



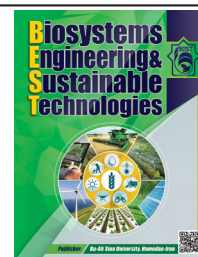
Bu-Ali Sina University

# Biosystems Engineering & Sustainable Technologies (BEST)

Journal Homepage: [www.best.basu.ac.ir/](http://www.best.basu.ac.ir/)

BEST J., 2(1) (2025) 39-50

DOI: <https://doi.org/10.22084/best.2025.31593.1011>



## Modeling and Optimizing an Aerobic Co-digestion Based on Optimal-Mixture Design

Ezatollah Azadi<sup>id</sup>, Majid Rasouli<sup>\*id</sup>, Moloud Jafari

Department of Biosystems Engineering, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran.

Department of Mechanical Engineering, École de technologie supérieure (ÉTS), Montreal, Canada.

### PAPER INFO

#### Paper history:

Received: Sep. 18, 2025

Revised: Nov. 02, 2025

Accepted: Nov. 26, 2025

Available Online: Dec. 30, 2025

#### Keywords:

Lignocellulosic Biomass

Response Surface Methodology (RSM)

Systematically Optimizing

Waste to Energy

Wastewater Sludge

### ABSTRACT

A mesophilic co-digestion of sugarcane straw and sewage sludge with long hydraulic retention time in lab-scale reactors has been studied. Anaerobic biodegradability was examined in a biochemical methane potential (BMP) testing apparatus using 500 ml bottles. Both the design of the experiment method and the I-optimal mixture design were used as a mixed design strategy to systematically optimize the substrate composition ratios and elucidate the possible synergistic effects for an anaerobic co-digestion system. A reduced cubic model was created by Design-Expert software as a function of substrate composition ratios. The model was experimentally validated by the ANOVA method. Based on the observations, all linear impacts and interactions between substrates showed synergistic effects on the biogas production rate. The optimum proportions of the feedstock were 0.28 % (w/w) of Primary sludge (A), 48.98 % (w/w) of Secondary sludge (B), and 50.73 % (w/w) of sugarcane straw (C). Also, according to the aforementioned optimum proportions, cumulative biogas reaches the maximum level of 8.581 L during 150 days.

## 1. Introduction

With increased industrialization, vast amounts of wastewater are produced annually by various industries, and a significant challenge in biological treatment is managing wastewater sludge (WS) from the treatment plants (Asaithambi et al., 2022). Increased human activity, industrialization, and urbanization result in the production of enormous quantities of wastewater. Generally, physicochemical and biological methods are employed to treat industrial effluent and wastewater and have demonstrated high efficacy in removing pollutants. However, some industrial effluent and wastewater contain contaminants that are extremely difficult to remove using standard physicochemical and biological processes. Previously, electrochemical and hybrid advanced oxidation processes (AOP). Sludge management is a highly time-consuming approach for environmentalists, and it may represent as much as 60% of all municipal wastewater treatment expenses. Therefore, great care is taken to reduce sludge generation and improve sludge processing (Rasouli et al., 2022; Siddiqui et al., 2021). Reducing environmental pollution through waste-to-energy conversion using lignocellulosic biomass and sewage sludge is one of the best strategies. Using a clean process to produce waste by-

products with high value-added is crucial. (Wang et al., 2022). Untreated, fresh sludge with many pathogens carries large amounts of water and high biochemical oxygen demand and is typically foul-smelling (Alexandre et al., 2016; Wei et al., 2003). Biocompatibility and low toxicity. In activated sludge systems, they reduce coalescence and disintegrate flakes, enabling more cells to have access to oxygen. At low concentrations, they may act as growth inhibitors. In this study, rhamnolipid was added to a bench scale sequential batch reactor operating in similar conditions as oil refinery wastewater treatment plants. Concentrations from 12 to 50 mg rhamnolipid/L were evaluated, the latter being the minimum concentration necessary to reduce sludge disposal. In this concentration, rhamnolipid reduces sludge disposal of up to 52%, maintaining COD removal of 81-97% and good sludge settling properties (SVI 120 mL/g). The most popular WS treatment method is anaerobic co-digestion biotechnology, while other possibilities exist. Researchers are concentrating on agricultural waste management, mainly recycling and turning trash into energy, due to the rise in agricultural waste. The world's most affordable and significant natural renewable resource is lignocellulosic biomass. Recycling these wastes has several economic, social, and environmental benefits (Elsayed et al., 2016).

\*Corresponding author's email: [m.rasouli@basu.ac.ir](mailto:m.rasouli@basu.ac.ir)



Biomass from lignocellulosic materials such as energy crops, corrosive organic materials, food and agricultural wastes, forestry wastes, and livestock faces can produce renewable fuels and chemicals through biological or chemical processes (Kang et al., 2020).

A small amount of these wastes is used as fuel or to create usable products. For example, according to (Amin et al., 2022) palm oil mill effluent (POME) Only 27% of the produced palm oil mill effluent is used in Malaysia. Among these lignocellulosic materials, sugarcane is one of the most abundant agricultural materials used for sugar making, bioethanol, and bioelectricity in tropical and subtropical regions of Brazil, India, Pakistan, Thailand, and China. In the last decades, sugarcane straw (SCS) was burned in old pre-harvesting systems. However, due to environmental, agronomic, and economic reasons, in many cases, the system of automated harvesting has been gradually replaced with older ones (Janke et al., 2017) SCS was previously homogenized by milling in 2 mm particle size and pretreated in NaOH solutions at various concentrations (0, 3, 6 and 12 g NaOH/100 g SCS. According to ("Sugarcane | Land & Water Food and Agriculture Organization of the United Nations | Land & Water | Food and Agriculture Organization of the United Nations), the under-cultivation area and production amount of sugarcane are  $1.3 \times 10^7$  ha and  $1.254 \times 10^9$  tons/year, respectively. The generation of agro-industrial waste is an essential part of processing agricultural production to provide food, which must be managed correctly (Li et al., 2018) additional of co-substrates may require additional energy inputs and thus affect the overall energy efficiency of the system. In this study, reactor performance and energy analysis of solid state anaerobic digestion (SS-AD. Sugarcane leaf waste makes up 40% of the whole plant, and because it has no particular use after harvest, it is considered a low-consumption source (Smithers, 2014). It can be a renewable source of fermentable sugars for biofuels (Moodley & Kana, 2015).

On the other hand, Anaerobic co-digestion is one of the most often used techniques for stabilizing sewage sludge. The simultaneous digestion of two or more substrates, or anaerobic co-digestion, is a possible way to get around the drawbacks of mono-digestion (the digestion of a single substrate). It can also boost methane production and anaerobic co-digestion facilities' profitability. (Hagos et al., 2017) there is increasing awareness that renewable energy and energy efficiency are vital for both creating new economic opportunities and controlling the environmental pollution. AD technology is the biochemical process of biogas production which can change the complex organic materials into a clean and renewable source of energy. AcoD process is a reliable alternative option to resolve the disadvantages of single substrate digestion system related to substrate characteristics and system optimization. This paper reviewed the research progress and challenges of AcoD technology, and the contribution of different techniques in biogas production engineering. As the applicability and demand of the AcoD technology increases, the complexity of the system becomes increased, and the

characterization of organic materials becomes volatile which requires advanced methods for investigation. Numerous publications have been noted that ADM1 model and its modified version becomes the most powerful tool to optimize the AcoD process of biogas production, and indicating that the disintegration and hydrolysis steps are the limiting factors of co-digestion process. Biochemical methane potential (BMP). The generation of biogas and the quality of biosolids have both increased thanks to the use of anaerobic co-digestion of sewage sludge with other organic wastes around the world (Athanasoulia et al., 2014; Rasouli et al., 2023). Extensive use of this technique is evidence of its potential benefits, which include a 30 to 50 % reduction in sludge volume and methane energy production that exceeds the amount required to run the process (Raheem et al., 2018; Villamil et al., 2020) dioxins, furans, heavy metals, etc.. For treatment and energy recovery from wastewater and food and agricultural waste, anaerobic co-digestion is an alternative option to dispose of and evaluate organic wastes like sugarcane, which is known as lignocellulosic biomass (Amin et al., 2017; Yaser et al., 2022) such as lignocellulosic materials (LM. There are two reasons that WS is a desirable co-digestion material to add SCS in the anaerobic co-digestion process:

It has been determined that the optimal C/N ratio of the substrate in the anaerobic co-digestion process to yield maximum methane without preventing degradation is 30:1. In contrast, the C/N ratio of SCS is high (118) (Janke et al., 2017) SCS was previously homogenized by milling in 2 mm particle size and pretreated in NaOH solutions at various concentrations (0, 3, 6 and 12 g NaOH/100 g SCS. On the other hand, the C/N ratio of WS is low (6-8) (Comesaña et al., 2018), which can optimize this parameter (Yaser et al., 2022).

2. Experiments have shown that bacteria are necessary for biodegradation in anaerobic co-digestion. There are no bacteria in the structure of SCS. However, there is a considerable amount of bacteria in WS, which can compensate for the lack of bacteria in SCS and contribute to the bacteria of the inoculum (Matheri et al., 2017).

Researchers have investigated the co-digestion of primary sludge and microalgae (75 % to 25 %) based on volatile solids in a continuous reactor with a hydraulic residence period of 20 days. The results showed that anaerobic co-digestion boosted methane output by up to 65 % and reduced ammonia toxicity compared to mono-digestion of microalgae (Solé-Bundó et al., 2019). Co-digestion also yields 4.5 times the energy consumed, according to energy analysis. In a different study, a lab-scale semi-continuous anaerobic membrane bioreactor was used to evaluate the biological performance and microbial population in the anaerobic co-digestion of raw microalgae (*Scenedesmus* & *Chlorella*) and primary sludge.

This study's retention time and temperature were 100 days and 35°C, respectively. Biodegradability was 73%, and high stability of the system in terms of pH and volatile fatty acids, as well as enhancing methane production, was observed (Serna-García et al., 2020). The synergetic effects of anaerobic co-digestion of sewage sludge, food

waste, and yard waste were studied (Mu et al., 2020). In this investigation, which was related to the more rapid development of archaea and bacteria, a semi-continuous method was used to transfer a mixture of anaerobic co-digestion from various urban organic wastes. Results show that methane output increased by 3.4 – 19.1 %. Considering appropriate organic materials for the anaerobic co-digestion leads to the balance and contribution of materials to achieve an ideal substrate for the digester's optimal performance. Also, it can provide nutritional material for the microorganisms, stabilize the system, and enhance methane production (Yaser et al., 2022). Many properties of the substrate that are expected to be enhanced include buffer capacity, pH, variation of bacteria, C/N ratio, and nutritional elements (Pellera & Gidarakos, 2017) namely cotton gin waste, winery waste, olive pomace and juice industry waste, in semi-continuous mode, conducting mono-digestion and co-digestion assays, using an artificial organic fraction sample as co-substrate. These assays were divided into two groups, in which different conditions were applied. Group I investigated the variation in two operational parameters, i.e. the organic loading rate (OLR).

This study aims to analyze the performance of the anaerobic co-digestion process of sewage sludge as an organic waste with sugarcane straw, analyze the synergy effects, and use a mixture design to optimize the response variable. Additionally, optimizing and modeling biogas production is another goal of this study to predict some bioreactor behavior. Response surface methodology was used in this work. Most studies choose the component proportions at random. There is no information on optimizing waste ratios to enhance digestive efficiency in a controlled and methodical manner.

**Table 1. The composition of sludge**

Property	Quantity	Unit
Phosphorus	$6.81 \times 10^{-1}$	g/L
Carbon	$6.75 \times 10^{-1}$	g/L
Nitrogen	$1.35 \times 10^{-1}$	g/L
Moisture	97.4	%
Volatile organic matter	86	%
pH	6.3	-

## 2. Materials and methods

### 2.1. Inoculum and substrates

Primary and secondary sludge were obtained from the wastewater treatment plant of Hamedan, Iran. SCS was collected from Khuzestan's sugarcane farms. The composition of the used sludge is listed in Table 1. SCS was grinded and separated into fine particles sieved through a 1 mm screen. The particles smaller than 1mm participated in this experiment (see Fig. 1).

The minuscule particles can potentially augment the particle availability for microbial digestion. The output substrate from the semi-industrial reactors in the Bu-Ali Sina University Renewable Energy Laboratory served as the insemination material. Cow manure emerged from the semi-industrial reactors, which took around six months to digest completely.



(a)



(b)

**Fig. 1. a) sugarcane leaves and b) sugarcane leaves that have been ground up.**





**Fig. 2. Biochemical methane potential (BMP) testing system.**

## 2.2. Experimental setup of anaerobic biodegradability

Anaerobic biodegradability was tested in a biochemical methane potential testing system with 500 ml bottles (see Fig. 2). An equal quantity of mixture was considered for every bottle with a final volume of 350 ml of liquid. Approximately 150 ml was allowed a headspace for the gas accumulation (Rasouli et al., 2023).

The pH of each bioreactor was set to 7.5. While the biomass was deteriorating, the pH was measured using a digital Metrohm pH meter. The pH decreased as a result of the formation of acid. This problem was resolved by adding a 4% NaOH solution to the mixture to balance the pH. The reactors were submerged in water and kept at a steady 37°C mesophilic temperature. Every day, all reactors were rocked routinely. There was a 150-day hydraulic retention period. The amount of biogas generated by the mixture of materials was calculated using the water displacement method.

## 2.3. Analytical methods

The characteristics of volatile solids (VS) and total solids (TS) were monitored during the activation of bioreactors under the Standard Methods for the Examination of Water and Wastewater (Federation, 1999). A predetermined amount of sample was obtained and placed in a dry crucible to calculate the amount of TS in mg/L. After the sample was placed in the crucible and heated to 150°C, it was thoroughly dried off. The dried sample was placed in a crucible, which was weighed, and the results of Eq. 1 were calculated:

$$TS = (W_2 - W_1)/V \quad (1)$$

Where  $W_1$  is the mass of the dry crucible (g),  $W_2$  is the mass of the dry crucible with dried sample (g), and  $V$  is the sample volume (ml).

A predetermined sample-specific volume was obtained and centrifuged to determine TSS. The centrifuge sediment was poured into a dry, empty, and weighed crucible. The sample was thoroughly dried by placing the crucible containing it in an oven set at 105 °C. Weighing the crucible containing the dried sediment allowed us to determine the TSS in mg/L.

$$TSS = (W_2 - W_1)/V \quad (2)$$

Where  $W_1$  is the mass of the dry crucible (g),  $W_2$  is the mass of the dry crucible with dried sediment (g), and  $V$  is the sample volume (ml).

The crucible used to measure the TS was placed in an oven set at 505 °C to burn to ashes before being used to measure TVS. The ash-filled crucible was weighed, and TVS was determined in mg/L using Eq. 3.

$$TVS = (W_2 - W_1)/V \quad (3)$$

Where  $W_1$  is the mass of the crucible with the ash (g),  $W_2$  is the mass of the crucible with dried sample (g), and  $V$  is the sample volume for the TS measurement (ml).

## 2.4. Experimental design and mathematical model

This study aimed to obtain the optimal combination of sewage sludge (primary and secondary) and SCS to

optimize biogas production. Therefore, the independent variables in this experiment are the different combinations of substrate and dependent variables, and the biogas produced. The response surface method (RSM) and Design Expert 13 software were used to create an appropriate mixture design for the experimental setup and data processing. This statistical methodology could be an appropriate approach when the response is affected by several variables. Based on this design, 24 alternative combinations of substrate materials were considered, each according to the software's output, with a center point and three repeating points.

This methodology allows the formulation of a third-order polynomial model to describe the process, as shown in Eq. 4 (Montgomery, 2020):

$$\begin{aligned} \gamma = & \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^n \beta_{ii} x_i^2 \\ & + \sum_{1 \leq i < j} \beta_{ij} x_i x_j + \sum_{i=1}^n \beta_{iii} x_i^3 \\ & + \sum_{1 \leq i < j} \beta_{iij} x_i^2 x_j + \sum_{1 \leq i < j} \beta_{iji} x_i x_j^2 \\ & + \sum_{1 \leq i < j < k} \beta_{ijk} x_i x_j x_k + \varepsilon \end{aligned} \quad (4)$$

Where  $k$  is the number of variables, and  $Y$  is the response variable's projected value,  $\beta_0$ ,  $\beta_i$ ,  $\beta_{ii}$ ,  $\beta_{ij}$ ,  $\beta_{iij}$ ,  $\beta_{iii}$ ,  $\beta_{iji}$ , and  $\beta_{ijk}$  are model parameters,  $x_i$ ,  $x_j$ , and  $x_k$  are coded factors, and  $\varepsilon$  is the remainder of the experiments.

## 2.5. Statistical analysis

Fitting the experimental model, evaluating the fit's accuracy, displaying the 2D contours and the 3D response surface, and modifying the mixture's component ratios are the subsequent steps in the mixture test design. Expert in Design 13 The response surface was designed, and the regression analysis of the experimental data was performed using statistical software from Stat-Ease, Inc. Furthermore, the statistical characteristics were estimated using the ANOVA method.  $R^2$  was calculated as the determination coefficient.  $R^2$  is a metric that shows how much data variability there is. The model explains it, and hence the model's grade of fit. P-values of parameter estimation can be used to validate the model. Model terms are significant when their p-values are less than 0.05. The interaction effects of the independent variables on the dependent variables were displayed using three-dimensional graphs.

## 3. Results and discussion

### 3.1. Biogas generation from sewage sludge and SCS co-digestion

The SCS comprises 36.7 % cellulose, 17.5% hemicellulose, 10.4% lignin, and 12.6 % ash. The C/N ratio of SCS hydrolysate was 14.8 times greater than that of anaerobic sludge hydrolysates (Sithikitpanya et al., 2021). As a result, sugarcane straw is an excellent alternative for balancing nutrients in the anaerobic co-digestion bed.

When the boundary limits are complex and have non-uniform sizes, an optimal approach makes sense. Tests were constructed around the D-optimal, A-optimal, and I-optimal methods, which comprise the third category of optimal strategies according to design-expert software. Across the whole experimental region, the mean prediction variance is smaller in I-optimal designs. Consequently, factors influencing the co-digestion of sewage sludge and SCS for biogas generation were optimized (Tahwia et al., 2023). Table 2 displays a summary of the design. The I-optimal design algorithm chooses sites that minimize the predictive integral of variation throughout the design space by varying the number and range of components. The model chose twenty-four points. Twelve points went to modeling, 5 to estimating non-fit, 5 to rerun the test, and 2 to centrality. Thus, 24 run conditions were offered by the proposed model. The response values were entered into the software's answer column after being acquired through the execution of the experiments that the model had in mind. The parameters for the 24 trials the model recommends are listed in Table 3, along with the experimental response values. The cubic model for the mixture's constituent parts, which was created to foresee the production of biogas, may be expressed using equation 5:

$$\begin{aligned} Y_{\text{Biogas}} = & 162.20A + 79.23B + 94.43C \\ & + 376.81AC + 798.79AC(A-C) \end{aligned} \quad (5)$$

Low levels of mixed components are coded as 0, whereas high levels are automatically recorded as +1. The coded Eq. helps identify the relative effects of the parameters by comparing the parameter coefficients.

The Design-Expert software performed an ANOVA to evaluate the coherence and significance of the model. ANOVA also demonstrates the effects of independent variables and variable interactions on the biogas produced by the mixture. The ANOVA findings are shown in Table 4. A more straightforward Cubic model was produced after the effects of the AB, BC, AC, ABC, and AB (A-B) components of the Cubic model were determined to be negligible.

The model's F-value of 8.24 indicates statistical significance. The likelihood that a value of this magnitude will occur due to noise is 0.05 percent. P-values for significant model terms are less than 0.0500. The model terms are

**Table 2. Design Summary.**

Study type: Mixture Design type: I-optimal Design model: cubic Runs: 24									
Component	Name	Units	Type	Min.	Max.	Coded Low	Coded High	Mean	Std. Dev.
A	Primary sludge	%	Mix.	0	100	+0 ↔ 0	+1 ↔ 100	32.86	30.24
B	Secondary sludge	%	Mix.	0	100	+0 ↔ 0	+1 ↔ 100	33.20	30.03
C	Sugarcane leaves	%	Mix.	0	100	+0 ↔ 0	+1 ↔ 100	33.94	28.77
Response	Name	Units	Obs.	Min.	Max.	ratio	Analysis	Mean	Std. Dev.
Y <sub>1</sub>	Biogas	ml	24	562	8581	15.27	Polynom.	3837.29	2467.50

**Table 3. Experimental parameters and response values for the proposed model.**

Run	Build Type	Space Type	Component 1 A: Primary sludge (%)	Component 2 B: Secondary sludge (%)	Component 3 C: sugarcane straw (%)	Response Biogas (L)
1	Model	Edge	69.5928	30.4072	0	4.826
2	Model	Vertex	0	0	100	1.876
3	Model	Interior	63.4384	18.5127	18.0489	7.179
4	Model	Edge	71.7522	0	28.2478	7.916
5	Replicate	Edge	71.7522	0	28.2478	6.199
6	Replicate	Edge	0	70.3383	29.6617	6.615
7	Replicate	Center	33.3333	33.3333	33.3333	2.395
8	Lack of Fit	Interior	47.0995	7.44111	45.4594	6.819
9	Replicate	Edge	28.8887	0	71.1113	1.227
10	Center	Center	33.3333	33.3333	33.3333	2.601
11	Model	Edge	28.8887	0	71.1113	1.188
12	Model	Vertex	100	0	0	3.445
13	Model	Edge	0	27.8518	72.1482	9.16E-1
14	Lack of Fit	Interior	0.283577	48.984	50.7324	8.581
15	Model	Interior	17.3778	65.602	17.0202	5.136
16	Lack of Fit	Interior	46.0794	45.9745	7.94615	4.318
17	Model	Edge	0	70.3383	29.6617	3.123
18	Model	Edge	30.2369	69.7631	0	4.033
19	Model	Vertex	0	100	0	2.112
20	Model	Interior	18.0726	17.872	64.0554	5.62E-1
21	Replicate	Edge	0	27.8518	72.1482	9.67E-1
22	Lack of Fit	Interior	82.0346	12.6163	5.34902	5.366
23	Center	Center	33.3333	33.3333	33.3333	7.03E-1
24	Lack of Fit	Interior	13.2322	83.2501	3.5177	3.992

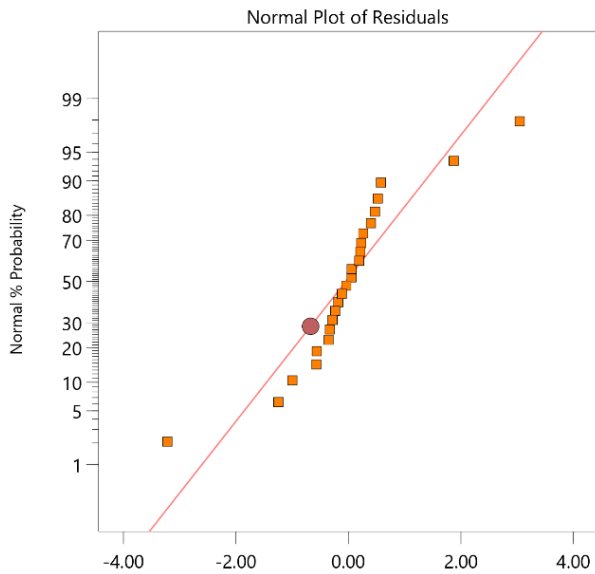
**Table 4. Reduced Cubic model using ANOVA. Biogas (Transform: Natural Log; Constant: 0) in response.**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	9.81	4	2.45	8.24	0.0005	significant
<sup>(1)</sup> Linear Mixture	5.02	2	2.51	8.42	0.0024	
AC(A-C)	2.76	1	2.76	9.26	0.0067	
BC(B-C)	2.29	1	2.29	7.68	0.0122	
Residual	5.66	19	0.2978			
Lack of Fit	4.27	13	0.3285	1.42	0.3478	not significant
Pure Error	1.39	6	0.2312			
Cor Total	15.47	23				

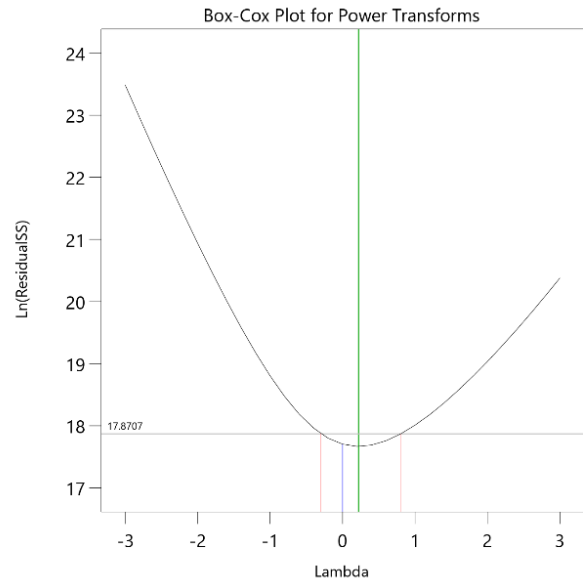
<sup>(1)</sup> Type I sums of squares are used in inference for linear mixtures.

L Pseudo is the code for the Mixture Component.

Type III - Partial sum of squares



(a)



(b)

**Fig. 3. Normal plot of residuals (a) and Box-Cox plot (b) are diagnostic tools.**

unnecessary if the value is more significant than 0.1000. Model reduction can help if a model has many concepts by removing concepts that have no meaning (aside from those essential to maintain hierarchy). The F-value of 1.42 indicates that the lack of fit is not statistically significant compared to pure error. A significant F-value for Lack of Fit has a 34.78 % probability of occurring due to noise. A non-significant lack of fit indicates that the model is reliable and accurately characterizes the biogas. The mixture components A, B, C, BC (B-C), and AC (A-C) are significant terms of the model, as shown by the ANOVA chart, showing that their P-values are less than 0.05.

### 3. 2. Diagnostic evaluation

The first step in a diagnostic test is a graphical analysis of the model. The residual standard plot (see Fig. 3a) is an essential diagnostic tool. It shows a normal residual distribution if the points of a 45° line can be located; hence, no data transfer is required. The residues' normal distribution is depicted in the standard probability diagram. Even with typical data, there will be some little dispersion. Specific curving patterns, such as "S-shapes," suggest that a more accurate analysis will be obtained by applying a transfer function to the dependent variable or model response.

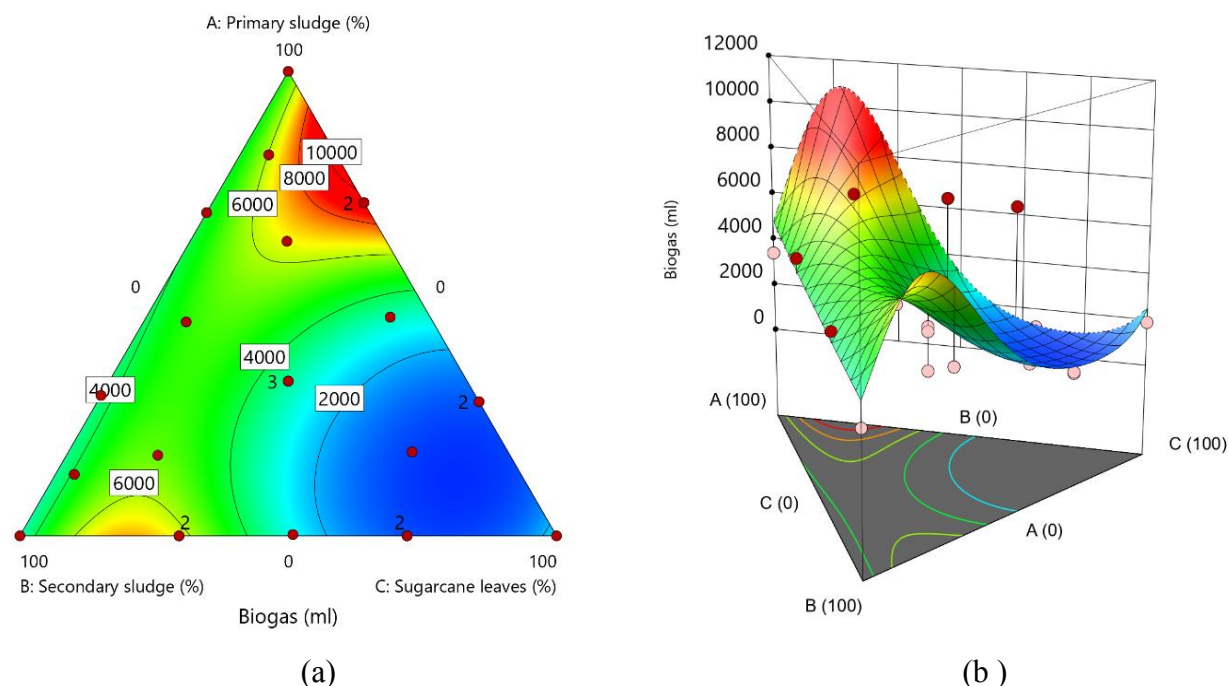
(Montgomery, 2020). The Box-Cox diagram, the second diagnostic tool, validates this standard. The Box-Cox chart is one tool for determining a particular response's optimal power transfer function. The smallest sum of residue squares in the converted model yields the lowest point in the Box-Cox diagram (see Fig. 3b), which displays the optimal value of lambda. When the highest to lowest response value ratio is more significant than 3, the power function is more likely to change the model. On this graph, a 95% confidence interval is also displayed. The Box-Cox diagram and the lambda values inside the given range indicated that the transfer function was not required.

### 3. 3. Contour plots and 3D response surface interpretation

Using Design Expert software, contour plots and 3D surface plots were made to establish the ideal anaerobic co-digestion meal composition ratio to maximize biogas output. Plots with a surface and a contour can help find the desired response values. In a two-dimensional depiction known as a contour plot (see Fig. 4a), all points with the same response are connected to produce contour lines with constant responses. A surface plot is a three-dimensional graphic depiction of the response surface that could help with comprehension. Fig. 4b displays the contour plots of the interaction between various parameters at optimal values and the three-dimensional response surfaces of the biogas production rate. Interactions between variables have significant effects on responses, according to the models; hence, results were presented and interpreted in terms of interactions. The feedstock proportions

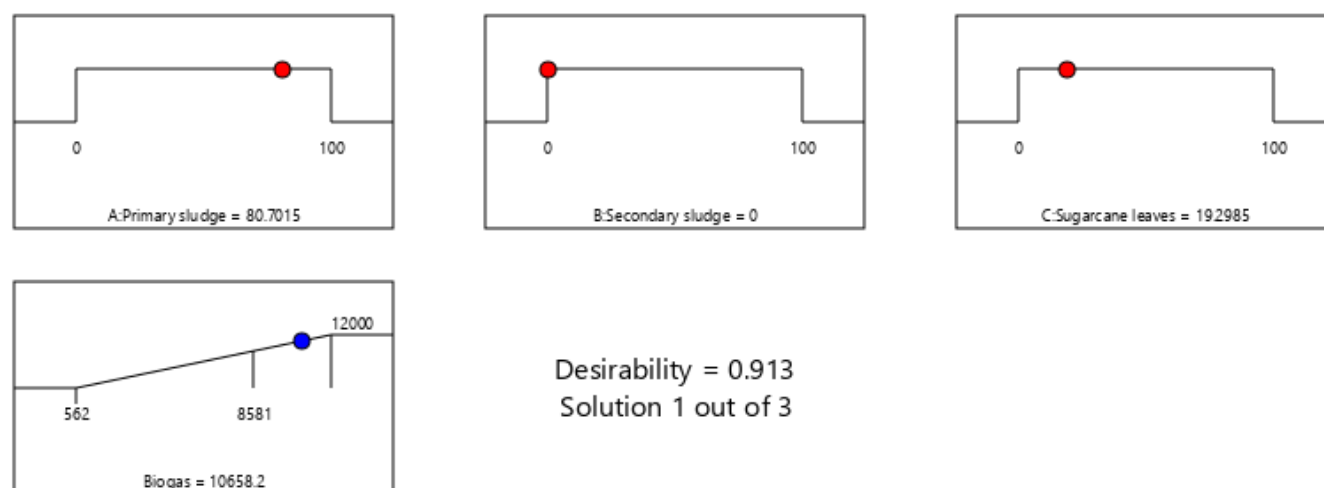
of 0.28, 48.98, and 50.73 (all in % (w/w)) were shown to be optimal for Primary sludge (A), Secondary sludge (B), and sugarcane straw, respectively. Experiments yielded a maximum Biogas of 8581 ml when these quantities were used.

Under optimal circumstances, the measured Y (8.581 L) exceeded the mono-digestion of primary sludge, secondary sludge, and sugarcane straw by 2.5, 4.06, and 4.57 times, respectively. The findings showed that, when carried out appropriately, the co-digestion process gave the anaerobic co-digestion system the optimal C/N ratio and nutrient balance. The C/N ratio of the substrate is essential for the growth and activity of methanogen bacteria. (Cai et al., 2022). Microorganism development and metabolism are hampered by excess C/N, which leads to ineffective substrate utilization and methane production. On the other hand, a low C/N ratio indicates that the substrate may contain too much nitrogen, which could prevent methane formation and result in ammonia inhibition (Gadow et al., 2022). The results of a literature search on sludge co-digestion with different kinds of lignocellulosic biomass were compared with the findings of this investigation. Research was done on the two-stage anaerobic co-digestion of mixed sludge in different volume ratios by (Sun et al., 2022). The highest cumulative CH<sub>4</sub> output (2.6436 L) was achieved at a volume ratio of 1:3 (primary sludge: secondary sludge). Using volatile fatty acids in two-phase anaerobic co-digestion and the organics produced in extracellular polymeric compounds could benefit from mixed sludge. Experiments were carried out in a different study to determine how much biogas could be produced



**Fig. 4. Contour plots (a) and Response surface plot (b) showing the interaction effects of the three composition factors on  $Y_{\text{Biogas}}$**





**Fig. 5. Ramps of the numerical optimization.**

by two combinations (A and B). The mixture composition for anaerobic co-digestion was developed using simplex designs. The best mixtures with the highest answers were 75.5 % sewage sludge and 24.5 % cow dung for sets A and B, respectively (Rao & Baral, 2011). In a study investigated by (Xie et al., 2017), the bio-methane potential evaluation of anaerobic mono-digestion and co-digestion of primary sludge with food waste or paper pulp rejection was used to clarify the synergistic effect. The specific methane yields during mono-digestion of primary sludge, food waste, and paper pulp reject were  $1.59 \times 10^{-1}$ ,  $6.52 \times 10^{-1}$ , and  $1.57 \times 10^{-1}$  L/g VS, respectively. Specific methane outputs from primary sludge co-digestion with food waste or paper pulp rejection were significantly higher, at  $7.99 \times 10^{-1}$  and  $3.68 \times 10^{-1}$  L/g VS, respectively. According to the results, the efficiency of methane generation is influenced by the C/N ratios, substrate types, and co-digestion processes. (Maragkaki et al., 2017) looked into co-digesting sewage sludge with cheese whey, olive mill effluent, and crude glycerol to increase the biogas output of a pilot-scale digester. This study shows that sewage sludge digesters can produce biogas rather effectively by adding a small amount (5%) of agro-industrial by-products, mainly crude glycerol.

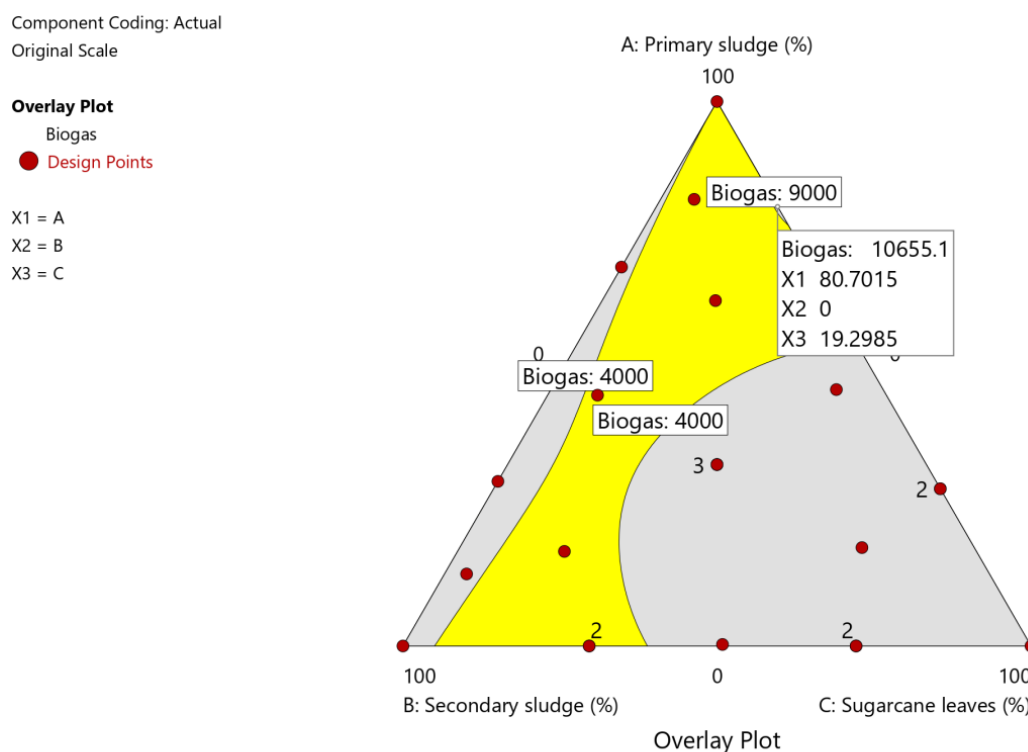
### 3. 4. Process optimization

Using numerical optimization, the models find the best trade-offs in the factor space to achieve various objectives. In order to predict the optimal factor levels that will maximize biogas production, the optimization function incorporates the maximum of Ybiogas. Design-Expert provided a numerical optimization for the RSM dataset, which was then subjected to a numerical optimization ramp. The numerical analysis will provide the ideal factor levels for obtaining the maximum biogas output. Simultaneously, the solutions tool examination ramps will produce a ramp that links the factor

levels to a significant target area defined by the user. The overall desirability and the attractiveness of each element and reaction are shown in Fig. 5. A highlighted (red and blue) point indicates the particular value of the component or response (horizontal movement of the point), together with the degree to which the target was fulfilled (how high up the ramp). The value of the advisability of process optimization was found to be 0.913, primarily dependent on how near the true optimum the lower and upper bounds are. The graphical optimization process uses the models to show the volume at which satisfactory response outcomes can be found. An overlay figure illustrating the region of the optimization process variable settings is presented in Fig. 6. For primary sludge (A), secondary sludge (B), and sugarcane straw, the optimal feedstock proportions were 80.701, 0.000, and 19.298 (all expressed as a percentage (w/w)), respectively. With these ratios, the highest biogas production was predicted to be  $1.065 \times 10^1$  L.

### 4. Conclusions

The optimal mixture design in an anaerobic co-digestion system can be used to calculate the best substrate composition. To produce biogas, the co-digestion of sewage sludge and SCS was optimized. The trials involving the anaerobic co-digestion of sewage sludge and SCS yielded better results; adding SCS to the system might stabilize it and boost biogas generation considerably. According to the observations, in the absence of the other two chemicals, primary sludge, secondary sludge, and SCS produced 3.445, 2.112, and 1.876 L of biogas, respectively. Also,  $7.03 \times 10^{-1}$  L of biogas was produced with an equal composition of all three chemicals. After the model was numerically adjusted, the optimal feed composition for the maximum biogas output (8.581 L cumulative) was determined to be 0.28% of feed A, 49.998% of feed B, and 50.73% of feed C. The results showed that a



**Fig. 6. The overlay plot for the process optimization region illustrates the variable settings.**

C/N ratio distortion caused a considerable decline in biogas output for compounds containing more than 50% sugarcane waste (Note the overlay plot). An eco-friendly and long-term sewage sludge treatment technique is co-digestion.

### Funding

This research was supported by Bu-Ali Sina University's Final Project Recognition Grant no. 1003381.

### Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

### References

- Alexandre, V.M.F., de Castro, T.M.S., de Araújo, L. V., Santiago, V.M.J., Freire, D.M.G., Cammarota, M.C., 2016. Minimizing solid wastes in an activated sludge system treating oil refinery wastewater. *Chem. Eng. Process. Process Intensif.* 103, 53–62. <https://doi.org/10.1016/J.CEP.2015.10.021>
- Amin, F.R., Khalid, H., Zhang, H., Rahman, S., Zhang, R., Liu, G., Chen, C., 2017. Pretreatment methods of lignocellulosic biomass for anaerobic digestion. *AMB Express* 7, 1–12. <https://doi.org/10.1186/S13568-017-0375-4/TABLES/2>
- Amin, M.A., Shukor, H., Yin, L.S., Kasim, F.H., Shoparwe, N.F., Makhtar, M.M.Z., Yaser, A.Z., 2022. Methane Biogas Production in Malaysia: *Challenge and Future Plan*. *Int. J. Chem. Eng.* <https://doi.org/10.1155/2022/2278211>
- Asaithambi, P., Yesuf, M.B., Govindarajan, R., Hariharan, N.M., Thangavelu, P., Alemayehu, E., 2022. A Review of Hybrid Process Development Based on Electrochemical and Advanced Oxidation Processes for the Treatment of Industrial Wastewater. *Int. J. Chem. Eng.* <https://doi.org/10.1155/2022/1105376>
- Athanasoulia, E., Melidis, P., Aivasidis, A., 2014. Co-digestion of sewage sludge and crude glycerol from biodiesel production. *Renew. Energy* 62, 73–78. <https://doi.org/10.1016/J.RENENE.2013.06.040>
- Cai, Y., Zheng, Z., Wei, L., Zhang, H., Wang, X., 2022. The characteristics of multi-substrates (low and high C/N) anaerobic digestion: focus on energy recovery and the succession of methanogenic pathway. *Bioresour. Technol.* 343. <https://doi.org/10.1016/J.BIORTECH.2021.125976>
- Comesaña, D.A., Comesaña, I.V., Iglesia, S.M. de la, 2018. Municipal Sewage Sludge Variability: Biodegradation through Composting with Bulking Agent. *Sewage.* <https://doi.org/10.5772/INTECHOPEN.75130>
- Elsayed, M., Andres, Y., Blel, W., Gad, A., Ahmed, A., 2016. Effect of VS organic loads and buckwheat husk on methane production by anaerobic co-digestion of primary sludge and wheat straw. *Energy Convers. Manag.* 117, 538–547.
- Federation, W.E., 1999. Standard Methods for the Examination of Water and Wastewater Standard

- Methods for the Examination of Water and Wastewater. *Public Health* 51, 940–940. <https://doi.org/10.2105/AJPH.51.6.940-a>
- Gadow, S.I., Estrada, A.L., Li, Y.Y., 2022. Characterization and potential of two different anaerobic mixed microflora for bioenergy recovery and decolorization of textile wastewater: Effect of C/N ratio, dye concentration and pH. *Bioresour. Technol. Reports* 17. <https://doi.org/10.1016/J.BITEB.2021.100886>
- Hagos, K., Zong, J., Li, D., Liu, C., Lu, X., 2017. Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. *Renew. Sustain. Energy Rev.* 76, 1485–1496. <https://doi.org/10.1016/J.RSER.2016.11.184>
- Janke, L., Weinrich, S., Leite, A.F., Terzariol, F.K., Nikolausz, M., Nelles, M., Stinner, W., 2017. Improving anaerobic digestion of sugarcane straw for methane production: Combined benefits of mechanical and sodium hydroxide pretreatment for process designing. *Energy Convers. Manag.* 141, 378–389. <https://doi.org/10.1016/J.ENCONMAN.2016.09.083>
- Kang, X., Zhang, Y., Li, L., Sun, Y., Kong, X., Yuan, Z., 2020. Enhanced methane production from anaerobic digestion of hybrid Pennisetum by selectively removing lignin with sodium chlorite. *Bioresour. Technol.* 295, 122289. <https://doi.org/10.1016/J.BIORTECH.2019.122289>
- Li, Yangyang, Xu, F., Li, Yu, Lu, J., Li, S., Shah, A., Zhang, X., Zhang, H., Gong, X., Li, G., 2018. Reactor performance and energy analysis of solid-state anaerobic co-digestion of dairy manure with corn stover and tomato residues. *Waste Manag.* 73, 130–139. <https://doi.org/10.1016/J.WASMAN.2017.11.041>
- Maragkaki, A.E., Fountoulakis, M., Gypakis, A., Kyriakou, A., Lasaridi, K., Manios, T., 2017. Pilot-scale anaerobic co-digestion of sewage sludge with agro-industrial by-products for increased biogas production of existing digesters at wastewater treatment plants. *Waste Manag.* 59, 362–370. <https://doi.org/10.1016/J.WASMAN.2016.10.043>
- Matheri, A.N., Ndiweni, S.N., Belaid, M., Muzenda, E., Hubert, R., 2017. Optimising biogas production from anaerobic co-digestion of chicken manure and organic fraction of municipal solid waste. *Renew. Sustain. Energy Rev.* 80, 756–764. <https://doi.org/10.1016/J.RSER.2017.05.068>
- Montgomery, D.C., 2020. Design and Analysis of Experiments, 10th Edition, Wiley. Wiley 1–682.
- Moodley, P., Kana, E.B.G., 2015. Optimization of xylose and glucose production from sugarcane leaves (*Saccharum officinarum*) using hybrid pretreatment techniques and assessment for hydrogen generation at semi-pilot scale. *Int. J. Hydrogen Energy* 40, 3859–3867. <https://doi.org/10.1016/J.IJHYDENE.2015.01.087>
- Mu, L., Zhang, L., Zhu, K., Ma, J., Ifran, M., Li, A., 2020. Anaerobic co-digestion of sewage sludge, food waste and yard waste: Synergistic enhancement on process stability and biogas production. *Sci. Total Environ.* 704, 135429. <https://doi.org/10.1016/J.SCITOTENV.2019.135429>
- Pellera, F.M., Gidarakos, E., 2017. Anaerobic digestion of solid agroindustrial waste in semi-continuous mode: Evaluation of mono-digestion and co-digestion systems. *Waste Manag.* 68, 103–119. <https://doi.org/10.1016/J.WASMAN.2017.06.026>
- Raheem, A., Sikarwar, V.S., He, J., Dastyar, W., Dionysiou, D.D., Wang, W., Zhao, M., 2018. Opportunities and challenges in sustainable treatment and resource reuse of sewage sludge: A review. *Chem. Eng. J.* 337, 616–641. <https://doi.org/10.1016/J.CEJ.2017.12.149>
- Rao, P.V., Baral, S.S., 2011. Experimental design of mixture for the anaerobic co-digestion of sewage sludge. *Chem. Eng. J.* 172, 977–986. <https://doi.org/10.1016/J.CEJ.2011.07.010>
- Rasouli, M., Babaei, H., Environmental, B. Ataeiyan, 2023. The effect of lignocellulosic waste on treatment of municipal wastewater in anaerobic digestion process. *icevirtuallibrary.com, Journal of Environ. Eng.* 18, 61–69. <https://doi.org/10.1680/jenes.22.00060>
- Rasouli, M., Dini, M., Ataeiyan, B., 2022. Anaerobic co-digestion of sewage sludge and *Cladophora* green algae: Investigation of synergistic effects and Optimization of substrate composition ratio. *Environ. Eng. Res.* <https://doi.org/10.4491/EER.2021.516>
- Serna-García, R., Zamorano-López, N., Seco, A., Bouzas, A., 2020. Co-digestion of harvested microalgae and primary sludge in a mesophilic anaerobic membrane bioreactor (AnMBR): Methane potential and microbial diversity. *Bioresour. Technol.* 298, 122521. <https://doi.org/10.1016/J.BIORTECH.2019.122521>
- Siddiqui, M.A.H., Pal, S.K., Dewangan, N., Chattopadhyaya, S., Sharma, S., Nekoonam, S., Issakhov, A., 2021. Sludge Formation Analysis in Hydraulic Oil of Load Haul Dumper 811MK v Machine Running at Elevated Temperatures for Bioenergy Applications. *Int. J. Chem. Eng.* <https://doi.org/10.1155/2021/4331809>
- Sittthikitpanya, N., Sittijunda, S., Khamtib, S., Reungsang, A., 2021. Co-generation of biohydrogen and biochemicals from co-digestion of *Chlorella* sp. biomass hydrolysate with sugarcane leaf hydrolysate in an integrated circular biorefinery concept. *Biotechnol. Biofuels* 14, 1–16. <https://doi.org/10.1186/S13068-021-02041-6/FIGURES/6>
- Smithers, J., 2014. Review of sugarcane trash recovery systems for energy cogeneration in South Africa. *Renew. Sustain. Energy Rev.* 32, 915–925. <https://doi.org/10.1016/j.rser.2014.01.042>
- Solé-Bundó, M., Garfí, M., Matamoros, V., Ferrer, I., 2019. Co-digestion of microalgae and primary sludge: Effect on biogas production and microcontaminants removal. *Sci. Total Environ.* 660, 974–981. <https://doi.org/10.1016/J.SCITOTENV.2019.01.011>

- Sugarcane | Land & Water | Food and Agriculture Organization of the United Nations | Land & Water | Food and Agriculture Organization of the United Nations [WWW Document], n.d. URL <https://www.fao.org/land-water/databases-and-software/crop-information/sugarcane/en/> (accessed 7.5.23).
- Sun, C., Guo, L., Zheng, Y., Yu, D., Jin, C., Zhao, Y., Yao, Z., Gao, M., She, Z., 2022. Effect of mixed primary and secondary sludge for two-stage anaerobic digestion (AD). *Bioresour. Technol.* 343. <https://doi.org/10.1016/J.BIORTECH.2021.126160>
- Tahwia, A.M., Hamido, M.A., Elemam, W.E., 2023. Using mixture design method for developing and optimizing eco-friendly ultra-high performance concrete characteristics. *Case Stud. Constr. Mater.* 18, e01807. <https://doi.org/10.1016/J.CSCM.2022.E01807>
- Villamil, J.A., Mohedano, A.F., San Martín, J., Rodriguez, J.J., de la Rubia, M.A., 2020. Anaerobic co-digestion of the process water from waste activated sludge hydrothermally treated with primary sewage sludge. A new approach for sewage sludge management. *Renew. Energy* 146, 435–443. <https://doi.org/10.1016/j.renene.2019.06.138>
- Wang, R., Lin, K., Ren, D., Peng, P., Zhao, Z., Yin, Q., Gao, P., 2022. Energy conversion performance in co-hydrothermal carbonization of sewage sludge and pinewood sawdust coupling with anaerobic digestion of the produced wastewater. *Sci. Total Environ.* 803. <https://doi.org/10.1016/J.SCITOTENV.2021.149964>
- Wei, Y., Van Houten, R.T., Borger, A.R., Eikelboom, D.H., Fan, Y., 2003. Minimization of excess sludge production for biological wastewater treatment. *Water Res.* 37, 4453–4467. [https://doi.org/10.1016/S0043-1354\(03\)00441-X](https://doi.org/10.1016/S0043-1354(03)00441-X)
- Xie, S., Wickham, R., Nghiem, L.D., 2017. Synergistic effect from anaerobic co-digestion of sewage sludge and organic wastes. *Int. Biodeterior. Biodegradation* 116, 191–197. <https://doi.org/10.1016/J.IBIOD.2016.10.037>
- Yaser, A.Z., Lamaming, J., Suali, E., Rajin, M., Saalah, S., Kamin, Z., Safie, N.N., Aji, N.A.S., Wid, N., 2022. Composting and Anaerobic Digestion of Food Waste and Sewage Sludge for Campus Sustainability: A Review. *Int. J. Chem. Eng.* <https://doi.org/10.1155/2022/6455889>