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MAXIMIZING BIOGAS YIELD IN ANAEROBIC DIGESTION: A RESPONSE SURFACE METHODOLOGY APPROACH

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Abstract: The aim of this study is to evaluate the eutrophication water and cattle manure wastes in the Güldürcek dam reservoir that meets the drinking water of Çankırı province in biogas production and to contribute to the economy of Çankırı province. Microwave pretreatment experiments were carried out but it was determined that it did not increase the biogas efficiency. In the study, it was determined that when eutrophication water was mixed with cattle manure by the RSM method and the mixing ratio was 1/1, the highest methane production was obtained at a rate of 83-86% in the first 8 and 24 hours under optimum conditions with anaerobic digestion at a temperature of 35 °C. The composition of the obtained biogas was measured as follows; carbon dioxide (CO_2) 7.65%, oxygen (O_2) 4.5%, hydrogen (H_2) 0.018% and hydrogen sulfide (H_2S) zero. Another point to be noted in the analysis results is that the H2S rate, which is formed as a result of anaerobic digestion and causes economic losses due to corrosion of metal equipment in the system, is zero. According to the RSM method, the most suitable model was found to be Quadratic. The high R^2 value of the selected model (0.9649) supports the accuracy of the model.

Keywords: Biogas production, Anaerobic digestion, Eutrophication water, Cattle waste, Response surface methodology

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1. Introduction

In recent years, the increasing prices of petroleum products, environmental pollution and the risk of depletion of fossil fuels have directed us to alternative and sustainable energy sources (Moosavian et al.,2024; Zhang 2024). When we look at the research on alternative energy sources, we see that there are studies conducted with various wastes such as food waste, animal manure, organic waste and solid municipal waste Hoyos-Sebá et al. (2024). Since these wastes cause environmental pollution, their conversion into biofuel is important in terms of environment, economy and sustainability (Gong and Aslam, 2024). These waste materials include a variety of food waste and animal manure, a significant amount of organic material that is fermented anaerobically. Anaerobic digestion (AD) has proven to be an effective method to reduce organic waste while recovering valuable by-products such as biogas and digestate Ahmad et al. (2024). The selection of the type of waste used as raw material in the AD process is effective in digestion performance. Using various wastes together has an important place in co-digestion due to its high ability to increase biogas and methane production compared to single-raw material AD methods (mono-digestionCo-digestion is known to be an effective method for determining the carbon-nitrogen (C/N) ratio, reducing the inhibitory effect of ammonia and overcoming the difficulties of mono-digestion Akbay (2024). In the study conducted by Singh et al., it was realized that eutrophic inland water bodies are one of the contributing factors to greenhouse gas (GHG) emissions Singh et al. (2023). Mainly nitrate and phosphate, promote the formation of algae and vegetation in water bodies Chanu et al. (2022). As a result, eutrophication causes more disturbances in the system, which mainly leads to the water becoming unhealthy for the aquatic habitat Mishra (2023). According to a study, it also affects the environment through greenhouse gas (GHG) emissions. It is predicted that eutrophication will increase in the next few decades due to population growth. It has also been found that digesting microalgae biomass together with carbon-rich substrates such as cattle manure and agricultural waste improves biomethanization Qian et al. (2025). In one study, it is observed that cow manure and waste algal biomass from Hauz Khas lake are used as auxiliary substrates for anaerobic digestion and up to 45% advanced biomethane production is carried out. Cattle manure is expressed as a superior auxiliary substrate that facilitates the further growth of anaerobic bacteria and archaea, which helps in the digestion of algal biomass Singh et al. (2023).

The AD process is carried out by fermentation to ensure a balanced nutrient composition and to produce biogas during the reaction process Pourrostami et al. (2024). In

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this process, the selection of waste used and the parameters affecting the performance of the AD process consist of various factors, including solid concentration, temperature, pH and retention time. To ensure effective biogas production, these restrictions need to be optimized (Tamilselvan and Selwynraj, 2024) . Temperature is an important parameter that affects AD. Psychrophilic (10-30 ° C), mesophilic (30-40 ° C) and thermophilic (50-60 ° C) can occur under various temperature conditions Kumar et al. (2024). In the study, if the temperature goes below 30 °C, an acidic environment occurs in the AD process, which has an adverse effect on the biogas yield. In addition, if the temperature rises above 70 °C, it is especially harmful to methanogenic bacteria (Wang et al., 2024; Monteiro et al., 2024). Optimization method such as RSM is used to increase the yield of biogas in anaerobic digestion (Efetobor et al., 2024; Bensegueni et al., 2025). For example, El Bari and Habchi (2024) have used the RSM method methodology to analyze biogas production by taking into account parameters such as pH, reactor temperature, process alkalinity and raw material retention. In this study, they found that pH and temperature changes significantly affect biogas yield and methane concentration (Humphrey et al., 2024; Jadhav et al. 2024; El Bari and Habchi 2024). In another study, Bhujbal et al. (2025) found that the optimal working conditions for good yield output were determined when RSM (CCD) was used to study the biogas production of anaerobic digestion of co-digestion.

Mohammadianroshanfekr et al. (2024) used the RSM-based CCD approach to analyze the effectiveness of AD on biogas production efficiency and chemical oxygen demand reduction. There is a study in which it is found that the pH, temperature and co-digestion rates of solid raw material affect the production of biogas and the reduction of chemical oxygen demand.

In this study, according to the chemical analysis of the organic wastes used, the most suitable waste pair is cattle manure and Güldürcek Dam Lake Decontamination water, which was selected because the C/N content is close to the value that should be between the desired 15-30 in the literature. In addition, the evaluation of the eutrophication water occurring due to the decrease of the water level in the summer months at the Çankırı Drinking Water Dam is important on the basis of Çankırı province. Since the use of this raw material, which is extremely weak in terms of dissolved oxygen, in biogas production has not been found in the literature, its use has been considered in the study. The RSM method has been used to increase the biogas yield. Independent variables such as solid concentration, pH, temperature and co-digestion were taken into account, while biogas yield was taken as a response variable. The study evaluated the optimal response variable compared to the experimental findings.

2. Materials and Methods

The organic wastes in question include cattle and chicken manure, eutrophic water from the Güldürcek Dam Lake, urban water treatment sludge and urban solid waste leachate, obtained respectively from agricultural enterprises operating within the province of Çankırı and from the Çankırı Municipality Urban Waste Processing and Drinking Water Treatment units. The waste samples used in the study were stored at 4 °C before further processing. The chemical analysis of cattle and poultry manure used in biogas production was conducted in an accredited laboratory according to TS EN ISO 17025 standard. The methods used were as follows: total organic carbon (TOC) by the Walkley-Black method, total nitrogen by Kjeldahl method, C/N ratio calculation, total suspended solids (TSS) by gravimetric method, organic matter by gravimetric method, volatile solids (organic dry matter) by gravimetric method, moisture (70°C) by gravimetric method. The analyses of urban water treatment sludge and urban solid waste leachate included total organic carbon (TOC) by high-temperature combustion, total nitrogen by Kjeldahl method, volatile suspended solids (VSS) by gravimetric method, total suspended solids (TSS) by gravimetric method, and organic matter (permanganate index) by titrimetric method. pH measurements were taken using an AE-PH502 pH Portable device. The nitrogen gas used was of 99.9% purity, and the gas composition resulting from anaerobic digestion was measured using an Optima 7 biogas gas analyzer. Fermented manure analyses included organic matter by gravimetric method, total nitrogen by Kjeldahl method, copper by ICP-OES, total phosphorus (P_2O_5) by spectrophotometric method, total potassium oxide (K_2O) by ICP-OES, C/N ratio calculation, pH by electrometric method, moisture (70°C) by gravimetric method, iron (Fe), zinc (Zn), manganese (Mn) by ICP-OES, and humic acid by gravimetric method. The microwave used for pre-treatment was a Samsung MS23F301 EAW household microwave.

2.1 Anaerobic Digestion Study

In the study, biogas potential was carried out using a batch reactor for monitoring and evaluation purposes due to its simple and easy installation. The experimental setup shown in Figure 1 is a system based on the liquid displacement principle. The biogas measurement setup consists of a biogas storage bottle connected to a small anaerobic digestion reactor with tubes, a 1000 mL dark glass bottle containing 0.5 M NaOH solution, a bottle used to displace water with the pressure of the produced gas and a bottle containing 1000 mL liquid (distilled water) used to measure the CH_4 volume. A 1000 mL double-walled glass reactor with four inlets and a leak-proof lid was used. A mechanical stirrer operating at 40 rpm was connected to the outlet on the lid, a hose used to pressurize the system with 5 bar nitrogen, and a tube going to the water displacement bottle for gas volume measurement. The fourth outlet was closed with an airtight cork for gas measurements. After the reactor was loaded at certain rates, anaerobic conditions were provided by pressurizing 99.9% pure nitrogen (N_2) gas to 5 bar for 2 minutes. The 1000 mL distilled water bottle and 0.5 M NaOH solution used in the system helped to separate impurities in the biogas and determine the efficiency. Due to biogas production, the pressure inside the 1000 mL reactor increased, causing water to be displaced from the 1000 mL laboratory glass bottle to the third bottle through a plastic tube. In the biogas production system, liquid (distilled water) displacement was observed from eutrophic water coming from the Güldürcek Dam Lake and cattle manure during the first 8 hours and 12 to 24 hours.

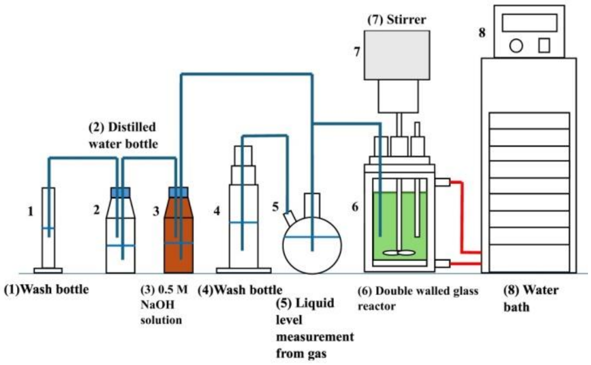

Figure 1. Biogas production system consisting of a batch-type reactor on a laboratory scale.

3. Results

3.1. Biogas Production from Cattle Manure and Eutrophic Water from Güldürcek Dam Lake

In the biogas production studies, a series of preliminary trials were conducted to determine the two most efficient waste types for anaerobic digestion and to determine the optimal production conditions. When the chemical properties of the organic wastes in Table 1 were examined, it was determined that the most suitable pair of organic wastes for anaerobic digestion was cattle manure and eutrophication water. Since the pH value of the binary mixture is 7.035, it is between 6.8-7.2, which are the desired pH values for biogas production. (Risberg et al., 2013; Nardi et al., 2021). On the other hand, it is known that the C/N ratio of this waste pair, which is an important factor in biogas production, is 15-30 and the optimum C/N ratio is 15.39 (El-Jalil et al., 2008; Wittwer and van der Heijden 2020). (Table 1). Biogas production studies with eutrophication water and cattle manure obtained from Güldürcek Dam Lake were carried out under mesophilic conditions at a constant temperature of 35±1 °C by loading 450 g of cattle manure and 450 g of eutrophication water obtained from Güldürcek Dam Lake. The study, which was carried out for 24 hours under the same and constant conditions, was carried out with serial gas measurement processes with Optima 7 biogas brand gas analyzer, which is directly connected to the reactor and provides information about the gas composition in the reactor by performing instantaneous gas analysis at 2nd, 4th, 8th, 12th and 24th hours. The analysis results subjected to the measurement processes are presented in Table 2. As understood from the analysis results, good efficiency was obtained in methane production in the first 8th hour and 12th and 24th hours. The obtained results gave results close to the methane production rates obtained as a result of anaerobic digestion with various wastes given in the literature research.

Table 1. Chemical properties of organic wastes to be used in biogas production

N	S	OC%	N%	C/N%	TSM%	TOM%	FS%	VS%	M%	pH
1	CM	32	3.53	9.06	9.43	72.8	27.2	72.8	90.5	6.87
2	EW	0.0016	0.0003	6.33	0.021	0.0002	0.020	0.0011	-	7.2

S= sample, OC= organic carbon, N=nitrogen, C/N= carbon/nitrogen, TSM=total solid matter, TOM= total organic matter, FS= fixed solid, VS= volalite solid, M= moisture, CM= cattle manure, EW= euphication water.

Table 2. Measurement results with Optima7 Biogas measurement detector in biogas production with cattle manure and Güldürcek Dam Lake eutrophic water

Parameter	Units	2 hour	4 hour	8 hour	12 hour	24 hour	Average
Methane (CH_4)	%	47.1	75.1	83.0	85.1	86.0	75.3
Carbon Dioxide (CO_2)	%	3.53	4.93	6.75	7.58	7.65	6.09
Oxygen (O_2)	%	6.6	3.9	4.8	4.6	4.5	4.88
Hydrogen (H_2)	ppm	10	12	15	16	18	14.2
Hydrogen Sulfur (H_2S)	ppm	0	0	0	0	0	0.00

3.1 RSM methodology in Biogas Production

The effects of factors such as cattle manure, eutrophication water and time on methane gas formation were investigated using the RSM method. When Table 4 (a,b,c) is examined, it is concluded that the second-order model proposed in RSM is the most appropriate choice. The Adjusted R^2 value of 0.9334 and the Lack of Fit p-value of 0.1082 provide evidence for the accuracy of the model. The 2FI model is the most appropriate choice against the second-order model, with the Mean Square value of 0.6701 and the p-value of 0.0004 indicating the accuracy of the model.

3. 2 Optimization of Biogas Production by Response Surface Methodology (RSM)

In this study, RSM was used to design the parameters affecting biogas production. The relationship between independent variables and biogas production was evaluated in RSM (Dağ et al., 2023; Afridi and Qammar 2020). The best model for numerical experiments is the central composite design. When the literature studies in this field are examined, it was found that RSM performance is the most effective technique and the best software that includes the best task in biogas process optimization. Optimum targets such as increasing biodegradation, optimum biogas yield and methane production, increasing total solids, reducing volatile solids and eliminating chemical oxygen demand were obtained for biogas production. The main advantage of RSM is that it provides time and cost benefits by reducing the number of experimental trials. In the last 20 years, 37 process parameters have been optimized using RSM. Five of these parameters are dominant: temperature, pH, retention time, pretreatment and loading rate. The main difficulties in using RSM for biogas production process optimization are limited experimental ranges. To address these issues, studies have been conducted by combining RSM with other optimization methods such as Taguchi, Kriging or Artificial Neural Network (ANN) (Aydoğmuş et al., 2022; Güven et al., 2008; Djimtoingar et al., 2022). The effects of factors such as cow dung, eutrophication water and time on methane gas formation have been investigated using the RSM method. These effects are graphically shown in the figures. Table 3 shows the experimental design schedule with the RSM method.

Table 3. RSM experimental design chart

Experiment	Hour	Cattle manure(g)	Eutrophic water(g)	Methane (%)
1	6	519	281	84.5
2	13	600	400	85.8
3	13	400	200	85.4
4	20	519	519	86.6
5	6.45	281	281	84
6	6.45	519	519	85.5
7	13	400	400	86.1
8	19.54	281	519	86.2
9	13	400	400	86
10	13	400	600	86.1
11	13	400	400	85.9
12	13	200	400	85.3
13	13	400	400	86.2
14	13	400	400	85.8
15	19.54	519	281	85.8
16	6.45	281	519	85
17	24	400	400	86.5
18	19.54	281	281	85.7
19	13	400	400	86

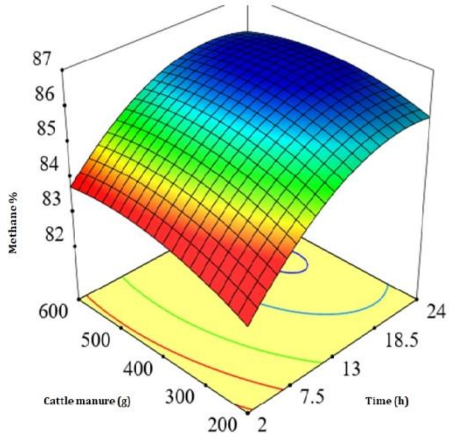

Figure 2. Examines the effect of cattle manure and eutrophication water, and time on methane (%) gas formation according to the RSM.

As shown in Figure 2 depicting the impact of cattle manure, time and duration on methane gas formation according to the RSM method, it is observed that methane gas production increases with an increase in the amount of cattle manure. The highest efficiency, approximately 86-87 %, is achieved with cattle manure quantities in the range of 500-600 g and durations in the range of 18.5-24 hours. Generally, an increase in duration (hours) leads to an increase in methane gas production, and an increase in the quantity of cattle manure results in increased efficiency. However, the plateauing of efficiency after a certain point suggests the influence of other factors on efficiency.

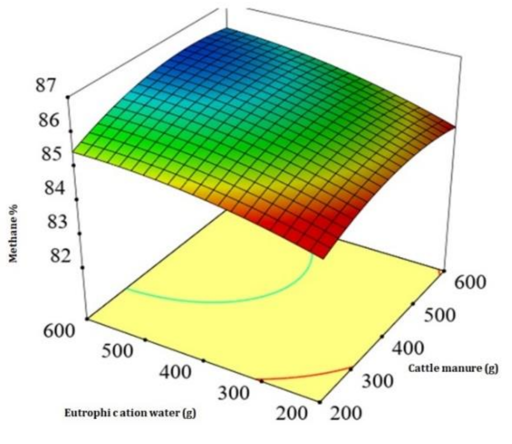

Figure 3. Effect of cattle manure and eutrophication water on methane (%) gas formation according to RSM.

In Figure 3, an increase in eutrophication water is associated with a gradual increase in efficiency, while the efficiency of cattle manure peaks at around 600 g. It is believed that certain bacteria present in eutrophication water contribute additionally to methane gas production. Working on a laboratory scale and designing experiments are prioritized for addressing some deficiencies. This is believed to hinder further increases in efficiency.

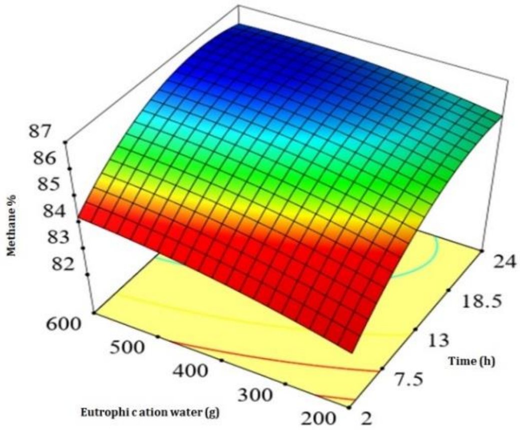

Figure 4. Effect of time and eutrophication water on methane (%) gas formation according to RSM.

Figure 4 illustrates the impact of eutrophication water volume and exposure duration on methane gas emissions. It shows that methane release increases with both higher volumes of eutrophication water and longer exposure durations. These two factors appear to play a significant role in methane production. While methane levels were initially low, they showed a noticeable increase over time. This low early-stage methane output is likely due to the low carbon-to-nitrogen (C/N) ratio in the eutrophication water. Below is the model equation derived from the experimental study conducted, based on the data obtained. This model indicates that factors such as time (t: hours), cattle manure (CM: grams), and eutrophication water (EW: grams) determine the percentage of methane gas (equation 1). The coefficients in the equation have been calculated through statistical analysis, demonstrating compatibility with experimental data.

$$Methane\,(\%) = +78.11538 + 0.406096 \bullet t + 0.010264 \bullet CM + 0.007997 \bullet EW - 0.000080 \bullet t \bullet CM - 0.000112 \bullet t \bullet EW + 2.65165 \bullet 10 - 6 \bullet (CM \bullet EW) - 0.008198 \bullet t2 - 0.000011 \bullet CM2 - 6.04795 \bullet 10 - 6 \bullet (EW)2 \qquad (1)$$

In the RSM method, the determined R^2 (0.9649) and standard deviation (0.2073) values show consistency between experimental data and model results.

When examining Table 4 (a,b,c), it is concluded that the quadratic model suggested in the RSM is the most suitable choice. The Adjusted R^2 value of 0.9334 and the Lack of Fit p-value of 0.1082 provide evidence of the model's accuracy.

Table 4 evaluates the statistical significance of the RSM model data. It suggests that the quadratic vs. 2FI model is the most appropriate choice, with a Mean Square value of 0.6701 and a p-value of 0.0004, indicating the model's accuracy.

From the statistical evaluation of the RSM model data presented in Table 4 (a,b,c), it is determined that the correct model choice is made based on the R^2 value of 0.9649 and Adjusted R^2 value of 0.9334. In addition to the experimental study, biogas optimization was conducted using the Response Surface Methodology (RSM). Figure 4 compares theoretical and experimental values obtained from the RSM. Table 4 gives the recommended solution table of the RSM model.

Table 4. Statistical evaluation of the data and results with the quadratic model proposed in RSM (a,b,c)

a

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.0078	0.7534	0.6834	
2FI	0.9052	0.0045	0.7089	0.4973	
Quadratic	0.0004	0.1082	0.9334	0.7824	Suggested
Cubic	0.0423	0.8708	0.9740	0.9777	Aliased

b

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	1.465E+05	1	1.465E+05			
Linear vs Mean	9.71	3	3.24	20.35	< 0.0001	
2FI vs Linear	0.1038	3	0.0346	0.1843	0.9052	
Quadratic vs 2FI	2.01	3	0.6701	15.60	0.0004	Suggested
Cubic vs Quadratic	0.3291	4	0.0823	4.91	0.0423	Aliased
Residual	0.1006	6	0.0168			
Total	1.465E+05	20	7327.12			

c

Std. Dev.	0.2073	R^2	0.9649
Mean	85.59	Adjusted R^2	0.9334
C.V. %	0.2422	Predicted R^2	0.8824
		Adeq Precision	19.2244

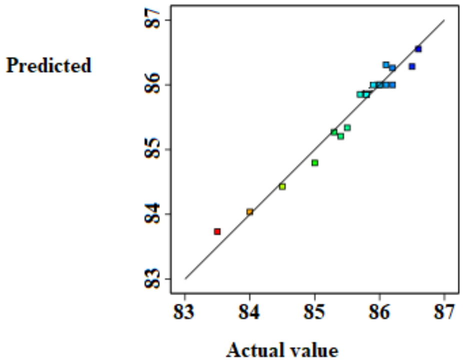

Figure 4. Comparison of Experimental (Actual Value) and Theoretical (predicted) Values in RSM.

4. Discussion

Due to its simple and easy installation, it was found appropriate to use a batch reactor for monitoring and evaluation (Rocha-Meneses et al., 2022). In this study, the 100 mL volume double-walled reactor is one of the laboratory-scale batch reactors described in Ellacuriaga et al. (2021). Batch reactor can be used in biogas production with AS from organic waste. The results obtained in biogas production with cattle manure and Güldürcek Dam lake eutrophication water showed that it can be used in biogas production.

Portable and small-scale batch reactors have been used in biogas production from animal wastes such as cow manure. There are studies in which biogas production has been optimized with additives such as molasses in these reactors. In this study, it is seen that chemical and oxidative studies were performed as pretreatment (Song et al., 2014; Ramos-Suárez et al., 2017). In our study, methane yield was 86% without pretreatment. This is important in terms of energy saving. Eutrophication water is obtained from water bodies enriched with nutrients and has a high organic matter content. The combination of such waters with cattle manure can increase biogas production. However, studies on this specific combination in the literature are limited.

Mesophilic conditions were chosen for energy saving purposes and because they offer more positive benefits as reported in the literature (Akindolire et al., 2022). The decrease in temperature has a detrimental effect on various AD operating parameters and has a strong negative effect on microbial growth and enzymatic activity, especially among mesophiles and thermophiles (Kalaiselvan et al., 2022). In our study, methane yield was high in the AD process carried out at 35°C.

In general, the aim of biogas optimization is to help increase the efficiency and sustainability of biogas production, thus transforming it into a more viable renewable energy source (Afridi and Qammar 2020). Our study shows that the RSM model explains the experimental data well and is appropriately adapted to the data. The R^2 (0.9649) and standard deviation (0.2073) values found by statistical analysis in the RSM method showed that the experimental data and the model results were compatible. According to the results, 456 grams of cattle manure and 494 grams of eutrophication water should be subjected to anaerobic co-digestion for approximately 17 hours to obtain maximum methane gas yield (86.5%).

5. Conclusions

In this study, anaerobic digestion studies were carried out by using cattle manure from an animal farm located in Eldivan district of Çankırı province and eutrophication water formed due to the decrease in water level in summer months for drinking water supply. The waste samples used in the study were kept at 4 °C before applying advanced treatment processes as stated in the literature. Biogas potential was evaluated using a batch type reactor due to its simple and easy installation for monitoring and evaluation purposes. After loading certain amounts into the reactor, anaerobic conditions were created by pressurizing it with 99.9% pure nitrogen (N_2) gas at 5 bar pressure. The reactor temperature was kept at 35±1 °C in mesophilic conditions. The system was operated at atmospheric pressure in a laboratory environment. The chemical analysis results of the two most efficient wastes in anaerobic digestion were examined and optimum production conditions for biogas production were determined by considering certain mixing ratios, C/N ratios and pH measurements. Experiments with eutrophication water and cattle manure showed that the pH value was 7.035 and the C/N ratio was 15.39 as suitable waste. The biogas formed was obtained by anaerobic digestion of eutrophication water obtained from Güldürcek Dam Lake with cattle manure according to the liquid displacement principle. The effects of factors such as cattle manure, eutrophication water and time on methane gas formation were investigated using the RSM method. In the studies seen in Table 4 (a, b, c), it was concluded that the second-order model proposed in RSM was the most appropriate choice. The Adjusted R^2 value of 0.9334 and the Suitability P-value of 0.1082 provide evidence for the accuracy of the model. The 2FI model is the most appropriate choice against the second-order model, the Mean Square value is 0.6701 and the p-value is 0.0004, which shows the accuracy of the model.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	K.G.	Z.G.Y.
K	40	60
T	40	60
Y	30	70
VTI	40	60
VAY	40	60
KT	40	60
YZ	40	60
KI	30	60
GR	10	90
PY	30	70
FA	30	70

K= concept, T= design, Y= administration, VTI= data collection and/or processing, VAY= data analysis and/or interpretation, KT= literature review, YZ= writing, KI= critical review, GR= submission and revision, PY= project administration, FA= funding acquisition.

Conflict of Interest Statement

The authors declare that they have no conflict of interest in this study.

Ethical Approval Statement

Since this study did not involve any studies on animals or humans, ethics committee approval was not obtained.

Declaration of competing interests
The authors declare that this work was supported by the Çankırı Karatekin University Scientific Research Projects Coordination Unit project number MF210621D02.

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