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Research article

Evaluation of Optimal bio-methane Potential of *Cuscuta japonica*: Weed Control

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ABSTRACT

Invasive weeds have been a constant threat to Kenyan ecosystem endurance with ecosystem with trees being greatly affected which might cause species loss. The growth of Cuscuta japonica is spreading fast, which could be attributed to factors such as climate variability, transmission agents i.e. water, people and birds making it even hard to control. Substrate collection was done mechanically before being chopped and chemically pre-treated. Elemental composition found was used in calculating theoretical methane potential found to be 577.71 mL CH4 VS added. TS, VS, TOC obtained were 13.76% (w/w), 12.60% (w/w) and 41% DM respectively. The BMP study was done in a batch assay process with the use of bioreactors where the carbon dioxide was removed, and the final gas produced was only methane. The number of runs used in this experiment was 9 and the factors were determined by Design Expert 13 i.e. Central Composite Design (CCD). The highest specific cumulative methane yield produced was 571.00 mL CH4 g- VS added at ratios 2.5:1 with the highest biogas produced per gram of VS being 112.00 mL CH4 g- VS added. (2:1) making it a prime substrate for bio gas production, making it one of the potential candidates for Cuscuta japonica control management.

Keywords: Invasive weeds, biogas, Cuscuta japonica, batch assay, Biogas yield, Climate variability

1. Introduction

Crop production is facing a challenge from invasive weed interference affecting over ten percent of global agricultural production (Khanh et al., 2008). Dodder weed invasion has become problematic to control once it establishes on host plants (Sarić-Krsmanović, 2017). With dodder weed invasion, global biodiversity and agricultural productivity have been put at stake with several developing countries being highly affected (Kobilinsky, 2016). Kenya depends mostly on farming as its source of income (Alessandro et al., 2015) and with this invasion, the production is expected to drop thus impacting greatly the already disadvantaged food security. Most parts of Kenya are composed of arid and semi-arid areas (ASALs) (Birch, 2018) and many of these regions depend on livestock farming based on shrub vegetation feeding (Elizabeth & Isaiah, 2019) which affects the food chain through loss of biodiversity (Linders et al., 2019). Dodder weed in Kenya has received less attention until recently when the harm was witnessed in several parts and its spread is fast. The affected parts include most of Western Kenya, Eastern and coast (Ngare et al., 2020: Chepkirui, 2020: Nunda, 2021). The forest ecosystem has received similarly great impact and this has contributed to the decline of forest cover percentage (Xuequan, 2017) and thus greatly impacts negatively food security and causes environmental damage (Wenning, 2020).

This has led to intervention from stakeholders concerned, such as Kenya Agricultural and Research Organization (KALRO), Kenya Forest Service (KFS) and Kenya Forestry Research Institute (KeFRI) that are working tirelessly on joint effort to find a long-lasting solution for the dodder problem(Williams & Constantine, 2019). They are currently performing chemotaxonomic studies, research on dodder selective contact herbicides and on the possibility of dodder being used as animal feed. There is an increased energy demand in Kenya causing an imminent threat to expand existing agricultural and ecosystem problems, but simultaneously offers a possibility for shifting to environmentally friendly energy sources and thus reducing demand on firewood (Rahnema et al., 2017). Dodder may play an important role in energy generation (Chiumenti et al., 2018). Namely, dodder weed possibly could be greatly utilized through the anaerobic digestion (AD) technique for biogas production. Biogas has recently gained world attraction due to its economic and environmental benefits. This study focuses on a sustainable method of controlling dodder *Cuscuta* which has parallel environmental benefits. Besides dodder control with biogas yield from anaerobic digestion of it.

2. Materials and Methods

2.1 Substrate collection

John Michuki Memorial park is a 12.3 ha which was gazette in 2008 to recreation center through dumpsite rehabilitation (Sonkoyo & Wasonga, 2020). It is situated in the central district of Nairobi and this land was reclaimed from a dumpsite. During the reclamation process, over 20,000 tons of waste which allowed over 6,357 indigenous trees was planted. On The part of the reclaimed land, a section was set for orchard where fruits are planted. Michuki Park is among the places in Kenya which has been greatly invaded by dodder weed with *Cuscuta campestris* and *Cuscuta japonica* being dominant. Sample wet biomass of 3.44 kg of *Cuscuta japonica* was picked from the hanging from a tree mechanically. The samples were then physically by milling before chemical pretreated with 0.3 M sodium hydroxide (NaOH).

Figure 1: Cuscuta japonica hanging on tree at Michuki Memorial Park

2.2 Samples preparation

Received samples were weighed and mass recorded. Physical and chemical pretreatment were done to the sample from reducing size to 30mm. Sodium hydroxide (NaOH) (0.3M) was then used in the chemical pretreatment. Physicochemical characteristics were done immediately to avoid some environmental related errors which could be associated with either gaining or draining of the parameters to be measured such as moisture content or dry matter percentage.

2.3 Characterization

Physicochemical characterization of the Cuscuta japonica sample done included pH, TOC, VS, TS, Ash content, Nitrogen Content and Moisture Content. The total solids and moisture content was determined after drying in an oven for an overnight at 1050C while the Volatile solids at 5500C and ash was done at 680 oC until it reached uniform color. Total organic Carbon was determined using EPA method 9060A on “total organic carbon” standard nitrogen content was determined using Kjeldahl method EPA method 1687. Dry matter mass consistency was able to be achieved after an hour using DIN EN 14774-2 until <0.4% consistency is achieved while elemental analysis was done using LECO analyzer.

2.4 Experimental design

Response Surface Model (RSM) determined the interaction effects and levels in experimental setup caused by parameters being tested through optimization. Mathematical models present in Design-Expert software help by using graphical and numerical solutions. First, the parameters used in the study were identified which varied ratio of substrate to inoculum affecting biogas yield. Temperature was kept constant at 350C while pH was raised to a constant 7.0 by adding sodium hydrogen carbonate ($NaHCO_3$) as a buffer solution. Central Composite Design (CCD) model was used in determining number of factors and levels which provided number of treatment combinations used for the experimental test. The factors studied were organic substrates being added (VS) and inoculum (UASB) by varying ratios to evaluate the optimal performance and yield of the Cuscuta japonica. The levels obtained by the CCD model was 9 runs which had 2 blank tests used in control i.e. hot (0.5ml 96% ethanol as inoculum) and cold control (with no inoculum added), while the other 7 runs had varying number of substrates and inoculum added.

2.5 Procedure

2.5.1 Experimental design setup

Bioreactor was used in the experiment. The bioreactor system contains three units which involved the incubator, carbon dioxide gas absorption and pressure measurement unit and gas collection unit. In the incubation unit, the samples placed in bioreactor BMP bottles are magnetically stirred. The produced gas is then passed through the carbon dioxide absorption unit which contains sodium hydroxide (NaOH) which absorbs CO_2 allowing only methane to be present in the bioreactor. A pH indicator is added to each of the CO_2 absorbing unit to control acid binding capacity. The volume of methane gas is measured by pressure change done with the piezoelectric system. Theoretical reference value for the hot control with ethanol: one mole of ethanol produces 2 moles of biogas with 75% CH_4, if 0.5 ml of 96 % ethanol is added, the expected methane volume is:

$$0.5ml \times 0.81 \times \frac{0.96}{46g} \times 2mol \times 22.4L \times 0.75 \times 1000 = 284ml\ CH_4$$

Where the molar mass of ethanol is 46g, density of ethanol being 0.81 whiles the volume of one mole of gas at standard temperature and pressure being 22.4l. In the cold control, no or minimum methane production may be measured.

3. Results and Discussion

3.1 Physicochemical characteristics

3.1.1 Substrate Characterization

The tests were done within the optimal pH of 7.0 with sodium hydrogen carbonate (NaHCO3) being added to the substrates as a buffer due to its ability to balance acidity formed during the digestion. The UASB granules provided by a wastewater plant in France worked well at pH 7.0. From the tests done, TS and VS were found to be 13.76% (w/w) and 12.60% (w/w) respectively with the TS: VS ratio was found to be 1.09. Moisture content has a direct correlation with both VS and TS which helps in the metabolic process, acting as an essential medium of microorganisms and nutrients and can displace water in porous spaces which improve the formation of anaerobicity the moisture content of the substrate was 86.24% (w/w). The amount of organic carbon found in Cuscuta japonica was 41% and this played a crucial role in biogas production. The nitrogen ratio was found to be 2.05% and in the determination of the C: N ratio it was found to be 20. This is within the optimal range for biogas production making the Cuscuta japonica an ideal candidate for anaerobic digestion.

Parameter	Results
Theoretical BMP	577.71g CH_4- VS
Total solids	13.76% (w/w)
Volatile solids	12.60% (w/w)
Total organic carbon	41% /DM
Moisture content	86.24% (w/w)
Ash content	1.16% (w/w)
TS/VS ratio	1.02
C:N ratio	20
Total Nitrogen content	2.05%/ DM
pH	5.23

Table 1: Physicochemical characteristics of Cuscuta japonica

3.1.2 Experimental

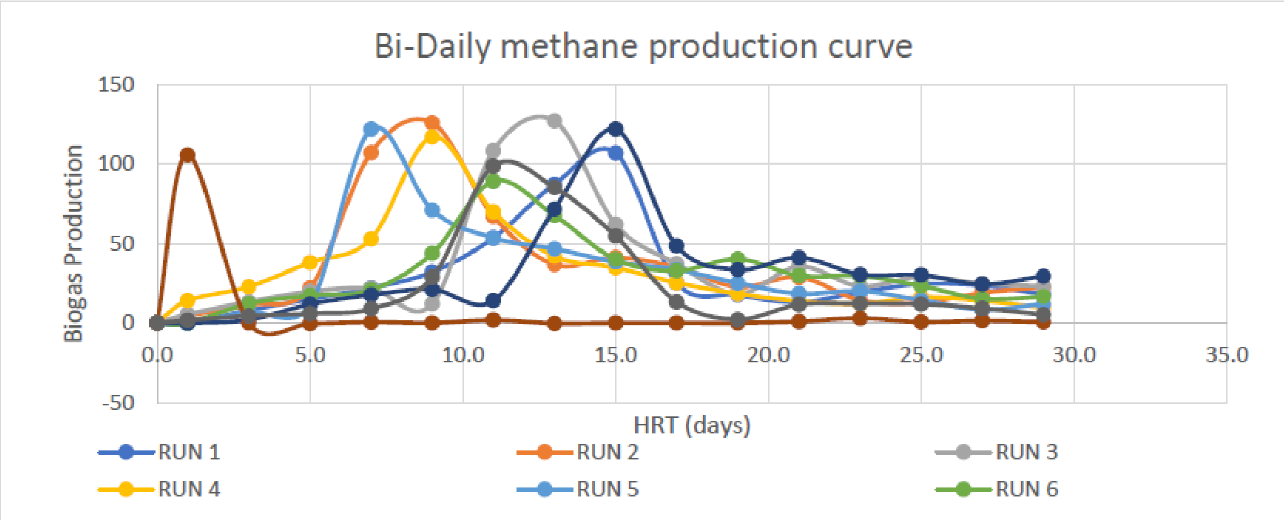


Figure 2: Bi-daily methane production

The biogas production was followed for 29 days. In the first period, the biogas production was poor excepting the cold control. This phenomenon might be because the temperature shifts in this bottle were more rapid than in the others (inoculum not added). From the third day, the digestion started in all the digesters. The daily increase in digestion became eminent on the 5th day of digestion in bottles 2, 4 and 5 with peaks reached on day 9th and 7th respectively (126, 117 and 122 mL CH4-VSadded), showing maximum microbial activity. Run 3, 6 and 9 started the steep slope on day 10 showing that microbes had stabilized, with most peaks of these being on days 11 and 13. Bottles 7 and one had the latest peaks starting from day 11 with its peak on the 15th day. Bottle 3 (560 mL CH4-V Sadded) had the highest biogas production while Bottle 8 had the lowest (114 mL CH4-VSadded) methane production, which is not a valid value because there was no inoculum added. When the substrate has depleted the methane, production started to drop. Controls which were used exhibited some characteristics expected during the process with cold control not producing any of the gases except during the first thermal shock introduced at the first instance which broke down some of the organic materials without addition of inoculum. Hot control data was found nearly within the range which from the calculation of the control calculation.

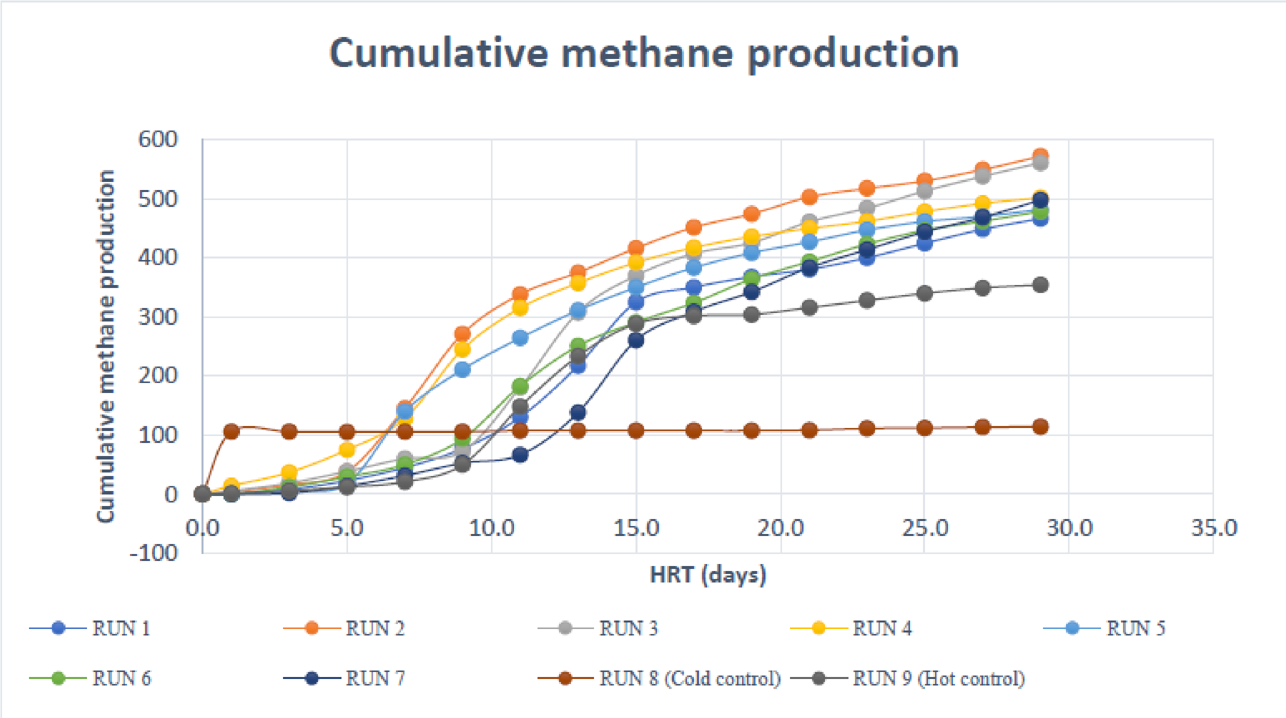


Figure 3: Cumulative biomethane potential of Cuscuta japonica

The gram production per gram of VS was higher on the ratio 2:1 with 10g of inoculum with 5 gram of substrates added with 112.00 mL CH4 /g VSadded while the lowest on gases produced being 25.05 mL CH4- /g VSadded of the VS added.

RUN	Cumulative methane ($mL\ CH_4 /g\ VS_{added}$)	Substrate added (g)	Inoculum	Ratio I/S	($mL\ CH_4$ / g VS_{added})
1	465	10	20	2.0	46.50
2	571	6	15	2.5	95.17
3	560	5	10	2.0	112.00
4	501	20	20	1.0	25.05
5	481	17	22	1.3	28.29
6	477	17	15	0.9	28.06
7	497	6	8	1.3	82.83
8	114	10	0	10.0	11.40
9	353	10	0.5 ml	20.0	35.30

Table 2: Table of cumulative methane and methane production per gram of VS.

3.1.3 Theoretical methane potential

	Elemental Composition	Molar mass	Buswell ratio
Carbon	41	12	3.417
Hydrogen	6.23	1	6.230
Oxygen	29.23	16	1.827
Nitrogen	2.05	14	0.146
Sulphur	0.45	32	0.014

Table 3: Buswell ratio and elemental composition

3.1.4 Buswell equation

$$CaHbOcNdSe+(a-b4-c2+3d4+e2)H2O \rightarrow (a2+b8-c4-3d8-e4)CH4+(a2-b8+c4+3d8+e4)CO2+dNH3+eH2S$$

In order to get chemical formulae of the feedstock, each element is divided by the molar mass in order to give the ultimate analysis in the ratio C: H:O:N:S (Achinas & Euverink, 2016)

$$a = \frac{a\ ultimate\ analysis}{12.0107}$$

$$b = \frac{b\ ultimate\ analysis}{1.0079}$$

$$c = \frac{c\ ultimate\ analysis}{15.999}$$

$$d = \frac{d\ ultimate\ analysis}{14.0067}$$

$$e = \frac{e\ ultimate\ analysis}{32.065}$$

$$BMP_{Theoritical} = \frac{\left[\frac{a}{2}+\frac{b}{8}-\frac{c}{4}-\frac{3d}{8}-\frac{e}{4}\right] \times 22.4}{12a+b+16c+14d+32e}$$

$$BMP_{Theoritical} = \frac{\left[\frac{3.417}{2}+\frac{6.230}{8}-\frac{1.827}{4}-\frac{(3 \times 0.146)}{8}-\frac{0.014}{4}\right] \times 22.4}{(12 \times 3.417)+(1 \times 6.230)+(16 \times 1.827)+(14 \times 0.146)+(32 \times 0.014)}$$

$$BMP_{Theoritical} = 577.71\text{mL}CH_4/\text{VS}$$

According to VDI 4630 guideline, experimental cellulose should produce about 80% of theoretical maximum yield (Himanshu et al., 2017)

Run	Cumulative methane (CB) (mL CH_4 / g VS_{added})	Theoretical methane (TB) (mL CH_4 /g VS_{added})	TB-CB	Efficiency (%)
1	465	577.71	112.71	**80.49**
2	571	577.71	6.71	**98.84**
3	560	577.71	17.71	**96.94**
4	501	577.71	76.71	**86.72**
5	481	577.71	96.71	**83.26**
6	477	577.71	100.71	**82.57**
7	497	577.71	80.71	**86.02**
8 (Cold control)	114	577.71	463.71	**19.73**
9 (hot control)	353	577.71	224.71	**61.10**

Table 4: Efficiency, experimental and theoretical methane potential of Cuscuta japonica

3.1.5 Biogas optimization

Biogas production was optimized with the SIR and OLR of the Cuscuta japonica being carried out in AMPTS II digester. Design of Experiment (DOE) was utilized and produced the BMP yield optimization, factors and levels which was used for the study, which led to two factors i.e. substrates organic loading rate and inoculum being added. Response surface model (RSM) using the CCD option provided 9 levels (runs) produced from different values or setup conditions with the response (R1) being BMP yield (mL CH4-VSadded).

3.1.6 ANOVA analysis

Analysis of Variance

Source	*Sum of squares*	*df*	*Mean Square*	*F-value*	*p-value*	
Model	1.549+05	5	30983.93	108.80	0.0014	significant
A-Substrates added	1374.65	1	1374.65	4.83	0.1155	
B-Inoculum	21907.87	1	21907.87	76.93	0.0031	
AB	2398.86	1	2398.86	8.42	0.0624	
A^2	70.33	1	70.33	0.2470	0.6534	
B^2	21539.15	1	21539.15	75.63	0.0032	
Residual	854.34	3	284.78			
Cor Total	1.558+05	8				

Table 5: Analysis of Variance (ANOVA)

3.1.7 Fit statistics

Std. Dev	*16.8755*	*R^2*	*0.9945*
Mean	446.6667	Adjusted R^2	0.9854
C.V. %	3.7781	Predicted R^2	0.9423
		Adeq Precision	32.0839

Table 6: Fit statistics table

"The Predicted R^2 of 0.9423 is in reasonable agreement with the adjusted R^2 of 0.9854; i.e. the difference is less than 0.2 which falls within the acceptable agreement. The actual value of R2 of 0.9945 is closer to 1 and this signifies a high correlation existing between experimental values and predicted values. The response model has 95% confidence level showing its significance with the model F-Value of 108.0 with only 0.14% chances that F-value will be large if noise occurs. P-values with less than 0.05 indicate that the model being used is significant showing that B, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. In Adeq Precision measures the signal to noise ratio, a ratio greater than 4 is desirable with ratio of 32.08 indicating adequate signal indicating that this model can be used in the design space." Design Expert 13

3.1.8 Diagnostics

In order to ensure that assumptions made by the ANOVA are met, residuals and diagnostics plots are need to ensure the feasibility of the model are met, normality vs. residual plots are supposed to be in a linear and straight line whereas residual vs. predicted response need to be a random scatter plot (MiniTab, 2021). Errors in normal vs. residual plot were normally distributed because most of the residuals fell on a straight linear line. Independent and variance assumptions proposed were met from the normal vs. predicted response plot due to random scattered with unpredicted pattern.

Residual vs. predicted plot showed scattered data points which are near to each other showing no common pattern and thus by increasing other factors which were not tested in this experiment could increase biogas yield and thus showing independence of the model. Actual vs. predicted model values in biogas corresponds to the operating conditions for biogas can be explained on the regression model and from experimental data showed data points close to line best of fit form the model and thus normal probability plot indicated a good validity and significance for approximation of the regression model.

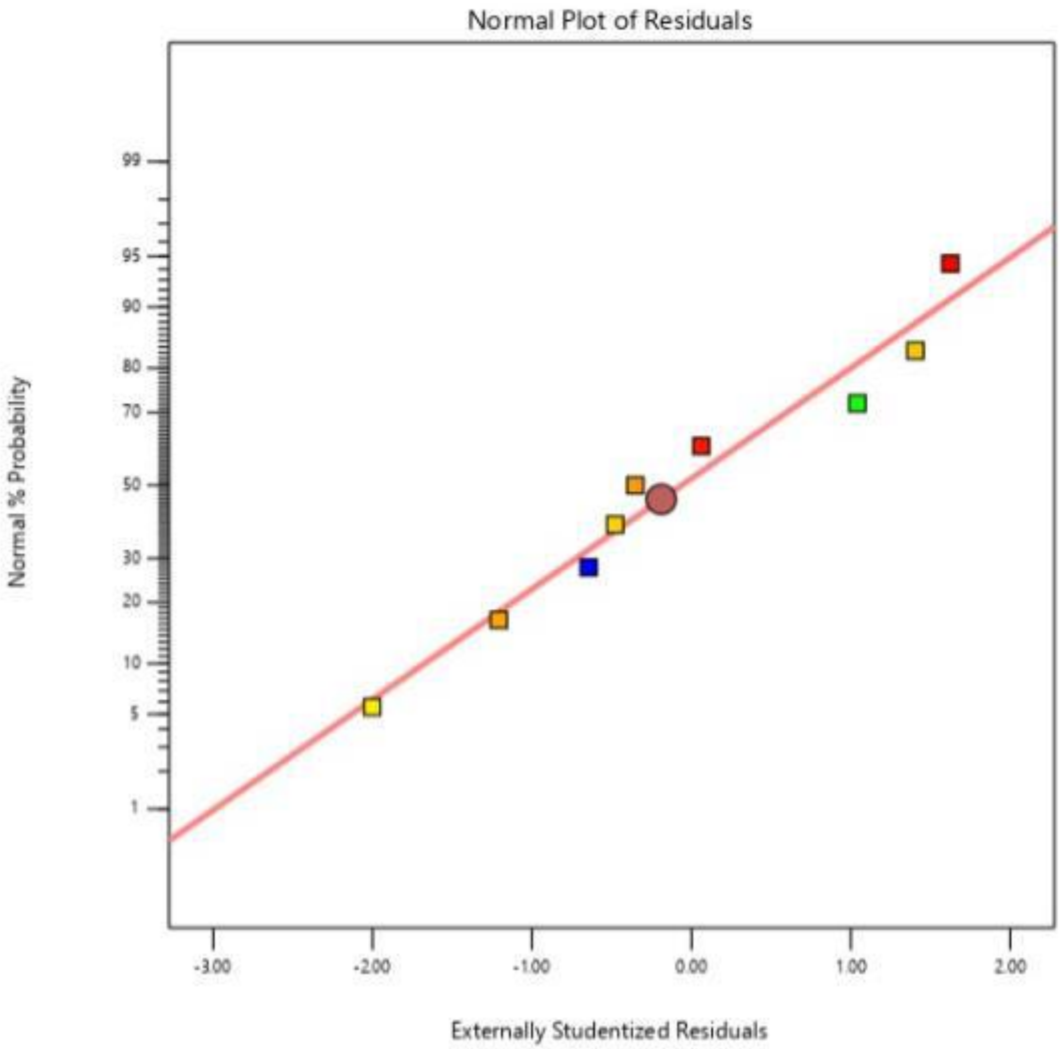
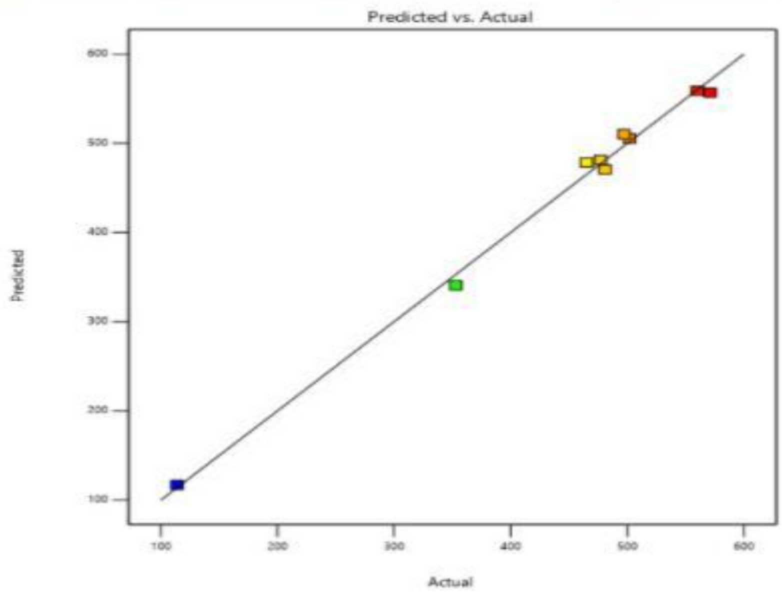


Figure 5: Normal vs. Residuals and predicted vs actual



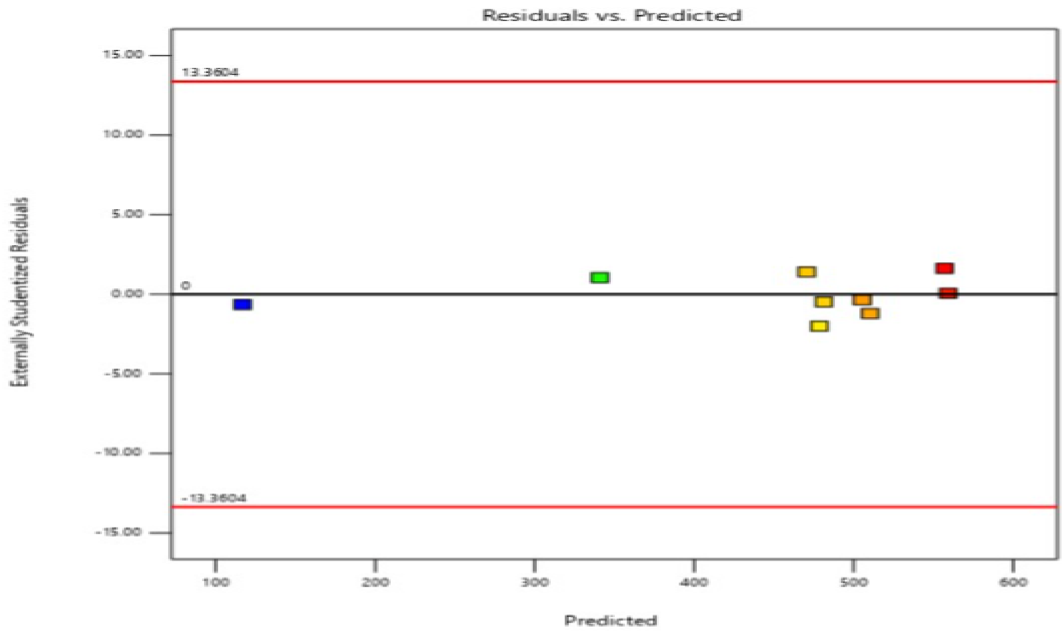
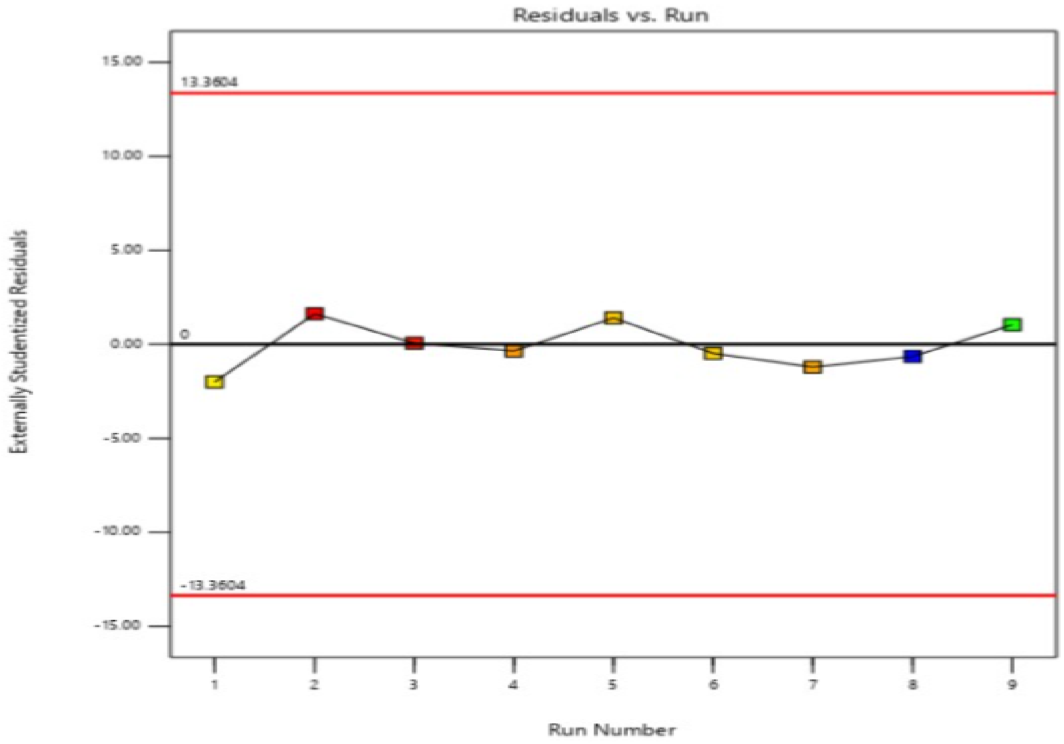


Figure 6: Residual vs. Prediction and residual vs. Runs



4. Conclusion

Cuscuta japonica showed a good potential for biogas production from the studies. The data obtained shows the importance of using biogas technology from Cuscuta japonica in meeting energy demand and curb food insecurity by utilizing the invasive weed as a way of managing it by farmers. This shows that with the increased agricultural threat from Cuscuta japonica, the benefits outweigh the challenges such that it can be used in overcoming the energy challenges which is a challenge to the

Kenyan ecosystem. In terms of sustainability, the ratios in which Cuscuta japonica has the greatest potential is when the ratio was 2:1 (inoculum to substrate ratio) thus supporting the challenge being faced now. From the findings, increasing the inoculum increased the biogas production and thus the substantiality.

COMMENDATION

Further studies need to be done especially on co-digestion to check optimal biogas yield with other substrates. Use of other pretreatment methods apart from chopping because might be a reason for experimental values varying greatly with theoretical methane potential.

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