Contents list available at IJRED website

Int. Journal of Renewable Energy Development (IJRED)

*Journal homepage: http://ejournal.undip.ac.id/index.php/ijred*

*Research Article*

# Anaerobic Co-Digestion of Water Hyacinth (*E. crassipes*) With Ruminal Slaughterhouse Waste for Biogas Production

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**ABSTRACT**. The use of biomass as renewable energy source is of interest in reducing dependence on fossil fuels and associated impacts of climate change. Water hyacinth (WH), an invasive aquatic plant of environmental concern has large biomass that is available for biogas production. Co-digestion of this largely lignocellulose biomass with other substrates may correlate process parameters and improve biogas production. This study evaluated co-digestion of WH biomass with various mix proportions of ruminal slaughterhouse waste (RSW) at 24, 32 and 37°C in order to assess the optimum proportion and temperature. The rate of biomethanation increased with temperature from 0.23 at 24ºC to 0.75 and 0.96 at 32ºC and 37ºC, respectively, and similarly methane yield improved from 14 at 24ºC to 40 and 52 L/kg air dried water hyacinth at 32ºC and 37ºC respectively. A WH: RSW ratio of 30% showed optimum acclimatization and methane yield in a residence time of 60 days. The duration of the initial drop in pH that indicates hydrolysis stage decreased with increase in proportion of RSW, indicating faster hydrolysis and fermentation processes. Longer and stable latter alkaline pH zone suggested improved biomethanation and greater biogas production. Co-digestion with 30% RSW at 24ºC improved biogas yield by 75% from 8.05 to 14.09L/Kg biomass, methane component of biogas by 9% from 59 to 68% and reduced the retention time for substrate by 36%, suggesting synergy in co-digestion with respect to biogas quality. Changing the temperature from 24 to 32ºC increased the yield by 186% and reduced retention time by 73%. The results demonstrated synergy in co-digestion of the two substrates and the process dynamics that are useful in a possible process commercialization. 

***Keywords:*** Co-digestion, Biomass, Biogas, Water hyacinth, C/N ratio, Ruminal Slaughterhouse Waste.

**Article History**: Received: July 19, 2019; Revised: Sept 22, 2019; Accepted: Oct 21, 2019; Available online: Oct 30, 2019

**How to Cite This Article**: Omondi, E.A., Njuru, P.G. and Ndiba, P.K. (2019). Anaerobic Co-Digestion of Water Hyacinth (*E.* crassipes) with Ruminal Slaughterhouse Waste for Biogas Production. International Journal of Renewable Energy Development, 8(3), 253-259
https://doi.org/10.14710/ijred.8.3.253-259

## 1. Introduction

The use of fossil fuels is increasingly expensive and poses serious health and environmental concerns especially climate change (Budiyano et al., 2010). Accordingly, biomass is increasingly of interest as a source of renewable energy. Short-cycle crops are the most commonly used source of biomass for energy production; however, the use of crops faces the challenge of competing demands for arable land (Svetlana and Johan, 2010).

Water hyacinth, an invasive aquatic plant with short doubling times of 7–12 days (Reddy and Debusk 1985; Tag El-Din 1992) grows on water and, therefore, does not compete for agricultural land with crops (Bett, 2012). O'Sullivan et al. (2010) obtained biogas production in the range 200-400 L biogas $kg^{-1}$ volatile solids (VS). Water hyacinth biomass has relatively high carbon to nitrogen ratio, a characteristic desired in substrates for biogas production (Subhabrata et al., 2013, Omondi et al., 2019). However, the lignocellulose nature of water hyacinth may slow down hydrolysis process and conversion to biogas (Yadviva et al. 2004). The intricate structure of lignocellulose (Bajpai 2016) can limit microbial degradation and result in slow digestion and reduced biogas yield (Li, 2015). Techniques that are available for improvement of bio digestion include using different pre-treatment methods (Ofuofule et al, 2009); optimization of dilution on biomethanation of fresh water hyacinth (Patil et al, 2011) and effects of particle size, plant nitrogen content and inoculum volume. A simple and inexpensive technology for enhancing microbial degradation of the biomass is correlating process parameters, for example, by co-digestion with other substrates (Callaghan et al, 1999; Kumar and Sharma, 2017).

Studies have shown that synergies in simultaneous processing of substrates through co-digestion result in better performance than with individual substrates (e.g. Li et al. 2011; Rao and Baral 2011; Dias et al. 2014). Co-digestion has numerous advantages for microbial digestion that include reduced concentration of toxic

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compounds, increased nutrients concentration, improved substrate loading, supply of buffer capacity and hygienic stabilization of enzymes (Tufaner and Avsar, 2016). These benefits are important for stability and performance of the anaerobic process (Esposito et al. 2012). Consequently, co-digestion has potential for reduced hydraulic retention times and increased biogas yield. In some previous studies, food waste and cattle manure in the ratio of 2:1 has been found to enhance methane yield by 41.1% and 55.2% corresponding to 388 mLg-1VS and 317 mLg-1VS in batch and semi-continuous reactors respectively (Zhang et al., 2012). Earnest and Singh (2013) observed that co-digestion of fruit and vegetable wastes with cow dung in the ratio of 1:1 and 1:2 yielded 245 and 230 ml of biogas respectively and Gomez et al., (2006) found that biomethanation potential of primary sludge and vegetable fraction of municipal garbage under mesophilic conditions resulted in biogas yield of 0.60 – 0.80 $Lg^{-1}$VS compared to 0.4 – 0.6 $Lg^{-1}$ VS for co-digestion and primary sludge alone respectively. Co-digestion of cattle manure and organic kitchen wastes in the ratios of 1:3, 1:1 and 3:1 was found to enhance biogas yield from 24.12 to 47.13% while improving cumulative biogas yield by 1.01 – 1.84 times (Aragaw and Andargie, 2013).

Slaughterhouse waste has significant concentration of nutrients that can complement the digestion of other substrates such as water hyacinth (Wei wu, 2010, Omondi et al., 2019). However, most of the slaughterhouse waste components with the exception of ruminal waste, have large concentration of proteins, which make them susceptible to ammonia toxicity (Callaghan et al., 2002; Edstrom et al., 2003; Cuetos et al., 2010, Chen et al., 2008). Similarly, volatile fatty acids (VFAs) tend to accumulate in the reactors causing progressive drops in pH that stress and inhibit the activity of methanogenic archaea (Siegert and Banks, 2005). Rumen contents which have limited protein concentration and occur in the largest proportion in the waste would therefore be the desirable component for co-digestion with water hyacinth. Furthermore, rumen waste contains cellulolytic anaerobic bacteria that are suitable as inoculum for degradation of cellulose (Aurora, 1983; Castillo, 1995). This study evaluated synergy in co-digestion of water hyacinth with ruminal slaughterhouse waste in biogas production.

## 2. Materials and Methods

### *2.1 Overview of methods*

The study investigated biogas production in co-digestion of water hyacinth (WH) from Lake Victoria with ruminal slaughterhouse waste (RSW). The co-digestion was conducted in batch digesters while biogas output was measured by displacement method.

### *2.2 Sample collection and preparation*

Water hyacinth used was obtained from the shores of Winam Gulf, Lake Victoria, in Kisumu City at coordinates 0° 5'39.71"S, 34045'2.44"E while ruminal slaughterhouse waste was collected from Nairobi's Dagoreti Slaughterhouse located at coordinates 1°17'3.71"S, 36°41'1.98"E (Figure 1). Fresh and healthy mature water hyacinth plants were obtained and packed in sampling bags and transported, within 12 hours, to the University of Nairobi's Environmental Engineering Laboratory, where they were stored in a cold room. Approximately 4kg wet samples of fresh ruminal slaughterhouse were placed in sampling bags and transported immediately to the laboratory, where they were stored in a cold room at 4ºC until processing for study.

Approximately, 5kg of whole water hyacinth plants including leaves, stems and roots, were cut to small sizes of about 2 cm. Approximately 50 g was kept for determination of total water content while the rest was dried under the sun for a period of 7 days. The sun-dried water hyacinth was ground to fine particles using a mortar and pestle, placed in plastic bags and stored in a refrigerator. Approximately 50 g of the fresh slaughterhouse waste sample was kept for determination of total moisture content while the remaining portion was dried in the sun for a period of 3 days to improve handling and ease storage. The sun-dried samples were kept in plastic bags and stored for biogas production.

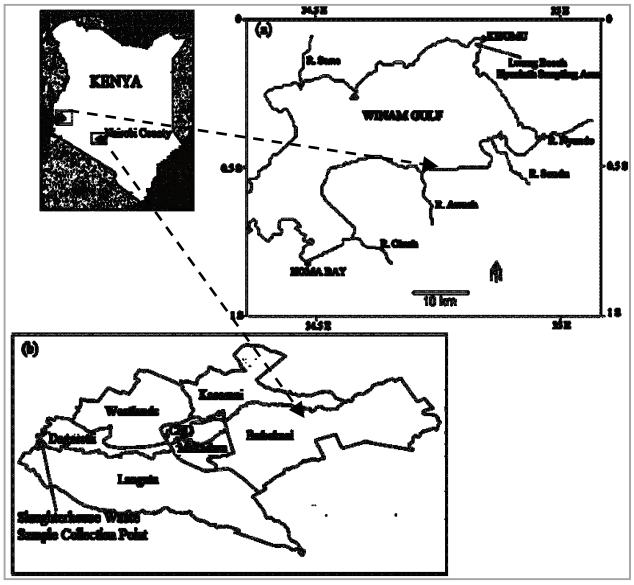


**Figure 1**. Map showing location of (a) WH Sampling Point in Winam Gulf, Kisumu, Kenya (b)Slaughter house Waste sampling Point, Dagoreti, Nairobi (From: Omondi et al. 2019))

### *2.3 Experimental set-up*

The experimental setup consisted of eight sets of three round bottom 1,000 ml flasks and a graduated measuring cylinder (Figure 2). All the flasks were fitted with tight fitting rubber cocks for airtightness. The first flask was used as the reactor for anaerobic digestion. The reactor was fitted with a thermometer and a pH meter, HI98103 checker pH tester from Hanna Instruments, for monitoring temperature and pH respectively. A balloon with a needle inserted into reactor headspace was set up to sample gas for characterization. The second flask contained a scrubber solution for $CO_2$ and other minor gases, comprising of an alkaline solution prepared using 1 molar sodium hydroxide solution, prepared by dissolving 40 g sodium hydroxide in 1 L of water. Three drops of phenolphthalein indicator were added for monitoring pH variation in the solution. The scrubber solution was replaced when the pink/violet colour of the indicator turned colourless. The change in colour is associated with a drop in pH below 8.2. The third flask was for gas

displacement of water for measurement of the volume of gas produced. Water in the displacement bottle was charged with a few drops of methyl orange to make it easier to read the volume in the graduated cylinder. The bottle was kept covered with an aluminium foil to minimize loss of water by evaporation.

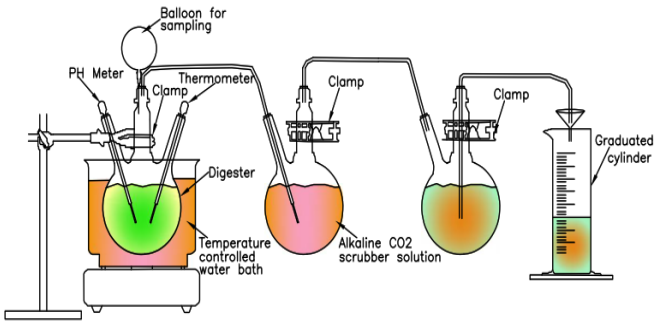


**Figure 2**. Biogas production set up (modified from: Omondi et al., 2019)

*2.4 Anaerobic digestion and biogas production*

Substrate for bio-digestion were prepared by mixing 150 g of WH and RSW in different proportion with 500 ml of water in 1000 ml round bottom reactor flasks; a total of eight reactor flasks labelled D1 to D8. The mix proportions used are shown in Table 1. The reactors were tightly sealed using rubber cocks and kept airtight to operate under anaerobic digestion mode for a residence time of 60 days. The biogas generated was passed through the scrubber solution. The volume of resultant methane gas was measured through water displacement method into the graduated measuring cylinder (Esposito et al., 2012). The cumulative volume of methane generated, pH and temperature were recorded daily at 9 am. Room temperature was also recorded throughout the test.

**Table 1.**
Mix Proportions of Dried Substrates

| Digester | Water Hyacinth (g) | Slaughter-house waste (g) | Percent of co-substrate (%) |
|---|---|---|---|
| D1 | 150 | Nil | 0 |
| D2 | 142.5 | 7.5 | 5 |
| D3 | 135 | 15 | 10 |
| D4 | 127.5 | 22.5 | 15 |
| D5 | 120 | 30 | 20 |
| D6 | 105 | 45 | 30 |
| D7 | 75 | 75 | 50 |
| D8 | 0 | 150 | 100 |

Gas for characterization was sampled in balloons through a needle in the headspace. Gas composition was determined, in triplicate for each parameter, using a gas chromatograph fitted with flame indication detector (GCFID) (Sugumaran et al., 2014). The reactors were operated at three different temperatures, room temperature of about 24ºC, 32 and 37ºC.

*2.5 Biogas characterization*

The quality of biogas depends mainly on the presence of methane in it where a good quality biogas has high percentage of methane and is therefore desirable for maximum energy production. The percentage of methane in biogas is generally determined by the Orsat apparatus, gas chromatograph etc. (Holman, 1995). The percentage of methane $CH_4$ can be estimated through recognition of $CO_2$ percentage from Equation 2:

$CH_4 = 100\% - [CO_2\% + 0.2\% H_2S]$ ..... vol. % (Konstandt 1976) (1)

In this estimation, methane content is measured by absorption of carbon dioxide with10%, 33% and 40% of KOH (Habel-Hadi, 2008) respectively. The assumption by using this method is that biogas is mainly constituted of methane and carbon dioxide gas, where the other gases produced during anaerobic process are neglected. Gas Chromatography (GC) is an optimal analytical instrument for the analysis of components such as $CH_4$, $CO_2$, $H_2S$ and siloxanes which are present in the gas (Anderson et al., 2010).This study adopted GC method in analyzing produced biogas.

## 3. Results and Discussions

*3.1 Variations of pH with duration of co-digestion*

The various stages of anaerobic digestion take place at different pH and hence the pH of the digesting substrates can give an indication of the dominant digestion stage at any time and its duration. Generally, the first digestion stage, hydrolysis of lipids and protein to volatile fatty acids and amino acids, resulted in a drop in pH while the onset of acidogenesis stage resulted in rise in pH due to production of $CO_2$ and $NH_3$ and the associated $CO_3HNH_4$ (e.g. Malakahmad et al., 2012). Further rise in pH occurred in the predominantly methanogenesis third stage because of ceased hydrolysis of volatile fatty acid and continued production of $CO_3HNH_4$.

The hydrolysis stage for RSW mix proportion of less than 15% had a pH less than 6.2 (Figure 3a) but increased to 6.8 to 7.5 for the RSW proportions of 20 -100%. Varying the RSW proportion from 15 to 20% resulted in greatest increase in the hydrolysis pH from about 6.2 to 6.8. Moreover, the reduction of duration of the hydrolysis stage with increasing RSW proportion, from 33 days for 5% RSW to 25 days for 50%, maybe an indication of prolonged acidogenesis and methanogenesis stages with co-digestion. The observation correlates with previous studies (e.g. Feng et al., 2009).

For the 30% RSW proportion, digestion at different temperatures (24, 32 and 37ºC) showed varied changes in pH with time (Figure 3b). After the seventh day, when pH was similar for all the reactor temperatures, there was a clear pattern of higher increase of pH with temperature, which may be a result of increased biological activity.

*3.2 Biogas production for various substrates mix proportion*

In Figure 4, variations in cumulative biogas production over 60 day for reactors with different proportions of WH and RSW operated at room temperature are presented. During the first 7 days, all the mix proportions except 50% and 100% showed some increase in cumulative biogas production. This indicates excellent but acclimatization of WH and WH with low portions of RSW. This may be attributed to high volatile solids originally present in WH biomass which leads to volatile organic acids produced

during hydrolysis of the substrate that tend to reduce the pH, an effect that is counteracted by destruction of the volatile acids and reformation of bicarbonate buffer during methane formation. The 50% and 100% showed a lag in the initial days, with no or minimal production of biogas which may also be attributed to low volatile solids originally present in RSW biomass which leads to slow acclimatization but quick hydrolysis of the substrate characterized by minimal pH drop with methane formers quickly outpacing the acid formers in the leading to a stable biogas yield. Achieving a balanced condition requires careful co-digestion to overcome the low growth rate of methane bacteria and achieve a stable AD process (Kugelman, 1971). In this study, the largest biogas cumulative yield was observed for slaughterhouse waste alone (100% RSW) (17.8 L $CH_4$/kg substrate) followed by 50 and 30% RSW while the smallest yield was for water hyacinth alone (0% RSW) at 8 L $CH_4$/kg substrate.

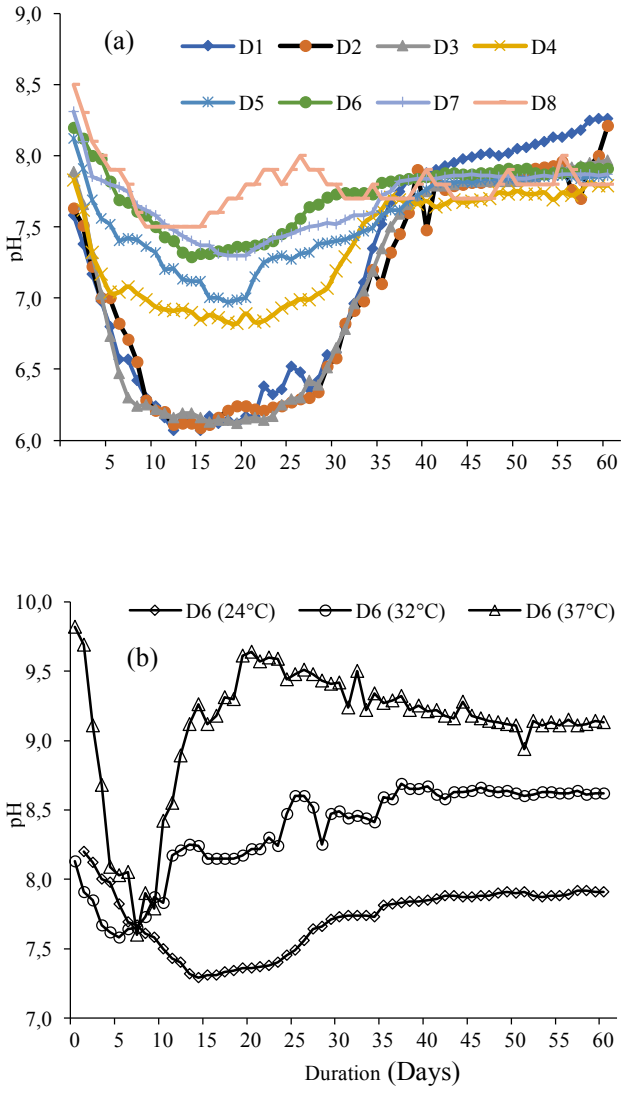


**Figure 3.** pH variations for (a) different RSW substrate proportions (D1=0%, D2=5%, D3=10%, D4=15%, D5=20%, D6=30%, D7=50%, D8=100%) at 24°C; and (b) 30% RSW mix proportion at 24, 32 and 37°C

### *3.3 Effect of co-digestion on retention time*

The effect of co-digestion on retention time (RT) was determined by relating the time it takes to produce equivalent volume of methane for WH alone (0%RSW) at 60 days. Thus, the time it takes to produce 8L/kg methane was determined for various mix proportions in co-digestion (Fig 4). From the results, co-digestion reduced the retention time by 9, 15, 18, 20, 22 and 26 days for 5, 15, 10, 20, 30 and 50% RSW respectively. The results indicate that, co-digesting WH biogas plant with 30% RSW for example will reduce RT by 22 days. Therefore, co-digestion of WH with 5-50%RSW has significant reduction on RT. Co-digestion with proportions greater than 50% RSW will have no further impact on RT as the reduction in RT for RSW alone coincides with that of 50% RSW.

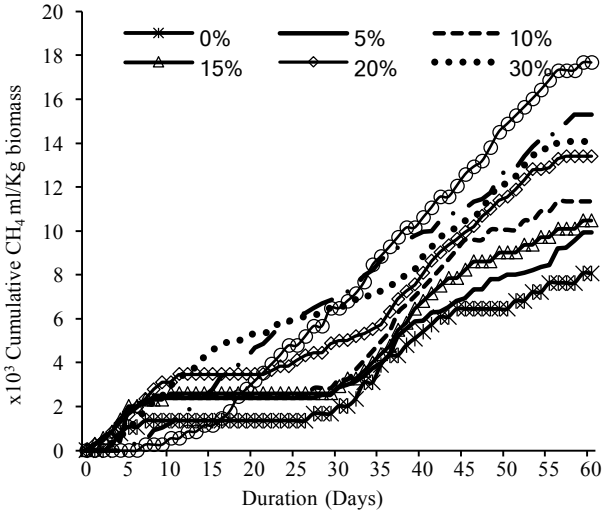


**Figure 4.** Biogas production for various mix proportions at 24°C

### *3.4 Influence of temperature on biomethenation*

Co-digestion of WH and RSW at 30% RSW proportion at various temperatures; namely, 24, 32 and 37°C showed the influence of temperature on biomethanation. For the higher temperatures, startup time reduced from three days to one day. The rate of biomethanation improved from 0.23 to 0.75 and 0.96 at 32 and 37°C respectively. Increasing the temperature from 24 to 32°C increased methane yield from 14 to 40 L/kg or 186%, but increasing the operating temperature to 37°C only increased the yield by a further 30% to 52 L/Kg (Fig. 5). Consequently, increasing the operating temperature from 32 to 37°C may not be merited unless the cost benefit of the gas production and reduced capital cost surpasses the extra cost of energy.

Apart from the increase in biogas yield, change in temperature from 24 to 32°C reduced the retention time by 44 days, from 60 to 16 days (Fig. 5). Further increase in temperature from 32 to 37°C only reduced the retention time by 2 more days, a reduction not very significant to justify the temperature increase.

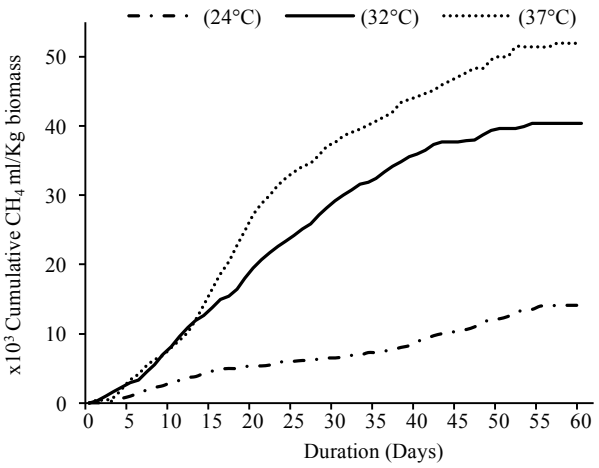


**Fig 5.** Cumulative methane gas production for 30%RSW at 24, 32 and 37°C temperature conditions.

*3.5 Biogas characteristics*

The percentage compositions of biogas produced at 24ºC for different RSW are presented in Figure 6. Methane gas proportion increased with increase in RSW mix proportion in the reactor mixture, from 59% for water hyacinth alone (0% RSW) to a maximum value of 68% for 20% and 30% RSW mix proportions and then decreased to a minimum value of 58% for RSW alone (100% RSW). In contrast the composition of $CO_2$ in the biogas decreased with increasing RSW mix proportion in the reactor mixture from 39% for water hyacinth alone (0% RSW) to about 30% for 15%, 20% and 30% RSW mix proportions before increasing to 58% for RSW alone reactor. The proportion of trace gases in the biogas was highest for the RSW alone reactor (4%), approximately double the amount in the other reactor mixtures (2%). Consequently, the co-digestion of WH with RSW improved the quality of biogas compared to digestion of either substrate alone. This demonstrates a synergy in improvement of biogas quality through co-digestion of the two substrates.

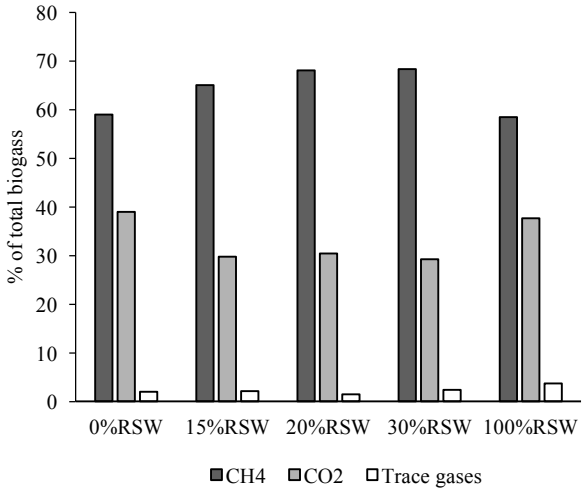


**Figure 6**. Percentage proportions of methane, carbon dioxide and trace gases in the biogas produced for different co-digestion mix proportions operated at 24°C

## 4. Conclusion

This study found that co-digestion of water hyacinth with slaughterhouse increased biogas production by between 25 and 66% for 5 to 20% RSW mix proportion and resulted in 8% improvement in biogas quality. A RSW of 20% produced the best weighted return per unit RSW used. Co-digestion of WH with RSW reduces the pH fluctuations during the hydrolysis and in turn increases acclimatization and biogas yield Varying temperature from 24 to 32ºC had a significant impact in the biogas yield for co-digested WH and RSW biomass at 30% RSW. Further increase in temperature from 32 to 37ºC demonstrated insignificant increase in biogas yield and impact on retention time. This study therefore recommends co-digestion of WH with 30% RSW at 32ºC. Future studies can determine the outcomes between 24 and 32ºC.

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