Biogas Production from Anaerobic Co-digestion of Water Hyacinth (Eichhornia crassipes) and Cow Manure

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Authors' contributions

This work was carried out in collaboration between both authors. Author KD supervised the study, wrote the final draft and approved the final manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Biogas production from anaerobic co-digestion of local, low-cost organic materials. Co-digestion of Water Hyacinth (Eichhornia crassipes) and cow manure, was performed with a designed laboratory anaerobic fermentation system at 39°C ± 2°C. Co-digestion was conducted with 100% cow manure as a basis, and cow manure and water hyacinth ratios of 25:75%, 50:50%, and 25:75% for complete water hyacinth plants, water hyacinth roots and water hyacinth leaves fractions.

Biogas production per gram volatile solid for anaerobic digested cow manure was between 134 ml to 355.59 ml.

Co-digestion of cow manure and water hyacinth showed the highest average biogas production per gram volatile solids for complete water hyacinth and water hyacinth root mixture ratios of 25% cow manure and 75% of water hyacinth and water hyacinth roots yielding 273.01 ml and 462.63 ml respectively.

The least biogas per gram volatile solids produced during co-fermentation was for complete water hyacinth and water hyacinth root with a ratio of 75% cow manure and 25% water hyacinth and water hyacinth root, yielding 163.77 ml and 250.28 ml respectively.

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Water hyacinth leave mixture at 50% cow manure and 50% water hyacinth leaves had the lowest average biogas production of 172.54 ml per gram volatile solids. The highest biogas production of 283.55 ml per gram volatile solids was achieved for a mixture of 75% cow manure and 25% water hyacinth leaves.
The biogas composition without CO₂ showed a biogas content for the cow manure between 54 and 65%.
The application of co-digestion utilizing cow manure and water hyacinth as a feedstock could help minimize the negative environmental impact of water hyacinth and help to restore biodiversity, water quality and habitat of infested sites.

Keywords: Anaerobic digestion; biogas; co-digestion; cow manure; energy production; fermentation; water hyacinth.

1. INTRODUCTION

New energy sources for production of electricity, heat and fuel are important for the social and economic growth today.

Of the various energy sources, fossil fuels were used to produce 82% of the global total primary energy supply in 2014 [1]. Furthermore, the U.S. Energy Information Administration projected a 28% increase in world energy consumption between 2015 and 2040 and more than three-quarters of the global energy consumption in this projection was expected to come from fossil fuels [2].

Despite this projected increase, there are many drawbacks to the world’s reliance on fossil fuels. The primary drawback of depending on fossil fuels is the processes of procuring, transporting, refining, andcombusting them each generate pollution by-products, which in turn generate a plethora of problems [3]. Therefore, finding sustainable resources for the production of energy is one of the priorities of the 21st century [4].

Biogas is an alternative, carbon-neutral, renewable fuel that can be easily generated from local, low-cost organic materials [5-9]. Biogas can be used to generate heat orelectricity or be used as a transportation fuel [5,6,10]. It is competitive with other alternative energy sources in terms of efficiency and minimal environmental impact [5]. Biogas is produced from anaerobic digestion (AD), which is a biological process in which microorganisms naturally degrade organic material in the absence of free oxygen [6,11,12]. Some examples of common AD feed stocks are food waste, yard waste, animal manures, food waste, and wastewater solids [13]. Thus, another major benefit to utilizing AD is sustainable waste management [7,14,15].

A variety of feedstocks can be utilized by AD technologies to generate biogas [7]. Common AD feedstocks include energy crops, plant biomass, agricultural residues such as animal manure and crop residues, industrial residues particularly from the food and beverage industries, wastewater treatment and sewage sludge, municipal organic waste, bio waste such as that from paper processing, and other organic waste sources [6,11,15,16].

To increase organic content, biogas yield and ultimately improve the performance of any anaerobic digester different substrates can be combined and co-digested [5,6,7,17]. Some organic wastes that are commonly co-digested include food wastes, municipal bio wastes, and/or agriculture-related industrial wastes (Achinas, Achinas, & Euverink, 2017). The types of substrates being co-digested determines the biogas composition and biogas yield [18]. The biogas and methane production and yield from the anaerobic co-digestion of various biological wastes has been assessed through several studies.

Water Hyacinths (WH) (Eichhornia crassipes) are a species of free-floating, vascular, invasive aquatic plant that grow very quickly [19,20,21]. The biodiversity, water quality, and habitat of infested sites are threatened by these pest plants [21,22]. In addition, WH increase evaporation rates and mosquito breeding sites and interfere with swimming, boating, and other recreational activities [19,22].

However, their ability to fix nutrients and grow rapidly in nutrient-rich waters also makes them a method for treating wastewater [19,24,25]. In addition to showing potential in water treatment, these plants are also excellent AD feedstock candidates because they consist of fermentable matter, nitrogen, and other nutrients [21,22].
Therefore, controlling and harvesting WHs would be a means to control their population while providing wastewater treatment and generating an AD feedstock [22,21,25]. Numerous studies have examined the amount of biogas and methane generated from the AD of WH.

WH using a pilot-scale batch reactor and 18 100-mL Biological Methane Potential (BMP) tests [22]. The BMP tests were performed using dry and wet WHs and the following mediums: water, a BMP nutrient solution, and a water and manure mixture. The pilot-scale batch reactor test yielded 267 L biogas/kg VS, with the biogas consisting of approximately 50% methane. The small-scale BMP tests revealed low variability in the methane production independent of media or additives used. The biogas generated during these tests was not significantly higher for those containing manure nor nutrient medium. Additionally, drying hindered the anaerobic degradability of the WH.

Patil et al. explored the AD of WH at mesophilic conditions using poultry litter as an inoculum [21]. A series of batch-mode digestions were carried out in 0.25 L. The reactor containing 4.0 g of WHs, 100 g of water, and 0.3 mL of acetic acid yielded 230 L of biogas/kg VS, whereas the reactor containing 4.0 g of WHs, 25 g of water, 75 grams of poultry litter inoculum, and 0.3 mL of acetic acid produced 480 L of biogas/kg VS.

Moorhead and Nordstedt performed AD on WHs in a batch reactor at 35°C under different conditions [25]. The goal of this study was to compare the effect of WH particle size, WH nitrogen content, and inoculation volume on the AD process. WH particles of size 6.4 mm yielded the greatest biogas production rate. At 15 days, plants with high nitrogen content showed increased biogas production rates as inoculum volume increased. The study also showed that after 60 days the biogas and methane accumulations were similar, independent of inoculum volume, particle size, and nitrogen content. The range of biogas yields was 200 to 280 L/kg VS.

A study by O’Sullivan et al. examined the AD of Today animal manure is used for AD commonly as feedstock and inoculum or co substrate in mono-digestive processes of waste biomass that contains undigested material which still can be degraded by microorganisms contained in the in the manure inoculum or co-substrate [26].

Cow manure (CM) in specific degrades naturally, leading to the emission of methane and carbon dioxide, which are greenhouse gases [6,12,17]. This makes CM as a ideal substrate as inoculum and co-digestate.

A variety of substrates have been studied for use in co-digestion with animal manure. Manures from dairy cows and other cattle, pigs, chickens and other poultry, llamas, horses, goats, and sheep have been documented in numerous studies and reviews as both substrates and inoculum sources [12,27-31].

WH as co-substrate was used by Yusuf and Ify co-digested wastepaper, CM, and WH an aerobically [30]. Using 5 different digesters at room temperature, they only varid the amount wastepaper feedstock and measured the resulting biogas yield. The presence of wastepaper in the digestion improved the biogas yield but the biogas yields also decreased as the quantity of wastepaper increased. A first order kinetic model was generated for each digester to estimate the maximum biogas yield generated from each set of biomass feedstock. Based on these results, 0.5% wastepaper was assumed to generate the maximum biogas yield, producing 340 L biogas/kg VS and 204 L methane/kg VS.

Priya et al. examined (i) the co-digestion of WH with food waste and (ii) the co-digestion of WH with waste activated sludge (WAS) derived from sewage treatment [24]. The WH were combined with food waste in a 1:1 ratio and WH were also combined with WAS in a 1:1 ratio. These mixtures were generated in triplicate and each mixture was inoculated with a seed inoculum. Tests were performed in 1000 mL batch reactors simultaneously. The WH and WAS generated 148 ± 5 L biogas/kg VS while the WH and food waste generated 394.6 ± 12 L biogas/kg VS. This result could be due to the WH and food waste mixture having a higher solids content.

Abdoli et al. investigated the co-digestion of cow dung and maize waste at two different ratios; the cow dung/maize waste ratios were 10:1 and 10:5 [5]. The digestion took place in a 5 L batch reactor operated under mesophilic conditions (36 ± 1°C). The 10:1 ratio yielded 250 L biogas/kg VS and 130 L methane/g VS whereas the 10:5 ratio yielded 480 L biogas/kg VS and 300 L methane/kg VS.

In this research project we are specifically interested in evaluating anaerobic digestion of
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Water Hyacinth (WH) as AD feedstock using CM as an inoculum and co-substrate for mono anaerobic digestion under mesophilic conditions in a single stage batch system, as many dairy farms today operate a single stage system due to investment cost needed [4].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Water Hyacinth (*Eichhornia crassipes*) plants

Water Hyacinth plants as shown in Fig. 1 came from a private fishpond were, they were used as ornamental plants from May to October. The plants were originally obtained from a nursery in Florid, USA. The collected Water Hyacinth plants are divided up in three 2 kg fractions. The first fraction contains the leaves, the second fraction the roots only and the third fraction the complete plants. Each fraction is cut in 1 cm long pieces and packed in Zip-log bags containing 0.5 kg. The Zip-log bags are then frozen in a freezer chest at -5°C.

![Water Hyacinth](image)

Fig. 1. Water Hyacinth (*Eichhornia crassipes*) plants [23]

2.1.2 Fermentation inoculate

Cow Manure inoculate (CMI) was obtained from the Stat University of New York (SUNY) Dairy Farm located in Morrisville, New York prior to performing each test set.

2.1.3 Barrier fluid

Preparation of the barrier fluid was based on DIN 38414 [32]. First, 1000 ml of deionized water was heated under stirring in a 1500 ml glass beaker using Thermo Scientific brand stirring hot plate and a magnetic stir bar. After a temperature of 40°C was reached, 30 ml of sulfuric acid (H₂SO₄; ρ=1,84 g/ml). Then 200 g of sodium sulfate dehydrate (Na₂SO₄) is added slowly to the diluted sulfuric acid solution. The solution is stirred till all sodium sulfate dehydrate is dissolved in the solution.

Second, in a 150 ml glass beaker 0.1 Methyl orange sodium salt is dissolved in 100 ml of distilled water under constant stirring at a temperature of 20°C.

Third, a few drops of the Methyl orange solution are added to the barrier fluid to allow for easier visualization. The color can be adjusted to either a lighter or a darker orange by adding more or less drops to the barrier solution.

Forth, the barrier solution should be stored at room temperature to prohibit crystallization. If crystallization occurs, the crystallization can be reversed easily by heating and stirring the barrier solution to of 40°C.

2.1.4 Absorbent fluid

The Absorbent fluid was prepared using a 1000 ml glass beaker filled with 500 ml of deionized water with 20°C. The beaker was placed on a Thermo Scientific brand stirring hotplate, and under stirring using a magnetic stirrer Sodium Hydroxide (NAOH) pellets were added till a final NAOH solution of 10% was achieved. The prepared adsorbent solution was filled in a clear PVC container and covered till used.

2.2 Laboratory Benchtop Anaerobic Fermentation System

A Laboratory Benchtop Anaerobic Fermentation (LBAF) system was designed, build and installed according to Fig. 2. The LBAF system consisted of a Fisher Scientific brand 7.25 x 7.25 digital heating-stirring hot plate (1). A 2.0 l glass beaker (2) filled with deionized water (12), heated by the stirring hot plate. The glass beaker serves as the heating vessel that provides the desired anaerobic fermentation temperature of approximately 39 °C. A 500 ml Erlenmeyer flask with a 40 mm magnetic stirrer serves as the Anaerobic Fermentation Vessel (AFV) (3). A rubber stopper (4) seals the reactor vessel. The rubber stopper contains a glued in 1/4”Outside Diameter (OD) x 1/8” Inside Diameter (ID) Male National Pipe Thread (MNPT) Nylon male adaptor fitting that is connected to a Polyvinyl Chloride (PVC) hose with 1/4” OD and 1/8” ID (5). The PVC hose contains a shut-off clamps (6) that allow to seal off the AFV if closed. If opened the produced biogas (13) from the biomass
suspension (11) can flow through PVC Tee (7) into a inverted installed 120 ml PVC graduated cylinder (9) if shut-off valve (8) is closed. The graduated cylinder (9) serves as the displacement vessel (9) for the barrier fluid (14). The graduated cylinder has a 1/4″ OD x 1/8″ ID MNPT Nylon male adaptor glued in on the bottom connected to a PVC hose having 1/4″ OD and 1/8″ ID (5). Displacement vessel (9) is located approximately 5 mm above the bottom of a 500 ml clear PVC beaker which serves as the barrier fluid reservoir (10). If shut-off valve 6 left of the tee (7) is closed and shut-off valve (8) in the PVC hose (5) line right of the tee (7) is opened, the barrier fluid can be moved back into the displacement vessel (9) using the attached 3-way rubber suction ball (15). If the suction ball (15) is replaced by a 50 ml PVC syringe biogas can be extracted using from the displacement vessel for analyses.

Fig. 2. Laboratory Benchtop Anaerobic Fermentation system: 1) Digital heating-stirring hot plate, 2) Heating vessel, 3) Fermentation vessel, 4) Rubber stopper, 5) PVC hose, 6) Shut-off valve, 7) Tee, 8) Shut-off valve, 9) Barrier fluid displacement vessel, 10) Barrier fluid reservoir, 11) Biomass suspension, 12) Heated water, 13) Biogas, 14) Barrier fluid, 15) 3-way rubber suction ball [33]

2.2.1 Operation of the LBAF

To operate the LBAF, approximately 350 g prepared biomass solution was filled into a pre-weighted digester vessel (3) with the magnetic stirrer. Next, the digester vessel was weighed to obtain the wet sample weight measurements. Then the digester vessel (3) was sealed with rubber stopper (4) and its attachments show in Fig. 2. Several layers of Parafilm were used to maintain a tight seal. The digester vessel was incubated in heating vessel (2) that is placed on a digital heating-stirring hot plate (1), containing 1200 ml distilled water at 39°C for the duration of the experimental trials. Valve (6) was closed at the start of the experiment. The rotation of the magnetic stirrer was adjusted that the biomass solution (11) turns slowly in the digestion vessel (2). Additional distilled water was added to the beaker until the water level reached the neck of the Erlenmeyer flask digestion vessel (3). This water served as a water jacket to control the temperature of the mixture in the digestion vessel (3). The temperature and volume of the water bath and the stirring speed were adjusted during the trial to be as close as possible to the initial set value.

Next, the barrier fluid (14) was succeed into the barrier fluid displacement vessel (9) using the 3-way rubber suction ball (15), till it reached the top of the barrier fluid displacement vessel (9). Then, clamp valve (8) was closed and clamp valve (6) was opened. As the produced biogas (13) was drawn from the headspace of the reactor into the barrier fluid displacement vessel (9) replacing the barrier fluid (14). Biogas generation was allowed to proceed, and biogas generation measurements were taken until biogas generation was deemed minimal or nonexistent. This time and length of each AD test varied between tests but was approximately five to eight days.

After each biogas measurement barrier fluid (14) was succeed back into the displacement vessel (9) until it reached the top, using the 3-way ball suction (15) by opening clamp valve (6) and opening clam vale (8). Thereafter clamp valve (8) is closed and clam valve (6) is opened, allowing biogas flow to the displacement vessel (9).

2.3 Laboratory Benchtop Methane Analyzer System

A Laboratory Benchtop Methane Analyzer (LBMA) system was designed, built, and installed according to Fig. 3. The LBMA system consisted of a 500 ml clear PVC Container which serves as the solvent fluid reservoir (1). An inverted installed 120 ml PVC graduated cylinder serves as the displacement vessel (2) for the adsorbent fluid (10). The displacement vessel (2) is located approximately 5 mm above the bottom of a 500 ml clear PVC beaker which serves as the solvent fluid reservoir (1). The graduated cylinder has a 1/4″ OD x 1/8″ ID MNPT Nylon male adaptor glued in on the bottom. A PVC hose having 1/4″ OD and 1/8″ ID (3) is connected
to either side of a Tee (4). The Tee (4) has on the left and right PVC hose line a shut-off clamp (5) and (6) installed. On the right side a 3-way rubber suction ball (7) is attached to the PVC hose. On the left side a 50 ml syringe (8) can be attached, containing the biogas (9) for analyzation.

Fig. 3. Laboratory Benchtop Methane Analyses System: 1) Solvent reservoir 2) Solvent displacement vessel 3) PVC hose, 4) Tee, 5) Shut-off valve, 6) Shut-off valve, 7) 3-way rubber suction ball, 8) 50 ml syringe, 9) Biogas, 10) Solvent [34]

2.3.1 Operation of the LBMA

If the left shut-off valve (5) is closed and the right shut-off valve (6) is open the solvent fluid can be moved into the displacement vessel (9) using the attached 3-way rubber suction ball (7) to the desired height. Then shut-off (6) is closed. Syringe (8) containing biogas (9) is attached and shut-off valve (5) is opened. Biogas (9) contained in syringe (8) is pressed into the displacement vessel (2) replacing the adsorbent fluid (10). The adsorbent fluid will then adsorb CO₂ contained in the biogas and move back into the displacement vessel. The difference between the biogas volume pressed into the displacement vessel and the volume of the adsorbent fluid moved back into the displacement vessel is the true biogas content without CO₂.

2.4 Testing Procedures

The following section describes the procedures used for each sample to determine the Total Solids Content (TSC), Ash content (AC) and Volatile Solids Content (VSC). All anaerobic co-digestion tests were run in dupliplicate. Analytical tests for TSC, AC and VSC were run in triplicate. A Denver Instrument SI-234 analytical balance was used to determine the sample weight. TSC of each test sample was determined based on modified TAPPI test method T412 om-06 “Moisture in pulp, paper and paperboard” [35] using a 70.7 l (2.5 cuft) Thelco drying oven set to 105°C. AC was determined for each test mixture using TAPPI test method T 211 om-02, “Ash in wood, pulp, paper and paperboard: combustion at 525°C” [36] using a Fisher Scientific Thermolyne 1.3 l (0.04 cuft) Muffle furnace set to 525°C.

VS content in % was determine by ((TSC-AC)/TSC*100).

Temperature and pH measurements were conducted using a portable Accumet AP85 pH/temperature/Conductivity meter.

2.4.1 Solids content, ash content measurement procedures

To evaluate the SC of a given test sample 50 ml aluminum sample trays were marked and weighted accordingly. Then approximately 30 to 45 ml of the prepared biomass suspension or test sample was added to each of the corresponding aluminum sample trays prepared for the given test sample. Next these samples were weighed to obtain their wet sample weight measurements and then placed in a ~105°C oven to dry for 24 hours. After drying the samples were weighed again to determine their dry weight measurements. The loss in mass was attributed to moisture. The remaining solids were the Total Solids Content (TSC) of the feedstock.

To determine the AC, 30 ml crucibles were labeled, weighed, and the remaining dried solids were scraped from their aluminum trays into their corresponding crucibles. The crucibles containing the samples were then weighed again and placed in a 525°C muffle furnace for approximately 6 hours for combustion. After combustion the crucibles with the remains were weighed to determine their ash weight measurements. The change in mass was attributed to the VS of the biomass material, which were ignited during the process. The remaining solids were the ash present in the sample.

2.4.2 Material preparation

Preserved frozen WH material fractions (leaves, roots, and complete plants) from the prep-reparation were defrosted prior to testing. Prior to testing, each WH component and the CM was diluted with tap water to a bendable biomass mixture. Each component was then blended for 2 minutes separately using a 1.5 l benchtop
laboratory blender/mixer to achieve better mixing and stirring in the fermentation vessel. Preliminary blending/mixing tests showed, that WH Complete (WHC) plants, WH Leaves (WHL) and WH Roots (WHR) at a solids content above 2.70%, 2.00% and 3.00% respectively is hard to stir with a magnetic stirrer in the AD vessel due to its long fibrous content if not blended/mixed. Therefore, AD was performed at a target solids content for the WHC, WHL, and WHR test at 2.50%, 1.75% and 2.75% respectively. In addition, it is expected that the AD microorganisms from the CM can access the WH substrate more easily in the mixture and thus expected to increase the rate of AD and biogas production. As shown in Fig. 4, biomass content of WH plants can vary greatly, pending on their growth stage and root structure. Roots can take up to 80% of the plant biomass providing more solid and hard to disintegrate biomass. Leaves tend to be more bulky, due to bulbous and or attenuated shaped petiole that contains air cells which keep the plant afloat [37].

![Figure 4: Water Hyacinth (Eichhornia crassipes) plant with different roots, leave size with bulbous petiole](image)

After mixing the CM to a mixable suspension solids content of the individual fractions was determined. The CM had a solids content of 6.39%, the WHC plants, WHL and WH had a solids content of 4.65%, 7.24%, and 3.97% respectively. After blending/mixing the suspension were stored in a refrigerator at 4°C.

2.4.3 Experimental procedure of the anaerobic fermentation experiment

A total of 24 AD experiments were conducted with CM, WHL, WHR, and WHC mixtures using the LBAF system. First, the mixtures were diluted with tap water to a target solids content for the WHC, WHL and WHR test at 2.50%, 1.75% and 2.75% respectively and adjusted to a pH of 8.0 with a 20% Calcium Hydroxide (Ca(OH)₂) solution. AD tests were performed in the mesophilic temperature area conditions which is 20°C to 45°C (68°F to 113°F) [39]. During the experiments the AD temperature was at kept at 39°C ± 2°C for the duration of the experimental trials. From each solution 350g of an AD solution was prepared by mixing the individual solution components into a 500 ml Erlenmeyer flask that contained a 40 mm magnetic stirrer. AD solutions with the following mixture rations were prepared: a) 100% CM, b) 75% CM and 25% WHL, WHR, WHC respectively, c) 50% CM and WHL, WHR and WHC respectively, and d) 25% CM and 75% WHL, WHR and WHC respectively. All 24 individual AD experiments with the LBAF system were run as described in section 2.3.1. with duplicate tests.

Measurements of the produced biogas volume per experiment was done as described in section 2.2.1. Biogas content was measured according to section 2.3.1.

3. RESULTS AND DISCUSSION

3.1 AD digestion of Water Hyacinth Leaves

Fig. 5 represents the AD experiment with 100% CM (Test 1 & Test 2) as a reference and CM and WHL ration of 75%:25% (Test 3 & Test 4), 50%:50% (Test 5 & Test 6), and 25%:75% (Test 7 & Test 8) respectively.

The 100% CM AD experiment revealed for Test 1 and Test 2 a Cumulative Biogas Production (CBG) of 170 ml and 163 ml and a VS consumption (VSC) of 0.62 g and 0.91 g respectively. The CBG per gVS (CBG/gVS) was 258.57 ml and 199.56 ml with an average VSC and CBG of 0.78 g and 219.06 ml respectively.

Test 3 and Test 4 with a ratio of 75% CM and 25% WHL show a CBG of 80 ml and 79 ml and a VSC of 0.26 g and 0.31 g respectively. The CBG/gVS was 309.83 ml and 257.28 ml with an average VSC and CBG/gVS of 0.28 g and 283.55 ml respectively.

Test 5 and Test 6 with a ratio of 50% CM and 50% WHL show a CBG of 164 ml and 147 ml and a VSC of 1.37 g and 0.65 g respectively. The CBG/gVS was 120.06 ml and 225.04 ml with an average VSC and CBG/gVS of 1.01 g and 172.54 ml respectively.
Test 7 and Test 8 with a ratio of 25% CM and 75% WHL show a CBG of 200 ml and 152 ml and a VSC of 0.79 g and 0.86 g respectively. The CBG/gVS was 252.21 ml and 176.11 ml with an average VSC and CBG/gVS of 0.83 g and 214.16 ml respectively.

Biogas samples of 50 ml was taken at the end of the study. The biogas composition without CO$_2$ showed a biogas content of 65% for the CM test, a 50% content for the 75% CM and 25% WHL test, a 54% content for the 50% CM and 50% WHL test, and a 56% content for the 25% CM and 75% WHL test.

### 3.2 AD digestion of Water Hyacinth Roots

Fig. 6 represents the AD experiment with 100% CM (Test 1 & Test 2) as a reference and CM and WHR ration of 75%:25% (Test 3 & Test 4), 50%:50% (Test 5 & Test 6), and 25%:75% (Test 7 & Test 8) respectively. The 100% CM AD experiment revealed for Test 1 and Test 2 a CBG of 382 ml and 280 ml and a VSC of 0.67 g and 1.92 g respectively. The CBG/gVS was 565.72 ml and 145.48 ml with an average VSC and CBG of 1.29 g and 355.59 ml respectively.

Test 3 and Test 4 with a ratio of 75% CM and 25% WHR show a CBG of 339 ml and 314 ml and a VSC of 2.38 g and 0.88 g respectively. The CBG/gVS was 142.46 ml and 358.11 ml with an average VSC and CBG of 1.63 g and 250.28 ml respectively.

Test 5 and Test 6 with a ratio of 50% CM and 50% WHR show a CBG of 189 ml and 225 ml and a VSC of 0.65 g and 0.98 g respectively. The CBG/gVS was 288.90 ml and 230.57 ml with an average VSC and CBG of 1.63 g and 259.73 ml respectively.

Test 7 and Test 8 with a ratio of 25% CM and 75% WHR show a CBG of 92 ml and 171 ml and a VSC of 0.17 g and 0.46 g respectively. The CBG/gVS was 549.96 ml and 375.30 ml with an average VSC and CBG of 0.23 g and 462.63 ml respectively.

Biogas sample of 50 ml was taken at the end of the study. The biogas composition without CO$_2$ showed a biogas content of 63% for the CM test, a 52% content for the 75% CM and 25% WHR test, a 55% content for the 50% CM and 50% WHR test, and a 54% content for the 25% CM and 75% WHR test.

### 3.3 AD Digestion of Complete Water Hyacinth

Fig. 7 represents the AD experiment with 100% CM (Test 1 & Test 2) as a reference and CM and WHC ration of 75%-25% (Test 3 & Test 4), 50%-50% (Test 5 & Test 6), and 25%-75% (Test 7 & Test 8) respectively.

The 100% CM AD experiment revealed for Test 1 and Test 2 a CBG of 298 ml and 256 ml and a VSC of 2.22 g and 1.91 g respectively. The CBG/gVS was 134.52 ml and 133.86 ml with an average VSC and CBG of 2.06 g and 134.19 ml respectively.

Test 3 and Test 4 with a ratio of 75% CM and 25% WHC show a CBG of 296 ml and 217 ml and a VSC of 1.66 g and 1.46 g respectively. The CBG/gVS was 178.73 ml and 148.83 ml with an average VSC and CBG of 0.73 g and 163.77 ml respectively.

Test 5 and Test 6 with a ratio of 50% CM and 50% WHC show a CBG of 312 ml and 187 ml and a VSC of 1.14 g and 1.19 g respectively. The CBG/gVS was 273.90 ml and 157.49 ml with an average VSC and CBG of 1.16 g and 215.69 ml respectively.

Test 7 and Test 8 with a ratio of 25% CM and 75% WHC show a CBG of 202 ml and 210 ml and a VSC of 1.04 g and 0.60 g respectively. The CBG/gVS was 194.42 ml and 351.61 ml with an average VSC and CBG of 0.82 g and 273.01 ml respectively.

Biogas samples of 50 ml was taken at the end of the study. The biogas composition without CO$_2$ showed a biogas content of 64% for the CM test, a 54% content for the 75% CM and 25% WHC test, a 56% content for the 50% CM and 50% WHL test, and a 51% content for the 25% CM and 75% WHL test.
Fig. 5. Cumulative biogas production over time for the CM and WHleaves test set. Tests were performed at 4 different CM to WHleaves ratios in duplicate.

Fig. 6. Cumulative biogas production over time for the CM and WHroots test set. Tests were performed at 4 different CM to WH root ratios in duplicate.
Fig. 7. Cumulative biogas production over time for the CM and Complete WH test set. Tests were performed at 4 different CM to WH rootratios in duplicate

4. CONCLUSION

The cumulative biogas production by anaerobic co-digestion of CM and WHC, WHL and WHR was performed with a designed LABF system at 100% CM as a basis, and CM:WH component ratios of 25%; 75%; 50%;50%, and 25%-75% for the WHC, WHL and WHR respectively. Each biomass component was prepared separately by blending/mixing and then mixed to the experimental ratios. The individual mixtures were diluted with tap water to a target solids content for the WHC, WHL, and WHR test at 2.50%, 1.75% and 2.75% respectively. The pH was adjusted to 8.0 with a 20% Ca(OH)$_2$ solution.

Each AD test lasted between 6 to 8 days at a temperature of 39°C ± 2°C for the duration of the experimental trials.

Biogas production per g/VS of the CM AD tests was between 134 ml to 355.59 ml.

Co-digestion of CM and WH showed the highest average biogas production per g/VS for WHC and WHR for a ratio of 25% CM to 75% WHC and WHR of 273.01 ml and 462.63 ml respectively. WHL showed the highest average biogas production 283.55 ml per g/VS for a ratio of 75% CM to 25% WHL.

The least biogas produced per g/VS by WH co-digestion was for a WHC and WHR for a ratio of 75% CM to 25% WHC and WHR of 163.77 ml and 250.28 ml respectively. WHL showed the lowest average biogas production of 172.54 ml per g/VS for a ratio of 50% CM to 50% WHL.

The biogas composition without CO$_2$ showed a biogas content for the CM between 54 and 65%. For the WHL, WHR and WHC tests a biogas composition without CO$_2$ between 50% and 56% resulted.

Overall, the use of co-digestion technology by utilizing CM and WH as a fast growing co-digestate feedstock. Harvesting WH for energy production using AD could help to minimize the negative environmental impact of WH and help to restore biodiversity, water quality and habitat of infested sites, as well as improve utilization of water ways for shipping and recreational activities.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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