake Abaya. Ethiopia is endowed with huge resources of water and wetland ecosystems such as rivers, lakes, swamps, floodplains, and man-made reservoirs.<sup>7</sup> These bodies of water are in danger of being infected by WH due to its rapid propagation if the condition remains unchanged. Attempts to control the weed have caused high costs and labor requirements, leading to nothing but temporary removal of the water hyacinths.<sup>8</sup> Therefore, this leads to the search for another alternative method of water hyacinth management, which can be easily implemented locally.

WH is rich in essential nutrients, nitrogen, and has a high content of fermentable matter and hence the locally grown WH is a potential biomass for biogas production. Apart from biogas, the digestate obtained after AD is rich in macronutrients needed for the healthy growth of plants; hence, it is a useful soil conditioner and manure with no detrimental effects on the environment.<sup>9</sup> Thus, the application of WH as a source of renewable energy to substitute fossil fuels will provide effective management of this invasive plant on water bodies, and in the

meantime, the effluent after AD could be used as a suitable organic fertilizer for plant nourishment. Therefore, this study aims to determine the biomethane potential and substance conversion during anaerobic digestion while analyzing the gradual changes in system stability from the water hyacinth collected from Lake Abaya, Ethiopia.

## 2. Materials and methods

### 2.1 Feedstock and inoculum

**2.1.1 Feedstock.** Water hyacinth was collected locally from Abaya Lake, Ethiopia (Fig. 1). After collection, the water hyacinth was cleaned with tap water 3 times to remove the soil and other unwanted materials. The leaves, stem, and root were taken as the substrate for AD, and then chopped into small sizes of about 2 cm, then the contents were dried to remove humidity under the open air for a period of 7 days; after that, the sample was further dried in an oven at 60 °C for 6 h. In this study, drying of WH removed extra moisture to conduct high solid anaerobic digestion. This oven-dried WH was then ground to fine particles to increase the surface area for subsequent pretreatment using a grinding mill (HYDD Nice 500g Grain Mill

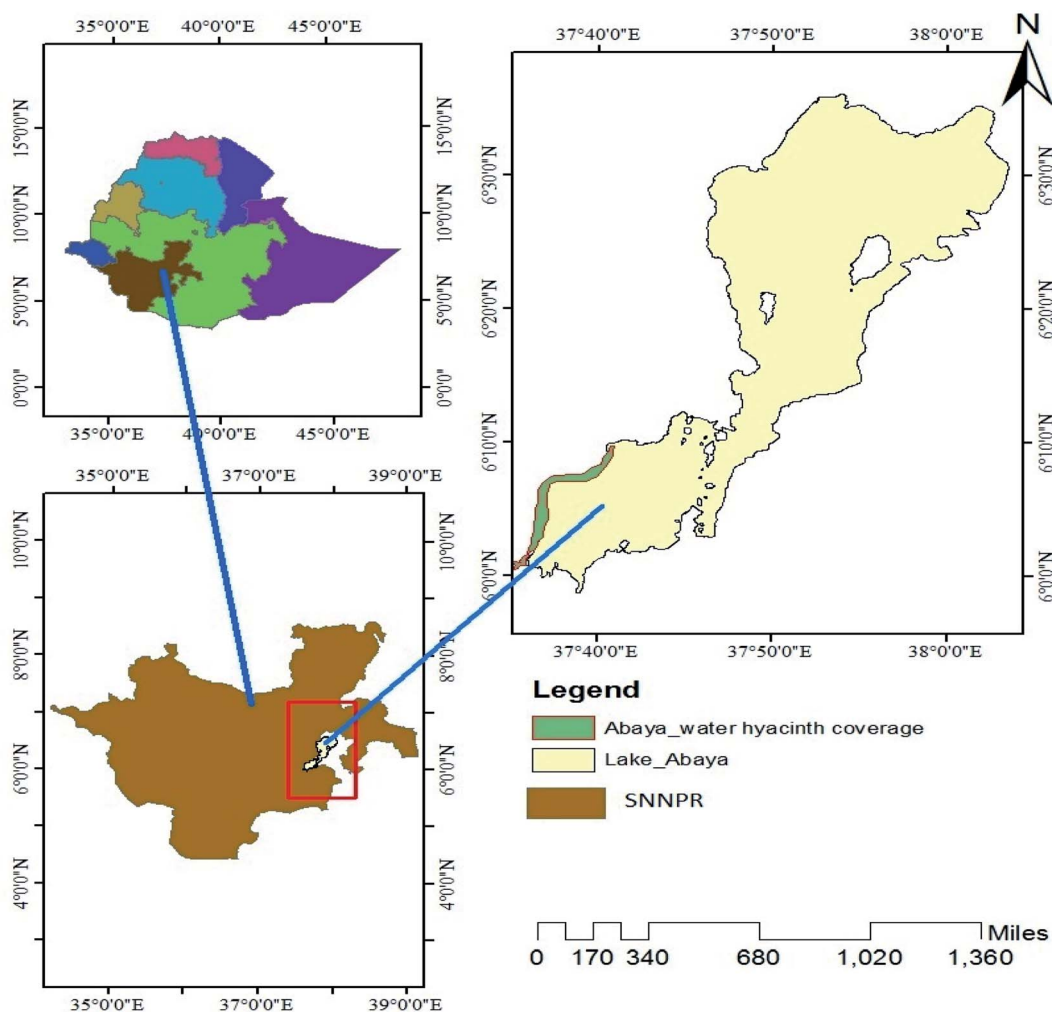


Fig. 1 Location of the study area.



Table 1 The Initial characteristics of the starting materials

Parameters	Water hyacinth	Inoculum
Total solids of dry basis (%)	91.9 ± 0.12	3.0 ± 0.07
Total volatile solids (%)	84.82 ± 13.04	56 ± 0.21
Ash contents (%)	18.49 ± 0.07	0.72 ± 0.04
Total nitrogen contents (%)	1.82 ± 0.32	1.96 ± 1.19
Total organic carbon (TOC)%	37.73 ± 2.00	—
COD (mg L <sup>-1</sup> )	11 780 ± 56.57	182 ± 14.14
pH	—	7.6
Ammonia nitrogen (NH <sub>3</sub> -N), mg L <sup>-1</sup>	767.2	641.2

Grinder 2500W) and passed through a 2 mm sieve.<sup>10</sup> The prepared sample was stored in an airtight plastic bag until next use. The specific characteristics of WH and inoculum are given in Table 1.

Preliminary tests were conducted before doing the main research to know about the optimum pretreatment chemical dose and optimum pretreatment time intervals that would be used. Four preliminary tests were conducted using urea pretreatment applied for hydrolysis of long chained lignocellulosic compounds in water hyacinth. Organic loading of 65 g L<sup>-1</sup> water hyacinth was mixed using distilled water in the ratio of 1 : 10 (w/w) to make a slurry according to the pervious study of ref. 11. Then, the urea solution with a different concentration was added to the slurry to make an optimum concentration for pretreatment ahead of the AD process. This was done by measuring the drop of pH until its changes became approximately constant. This hydrolysis experiment was done using 2%, 3%, 4%, and 6% urea solution based on the dry matter content of water hyacinth. Daily changes in pH were measured to increase the accuracy of preliminary alkaline hydrolysis experimental results. In this study, urea was used as a pretreatment reagent because it has an additional benefit in improving the C/N ratio in AD reactors, increases digestate quality to use as fertilizer, and is less corrosive to the equipment.<sup>12</sup>

Based on the preliminary results of the experiment, the optimum pretreatment time and optimum dose of urea suitable to hydrolyze lignin bond was found to be five days and 4% urea. Accordingly, the required amount of urea was dissolved in distilled water prior to the actual experiment. The solution was then added into different 1.5 L anaerobic digesters and thoroughly mixed with water hyacinth in the prepared digesters. These digesters were then sealed and kept in the laboratory at ambient temperature for five days.

**2.1.2 Inoculum.** The inoculum used as bio starter can facilitate the biogas production process. The anaerobic digestion process is one of the complex processes requiring the presence of several microorganisms. The anaerobic inoculum used for starting up the digesters was obtained from the Wolayita Soddo household level biogas plant, which receives municipal wastewater (20%) and animal manure (80%). The mixed liquor suspended solids (MLSS) of the active anaerobic inoculum was collected and incubated in the water bath at 37 ± 1 °C for 5 days for inoculation and further incubated in the same design condition for 5 days to eliminate the background gas production before seeding to actual reactor.<sup>13</sup>

## 2.2 Experimental design

The experimental design of this study was structured to measure the daily base biogas and methane production in an anaerobic reactor while determining substance conversion in lab-scale batch digesters. Based on a ref. 14, 15 g MLSS L<sup>-1</sup> inoculum was added to start up the anaerobic digestion system. After transferring the substrate, inoculum, and required amount of dilution, the reactor was closed with a thick butyl rubber stopper, which is held in place by sealing with a hot glue gun to maintain the anaerobic condition. The anaerobic digestion of WH was carried out with the glass bottle reactors having 1.5 L total volume, and the active working volume was 80% of the total volume. The remaining volume of the reactors was left empty. The reactor was worked in mesophilic conditions at 37 ± 1 °C and the hydraulic retention time of the AD process was 45 days. The reactors were flushed with N<sub>2</sub> gas for 5 min to create an anaerobic condition inside the digesters, and the digesters were maintained in a temperature-controlled water bath at 37 ± 1 °C. The homogeneity of the substrate was maintained by shaking the reactors manually once per day. The experiment was based on the previous study that showed high mesophilic temperatures had better digestion performance for lignocellulosic substance conversion.<sup>15</sup>

The system was checked for airtightness before starting the experiment in order to prevent the leakage of gas into and out of the reactor system, and it was fed and withdrawn once in the entire reaction period.

Fig. 2 shows the experimental set-up used to measure the volume of biochemical methane potential in the anaerobic reactor through adsorption of CO<sub>2</sub> in alkaline solution; in the reactor, a 1 L graduated measuring cylinder was inverted in the container, and in the intermediate container filled with 0.1 M Ca(OH)<sub>2</sub>, which traps CO<sub>2</sub> to produce CaCO<sub>3</sub> precipitate and allow CH<sub>4</sub> to pass through the glass bottle having a volume of 1.5 L. Ca(OH)<sub>2</sub> was used to trap CO<sub>2</sub> because of its economic feasibility and less corrosive nature for the testing environment.

## 2.3 Analytical methods

The pH and temperature of the substrate and inoculum were measured using Jenway 3510 pH meter, USA and mercury thermometer, Germany, respectively. The analytical procedures for the parameters were based on ref. 16 standard method. The total solids (TS) and total volatile solids (TVS) were gravimetrically determined using the standard method, whereas the measurement of COD was based on the “open reflux, colorimetric method” described in Standard Methods, and also total phosphorous was analyzed using molybdate ascorbic acid method according to ref. 16.

Moisture content in the sample was determined gravimetrically by hot air oven-drying at 80 °C, according to ref. 17.

Moisture content (%) =

$$\frac{\text{masses of moisture sample mass of dry sample}}{\text{masses of dry sample mass of container}} \times 100 \quad (1)$$



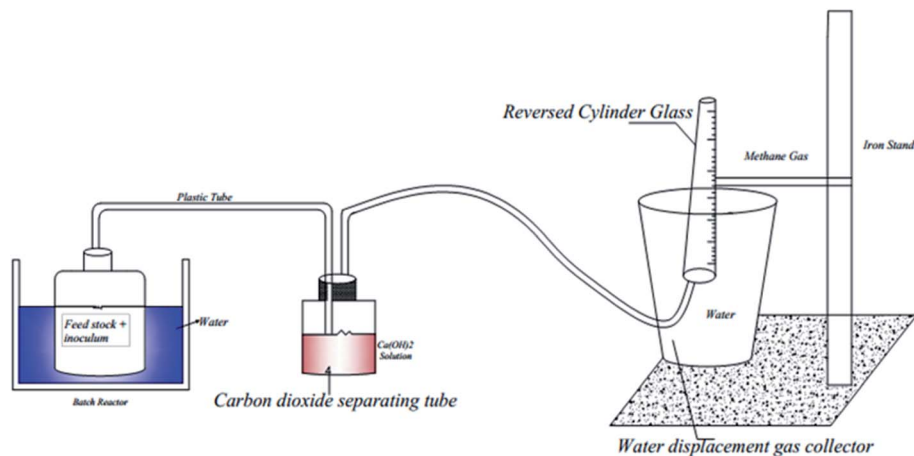


Fig. 2 Experimental set up in a lab-scale anaerobic batch reactor.

Analysis of lignocellulosic components was conducted using the direct method adapted from ref. 18. Two g of sample was taken and boiled with ethanol (4 times) for 15 min, after that thoroughly washed with distilled water, and retained in the oven for dry weight at 40 °C overnight, then allocated into two parts in which one part was considered as fraction "A". The other part of the residue was treated with 24% potassium hydroxide for 4 h at 25 °C, washed thoroughly with distilled water, dried at 80 °C overnight, and the dry weight was taken as fraction "B". 2 g of samples was taken again and treated with 72% H<sub>2</sub>SO<sub>4</sub> for 3 h to hydrolyze the cellulose and refluxed by 5% H<sub>2</sub>SO<sub>4</sub> for 2 h. Distilled water was used to remove H<sub>2</sub>SO<sub>4</sub> by washing it completely, dried at 80 °C in the oven overnight, and the dry weight was taken as fraction "C".

$$\text{Cellulose} = B - C \quad (2)$$

$$\text{Hemicellulose} = A - B \quad (3)$$

$$\text{Lignin} = C \text{ itself} \quad (4)$$

The determination of organic carbon (OC) in the sample was performed using Walkley & Black chromic acid wet oxidation method ref. 19. Total organic carbon content in the sample was determined using eqn (5) as shown below:

Organic carbon (% OC) =

$$\frac{(V_{\text{blank}} - V_{\text{sample}}) \times M \text{ of titrant} \times 0.003 \times 100 \times f}{W} \quad (5)$$

where:  $V_{\text{blank}}$  is taken as the volume of titrant in the blank, mL, whereas  $V_{\text{sample}}$  is taken as the volume of titrant in the sample, mL, while  $M$  = concentration of standardized FeSO<sub>4</sub> or (NH<sub>4</sub>)<sub>2</sub>Fe(SO<sub>4</sub>)<sub>2</sub>·6H<sub>2</sub>O solution, molarity, and 0.003 = carbon oxidized,  $F$  = correction factor, 1.3, and also  $W$  = weight of the sample, g.

Total nitrogen content was determined by digestion with strong sulfuric acid in the presence of a catalyst within Kjeldahl digestion setup, and ammonium nitrogen was distilled.

Ammonium nitrogen was measured using acid titration from the distillate.<sup>20</sup>

The content of crude protein was determined using the modified micro-Kjeldahl method using the formula below ref. 21.

$$\text{Crude protein content (\%)} = \text{micro-Kjeldahl nitrogen content (\%)} \times 6.25 \quad (\text{based on the assumptions that nitrogen contains 16\% of protein}) \quad (6)$$

## 2.4 Biogas measurement techniques

The daily biogas production from each reactor was measured using the liquid displacement method. In this method, the biogas produced is allowed to replace water at an equal volume of water displaced, and this was used to quantify the volume of biochemical methane potential produced daily. The volume of biochemical methane potential was measured using barrier solution through adsorption of CO<sub>2</sub> in alkaline solution as shown in Fig. 2, using the method adapted from ref. 22.

The method consists of allowing the biogas produced to pass through a 0.1 M Ca(OH)<sub>2</sub> solution, which was filled in a separate container in order to capture and convert the CO<sub>2</sub> fraction present in the biogas into CaCO<sub>3</sub>, and then, the measuring cylinder was submerged in a container having the same liquid, inside of the measuring cylinder was attached a plastic tube, which was connected with a separate reactor at the other end through which volumetrically the fraction of methane produced bubbles in the liquid and filled the cylinder, replacing the liquid, and the gas volume can then be read as shown in Fig. 2. Moreover, the cylinder was supported by an iron stand. The amount of liquid replaced corresponds to the volume of methane produced.<sup>23</sup> Furthermore, phenolphthalein solution was added to the barrier solution. Phenolphthalein imparts pink color in alkaline solutions, and a change in color indicates whether Ca(OH)<sub>2</sub> is available to absorb CO<sub>2</sub> or not.<sup>22</sup>

Once the cumulative biogas and methane production were determined, then the composition of biogas was calculated stoichiometrically as CH<sub>4</sub> and CO<sub>2</sub> in liters and converted to normalized volume using standard temperature and pressure





(STP).<sup>24</sup> The CH<sub>4</sub> yield was calculated to measure the volume of methane produced relative to the amount of volatile solids added (VS) based on the formula given in (eqn (5)).

$$\text{Methane yield (mL CH}_4\text{ g VS}^{-1}) = \frac{\text{cumulative volume of methane (mL CH}_4\text{)}}{\text{amount of original volatile solids added (g VS)}} \quad (7)$$

### 3. Results and discussion

#### 3.1 Continuous trends of biogas, bio-methane, and CO<sub>2</sub> production

The primary indicator of the quality of biogas is CH<sub>4</sub> contents, *i.e.*, higher CH<sub>4</sub> content in biogas indicates good quality of biogas produced during AD. In this study, carbon dioxide and the methane contents of the produced biogas were measured daily throughout the digestion period. The biogas composition from WH in this study consisted of 63.2% CH<sub>4</sub> and 36.7% CO<sub>2</sub>, and the methane production potential was higher than reported by a similar study,<sup>25</sup> which were 58% CH<sub>4</sub> and 42% CO<sub>2</sub>. This methane production increment is due to the selection of a suitable pretreatment approach using urea before the actual test, so it shows pretreatment of this substrate is important to improve methane production potential, and also it was comparable with H<sub>2</sub>SO<sub>4</sub> pretreated water hyacinth reported by ref. 26, which is 64.38% of methane.

Mineralization of lignocellulose takes place in an anaerobic digestion process under complex biochemical conditions. In this work, evaluation of the daily performance of the anaerobic reactor showed that there were irregularities in process routines, which caused fluctuating biogas production trends. Anaerobic digestion of urea pretreated water hyacinth at mesophilic conditions (37 ± 1 °C) was performed to assess continuous gas production trends and related featuring objects during the process. Fig. 3 shows daily biogas production, and Fig. 4 shows cumulative biogas yield of water hyacinth.

The daily biogas production fluctuates during the entire digestion period, and the system achieves biogas production in between two major peak points. The daily biogas production showed rapid increment on the first day to the 5<sup>th</sup> day and then

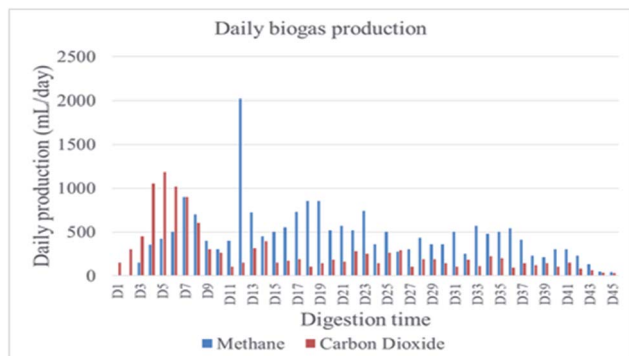


Fig. 3 The average daily volume of biogas and methane gas generated from day 1 to day 45 at STP. The experiment was conducted at 37 °C.

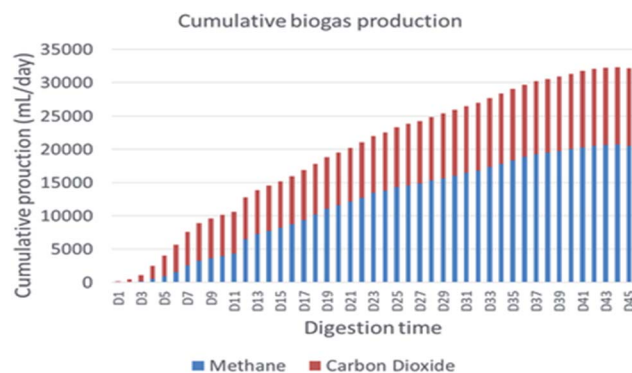


Fig. 4 Cumulative biogas production from urea pretreated WH.

reached a minor peak point on the 7<sup>th</sup> day. The change continued, and it decreased from the 8<sup>th</sup> day to the 11<sup>th</sup> day before it reached a major peak point on the 12<sup>th</sup> day, and then it was gradually decreased up to a stable point. More specifically, the first minor peak biogas production (1800 ± 28.28 mL d<sup>-1</sup>) was recorded on the 7<sup>th</sup> day of the anaerobic digestion period. However, the reactor performance directly came down after 4 days to the biogas production volume of 500 ± 70.71 mL d<sup>-1</sup> on day 11. The good thing was the process recovered quickly from the shocking decrease in biogas production and raised the production up to the major peak point (2170 ± 28.28 mL d<sup>-1</sup>) of daily biogas production on day 12. The daily methane yield followed the same style in the parallel way like daily biogas production except in the first six days, where the methane production was very slow compared to the rest of the digestion periods. Carbon dioxide is the 2<sup>nd</sup> abundant gas constituent in biogas produced from AD of lignocellulose.<sup>27</sup> In the present study, the composition of CO<sub>2</sub> dominated in the initial stage of digestion periods to reach the highest value on day five (1180 mL d<sup>-1</sup>), but after day 6, the CO<sub>2</sub> concentration gradually decreased in biogas and became relatively constant after the 35<sup>th</sup> day of digestion time.

The cumulative biogas production within the digestion period is shown in Fig. 4. Thus, the biogas production was continued up to 45 days. The total volume of methane gas production within 45 days' digestion period was 12 000 mL at STP. Whereas the corrected cumulative biogas yield of the experiment was 474.92 (mL g<sup>-1</sup> VS<sup>-1</sup>), while the cumulative methane potential yield of the experiment was 213.92 (mL g<sup>-1</sup> VS<sup>-1</sup>). This result was comparable with a similar study reported by ref. 28, and it was done in the Silpakorn University of Thailand where biogas and methane yields (458.44 L kg<sup>-1</sup> of VS and 237.37 L kg<sup>-1</sup> VS, respectively) and the components of CH<sub>4</sub> and CO<sub>2</sub> were 63.2% and 36.7%, respectively, from water hyacinth. The percentage composition of this study is comparable with a similar study reported by ref. 29 with the composition of CH<sub>4</sub> and CO<sub>2</sub> (68.89% and 29.08%, respectively).

#### 3.2 Evaluating system sensitivity during anaerobic digestion period

**3.2.1 pH, total alkalinity, and ammonia.** The system sensitivity parameters such as pH, total alkalinity, and



ammonia-nitrogen concentration are very essential for maintaining sustainable and stable performance during the AD process. Every fifth day of anaerobic digestion, one reactor was discarded for laboratory analysis of sensitive parameters. The total alkalinity, pH, and ammonia-nitrogen of the sample were investigated.

pH is one of the significant parameters used to evaluate the performance of anaerobic microorganisms during the process of bio-methane production. There are different kinds of microbial groups taking parts in the AD process, which have varied optimum pH values for their optimal-growth rates. For example, a pH range of 5.0 to 6.0 is suitable for acidogens, while pH from 6.5 to 8.5 is more convenient for the methanogens group.<sup>30</sup> In most cases, pH value generally drops after a few days of microbial incubation. The pH of WH was in the acidic range, whereas inoculum was in the neutral range, so the pH of the substrate was adjusted to the optimum range before AD with 1 N Ca(OH)<sub>2</sub> solution.

In this study, pH of the water hyacinth at the initial stage was 5.91, so the initial pH was adjusted before AD by 1 N Ca(OH)<sub>2</sub> solution. There was a partial change in the pH value during the digestion period, at the initial AD period, which was in the range of 7.0–7.2. To investigate the system stability, nine additional reactors were arranged in the same condition to check the system sensitivity every five days, discarding one reactor and measuring the sensitive parameters throughout the digestion period.

In the beginning, a slight decrement in pH was shown but the system sustained its stability for methane conversion, and the pH at the end of AD was 7.9. This might be the influence of inoculum and urea pretreatment that played significant roles in sustaining the pH-buffering of the reactor without the addition of alkali to the reactor.

The alkalinity of the anaerobic reactor can be defined as the tendency of the intermediate solutions to maintain stable buffer capacity caused by existing organic acids.<sup>31</sup> To achieve the constancy and neutralize VFAs, the total alkalinity of the system must be higher than 2000 mg L<sup>-1</sup>,<sup>32</sup> unless it could affect the action of microbial groups during methane production. In this study, the alkalinity of the system at the beginning was 2200 mg L<sup>-1</sup>, and it was in the range, but it fluctuated during the digestion period, as shown in Fig. 5.

The optimum content of the ammonium ion (as well the ammonia) inside the reactor has a great role in the stability of the AD process. The optimal ammonia concentration increases the stability process by ensuring adequate buffer capacity of the methanogenic medium.<sup>33</sup> Due to the use of urea as a pretreatment chemical, it buffers pH drops during digestion periods. In this study, ammonia concentration before AD was 762.5 mg L<sup>-1</sup>, and it fluctuated during the digestion period and after AD was 785.8 mg L<sup>-1</sup>, as shown in Fig. 5.

### 3.3 Characteristics of raw and treated water hyacinth

In this section, the specific characteristics of the starting materials based on dry matter of water hyacinth, inoculum, and the digestate after AD are presented, and then the substance

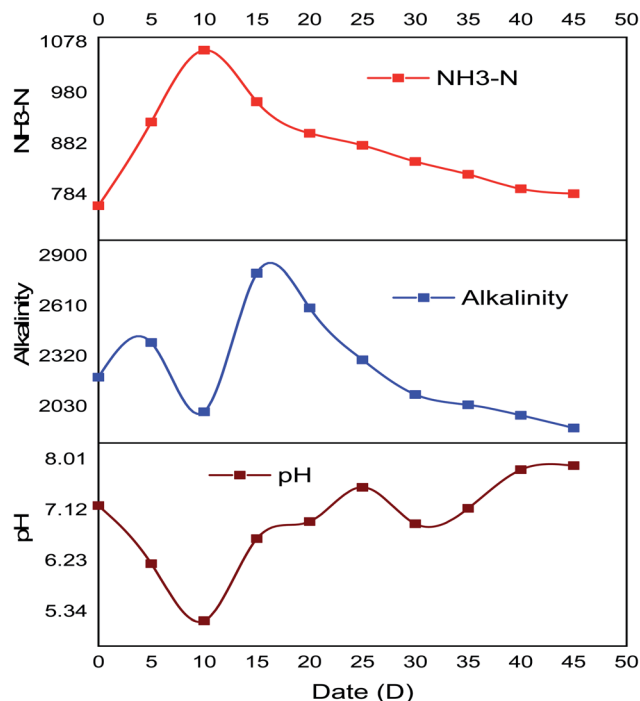


Fig. 5 System stability and sensitivity indicating parameters measured every five days during anaerobic digestion periods.

conversion efficiency of the digestion process is discussed. The compositional change of substances in WH before and after urea showed significant changes (Table 4), which indicates the effectiveness of urea pretreatment for the substance degradation.

The total solid (TS) and volatile solid (VS) at dry basis of WH and inoculum were 91.9% and 84.82%; and 3.0% and 56%, respectively. Furthermore, the detailed properties of the starting materials are shown in Tables 1 and 2.

The initial physicochemical analysis of the inoculum and substrate are important factors to be analyzed as it influences the overall anaerobic system stability and biogas production.

The high contents of volatile solids and COD of water hyacinth indicate that water hyacinth is favorable for the AD process to biogas production.

**3.3.1 Physicochemical composition of WH.** The composition of water contents in the water hyacinth, physico-chemical and structural composition are given in Table 2.

Carbon/nitrogen (C/N) is an essential parameter that determines biogas production. The C/N ratio should be in the range of 15 to 30 for biogas production operating at full potential. The carbon/nitrogen ratio of WH in this study was 20.73, and it was found to be in the optimal range for bio-methane production as recommended by ref. 34. This shows that WH collected from Lake Abaya can serve as a sole feedstock for bio-methane production without co-digestion or mixing it with other substrates.

The results showed that the total solids and total volatile solids of the WH were  $91.9 \pm 0.12$  and  $84.82 \pm 13.04\%$ , respectively. The volatile solid content was higher than the



Table 2 Comparison of physiochemical characteristics of WH with available literature values

Parameter	Water hyacinth	Literature results	Reported by
Moisture content (%)	89.31 $\pm$ 0.12	90.00 $\pm$ 5%	41
Dry matter (DM) (%)	8.81 $\pm$ 0.14	8.56	26
Total solids of dry basis (%)	91.90 $\pm$ 0.12		
Total volatile solids (%) as percentage of TS	84.82 $\pm$ 13.04	89.13%	37
Ash content (%) as percentage of TS	18.49 $\pm$ 0.07	20.2	46
pH	5.91 $\pm$ 1.00	5.80	41
Cellulose (%)	25.39 $\pm$ 0.73	24	47
Hemicellulose (%)	26.89 $\pm$ 2.51	30	47
Lignin (%)	19.05 $\pm$ 2.64	16	47
Total organic carbon (%)	37.73 $\pm$ 2.00	38.4	47
C/N	20.73 $\pm$ 1.20	25	48
Crude protein contents (%)	11.38 $\pm$ 0.70	11.8%	49
Food to microbe ratio, TVS (g kg <sup>-1</sup> )/inoculum (TVS g kg <sup>-1</sup> )	1.51 $\pm$ 0.12	1.5	50
Sodium (%)	0.78 $\pm$ 0.01	0.58 $\pm$ 0.40	18
Potassium (%)	2.60 $\pm$ 0.02	2.78	47
Calcium (mg L <sup>-1</sup> )	32.10 $\pm$ 2.20	—	—
Magnesium (mg L <sup>-1</sup> )	48.10 $\pm$ 4.21	—	—
Chloride (mg L <sup>-1</sup> )	728.74 $\pm$ 5.89	—	—
Total alkalinity as CaCO <sub>3</sub> (mg L <sup>-1</sup> )	2200.00 $\pm$ 56.50	—	—
Phosphate (PO <sub>4</sub> <sup>3-</sup> ) (%)	12.00 $\pm$ 0.02	15.07	46
Nitrate-nitrogen (NO <sub>3</sub> <sup>-</sup> -N) (%)	0.37 $\pm$ 0.01	—	—
COD (mg L <sup>-1</sup> )	11 780 $\pm$ 56.57	15 942	29

biodegradable range of 75–80% stated by ref. 35. Volatile matter (VM) is the most flammable biomass component.<sup>36</sup> This indicates that large content of WH was biodegradable during the AD process, and hence it can serve as an important substrate for bio-methane production. The content of total solids and VS in WH is comparable with a similar study reported by ref. 37, which is 7% and 89.13%, respectively. The contents of total solids and VS of rice straw were 93.90% and 84.31%, respectively, as reported by ref. 38. The ash content of this biomass is the second largest content with the value of 18.49  $\pm$  0.07% in this study. The ash content is influenced by the inorganic elements, which are part of biomass.<sup>39</sup> The result obtained in this study was comparable with a similar study reported by ref. 40, which is 20.1%.

Physically, water hyacinth is rich in terms of moisture content. In this study, the moisture content was 89.31  $\pm$  0.12% of its total solids, and it is comparable with a similar study reported by ref. 41, which is 90  $\pm$  5%.

The components of lignocellulosic compounds in WH (lignin, hemicellulose, and cellulose) were measured based on the direct method. From the result, the hemicellulose content is 26.89  $\pm$  2.51%, and hemicellulose is associated with cellulose and contributes to the structural component of the biomass, and cellulose content from the result is 25.39  $\pm$  0.73%. Cellulose is the main structural component in the cell wall, which shows that WH has a good potentiality for biogas production, and it is comparable with a similar study reported by ref. 25, in which the hemicellulose content is 30%, and cellulose content is 24%.

### 3.4 Substance conversion

The anaerobic digestion process increases the consumption of the feedstock for their metabolic process and development,

which decreases the main components of the substrate. The overall removal rates of VS and COD of WH after AD are shown in Table 3. The total solids in the substrate and in the digestate were 91.9% and 4.3%, respectively. The removal efficiency of total solids was 95.3%. The volatile solids in the substrate and in the digestate were 84.82% and 3.3%, respectively. The removal efficiency of volatile solids was 96.1%.

The removal efficiency of total volatile solids and COD is an essential parameter to determine the stability and efficiency of the AD system. Normally after hydrolysis, cellulose, and hemicellulose of the lignocellulosic substrate are converted into lower molecular mass organic compounds or COD (usually into simple soluble sugars). The higher the removal, the higher the

Table 3 Overall conversion efficiency of main components of water hyacinth to biogas

Parameter	After AD	% removal efficiency
Total solids (%)	4.30 $\pm$ 0.06	95.3
Total volatile solids (%)	3.30 $\pm$ 0.11	96.1
Cellulose (%)	1.91 $\pm$ 0.09	92.5
Hemicellulose (%)	3.17 $\pm$ 0.66	88.2
Lignin (%)	16.59 $\pm$ 2.11	12.9
Crude protein contents (%)	0.16 $\pm$ 0.02	98.6
COD (mg L <sup>-1</sup> )	82.00 $\pm$ 1.22	99.3
Phosphate, PO <sub>4</sub> <sup>3-</sup> (%)	5.00 $\pm$ 2.02	58.3
Nitrate-nitrogen, NO <sub>3</sub> <sup>-</sup> -N (%)	0.23 $\pm$ 0.01	37.8
Calcium (mg L <sup>-1</sup> )	16.03 $\pm$ 3.11	50.1
Magnesium (mg L <sup>-1</sup> )	1.64 $\pm$ 0.24	96.6
Potassium (%)	2.10 $\pm$ 0.2	19.2
Chloride (mg L <sup>-1</sup> )	339.40 $\pm$ 44.00	53.4
Total alkalinity as CaCO <sub>3</sub> (mg L <sup>-1</sup> )	420.00 $\pm$ 11.02	80.9
Ammonia nitrogen (NH <sub>3</sub> -N) (mg L <sup>-1</sup> )	641.20 $\pm$ 12.03	16.4
pH	7.89 $\pm$ 0.01	—



**Table 4** The compositional changes of WH before and after urea pretreatment

Lignocellulose components	Before pretreatment	After pretreatment
Cellulose	25.39 ± 0.73	23.41 ± 0.42
Hemicellulose	26.89 ± 2.51	22.67 ± 0.91
Lignin	19.05 ± 2.64	14.24 ± 1.1

stability of the system.<sup>41</sup> In this study, total volatile solid and COD removal efficiencies were 96.1% and 99.3%, respectively. It is indicated that the higher removal rate of COD/TVS had higher methane production.<sup>30</sup>

The main components of carbonaceous compounds in water hyacinth are cellulose, hemicellulose, and lignin. The effectiveness of the pretreatment applied and AD performance can be evaluated based on the rate of lignocellulose degradation.<sup>42</sup> But the hydrolysis and cellulose crystallinity was basically hindered by an unmanageable lignin network. The differences in lignocellulose concentration before and after AD are given in Table 3. The total composition of lignocellulose in water hyacinth was 71.33%. Out of this value, hemicellulose, cellulose, and lignin accounted for 26.89%, 25.39%, and 19.05%, respectively. However, the bio-methane production would be more affected by the accessibility and degradability of hemicellulose, cellulose, and the extent of lignin inter cross-linkage. In the present study, the conversion rate of hemicellulose, cellulose, and lignin were 88.2%, 92.5%, and 12.9%, respectively. The substance conversion efficiency of AD of WH was calculated as per the equation below:

$$\% \text{ removal efficiency} = \frac{(C_{\text{inf}} - C_{\text{eff}})}{C_{\text{inf}}} \times 100 \quad (8)$$

where,  $C_{\text{inf}}$  = concentration of the raw influent,  $C_{\text{eff}}$  = concentration of the material after anaerobic treatment system.

### 3.5 Physicochemical characterization of digestate

One of the advantages of anaerobic digestion was the use of digestate as organic fertilizer. As a result, the pH and macronutrients in the digestate from reactors were determined. The pH of the WH digestate after AD was found to be 7.89. The pH of the digestate was between the minimum and maximum accepted values of FAO standard for plant growth, which are 6.0 and 8.5, respectively.

**3.5.1 Nutrient composition of the digestate.** Water hyacinth is considered as one of the fastest invading water weeds and depletes nutrients rapidly from water bodies since anaerobic digestion only removes organics, and the main mineral material and almost all nutrients remain in the slurry. The chemical components of the WH depend on the nature of water bodies where it is collected. Almost 100% of the phosphorus and about 50 to 70% of the nitrogen as ammonium was still found in the digested slurry.<sup>43</sup> Following the completion of gas production, the nitrate, phosphorous, and potassium of the digestate were determined. Nitrogen, phosphorus, and potassium are the most required nutrients for plants. Nitrate,

phosphorus, and potassium contents in the digestate were 0.23%, 5%, and 2.103%, respectively, so these can be considered as an alternative to chemical fertilizer. The results show that WH manure had the highest P and K than other forms of manure like cattle manure and green manure, which are 0.15% and 0.78%, respectively.<sup>44</sup> Bio-fertilizers are soil conditioners containing the minimum contents of nitrogen, phosphate, and potash (NPK) that is derived solely from the leftovers or derivatives of an organism.<sup>44</sup>

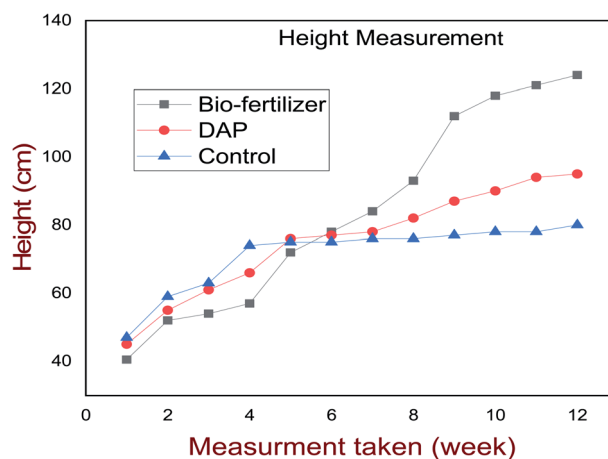
All major plant nutrients such as nitrogen, phosphorous, and potassium were retained in the reactor during the AD process. The previous study conducted by ref. 45 has shown that the digestate of WH can be taken as a bio-fertilizer when incorporated into the soil, which increases the performance of plant growth. With high nutrient content, the digestate from AD was considered to be an effective alternative fertilizer and environmentally friendly relating to chemical fertilizer.<sup>29</sup>

### 3.6 Bio-fertilizer quality evaluation

Determination of plant responses using WH digestate as bio-fertilizer was managed sideways with the diammonium phosphate (DAP) to tomato vegetable planted in pots and control for comparison.

Three different groups were designed, and these were replicated ten times, giving a total of thirty experimental groups. Replication was done to increase the accuracy of the experiment.<sup>43</sup> The tomato crop was planted using digestate as organic fertilizer, inorganic fertilizer (DAP), and control. The control treatment was without both bio-fertilizer and DAP application. The performance of water hyacinth as a bio-fertilizer was compared to the chemical fertilizer (DAP). The indicators of physical appearance performance were studied like plant height, the yield of fruit at maturation, and weight of fruits per plant (kg).

The height of the plant increased consistently from week one to week twelve for all the treatments, as shown in Fig. 6. Even though the control had shown the highest increment in height



**Fig. 6** Comparison of the growth performance among bio-fertilizer, DAP, and control on tomato height.





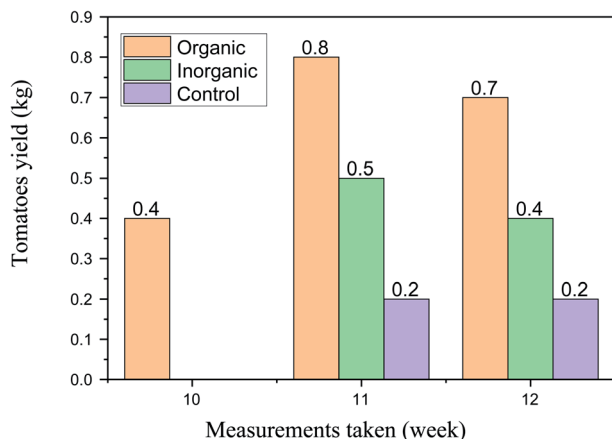


Fig. 7 Mean tomato production yields (kg) at different harvesting times.



Fig. 8 Experimental pots with tomato plants after ten weeks: (A) bio-fertilizer and (B) inorganic fertilizer.

within four weeks, but the stem was very thin and had a probability of breaking compared with both treatments, having an average height of 80 cm. After week four, the treatment (using bio-fertilizer) had shown an increment in growth stage up to 124 cm, and it proved to be more effective with high strength, branched, and high yield than using chemical fertilizer. Application of bio-fertilizer statistically led to improvement of the plant height and strength.

The harvesting stage of the tomato plant started in the 10<sup>th</sup> week. The average yield of the plant treated by bio-fertilizer per plant was 0.4 kg, whereas the yield of the plant treated with DAP and control started in week eleven. In week eleven, the yields treated with bio-fertilizer, DAP, and neutral were 0.8, 0.5, and 0.2 kg, respectively, per plant and also in week twelve, the yields treated with bio-fertilizer, DAP, and neutral were 0.7, 0.4, and 0.2, respectively, as shown in Fig. 7.

In general, the result related to physical appearance (Fig. 8) and the product yield showed that the application of digestate from water hyacinth significantly influenced the plant height and production yield among the treatment performance of tomato plants in all the parameters evaluated. In general, the performance of the crops using digestate was very good.

## 4. Conclusions

Based on the results of this study, it can be concluded that urea pretreated water hyacinth collected from Lake Abaya can be

applied as a suitable substrate for anaerobic digestion. The water hyacinth pretreated with 4% urea and solid loading rate of 65 g L<sup>-1</sup> of total solid produced cumulative biogas yield of 474.92 mL g<sup>-1</sup> VS and cumulative methane yield of 213.92 mL g<sup>-1</sup> VS, whereas biogas composition from WH in this study consist of 63.2% CH<sub>4</sub> and 36.7% CO<sub>2</sub>. From the result, the volatile solid content of the WH was 84.82% of TS. The process of anaerobic digestion has converted a significant amount of the raw materials (96.1% of TS and 99.3% of COD) into biogas. Additionally, the digestate used as organic fertilizer produced a higher yield of tomatoes than the inorganic fertilizer. Therefore, this study tried to solve the water hyacinth management problem in Lake Abaya as well as produce renewable sources of energy in the form of biogas and soil-friendly bio-fertilizer.

## Conflicts of interest

There are no conflicts to declare.

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