Recovering biomethane and nutrients from anaerobic digestion of water hyacinth (*Eichhornia crassipes*) and its co-digestion with fruit and vegetable waste

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**ABSTRACT**

The potential to recover bioenergy from anaerobic digestion of water hyacinth (WH) and from its co-digestion with fruit and vegetable waste (FVW) was investigated. Initially, biogas and methane production were studied using the biochemical methane potential (BMP) test at 2 g volatile solids (VS) L\(^{-1}\) of substrate concentration, both in the digestion of WH alone and in its co-digestion with FVW (WH-FVW ratio of 70:30). Subsequently, the biogas production was optimized in terms of total solids (TS) concentration, testing 4 and 6% of TS. The BMP test showed a biogas yield of 0.114 m\(^3\) biogas kg\(^{-1}\) VS\(_{\text{added}}\) for WH alone. On the other hand, the biogas potential from the WH-FVW co-digestion was 0.141 m\(^3\) biogas kg\(^{-1}\) VS\(_{\text{added}}\), showing an increase of 23% compared to that of WH alone. Maximum biogas production of 0.230 m\(^3\) biogas kg\(^{-1}\) VS\(_{\text{added}}\) was obtained at 4% of TS in the co-digestion of WH-FVW. Using semi-continuously stirred tank reactors, 1.3 m\(^3\) biogas yield kg\(^{-1}\) VS\(_{\text{added}}\) was produced using an organic loading rate of 2 kg VS m\(^{-3}\) d\(^{-1}\) and hydraulic retention time of 15 days. It was also found that a WH-FVW ratio of 80:20 improved the process in terms of pH stability. Additionally, it was found that nitrogen can be recovered in the liquid effluent with a potential for use as a liquid fertilizer.

**Key words** anaerobic digestion, biochemical methane potential (BMP), fruit and vegetable waste, renewable energy, water hyacinth

**INTRODUCTION**

Water hyacinth (*Eichhornia crassipes*) is an aquatic plant belonging to the pickerelweed family (Pontederiaceae). Native to Brazil, it occurs in several waterways and water storages in most countries of the world. It is a free-floating aquatic plant well known for its production abilities and removal of pollutants from water and as a reservoir of both energy and nutrients (O’Sullivan et al. 2010). This plant is considered as a noxious weed. According to (Malik 2007), it can quickly grow to very high densities (over 60 kg m\(^{-2}\)), even to completely clog water bodies, which leads to serious problems in the preservation of water ecosystems and human activities like fishing, navigation, irrigation and power generation (Fernández et al. 1990; Epstein 1998). In order to control its aggressive growth, various efforts have been made, based on weed management methods such as physical removal, chemical methods (application of herbicides) and release of biological control agents. However, water hyacinth (WH) has successfully resisted all attempts to its eradication (Malik 2007).

Direct landfilling of biodegradable material from municipal solid waste (MSW), as in the case of Colombia, poses lasting detrimental impacts to the environment and human health. Among the major issues are the consequential emissions of greenhouse gases to the atmosphere and water and soil contamination (Nguyen et al. 2007). According to the Ministry of Environment and Housing and Territorial Development (MAVDT 2008), on average MSW in Colombia contain an important fraction of biodegradable material, about 65% (wet weight), and the generation rate of this waste is increasing in time.

Several efforts have been made in order to give effective solutions to these problems. In the case of WH, recent research has been focused not only on control methods but also on the utilization of technologies with significant...
achievements. It has been shown that in rural areas WH can play an important role in decentralized wastewater treatment systems coupled to biogas and compost production (Malik 2007). In the same way, environmental authorities in Colombia are committed to promoting the use of biodegradable waste in the framework of integrated waste management, and again biogas production and composting are emerging as sustainable technologies to be implemented.

Anaerobic digestion might be a feasible method to decrease disposal costs of MSW and recover renewable energy in the form of methane from both WH and MSW individually. Biogas is a desirable product of digestion as it contains significant energy potential, approximately 25 MJ kg$^{-1}$ for biogas with 75% methane content (Wang & Calderon 2012). Additionally, anaerobic digestion releases nutrients in both solid and liquid effluents, which can be subsequently used as fertilizers (Kim et al. 2003; Dong et al. 2010).

The aim of this project is to investigate the potential to recover renewable energy from anaerobic digestion of WH and co-digestion of WH and fruit and vegetable waste (FVW), and additionally to optimize the total solids content in a semi-continuous process. The experiments were specifically designed to reveal the rate of degradation of that mixture, the biogas yield and the quality of the biogas (% methane).

**MATERIALS AND METHODS**

**Water hyacinth and fruit and vegetable waste sample collection and inoculum**

Water hyacinth was harvested from the natural reserve ‘Laguna de Sonso’ located in Valle del Cauca, Colombia, with coordinates 3°52’12”N 76°2’13”W. Between 40 and 50 kg (wet mass) of WH were collected and taken to the laboratory.

The FVW was collected from typical market places and major supermarkets in the municipality of Palmira, Valle del Cauca. The raw material (uncooked) was taken fresh after a typical market day. Figure 1 shows the composition of FVW.

Once in the laboratory both substrates were shredded in order to make them more homogeneous and increase their surface area. After size reduction, substrates were kept in cold storage at 4°C until use. The characterization of WH and FVW used is presented in Table 1. The elemental analysis was carried out according to the Standard Methods for Instrumental Determination of Carbon, Hydrogen, and Nitrogen in Laboratory Samples of Coal and Coke (Designation: ASTM D 5373-93), while fiber was determined using the Van Soest method.

The inoculum used was a mesophilic anaerobic sewage sludge, collected from an upflow sludge blanket reactor located in the municipality of Ginebra (Valle del Cauca, Colombia), treating domestic wastewater. Inoculum volatile solids (VS) were analyzed as quality criteria. Inoculum acclimatization at 37°C and feeding with the substrate were carried out for 1 month before the experiments.

**Experimental procedure**

The biochemical methane potential test (BMP) was used to evaluate the rate of degradation and the biogas yield from the digestion of WH and co-digestion of WH–FVW. The test was performed according to Owen et al. (1979) with nutrient...
media modifications as described by Kim et al. (2003). Duran glass bottles (24 in total) with a capacity volume of 500 mL with hermetically sealed stoppers and controlled opening valves were used as reactors (Figure 2). The effective volume was 400 mL and the headspace gas volume was 100 mL. The bottles were incubated at 37°C for 60 days. Triplicates of treatments and control bottles without the substrate were also included to improve the accuracy of the experiment and correct the inoculum methane production.

The substrates evaluated were WH alone and co-digestion of WH and FVW in a 70:30 ratio on a VS basis. Previous research has demonstrated that anaerobic digestion of FVW as the only substrate was very difficult to run due to the serious accumulation of volatile fatty acids (VFAs) (Mata-Alvarez & Cecchi 1992; Lin et al. 2011); for this reason a higher amount of WH was selected in order to avoid acidification problems. In a first BMP test, a substrate concentration of 2 g VS L⁻¹ was tested to determine methane potential. In a second experiment, total solids content was optimized under BMP conditions using total solids (TS) concentrations of 4 and 6%.

The gas production was determined daily using the liquid displacement technique and representative samples were analyzed by gas chromatography for methane content.

### Anaerobic co-digestion in semi-continuous CSTR

For semi-continuous digestion experiments, two completely stirred tank reactors (CSTRs) with a total volume of 2.4 L and a working volume of 2 L were used. Due to pH reduction related to VFA accumulation during the first experiment, it was decided to reduce the amount of FVW for the present experiment. Thus, in this case, feedstock was a WH:FVW mixture of 80:20 on a TS basis, corresponding to a carbon/nitrogen (C/N) ratio of 20. The TS of feedstock was adjusted to 4%. Top-mounted mechanical stirrers were intermittently operated at 60 rpm (15 min on and 45 min off) to ensure close to ideal mixing conditions.

In order to evaluate optimum operating conditions, three organic loading rates (OLRs) (2, 2.5 and 3 g VS L⁻¹ d⁻¹) and two hydraulic retention times (HRTs) (10 and 15 days) were studied. Biogas production was measured daily using the liquid displacement technique. The experiment was conducted for a 90 day period.

### Analytical methods

Samples were regularly taken from each reactor and after centrifuging the samples at 4,000 rpm for 20 min, analyses of pH, TS, VS, soluble chemical oxygen demand (COD), ammonia, alkalinity, VFAs and total Kjeldahl nitrogen were conducted following the methods outlined in Standard Methods (APHA 2005).

The composition of the biogas (CH₄ and CO₂) was measured on an SRI gas chromatograph equipped with a thermal conductivity detector and a 2.44 m stainless steel column packed with Haysep (80/100 mesh).

### RESULTS AND DISCUSSION

#### Biological methane potential tests

### Methane potential

At the beginning of the experiment the production of biogas initiated more rapidly for the co-digestion of WH and FVW than for the WH alone, between days 10 and 20 both experiments showed similar methane potential, but after that point, co-digestion showed a higher production until the end of the experiment (Figure 3). Each point represents an average of the biogas produced daily in the three reactors; in all cases the standard deviation was always lower than 15%.

The biogas potential recorded for WH alone was 0.114 m³ biogas kg⁻¹ VS added. Many studies have reported yields in the range of 0.200–0.300 m³ biogas kg⁻¹ VS and around 0.140–0.200 m³ methane kg⁻¹ VS (Anand et al. 1991; Moorhead & Nordstedt 1993; Kumar 2005; Gunnarsson & Petersen 2007; Malik 2007; Ferrer et al. 2010). The biogas yield obtained in this study is slightly below the range reported in the literature, and there are two possible causes. On the one
hand, the BMP nutrient media could have been inhibitory to the digestion of WH, as was described by O’Sullivan et al. (2010). On the other hand, low quality of the inoculum used could have affected the digestion process since the VS content was below 60%.

The results showed a positive effect of the co-digestion of WH and FVW, exhibiting a higher production of biogas (0.141 m$^3$ biogas kg$^{-1}$ VS$_{\text{added}}$). This means that the biogas potential of WH-FVW co-digestion was 23% higher than that of WH alone. Regarding quality of the produced biogas, it was observed that the methane content for WH alone was 57.5% while for WH-FVW it was 60.5%; however, when analysis of variance (ANOVA) was conducted no significant differences were found between averages of the treatments, with 95% of confidence. The poorer performance of the WH alone can be explained by the composition of the plant cell wall, which contains cellulose, hemicellulose and lignin as main supporting tissues; these are complex substances and difficult to biodegrade. The polymer organic material cannot be used directly by microorganisms unless soluble compounds are hydrolyzed (Chandra et al. 2012). The lignin content is similar in both substrates. However, hemicellulose and cellulose contents are around three times higher in the WH than in the FVW. This high concentration of long polysaccharides could limit and reduce the velocity of the hydrolysis process (Cadavid-Rodríguez & Horan 2013). On the other hand, the addition of FVW seems to stimulate the production of enzymes that facilitate the hydrolysis process.

pH, VFAs, COD, ammonia and alkalinity

As a result of the digestion process, the pH, initially very close to 7, showed a slight decrease during the first 4 days (until 6.5 ± 0.2), whereupon it started to increase until day 14 when it stabilized around 7 ± 0.05.

In the co-digestion treatment, VFAs rapidly increased from 410 to 446 mg acetic acid L$^{-1}$ from the beginning of the experiment until day 3; after this, it started to decrease (Figure 4(a)), probably by the presence of FVW that has a faster degradation rate than WH. Once the hydrolytic stage of WH started, a second peak appears on day 30. The hydrolysis and fermentation of both treatments resulted in an increase in the soluble COD in the first days of the test (Figure 4(b)), primarily due to the formation of VFAs as explained by Anand et al. (1991). The high production and subsequent consumption of VFAs indicated a great production of biogas between days 25 and 40. Even though there were changes in the amount of VFAs along the experiments, the pH was always around 7, indicating that there was also an increase in the alkalinity and thus an optimal buffer capacity to avoid abrupt changes in the pH and subsequent inhibition of the process (Figure 5(a)).

Regarding ammonia, it increased during the first 7 days; after that it seems that there was ammonia consumption to Figure 3 | Biogas potential of WH and co-digestion of WH-FVW.

Figure 4 | Volatile fatty acids (a) and chemical oxygen demand variations (b) in the BMP test.
neutralize VFA production, and finally there was another increase until day 60. The final ammonia concentrations were 225 and 250 mg L\(^{-1}\) for WH and co-digestion WH-FVW, respectively (Figure 5(b)). As can be observed, ammonification adds to the alkalinity, since these two parameters present a correlated behavior (Figure 5).

**Optimization of total solids content**

In order to explore the optimum condition for a conventional anaerobic digestion process of WH and WH-FVW co-digestion, two TS concentrations were tested to find the best condition for biogas production. In this case, TS of 4 and 6% were tested for both treatments: WH alone and WH-FVW co-digestion (Figure 6).

For both TS concentrations, once again the co-digestion of WH-FVW exhibited a better performance compared to the digestion of WH alone. The maximum biogas potential of 0.230 m\(^3\) kg\(^{-1}\) VS\(_{\text{added}}\) was reached with 4% of TS from co-digestion WH-FVW, proving that co-digestion of WH with FVW improves the digestion process of WH. A relatively low TS concentration of 4% favored a rapid conversion of acids into biogas, while a higher solids concentration of 6% showed a reduction of 12% in biogas production; consequently, there was a drop in VS destruction (less than 16%). Conversely, for digested WH alone, it was found that the treatment with 6% of TS resulted in a slightly higher production of biogas than the treatment with 4%.

During the first 7 days of the test, a decrease of pH (to around 6.2) was observed in the WH-FVW co-digestion, which had to be controlled by addition of NaOH. The reason may be the rapid VFA production caused by the hydrolysis and acidogenesis of FVW. This effect could be increased with higher TS concentrations as stated by Chen et al. (2008). Therefore, reducing the amount of FVW could be a good option to reduce pH control during the conventional anaerobic digestion process. Another alternative could be to explore a two-stage process or a solid bed reactor in order to separate acidogenesis from methanogenesis.

**Nutrient recovery**

The WH releases significant amounts of nitrogen into the water column and sediments (Gamage & Asaeda 2005). Therefore, it may be possible to retain much of the nitrogen and phosphorus in the liquid effluent and the solid digestate after its anaerobic digestion. These nutrients could be used as fertilizers instead of releasing them into the waterways, causing environmental problems.

Nitrogen from the substrate is mainly transformed to ammonia which remains in solution after the digestion process. In the present study, treatments with WH alone exhibited a
slightly greater release of ammonia, 414.4 mg NH₃ L⁻¹ and 503.3 mg NH₃ L⁻¹ for the digestion of WH compared to 375.9 mg NH₃ L⁻¹ and 483.4 mg NH₃ L⁻¹ for the co-digestion, with 4% and 6% of TS, respectively (Figure 7). Given these ammonia concentrations, the liquid effluent could have potential to be used as a liquid fertilizer. However, further investigation is required to ensure a feasible and safe nutrient utilization from anaerobic digestion of WH.

Continuous digestion tests

In a continuous digestion process, OLR and HRT determine the rate of production of intermediary metabolites, and they also play a role in terms of the efficiency and economy of the process (Li et al. 2014). The digester size and form and the amount of material to be processed per unit of time depend on them. Figure 8 shows the biogas production under different conditions of OLR and HRT. Using an OLR of 2.0 kg VS m⁻³ d⁻¹ and HRT of 15 days, the biogas production was 1.3 ± 0.3 m³ kg⁻¹ VS added compared with 0.5 ± 0.07 m³ kg⁻¹ VS added reached at 2.0 kg VS m⁻³ d⁻¹ and 10 days, indicating an increase in the biogas production of 60% with a higher HRT. WH has high concentrations of hemicellulose and cellulose that limit and reduce the velocity of the hydrolysis process. Using higher HRT, the hydrolysis phase releases a higher quantity of components which will be used by methanogenic bacteria to improve the biogas yield. OLRs of 2.5 and 3.0 kg VS m⁻³ d⁻¹ drastically affected the performance of the process, reducing the biogas production by 80% compared with an OLR of 2.0 kg VS m⁻³ d⁻¹. There was a significant difference (95% confidence) between the low (2.0 kg VS m⁻³ d⁻¹) and the high OLR (3.0 kg VS m⁻³ d⁻¹) evaluated for each HRT. These results could be explained by an inhibitory acidification that was found during the evaluation of higher OLRs (pH decrease from 7.2 to 5.5).

CONCLUSIONS

The results showed that it is possible to use WH to produce biogas and obtain energy recovery through an anaerobic digestion process. However, better results can be obtained if co-digestion with FVW is implemented; a TS proportion of 80:20 (WH/FVW), corresponding to a C/N ratio of 20, is recommended in order to avoid pH correction.

In a conventional digestion process, the optimum TS concentration for biogas production was found to be 4% in a co-digestion (WH-FVW) process. For a continuous co-digestion process an optimum biogas production was found with an OLR of 2.0 kg VS m⁻³ d⁻¹. Additionally, this study showed a potential for nitrogen recovery from the liquid effluent, which can be used as a liquid fertilizer.
Finally, more research is needed in order to study and optimize operating conditions that allow full-scale implementation of WH-FVW co-digestion.

ACKNOWLEDGEMENTS

The authors wish to thank COLCIENCIAS and particularly the Young Researchers and Innovators Program ‘Virginia Gutierrez Pineda’, and the Universidad Nacional de Colombia, especially DIPAL for providing the financial support through project QUIPU 20201001103 and 20201001336. We also wish to thank the staff of Nutrición Animal, Análisis Ambiental and Investigaciones Ambientales laboratories for their kind collaboration on the experimental work.

REFERENCES


APHA 2005 Standard Methods for the Examination of Water and Wastewater, 22nd edn. American Public Health Association (APHA)/Water Pollution Control Federation/Water Environment Federation, Washington, DC, USA.


Kumar, S. 2005 Studies on efficiencies of biogas production in anaerobic digesters using water hyacinth and night-soil alone as well as in combination. Asian Journal of Chemistry 17, 934–938.


O’Sullivan, C., Rousefell, B., Grinham, A., Clarke, W. & Udy, J. 2010 Anaerobic digestion of harvested aquatic weeds: water hyacinth (Eichhornia crassipes), cabomba (Cabomba Caroliniana) and salvinia (Salvinia molesta). Ecological Engineering 36, 1459–1468.


First received 26 May 2015; accepted in revised form 16 September 2015. Available online 28 September 2015