

Ultrasound assisted alkaline pre-treatment of sugarcane filter mud for performance enhancement in biogas production

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Abstract: Sugar cane filter mud pre-treated by combined ultrasound-alkaline pre-treatment at different NaOH loading rates (0.25%-6%) and pre-treatment time (5 min to 60 min) performed in batch reactors under mesophilic conditions ((37±1)°C). Central composite design (CCD) was used to assess the effects on the chemical oxygen demand (COD) solubilisation, volatile fatty acid (VFA) concentration and methane yield. All pre-treatments were found significant ($p < 0.01$) to enhance methane yield (up to 39.49%) compared to the untreated filter mud. In general, results demonstrated the effectiveness of ultrasound assisted alkaline pre-treatment while increasing both NaOH loadings and pre-treatment time. Pre-treatment also increased the CODs solubilisation as well as VFA concentration, the optimal Initial CODs and VFA of 12 212 mg/L and 5830.97 mg/L, as determined by response surface methodology were noted on E9. Finally, modified Gompertz model was applied successfully to study kinetic of biogas production data, and showed an excellent fit.

Keywords: anaerobic digestion, ultrasound-alkaline pre-treatment, biogas production, filter mud, enzymatic hydrolysis

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1 Introduction

The significant increase in energy utilization especially in rapidly developing countries has generated interest in the development of alternate fuels. One of the concepts used for recovering the energy is the biological degradation of organic wastes by anaerobic digestion for the generation of methane as a substitute for fossil fuels^[1]. The Sugarcane Industry is responsible for production of different types of organic wastes. Cane straw left in the field after harvesting, bagasse, and filter mud, bagasse and a small share of straw have already been used as solid fuel in cogeneration systems in general. Meanwhile, the filter mud has been completely unused from the energy point of view^[2].

About 3–4 kg of filter mud per 100 kg of sugarcane crushed is left behind as by-product from filtration of the cane juice^[3]. The

residual mud is a soft, semi-solid, dark brown to black colored material, composed of inorganic soil particles, residual sugars and small pieces of sugarcane bagasse. It contains 60%–85% moisture, fiber lignocellulose, crude protein, sugar, wax, fat and ash^[4,5]. Usually, the filter mud spread on the field as fertilizer or sold as immature compost to farmers. However, this practice is not economically suitable and pollute the environment^[6]. According to the characteristics mentioned, anaerobic digestion is a promising strategy to making use of filter mud for the production of biogas. Early studies have been report on the anaerobic digestion of filter mud^[7]. Some researchers have also been made to produce the biogas from filter mud by mixing it with bagasse and other wastes^[8,9].

As filter mud contains a considerable amount of fibre, applying different pre-treatment is expected to be useful in making the substrate particles utilizable to microorganisms. However, numerous pre-treatment methods based on the enzymatic hydrolysis of lignocelluloses materials including physical, physicochemical, biological as well as chemical methods have been develop. Only a few studies have assessed the effects of different pre-treatment methods on methane production from filter mud^[10–12]. To our knowledge, pre-treatment of filter mud by ultrasound assisted alkaline for biogas production in patch reactor has not been studied yet.

Alkaline, usually employing NaOH, is known to break bond linkages between lignin in the lignocelluloses material, causing cellulose swelling which results in greater accessibility of the cellulose fraction^[9]. At the same time, the sodium concentration of filter mud was found to be 8.15 mg/L which is very low than

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recommended values^[13], the use of NaOH for substrate pre-treatment could overcome these possible drawbacks. Ultrasound produces a rapid cycling of pressure. During rarefaction phase of sonication, the pressure differentials within a solution result in the formation and growth of cavitation microbubbles in the liquid being sonicated. Bubble collapse is leading to the disruption of cell wall which increases the usability of biomass^[14]. Studies concerning a proper combination of ultrasound and alkaline pre-treatments applied to other biomass such as agriculture waste^[15], newspaper as a lignocelluloses biomass^[16] and sugarcane bagasse^[17].

Therefore, in this study the effect of ultrasound-alkaline pre-treatment was investigated in batch experiments to enhance the anaerobic digestion of filter mud regarding methane yield, using a central composite design (CCD) statistical experimental design. The effects of the NaOH loading and pre-treatment time on chemical oxygen demand solubilisation CODs and volatile fatty acids (VFAs) production were determined for different experimental conditions.

2 Materials and methods

2.1 Substrate and inoculum

Sugarcane filter mud obtained from a White Nile Sugar Company factory (WNSC) in the White Nile state, Sudan during the 2015/2016 season, dried and transported to China in sealed plastic bags and stored at 4°C until its use. The inoculum collected from an anaerobic digestion plant 'Kaiping Family Farm, Poukou, Nanjing, China' uses pig waste as substrate and adapted during two weeks to filter mud. The inoculum used in the tests contained 2.01% total solids (TS); 47.90% volatile solids (VS) and pH 7.76.

2.2 Analytical methods

All analyses were duplicated, and the results given are mean values. TS, VS, ashes, total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), and chemical oxygen demand (COD) were determined according to the procedures in standard methods^[18]. The pH was monitored in samples using digital pH meter (FE20K, Mettler-Toledo, Switzerland) capable of measurement in liquid substrates. Samples for analysis chemical oxygen demand solubilisation (CODs) and VFA were centrifuged at 10 000 r/min for 4 min in a centrifuge. After centrifugation, only the supernatant was used. VFA was determined by gas chromatography (GC-2014, Shimadzu, Japan), having a column of (DA, 30 m × 0.53 mm × 1 μm Stabilwax) and flame ionization detector (FID), while injector and detector temperature was 150°C and 240°C respectively. The CH₄ content measurement was conducted through biogas sampling from reactors by a special syringe and injection to the thermal conductivity detector (TCD) of a gas chromatograph (Agilent 7820A) equipped with PQ 80-100 Mesh column. The operation condition was: 25 mL/min He as the carrier gas, detector temperature 250 °C and 90 °C of column temperature. The % CODs removal was calculated as a percentage between initial and final CODs.

2.3 Pre-treatment conditions

The pre-treatment process was conducted on a 1 L glass flask. Filter mud (48 g TS) was pre-soaked in a NaOH solution at room temperature for 15 min, solid to liquid ratio was 1:8 and treated with ultrasound by using a probe sonicate (HC-SHL-800, Zhejiang, China). The operating frequency and power were 28 kHz and 440 W, respectively. The ultrasonic irradiation was transferred through a titanium cylindrical horn 2 cm diameter, introduced into

the flask through the side neck and submerged 1.0 cm into the mixture. The temperature was controlled using a water bath. Different conditions of NaOH loading (0.25%, 1.09%, 3.13%, 5.16% and 6%) and pre-treatment time (5 min, 13.05 min, 32.5 min, 51.95 min and 60 min) were used according to a CCD setup in Table 1 (empty cells not tested in the CCD). After pre-treatment, the pre-treated filter mud slurry was neutralized with hydrochloric acid and immediately used for subsequent biochemical methane potential test.

Table 1 CCD setup

Time/min	NaOH loading/g NaOH (100 g TS) ⁻¹				
	0.25	1.09	3.13	5.16	6
5			E4		
13.05		E2		E7	
32.5	E1		E5		E9
51.95		E3		E8	
60			E6		
Total runs	2	4	10	4	2

Note: Duplicated experiments are indicated.

2.4 CCD and evaluation by response surface methodology (RSM)

The Central composite design of response surface methodology was used to evaluate the optimum response regions of CODs and to optimize the corresponding variables. The CCD for two factors utilized in the study, presented in Table 1. A total of 22 experimental runs including eight tests for factorial points, eight tests for axial points, also, six center points were included along the experimental blocks to provide orthogonality and to estimate the experimental error^[19]. The effect of two pre-treatment operating variables NaOH concentration (x_1) and pre-treatment time (x_2) on the two response variables, COD solubilisation, and VFA was determined. The response variable was fitted using a second-order quadratic equation to correlate the response variable to the independent variables^[20].

$$y = b_0 + \sum_{i=1}^k b_i x_i + \sum_{i=1}^k b_{ii} x_i^2 + \sum_{i=1}^k \sum_{j=1}^k b_{ij} x_i x_j + e \quad (1)$$

where, y is the measured response; b_0 is the free or offset term, b_i is the first-order (linear) main effect; b_{ii} is the quadratic (squared) effect; b_{ij} is the interaction effect; x_i and x_j are levels (codes values) of independent variables; e is random error.

2.5 Anaerobic digesters

The anaerobic digestion experiments for measuring methane potentials from untreated and pre-treated filter mud were carried out in 1 L bottles used as batch reactors, sealed with two holes rubber stoppers. The total working volume was 800 mL, with 6% TS concentrations. The bottles were filled in with the slurry of treated filter mud sample about 400 mL and complete the volume to 800 mL with the inoculum, which met an inoculum to substrate ratio of about 1:1 in volume basis. One of the holes in the rubber stopper was used to withdrawal sludge sample to analyze the process parameters during anaerobic digestion, and the second hole was connected with the water bottle through a pipe having a port for taking gas samples for GC analysis. Biogas production was measured by water displacement technique which the water was saturated with a brine solution that was prepared with 10% NaCl (w/v) to minimize the dissolution of produced gasses from the reactor. The pH of the substrate after ultrasound-alkaline pre-treatments was found to be higher than 10. It was adjusted one time before starting fermentation in 7.0-8.0 by using HCl.

Biogas composition and total biogas production were measured on a daily basis while pH, VFA and COD were done after each three days. Reactors were kept at $(37 \pm 1)^\circ\text{C}$ in a water bath and shaking manually once daily.

2.6 Kinetic evaluation (Modified Gompertz model)

In this study, modified Gompertz model were applied to determine the biogas production potential of the substrate, maximum biogas production rate and the lag phase of the reaction with available experimental results. Gompertz model has been identified as a good empirical non-linear regression model commonly applied in the simulation of CH_4 accumulation, because of its good enough precise prediction for different substrates. The equation is given by:

$$G(t) = G_0 \cdot \exp \left\{ -\exp \left[\frac{R_{\max} \cdot e}{G_0} (\lambda - t) + 1 \right] \right\} \quad (2)$$

where, G_0 is the potential methane production, mL/g VS ; R_{\max} is maximum methane production rate, mL/(g VS d) ; λ is the lag-phase, d; $G(t)$ is the accumulated bio-methane at the time t , mL/g VS ; t is measured time, d and e , $\exp(1)=2.718282$. The model's parameters were determined using the curve fit function in MATLAB R2016a (9.0.0.341360) software, which solves nonlinear curve fitting problems using the least squares method.

2.7 Statistical analyses

All the experiments were conducted by a set of conditions provided through Deign Expert v7. Two-way analysis of variance (ANOVA) was used to test significant differences in mean values for the response variables CODs, VFA and $G(t)$. Furthermore, the data sets were analyzed, and graphical representation was provided by using Analytical Software package (Graph Pad, Prism 6.01.) with a confidence interval of 95%.

3 Results and discussion

3.1 Characteristics of the substrate

Filter mud used as a substrate for biogas production in this study was analyzed for TS, VS, and TOC, pH, TN and sodium contents (Table 2). The moisture content for new sample varied from 54.9% to 58.6% (averaging 56.7%). Dry filter mud contained TS and VS values (95.90% and 45.33% of TS). Depends on the cane variety, soil conditions, and nutrients applied in the field, the process of clarification adopted by WNSC and other environmental factors, filter mud contains various micro-nutrients such as nitrogen and phosphorous (3.09 g/kg and 0.87%), respectively. The organic matter equal to 239.53 g/kg. TOC content was higher in comparing to nitrogen, which leads to C/N ratio of 44.95 greater than the optimal value recommended for anaerobic digestion (20-40)^[21]. The pH of filter mud slurry before pre-treatment was 5.77.

Table 2 Composition of untreated sugarcane filter mud

Parameters	Filter mud
pH	5.77
Total Solids (TS) %	95.90±0.01
Volatile Matter (VS) %	45.33±0.02
Organic matter /g·kg ⁻¹	239.53 ±3.09
Total Organic Carbon (TOC) /g·kg ⁻¹	138.90±1.81
Total-N /g·kg ⁻¹	3.09±0.02
Na /mg·L ⁻¹	8.15±0.08
Total-P %	0.87 ±0.05
C/N Ratio	44.95

Note: Each value represents mean ±STDEV of two replications.

3.2 Effects of pre-treatment on anaerobic digestion

Different parameters for the anaerobic digestion process of filter mud were tested to study the effects of pre-treatment (NaOH loadings and time of ultrasound irradiation) on CODs, VFAs and bio-methane production BMP. Experiments were conducted according to CCD as explained before. In general, results demonstrated the effectiveness of ultrasound assisted alkaline pre-treatment while increasing both NaOH loadings and pre-treatment time (Table 3).

Table 3 Effects of pre-treatment on VFAs, COD solubilisation and accumulated BMP

	BMP /mL (g VS) ⁻¹	Initial CODs /mg L ⁻¹	Initial VAF /mg L ⁻¹	COD removal /%
Untreated	81.54±1.26	5,100±03.18	N.D.*	69.88
E1	127.64±6.11	6,152±1.31	4109.61±340.56	85.11
E2	127.94±7.71	6,912±45.25	4155.76±234.28	87.62
E3	116.01±9.96	6,232±79.19	3909.63±175.99	83.12
E4	116.90±2.03	6,336±84.85	4361.93±437.32	80.87
E5	128.39±8.47	7,648±97.19	4582.78±488.98	79.65
E6	123.70±0.39	9,260±84.85	3782.51±348.94	81.08
E7	111.35±2.13	8,480±135.76	3883.47±110.18	87.69
E8	123.65±0.13	12,076±28.28	3816.28±193.42	85.43
E9	134.77±3.33	12,212±96.16	4052.42±22.79	84.34

Note: *N.D.: not detected. Data expressed as mean ± standard deviation.

3.2.1 COD solubilization and removal

The behavior for CODs values was examined deeply during the anaerobic digestion process after each three days interval, as shown in Figure 1. In all cases, values for CODs had shown increasing pattern than the CODs for untreated filter mud, due to the effect of pre-treatment in changing the composition of hardly degradable compounds and increase the biodegradable CODs. At the beginning of the experiments, the filter mud was hydrolysed with high strength adapted inoculum, this hydrolysed substrate further acidified and converted into CH_4 and CO_2 with the help of methanogens. The initial COD values ranged between 6152 mg/L and 11 576 mg/L, in comparison with 5100 mg/L for the control. The highest initial COD solubilisation reached in the current work was obtained for reactor E9, resulting in an increase of 58.24% with respect to untreated filter mud. Lowest solubilisation were found with E1, E2, E3 and E4 reactors. the initial COD values were 6152 mg/L, 6912 mg/L, 6232 mg/L and 6336 mg/L, respectively, which is in accordance with previous studies conducted by Jank et al.^[12], who found that, the lower NaOH concentration did not show any improvement COD release from filter mud. Generally speaking, the level of COD concentration started to decrease since the first day of the fermentation process as shown in Figure 1.

Futhermore, the COD removal efficiency of all pre-treatments and control presented in Table 3. As the results-oriented, the % of COD removal in all the treatments increased at different rates, the highest COD removal of 87.69% was observed for E7 reactors while the least was 69.88% for untreated filter mud.

Higher levels of delignification (80%) have been reported at a NaOH concentration of 1 N, paper loading of 0.5% (w/v), sonication power of 100 W^[16]. During the combination of alkaline and ultrasonic pre-treatment of sewage sludge, the COD solubilisation was increased from 1200 mg/L to 11 000 mg/L after such treatment^[22]. The ratio of soluble COD to total COD was increased from 47% in raw leachate to 63% after 45 min sonication at 600 W/L^[23]. Cellulose content was also increased by the

combination of alkaline and ultrasonic pre-treatment of bagasse at 1% NaOH with ultrasound 100% duty cycle^[15].

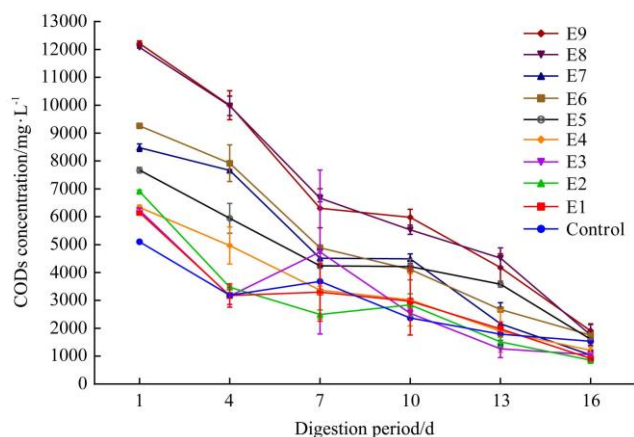


Figure 1 COD concentration profile at different time periods of anaerobic digestion process

The CCD of RSM was used to evaluate the effect of ultrasound time and NaOH loading on initial CODs (Figure 2). The mathematical regression for initial CODs as a function of NaOH loading (A) and sonication time (B) was obtained as follows:

$$\text{CODs} = 7648 + 1997.77 \times A + 881.40 \times B + 1069 \times A \times B + 750.75 \times A^2 + 58.75 \times B^2 \quad (3)$$

The ANOVA of the quadratic equations for CODs was used to determine the adequacy and significance (Table not shown), which indicated that the interactive model term is insignificant ($p > 0.05$). The main first-order effects of the independent variables A (NaOH loading) and B (ultrasound time) had a positive effect on initial CODs, with factor A exhibiting the highest effect on the response and more significant than their respective quadratic effects.

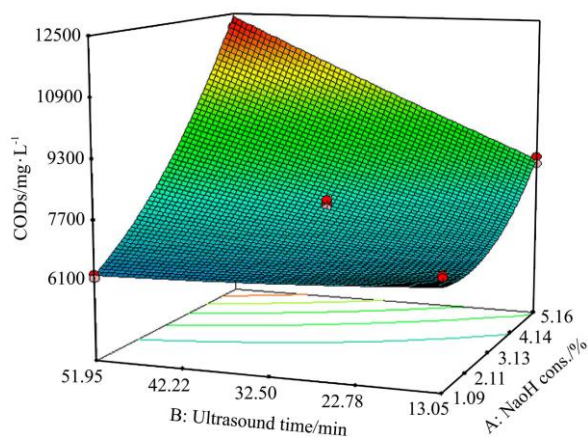


Figure 2 Response surface plot for the effect of NaOH loading (A) and ultrasound time (B)

RSM was made to optimize the process parameters for maximizing the CODs. Point optimization for CODs was attempted with Design Expert version 7.0, which predicted the highest yields of CODs equivalent to 14 072.3 mg/L after enzymatic hydrolysis of the filter mud pre-treated with 6% of NaOH in the presence of ultrasound for 49.19 min.

The correlation coefficient (R^2) value determines the quality of the quadratic model. The value of R^2 is always between 0 and 1. It is known that the R^2 value greater than 0.75 indicates the accuracy of the model. The R^2 and the adjusted correlation coefficient (R^2 adj.) for the model were 0.9913 and 0.9884 respectively, which indicated that only less than 1% of the total

variation remained unexplained by the quadratic model.

3.2.2 VFA concentration

VFAs are important mid-products in the production of methane, and their concentrations affect in methanogenic population, pH fluctuation, and anaerobic digestion efficiency. To address these effects, acetic acid, propionic acid, butyric acid, iso-butyric acid, valeric acid and iso-valeric acid were intensely observed at each three days interval of the whole digestion period. Figure 3 presented the VFAs concentration level. In the first ten days of the digestion, all reactors showed the highest VFAs production. Maximum VFA was recorded for reactor E9 on the 10th day of digestion that was 5830.97 mg/L while least TVFAs, 300.96 mg/L belonged to the E3 reactor as showed in Figure 3, these VFAs degraded and consumed by methanogens to produce more than 81% of the total methane generated during whole digestion period. In the first week of digestion, acetic acid production was higher than the other acids, but on the 10th day of digestion, propionic acid concentration was found increasing rapidly, which affect negatively in the anaerobic digestion process, and the total methane yield consequently became very low (<5 mL). Another study showed that, when the propionic acid concentration was increased to 900 mg/L, significant inhibition effect on methanogenic bacteria growth appeared, these effects resulted in the accumulation of ethanol and VFAs, and the total methane yield consequently became very low^[24].

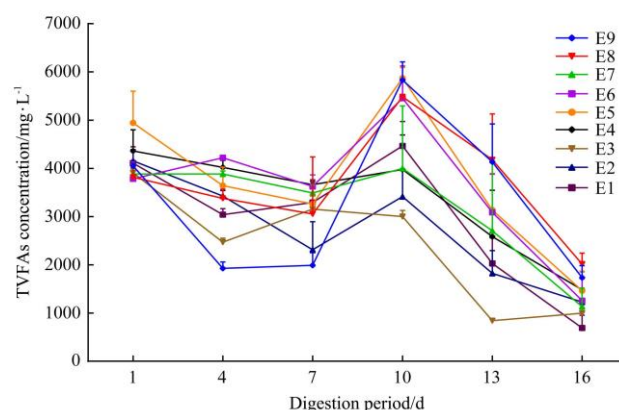


Figure 3 VFAs production by filter mud during aerobic digestion period

3.2.3 Biogas and methane production

All pre-treatments of filter mud used in the present study had significantly increased methane production ($p < 0.01$) as compared with the untreated filter mud, daily methane production and cumulative methane yield were shown in Figure 4, respectively. Methane yield increased by 39.49% from untreated filter mud for pre-treatments E9, followed by E5 with 36.49% methane enhancing capability. There is a significant difference between the results obtained by the highest pre-treatments (E5 and E9) compared to the untreated reactor. The best pre-treatment is, therefore, E5, because fewer reagents as well as less pre-treatment time are required. Methane production by the remainder other pre-treated reactors found significant to enhance the methane, but not significant when compared with each other (Table 3).

To our knowledge, combined ultrasound-alkaline pre-treatment of filter mud has not yet been reported. Ultrasound alone pre-treatment has been extensively studied to increase biogas yield from waste activated sludge^[25]. Recently, several studies have been conducted on the use of ultrasound to pretreat lignocellulosic biomass and other wastes for biogas production; it was shown

improvement in the accumulated methane production over untreated biomass^[23,26,27]. Combined ultrasound-alkaline pre-treatment has however been applied to others substrates with positive results, Wang et al.^[28] observed that ultrasound assist low concentration alkaline pre-treatment led to a 68% to 77% increase in biogas yield of rice stalks as compared with untreated stalks.

3.3 Kinetic study using Gompertz model

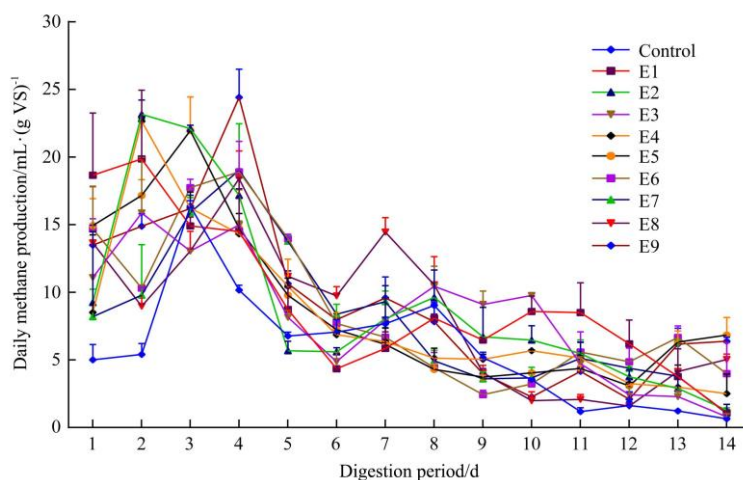
Biogas production from anaerobic digestion of treated and untreated filter mud was modeled based on modified Gompertz equation. The results of a kinetic study for an experiment using the modified Gompertz model were given in Table 4, which indicated that the modified Gompertz equation can be used to

predict biogas yield potential, maximum daily biogas production, and duration of lag phase. To evaluate the model, the predicted cumulative biogas values were plotted against the measured values, as shown in Figure 4b. The lag phase's λ was calculated to be in between 0.07 d and 1.37 d, because of the adapted inoculum added, and the active biodegradation component available in the pre-treated filter mud. The $RMSE$ value fell within the range of 2.086-5.458 and the R^2 value fell within the range of 0.9798-0.9946. The difference between the predicted and measured methane yields was in the range of 0.67%-10.86%. Thus, the modified Gompertz model was found to have the better fit to the experimental data for all reactors used in this study.

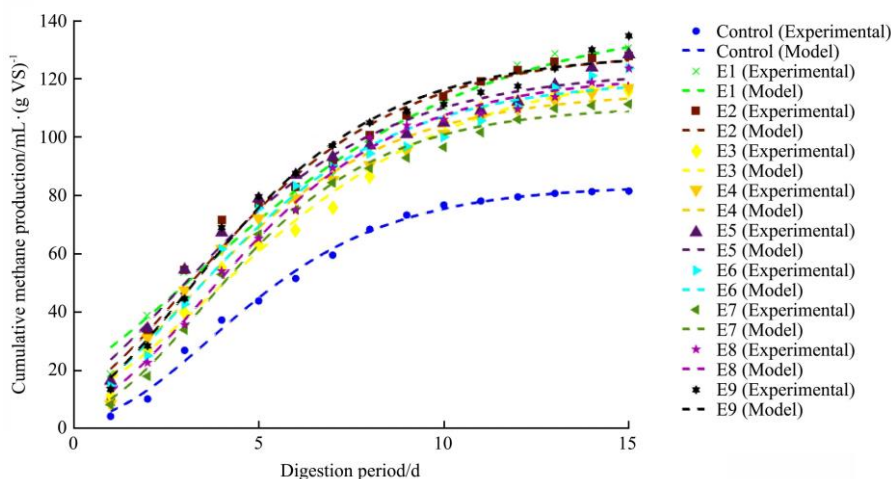
Table 4 Results from using a modified Gompertz model

	λ	R_{max}	Measure methane yield /mL (g VS) ⁻¹	Predicted methane yield /mL (g VS) ⁻¹	Difference between measured and predicted/%	R^2	$RMSE$
Untreated	0.93	11.13	81.54±1.26	83.50	2.40	0.9945	2.08
E1	1.37	11.30	127.64±6.11	141.50	10.86	0.9845	4.69
E2	0.26	14.56	127.94±7.71	129.70	1.37	0.9808	5.46
E3	0.18	11.77	116.01±9.96	124.80	7.58	0.9909	3.57
E4	0.20	13.55	116.90±2.03	115.70	1.03	0.9851	4.31
E5	0.66	13.59	128.39±8.47	123.00	4.20	0.9798	5.04
E6	0.11	13.86	123.70±0.39	119.90	3.07	0.9822	4.92
E7	0.65	14.71	111.35±2.13	110.60	0.67	0.9938	2.92
E8	0.43	14.49	123.65±0.13	121.40	1.82	0.9946	2.87
E9	0.07	15.68	134.77±3.33	128.70	4.50	0.9864	4.7

Note: Abbreviations used explained before.



a. Daily methane production of the filter mud by different pre-treatments



b. Cumulative biogas production – experimental and modified Gompertz model

Figure 4 Daily methane production and cumulative methane yield

4 Conclusions

Filter mud was found to be a potential substrate for biogas production. The combined alkaline and ultrasonic treatment, with different NaOH loading and pre-treatment times, were applied to enhance the anaerobic biodegradability of filter mud. Parameters like methane production, VFA, and CODs were monitored during digestion. 39.49% increase in methane yield from untreated filter mud was obtained. Pre-treatment increased the COD solubilisation as well as VFA concentrations. Hence, it was possible to increase methane production through the combination of ultrasound and alkaline pre-treatment followed by subsequent enzymatic hydrolysis.

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