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Enhancing the Economic Viability of Anaerobic Digestion by Exploiting the Whole Biomass of Mango Waste and Its Residues after Digestion

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Abstract: A significant expansion of anaerobic digestion (AD) processes would certainly result in a reduction in the current dependence on fossil fuels. The operational costs, the large amounts of digestate generated and the expenses of dealing with it and the volatility of the fuel indexes represent major environmental and economical challenges to the diffusion of AD. Increasing the bio-products of AD could possibly help in increasing its profitability and limit these challenges. This study investigates the influence of mango starch and seed coats on the biogas produced from mango waste. To overcome the environmental challenges, the digestate was tested and its bio-fertiliser potential proven. The study reached the conclusion that the effect of the starch on the AD biogas of mango waste is low while the effect of the seed coats is quite high. This finding supports further investigations to evaluate the effect of the production of mango starch and seed coat-based products on the profitability of AD. The highest energy balance achieved was 65% at 32 °C, 3.93 g-VS organic concentration and 37% sludge concentration, which yielded a maximum CH_4 yield of 62.5%. This finding encourages the application of gate fees for accepting bio-waste, which may help in overcoming its economic challenges.

Keywords: anaerobic digestion; mango waste; waste management; integration approach; response surface methodology

1. Introduction

Fossil fuels and their derivatives have adverse impacts on humans, animals and the environment. The huge amounts of the waste generated every day also represent a major threat and can cause major negative environmental consequences. Because of that, interest in exploiting renewable resources in the production of renewable energy and bio-products is gaining much attention [1]. Anaerobic digestion (AD) has proven its potential in converting many types of biomass into value-added products, however currently investment in AD is not very attractive due to some operational issues, the volatility of the fuel indexes and the large amounts of waste generated when it is applied at large scale [2–4], whereby the large amounts of digestate generated post-AD can pose a major environmental threat if are not properly handled [5].

Furthermore, food-based digestate typically contains the main elements of fertilisers such as N, P and K in varied concentrations. It also contains low amounts of other nutrients and trace elements which can help in maintaining soil fertility. AD digestate has broadly confirmed its potential in agriculture applications as a bio-fertiliser, soil amendment, etc. [6]. Moreover, the generation of digestate in large quantities and therefore storing it and transporting it, constitute a financial burden for AD plants. If the whole digestate must be separated into liquid and solid, an additional cost would be incurred. Due to all that and to dispose of it in a proper way, many AD plants are selling the digestate to farmers or giving it away for free as its value is quite low and to encourage farmers to substitute their commercial fertiliser with the digestate (bio-fertiliser) [7].

However, these operational issues can be controlled if they are considered at an early stage [8,9]. One of the recommended solutions to radically overcome the environmental and economical issues and therefore improve the investment in AD, is the application of an integrated approach by making full use of the entire biomass. An integrated approach is a relatively new method. It refers to the processing of biomass to produce multiple bioproducts such as biochemicals, biofuels, biomaterials etc. In terms of AD, it involves incorporating it with other bioconversion processes or bioproduct production processes in order to produce multiple bioproducts alongside with the AD products. It corresponds to the biorefinery concept [2,10]; this approach is still at an early stage and requires more assessments to prosper and increase its application in AD plants [11].

Mango is a common tropical stoned fruit and one of the most popular fruits worldwide. In terms of consumption and production, it is the fifth most consumed fruit and the second most produced fruit in the world. An estimation indicated that, in the period of from 1960 to 2008, the production of mango increased from about 10 to 35 million tons [12]. Additionally, according to an estimation in 2008, the Asian continent was the continent producing more mango, at a rate of 75% of the total [12,13]. In 2015, the projected mango production was approximately 42 million tons per annum [14]. India was the largest producer, with a production at approximately 1.52 million tons per annum [14,15], while Europe represents only to 0.02% of the mango production [15].

In addition, mango mainly consists of three fractions: the edible tissues (33–85% of the total fruit), peel (7–24%) and kernel (9–40%) [16]. It is mostly consumed fresh and rarely processed by industry. In any case, mango skins and kernels are disposed of as waste residues [13,17]. They represent 35–60% of the total mango weight [18]. According to Leanpolchareanchai et al. [19], approximately 1 million tons of mango seeds are generated annually and not utilised in any industrial application. As a solution to this problem, Sonthalia and Sikdar [20] revealed that the conversion of the seed kernel to starch is one solution. Mango kernels are composed of multiple nutritional compounds such as starch, fat, flour and antioxidants [21,22]. Starch constitutes approximately 21% of the seed [23], and as biomass byproduct and a natural raw material for many bioproducts, it has confirmed its potential in many industrial applications [24,25]. It exists in the roots, pulps, seeds and tubers of plants at different concentrations. It is produced by plants during photosynthesis [26,27]. Furthermore, the properties of mango starch are much like those of cassava starch or as it is commercially known, tapioca starch. Its solubility is relatively high and its viscosity is a little bit lower than the viscosity of the other common starches [23]. The amylose and amylopectin in mango starch represent approximately 39.9% and 60.1% of the total, respectively [28].

Instead of performing numerous experimental runs, the use of a design of experiments (DOE) technique reduces the number of the experimental runs, therefore facilitating the collection of data. Beside its statistical analysis of the results and its measurement of the adequacy of the developed model, it allows for the identification of the influences on the studied factors and their interactions with the responses. Additionally, engineers and scientists typically seek to optimise the process they are studying. This involves specifying the values of the input parameters at which the responses reach their optimum. Response surface methodology (RSM) is an optimisation technique used in DOE. It is normally applied in describing and assessing the performance of the process and obtaining the optimum results. The Box-Behnken design (BBD) is one of the most popular approaches amongst the RSMs. BBD was applied in the proposed study for generating the design matrix, statistically analysing the results, measuring the adequacy of the data, specifying the influence of each parameters and their interactions (if they exist) on the biogas quantity and quality produced from mango waste and obtaining optimum results.

Furthermore, temperature, organic concentration and sludge concentration are important factors which must be carefully considered in any AD as they could cause major changes in the biogas quantity and quality. Due to that and the following points as well, the following facts were considere in the current study: (1) the microbial activities are sensitive to temperature [29,30], (2) the improper balancing of organic and sludge concentrations could cause imbalancing of the bacterial population [31], (3) the larger the amounts of the wastes utilised, the higher the contribution of AD to waste management, and (4) the sludge is better kept at the lowest level, because it is considered an expense, limiting the accumulation of the digestate in large amounts and minimising the costs for transporting it, storing it, etc. [7,32].

The current study aims to increase the production of AD bioproducts through making full use of the biomass and the digestate generated from it in order to limit the environmental and economical obstacles associated with the use of AD when it is applied at a large scale. Therefore, the study investigates the influences of the mango coats and mango starch as the mango residues on the quantity and quality of the biogas produced from mango waste. This was carried out by comparing the biogas produced from the peels and the leftovers of the process of isolation of starch from the seeds with the peels and the whole kernels. In addition, the optimum biogas in terms of the quality and cost at the optimal studied factors (temperature, organic concentration g-VS and sludge %) were obtained by applying the RSM technique.

2. Materials and Methods

2.1. Experimental Setup, Substrates and Inoculum Characterization and Preparation

2.1.1. Experimental Setup

Prior to conducting the AD experiments, some preliminary tests were performed to set the ranges for all factors. Based on the tests, the range of each factors were set to be as follows: temperature (A) 32–38 °C, organic concentration (B) 1.6–6.5 g-VS and sludge concentration (C) 20–50% of the weight of the materials inside the digesters. Table 1 shows the studied factors and levels in both actual and coded values.

Table 1. Independent variables with their levels.

Variable Coded	Units	Limits		
		−1	0	1
Temperature (A)	°C	32	35	38
Organic concentration (B)	g-VS	1.6	4.05	6.5
Sludge concentration (C)	%	20	35	50

Moreover, the experiments were implemented in a batch system at a lab scale. Five water baths were used and set at the three different temperature levels. The temperature levels were chosen to be at the mesophilic range as most AD plants carry out the digestion process under mesophilic conditions, where the mesophilic bacteria are less sensitive to temperature fluctuations of ±3 °C [33,34]. On the day of the experiment, the required amount of digested sludge (inoculum) was collected directly from a fermenter located at Green Generation Ltd. (Kildare, Ireland). This plant is processing mixed food waste operating within the mesophilic range.

2.1.2. Substrates and Inoculum Characterization

The sludge was directly utilised in the experiment. The pH of the sludge was measured and recorded to ensure the equilibrium of the system and the stability of the digester and was found to be 7.9 ± 0.1. The contents of the sludge from the total solids (TS) and volatile solids (VS) perspective were also measured and found to be 5.5 g/100 g and 86.36% of the (TS), respectively. A predetermined quantity of a popular mango variety named Kent, was obtained from a local shop in Dublin and processed on the same day of the experiment. Because green mangoes contain higher amounts of starch, only green mangoes were carefully selected and used. They thoroughly washed in clean water and peeled. The kernels were opened to extract the seeds. After isolation of the seed skins, the seeds were cut into quarters and prepared for separation of the starch. Meanwhile, the coats were also cut in smaller slices.

2.1.3. Preparation

In order to investigate the influences of the coats and starch on the biogas, the residues were classified into three groups: (1) mango peels and seed skins, (2) seeds and (3) seed coats. After mixing group 2 with clean water, samples were mechanically pre-treated in a beating device called a Hollander beater for 5 min (see Figure 1).

Figure 1. Hollander beater and mango residues, (**a**) Hollander beater, (**b**) mango peels, (**c**) mango seeds, and (**d**) mango seed coats.

This was done to separate the starch from the seeds. Post beating pre-treatment of the seeds, the mixture was filtered into a container using a strainer and left for one hour until the starch settled on the bottom of the container. The cake remaining after filtering the starch was added to group 1. The remaining processed water (which stayed on the top) was further mixed with group 1 samples and the remaining cake in the beater in a ratio of (4:1) % *w/w* and beaten for 5 min. The weights of the leftover cakes were taken into consideration before blending it with group 1 and water. The same procedure was also followed with the controls (the entire biomass including starch and mango coats). Bio-digesters were fed with 400 mL of the pre-treated feedstock and sludge at an inoculum to sludge ratio (ISR) of 1 and distributed between the water baths at the different temperatures according to the design matrix shown in Table 2. Each run in the design matrix was conducted in triplicate and an average was taken. The digestion process ran for 21 days hydraulic retention time (HRT). In this paper, the resulted biogas quantity per g-VS and the concentrations of the CH_4 and CO_2 were the only responses considered. Gas measurement apparatus such as a volumetric flask, round bottom flask, and an electric pump and a biogas detector were used to measure the biogas yield and the gases concentrations, respectively.

In order to compare between the biogas produced with and without the starch and coats, nine reactors as controls were fed by beaten mango residues (mango peels, seed skins, seed coats and seeds) and sludge at 6.5 g-VS organic concentration and 50% sludge concentration. Three controls were digested at each of the three different temperature level. The same procedure was followed with the controls.

Table 2. The design matrix in actual values.

Std	Run	Parameter 1 A: Temperature (°C)	Parameter 2 B: Organic Concentration (g-VS)	Parameter 3 C: Sludge Concentration (%)
1	12	32	1.6	35
2	9	38	1.6	35
3	13	32	6.5	35
4	4	38	6.5	35
5	8	32	4.05	20
6	6	38	4.05	20
7	15	32	4.05	50
8	10	38	4.05	50
9	1	35	1.6	20
10	2	35	6.5	20
11	3	35	1.6	50
12	7	35	6.5	50
13	5	35	4.05	35
14	14	35	4.05	35
15	11	35	4.05	35
16	17	35	4.05	35
17	16	35	4.05	35

The powder remaining on the bottom of the container (containing the starch) was spread on a tray and air-dried until it was totally dry. The darker colour powder on the surface of the starch powder was removed using a laboratory scraper. Thereafter, starch powder was sieved using a 1 mm grid siever in order to obtain fine starch and its weight was measured and recorded.

On the other hand, the application of the digestate in agriculture and other uses is beneficial not only to the AD plants but to farmers, the environment, etc. Hence, the digestate generated from the bioreactor which produced the highest biogas volume and CH_4% was sent to a chemistry laboratory to check its content for the three basic nutrients of fertiliser (N, P and K) and their quantities.

2.2. Measurement of the TS, MS and VS

The moisture content (MS), total solids (TS) and volatile solids (VS) were measured according to the standard method (NREL/MRI LAP 1994, 2008) [35,36]. A laboratory oven and muffle furnace were used to measure the mango residues and controls. Three samples of both the residues and controls were taken from the beater.

The organic concentration g-VS or as is better known the VS% of the beaten mango residues after the isolation of starch and coats were evenly distributed in three containers. The organic concentration g-VS of each mango residue in the containers were set to one of the organic concentration levels. The concentrations were adjusted by water dilution to 6.5, 4.05 and 1.6 g-VS using Equations (1) and (2) below. The organic concentration g-VS of the controls were only set to 6.5 g-VS. This was in order to compare it with the predicted results of the biogas produced from the mango residues after the separation of starch and coats at the highest organic concentration g-VS and sludge concentration and each of the three temperature levels:.

$$C1\,[\mathrm{g}] \times VI\,[\mathrm{mL}] = C2[\mathrm{g}] \times V2\,[\mathrm{mL}] \tag{1}$$

$$V2\,[\mathrm{mL}] = V1\,[\mathrm{mL}] + D\,[\mathrm{mL}] \tag{2}$$

2.3. Optimisation

The idea behind increasing the production of bioproducts in an AD process is to overcome the economical and environmental challenges associated with it and therefore, increase the dependence on it and make its investments more attractive. That is mainly due to its effectiveness in reducing our dependence on fossil fuels. However, to reach the desired goal of the current study, three optimisation criteria were set in terms of the quality and cost. Table 3 shows the three optimisation criteria. As is clear from the table, the 1st criterion was the quality criterion. It is aimed at finding out the optimal biogas with no limitation on the process factors at a minimum CO_2% and maximum biogas volume per g-VS, and CH_4%. In addition, the other two criteria were set in terms of cost. They were set almost the same with only slight changes in the restrictions of the organic concentration g-VS. In the setting of the second and third criteria, the major revenue and expenses of AD plants were taken into account. Furthermore, in addition to the sale of the heat, electricity and digestate, the gate fees are also considered one of the main revenues of some AD plants but not in all. Due to that, in the third criterion the gate fees were considered as a revenue source thus, the restrictions of the organic concentration were changed to "maximise". On the other hand, the energy consumed in the pre-treatment and digestion processes as well as the sludge are all considered expenses and must be kept as low as possible. When setting the cost criteria, only the energy consumed in the digestion process was considered where the energy consumed by the pre-treatment process was quite low (0.15 kWh/5 min for processing of 5 kg). The sludge as an input (as some AD plants are buying it from external suppliers) were also considered as an expense. In all criteria, the importance of all responses was set to 3 except for CH_4% which was 5 (most important).

Table 3. Optimisation criteria.

Name	Goals			Lower Limit	Upper Limit	Importance
	1st Criterion	2nd Criterion	3rd Criterion			
Temp., °C	is in range	minimise	minimise	32	38	3
Organic Concentration, g-VS	is in range	is in range	maximise	1.6	6.5	3
Sludge Concentration, %	is in range	minimise	minimise	20	50	3
Biogas, cc/g-VS	maximise	maximise	maximise			3
CH_4, %	maximise	maximise	maximise			5
CO_2, %	minimise	minimise	minimise			3

2.4. Energy Balance

The energy balance was calculated at a lab scale based on the optimum results. The energy consumed by the digestion process was the only one considered, while, the energy consumed by the Hollander beater was neglected as it was quite low. The energy consumed by each water bath was measured using electric energy meters throughout the whole duration of the experiment (21 days). The full capacity of each water bath was not fully used. As the water baths were running at full performance at the pre-determined temperature levels, the electric energy consumed will be the same whether only one reactor was placed in the bath or more. According to the water baths' manual, the dimensions of each water bath is 590 × 220 × 350 mm and the full capacity is 45 L. The electric energy which was consumed by each water bath at each of the three temperature levels is based on using the full capacity of the bath. Therefore, a number of assumptions were set for calculating the energy balance, which were:

- The total capacity of the water bath was utilised.
- Only one reactor with a volume of 2/3 of the total volume of the water bath was utilised. The volume of the reactor used was 400 mL thus, the assumed reactor was equivalent to the volume of 75 reactors of the reactors have been used in the experiment (30 L).

- The remaining capacity (1/3) was the water volume which is used to heat up the reactor in the bath.

In order to calculate the energy gain/loss, Equations (3)–(7) were applied to the optimum results based on the three criteria [37,38]:

$$B_S = CH_4\,[\%] \times 9.67 \tag{3}$$

$$E_P = B_S \times B_P \tag{4}$$

$$E_C = \frac{E_{PT}}{VS_m} \tag{5}$$

$$\text{Net } E_P = E_P - E_C \tag{6}$$

$$\text{Energy balance} = \frac{E_P - E_C}{E_C} \times 100 \tag{7}$$

where, B_S is the energy content of the biogas produced from mango waste [kWh m^{-3}], CH_4 is the average of methane content [%], 9.67 is the energy content of 1 Nm^3 of biogas [kWh/Nm^3], B_S is the quantity of biogas produced for each gram of VS of the mango waste [m^3g^{-1}VS], E_P is the energy of the biogas produced from 1 g of VS of the mango waste [kWh g^{-1}VS], E_{PT} is the electric energy consumed in the digestion process [kWh], VS_m is the total quantity of VS into the reactor [g], E_C is the is the energy consumed during the digestion to digest 1 g of VS of mango waste [kWh g^{-1}VS], Net E_P is the total energy resulted by one g of VS of the pretreated mango waste and the energy gain/loss is the difference between (E_P) and (E_C). If the (E_C) is > than the (E_P), that means the AD of the mango wastes is not economically feasible.

3. Results and Discussion

3.1. Results

3.1.1. Quantity and Quality of the Biogas Produced before/after the Separation of Starch and Mango Seed Coats

The weights of all main mango wastes were measured separately. The results of the measurements revealed that mango wastes represent approximately one third of the total weight of the mango. This finding is in accordance with O'Shea et al. who stated that between 35–60% of the total mango weight is discarded after processing. The weights of the peels, seed and seed coats were 90, 25, 30 g per mango, respectively. This means that, the seed represents approximately 18% of the total weight of the mango waste. The starch weight was also measured and found to constitute 4 ± 1% of the total weight of the mango waste and 19 ± 2% of the seed weight. This finding is in accordance with Saadany et al. [23]. As previously described, the starch was only included in the controls along with whole mango wastes and excluded from the other reactors. Table 4 illustrates the general results of the AD of mango wastes after the separation of starch and coats. The table also includes the pH levels of each run after the digestion process. To simplify the comparison of the results and obtain more accurate results, the organic concentration g-VS of the controls were set to the highest concentration (6.5 g-VS). Table 5 shows the differences between the results of the controls and the predicted results of the biogas produced from the mango wastes after the separation of starch and coats at 6.5 g-VS and 50% sludge at the three temperature levels.

According to Tables 4 and 5, the pH levels of the controls and all runs were in the range of 6.5 to 8. This indicates the equilibrium of the systems and the stability of all digesters. It worth also noting that, prior to feeding the digesters, the pH of the sludge was measured and found equal to 7.9 ± 1. From Table 4, a relationship between the VS concentration (B), sludge concentration (C) and the pH level can be observed. Therefore, the pH level decrease as the organic concentration increases and the sludge concentration decreases. In regards to the volume of the biogas produced from the one g-VS, the highest was found at 35 °C (A), 1.6 g-VS (B) and 50% (C). While, the lowest was obtained at 35 °C (A), 6.5 g-VS (B) and 20% (C). On the other hand, the largest CH_4% was found at the centre point and was 68.9%. Run 2 which has recorded the lowest biogas volume/g-VS has also recorded the greatest level of CO_2 (61%).

Table 4. The pH levels and the results of all responses of the biogas produced from each sample.

Std	Run	pH Level	Biogas Vol. cc/g-VS	CH_4 %	CO_2 %
1	12	7.7	681.7	54.9	32.8
2	9	7.7	745.9	59.6	25.6
3	13	6.9	274.9	44.8	44.9
4	4	7.0	379.8	51.3	38.2
5	8	7.0	319.4	47.0	36.8
6	6	7.3	426.3	56.9	32.4
7	15	7.8	701.9	52.0	29.5
8	10	8.0	834.5	61.9	28.8
9	1	7.4	440.1	53.8	30.3
10	2	6.5	132.6	20.4	61.0
11	3	7.9	984.0	42.8	40.8
12	7	7.9	573.0	54.6	30.1
13	5	7.8	473.1	68.9	25.8
14	14	7.7	490.9	65.9	25.9
15	11	7.7	497.1	65.4	26.1
16	17	7.6	503.4	65.8	26.2
17	16	7.6	464.6	65.5	22.8

Table 5. A comparison between the results of the controls and the predicted results at the same conditions.

	Sample no.	Temp., °C	Organic Conc., g-VS	Sludge Conc., %	pH Level	Biogas, cc/g-VS	CH_4, %	CO_2, %
Actual (Controls)	1	32	6.5	50	7.9	336.8	48.1	38.3
	2	35			7.8	434.8	62.5	27.4
	3	38			7.8	492	65.4	30.2
Predicted	1	32	6.5	50		528.1	48.9	34.6
	2	35				542.9	52.8	32.2
	3	38				630.3	56.7	29.9
Difference %						−56.82	−1.71	9.65
						−24.85	15.52	−17.62
						−28.12	13.35	1.15

In reference to Table 5, it is clear that the incorporation of the starch and coats negatively influenced the biogas quantity, while, its influence on the quality of the biogas was also negative but less than its influence on the biogas quantity. As the starch weight were relatively low in the controls compared to the total weight of the waste, this finding can be mainly attributed to the stiffness of the mango seed coats. Therefore, they were difficult to digest by the microorganism.

The followings show the analysis of each response separately. They were carried out using the DOE. Each analysis of response, provides a statistical analysis of the developed model, measures the adequacy of the model, depicts the behaviour of each factor on the response and shows the significant influences of each factor and their interactions (if any) on the response.

(1) Biogas volume per each gram-VS

In the proposed study, if the *p*-value of the model and of any term does not exceed the level of significance ($\alpha = 0.05$), they are considered statistically significant within the confidence interval of $(1 - \alpha)$. Table 6 show the ANOVA analysis generated by Box-Behnken design (BBD) as a RSM approach for the biogas volume produced from one gram-VS of the mango waste after the separation of the starch and coats. The analysis has proven that the model was significant, the lack of fit was insignificant and the regression was good. It has also illustrated that the coded model terms: A, B, C, A^2 and C^2 had significant impacts on the biogas yield produced from each g-VS. It can be observed from the analysis also that, C (%) has the most significant influence, following by the influence of the B (g-VS). While, the influence of A (°C) was less significant than the influences of the other factors.

Table 6. The ANOVA table for the biogas volume produced from g-VS response.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	***p*-Value Prob > F**	
Model	711,662.02	6	118,610.34	161.60	$2.29^{\text{E-09 } 2.29 \times 109}$	significant
A-Temperature, °C	20,869.25	1	20,869.25	28.43	0.000332	
B-Organic Conc., g-VS	278,034.25	1	278,034.25	378.81	$2.8^{\text{E-9 } 2.8 \times 109}$	
C-Sludge Conc., %	393,828.13	1	393,828.13	536.57	$5.08^{\text{E-9 } 5.08 \times 109}$	
BC	2678.06	1	2678.06	3.65	0.085183	
A^2	5575.69	1	5575.69	7.60	0.020262	
C^2	9804.95	1	9804.95	13.36	0.004426	
Residual	7339.77	10	733.98			
Lack of Fit	6265.58	6	1044.26	3.89	0.104812	not significant
Pure Error	1074.19	4	268.55			
Cor. Total	719,001.79	16				
	$R^2 = 0.99$				*Pred.* $R^2 = 0.95$	
	Adj. $R^2 = 0.98$				*Adeq. Precision* = 46.97	

Equation (8) illustrates the final mathematical equation in terms of actual factors as obtained by ANOVA for biogas volume/g-VS:

$$\begin{aligned} \text{Biogas, cc/gVS} = -280.41 + 17.02 \times (\text{Temperature}) - 76.1 \times \\ (\text{Organic concentration}) + 14.79 \times (\text{Sludge concentration}) \end{aligned} \tag{8}$$

The perturbation plot (Figure 2) illustrates the behaviour of each factor on the response. As it can be noted, the temperature (A) has a slight positive influence on the volume. However, increasing the organic concentration (B) decreases the biogas volume. It is also clear that, the sludge concentration (C) is in direct proportion with the biogas volume produced from g-VS.

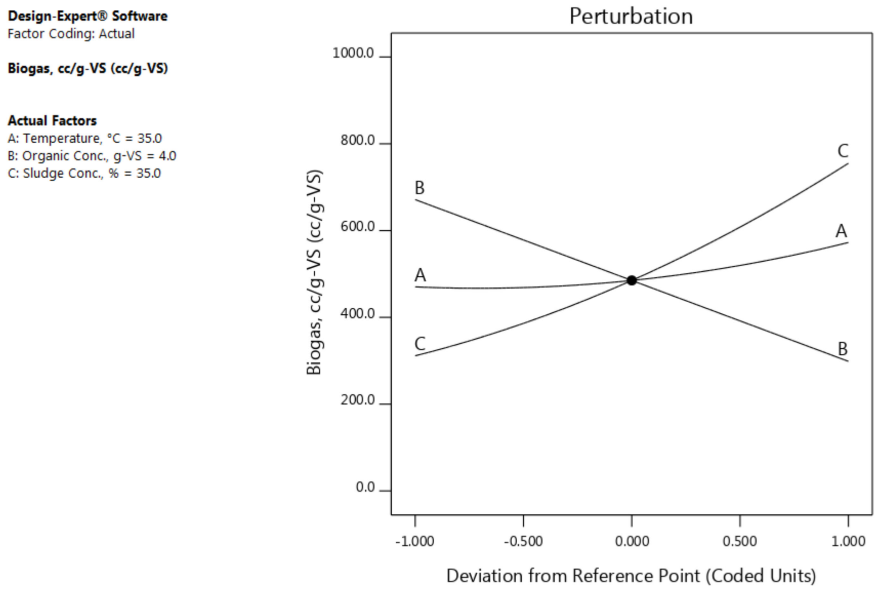

Figure 2. The perturbation plot of the biogas volume produced from g-VS.

(2) Methane concentration

The ANOVA analysis of the CH_4% is given in Table 7 and depicts that the developed model and the lack of fit were significant. It also shows that the model terms A, B, C, BC, B^2 and C^2 have significant influences on the CH_4%. The "Adeq. Precision" as one of the adequacy measurement tools was greater than 4, therefore, the model can be used to navigate the design space.

Table 7. The ANOVA table for CH_4% response.

Source	Sum of Squares	df	Mean Square	F Value	*p*-Value Prob > F	
Model	2210.69	6	368.449	79.446	$7.43^{\text{E-087.43} \times 108}$	significant
A-Temperature, °C	120.13	1	120.125	25.902	0.000471	
B-Organic Conc., g-VS	200.00	1	200.000	43.125	$6.34^{\text{E-5 } 6.34 \times 105}$	
C-Sludge Conc., %	137.78	1	137.780	29.709	0.000281	
BC	510.76	1	510.760	110.132	$1.02^{\text{E-6 } 1.02 \times 106}$	
B^2	676.21	1	676.213	145.807	$2.75^{\text{E-7 } 2.75 \times 107}$	
C^2	497.53	1	497.533	107.280	$1.15^{\text{E-6 } 1.15 \times 106}$	
Residual	46.38	10	4.638			
Lack of Fit	37.76	6	6.293	2.920	0.159492	not significant
Pure Error	8.62	4	2.155			
Cor. Total	2257.07	16				
$R^2 = 0.98$				*Pred.* $R^2 = 0.90$		
Adj. $R^2 = 0.97$				*Adeq. Precision* = 31.81		

In addition, the actual mathematical model resulted from this response is illustrated in Equation (9):

$$
\begin{aligned}
\mathrm{CH_4}, \% = {} & -30.87 + 1.291667 * (\mathrm{Temperature}) + 4.27 \\
& \times (\mathrm{Organic\ concentration}) + 2.41 \times (\mathrm{Sludge\ concentration}) \\
& + 0.31 \times (\mathrm{Organic\ concentration}) \times (\mathrm{Sludge\ concentration}) \\
& - 2.11 \times (\mathrm{Organic\ concentration})^2 - 0.05 \\
& \times (\mathrm{Sludge\ concentration})^2
\end{aligned}
\tag{9}
$$

Moreover, Figure 3 shows the predicted results versus the actual results plot. The plot helps in validating the strength of the generated model and shows the correlation between the actual and predicted response values. The distribution of most of the points in Figure 3 on the diagonal line or closer to it implies that, the model predicted the results very well and thus, there was a good correlation between the model's predicted results and the actual ones.

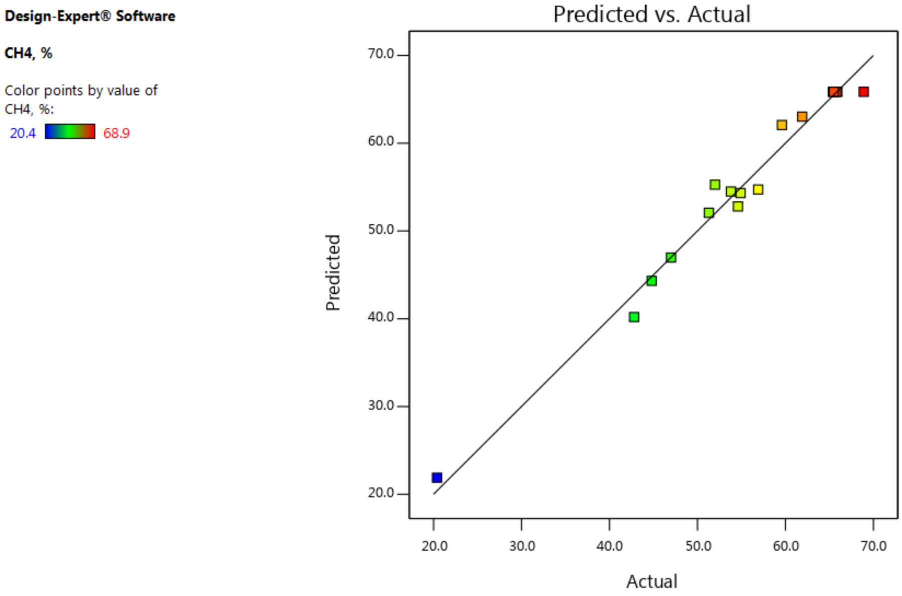

Figure 3. Scatter plot of the predicted results versus the actual ones of the $\mathrm{CH_4}$% response.

According to the perturbation plot in Figure 4, the $\mathrm{CH_4}$% increases as the temperature increases in the studied range. While, it is increases as the organic concentration (B) increases until it is reaches just before the reference point (4.05 g-VS) and begins to decline gradually. In terms of the sludge concentration (C) influence, the $\mathrm{CH_4}$% increased as the concentration of the sludge increased until the concentration of the sludge reached approximately 40% and then the $\mathrm{CH_4}$% started to decrease slightly.

The interaction and contour plots are provided to illustrate the influences of the interactions of the factors. Figures 5 and 6 show the interaction and contour plots of the influence of the interaction of the organic concentration and sludge concentration on the $\mathrm{CH_4}$%. It is obvious that, when the organic concentration is at the lowest level, changing in the sludge concentration does not make a big difference on the $\mathrm{CH_4}$%, but when the organic concentration is at the highest level, a significant variation in the $\mathrm{CH_4}$% can be observed when the sludge concentration is increased or decreased. In addition, when the sludge concentration is kept at the lowest level and the organic concentration increases in the studied range, thus the $\mathrm{CH_4}$% stays at approximately a constant level and then remarkably decreases after the organic concentration has reaching approximately 2.8 g-VS.

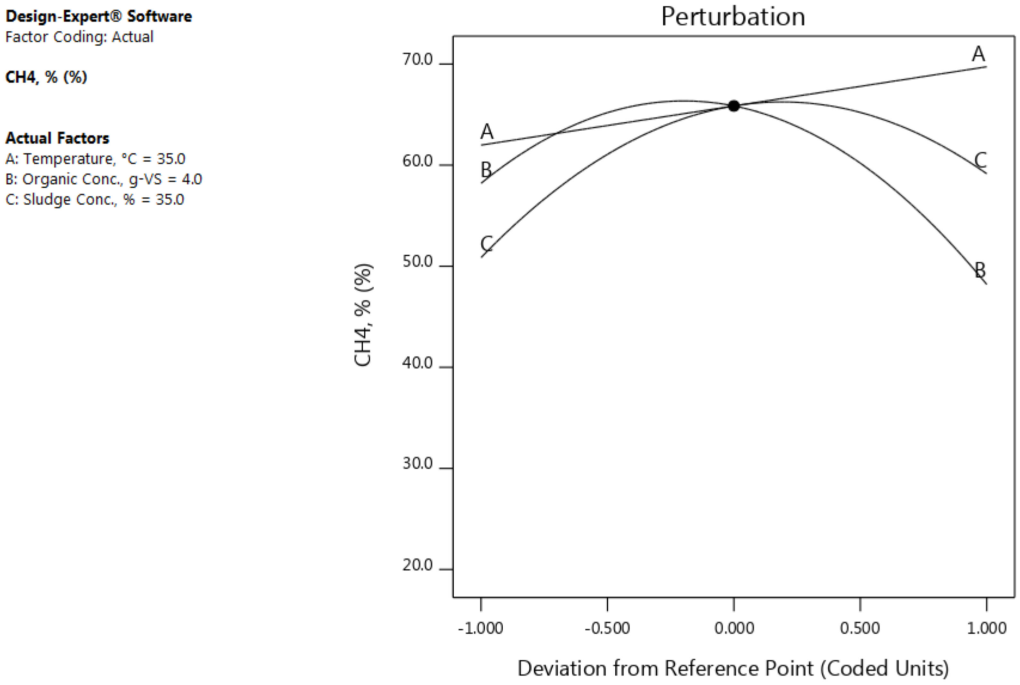

Figure 4. The perturbation plot of CH_4% response.

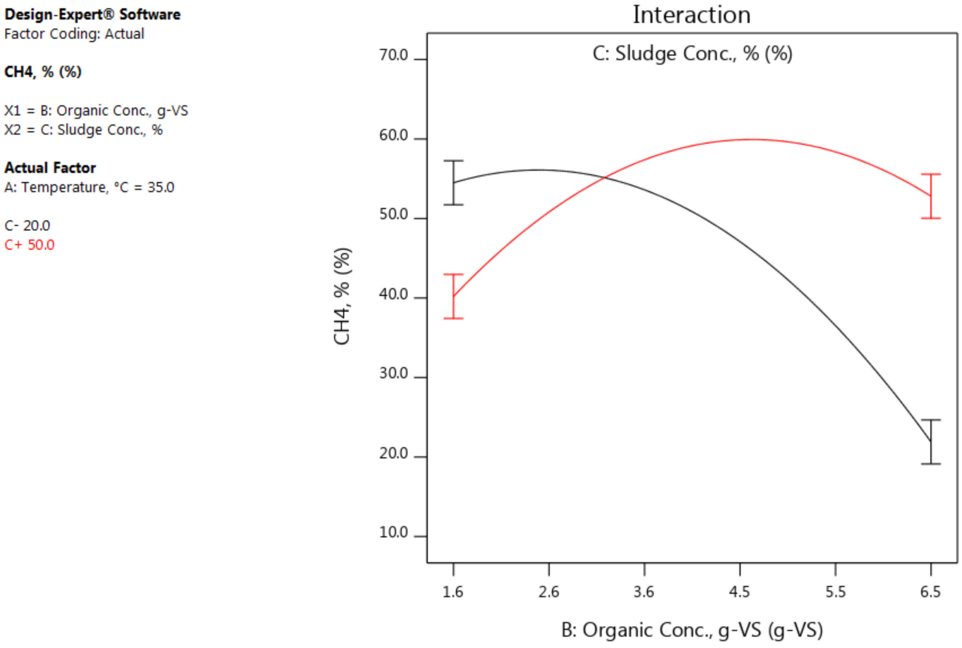

Figure 5. An interaction plot illustrating the influence of (BC) on the CH_4%.

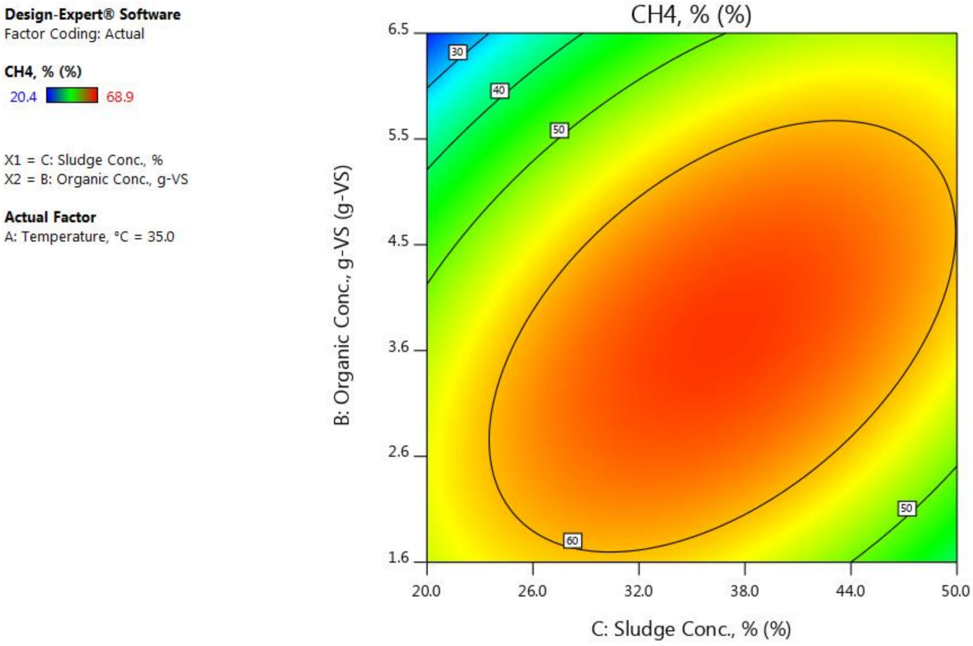

Figure 6. A contour plot illustrating the influence of (BC) on the CH_4%.

(3) Carbon dioxide concentration

The results of the ANOVA analysis of the developed model which was generated based on the CO_2% has confirmed that the validity of the model was significant and found the "lack of fit" to be insignificant (see Table 8). It worth noting that, the CH_4% and CO_2% responses were significantly influenced by the same terms. The analysis has also proved that the "Adeq. Precision" of the model was greater than 4 and therefore the model can be used to navigate the design space. It was also shown that the regression of the model was good as the R^2, adj. R^2, and pred R^2 were all close to 1 and the "pred R^2" of 0.89 was in reasonable agreement with the "adj. R^2" of 0.96.

Table 8. The ANOVA table for CO_2% response.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	***p*-Value Prob > F**	
Model	1390.90	6	231.817	62.00	$2.^{47\text{E-}072.47} \times 10^{7}$	significant
A-Temperature, °C	45.13	1	45.125	12.07	0.005981	
B-Organic Conc., g-VS	249.76	1	249.761	66.80	$9.76^{\text{E-}6\ 9.76 \times 106}$	
C-Sludge Conc., %	122.46	1	122.461	32.75	0.000192	
BC	428.49	1	428.490	114.60	$8.48^{\text{E-}7\ 8.48 \times 107}$	
B^2	371.51	1	371.511	99.36	$1.64^{\text{E-}6\ 1.64 \times 106}$	
C^2	145.99	1	145.994	39.05	$9.52^{\text{E-}5\ 9.52 \times 105}$	
Residual	37.39	10	3.739			
Lack of Fit	29.10	6	4.850	2.34	0.215096	not significant
Pure Error	8.29	4	2.073			
Cor. Total	1428.29	16				
$R^2 = 0.97$				*Pred.* $R^2 = 0.89$		
Adj. $R^2 = 0.96$				*Adeq. Precision* = 28.63		

Equation (10) shows the actual mathematical model of this response according to the ANOVA:

$$\begin{aligned} CO_2, \% = {} & 70.97 - 0.79 \times (\text{Temperature}) - 0.52 \times (\text{Organic concentration}) - 0.95 \\ & \times (\text{Sludge concentration}) - 0.28 \times (\text{Organic concentration}) \\ & \times (\text{Sludge concentration}) + 1.56 \times (\text{Organic concentration})^2 \\ & + 0.03 \times (\text{Sludge concentration})^2 \end{aligned} \tag{10}$$

Figure 7 indicates that, there was a reasonable correlation between the model's predicted results and the actual ones. The perturbation plot (Figure 8) has also shown that the behaviours of all factors with the CO_2% are almost opposite to the behaviours found for the CH_4%.

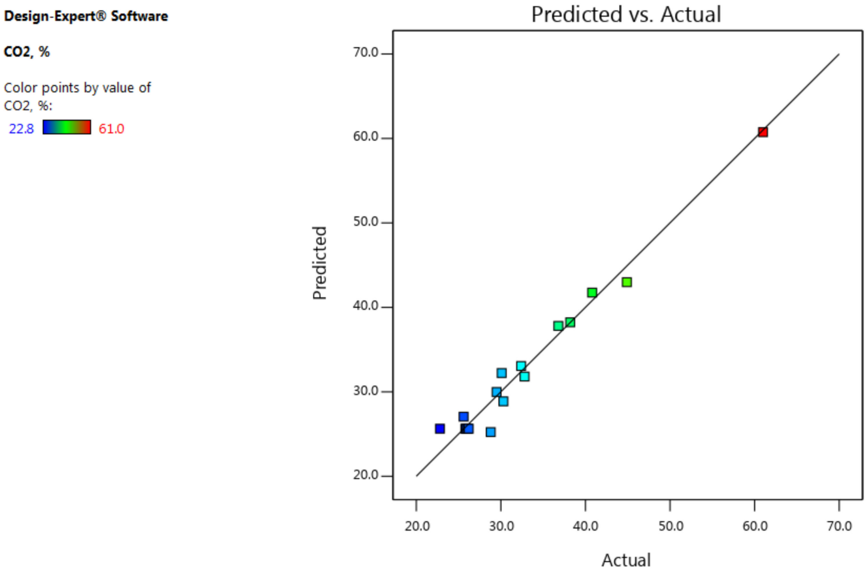

Figure 7. Scatter plot of the predicted values versus the actual values of the CO_2% response.

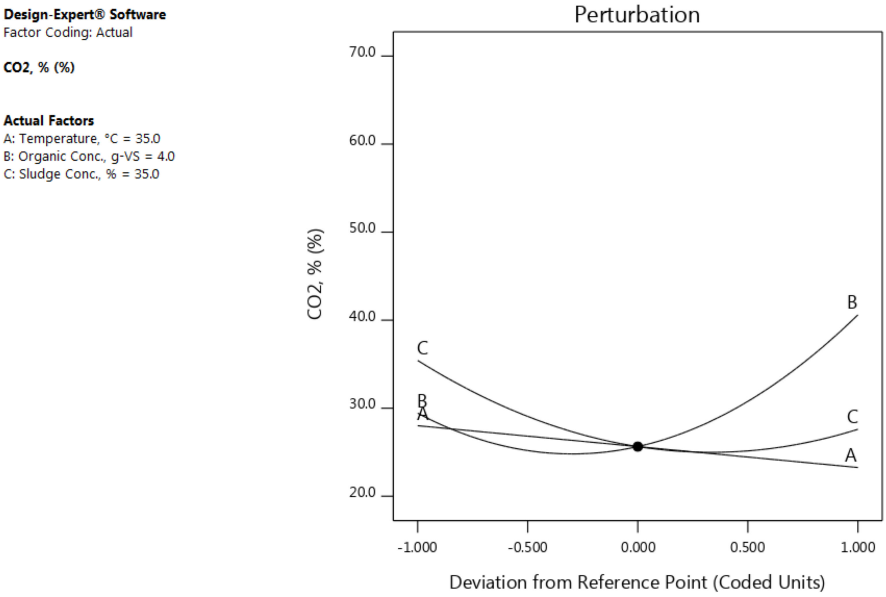

Figure 8. The Perturbation plot of the CO_2 % response.

Relatively, the same concept of the influence of the (BC) on CH_4% is applicable to its effect on the CO_2% but in an opposite manner. For instance, when the organic concentration is at the highest level, a significant variation in the CO_2% can be observed when the sludge concentration is moves from its lowest to its highest levels (see Figures 9 and 10). However, based on the analysis of the CO_2% response and by looking back into the analysis of the CH_4% response, the same terms have a significant effect on both responses but to varying degrees. In comparison between the results of the concentrations of both CH_4% and CO_2% which have been measured from each run, it is evident that the increase in CH_4% is associated with a decrease in CO_2% and vice versa.

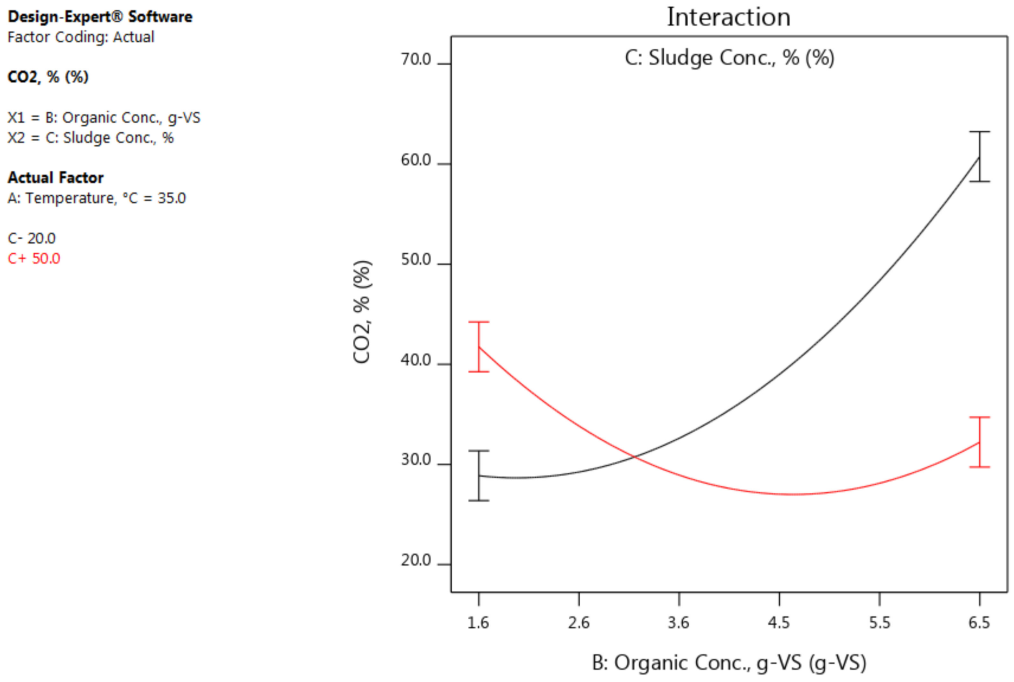

Figure 9. An interaction plot illustrating the influence of (BC) on the CO_2 %.

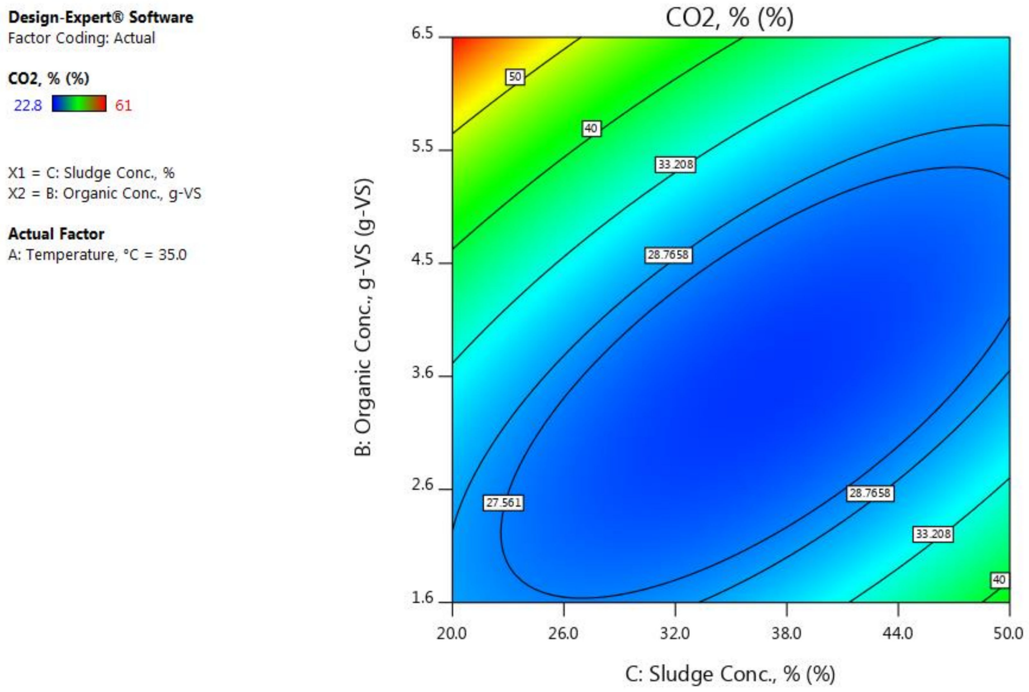

Figure 10. A contour plot illustrating the influence of (BC) on the CO_2 %.

3.1.2. Optimisation

Table 9 shows the optimum results based on the three criteria in numerical form. Figures 11–13 show the optimum results graphically in overlay plots. In terms of the biogas quantity and quality, the optimal results based on the 1st criterion were higher than the other optimal results based on the second and third criteria. The optimal biogas based on the cost criteria were relatively closer to each other. As is evident from Table 9, the changing of the goal of the temperature parameter in the 2nd and 3rd criteria to minimise, resulted in a drop of the optimum temperature from its highest level to the lowest level (32 °C). This indicates that, the influence of the temperature is less than the influences of the other two factors on all responses.

Table 9. The optimum results which were obtained by RSM.

Criterion	Temperature, °C	Organic Conc., g-VS	Sludge Conc., %	Biogas, cc/g-VS	CH_4, %	CO_2, %
1st	38.00	3.53	46.00	804.88	65.66	24.41
2nd	32.00	2.51	33.36	561.96	60.33	27.99
3rd	32.00	3.93	37.02	510.59	62.49	27.41

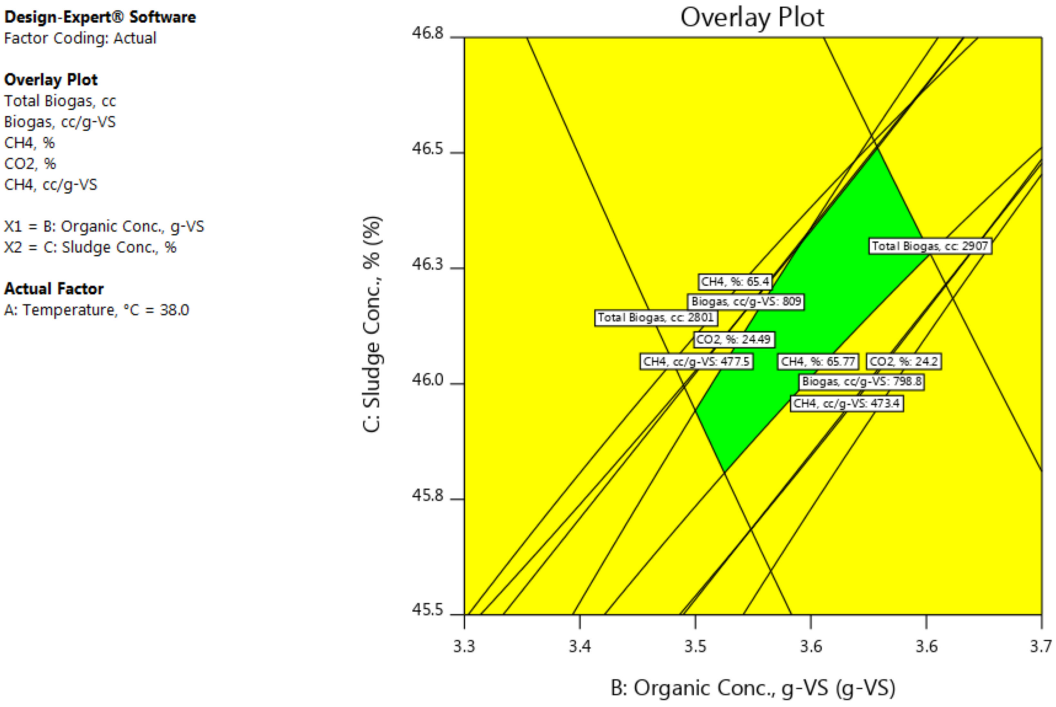

Figure 11. An overlay plot illustrating the optimum biogas based on the quality criterion.

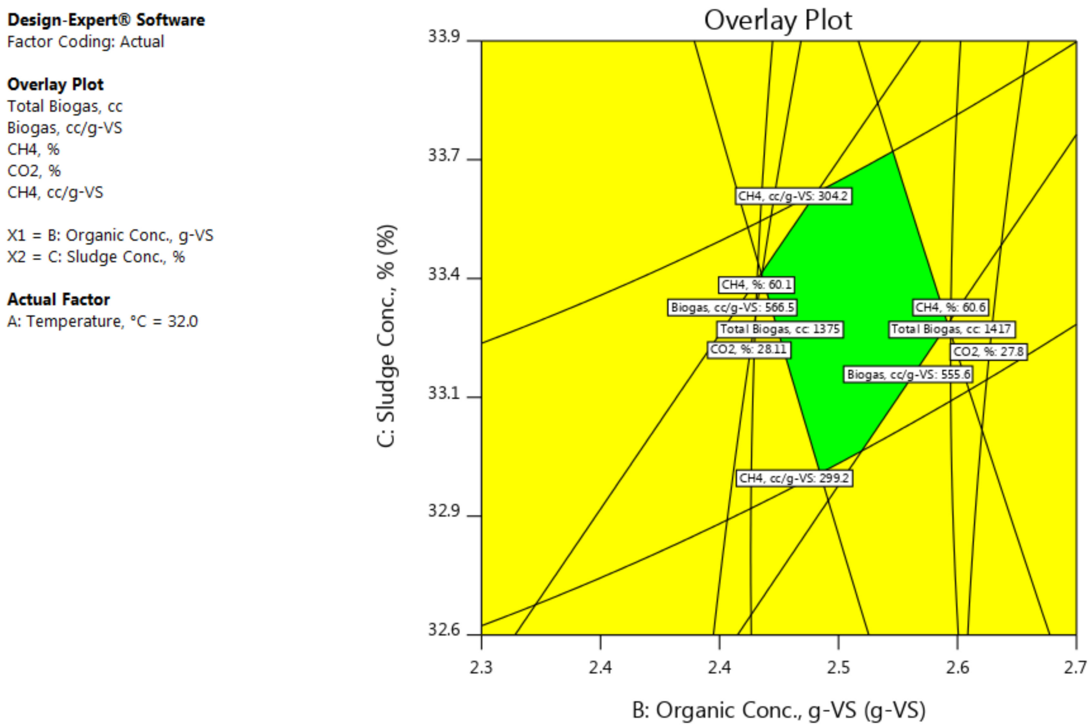

Figure 12. An overlay plot illustrating the optimum biogas based on the 2nd criterion (cost).

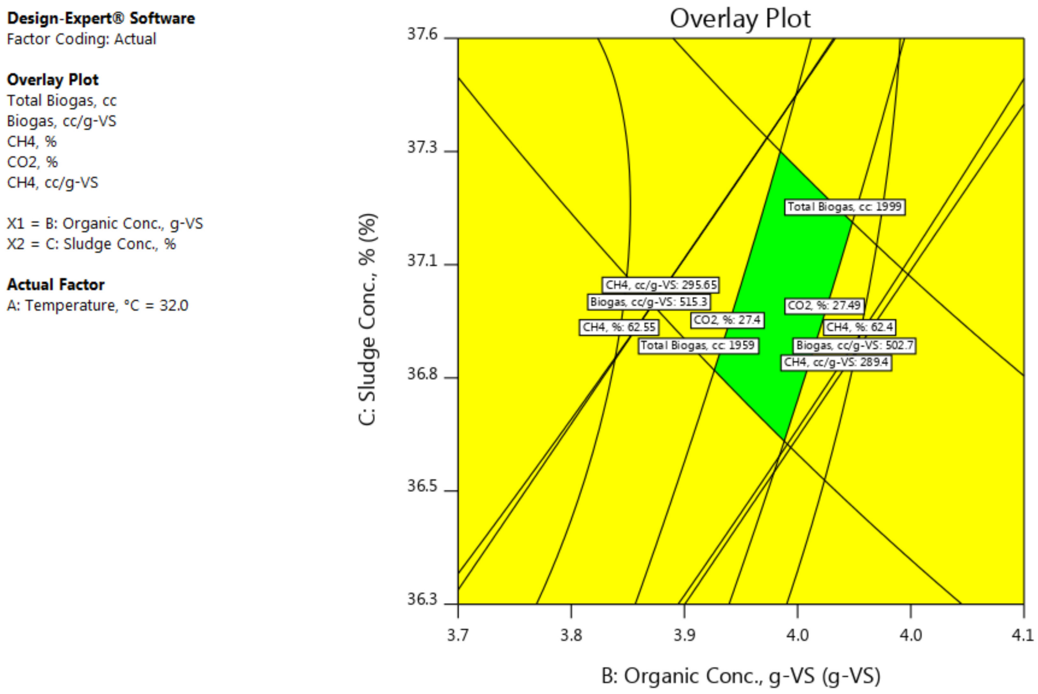

Figure 13. An overlay plot illustrating the optimum biogas based on the 3rd criterion (cost).

3.1.3. Energy Balance

Figure 14 shows the average electric energies consumed at each temperature level.

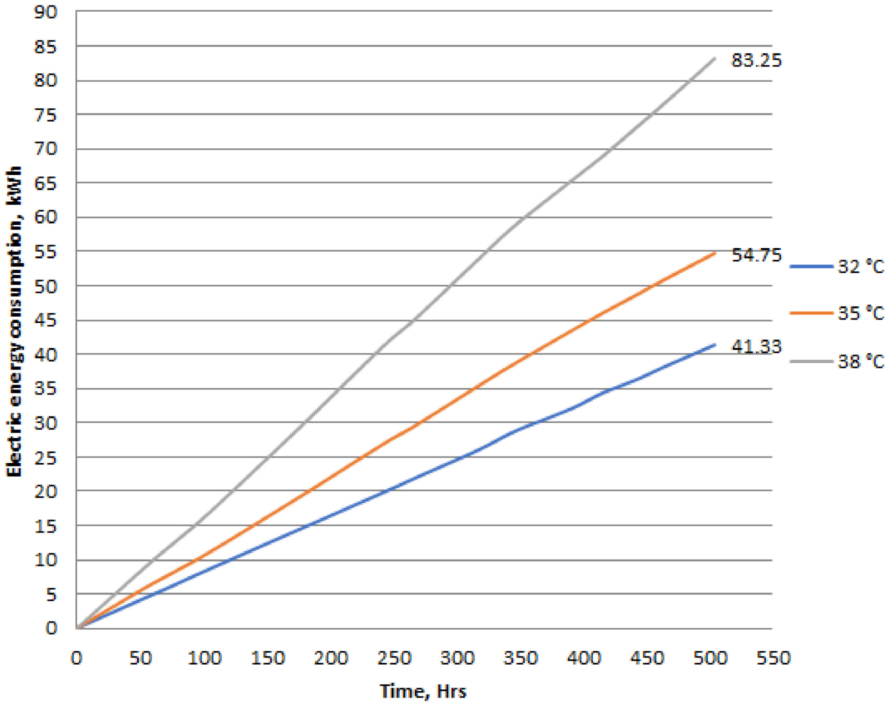

Figure 14. The average electric energy consumed at each temperature's level.

Table 10 provides the energy balance obtained from the optimal results based on the three criteria. The results revealed that, the highest energy gain in terms of the cost was approximately 65% and about 22% when the quality was taken into consideration. It is evident from the table that, when changing the goal of the organic matter concentration to "maximise", energy gains increased remarkably. In contrast, when the goals of the temperature and sludge concentration were set to "minimise", large losses in the energy produced (E_P) were noticed. According to Table 10, it can be said that, the highest energy gains can be achieved when the temperature and sludge are set at "minimise" value and the organic concentration at a "maximise" value.

Table 10. The energy balance based on each optimisation criterion.

Criterion	Energy Consumed, kWh	*VS* Weight, g	B_s, kWh/m^3	E_p, kWh/g-VS	E_c, kWh/g-VS	*Net* E_p, kWh/g-VS	Energy Gain/Loss, %
1st	83.3	3.53	6.35	0.38	0.31	0.069	21.9
2nd	41.3	2.51	5.83	0.25	0.22	0.026	12.0
3rd	41.3	3.93	6.04	0.23	0.14	0.091	65.0

3.1.4. The Validation of Using the Digestate in Agriculture

One of the anaerobic reactors (run 7) which was digested at 35 °C, 6.5 g-VS and 50% sludge was selected and tested in a chemistry laboratory to verifying the content of the digestate for the three basic nutrients found in conventional fertilisers. The test confirmed that the digestate contains the three main nutrients of fertiliser: N, P and K. It also found that the dry matter weight is low. Table 11 shows the quantity of each nutrient in the digestate as well as the dry matter weight. As is well known, the pH of the fertiliser and soil are very important, in terms of the absorption of nutrients for plants and plant growth. Therefore, the pH of the digestate was measured and found to be 7.9. Hence, the resulting digestate can be used as it is in agriculture lands or separated into liquors and solids and sold separately. For more assurance of the quality and validity of the resulting digestate, the contents of the digestate to other elements such as: total humic and fulvic acid and heavy metals (Zn, Cu, Sn, Cd, Ni, Pb, Hg, Cr) can be also measured. The digestate content of each of element is then compared to the permissible ratio for the element.

Table 11. The quantity of the three basic nutrients of fertiliser and dry matter weight in the digestate.

Item	Quantity	Unit
Total Phosphorous (P)	762	mg/kg
Potassium (K)	1251	mg/kg
Total Nitrogen (N)	3951	mg/kg
Dry matter	3.2	g/100 g

3.2. Discussion

AD has proven its potential in converting many types of biomass into biogas [39,40]. Beside its contribution in producing bio-energy, it also contributes greatly into waste management. Therefore, an increase of the dependence on AD would significantly help in reducing our dependence on fossil fuels. This increase cannot be achieved and its benefits reaped until the economical and environmental challenges of AD are overcome. As previously mentioned, mango is a highly consumed fruit worldwide [12]. Between 35–60% of the total weight of mango fruits are discarded after processing [18]. The waste contains multiple compounds such as starch, fat, flour, etc. [21,22,41]. Consequently, the exploitation of the mango waste will not only cause a reduction in its environmental pollution, but it would also help in minimising the cost of waste disposal for mango processing industries [42]. An integration approach is the proposed solution for these challenges. It has confirmed its potential in previous studies. It is aimed at producing more bio-products along with AD biogas to increase the profitability by making full use of the biomass [2,10].

Due to all of that, the current study investigated the influences of the mango seed coats and starch on the quantity and quality of the biogas produced from the mango wastes. The research carried out in the present study to investigate the impact of them on the biogas, is in order to investigate later the impact of the production of bioproducts along with the biogas and bioslurry and to compare these to the environmental and economical challenges associated with AD. The study revealed that the impacts of the starch on the biogas are low due to its low weight compared to the total weight of the waste, as its weight represents only 4 ± 1% of the total weight of mango waste. On the other hand, mango seed coats were not easily accessible by the hydrolytic enzymes and therefore, they were not fully digested by the microorganism inside the control digesters. Thus, beating pre-treatment of the mango seed coats was not sufficient.

Moreover, an observation has been drawn from the results that, the sludge concentration (C) as well as the organic concentration (B) have significant influences on the quantity and quality of the biogas produced. As it can be noted from the results of the biogas produced from each bioreactor, excessive feeding of the digester with the substrate did not increase the biogas quantity and quality. This implies, the excessive feeding makes the bacteria idle and therefore, they do not digest the whole substrate inside the digester. Thus, the concentrations of the fedstocks and the inoculum must be well balanced inside the digester. In addition, the careful determination of the concentrations of the inoculumand fedstock greatly helps avoiding the inhibition of the growth of the bacteria and the distortion of their metabolism. That would also help avoiding the imbalance of the bacterial population, VFA accumulation and reactor failure [43,44]. It was also observed that the temperature has a lower significant influence on the quantity and quality of the biogas produced than the influences of the organic concentration g-VS and sludge concentration.

It is worth mentioning that, the highest energy gain that was achieved was based on the third criterion. This key finding supports the maximisation of the organic matter concentration and therefore increase the contribution of AD in waste management by taking advantage of more amounts of mango waste. It would also encourage AD plants to apply gate fees for accepting the waste from other industries and others so as to increase the profitability of their AD plants.

The chemistry laboratory results have confirmed the biofertiliser potential of the digestate generated from the AD of mango waste. This result could motivate the AD plants to sell the digestate to farmers and others which would allow getting rid of it faster and reduce the cost of storing it. In order to meet this target, AD plants are crucially required to encourage farmers to use the digestate instead of conventional fertiliser. Therefore, the digestate should be subjected to more tests to ensure it is free from substances that may negatively affect plants and increase farmers' awareness of the digestate and its fertiliser potential to increase confidence and reliance on it.

4. Conclusions

The increase of AD profitability through increasing the bioproducts and the application of gate fees for accepting waste, could greatly help in overcoming the environmental and economical challenges associated with AD. The prosperity of AD will significantly increase its contribution in waste management and minimise the reliance on the fossil fuel and its derivatives. The major conclusions reached in the study are summarised in the following points:

- The effect of the starch on the quantity and quality of the biogas produced from the AD of mango waste is quite low and can be neglected.
- The digestate based on the AD of mango waste contains the basic nutrients of a conventional fertiliser in varied amounts and can be used in agricultural applications as another AD product. In order to fully assured its quality, further tests on the digestate are suggested.
- The employment of the Hollander beater for pre-treating mango seed coats is not sufficient. The coats require further pre-treatment to increase the accessible surface area and size of pores available for the hydrolytic enzymes. Alternatively, it can be utilised in waste-to-energy plants or in other industrial or commercial applications as a biofiller, biofibre, etc.
- The amounts of the substrates and sludge must be carefully balanced. Extremely high or low feeding of the digester negatively influencing the biogas production and quality.

Moreover, increasing the dependence on digestate in agriculture applications or others helps greatly reducing the costs spent on it. As a recommendation of this study, AD plants should seek to increase farmers' awareness about digestate and the reliability on it. On the other hand, the findings reached by studying the impact of the starch and the seed coats on the AD biogas of mango waste motivate exploiting the mango starch and coats in the production of more bioproducts along with biogas and bioslurry. Therefore, the research will continue to evaluate the incorporation of the AD of the mango waste process with the production process of the mango starch/coats-base bioplastic on the economical and environmental challenges of AD. They are encouraging also for investigating the waste of other fruits and vegetables for producing multiple bioproducts along with AD products.

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Conflicts of Interest: The authors declare no conflict of interest.

Nomenclature

Symbol	**Description**
AD	Anaerobic digestion
Adeq. Precision	Adequate Precision
Adj. R^2	Adjusted R^2
ANOVA	An analysis of variance
BBD	Box-Behnken design
Cor. Total	Total sum of the squares corrected for the mean
C1	The organic concentration resulted
C2	The concentration required
df	Degree of freedom
DOE	Design of experiment
D	The amount required to be added/removed to adjust the organic concentration at the predetermined concentration
HRT	Hydraulic retention time
ISR	Inoculum to substrate ratio
Ms	Moisture content
Pred. R^2	Predicated R^2
RSM	Response surface methodology
TS	Total solid
VFA	The volatile fatty acid
VS	Volatile solid
V1	The volume of the mixture at (C1)
V2	The volume of the mixture at (C2)

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