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Unleashing Dairy Manure's Biogas Potential: A Michaelis-Menten Modeling Approach

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ABSTRACT

In the quest for improved anaerobic digestion (AD) efficiency and stability, iron-based additives and drinking water treatment sludge (DWTS) have emerged as promising components. This study explores the kinetics of methane production during AD of dairy manure under various concentrations of iron shavings (IS) and Fe3O4 (10, 20, and 30 mg/L) and DWTS (6, 12, and 18 mg/L). The experimental data were employed to assess the suitability of the Michaelis-Menten model as a non-linear regression (NLR) equation for evaluating the kinetics of dairy manure AD with these additives. The results demonstrate that the Michaelis-Menten model exhibits sufficient predictive capability for estimating cumulative methane production during the digestion process. The model was then utilized to compare the average cumulative methane production across the investigated treatments using the least significant difference (LSD) method, as well as to calculate the quantity of methane production at 25%, 50%, 75%, and 90% of the final methane yield. Notably, the findings revealed a significant difference (P > 0.05) in biomethane production among the different levels of DWTS, IS, and Fe3O4. Additionally, treatments containing varying levels of DWTS exhibited significantly shorter time durations to achieve 25% and 50% of their maximum methane yield compared to treatments containing Fe3O4. The most pronounced changes in these parameters were observed between distinct levels of IS.

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INTRODUCTION

With the continuous acceleration of social development and the rapid growth of global population, energy demand has increased dramatically. Therefore, energy crisis and its environmental pollution have become two key problems of global sustainable development (Yan et al., 2012). Importantly, many global organizations have agreed that the application of new technologies in seeking new alternative energy sources is the way to resolve both of the two problems (Srivastava et al., 2015). During last decades, two forms of potential global bio-energy and alternative sources include energy crops, lignocellulosic complexes and algae crops (Srivastava et al., 2015), which have been defined as first, second and third-generation biomass resources, respectively. In this way, animal manure is one of the most important second-generation biomass resources due to its plentiful supply, lower cost, and a rich source of lignocellulosic and mineral compounds (Naik et al., 2010).

Dairy manure comprises bounteous amounts of nutrients. Actually, it contains substantial amounts of Nitrogen (N), Phosphorus (P), and Potassium (K) as well as small amounts of trace minerals. Generally, the upsurge in production and concentration of dairy industry has resulted in greater awareness and concern for the proper storage, treatment, and utilization of dairy manure (Khademi & Masomi, 2022; Zhu et al., 2021).

Recently, anaerobic digestion (AD) is a well-described and common process in dairy manure treatment. It represents the two-fold benefits – minimizing the environmental effect, while simultaneously producing biogas for local energy needs (Li et al., 2018).

Anaerobic digestion is a biochemical process that converts a variety of organic matter using naturally occurring microorganisms under oxygen depleted conditions to produce a gaseous mixture mainly composed of methane and carbon dioxide, known as biogas. The overall AD process is a blend of physicochemical and biochemical reactions. These can be basically categorized as disintegration, hydrolysis, fermentation (acidogenesis), acetogenesis (acetate generation) and methanogenesis (methane generation) (Uddin & Wright, 2023).

Despite the rapid development of AD technology, some of its drawbacks, such as low biodegradation efficiency, poor stability, and environmental sensitivity, have hindered its commercial application. To address these challenges, promising approaches such as co-digestion, pretreatment, and new reactor designs, as well as the use of additives have been proposed. The additives stimulate bacterial growth and reduce inhibitory effects which can help control microbial generation time, degradation rate and gas production (Choong et al., 2016). Studies conducted by Al Seadi et al. (2008) and Cheng et al. (2020) emphasize the significance of incorporating trace elements or micro-nutrients, like Iron (Fe), cobalt (Co), nickel (Ni), into the anaerobic digestion process. These additives play a crucial role in facilitating the digestion process. While trace elements have proven to be beneficial, their widespread implementation remains limited primarily due to the high cost associated with these chemicals.

However, exploring more affordable sources of micro-nutrients could render their utilization economically feasible. Several studies (Ebrahimi-Nik et al., 2018; Huiliñir et al., 2015; Huiliñir et al., 2017) have highlighted the successful utilization of fly ash and drinking water treatment sludge (DWTS). DWTS is composed of alkaline, trace, heavy metals, and clay, arising from the treatment of surface water for drinking purposes.

In addition to this, as in any biological/chemical process, studying AD kinetics is essential to evaluate the feasibility of the process (as well as the design of a biogas production plant). It means that, the application of mathematical modeling in AD proves to be a rapid and cost-effective approach for predicting and optimizing fuel processing engineering and waste industry design (Andriamanohiarisoamanana et al., 2020).

Amidst this context, AD processes exhibit compatibility with non-linear models, as the growth of microorganisms and subsequent production kinetics are frequently non-linear in nature (Khamis, 2005). Within this framework, numerous non-linear regressions (NLRs) were derived from AD experiments, emphasizing the significance of making appropriate selections from an extensive library of functions (Archontoulis & Miguez, 2015). Generally, it provides information concerning the biodegradability rate of the substrates and also bottlenecks of the process that might affect digestibility and consequently methane yield (Allen et al., 2015; Karki et al., 2022; Tabassum et al., 2018). Kinetic models are useful to optimize, simulate and monitor the performance of the process under different conditions, especially when different additives such as trace element used in AD process of various feedstock, (Hassaan et al., 2021; Pramanik et al., 2019), because the kinetics of biogas production vary from one substrate to another.

According the extensive research in the field, there is currently a few published studies exploring the application of suggested kinetic models in evaluating of enhancing biogas yield by incorporating DWTS into the anaerobic digestion process of dairy manure and comparing it with iron-based additives. Thus, the present project seeks to fill this knowledge gap and aims to evaluate the impact of iron-based additives, IS, Fe_3O_4, and DWTS, as trace elements and additives (nine treatments) for biogas production during the anaerobic digestion process of dairy manure via the best suggested kinetic model. For this, we compared the quantity of methane production for each of the nine treatments at various points during the anaerobic digestion process. Also, the average cumulative methane production among the studied treatments compared after the completion of the anaerobic digestion process using the LSD method.

MATERIALS AND METHODS

Anaerobic digestion assays

In this study, dairy manure (sourced from the livestock farm) was used as substrate and the essential inoculum for the AD tests was procured from an active digester, receiving daily feedings of food waste, primarily consisting of rice, within Ferdowsi University of Mashhad's biogas laboratory. Fe_3O_4 and iron shavings served as the trace elements in this digester. The iron shavings, measuring less than 1 mm, were procured from the mechanic laboratory. Additionally, the experiments involved the application of drinking water treatment sludge (DWTS) as an effective additive, obtained from a drinking water treatment plant. The key components of DWTS included Fe_2O_3, SiO_2, CaO, and Al_2O_3, respectively. The composition of DWTS used in this research closely resembled that described in our previous study (Ebrahimi-Nik et al., 2018).

The process was conducted under mesophilic conditions at 37°C, we performed three independent experimental replicates following the procedure outlined by Holliger et al. (2016). The experiments were carried out using 500 mL bottles, with a working volume of 400 mL, ensuring each bottle's gas-tightness. To facilitate gas collection, the bottles were connected to 2 L gas collection bags via pneumatic mediators attached to their lids through plastic tubes. Both inlet and outlet were present on the gas bags, with a heparin cap connected to the outlet, enabling methane measurement using a syringe.

IS and Fe_3O_4 were selected at three levels: 10, 20, and 30 mg/L, whereas DWTS was utilized at 6, 12, and 18 mg/L. For clarity, Table 1 illustrates the experimental treatments and their corresponding symbols. Daily measurements of biogas and methane production resulting from the treatments were carried out using a 60cc syringe (Raposo Bejines et al., 2012). The anaerobic digestion process spanned 43 days and concluded when the rate of methane production during three consecutive days dropped below 1% of the total cumulative methane production (Holliger et al., 2016). More details about this process and its measurements can be find in our previous manuscript (Rezaeifar et al., 2023).

Table 1. Experimental treatments and their relevant symbols

Additives	Unit (mg/L)	Treatment(symbol)
	6	T1
DWTS	12	T2
	18	T3
	10	T4
IS	20	T5
	30	T6
	10	T7
Fe_3O_4	20	T8
	30	T9

Kinetic modelling

In order to examine the production of biogas through the anaerobic digestion of dairy manure and determine the relevant kinetic parameters, the utilization of nonlinear regression (NLR) models was implemented. In order to evaluate the NLR model and its coefficients importance, researchers often employ the analysis of variance (ANOVA).

The procedure of fitting nonlinear models involves multi-steps. The principal characteristics of nonlinear models are parsimony, interpretability, and prediction. On the other hand, key drawbacks are reduced flexibility compared to linear models and lack of an analytical solution for estimating the parameters. Also, an appropriate selection in a large library of functions is of great importance (Archontoulis & Miguez, 2015). More to the point, samples must be representatively large as well as accurate to obtain the desired results through the regression model. Therefore, this method is highly sensitive and may lead to errors (Wang et al., 2011).

For this study, the model coefficients were acquired by using the MATLAB function fitnlm, which is a built-in function capable of fitting a diversity of NLR models to data. For the obtained result from experimental section, a comprehensive summary of the NLRs analyzed is evaluated by Rezaeifar et al. (2023), they analysis an extensive range of data patterns, including exponential, logarithmic, polynomial, sinusoidal, generalized Mitscherlich, Michael Menten, and power-law functions.

Moreover, to assess the fitness of nonlinear regression models, the coefficient of determination (R^2), root mean square error (RMSE), and minimum value predicted by the model (MP) is employed as the following equations:

$$R^2 = 1 - \frac{\sum_1^N \left(B_{ai} - \hat{B}_{pi}\right)^2}{\sum_1^N \left(B_{ai} - \overline{B_a}\right)^2} \tag{1}$$

$$RMSE = \sqrt{\sum_{i=1}^{n} \frac{\left|B_{ai} - \hat{B}_{pi}\right|^2}{N}} \tag{2}$$

Where B_a and B_p denote the experimental and predicted values, respectively. The $\overline{B_a}$ represents the average value of the experimental values, and N denotes the sample size (Wang et al., 2011). By utilizing these criteria, we were able to identify the models that most precisely depict the fundamental biogas production kinetics. A good fit with experimental data is indicated by a low RMSE value and a high R^2 value when selecting the best time model ideally.

Although there are a wide range of equations can be used in modeling the kinetic of biogas production (Rezaeifar et al., 2023), in this study, we proceeded with the Michaelis-Menten non-linear regression model for further analyses. Generally, Michaelis-Menten model can explains how an enzyme can cause kinetic rate enhancement of a reaction and explains how reaction rates depends on the concentration of enzyme and substrate (Choi et al., 2017). Therefore, it may be practical in modeling the kinetic of biogas production on different substrate, while the past studies show that it has not been evaluated for the mentioned substrate in this study.

The formula of chosen model, Michaelis-Menten (MM), is as following, in this equation, a, b and c are final volume of produced gas, time at a/2 and a dimensional parameter, respectively.

$$f(t) = a\frac{t^c}{t^c + b^c} \tag{3}$$

RESULTS AND DISCUSSION

Prediction performance evaluation

Based on the R^2 and RMSE criterion, it is reported that the Michaelis-Menten model demonstrated great predictive ability for all treatments. The results of RMSE and R^2 for each of the nine treatments are reported in Table 2. Moreover, Table 2 presents the coefficients of the Michaelis-Menten nonlinear regression model, along with their standard deviation, p-values, coefficient of determination (R^2), and adjusted coefficient of determination for each of the studied treatments. The p-values for all cases are equal to zero, indicating that the coefficients of the models are significant at a significance level of one percent. The small standard deviation values of the coefficients, compared to the coefficient values, provide further evidence that the models' estimations can be trusted. With the exception of the T4 treatment, all other treatments have an R^2 value greater than 0.94, confirming the prediction reliability of the models. Hence, the results will be interpreted based on the estimations of the models.

In addition to these, a comparison between experimental and predicted value of methane production via the suggested mode is shown in figure 1 for T3, T4 and T8 as the highest, lowest and moderate sample of three different treatment.

Table 2. Coefficients, significance results, and coefficient of determination values for the Michaelis-Menten model

		T1	T2	T3	T4	T5	T6	T7	T8	T9
Michaelis-Menten	RMSE	90	118	130	187	134	138	91	85	81
	R^2	0.98	0.97	0.95	0.75	0.92	0.87	0.97	0.94	0.92
Coefficients	a	2566.0	2280.0	2158.5	1275.3	1562.9	1273.9	1325.3	893.0	736.1
	b	1.64	1.95	1.59	1.56	1.98	2.37	5.06	5.77	5.35
	c	11.50	9.83	10.76	17.46	8.41	6.50	13.31	12.90	12.62
Std	a	50.95	38.94	71.30	208.83	34.37	24.16	12.70	9.94	12.25
	b	0.07	0.10	0.12	0.35	0.16	0.26	0.29	0.44	0.59
	c	0.37	0.27	0.60	4.39	0.34	0.30	0.17	0.19	0.29
p-value	a	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	b	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	c	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R^2	R^2	0.99	0.98	0.97	0.80	0.96	0.93	0.98	0.97	0.94
	$R^2_{Adj.}$	0.99	0.98	0.97	0.80	0.95	0.92	0.98	0.97	0.94

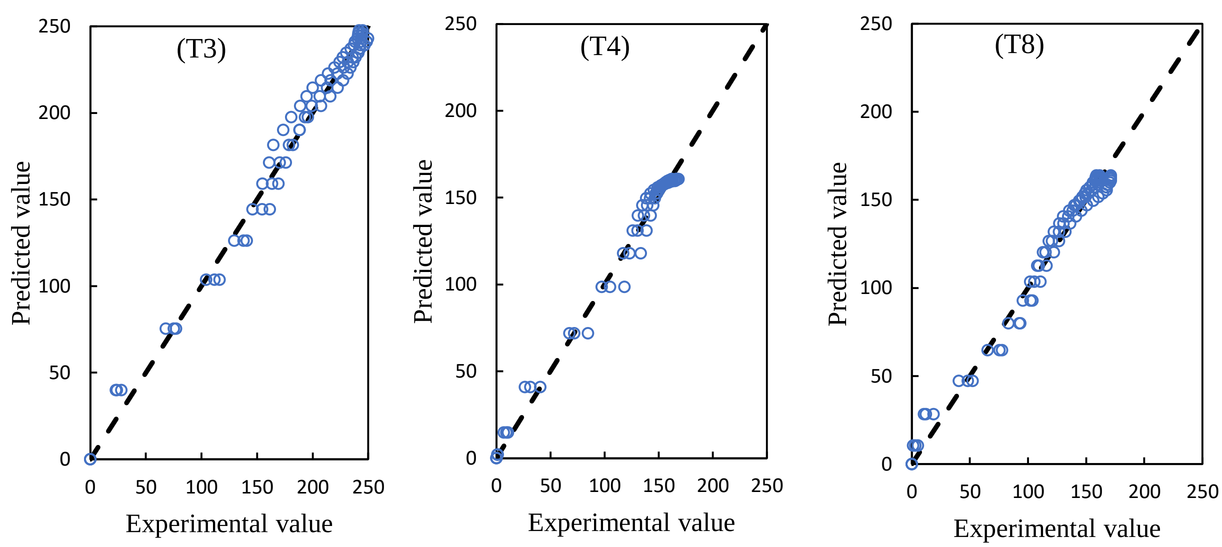

Figure 1. Comparison between predicted and experimental value of methane production for a) T3 b) T4 and c) T5

Biogas performance

Within the realm of anaerobic digestion, nonlinear regression proves to be a robust instrument for estimating significant parameters, including the degradation rate, the gas volume generated per nutrient degradation, and the fermentation process's lag phase and others. Also, we can calculate some other parameters those can be hard and time-consuming to determine during experiments. The suggested model can be practical to run an analysis of variance or do an LSD test.

It is clear by running an ANOVA test and get a significant result that means at least one of the groups tested differs from the other groups. However, it cannot be shown by the ANOVA test which group differs. In order to address this, the Fisher least significant difference (LSD) test can be helpful, which is only used when the null hypothesis is rejected as a result of your hypothesis test results. The LSD calculates the smallest significant between two means as if a test had been run on those two means (as opposed to all of the groups together). This enables researcher to make direct comparisons between two means from two individual groups. Any difference larger than the LSD is considered a significant result.

As can be seen, figure 2 present the comparison of average cumulative methane production using the LSD method at 5% level after the completion of the anaerobic digestion process. Figure 2(a) indicate the comparison of average the cumulative methane production among the studied treatments (with different level of DWTS) using the LSD method. As it can be seen, there isn't a statistically significant difference between these treatments.

In addition, this comparison between different levels of IS in figure 2(b) shows that the difference between IS10 with IS20 would be significant while IS20 has the highest amount of cumulative methane production. Furthermore, comparison between the treatment with different level of Fe_3O_4 in figure 2(c) shows the lowest level meaning $Fe_3O_4$10 shows a statistically significant difference with higher level of this additives ($Fe_3O_4$20 and $Fe_3O_4$30), while this treatment has the biggest amount of the cumulative methane production between these treatments.

Finally, figure 3 presents the comparison of average cumulative methane production among the studied treatments using the LSD method after the completion of the anaerobic digestion process. Notably, the figure highlights a significant difference ($P > 0.05$) in biomethane production between the different levels of DWTS, IS, and Fe_3O_4. As observed, the treatment with DWTS6 exhibits the highest level of average cumulative methane production, and there is a statistically significant difference between this treatment and the others, except DWTS12. This suggests that DWTS6 stands out as a particularly effective treatment for promoting methane production during the anaerobic digestion process, warranting further consideration for practical applications. Ebrahimi-Nik et al. (2018) demonstrated that the addition of 6 mg/kg DWTS to the anaerobic digestion of food waste resulted in a significant increase of 65% and 58% in biogas and methane yield, respectively

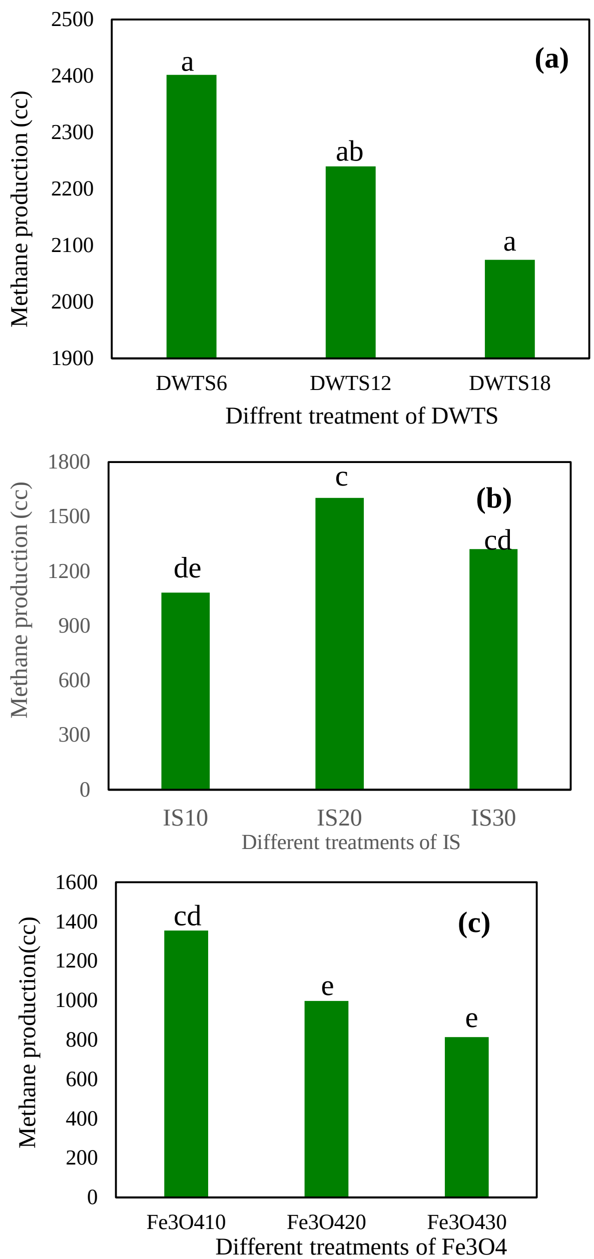

Figure. 2. Comparison of average cumulative methane production using LSD method among studied treatments with different level of a) DWTS b) IS c) Fe_3O_4

Furthermore, using the results of the modeling analysis, we computed the quantity of methane production for each of the nine treatments at various points during the anaerobic digestion process. Specifically, we calculated methane production when it reached 25%, 50%, 75%, and 90% of the final amount achieved at the end of the process. The computed values for above mentioned condition are shown with TV25, TV50, TV75, and TV90 for each treatment and presented in Table 3. By examining these values for each treatment, we can determine which treatments achieve their maximum methane production more rapidly or slowly. Opting for a treatment that reaches its maximum methane production earlier with a higher percentage would be preferable, as it indicates a more efficient and effective process.

The comparison of these results for the treatments of this study shows that although the treatments containing different levels of DWTS spent much less time to reach 25% and 50% of their maximum value compared to the treatments containing iron oxide, the time required to reach the 90% the maximum values for these treatments was much higher than the treatments containing Fe_3O_4. Also, as can be seen, the higher levels of all the additives used in this study generally reduced the time to reach 25, 50, 75 and 90% of the maximum, and the most changes for this parameter between these three types of additives were between different levels of IS.

In fact, higher levels of IS could double the time to reach the desired maximum in some cases.

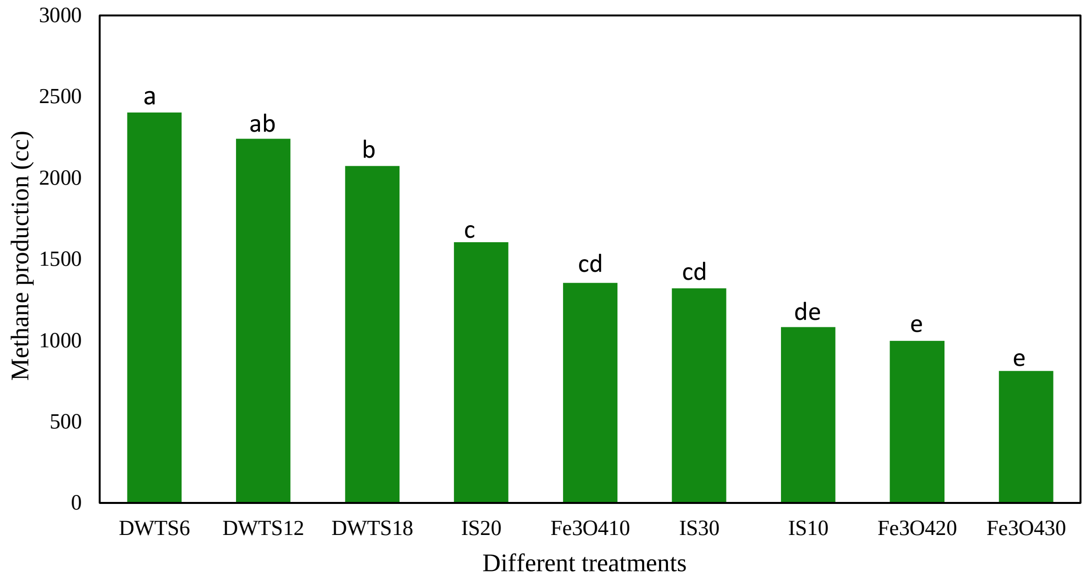

Figure 3. Comparison of average cumulative methane production among studied treatments using LSD method

Table 3. Calculated methane production values for TV25, TV50, TV75, and TV90 for each treatment

		TV25	TV50	TV75	TV90
DWTS	DWTS 6	5.65	11.29	21.77	38.89
	DWTS 12	5.64	9.65	16.32	26.93
	DWTS 18	5.28	9.40	16.49	28.03
IS	IS 10	8.48	17.65	23.05	32.61
	IS 20	4.73	7.94	13.20	21.57
	IS 30	4.21	6.64	10.41	16.14
Fe3O4	Fe3O4 10	10.41	13.22	16.72	21.06
	Fe3O4 20	10.76	13.02	15.71	18.90
	Fe3O4 30	10.40	12.16	14.20	16.53

Notes: TV25, TV50, TV75, and TV90 represent the times when methane production reaches 25%, 50%, 75%, and 90% of the maximum amount achieved at the end of the anaerobic digestion process, respectively.

CONCLUSIONS

In this study, we investigated the impact of iron-based additives, including Fe, Fe_3O_4, and DWTS, at various levels, on the anaerobic digestion of dairy manure by the Michaelis-Menten model as a non-linear regression. This model presents the best performance in estimating methane production kinetics for all nine treatments over time. The results revealed that DWTS at level 6 achieved the highest average cumulative methane production among the studied treatments using the LSD method at a 5% significance level, while there isn't a statistically significant difference between different levels of these treatments. Also, the difference between the higher level of IS and Fe_3O_4 were not statistically significant. In addition, computing the quantity of methane production at various points shows that the treatment reaches its maximum methane production earlier with a higher percentage would be preferable, as it indicates a more efficient and effective process. Generally, the higher levels of all the additives used in this study reduced the time to reach 25, 50, 75 and 90% of the maximum methane production.

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