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Research Article

OPTIMIZATION OF CATTLE MANURE LIQUID FRACTION ANAEROBIC DIGESTION AT DIFFERENT TEMPERATURES: MODELLING BY TAGUCHI METHOD

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ABSTRACT

In this study, the effect of different parameters (mixing speed, temperature, substrate/inoculation ratio) on the anaerobic digestion of cattle manure liquid fraction was analyzed and optimized with Taguchi method. As a result, it was concluded that mixing speed has an increase of 29% on methane gas production. Optimum operating conditions were determined as 120 rpm, 35 ˚C, and 1.5 S/I ratio. Under these conditions, 465 mL methane in 30 days was produced. 84% COD removal was provided. It has been observed that the liquid fraction of cattle manure can be decomposed under anaerobic conditions and transformed into energy rather than leaving it to the environment. It can be said that the farmers can efficiently digest the liquid part together with the municipal sewage sludge in the farms where solid-liquid separation is performed. Thus, environmental pollution will be prevented.

Keywords: Bio-methane, cattle manure, anaerobic digestion, liquid fraction, Taguchi.

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1. INTRODUCTION

Waste will be produced as long as human beings exist; the need for energy will increase with the development of industry and technology, and the increase in population. The number of increased people and the development of industry cause to increase the production of wastewaters; domestic and/or municipal sewage sludge are produced as a result of the treatment of wastewaters with appropriate methods. As the number of people in the world increases, so does the number of animals. Naturally, the amount of animal waste produced is also increasing. In the Food and Agriculture Organization of the United Nations (FAOSTAT), according to 2018-year data, there are approximately 1.5 billion cattle heads in the world. According to the regional distribution in the world, America, Asia, Africa, Europe, and Oceania are 35.7%, 31.9%, 19.7%, 10.0%, and 2.7%, respectively. The countries in the top five are Brazil, India, the United States of America, China, and Argentina, respectively [1]. According to Turkey Statistical Institute (TSI), in 2020, there are 18 million 615 thousand cattle heads in Turkey. It has increased by 4.2% compared to the previous year [2]. With the assumption that cattle will produce 10-20 kg of manure (wet) per day [3], approximately 186-372 thousand tons of cattle waste is generated per day in Turkey.

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Animal waste should not be disposed of on nature at random in order to achieve sustainable animal waste management. Moreover, direct dispose of to landfills increase the soil and/or groundwater contamination potential due to their high water content. For this reason, they may be used as a natural fertilizer in agricultural areas, gardens, and so on after composting in a controlled manner and/or digesting in anaerobic conditions. Animal wastes contain nutrients such as C, N, and P. Thus, these nutrients by anaerobic digestion (AD) are evaluated as also a fertilizer. AD is the process of decomposing waste/wastewater with organic content in an oxygen-free environment and converting it to colorless odorless biogas. The composition of the biogas contains approximately 40-70% methane, 30-60% carbon dioxide, 0-3% hydrogen sulfide, and a small amount of nitrogen and hydrogen depending on the composition of organic substances [4]. AD may be performed under psychrophilic (12-16 ˚C), mesophilic (35 37 ˚C) and thermophilic (55-60 ˚C) temperature conditions. It is performed psychrophilic decomposition in landfills, swamps, and sediments; mesophilic decomposition in the rumen and AD systems; and thermophilic decomposition in AD systems and geothermal heated ecosystems [5]. AD is a method that provides both stabilization and volume/mass reduction (30-50%) of wastewater [6] as well as the production of bio methane. It helps to reduce greenhouse gas emissions and also reduces the landfill load [7, 8]. Moreover, solid and/or liquid fertilizer is produced by this method.

When mesophilic (M-AD) and thermophilic (T-AD) anaerobic digestions are compared, M-AD is more widely used than T-AD. Although the decomposition of volatile solid matters at M-AD takes about 30-40 days, this temperature range is optimal for many methane-forming microorganisms. Furthermore, mesophilic temperatures require lower energy use (lower operating costs) and provide a better stability process than thermophilic temperatures. Also, at M-AD, nitrogen, phosphorus, and pH are approximately same in liquid products after decomposition. Mainly disadvantages of it are to provide lower chemical oxygen demand (COD) and methane yield, to be more dominant of acetic acid in the system, to be low bacterial removal (E. coli), and to be sensitive to toxic compounds.

Thermophilic temperatures provide higher removal of both infectious animal viruses and bacteriophages [9]. T-AD compared to M-AD has the ability to work at high organic loads, provides higher COD removal and methane production, and is about 2 times less suspended solid concentration in effluent. It provides better dewaterability of the digested product, requires smaller reactor volume and less a land by realizing faster decomposition, and produces less H_2S and less odor. Although T-AD has more advantages, the main reasons for its limited use are poor process stability (more sensitive to temperature change and to toxic compounds), worse supernatant quality [10], and high operating cost. Less stable T-AD means less microbial population, the stability of propionate acid, and increased toxicity [11]. For this reason, the properties (nitrogen-like constituents such as urea, protein, etc., acid, microbial content, etc.) of the feedstock are the most important parameters affecting performance and stability [11]. The feedstock with high nitrogen content causes high ammonia as a result of decomposition especially under thermophilic conditions; and high ammonia (NH_3) has a negative effect on the system by causing volatile fatty acid (VFA) accumulation. High NH_3 also causes an increase in pH [9]. After T-AD, effluent has the same VFA types and higher NH_3 content. Qi et al. [12] studied the digestion of cattle manure at thermophilic and mesophilic temperatures. The contents of liquid products after digestion were determined by analyzing indicator bacteria and heavy metals. In the study, a decreasing in indicator bacteria and low content of heavy metals were determined. It was measured that nitrogen was higher in the thermophilic product, and the amount of Bacillus and Pseudomonas was higher in the mesophilic product. Other nutrients were stated to have similar content [13, 14].

AD studies in the literature discuss different operating conditions; and some of these are temperature, mixing speed, using different inoculation and substrates, and different particle size of substrates. Co-digestion of different substrates and/or by adding inoculation may increase the dilution of toxic compounds or the number and type of nutrient/microorganism. This causes an increase in the stabilization of the system and thus the increased production of methane [5]. Some studies have been conducted on AD of different wastes [15-20]. In most of these studies, various wastes such as pig manure, poultry manure, milk manure, sludge, industrial wastes, food waste, slaughterhouse wastes, algae sludge wer e used as raw material and/or inoculation. It has been stated that digestion has a positive effect due to the co-digestion of wastes in appropriate proportions [18, 19, 21]. Mixing studies were conducted by some researchers [22-26]. In mixing studies, some researchers argued that mixing has a positive effect, while others argued that mixing has a negative effect. Wang et al. [26] obtained higher yields of cattle manure containing 5% total solids (TS) at mesophilic temperatures when compared to non-mixing one in continuous or intermittent mixing systems. It was stated that the sedimentation of solids was prevented by mixing and most methanogen species were more abundant in continuous mixing systems, thereby more methane was obtained. But, the full mixed reactor generally provides a homogeneous medium and accelerates material transfer. In other words, it makes it easier for bacteria to reach food. Moreover, it keeps solids suspended. These advantages lead to an increase in the efficiency of the system [27]. Mixing also allows the system to be stable in a shorter time under mesophilic conditions [28].

Cattle manure on farms can be separated from solid-liquid for its composting as above the mentioned, making it easier to transport and store the solid fraction (SF). And, the SF are composted or digested anaerobically. The produced product is used as a fertilizer in related areas. It can also easily send to people who need solid fraction. The liquid fraction (LF) can either be stored after a certain period of time and discharged into the environment or fertilizer can be obtained by digesting under anaerobic conditions with single or different wastes. Since the LF contains fewer solids and contains more than 80% biodegradable components, its anaerobic digestion ensures continuous operation and it takes place in a short time. When the literature research was carried out, it was seen that there are a limited number of studies on the LF of manure. These studies have carried out by Rico et al. [21, 29, 30] in different years. In these studies, mesophilic or psychrophilic temperatures have generally preferred. When the owner of the farm chooses to compost the SF of the manure, the LF of the manure is also a problem for the farmer due to the increasing environmental standards. Since the LF has a larger volume, storage for a while requires a large space. It can also cause environmental problems such as odor, aesthetic, etc. For this reason, it should be managed appropriately in the LF in terms of environment and human health.

Biochemical Methane Potential (BMP) studies are conducted easier than continuous experiments. Therefore, waste/waste waters are considered to be innovative technologies in determining the potential for producing methane from wastewater [31]. It has stated that significant economic profits are provided as a result of the conversion of methane gas into energy when the appropriate conditions are provided and the energy obtained may reduce the plant's operating cost (energy consumption) at the rate of 28% [4]. As a result of BMP analysis of olive black water, it was calculated that methane was 335 mL in 25 days [32], the amount of biogas produced in milk wastewater was measured 410 mL in 35 days [4].

Within the scope of the study, M-AD and T-AD were also evaluated by comparing it in terms of different criteria. The methane production potential of the remaining liquid fraction (constitutes a large part of the manure) after solid-liquid separation for composting of cattle manure was optimized under different operating conditions and modeled it with Taguchi method that is based on the principle of least experimentation to keep the costs to a minimum level. Some properties of cattle manure liquid fraction (CMLF), which is less studied in anaerobic digestion, were analyzed and it was tried to determine its methane production potential at mesophilic and thermophilic temperatures using the BMP, which is easy to operate in determining the methane production of raw materials. In this study, the reason for considering CMLF is that they have the highest biogas production capacity with a production share of 71.1% [25]. Besides the temperature, the effect of the substrate/inoculation (S/I) ratio and mixing speed was also investigated. Municipal sewage sludge was added as an inoculation to increase the organic/nutrient content and microorganism species/number in CMLF. The effect of mixing speed was studied because there is a limited study on determining the effect of mixing speed. Moreover, some properties of the liquid products formed were analyzed and also explained based on literature search.

2. MATERIALS AND METHODS

2.1. Chemicals

In this study, nitrogen gas was used to provide an anaerobic environment in BMP bottles. $NaHCO_3$ was used to prevent pH inhibition in the mixtures. The gas content produced under different operating conditions was determined according to the principle of displacement with water. 5% KOH was used to detect the methane portion of the produced gas, in other words, to occlude CO_2 and H_2S gas. The chemicals used are of high purity and obtained from Sigma Aldrich. In this study, no nutrients were added.

2.2. Characterization of Cattle Manure Liquid Fraction and Inoculation

The municipal sewage sludge as inoculation was used and it was taken from the anaerobic reactor in which domestic and textile wastewater treated by the National Membrane Technologies Application Center (Istanbul, Turkey). The sludge was precipitated and the lower phase was used in the study. pH, conductivity (EC), total solids (TS), and volatile suspended solids (VSS) analyzes were performed. pH, EC, TS, VSS and TS/VSS values were 5.24 ± 0.03, 1515 ± 3.54 µS/cm, 1.3 ± 0.03%, 91 ± 0.71% and 70, respectively. The addition of inoculation was preferred to shorten the commissioning of the system and as a source of essential nutrients/microorganisms.

CMLF was taken from the facility where dairy cattle raising are performed by Kain Farm Agriculture and Livestock Inc. in Yalova province (Turkey) and stored at 4˚C during the study. As soon as the CMLF was provided, it could not be fed into the system. Before, it was carried out some analyses. The solid-liquid separation from the raw manure is made by separator. The CMLF provided was not subjected to any pre-treatment. This company evaluates SF by composting. Characterization analyzes of CMLF were carried out before the system was started. All analyzes were performed in 3 replications according to APHA standard methods [33] and the results have given in Table 1.

Table 1. Characteristics of cattle manure liquid fraction

Parameter	Value	Parameter	Value
pH	7.3±0.04	Alkalinity (mg/L)	2244.5±2.83
EC (µS/cm)	9680±28.28	COD (mg/L)	23400±141.42
Salinity (‰)	5.5±0.01	VFA (mg/L)	1439.3±27.79
TS (%)	2.5±0.01	Ammonia (mg/L)	170.8±1.56
VSS (%)	56.7±0.52	Orthophosphate (mg/L)	34±2.83

2.3. Lab-Scale System and Operation

Table 2 shows the experimental conditions created with Taguchi Method. CMLF was put into BMP bottles with a total volume of 250 mL. The inoculation was then added to BMP bottles according to different substrate/inoculation (S/I) ratio. 50 gr/L $NaHCO_3$ solution was added to prevent pH inhibition and pH measurements were made. pH of all samples was measured between 7.1-7.2. The bottles were closed by gassing nitrogen gas for 5 minutes to remove oxygen. The prepared bottles were placed in the following BMP apparatus. Gas production was run until reduced (30 days). This set was carried out in two replicates and average results were given. In anaerobic systems, the active inoculation concentration is recommended to be between 3-5 g VSS/L. In this study, by selecting it 3 gr VSS/L, the S/I ratio was calculated and added to the system [34]. The S/I ratio was also determined as 1.5 and 4.5. The system used in the study is given in Figure 1. While the system was started, the bottles were wrapped with aluminum foil to allow the reaction to take place in the dark. Moreover, the bottles were operated in a beaker filled with water to minimize the temperature fluctuation.

Ghanimeh et al. [24] conducted on the effect of mixing on thermophilic AD. It was stated that mixing densities above 200 rpm may lead to the destruction of microbial populations; therefore slow mixing (100 rpm) provides better yields than vigorous mixing. According to this information, 90 and 120 rpm were selected as the mixing speed in this study.

Table 2. Experiments conditions

Sample	Mixing speed rpm	Temperature ℃	S/I Ratio
1	90	35	1.5
2	90	35	4.5
3	90	55	1.5
4	90	55	4.5
5	120	35	1.5
6	120	35	4.5
7	120	55	1.5
8	120	55	4.5

Figure 1. The system used in the study

The measurement of gas was carried out according to the principle of displacement with water. KOH was added to the solution prepared to occlude CO_2 gas produced. Thus, all of the gas produced was determined as CH_4. The analyses in the digested samples were carried out in the upper phase after 1-hour precipitation.

Optimization and statistical analysis by Taguchi method were performed by using Minitab 18 program. Data were transformed by Box-Cox transformation and one-way analysis of variance was performed. Taguchi method is one of the methods based on the principle of least experimentation to keep the costs to a minimum level developed by the Japanese Scientist Genichi Taguchi. It is an experience design that uses an orthogonal sequence [35]. The results obtained from the experiments have been converted to the signal/noise ratio (S/N). S and N represent the signal factor and noise factor, respectively. The signal factor refers to the actual value received from the system, and the noise factor refers to the factors that cannot participate in the design of the experiment but affect the result of the experiment. Noise sources are all variables that cause the performance characteristics to be obtained to deviate from the target value. Thus, the smaller the N value expressing the noise factors in the S/N ratio, the closer to the desired target value is. Therefore the purpose of this analysis is to maximize the S/N ratio and the “biggest best” (1) criterion was used for Nussetl [36].

$$^{S}/_{N} = -10 * log\left[\frac{1}{n}\sum_{f=1}^{n}\frac{1}{y_i^2}\right] \tag{1}$$

where, y_i denotes the performance characteristic value (Nusselt number), and n denotes the number of Y values.

In this study, 3 parameters (all are 2 levels) were selected for BMP tests. In this study, L8 (2^7) orthogonal sequence and the largest is best approach are used. The temperature was chosen as 35-55˚C, S/I as 1.5-4.5, and mixing speed as 90-120 rpm to model by Taguchi method the results obtained in determining the methane production potential of CMLF in different operating parameters.

3. RESULTS

3.1. Biogas/Bio-methane Production

Different mixing speeds, temperatures, and S/I ratios from the important operating parameters in anaerobic digestion were studied. The results obtained with Taguchi method are given in Figure 2.

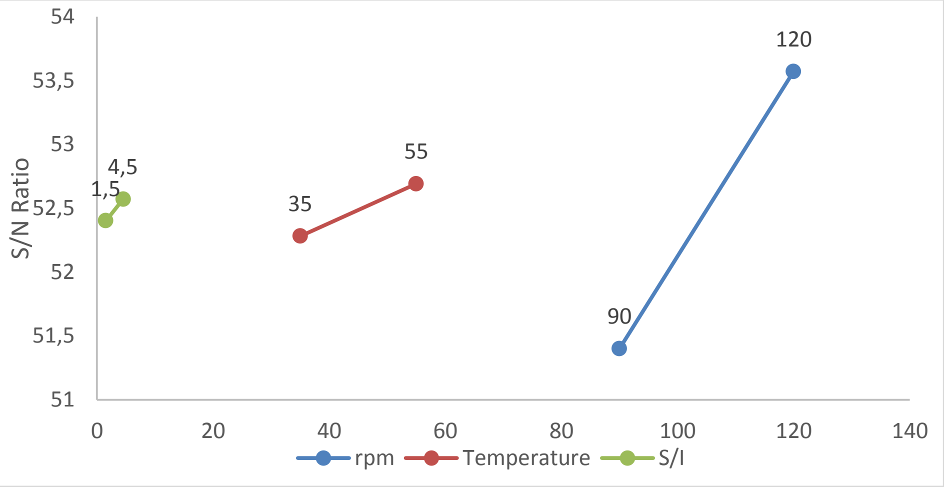

Figure 2. S/N ratios of operating parameters

According to S/N ratios figure, it is seen that as mixing speed, temperature and S/I ratio increase, methane production increases. The thermophilic temperature has increased methane yield. However, it is stated that gas production decreases above 60 ˚C and stops above 70 ˚C [37]. Therefore, operating above 55±0.5 ˚C may not be suitable depending on the properties of the raw substrate. Suhartini et al. [38] argued that thermophilic conditions have more advantages with high methane production, increased buffering capacity, and decreased foaming and dewatering of increased digested products in the study in which they stabilized sugar pulp under mesophilic and thermophilic conditions. This was because there was no formation of extracellular polymeric substances (EPS) as a result of better substrate hydrolysis [38]. Labatut et al. [11] operated cattle manure and dog food by continuously mixing under mesophilic and thermophilic conditions. It was stated that the stability of the system under thermophilic conditions was highly dependent on the composition of the feedstock. It can be said that it is suitable for digestion of LF at mesophilic and thermophilic temperatures. It must be forgotten that the higher the temperature is, the higher cost is. For the cost, mesophilic temperatures may be preferred. In the study conducted by Tufaner and Avsar [39] in which biogas was produced from cattle manure at mesophilic temperatures, (effective reactor volume 6.15 L), 17 L of biogas was produced in 31 days. A laboratory-scale upstream anaerobic reactor was used in this study. Optimum biogas was produced with the addition of 20-30% different wastes in the digestion of different ratios of cattle manure and different wastes together [40].

In the literature, it has been stated that the use of inoculation at different rates has a positive effect since it allows different microorganism communities. As can be seen from Figure 2, increasing this ratio from 1.5 to 4.5 does not have the same effect as the mixing speed, but still has increased the gas formation. The reason why inoculation rates do not have a major effect may indicate that LF provides adequate properties. For a clearer result, both different rates should be studied and microorganism analyzes should be conducted. In the literature, the effects of different inoculation contents on anaerobic digestion of different waste and/or wastewater have been studied [41-44]. Inoculation characteristics are important for AD performance. The use of it has been reported to alter degradation rate, biogas composition, and reactor stability. It has observed that co-inoculation increased the methane yield more [43]. It may be studied the effect of co-inoculation addition on methane yield.

From operating parameters studied, it was observed that mixing has a higher effect on methane yield. Increasing the mixing speed from 90 rpm to 120 rpm increased methane yield. Mixing results in additional investment and operating costs for the AD system. However, efficient mixing will reduce the energy requirement by providing higher methane [26]. Mixing should prevent solid matters from sedimentation (provide homogeneous media), increase contact between nutrients and microorganisms, increase the activity of the microbial population, facilitate the separation of organics, reduce stratification, minimize temperature gradients and reduce particle size. It was stated that slow mixing improves fermentation and vigorous mixing delays fermentation and causes low methane production [23, 45]. A high mixing speed means more energy consumption. Therefore, high-speed mixing may not be preferred. Ghanimeh et al. [24] studied the effect of mixing on thermophilic (55˚C) AD of organic fraction of municipal solid waste and fresh manure as inoculation. An average of 2819 L and 2504 L methane per day were produced in the reactors (14 L volumes; 9 L working volumes) mixed continuous and intermittent mixing at 100 rpm. It was stated that mixing velocities above 100 rpm may lead to destruction of microbial communities, and therefore slow mixing (100 rpm) provides better efficiency than vigorous mixing. Lindmark et al. [25] reported that more biogas was produced at 25 rpm compared to 150 rpm. The vigorous mixing does not recommend as it can be harmful to microbial consortia [26] and reduces contact between the nutrient and microorganism. It was stated that mixing should be done on substrates with foaming and/or solid-liquid separation [46]. Optimum mixing is still a matter of debate. Because there is no clear information about which intermittent or continuous mixing is more convenient [47]. Intermittent mixing is suggested to be economical for energy consumption [46]. It was stated that mixing on digesters operated at lower TS and longer hydraulic retention time (HRT) has no significant effect and that mixing on digesters operated at higher TS has a significant effect on biogas yield [47]. Karim et al. [22] studied the effect of mixing on AD of manure at mesophilic temperatures. In the study, it was stated that mixing in 5% TS content has no significant effect and increased methane content by 10-30% in digestion of substrate with 10% and 15% TS content. It is especially recommended for substrates with solid matter content higher than 5% [48, 49]. Slow mixing has been observed to increase the capacity of the digester, the stability of the system and the treatment efficiency. Mixing is an important parameter because it prevents hydrogen accumulation, but slow or intermittent mixing compared to no mixing and vigorous mixing has been suggested by various researchers [22-24]. The effect of intermittent mixing can be studied in the future.

It can be said that the dead volume is reduced, sufficient contact between the microorganism and substrate is ensured, solid-liquid separation is minimized, the temperature distribution is ensured and gas outflow is facilitated in 90-120 rpm mixing speeds. Furthermore, the accumulation of VFA with continuous mixing may have been minimal. For this reason, 90-120 rpm mixing velocities have been a positive effect on methane yield.

Regression analysis was performed according to Box-Cox transformation. The results obtained are given in Table 4.

Table 4. Regression analysis

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	3	13.6899	4.5633	122.59	0.000
Mixing, rpm	1	13.1289	13.1289	352.69	**0.000***
Temperature, ◦C	1	0.4659	0.4659	12.51	**0.024***
S/I	1	0.0951	0.0951	2.55	0.185
Error	4	0.1489	0.0372		
Total	7	13.8388			

*0.05

P values less than 0.05 indicate that coefficient estimates are significant. The fact that F values are high and P values are low shows that the regression coefficients are holistically significant. As a result of the regression analysis, R^2 value was calculated as 98.92. R^2 value is suitable as it is greater than 95. As a result of the calculations, the following regression equation has obtained.

$$Methane^{0.5} = 10.286 + 0.08540 \times Mixing + 0.02413 \times Temperature + 0.0363 * {}^{S}/_{I} \qquad (2)$$

This equation indicates that on methane yield, mixing has the highest effect and temperature has the lowest effect. Temperature is a parameter that affects the optimum reproduction of microorganisms and the decomposition rate/solubility of organic wastes. It can be said that mixing increased homogeneous distribution of total solids content, no dead zone formation, and microorganisms' access to food. Less effect of mesophilic and thermophilic conditions (temperature) may be due to the use of CMLF with more than 80% biodegradability [21]. It can also be related to both total solids content of this mixture and the type/number of microorganisms in the medium as a result of inoculation addition. The temperature may not have been had a major impact on the biogas yield, as it was studied at the optimum temperature values (35-55 C). Figure 3 shows the cumulative biogas and final bio-methane content. It is seen that higher biogas production and methane production in 5, 6, 7 and 8 samples than others. This result shows that mixing speed compared to S/I ratio and temperature is a more effective parameter on gas production under these conditions.

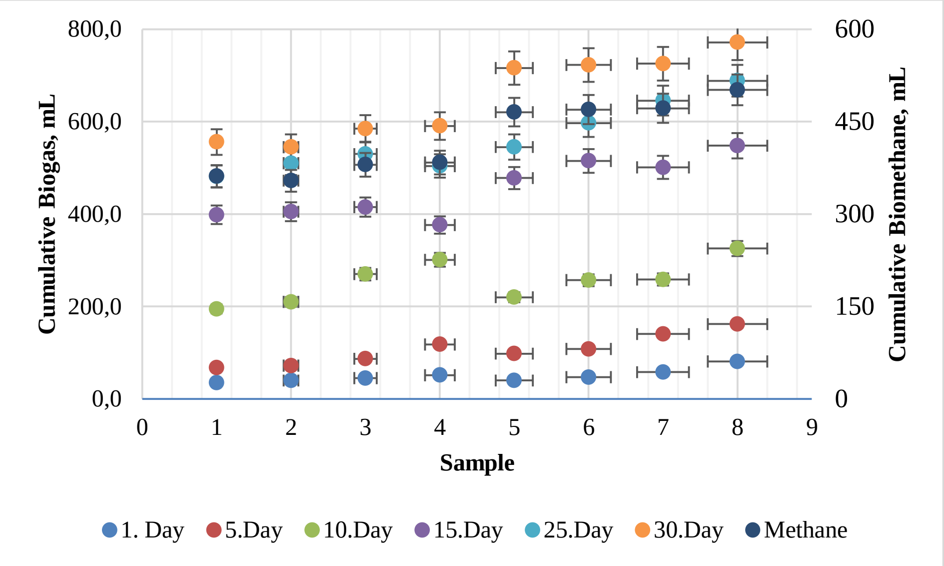

Figure 3. Cumulative biogas and final methane content

As a result of the digestion of CMLF having inoculation addition under different operating conditions during 30 days, methane and biogas production have observed in the ranges of 354-502 mL and 544-772 mL, respectively. Approximately 64% of the biogas has composed of methane. Increasing the mixing speed from 90 rpm to 120 rpm increased the methane yield by 29%. In terms of methane content, the highest was obtained in sample 8 (120 rpm, 55˚C, 4.5 S/I ratio). However, sample 5 conditions (120 rpm, 35˚C, 1.5 S/I ratio) has determined as optimum in terms of cost, methane production, and liquid products' properties. Cost analysis should be carried out to make a more accurate decision. In the literature, different values had obtained in the studies conducted with different substrates. For example, the methane yield by the co-digestion of swine manure and 25% winery wastewater measured 653 mL/day while methane yield of alone swine manure 256 mL/day in a semi-continuous reactor. In the batch reactor, the methane yield of alone swine manure determined less compared co-digestion of feedstocks [50]. These results indicate that mixing and co-digestion have a positive effect on methane production.

The amount of methane produced is highly affected by the type of substrate and operating conditions. In this study, the reason for obtaining high values is due to CMLF using and inoculation addition. CMLF has passed through the separator and municipal sewage sludge used as inoculation has had a small particle size and high VSS content (91%). In other words, the size of VSS/TS ratio of inoculation (91/1.3 = 70) indicates the low of inert components. Moreover, it can be said that animal wastes such as cattle manure were suitable in terms of the nutrient environment and necessary methanogenic bacteria to produce efficiently methane [51, 52]. Cattle manure contains lignin compounds because cattle fed with grass. Thus, methane yield is low due to its low decomposition under anaerobic conditions [52]. This disadvantage has minimized by digesting the liquid fraction in the present study.

After the liquid fraction is stored for a while (requires a large area), it is released into the environment. By digesting anaerobically, its volume can be reduced, fertilizer can be obtained, and the liquid product quality can be increased. In addition, due to the low particle content, it will also give the opportunity to continuous operation. Otherwise, it will cause to surface water and groundwater pollution, odor formation, and emission. In short, uncontrolled management will adversely affect the environment, human, and animal health socio-economically. Therefore, controlled and sustainable management of the liquid fraction of manure is of great importance. Tufaner and Avsar [3] were stated that according to cost-benefit analysis, it is possible to establish biogas facilities for farms larger than 50 cattle. Looking at these results, it can be said that the farmers can efficiently be digested the liquid part together with the domestic sewage sludge in the farms where solid-liquid separation is performed. In short, anaerobic digestion both reduces pollution and generates renewable energy (methane). Besides methane, hydrogen can also be produced using hybrid systems [53].

3.2. Properties of liquid products

pH of the samples was adjusted to 7.1 - 7.2 before starting the study, pH values of the liquid products were measured in the range of 7.73-8.18 after the digestion. When the results in Table 5 are examined, it can be said that the ratio of the S/I rather than the mixing speed and temperature affects pH more. Moreover, pH may have changed as a result of the conversion of nitrogenous compounds to ammonia [54] and the change in CO_2 solubility [5]. It was observed that pH does not fluctuate much in systems where cattle manure is used [55]. In this study, it can be said that there is not a great fluctuation and that the observed change may be due to the CMLF use and S/I ratio. COD removal was achieved by over 81% in all samples. The highest removal efficiency was 88.8% in sample 8. It can be said that the mixing speed has a higher effect on COD removal efficiency. It was stated that mixing increases soluble COD removal [24]. The use of only the liquid fraction of the cattle manure after it has passed through the separator has resulted in a low number of colloidal substances. Moreover, the fact that the particle size of the municipal sewage sludge used was very small increased the decomposition efficiency. The low content of colloidal matter also indicates in the low level of insoluble/difficult to decompose components [6].

Table 5. Properties of the supernatant in liquid products after anaerobic digestion

Sample	Mixing speed (rpm)	Temperature (℃)	S/I Ratio	pH	COD (mg/L)	COD Removal (%)
1	90	35	1.5	8.05±0.02	4375±35.36	81.3
2	90	35	4.5	7.83±0.01	4262±88.39	81.8
3	90	55	1.5	8.13±0.03	4268±44.55	81.8
4	90	55	4.5	7.90±0.03	4100±70.71	82.5
5	120	35	1.5	8.13±0.04	3692±11.31	84.2
6	120	35	4.5	7.83±0.04	3623±38.18	84.5
7	120	55	1.5	8.18±0.05	3259±57.98	86.1
8	120	55	4.5	7.87±0.04	2621±82.73	88.8

4. CONCLUSION

In this study, the bio-methanation potential of cattle manure liquid fraction under different operating conditions was optimized and modeled with Taguchi method. As a result of the study, it has been concluded that the effect on the methane yield from the operating conditions considered is mixing speed> S/I ratio> temperature. The optimum operating conditions were determined as 120 rpm, 35 ˚C, and 1.5 S/I ratio. Under these conditions, 465 mL of methane was produced in 30 days and 84% COD removal was provided. It can be said that co-digestion cattle manure liquid fraction and municipal sewage sludge as inoculation were suitable in terms of the nutrient environment to produce efficiently methane. Thus, besides energy production (mainly methane), environmental pollution will be prevented. For future studies, continuous and intermittent mixing's effect on the methane yield, intermediate products (especially antibiotics) to microbial properties of the liquid product, and the system's economic assessment may be suggested. The usability of digested liquid fraction in agriculture can be studied.

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