Circularity into Biogas Production : Characterization of The Principal Component; Rabbit Droppings Case

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Abstract— Circular economy is an economic system that aims to minimize waste and maximize the use of resources. For anaerobic mono digestion food waste alone was used as the substrate while for anaerobic co-digestion, two mixing ratios of rabbit droppings with macadamia nutshells were used (90:10, 80:20, 70: 30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90% respectively). To the researchers' knowledge the optimum amount of CH_4 produced by the anaerobic co-digestion of rabbit dropping and macadamia nutshell has not been predicted using Response Surface Methodology. The co-digested samples with different loading ratios of (90:10, 80:20, 70: 30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90% were characterized in terms of C/N ratio, total solids (TS), volatile solids (VS), moisture content and pH. They were measured by the standard method. For proximate analysis moisture, volatile solid, fixed carbon was found to be 4.7526%, 74.97%, 11. 46% wt respectively. The time required to dry the rabbit droppings under open sun drying conditions from the initial mass content of 50 ± 0.1 g and 100± 0.1g water/g dry sample to the final mass content of 34± 0.001 g and 70± 0.001g water/g dry sample was 150 minutes. The coefficient of determination (R^2) value was determined to be $R^2 = 0,9986$ and $R^2 = 1$ for 50g and 100g respectively. A four-factor central composite design was used for the anaerobic co-digestion experiments. The results showed that the optimized conditions which could yielded a high-purity/yield product were carbon to Hydrogen ratio (78-79), pH of (6.0-6.6), volatile solid ratio of (78-79), and retention time of 15 days, respectively. This combination yielded a methane product with ~300 ml yield and ~57% purity. Furthermore, the coefficient of determination (R^2) value of 0.7010 and 0.6689 for methane yield and purity respectively in the present study proves that the model was adequate and suitable. Therefore, this model may be used to navigate the design space.

Keywords—Anaerobic co-digestion, Rabbit dropping, macadamia nutshell, Response Surface Method.

I. Introduction

The anaerobic digestion (AD) process is a biological process that converts organic solid wastes, such as agricultural residues, food waste, and animal manure, into biogas through the action of microorganisms in the absence of oxygen [1] (Mata-Alvarez et al., 2000). This process involves a series of microbial reactions, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Mata-Alvarez et al., 2000). During anaerobic digestion, complex organic compounds are broken down into simpler compounds, such as volatile fatty acids and alcohols, which are then converted into methane (CH_4) and carbon dioxide (CO_2) by methanogenic bacteria (Mata-Alvarez et al., 2000). In the case of rabbit manure, anaerobic digestion can be employed to convert the organic waste into biogas, which primarily consists of methane and carbon dioxide. The process typically involves collecting the rabbit manure and transferring it to an anaerobic digester, which is a sealed container or system where the digestion process takes place. The manure is mixed with water and maintained under anaerobic conditions, allowing the microorganisms to break down the organic matter and produce biogas. The produced biogas, which mainly consists of methane and carbon dioxide, can be collected and utilized as a renewable energy source. It can be used for various purposes, such as heating, electricity generation, or as a fuel for vehicles. The remaining digested material, known as digestate, can be further processed and used as a nutrient-rich fertilizer. Anaerobic digestion offers several benefits, including the production of renewable energy, reduction of organic waste, and the potential for nutrient recovery. It contributes to the circular economy by converting organic waste into valuable resources and reducing greenhouse gas emissions.

The co-digestion of manure and crops has been demonstrated to produce a synergistic effect since it promotes the adequate balance of the carbon-to-nitrogen (C/N) ratio[1], [2], [3].(Li et al. 2013b; Mata-Alvarez et al. 2014; Schnurer et al. 2016).To the researchers' knowledge the optimization of anaerobic co-digestion of Rabbit dropping and macadamia nutshell for methane production has not been predicted using Response Surface Methodology. The response surface model (RSM) is a simplified relationship that can be used for practical engineering purposes, where high costs, high energy and power consumption of engineering processes is not desirable (MAXIMISING/ MINIMISING). RSM is a set of statistical and mathematical techniques used in order to: Establish a series of experiments to accurately predict a response. Determining optimum conditions on the input variables of the model, which result in maximum or minimum response within a region of

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interest with the aim to optimize the response. Response surface design methodology is often used to refine models after you have determined important factors using screening designs or factorial designs; especially if you suspect curvature in the response surface. References [4][3][5][6][7] Tetteh et al. (2018), Lin et al., 2021; , Sukhesh et al., 2019; , Menon et al., 2015), and Deng et al. (2019) demonstrate the application of response surface methodology (RSM) in optimizing biogas production from various substrates, including cow dung, swine manure, citrus pulp, lawn grass, chicken manure, food waste, peanut hulls, and swine manure. RSM is used to determine the optimal operational parameters and substrate mixing ratios to maximize biogas production.

In these study the researchers want to obtain the optimum condition for methane production by anaerobic co-digestion of Rabbit dropping and macadamia nutshell. There are several operational parameters that impact on the biodigester performance, including organic loading rates, biodigester mixing, temperature control, pH, nutrient and trace elements, and C/N ratios should be taken into account [3] (Schnurer et al. 2016). In this context Response Surface Methodology is a DOE that constitute a key tool in order to determining optimum conditions on the input variables of the model especially when the the optimum condition for the optimum amount of CH_4 by the anaerobic co-digestion is to be obtained. The anaerobic process performance will be evaluated by means of the determination of the theoretical methane potential, the specific methane yield, and the degree of degradation of the substrate.).

II. Literature Review

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TABLE I Summarized outcomes form a review of the literature.

Input materials	Method	Biogas Yield	%CH_4	References
Rabbit waste, pig waste and rabbit wastes with pig manure	**Experiments on anaerobic digestion were made in 6-litre digesters at 37°C with manual loading once a day and mixing for 3 min each hour.**	**215 litres kg−1 VS**	**(58.03 ± 2.57), (68-74 ± 1.10 %) and (68.08± 1.16%) respectively.**	**[8] (Aubart & Bully, 1984)**
food waste with cow dung	**Three digesters of 500 ml were used and placed into a water bath at 45°C through a Biochemical Methane Potential (BMP) Test using the Automatic Methane Potential Test System II (AMPTS II)**	**The anaerobic mono-digestion of FW yielded 405.1 Nml of biomethane while the anaerobic co-digestion of FW with cow dung yielded 267.4 and 274.8 Nml at 2:1 and 1:2 mixing ratios correspondingly.**	**Mono-digestion 32.16% Co-digestion 33.99%**	**[9] (Bambokela Empomopo et al., 2017)**
rabbit manure and sorghum crops	**The biodigester was set up in a region where temperature varies significantly during the year, and was operated under semi-batch conditions with non-thermal control for 16 months.**	**134 ml**	**60**	**[10] (Adrover et al., 2020)**
horse, rabbit and goat	**Anaerobic digestion batch assay in 0.5 L reactors, was performed under mesophilic conditions (35±2 °C) during 40 days.**	**245, 326 and 112 L CH_4 kg/VS**		**[11] (Carabeo-Pérez et al., 2021)**
corn-chaff and cow dung		**biogas yield 6.19 L**	**68**	**[12] (Iweka, Owuama, Chukwuneke, & Falowo, 2021)**

III. Research Methodology/Materials/Methods/Approach

The study was limited to two substrates: Rabbit droppings (Rd) as the main substrate and Macadamia nutshell (Mn) as the co-substrate. The rabbit dropping and macadamia nutshell used in this study were quantified at the University of Johannesburg, Gauteng Province, South Africa, respectively. The rabbit was taken from the residential urban farm in Johannesburg. The macadamia nutshell was also collected in a 1kg plastic bag. The rabbit dropping substrates were stored in a refrigerator at 4 ◦C for further processing. The investigation aimed to determine the optimal proportion of the co-substrate that would improve the biogas yield. To achieve the objective of this study, different loading ratios of rabbit dropping and macadamia nutshell were used, namely 90:10, 80:20, 70: 30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90. The co-digested samples with different loading ratios were characterised for moisture content, C/N ratio, total solids content, chemical oxygen demand and volatile solids.

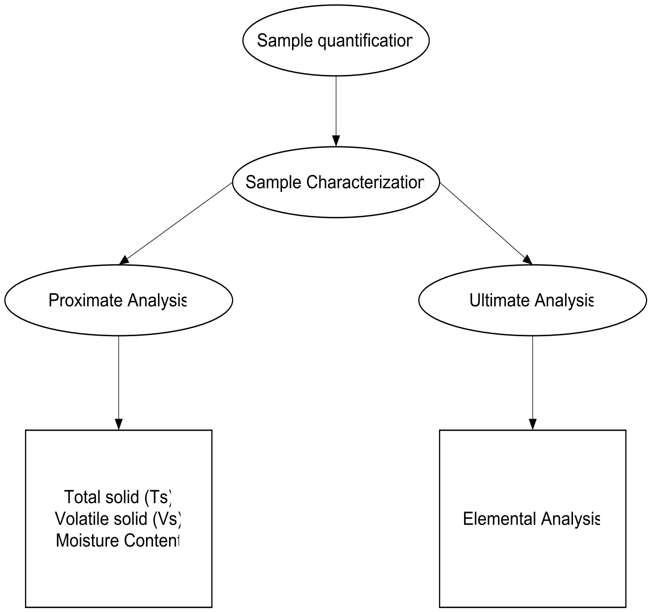

Fig 1.. Crystallization of the research methodology employed

Characterisation was carried out to determine the composition of the substrate and co-substrate. Primary analyses of volatile solids, total solids and moisture content were carried out. The characterisation was carried out according to the standard methods developed by APHA in 1995 [21]. In addition, a final analysis was performed for each substrate (rabbit drooping and macadamia nutshell) to determine the CHNS present in the dry sample. This analysis was performed according to ASTM E870. All procedures followed the standard given in the user manual. The procedure involved burning the dry substrate to break down the components into simple compounds, which were then quantified by infrared spectroscopy. The composition was expressed as a percentage of the total solids.

Furthermore open sun drying process was carried out in September at the, University of Johannesburg. Rabbit dropping were separated from the stalks manually. Thereafter, the gross weight of the sample (50 ± 1 g) and (100 ±1g) was determined using a mass balance laboratory scale with readability to 0.001 g. Afterwards, the average initial mass content of the 50 g and 100g samples were recorded respectively. The weight of the samples was taken using a mass balance equipment at 30 min interval until the weight was constant (samples attained equilibrium with the drying air conditions). Before exposing the samples to open sun, the ambient air temperature, was recorded. The samples were spread uniformly on a single layer sample tray and then exposed immediately to the open sun. The experiment started about 13:00 h and continued until 16:00 h. The loss in moisture content from the samples was determined using the weight changes at 30min intervals throughout the drying process. The samples were dried until their mass content was constant water/g sample.

In preparing this experiment, the ratio of inoculum to substrate was chosen based on the VS of the sample. The inoculum used was from the previous experiment, which was fermented for 21 days to expel the biogas and feed the microbes. The 11 digesters of 500 ml each with their lids were used and marked with a working volume of 400 ml, leaving a headspace of 100 ml for gas production. The reactors were then fed with inoculum, monoculture rabbit dropping and macadamia nutshell, as well as the co-digestion ratios mentioned above. Conditions were set at mesophilic temperatures of 37 ◦C and a working pH of 6.5–7.5. The thermostatic water bath was filled with 8 litres of water to cover the digesters. All reactors/digesters were placed in the water bath and connected to the CO_2 fixation bottles and flow cells. The contacts for stirring were connected to the individual motors and to the gas volume metre. The digesters were purged with nitrogen gas to create an anaerobic condition by removing the oxygen. The gas that came out of the CO2 fixation unit was fed into the flow cell (gas collection unit) and evaluated daily until the residence time was completed.

A. RSM modelling

Experimental design for statistical analyses and optimisation

The RSM was used for the selected parameters to determine the optimal combination that would yield the highest methane yield and purity, and the statistical software Minitab 19® was used. A central composite design (CCD) was used to design the experiment for optimized methane production from rabbit dropping and macadamia nut shells. Table 1 shows the chosen parameters, which were Bacteria, pH, retention time, and carbon to nitrogen ratio. Each chosen variable was studied at two levels (− 1 and 1) on the CCD. The output variable was methane yield and purity.

TABLE II. DESIGN APARAMETERS.1**.**

Factors	**(-1)**	**(1)**
Volatile solid	77,69	79,69
pH	6,00	7,00
Retention time	14,00	20,00
C:H ratio	6,23	8,23

IV. Results

A. Proximate analysis

The results of the characterization of monocultures and co-digestion of rabbit droppings and macadamia nutshell are shown in Fig. 4. The study shows that the monocultures Rb and Mn had a % VS of 74.77% and 84.23% respectively. Co-digestion of Rd:Mn in the ratio of ((90:10, 80:20, 70: 30, 60:40, 50:50, 40:60, 30:70, 20:80 and 10:90%) gives a % VS of 75,71%, 76,67%, 77,61%, 78,55%, 79,51, 80,45%, 81,34%, 82,33%, and 83,29% respectively. This means that the Mono anaerobic digestion of macadamia nutshell has the highest % VS and the co-digestion ratio of 10:90 of Rb:Mn has the highest % VS. The higher the VS of the substrate, the higher the organic content. This is favorable for biomethane production and shows that mono- and co-substrates have the potential to convert the investigated feed into biomethane.

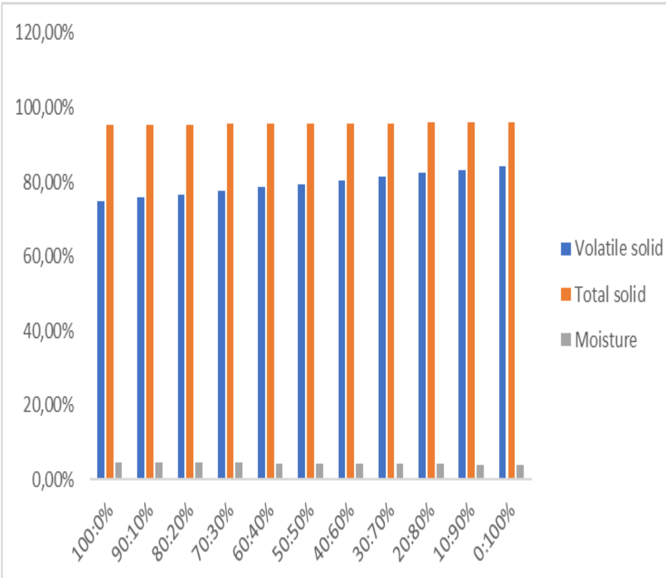

Fig. 1 . Ultimate analysis

Fig. 2 shows the results of the final analysis of the substrate. The ratio of carbon to nitrogen is another important factor that can influence AD. A low nitrogen content would lead to an inhibition of AD, as anaerobic microbes need enough nitrogen for their growth. At the same time, organic carbon is considered the only source of anaerobic activity [22]. Fig. 5 shows the final analysis of CHNS and C/N ratio of mono- and co-digestion substrates. The C/N ratio of mono-Rb and Mn are 24,17% and 107,02%, respectively. For co-digestion, the highest C/N ratio (82,27%) was observed at a co-digestion ratio of 10:90% (Rb: Mn). The lowest C/N ratio was observed in the rabbit dropping, which could be due to a slightly increased Ph value, as the increase in pH can be the result of a low C/N ratio. Co-digestion ratio of 80:20%Rb:Mn has the highest required C/N ratio of 29,31%, which is within the acceptable C/N range of 20.1 to 30.1 [23]. The literature points out that a high C/N ratio, i.e., above 30.1, is unfavorable for an AD system because it directly leads to rapid conversion of nitrogen, resulting in low biogas production [22]. The results also show the effects of co-digestion of Rb with Mn. The rabbit dropping has a required C/N ratio in mono-digestion but improves to a C/N ratio of 29,31% in co-digestion with macadamia nutshell, which is within the acceptable C/N ratio.

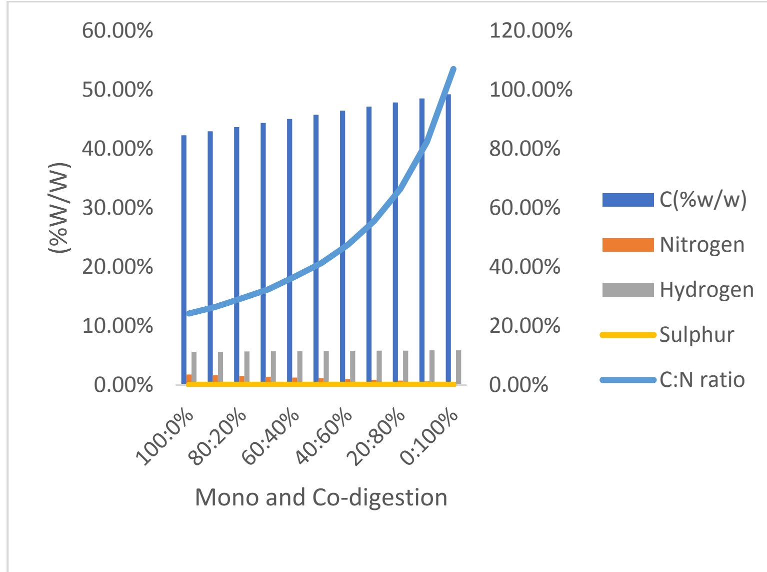

Fig. 2. Response surface analyses

RSM is a mathematical and statistical technique used to develop, improve, and optimise processes (Carley et al., 2004). It is commonly used to analyse the effect of the interactions between multiple input variables on the response. The six interactions studied in the present study were pH vs. C/H, pH vs. volatile solid, pH vs. retention time, C/H vs. volatile solid, C/H vs. retention time, and volatile solid vs. retention time.

Rabbit droppings methane purity optimisation prediction

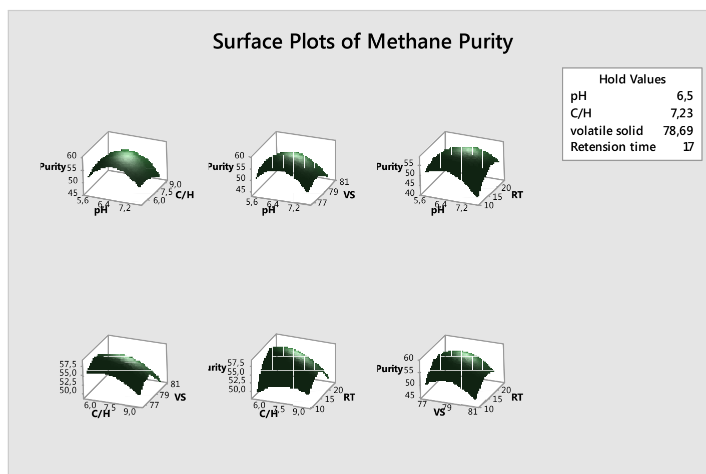

Fig. 4. Surface plots of pH vs. C/H and retention time

The surface plots of methane purity as a function of pH vs. C/H and pH vs. volatile solid are illustrated in Fig 4. It was plotted using a pH range of 5.4 to 7.2 and a bacteria amount range of 0.6 to 2.4 as the input variables. The results suggested that the optimum working conditions would be the pH range of 6.6 to 7.2 and the bacteria range of 1.2 to 1.8 (Fig. 2A).The optimum working conditions for bacteria were between 1.2 and 1.8 and between 15 and 20 days for the retention time, as illustrated by the plot using the retention time range of 10 to 20 days and bacteria amount range of 0.6 to 2.4 as the input variables respectively (Fig. 2B). The output variable for both figures was the expected product purity range of 50 to 55%, as stated in the objectives. Surface plots of C:N ratio vs. bacteria and CN ratio vs. pH. The surface plots of methane purity as a function C:N ratio vs. Bacteria and Retention time (min) vs. pH are illustrated in Fig. The surface plot was plotted using a C:N range of 10 to 30 and a bacteria range of 0.6 to 2.4 as the input variables. The results suggested that the optimum conditions would be the C:N ratio of 20 to 30 and the bacteria range of 1.2 to 1.8 (Fig. 3A). While the optimum conditions to work at for the reaction time were between 15 and 20 days and between 6.0 and 6.6 for the pH for the surface plot using a retention time range of 10 to 20 days and a pH range of 5.4 to 7.2 as the input variables respectively (Fig. 3B). The output variable for both figures was the expected methane purity range of 50 to 60%, as stated in the objectives.

Surface plots of CN ratio vs. pH and CN ratio vs. Retention time. The surface plots of product purity as a function of C:N ratio vs. pH and C:N ratio vs. retention time (days) are illustrated in Fig. 4A and B. The surface plot was plotted using a C:N ratio range of 10 to 30 and a pH ratio range of 5.4 to 7.2 as the input variables. The results suggested that the optimum conditions to work at would be the C:N ratio range of 20 to 30 and the pH range of 6.0 to 6.6 (Fig. 4A). While the optimum conditions to work at for the retention time would be between 15 and 20 days and between 20 and 30 for the C:N range for the surface plot using a retention time range of 10 to 20 days and a C:N range of 5.4 to 7.2 as the input variables respectively (Fig. 4B). The output variable for both figures was the expected methane purity range of 50 to 60%, as stated in the objectives.

Surface Plot for Methane Yield

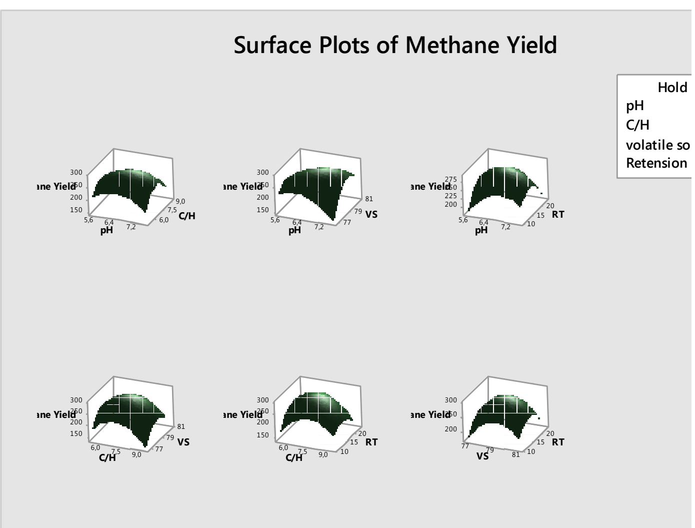

Fig. 5. Surface plots of bacteria vs. pH and bacteria vs. retention time

The surface plots of methane purity as a function of pH vs. bacteria and retention time vs. bacteria are illustrated in Fig. It was plotted using a pH range of 5.4 to 7.2 and a bacteria amount range of 0.6 to 2.4 as the input variables. The results suggested that the optimum working conditions would be the pH range of 6.6 to 7.2 and the bacteria range of 1.2 to 1.8 (Fig. 2A).The optimum working conditions for bacteria were between 1.2 and 1.8 and between 15 and 20 days for the retention time, as illustrated by the plot using the retention time range of 10 to 20 days and bacteria amount range of 0.6 to 2.4 as the input variables respectively (Fig. 2B). The output variable for both figures was the expected product purity range of 50 to 55%, as stated in the objectives.

Surface plots of CN ratio vs. Bacteria and Retention time vs. pH

The surface plots of methane purity as a function C:N ratio vs. Bacteria and Retention time (min) vs. pH are illustrated in Fig. The surface plot was plotted using a C:N range of 10 to 30 and a bacteria range of 0.6 to 2.4 as the input variables. The results suggested that the optimum conditions would be the C:N ratio of 20 to 30 and the bacteria range of 1.2 to 1.8 (Fig. 3A). While the optimum conditions to work at for the reaction time were between 15 and 20 days and between 6.0 and 6.6 for the pH for the surface plot using a retention time range of 10 to 20 days and a pH range of 5.4 to 7.2 as the input variables respectively (Fig. 3B). The output variable for both figures was the expected methane yield range of 200 to 300%, as stated in the objectives.

Surface plots of CN ratio vs. pH and CN ratio vs. Retention time

The surface plots of Methane Yield as a function of C:N ratio vs. pH and C:N ratio vs. retention time (days) are illustrated in Fig. 4A and B. The surface plot was plotted using a C:N ratio range of 10 to 30 and a pH ratio range of 5.4 to 7.2 as the input variables. The results suggested that the optimum conditions to work at would be the C:N ratio range of 20 to 30 and the pH range of 6.0 to 6.6 (Fig. 4A). While the optimum conditions to work at for the retention time would be between 15 and 20 days and between 20 and 30 for the C:N range for the surface plot using a retention time range of 10 to 20 days and a C:N range of 5.4 to 7.2 as the input variables respectively (Fig. 4B). The output variable for both figures was the expected methane yield range of 200 to 300%, as stated in the objectives.

Data analysis and computations

The RSM was used to design the gelatinisation experiments to develop the mathematical model to describe the relationship between the input variables and the response (dry purity in%). Statistical softwares Minitab 19® and Design-Expert ® Softwares were used to design and analyse the experiments. A four-factor CCD was used to design the experiment to gelatinise CTG and LBG. They were studied at two different levels (− 1 and 1). Table 2 shows the randomised coded and actual factors for gelatinisation experiments. Thirty-one experimental runs were conducted according to the design presented in Table 2. Response surface analysis was used to evaluate the relationship between the input variables from the experimental data generated. The input variables were water amount, NaOH amount, polymer amount, and gelatinisation time. After subjecting the experimental data generated to response surface

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analysis, the functional relationship between dry product purity and the input parameters was developed.

Design of experiments and optimization

The experimental data obtained were subjected to response surface analysis to evaluate the relationship between the input variable. A functional relationship was developed between the input variables and the response, viz., the product dry purity. Thus, multiple regression analysis for product purity (%) as a function of C:H, pH, Retention time, and volatile solid was performed on Minitab 19® and Design-Expert software to generate the second-order polynomial model Eq. (6). Furthermore, the evaluation of lack of fit and inaccuracy in the experiment was measured by a centre run. The equation in terms of actual factors can be used to predict the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor, and the intercept is not at the centre of the design space.

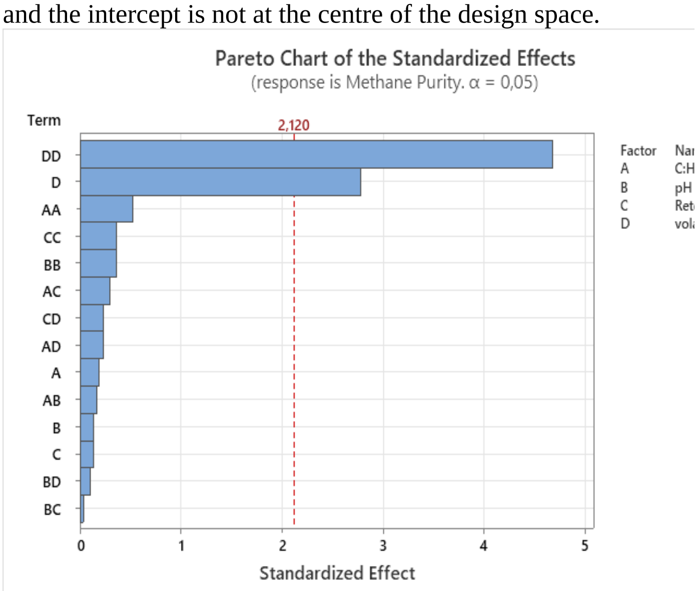

Fig.6 a).

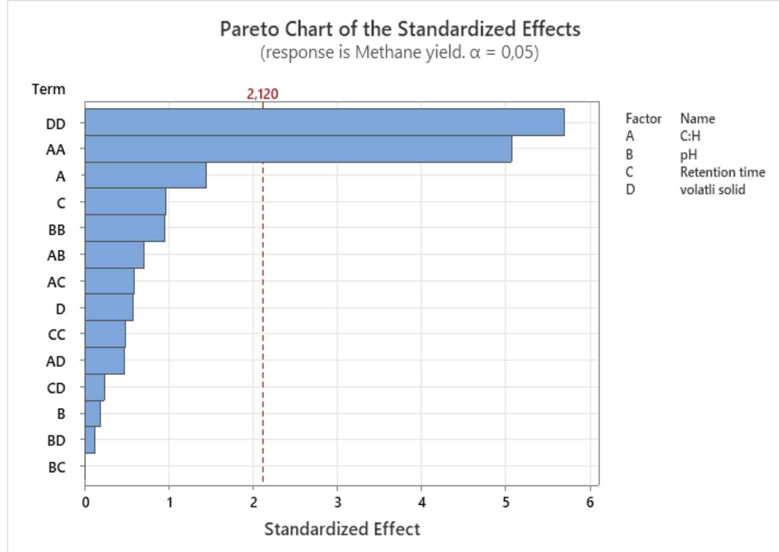

Fig.6 (b).

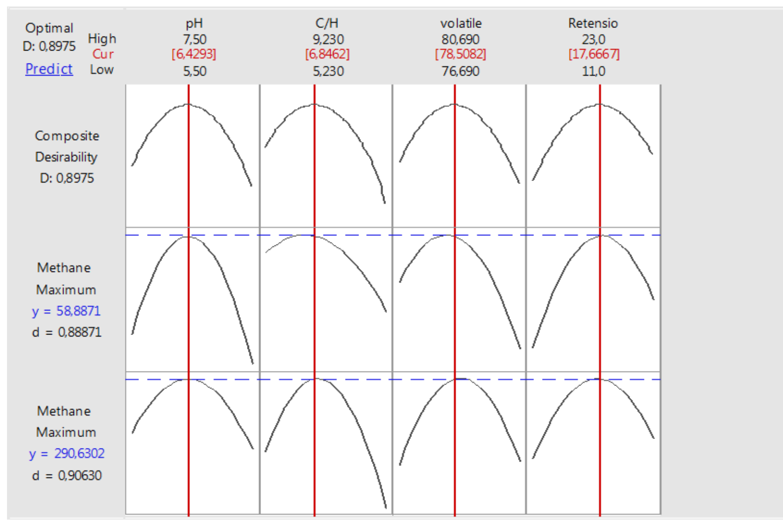

Fig. 7. Sensitivity analysis

MATHEMATICAL EXPRESSION OF THE OPTIMIZATION OF METHANE PRODUCTION

Methane Yield = -51448 + 376 C:H + 488 pH + 1215 Vs + 126 Retention time - 18,13 C:H*C:H - 27,5 pH*pH - 7,51 Vs*Vs - 2,223 Retention time*Retention time + 3,7 C:H*pH - 1,25 C:H*Vs - 2,08 C:H*Retention time - 2,5 pH*Vs + 2,50 pH*Retention time - 0,62 Vs*Retention time

Model Summary

R-sq 70,10%

Methane Purity = -5203 + 45,2 C:H + 121,3 pH + 115,8 Vs + 18,8 Retention time - 1,562 C:H*C:H - 3,75 pH*pH - 0,688 Vs*Vs - 0,1875 Retention time*Retention time - 1,50 C:H*pH - 0,125 C:H*Vs - 0,167 C:H*Retention time - 0,75 pH*Vs - 0,167 pH*Retention time - 0,125 Vs*Retention time

Model Summary

R-sq
66,89%

V. Conclusion

The study provided valuable insights into the optimal parameters for co-digestion process and validated the effectiveness of the developed model. Further exploration and implementation of this model in practical applications may significantly enhance the efficiency and productivity of co-digestion processes, opening doors for improved quality, quantity control and industrial-scale production. In conclusion, this study successfully used response surface methodology to identify the optimized conditions to produce a high-purity/yield co-digestion product. Through a systematic investigation, the individual and interactive effects of bacteria, pH, retention time, and carbon to nitrogen ratio on product yield and purity were investigated. The individual and interactive effects of bacteria, pH, retention time, and carbon to nitrogen ratio on product yield and purity were investigated. A four-factor central composite design was used for the anaerobic co-digestion experiments. The results showed that the optimized conditions which could yielded a high-purity/yield product were carbon to Hydrogen ratio (78-79), pH of (6.0-6.6), volatile solid ratio of (78-79), and retention time of 15 days, respectively. This combination yielded a methane product with ~300 ml yield and ~57% purity. Furthermore, the coefficient of determination (R^2) value of 0.7010 and 0.6689 for methane yield and purity respectively in the present study proves that the model was adequate and suitable. Therefore, this model may be used to navigate the design space. Based on these results, it may be concluded that the developed model holds great promise for effectively navigating the design space of co-digestion processes. The model's ability to accurately predict the ideal conditions for obtaining high-purity products suggests its potential applicability in industrial settings. By utilizing this model, researchers and engineers may be able to streamline the optimization process, saving time and resources while ensuring the production of high-purity co-digestion products. Furthermore, the time required to dry the rabbit droppings under open sun drying conditions from the initial mass content of 50 ± 0.1 g and 100± 0.1g water/g dry sample to the final mass content of 34± 0.001 g and 70± 0.001g water/g dry sample was 150 minutes. The coefficient of determination (R2) value was determined to be $R^2 = 0{,}9986$ and $R^2 = 1$ for 50g and 100g respectively.

Acknowledgment

Contribution of authors

Antoine F. Mulaba - Bafubiandi initiated, conceptualized and supervised the research project. Blessing Nkuna conducted the research work under the supervision of Antoine F. Mulaba – Bafubiandi.

Declaration of interest

The authors have no financial nor personal interest in the content of the work here presented.

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