

# Effect of co-digestion with water hyacinth, inoculum concentration and dilution on biogas production of fish waste

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## ABSTRACT

A report from the FAO (Food and Agriculture Organization) estimates that 9.1 million tons of Fish wastes (FW) are thrown away each year. Water hyacinth (WH) is the most invasive water weed that thrives in freshwater water and has impacts on the environment, ecology, and society. The waste generated is either discarded onto the open ground or disposed into pits which result in environmental pollution and health hazards. FW and WH can be used for biogas production. However, FW digests quickly and produces a lot of ammonia and Volatile fatty acids (VFAs) which inhibit the digestion when digested alone, it has also a low carbon to nitrogen (C/N) ratio; consequently, it is not directly suitable for anaerobic digestion (AD). In this regard, co-digestion with WH (high C/N ratio) prior to AD is necessary. To examine the effects of the operating factors: Substrate ratio (WH: FW), Inoculum concentration (IC) and Dilution, Central composite design (CCD) in Design Expert 13 software was used to design experiments of three factors at five levels which resulted in 17 runs. Laboratory scale experiments were carried out under mesophilic temperature (37 °C). The maximum biogas with the highest methane content of 68.15% was produced at the substrate ratio of 25:75 g/ 250 mL (WH: FW), 15 g/ 250 mL of IC and dilution of 95 mL. The biogas yield increased by 16.1% and 32.4% compared to pure WH and FW respectively. This implied that the operating conditions were efficient in maximising the biogas yield. FW had a lower C/N ratio; further study needs to consider co-digestion with other higher C/N ratio substrates.

## 1. Introduction

Most of the world's energy originates from fossil fuels which are exhaustible and cause environmental pollution through the emission of greenhouse gases (Baredar et al., 2016; Tsavkelova and Netrusov, 2012). Organic wastes from animal, agricultural, fish, water hyacinth, etc. are digested by bacteria in anaerobic environment for biogas production (Emmanuel Pax et al., 2020; Marchaim, 2007; Nadu, 2017; Oke, 2016). The conversion of wastes to energy provides an answer to environmental pollution, waste treatment and management, and rising energy costs (Vijay Kumar et al., 2013; Olatunde, 2016; Folorunsho et al., 2016; Araoye et al., 2018).

Fish by-products are now a global issue and cause a problem to the long-term viability of fish aquaculture. A report from the FAO (Food and Agriculture Organization) estimates that 9.1 million tons of fish waste (FW) are thrown away each year (Kandyliari et al., 2020). Water Hyacinth (WH) is the most invasive water weed that thrives in freshwater water and has impacts on the environment, ecology, and society (Almoustapha and Kenfack, 2019; Armah et al., 2017; Bote et al., 2020; Katima, 2001; Rozy, 2016). Its mats block waterways, make fishing impossible, limits sunlight and lowers oxygen levels in water, and wipes out aquatic life. Millions of dollars are spent to manage its growth (Bote et al., 2020; Chanathaworn, 2017; Njogu et al., 2015; Tham, 2012). The waste generated is either discarded onto the open ground or disposed

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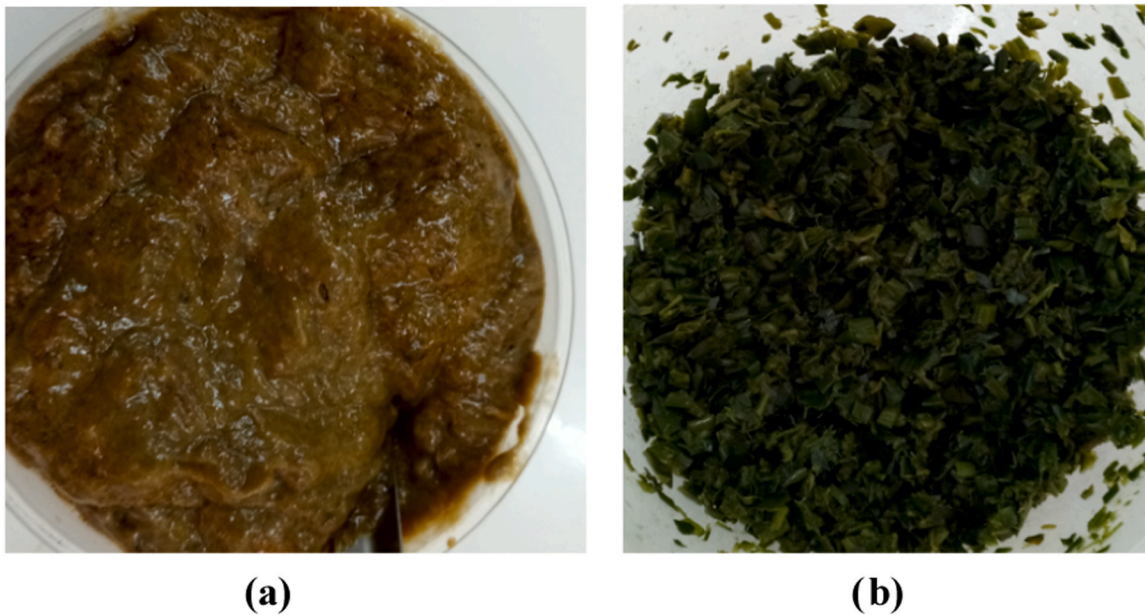


Fig. 1. Substrate samples for (a) Fish waste and (b) Water hyacinth 2.2. Analytical analysis.

into pits which result in environmental pollution and health hazards (Oke, 2016; Kandyliari et al., 2020; Kafle and Kim, 2012; Maghaydah, 2003; Pina et al., 2018). However, FW and WH can be used for biogas production. Fish waste is rich in lipids, proteins and contains easily biodegradable organic matter while WH has a high cellulose, low lignin and high C/N ratio (Marchaim, 2007; Bote et al., 2020; Kafle and Kim, 2012). Fish waste digests quickly and produces a lot of ammonia and VFAs which inhibit the anaerobic digestion when digested alone, it has also a low C/N ratio, consequently it is not directly suitable for AD (Pina et al., 2018). To increase the biogas production, FW can be co-digested with additional substances (Kafle and Kim, 2012; Pina et al., 2018). Co-digestion of a low C/N ratio substrate with a high C/N ratio substrate has been shown to enhance biomethane production.

Tsavkelova and Netrusov (2012) Moreover, the anaerobic co-digestion of FW and WH has been used to increase biogas energy (Nalinga and Legonda, 2016). Biogas yield can be also enhanced by evaluating variables such as inoculum concentration (IC), pH, dilution, temperature, substrate ratio, retention time and organic loading rate (OLR) (Nadu, 2017; Vijay Kumar et al., 2013). Emmanuel Pax et al. (2020) did research on the impact of co-digestion of food waste and cow dung; biogas production has increased due to co-digestion. It is important to use adequate amounts of substrate in co-digestion to maximise microbial activity in the digester.

Girmaye Kenasa and Kena (2019) IC is an important factor as it determines how quickly organic waste decomposes in AD (Jnr, 2011; Madondo, 2017; Owamah, 2010; Yadvika, 1, 10, Oct. et al., 2004; Slimane et al., 2014). The biogas and methane yield has increased for the IC between 10% and 40% (Owamah, 2010). Water reduces the concentration of toxic compounds such as ammonia and hydrogen sulphide which can hinder the AD process (Madondo, 2017; Slimane et al., 2014; Hhaygwawu, 2016). The degradation rate of waste increased as dilution increases, which resulted in high biogas yield (Jnr, 2011). Substrate concentration, particle size, and incubation period were evaluated for biogas production from WH. Highest methane was produced within 5 days at substrate concentration of 25 g and particle size less than 1 mm of WH (Katima, 2001). Based on various literature reviews, it was noted that the anaerobic co-digestion of FW and WH has not been evaluated for any operating parameter. Biogas can be used for cooking, lighting, heating and as fuel for machines and automobiles (Rozy, 2016; Hhaygwawu, 2016). The by-products of AD can be used in agriculture as a bio-fertilizer (Jnr, 2011; Madondo, 2017). Currently, large amounts of

**Table 1**  
Characteristics of the feedstock.

Parameters	FW	Substrate WH	Inoculum
MC (%)	61.58 ± 2.1	94.22 ± 2.20	89.57 ± 2.15
TS (%)	38.11 ± 1.9	5.58 ± 0.06	10.23 ± 0.01
VS (%)	93.84 ± 1.0	99.48 ± 1.11	74.82 ± 1.95
AC (%)	0.51 ± 0.05	16.65 ± 0.35	25.07 ± 0.24
TC(%)	54.10 ± 1.50	42.67 ± 1.13	35.81 ± 1.12
TN(%)	9.11 ± 0.09	2.01 ± 0.02	1.43 ± 0.00
C/N ratio	5.79 ± 0.12	21.25 ± 0.25	23.37 ± 0.32
pH	6.42 ± 0.1	7.19 ± 0.12	6.79 ± 0.11

FW and WH are produced in Kenya and many other countries. They are available, affordable, renewable and sustainable; consequently, the successful utilisation of FW and WH for biogas production can have a significant impact. The long-term economic benefit of knowing the right parameters to produce maximum biogas and methane yields will also benefit fish processors. The research work evaluated the effect of substrate ratio (WH: FW), inoculum concentration (IC) and dilution as operating factors to convert these wastes into biogas for maximum energy generation.

## 2. Material and methods

### 2.1. Feedstock collection

FW used in this experiment was collected from the fish point, Eldoret, Kenya. Water Hyacinth was collected from Lake Victoria in Kisumu County, Kenya, and the inoculum was collected from the Moi University biogas plant. Substrates were cut into small pieces, and blended using a blender to increase its biodegradability for microbial activity. Substrate samples used are shown in Fig. 1.

FW, WH, and inoculum were characterised for Moisture content (MC), Total solids (TS), Volatile solid (VS), Ash content (AC), pH, Total carbon (TC), Total nitrogen (TN) and carbon to nitrogen (C/N) ratio using standard methods (Bakr and El-ashry, 2018; Maurice et al., 2021).



Fig. 2. Overview of biogas production setup.

2.2. Operating procedure for biogas production

Experimental variable levels for biogas were substrate ratio (WH: FW, 25–75 g/250 mL), IC (5–15 g/250 mL), and dilution (85–95 mL). To examine the effects of the operating factors, CCD in Design Expert 13 software was used to design experiments of three factors at five levels. Using the expression  $2^n + 2n + 3$ ,  $2^n$  ( $2^3 = 8$  factorial points),  $2n$  ( $2 * 3 = 6$  axial points) and 3 centre points of replications, resulted in 17 runs. The substrates were fed into the bio-digesters at varied substrate ratios (8–92 g/250 mL), IC (1.6–18.4 g/250 mL), and dilution (81.6–98.4 mL). Laboratory scale experiments were carried out under mesophilic temperature (37 °C). Biogas production was measured using a water displacement method and biogas composition was determined using portable gas detectors. The setup used for biogas production is shown in Fig. 2.

3. Results and discussion

The biogas yield increased by 16.1% and 32.4% compared to pure WH and FW respectively. This implied that the operating conditions were efficient in maximising the biogas production. The biogas yield

from FW, WH and Optimised biogas were compared as shown in Fig. 3.

Daily biogas production from 17 bio-digesters were computed as shown in Fig. 4. The maximum biogas yield was produced at substrate ratio of 25:75 g/250 mL (WH: FW), 15 g/250 mL of IC and dilution of 95 mL (Run 6).

The high decomposition rate in the first days was due to increased hydrolysis rate, softness and presence of quickly degradable organic materials (Marchaim, 2007; Oke, 2016; Pina et al., 2018). The high biogas yield was due to the presence of enough active methanogens. Also, was due to the balanced nutrients (C/N ratio) needed by microorganisms, optimal MC, increased capacity of buffer, and reduced effects of toxic compounds (Chanathaworn, 2017; Madondo, 2017; Owamah, 2010; Hanghome, 2014; Maile et al., 2016; Rabii et al., 2019). The low rate of biogas yield in the last days was due to an increased number of microorganisms and decreased amount of food available, hence less food being converted into biogas or some remaining materials were more

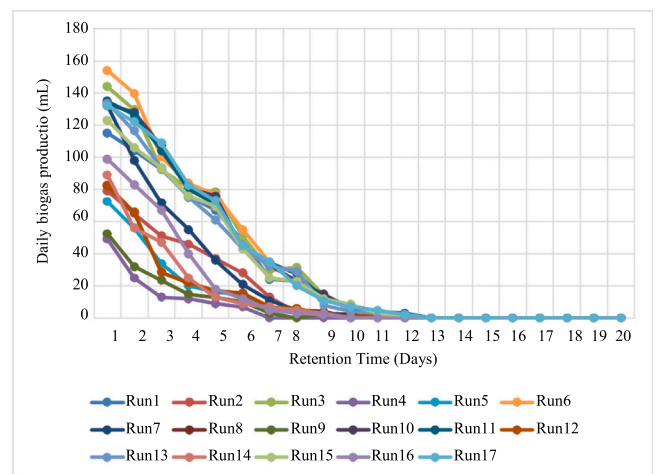
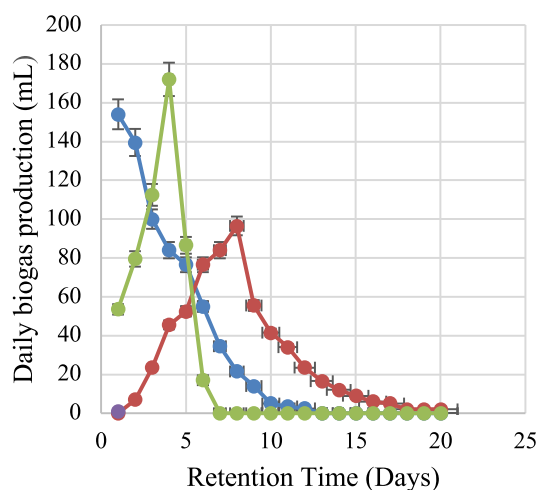
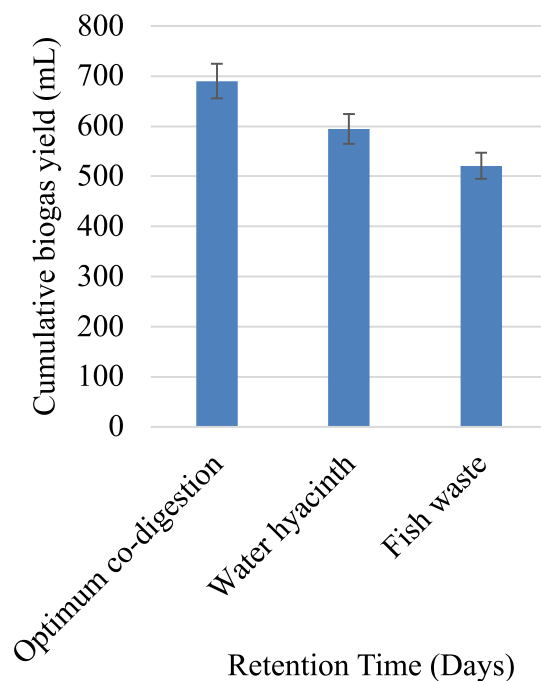


Fig. 4. Daily biogas production from bio-digesters.



(a)



(b)

Fig. 3. Daily (a) and Cumulative (b) biogas yield from pure FW, pure WH and Optimized biogas.

**Table 2**  
The Biogas Compositions.

Compositions	Fish waste	Water Hyacinth	Co-digestion
CH4 (%)	50.22 ± 0.03	56.68 ± 0.02	68.17 ± 0.04
CO2 (%)	38.82 ± 0.04	32.43 ± 0.03	27.96 ± 0.02
O2 (%)	0.44 ± 0.01	1.12 ± 0.02	0.12 ± 0.02
H2S (ppm)	235 ± 0.30	120.20 ± 0.02	105.20 ± 0.40
Others (%)	8.95 ± 0.03	8.75 ± 0.03	2.76 ± 0.04

resistant to bacteria (Katima, 2001; Hhaygwawu, 2016).

### 3.1. Biogas composition

Table 2 shows the content of biogas produced from pure FW, WH and the optimised biogas. The operating factors (substrate ratio, IC, and dilution) were efficient in maximising the biogas production and methane content as the highest methane (68.15%) yield was observed in the optimised biogas. Methane content in pure FW and WH was 50.12% and 55.67% respectively, as shown in Table 2.

The results are in range with other literature where they found that the CH4 content of WH, FW, and their co-digestion was between 45.18% and 73.3% (Bote et al., 2020; Katima, 2001; Njogu et al., 2015; Nalinga and Legonda, 2016).

The methane content increased as carbon dioxide decreased as shown in Table 2. This might be explained that organic matter removal was related to methane production (Chanathaworn, 2017).

### 4. Conclusion and recommendations

The biogas yield increased by 16.1% and 32.4% compared to pure WH and FW respectively. This implied that the operating conditions were efficient in maximising the biogas yield. The maximum biogas with the highest methane content of 68.15% was produced at the substrate ratio of 25:75 g (WH: FW), IC of 15 g/ 250 mL and dilution of 95 mL. FW had a lower C/N ratio; further study needs to consider co-digestion with other higher C/N ratio substrates. The biogas can be upgraded for carbon dioxide (CO2) removal. Also, a simplified techno-economic assessment considering substrate availability and total methane production in full-scale operations could be conducted.

### CRedit authorship contribution statement

**Conception and design of study:** Hortence Ingabire and Milton M. Marimi. **Acquisition of data:** Hortence Ingabire and Milton M. Marimi. **Analysis and/or interpretation of data:** Hortence Ingabire and Maurice Twizerimana. **Drafting the manuscript:** Maurice Twizerimana and Hortence Ingabire. **Revising the manuscript critically for important intellectual content:** Maurice Twizerimana and Hortence Ingabire. **Approval of the version of the manuscript to be published:** Hortence Ingabire, Maurice Twizerimana, and Malton M. Marimi.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data Availability

The research contains all relevant data. The corresponding author would provide any additional data upon request.

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