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Effect of Biological Pretreatment on Anaerobic Sewage Sludge Digestion: Using Growth Media for Methanogenic Bacteria and Kinetic Studies for Biogas Yield

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ABSTRACT
The valorization of sewage sludge, a by-product of wastewater treatment by anaerobic digestion (AD), is getting more attention as a result of the advantages it provides for the environment and economy. The current investigation is an experiment performed in a lab setting using a batch-operated anaerobic digestion reactor with a mesophilic temperature of 35°C. This study examined the generation of experimental biogas and biodegradability .The effect of pretreatment by methanogenic bacteria growth medium on anaerobic digestion of sewage sludge was studied on three different concentrations of growth media, a control, and a low, medium and high concentration of culture medium, with cumulative biogas production of 610N ml /gVS added, 750 N ml /gVS added, 900 N ml /gVS added, 10 N ml /gVS added, respectively, with biodegradability rate of 52.16%, 56.5%, 74.04%, 28.70% respectively. Biogas production was enhanced at a medium concentration of culture medium and inhibited at a high concentration during anaerobic digestion of sewage sludge. Additionally, a theoretical biogas estimate was evaluated using four kinetic models (Logistic function, Modified Gompertz, Transference function, and First order); which were utilized to match the experimental biogas generation process involving the anaerobic digestion of untreated and pretreated sewage sludge by various concentrations of growth media of methanogenic bacteria . The kinetic findings demonstrated that both models, Modified Gompertz and Logistic function, were useful for predicting biogas output and matched experimental biogas production.

Keywords: anaerobic digestion, sewage sludge, pretreatment, growth media, biodegradability, kinetic models.

INTRODUCTION

Worldwide, the most common method of sewage sludge from wastewater treatment is anaerobic digestion treatment (AD) which is an exciting method that could meet local and global energy needs and provide environmental benefits (Li et al., 2022; Elasri and El amin Afilal, 2016). It is a natural process that converts organic matter in waste into a usable product under the influence of two major kinds of microbial activity (Narihiro and Sekiguchi, 2007; Admasu et al., 2022). The first type of bacteria known as acidifying bacteria breaks down complex organic substances (proteins, lipids and carbohydrates) into simpler components. The second type of bacteria, the methanogenic bacteria, which transform organic acids into methane as well as carbon dioxide and other gasses, feed on these simpler organic materials. In the absence of oxygen, a mixture of biogas is obtained containing methane and CO_2 and other gasses (Menon et al., 2017; Hendriks et al., 2018). Sewage sludge is created as a by-product of wastewater purification (Gagliano et al., 2018); allowing its subsequent use as a source of energy and biomass (Belaid et al., 2019). In view of the multiple studies on anaerobic digestion, some earlier attempts to boost gas generation through microbial activity have been made (Hoshiko et al., 2022). By using biological and chemical pretreatment procedures under different operating conditions (Cyprowski et al., 2018). As such, they are generally of lesser importance in terms of use in the habitat, but if used as additives in the biogas plant, they could significantly improve its performance (Deena et al., 2022). However, it is difficult to define the favorable culture medium for optimal bacterial growth, not only because of the variety of processing conditions used. But also because of different concentrations of growing media used (Hendriks et al., 2018). A growth media considered a biological pretreatment; is often a medium that contains all the components required for cell growth. (Or vitamins) Elements that are used by organisms for the creation of energy (Andreesen and Ljungdahl, 1973), growth, and maintenance of cell activities are known as necessary elements. The concentration of macronutrients and micronutrients in a growth medium varies based on the quantity needed (Hendriks et al., 2018). Macronutrients are made up of the elements calcium, magnesium, phosphorus, potassium, sulfur, oxygen, hydrogen, and nitrogen (Gagliano et al., 2018; Menon et al., 2017). The manufacture of enzymes or cofactors takes up the majority of the consumption of all other nutrients, including vitamins and the trace metals iron, cobalt, and nickel (Menon et al., 2017). They are regarded as micronutrients due to the fact that significantly less of them are required. During anaerobic digestion processes, a lack of trace metals can result in a decline in the production (rate) of biogas and process instability (Muñoz Sierra et al., 2017; Roy et al., 2022). Additionally, it has been noted that the inclusion of trace elements including cobalt, nickel, molybdenum, and tungsten can accelerate the methanogenesis phase (Andreesen and Ljungdahl, 1973). Numerous experiments on methanogenesis and anaerobic digestion have used wastewater and nutrients to promote growth. Similarly, many wastewater systems lack growth nutrients and demand their addition prior to anaerobic treatment. Given the distinct roles that macro- and micronutrients play in the growth of crops, growth media should include both types of nutrients (Li et al., 2022).The studies carried out on anaerobic digestion for the evaluation growth media and consequently the improvement of the performance of growth media were supported by kinetic studies that became famous in the field of bacterial growth to estimate the cumulative biogas rate and lag phase and quickly understand the useful production of biogas(Kerrou et al., 2021).

To improve the production of biogas from the anaerobic digestion of sewage sludge, this study used a growth medium for methanogenic bacteria as a biological pretreatment to confirm that the growth medium influences the anaerobic digestion process. This article explains the role(s) of the various macro- and micronutrients, as well as the choices of nutrient concentrations for optimal bacterial growth, the study also aims to evaluate the suitability of kinetic models, Modified Gompertz, First order, Transference function and Logistic function for anaerobic digestion of untreated and pretreated sewage sludge. This can help to evaluate the performance of pilot-scale bioreactors and predetermine the suitability of nutrients for anaerobic digestion to some extent which makes this study novel.

MATERIAL AND METHODS

Inoculum preparation and anaerobic digestion batch tests

The sewage sludge was collected from the anaerobic digester in a wastewater treatment plant. In this research, the biogas production from anaerobic digestion of sewage sludge will be investigated according to different concentrations of growth media of methanogenic bacteria; batch experiments were performed in serum bottles and biogas production was determined using the water displacement method. This experiment was carried out on a laboratory scale; the digesters used are sealed 250 ml bottles containing 10% sewage sludge, or 80g sludge in 100 ml tap water with 2 ml of various growth media concentrations (Hendriks et al., 2018) shown in Table 1. Biogas production was monitored for 30 days; the physical and chemical parameters of the untreated and pretreated sewage sludge are detailed in the (Table 2).

Experimental setup

The experimental setup at the laboratory scale, as shown in (Figure 1), the batch anaerobic digestion optimization tests operate at mesophilic temperature; in this experiment used different laboratory equipment: water bath, serum bottles, plastic pipe, gradual test tube 500ml .The experiment was carried out in serum bottles and incubated in a water bath at 35°; for 30 days to describe the production of biogas. The batch digester is sealed from the inside to prevent biogas leaks and a water

Table 1. The different concentrations of the growth media used in experiment adapted from (Sarmiento et al., 2011; Belaid et al., 2019).

Test of anaerobic digestion of sewage sludge without pretreatment (the control)	Test of anaerobic sewage sludge digestion with pretreatment		
	The serum bottles were fed with different concentrations of the growth media		
	Low concentration (g/L)	Medium concentration (g/L)	High concentration (g/L)
The serum vials used in this trial are sealed 250 ml vials with 10% sewage sludge, that is, 80 g of sewage sludge in 100 ml of tap water.	K_2HPO_4 : 2 $NaHCO_3$: 4 Kcl : 0.6 $MgSO_47H_2O$: 0 .02 NaCl : 0.02 $MoNa_2O_4$: 0.021 $FeCl_2$: 0.08 $MnSO_4H_2O$: 0 .02 Zn SO_47H_2O : 0 .02 Ni : 0.02 Cu : 0.02	K_2HPO_4 : 4 $NaHCO_3$: 8 Kcl : 1.2 $MgSO_47H_2O$: 0 .04 NaCl, 0.04 $MoNa_2O_4$, 0.04 $FeCl_2$: 0.16 $MnSO_4H_2O$: 0 .04 Zn SO_47H_2O : 0 .04 Ni : 0.04 Cu :0.04	K_2HPO_4 : 6 $NaHCO_3$: 12 Kcl : 1.8 $MgSO_47H_2O$: 0 .06 NaCl, 0.06 $MoNa_2O_4$, 0.06 $FeCl_2$: 0.24 $MnSO_4H_2O$: 0 .06 Zn SO_47H_2O : 0 .06 Ni : 0.06 Cu :0.06

bath is fully inserted to keep the temperature; the content of the digester is shaken by shaking with hands. Biogas production was determined using the water displacement methodology.

Analytical methods

In this article, we determined a number of chemical characteristics for sewage sludge of wastewater, such as total solids (TS), volatile solids (VS), alkalinity (ALC), the volatile fatty acids (VFA), global nitrogen (NGL), total Kjeldahl nitrogen (TKN), ammonium (NH_4), total phosphorus (TP) and chemical oxygen demand (COD). The total solid (TS) and the volatile solid (VS) were determined according to lihi et al (Lihi et al., 2023). The total solids were determined by drying the sample at 105°C for 24 hours. The VS were determined by burning the dried sample at 550°C for 2 hours. According to (Ourradi et al., 2022). The pH value was obtained using a pH meter and the alkalinity (mg $CaCO_3$/L) and volatile fatty acids (mg/L) were measured using a titration method. The COD was determined for the sewage sludge (Elasri and El amin Afilal, 2016), taking into consideration that the sewage sludge used in this experiment is not in a liquid state we performed a centrifugation for 10 minutes to remove the pellet and keep the residual supernatant according to (Elasri and El amin Afilal, 2016). To determine COD mg/L, global nitrogen (NGL) mg/L, ammonium (NH_4) mg/L, total phosphorus (TP) mg/L of the supernatant, we used kits (LCK) with own ranges for each analysis, (LCK kit, range of measurement 100–2000 mg/L), (LCK kit, measuring range 5- 40 mg/L), (LCK kit, measuring range 2–47 mg/L), (LCK kit, measuring range 2–20 mg/L) respectively. And for the determination of total Kjeldahl nitrogen (TKN) mg/L we used the following formula (TKN = global nitrogen NGL – (nitrites NO_2 + nitrates NO_3).

Table 2. Characteristics of the sewage sludge without and with pretreatment

Parameter	Sewage sludge without pretreatment	Sewage sludge with pretreatment
pH	8.17	7.93
Temperature °C	28.16	28.32
ALC	17500	18300
VFA	960	964
TS (g/kg) (total solids)	25.81	31.43
SM (g/kg)	10.64	12.65
VS (g/kg) (volatile solids)	15.16	18.78
VS (TS)%	58.81	59.75
COD mg/L	2500	–
Global nitrogen (NGL) mg/L	776	–
Total Kjeldahl nitrogen (TKN) mg/L	771	–
Ammonium (NH_4) mg/L	854	–
Total phosphorus (TP) mg/L	24.4	–

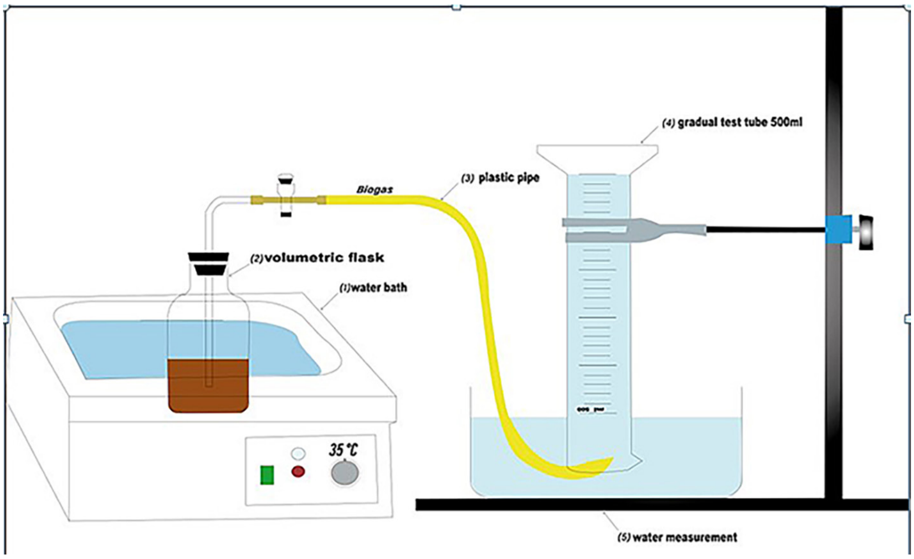


Figure 1. Schematic of the batch experimental setup

Biodegradability

At standard temperature and pressure (STP); the biogas volume was standardized. The amount of VS added (NmL/gVS) was used to calculate the potential of biogas produced by the sewage sludge under experimental conditions (Eq. 1) (Vannarath and Thalla, 2022).

$$TMP \text{ (biogas)} \frac{Nml}{gVS} = \frac{\text{P biogas } (Nml)}{\text{added VS}(gVS)} \quad (1)$$

The percentage of biodegradability was determined by dividing the difference between the initial volatile solid and the final volatile solid of the substrate over the final VS of the substrate, as given in (Eq. 2) below:

$$(\%) = \frac{(Initial\ VS - Final\ VS)}{Final\ VS\ of\ the\ substrate} \times 100 \quad (2)$$

Kinetic model of anaerobic digestion

Four kinetic equations were utilized in this study to fit the experimental data to the batch biogas production kinetic model and estimate the amount of biogas produced: Modified Gompertz model, which presupposes that the rate of methanogen bacterial development determines how much biogas is produced. The second model the Logistic function demonstrates a linear relationship between the biogas production rate and biomass concentration (Vannarath and Thalla, 2022). The third model Transference function describes the relationship between the output signal and the input signal of a control system for all possible input values. Finally, the first order model is used to progressively scale up experimental biogas production volumes for low substrate concentrations. Equations 3–6 were used to characterize the four models, according to panigrahi et al; vannarth et al (Panigrahi et al., 2020; Vannarath and Thalla, 2022).

- Modified Gompertz:

$$P(t) = A \cdot exp(-exp(\mu e\ A(\lambda\text{-}t)+1)) \quad (3)$$

- Logistic function**:**

$$P(t) = \frac{A}{1 + exp(\frac{4\mu}{A}(\lambda - t) + 2)} \quad (4)$$

- Transference function:

$$P(t) = A \cdot \left[1 - exp\left(\frac{\mu * (\lambda - t)}{A}\right)\right] \quad (5)$$

- First order:

$$P(t) = A \cdot (1 - exp(-K \cdot t)) \quad (6)$$

where: P is the cumulative biogas production at time (NmL/gVS added),
A is the maximum cumulative biogas (NmL/gVS added),
μ is the biogas production rate of (NmL/gVS.d),
e is a mathematical constant,

λ is the lag phase time (d),
K is the specific kinetic constant of methane production.

RESULTS AND DISCUSSION

Impact of growth media of methanogenic bacteria on cumulative biogas production

Figure 2 shows the cumulative biogas curve during 30 days of anaerobic digestion of untreated and pretreated sludge with different concentrations of growth medium. As shown in Figure 2, the biogas yield that was found to be the highest among the different pretreated sludge samples equals to 900 Nml/gVS added, it is obtained with medium concentration pretreatment of growth medium, the lowest biogas yield is obtained in the case of high concentration pretreatment, with a yield of 10 NmL/gVS added. Slightly low biogas production is observed in the case of sludge without growth medium ((diamonds), Fig. 2) its cumulative biogas is 610 NmL/gVS added. However, the biogas production increased in the presence of low (triangles) and medium (circles) concentration of growth medium by observing that the cumulative biogas production in the case of medium concerted pretreatment is higher than at low concentration with cumulative biogas production of 750 NmL/gVS added, 900 NmL/gVS added respectively (Fig. 2). These results indicate that high concentration of growth medium inhibit biogas production due to the inactivation of the methanogenic phase. Inhibition of methanogenic activities is often caused by high concentration of growth medium, resulting in disruption of the anaerobic digestion process. This allows us to know the impact of high concentrations of growth media on methanogenic activities. In the case of methanogenic bacteria, the concentration of growth media should be taken into consideration, as the trace element requirements (iron, nickel, cobalt, and zinc) of methanogenic bacteria are lower than those of acidogenic bacteria, thus allowing for any limitations (Hendriks et al., 2018). Growth media are necessary with medium concentrations (Table 1) but with high concentrations, they can become inhibitory or even toxic. Methanogenic bacteria in anaerobic sludge are particularly susceptible to poisoning by trace elements. The results obtained affirm that the increased activity of methanogenesis, which is a serious methane production pathway for the activity of methanogenic bacteria, is one of the reasons for the improvement of biogas production under the condition of an average concentration of the growth media of methanogenic bacteria.

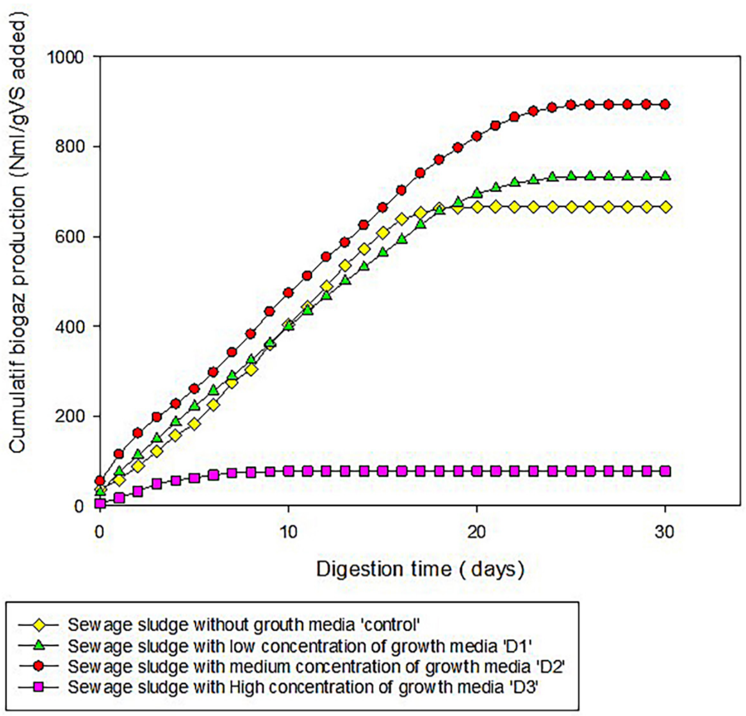


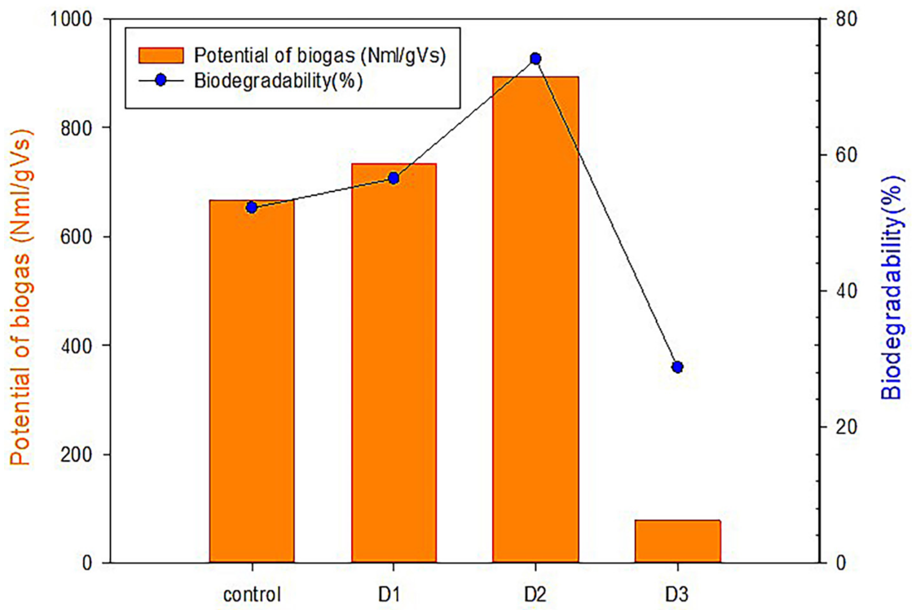
Figure 2. Cumulative biogas production over the 30-day digestion period for different growth media concentrations

Biodegradability

The biodegradability (Bd) in the case of medium concentration of growth medium D2 reaches a value of 74.04%, a higher percentage than that of the biodegradability of sludge without growth medium (control) which is 52.16%. In contrast to the low concentration of the growth medium (D1) the biodegradability does not exceed 56.5% and remains approximately the same as that of the control, with a difference of 2% (Fig. 3). Pretreatment with a high concentration of growth medium (D3) had no effect on the increase in biodegradability; its value is 28.70%. The failure of biodegradability in the high-concentration pretreatment was a consequence of inhibition by high concentrations of oligo- elements. Furthermore, as shown, biodegradability and biogas potential are similar in the sense that biogas production increases with the biodegradability.

Kinetic results

The use of growth kinetics was for the purpose of characterizing biogas production in a bioreactor, there are different kinetic models that work with different predictive methodologies and depending on the selected model, kinetic variables such as maximum biogas production rate and the lag phase may vary. The experimental data from the cumulative biogas tests as well as the results of the kinetic analysis obtained from these kinetic models were compared. The Microsoft (MS) Excel "Solver" tool was used to fit the model to the experimental data. In order to verify the adequacy of the kinetics models, the measured cumulative biogas as a function of digestion time was evaluated with the maximum cumulative biogas predicted by these four kinetic models. For each of the four models used in this study, Table 3. shows the kinetic parameters included in the anaerobic digestion process. To have the perfect model fit for the anaerobic digestion process, several approaches were conducted to fit the kinetic models to the experimental data. The results show that all models fit well (R^2 = 0.99) for pretreatment with a medium concentration of growth medium. At the same time, the gap between the daily values of produced and theoretical biogas, although, narrows as R^2 grows. The biogas production rate "μ" (Figure 4.) of the sewage sludge treated with (low and medium growth medium concentrations) and untreated sewage sludge was higher than



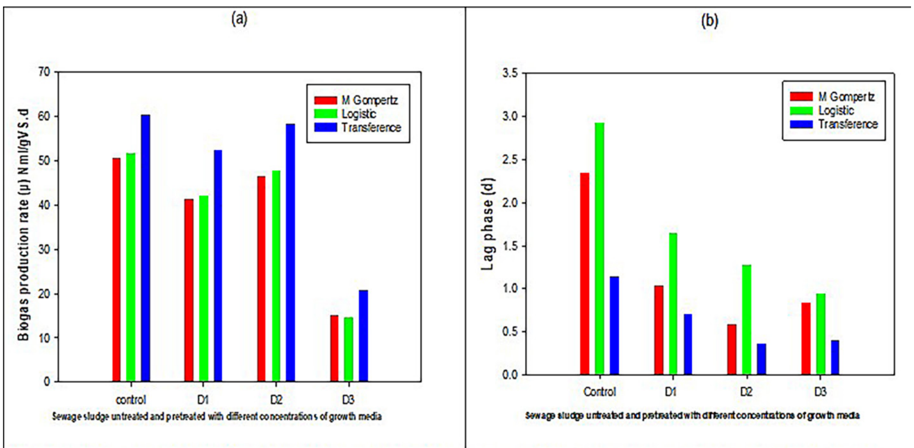
Control: Sewage sludge without growth media
D1: Sewage sludge with low concentration of growth media
D2: Sewage sludge with medium concentration of growth media
D3: Sewage sludge with high concentration of growth media

Figure 3. Curve of potential for biogas production and biodegradability of sewage sludge depending on various concentrations of the growth media

Table 3. Kinetic parameters used in several biogas testing models

Models	Parameters	Control	Low concentration of growth media	Medium concentration of growth media	High concentration of growth media
M Gompertz	P biogas (Nml/gVS)	689.54	756.30	923.09	77.58
	A(Nml/gVS)	697.60	790.25	982.41	77.58
	μ (Nml/gVS.d)	50.64	41.41	46.40	15.14
	λ (d)	2.35	1.04	0.59	0.84
	R^2	0.9927	0.9964	0.9961	0.9985
	Error (%)	3.57	3.19	3.37	0.10
Logistic	P biogas(Nml/gVS)	676.65	741.09	905.40	77.20
	A (Nml/gVS)	677.94	750.30	919.19	77.20
	μ (Nml/gVS.d)	51.74	42.28	47.83	14.72
	λ (d)	2.93	1.65	1.28	0.94
	R^2	0.9968	0.9943	0.9947	0.9943
	Error (%)	1.63	1.18	1.39	0.38
Transference	P biogas(Nml/gVS)	724.339	785.91	955.91	70.97
	A (Nml/gVS)	825.712	1002.8	1301.8	79.00
	μ (Nml/gVS.d)	60.40	52.42	58.24	20.87
	λ (d)	1.15	0.71	0.37	0.41
	R^2	0.9645	0.9892	0.9905	0.9748
	Error (%)	8.79	7.23	7.04	1.89
First order	P biogas(Nml/gVS)	733.530	792.59	960.13	79.28
	A (Nml/gVS)	895.162	1083.3	1364.4	79.35
	R^2	0.9563	0.9880	0.9902	0.9717
	K0	0.05	0.04	0.04	0.237
	Error (%)	10.177	8.14	7.51	2.30

that of the sewage sludge treated with high growth medium concentration. Noting that the transference function, the biogas production rate, was higher than that of Modified Gompertz. The values of the lag phase 'λ' for the Modified Gompertz (0.59–2.35) and the logistic function (0.64–2.93) and the transference function (0.37–1.15) (Figure 4.) fit well with the experimental data. In general, according to (Vannarath and Thalla, 2022) the values of λ range from 0–2.54 days represent the short adaptation period. This suggests that methanogens can adapt more quickly and produce biogas. For the first-order model, the value of k for treated (low and medium concentrations of growth medium) and untreated sewage sludge was shown to be 0.04–0.05/day, which is identical to the range of 0.038–0.063/day found in other study (Vannarath and Thalla, 2022). On the other hand, in the case of high-concentration pretreatment, the value of k was not found in the range was 0.2/day. The analyzes revealed that the value of k decreases as the severity of pretreatment increases. Errors of less than 10% difference between the experimental and fitted results indicate that the



Control: Sewage sludge without growth media ; **D1:** Sewage sludge with low concentration of growth media **D2:** Sewage sludge with medium concentration of growth media ; **D3:** Sewage sludge with high concentration of growth media

Figure 4. Effect of growth media on kinetics parameters for three models; (a) μ: biogas production rate; (b) lag phase

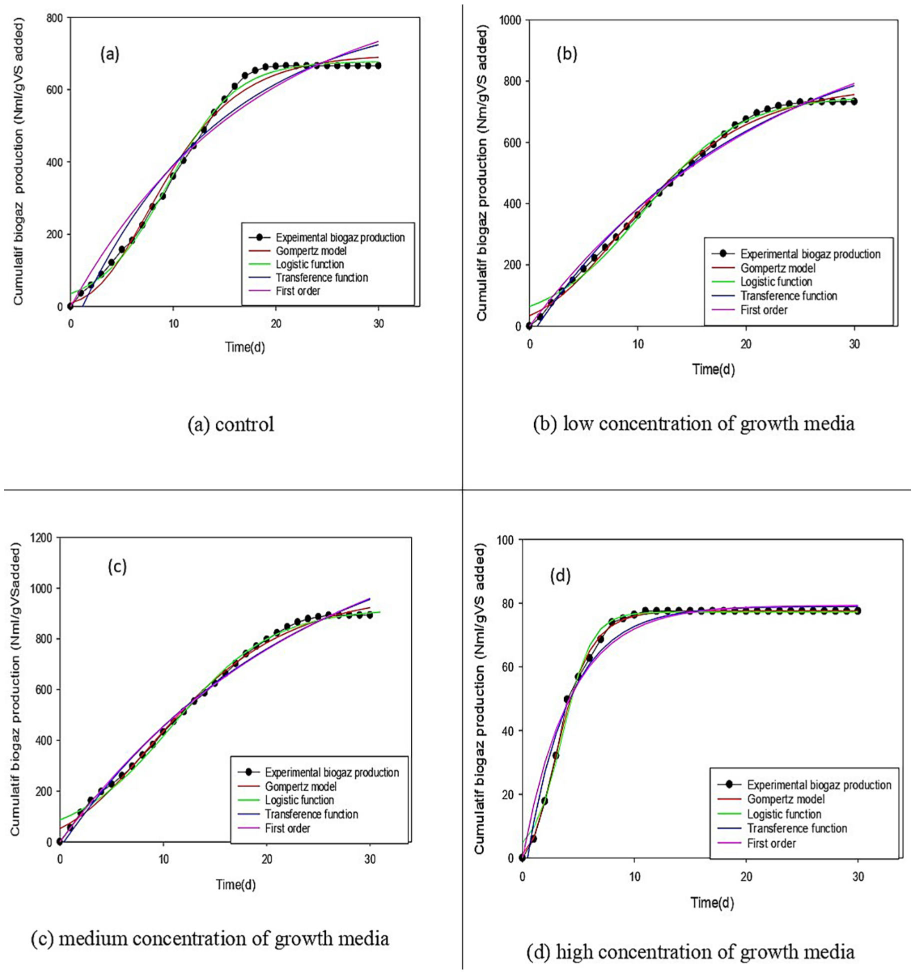


Figure 5. The fitting curves of Adjustment of Cumulated Biogas Production in Relation to Time for Models Adopted on Biogas Trials with Different Pretreatments "(a) control (b) low concentration of growth media (c) medium concentration of growth media (d) high concentration of growth media"

fitted models can be used to predict biogas potential. Figure 5. shows the fit curve of cumulative biogas production versus time (day) for the models that were used on the biogas tests of different pretreatments. As shown in Figure 5, the logistic function and Modified Gompertz are the best-fitting kinetic models that are closest to the experiment.

CONCLUSIONS

To evaluate the biogas potential, anaerobic digestion in batch mode of the sewage sludge without and with pretreatment with the growth medium was performed at 35°C. Increased biogas production and high biodegradability were observed after treatment of the sewage sludge with the methanogenic bacteria growth medium. The growth medium used had an average concentration and biodegradability of 74.04%, which led to the best increase in biogas production of 900 NmL/gVS. Therefore, pretreatment with average growth media concentration increased the biogas potential throughout the 30-day digestion period. The Logistic function and Modified Gompertz models fit the experimental results better than the first-order and transference function models. Because the calculated values were close enough to the actual values to reflect them, both of these models (Logistic function and Modified Gompertz) are applicable to all pretreatments with constituents comparable to sewage sludge, which affect bioreactor performance. The current work may be the best method for improving biogas production in sewage sludge treatment facilities, as it provides an effective biological pretreatment for the growth of methanogenic bacteria, which increases the efficiency of biogas production while providing real economic benefits.

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