Process optimization and effect of thermal, alkaline, H2O2 Oxidation and combination pre-treatment of sewage sludge on solubilisation and anaerobic digestion

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Abstract

Background

This study investigated the feasibility of enhancing anaerobic digestion of sewage sludge with triple, dual, and individual pre-treatment of waste activated sludge with heat, alkalinity, and hydrogen peroxide. These pre-treatments disrupt sludge flocs, organisms' cell walls, extracellular polymeric substance, and intracellular organic matter, which increase biodegradability and hydrolysis rate of organic matter. In addition, the influence of various variables on methane production was analyzed using the response surface methodology with the quadratic model. Eventually, an optimized temperature and chemical concentration for the highest methane production and lowest chemical usage is suggested.

Results

The highest amount of methane production was obtained from the sludge pretreated with triple pretreatment (heat (90°C), alkalinity (pH=12), and hydrogen peroxide (30 mg H2O2 /g TS)), which had better performance with 96% higher than that of the control sample with Temperature of 25°C approximately and alkalinity of pH=8. Response surface methodology with the quadratic model was also used for analyzing the influence of temperature, pH, and hydrogen peroxide concentration on anaerobic digestion efficiency. It was revealed that the optimized temperature, pH, and hydrogen peroxide concentration for maximizing methane production and solubilisation of organic matter and minimizing thermal energy and chemical additives of the pre-treatments are 83.2°C, pH=10.6 and 34.8 mg H2O2 /g TS, respectively, has the desirability of 0.67.

Conclusion

This study reveals that triple pre-treatment of waste activated sludge performs better than dual and individual pre-treatment, Respectively. The enhanced methane production can be used as an important renewable energy resource in wastewater treatment plants for producing electrical and thermal energy. Furthermore, exploiting a higher amount of methane in the anaerobic digestion stage decreases methane emission to the atmosphere in dewatering and landfilling stages and enhances the quality of digested sludge, bringing about environmentally friendly and economically attractive sewage sludge treatment process.

Background

Energy supply is one of the most important challenges in today's world. Nowadays, the most important source of energy production, especially in developing countries, is fossil fuels due to their easy availability and low cost. However, fossil fuels have many problems, including environmental problems and non-renewability. Therefore, the need for renewable and clean energy is rapidly increasing [1]. On the other hand, contamination caused by human activity has created numerous problems for the environment such as climate change, ozone depletion, plant and aquatic species extinction, and water contamination [2]. One way to address these issues is the use of wastewater treatment plants, in which wastewater is treated prior to being released into water streams. An important challenge associated with wastewater treatment plants is the management of sludge produced from the treatment process. This is important because sludge treatment units are the most cost-intensive parts of wastewater treatment plants, accounting for up to 60% of their total operating costs [3–5].

As a practical solution for simultaneous renewable energy production and contamination removal from the sludge treatment process, anaerobic digestion of sewage sludge has been used widely in wastewater treatment plants [6]. Anaerobic digestion of sewage sludge includes four predominant reactions, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolysis reaction plays an important role in converting organic matter to readily biodegradable organic matter for organisms' consumption, in which high molecular weight compounds such as protein, carbohydrates, and lipids are converted to a soluble phase. However, this stage is often restricted because of poor biodegradability of organic matter especially in anaerobic digestion of waste activated sludge [7]. To address this issue, recent researches have focused on different methods of enhancing the hydrolysis rate of anaerobic digestion of waste activated sludge with the thermal, chemical, and mechanical treatment of the sludge prior to anaerobic digestion [8–12]. These pre-treatments disrupt sludge flocs, organisms' cell walls, extracellular polymeric substance (EPS), intracellular organic matter, and bipolar cations, which increase biodegradability and hydrolysis rate of organic matter [13, 14]. According to recent studies, thermal pretreatment at a low temperature, alkaline pretreatment, and hydrogen peroxide pretreatment of sewage sludge have been considered as high-performance pretreatments in enhancing anaerobic digestion [10, 15].

Alkaline pretreatment is an effective method known in pretreatment methods, which can lead to the solubility of lignin and the types of neutralized acids produced by lignocellulos complexes. In addition, the presence of a small amount of remaining alkali after pre-treatment will help to stabilize pH during the acidogenesis process [16].

Heat pretreatment is initially used to improve sludge dewaterability by destroying the gel structure, which can destroy cell walls to release organic chemicals into the soluble phase, resulting in higher biodegradation and lower sludge viscosity[17]. Thermal pre-treatment is divided into two cases: low temperature (lower than 100 °C) and high temperature (higher than 100 °C). The pre-treatment method, in which a lower temperature is used, is considered more economically attractive and environmentally friendly.

Hydrogen peroxide (H_2O_2) has been successfully used for disintegration of anaerobic biomass [18]; this is due to the production of free radicals such as $NO•_2$ and HO• that disrupt cell walls, proteins, membrane phospholipids and EPS [19–21]. Interestingly, it has been revealed that H_2O_2 could be produced in situ from wastewater through a bio-electrochemical system [22]. Furthermore, Zhang et al. [23] reveal that H_2O_2 as a potential substance for combined pre-treatments can increase methane production from waste activated sludge by 23%.

This is the first study to analyze and optimize combined thermal, alkaline, and hydrogen peroxide pre-treatment of waste activated sludge for enhancing anaerobic digestion of sewage sludge. In addition, the influence of various variables on methane production was analyzed using the response surface methodology (RSM) with the quadratic model. Eventually, an optimized temperature and chemical concentration for the highest methane production and lowest chemical usage is suggested.

Results

Source and characterization of sludge

Primary sludge, activated sludge, and inoculum were collected from South Tehran's wastewater treatment plant. This plant is the largest wastewater treatment plant in the Middle East with the capacity of 450,000 m^3/day to receive and process a sewage flow. Primary sludge from gravitational sedimentation, activated sludge from a belt thickener, and inoculum from mesophilic anaerobic digesters were collected for subsequent use. Primary and waste activated sludge were maintained at 4 °C and inoculum at 37 °C before being used in the experiments. Table 1 lists the main characteristics of primary sludge, waste activated sludge, and inoculum.

Table 1
Characteristics of sludge used in the experiment

Parameters	PS	WAS	Inoculum
TCOD (gL^{-1})	35.94 1.6	63.51 3.5	37.00 0.8
sCOD (gL^{-1})	5.25 0.2	4.81 0.1	4.23 0.1
TS (gL^{-1})	27.91 1.4	44.20 1.6	29.06 1.4
VS (gL^{-1})	20.12 0.4	35.41 1.4	17.40 0.3
TSS (gL^{-1})	23.82 1.1	39.30 1.9	22.14 0.9
VSS (gL^{-1})	17.76 0.9	30.11 1.3	14.58 0.2
pH	6.36 0.1	6.52 0.3	7.33 0.2

TCOD: total chemical oxygen demand, sCOD: soluble chemical oxygen demand, TS: total solid,

VS: volatile solids, TSS: total suspended solids, VSS: volatile suspended solids

Experimental design

RSM was used to evaluate the independent, interactive, and quadratic input parameters (temperature, pH, and hydrogen peroxide concentration). The I-optimal method was also used for finding optimum points for the highest methane production and organic matter solubilisation. In mixture experiments, the proportions of the components of a mixture are the studied factors. The special nature of the factors makes special kinds of regression models necessary and special kinds of experimental designs. Although mixture experiments usually are to foresee the response(s) for all potential formulations of the mixture and to recognize optimal amounts for each of the ingredients, little research has been done regarding their I-optimal design. That I-optimal designs lower the average variance of prediction is so surprising and, consequently, looks more suitable for mixture experiments than the commonly used D-optimal designs, focusing on an exact model estimation rather than exact predictions[24].

Considering the results of previous studies [4, 25, 26] and the effectiveness of the pre-tests performed in this study, the proposed input range, as shown in Table 2, was applied. For statistical analysis, variable levels were normalized to three levels low (0), medium (1), and high (2).

Table 2
Levels and code of variables

Variables	Symbols	Range and value of variables		
		0 (Low)	1 (Medium)	2 (High)
Temperature (°C)	A	25	75	90
Alkalinity (pH)	B	8	10	12
H_2O_2 Concentration (mg/ g TS)	C	0	30	60

Table 3: Experimental design with response surface methodology and the results

No.	Input Variables			Results						
				Methane Production		Microbial				
	Temp. (°C)	pH	H_2O_2 concentration (mg/ g TS)	Cumulative MP (mL/ g VS)	Increase MP (%)	COD Solubilization (%)	Increase of sCOD (g/L)	Decrease of VSS (g/L)	Increase of Protein (g/L)	Increase of Polysaccharide (g/L)
1	25	8	0	314	0	3.65	2.32	1.95	0.52	0.01
2	25	8	30	373	18.79	8.09	5.14	5.11	0.95	0.03
3	25	8	60	358	14.01	9.05	5.75	4.25	1.35	0.04
4	25	10	30	410	30.57	18.67	11.86	10.61	2.16	0.27
5	25	10	60	426	35.67	18.97	12.05	12.24	2.53	0.22
6	25	12	0	444	41.4	14.56	9.25	8.67	1.66	0.17
7	25	12	60	471	50	22.59	14.35	13.88	2.94	0.18
8	75	8	30	402	28.02	16.72	10.62	10.98	1.68	0.22
9	75	8	30	415	32.17	16.34	10.38	11.21	1.84	0.2
10	75	10	30	480	52.67	21.67	13.76	14.35	2.87	0.38
11	75	10	30	469	49.36	21.82	13.86	14.51	2.46	0.34
12	75	10	30	488	55.41	21.56	13.69	14.2	2.81	0.28
13	75	10	60	506	61.15	23.43	14.88	15.72	2.97	0.33
14	75	10	60	502	59.87	23.51	14.93	15.99	3.02	0.23
15	75	12	0	492	56.69	17.86	11.34	12.01	2.39	0.17
16	90	8	0	418	33.12	15.24	9.68	8.08	2.14	0.18
17	90	8	60	524	66.88	22.17	14.08	15.64	2.85	0.31
18	90	10	0	479	52.55	21.1	13.4	14.55	2.74	0.36
19	90	12	30	615	95.86	30.37	19.29	19.47	3.81	0.52
20	90	12	30	621	97.77	30.17	19.16	19.71	3.91	0.59

Design-Expert® software (version 11.0.5.0) was used for statistical analysis and to find the optimal answer. The three considered factors and their interactions were analyzed using the ANOVA table.

Pretreatment effect on organic matter solubilisation

Table 3 shows the effects of different pretreatments on the solubility of organic matter in waste activated sludge. A significant increase in COD solubility in all treated samples was observed compared to the controls, varying from 3.65–30.37%. The highest increase in sCOD was due to the triple combination of A2 + B2 + C1 (19th test) pretreatment, which increased the sCOD to 19.29 g/L. Similar results are recorded in previous research [25, 27]. On the other hand, VSS variations are closely correlated with the sCOD. The highest VSS reduction, which was equal to 19.71 g/L, was also obtained from the combined pretreatment of A2 + B2 + C1. According to the ANOVA table for COD solubility, which is provided in the supplementary file, a second-order model with respect to $R^2 > 0.98$ and p-value = 0.0003, which is smaller than the acceptable value of 0.05 has been suggested. According to the analysis and by eliminating the unwanted terms, the following equation is proposed for COD solubility:

COD solubilisation (%) = -67.011–0.166 x A + 13.481 x B + 0.210 x C

– 0.017 x A x B + 0.004 x A^2 – 0.509 x B^2 – 0.003 x C^2 (1)

According to the ANOVA table for VSS, each of the input variables had a significant effect on VSS solubility with $R^2 > 0.98$ and p-value < 0.05. Interaction effects of BxC and CxA were removed because of their inappropriate p-value (> 0.05). According to the statistical analyses performed by the RSM and by eliminating the unwanted terms, the following equation is proposed for the VSS solubility:

VSS Solubilisation (%) = -130.034–0.180 x A + 25.442 x B + 0.343 x C

– 0.023 x A x B + 0.006 x A^2 – 1.001 x B^2 – 0.005 x C^2 (2)

In this study, the amount of soluble protein and soluble polysaccharide were measured before and after the treatments. According to the data obtained, the amount of soluble protein and soluble polysaccharide increased significantly when the pre-treatments were applied to waste activated sludge. This enhancement was more considerable when a higher concentration of chemicals and higher temperature were used. The highest enhancement in soluble protein and soluble polysaccharide obtained from the bioreactor pre-treated with 20th test, in which they respectively reached to 3.91 g/L and 0.59 g/L. These values are considerably higher than those obtained from the control bioreactor with 0.52 g/L (soluble protein) and 0.01 g/L (soluble polysaccharide). The amount of soluble protein and soluble polysaccharide experienced a slight increase in the control bioreactor without any chemical addition or pH control, confirming a slight solubilisation in the control due to natural activity of the organisms. A similar enhancement was observed in previous studies [12, 28]. For protein changes, the quadratic model is proposed with $R^2 > 0.97$ and p-value = 0.0008, which is a very good value that represents the power of the model. The proposed model with the elimination of unwanted terminology is equal to:

Increase of Protein (g/L) = -8.071–0.042 x A + 1.728 x B + 0.012 x C

+ 0.002 x B x C + 0.001 x A^2 – 0.070 x B^2 – 0.0002 x C^2 (3)

Figure 1 shows the normal probability figures of COD and protein solubility. According to these forms, the less the dispersion of the existing data, the closer the results are to the normal line. In other words, when the R^2 value tends to 1, the proposed model is stronger. Contour and 3D curves for COD and protein solubility at various concentrations of 0, 30, and 60 mg H_2O_2/g TS are shown in Figs. 2 and 3. According to Fig. 2, COD solubility is predicted to increase up to 32% in combined pre-treatment of 60 mg H_2O_2/g TS, 90 °C, and pH = 12, while the highest solubility enhancement from experimental results was 30.4% (19th test). The highest predicted VSS solubility was 55% in combined pre-treatment of 60 mg H_2O_2/g TS, pH = 12, and 90 °C, while the highest amount obtained from experimental results was 50% obtained from the 20th test (30 mg H_2O_2/g TS, pH = 12, and 90 °C). The addition of hydrogen peroxide to pretreatment demonstrated its function in breaking down the cell walls, as well as the EPS, caused by the release of radicals (hydroxyl radicals, hydroperoxyl radicals), converting organic matter to soluble phase [14].

Daily biogas production

As shown in Fig. 4 and Fig. 5, the highest daily biogas production was obtained between third and fifth days through the bioreactors. During the first days of the digestion process, the amount of biogas production in the control bioreactor was higher than most of the pre-treated bioreactors. This can be either attributed to the presence of inhibitory factors of the pre-treatment or due to the high organic loading available to anaerobic organisms and excessive volatile fatty acid [29].

Cumulative methane production

The amount of methane production from different bioreactors was measured regularly during the anaerobic digestion process. For normalizing the data, the produced methane (mL) was divided by the added volatile solids (gram) to each bioreactor. The effects of different pretreatment types on the cumulative yield of biogas production are shown in Table 3. The amount of cumulative methane yield was considerably enhanced when combined pre-treatment methods were used. The highest enhancement in methane production (97.77%), compared to the control, was obtained when the combination of A2 + B2 + C1 pre-treatments was employed. This is 30.89% higher than the highest increase achieved from the bioreactors with dual treatments (A2 + C2), corroborating the effectiveness of triple pre-treatment compared to individual and dual pre-treatments. As in previous studies, the methane enhancement from the individual pre-treatment used in this study was between 10% and 30% [13, 26, 30]. In this study, the methane increase from individual pre-treatment was between 14% in C2 and 33% in A2. Methane enhancement from dual pre-treatments was between 28% and 66%, while in previous studies, it was around 20–70% [12, 25].

Analysing the results of cumulative biogas production in Table 3 using RSM, a quadratic model with $R^2 > 0.98$ and p-value < 0.0001 was suggested. By eliminating unacceptable terms and incorporating acceptable terms, the following equation was achieved:

Increase MP (%) = -39.897–1.798 x A + 8.408 x B + 0.678 x C

+ 0.005 x A x C + 0.0181 x A^2 – 0.006 x C^2 (4)

Figure 6 shows contour and 3D graphs related to the increase of cumulative methane production in different concentrations of hydrogen peroxide. According to these diagrams, the highest percentage of cumulative methane production is about 115%, which abstained from the bioreactors pre-treated with 90 °C, pH = 12, and 60 mg H_2O_2/g TS. It is important to note that the highest increase in cumulative biogas production, which was 98%, was observed in the 20th test.

Optimum pretreatment condition

A scenario was written for achieving the optimum condition, whereby temperature, pH, and H_2O_2 were minimized and the methane production, sCOD, soluble protein, and soluble polysaccharide were maximized. In the scenario, methane production was allocated the highest importance factor. The best-suggested desirability was 0.673. Table 4 shows the information pertaining to the optimization process. Another test with the suggested inputs was carried out for verifying the predicted phenomena in the model, in which the methane enhancement of 71% was achieved in the pre-treated bioreactor (Table 5). The difference achieved can be attributed to different sludge characterizations in the verification test. Despite the important results this study represents, applying new systems to anaerobic digestion of sewage sludge entails precise economic and feasibility assessments. Thus, in prospective studies, economic assessments for full-scale application of the pre-treatments are crucial, in addition to investigating possible side-effects of the pre-treatments on microbial communities and behavior in long-term exposure.

Table 4. Constraints of optimum condition

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Temperature	Minimize	75	90	1	1	4
B:pH	Minimize	8	12	1	1	4
C:H_2O_2 Concentration	Minimize	0	60	1	1	4
Cumulative MP	Maximize	314	621	1	1	5
Increase MP	Maximize	0	97.77	1	1	5
Increase of sCOD	Maximize	2.32	19.29	1	1	4
COD solubilisation	Maximize	3.65	30.37	1	1	4
Decrease of VSS	Maximize	1.95	19.71	1	1	3
VSS solubilisation	Maximize	4.96	50.15	1	1	3
Increase of Protein	Maximize	0.52	3.91	1	1	5
Increase of Polysaccharide	Maximize	0.01	0.59	1	1	2

Table 5
Predicted and actual observation data at the achieved optimum operated condition

Input Variables			Result															
Temp (°C)	pH	H_2O_2 Concentr-ation (mg/g TS)	Cumulative MP (mL/g VS)		Increase MP (%)		Increase of sCOD (g/g VS)		COD solubilize-tion (%)		Decrease of VSS (g/g VS)		VSS solubilisation (%)		Increase of Protein (g/L)		Increase of Polysac-charide (g/L)	
83.2	10.6	34.8	Pre	Act	Pre	Act	Pre	Act	Pre	Act	Pre	Act	Pre	Act	Pre	Act	Pre	Act
			542	529	72.6	71.1	17.1	17.9	26.9	28.2	17.9	18.1	45.4	45.7	3.35	3.55	0.46	0.42

Discussion

The main objective of this research was to investigate the effect of combined and individual thermal, alkaline, and hydrogen peroxide pre-treatment of waste activated sludge on anaerobic digestion efficiency.

The significant increase of COD solubility can be attributed to the breakdown of cellular and microbial walls and the release of organic materials such as polysaccharides and proteins to the soluble phase [31, 32]. All three input variables of temperature, pH, and H_2O_2 concentration individually affected the COD solubility significantly, whose p-values were less than < 0.0001. For determining the effects of interactions, the term AxB had a positive effect with p-value<0.05 and appropriate F-value, but the effect of CxB and CxA was insignificant with p-value>0.1 and the very low and inappropriate F-value, so they were deleted. For the second-order equation, A^2, B^2, and C^2 had significant effects (p-value<0.05). Also, according to ANOVA table, the model is very suitable because of the lack of fit p-value and fit F-value. Organic matter is generally divided into two parts: biodegradable organic matter, which is consumed by microbial community, and unbiodegradable organic matter, which is not decomposed by organisms. Protein and polysaccharide, the two readily biodegradable components of COD, account for around 60% of organisms constituents in sludge [3]. Therefore, enhancement of soluble protein and polysaccharide means that a higher amount of nutrients is available to the anaerobic organisms. The increase in amount of soluble protein was considerably higher than that of soluble polysaccharide after treatments can be attributed to a higher proportion of protein in organisms constituents (around 50%) [3]. Thus, the pretreatments disrupted cell walls and EPS in waste activated sludge due to their biocidal effect, which affects protein much more than polysaccharide. This agrees with previous studies, in which a similar trend is observed [12, 33]. All three input variables, with p-value<0.05, had a significant effect on the model, while the effect of the input parameters on each other will be ignored due to p-value > 0.05. Meanwhile, second-order semigroups of input parameters, due to p-value < 0.05 and high F-value, will have a positive effect on protein changes.

Considerably higher soluble chemical oxygen demand in pre-treatment reactors provided a higher amount of readily biodegradable organic matter for anaerobic organisms leading to an increase of biogas and methane production [12, 29]. The enhanced methane is of paramount importance because not only does it enhance renewable energy generation in wastewater treatment plants, but it also reduces methane emission to the atmosphere, as a major greenhouse gas emission [34]. According to ANOVA table, all three input variables significantly affect methane production, yet their interactions did not affect the methane production substantially. The second-order effects of the terms A^2 and C^2 had a particular effect on the increase of methane production with p-value < 0.05, while B^2 was eliminated from the model with p-value > 0.1.

Conclusion

This study investigated the possibility of enhancing anaerobic digestion of sewage sludge with different pre-treatments. It was revealed that the triple pre-treatment of waste activated sludge with heat, alkalinity, and hydrogen peroxide increases soluble fractions of organic matter considerably more than dual and individual pre-treatments. This led to significantly higher daily biogas and methane production from the anaerobic digestion, as a higher amount of biodegradable organic matter was available to the anaerobic microbial community. In addition, the effect of input variables and their interactions on methane production were analyzed with response surface methodology and optimized input variables were suggested in the end. The enhanced methane production can be used as an important renewable energy resource in wastewater treatment plants for producing electrical and thermal energy. Furthermore, harnessing a higher amount of methane in the anaerobic digestion stage decreases methane emission to the atmosphere in dewatering and landfilling stages and enhance the quality of digested sludge, bringing about environmentally friendly and economically attractive sewage sludge treatment process.

Methods

Pretreatment methods

The different types of pre-treatment methods such as thermal, alkaline and hydrogen peroxide pretreatments were applied individually and in combination with the experimental design shown in Table 3, in accordance with the previous studies as well as their performance. Thermal pretreatment was performed for 5 hours in a warm water bath that was set at two temperatures of 75 °C and 90 °C. For this purpose, 1-liter sealed glasses containing activated sludge were used. For alkaline pretreatment, since the pH of the primary activated sludge is about 7, 1N NaOH solution, which represents a high impact alkalinity, as well as a reasonable price, should be used to increase the pH of the activated sludge. Thus, by adding a sufficient amount of this solution to the activated sludge which was filled in 1-liter glasses, the pH of the containers will be adjusted to 8, 10 and 12. After adjusting the desired pH, each container will be placed on the shaker at 150 rpm for a 24 hour residence time to keep the sludge uniform over the retention time. In pre-treatment with hydrogen peroxide, values of 30 and 60 mg of H_2O_2/g TS were added to the activated sludge in the 1-liter glasses. Then, the glasses, which contain the specific amounts of hydrogen peroxide, were placed on a shaker at the 150 rpm for 24 hours to keep the sludge uniform over the retention time. Also, in combined pretreatments, the types of pretreatments are permitted to be performed in a specific order in accordance with what was done for the single pretreatment.

Anaerobic digestion

The designed biochemical methane potential test consisted of a 1-liter glass reactor containing 600 ml of mixed primary and waste activated sludge with a ratio of 70%:30%. The same ratio between primary and waste activated sludge is considered in the South Tehran wastewater treatment plant. Liquid displacement method was used for measuring the volume of biogas production [35]. Strict anaerobic conditions were provided for better organism performance. The reactors were kept in a mesophilic environment at 37 °C, using a hot water bath heated by automatic heaters. During anaerobic digestion, the mixture was stirred at 100 rpm using magnetic stirrers for retaining uniform temperature and nutrient distribution. All measurements were carried out in triplicate with standard error. Anaerobic digestion process continued for 30 days when biogas production was negligible. A control reactor without any pre-treatment being implemented was also considered for comparing various phenomena of anaerobic digestion.

Analytical method

Chemical oxygen demand (COD), total solid (TS), total suspended solid (TSS), volatile solid (VS), and volatile suspended solid (VSS) were measured according to standard methods for examinations of water and wastewater [36]. The COD and VSS solubility after pretreatment were obtained through the following equation:

$$\text{COD solubilisation} = \frac{sCOD_t - sCOD_0}{TCOD_0} \times 100 \quad (5)$$

$$\text{VSS solubilisation} = \frac{VSS_0 - VSS_t}{TSS_0} \times 100 \quad (6)$$

Where t represents the value after pretreatment and 0 indicates the initial state.

To separate soluble solids from suspensions, centrifuges were used at a speed of 10,000 rpm for 30 minutes, and filters with a porosity of 0.45 micron were used. Soluble proteins were analyzed with Folin Phenol in accordance with Lowry's study [37], and soluble polysaccharides were analyzed with phenol and sulfuric acid based on the Dubois study [38]. The percentage of methane was measured using gas chromatography (GC) with a Thermal conductivity detector (TCD) at 100 °C and an oven at 60 °C. For the tests, 1 mL of the samples were injected into the GC device.

Abbreviations

EPS
extracellular polymeric substance
RSM
response surface methodology
PS
primary sludge
WAS
waste active sludge
TCOD
total chemical oxygen demand
sCOD
soluble chemical oxygen demand
TS
total solid
VS
volatile solid
TSS
total suspended solid
VSS
volatile suspended solid
MP
methane production
GC
gas chromatography
TCD
thermal conductivity detector

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Data and materials of this study were included in this article and its supplementary files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

S.S., B.A., and S.M.H originated and planned the idea and experiments. S.S., and S.M.H. carried out the experiments. S.S. planned and carried out the simulations. B.A. and S.M.H guided S.S. for better design and probable drawbacks. S.S., S.M.H., and B.A. contributed to the interpretation of the results. All authors provided critical feedback and assisted form the research, analysis and manuscript.

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Figures

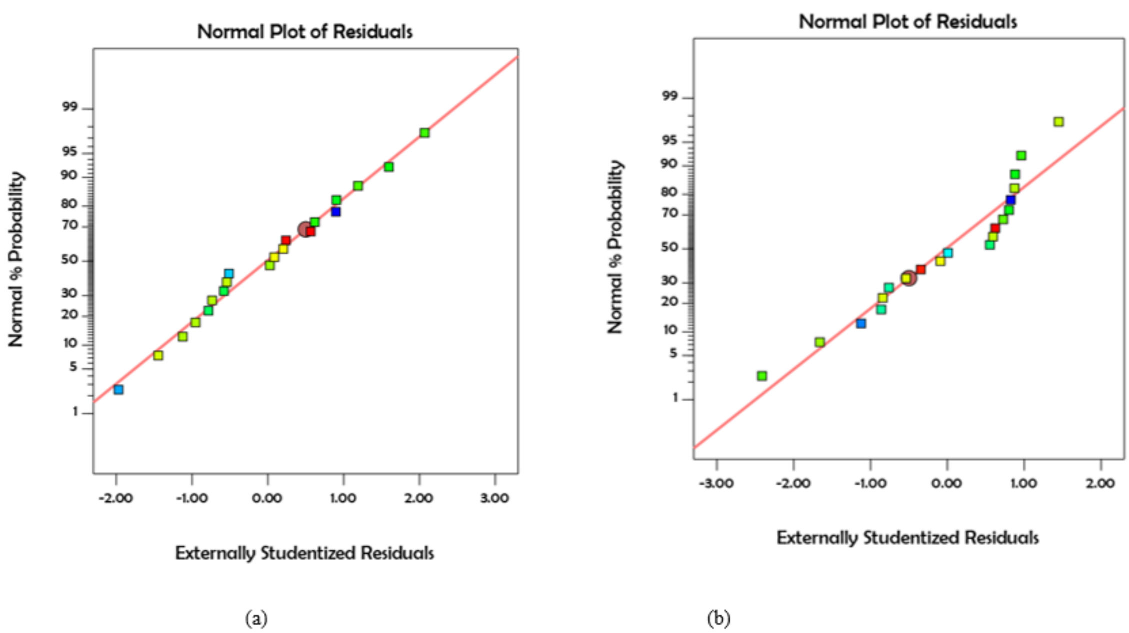

Figure 1

Normal plots of Residuals for (a) COD solubilisation (b) Increase of Protein

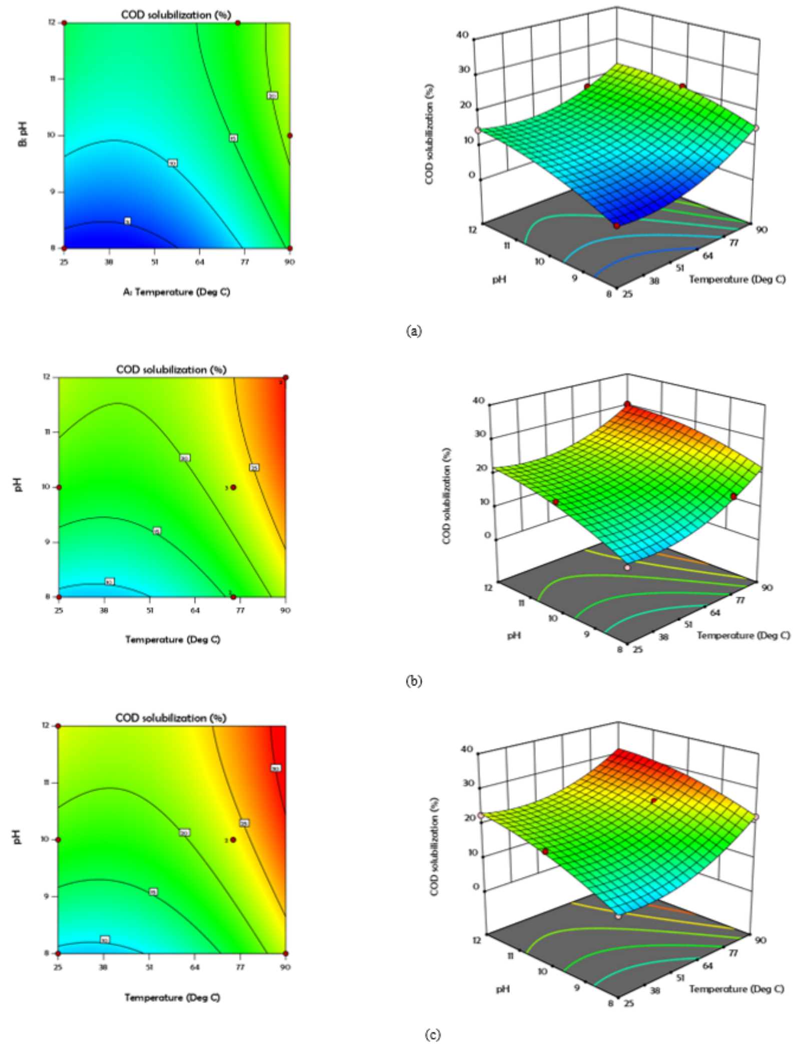

Figure 2

Contour and 3D plots for COD solubilisation at different concentration of H2O2 (a) 0 (b) 30 and (c) 60 mg/g TS

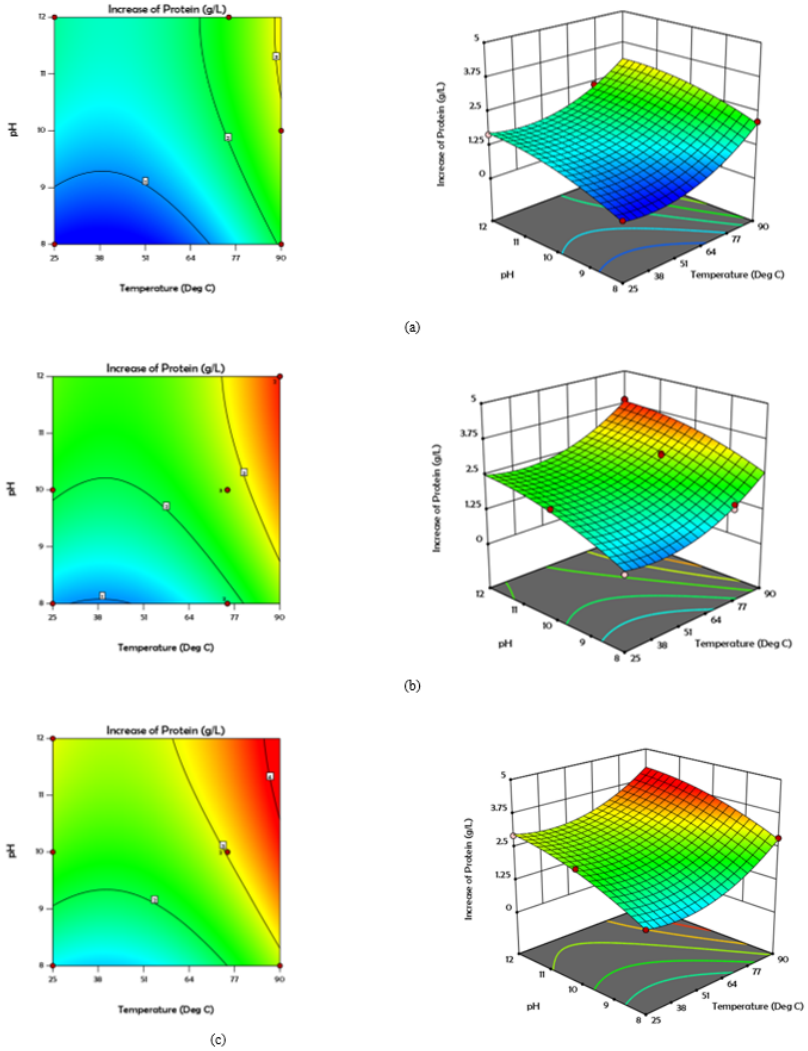

Figure 3

Contour and 3D plots for Increase of Protein at different concentration of H2O2 (a) 0 (b) 30 and (c) 60 mg/g TS

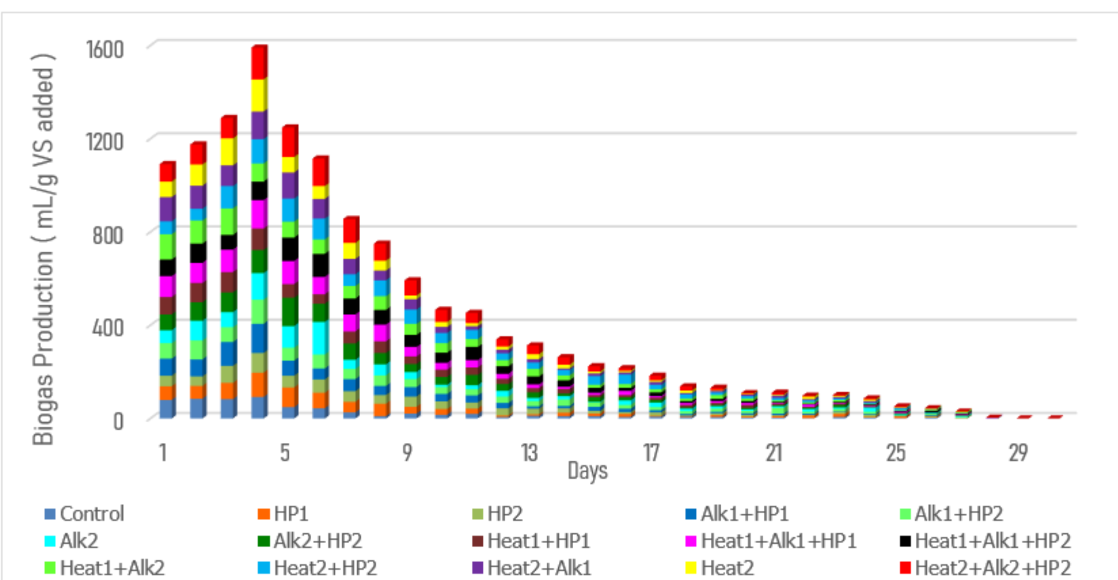

Figure 4

Biogas production divided by Days

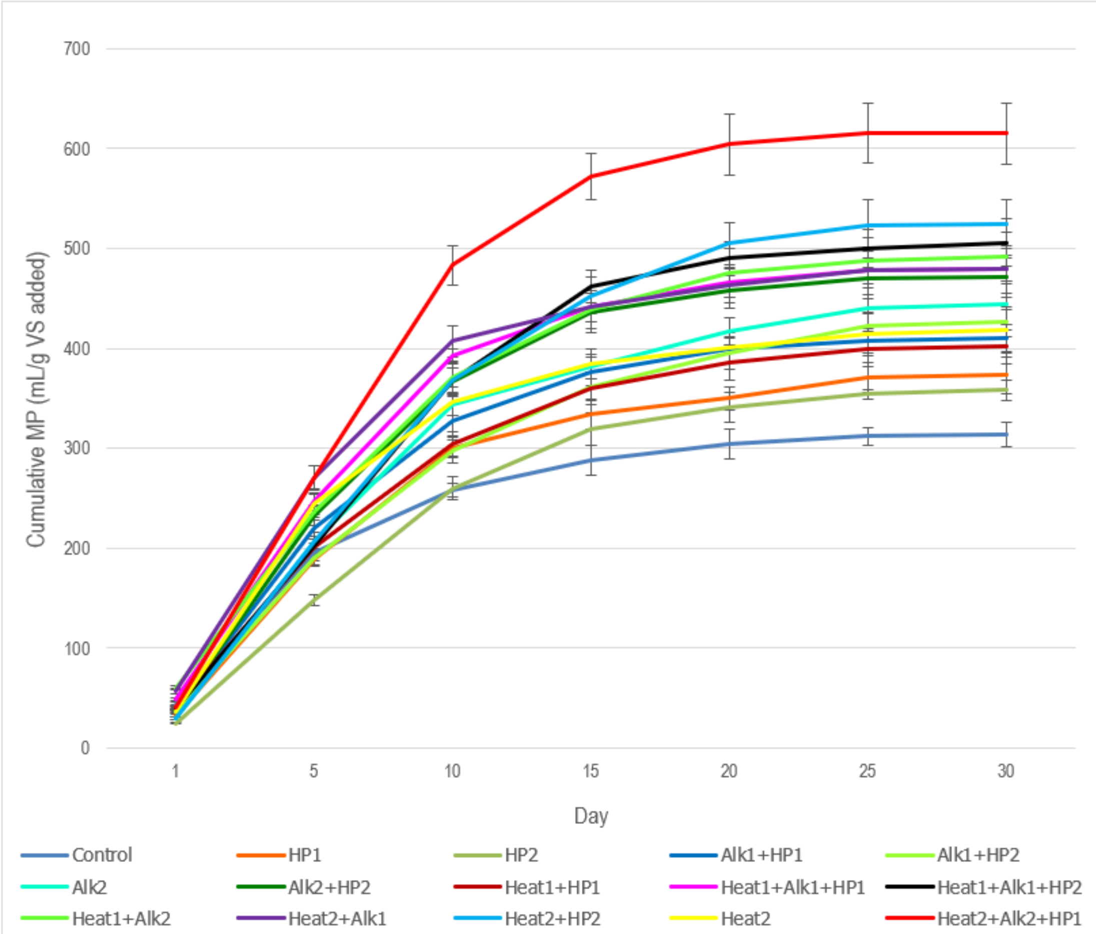

Figure 5

Effect of pretreatments on Cumulative methane production

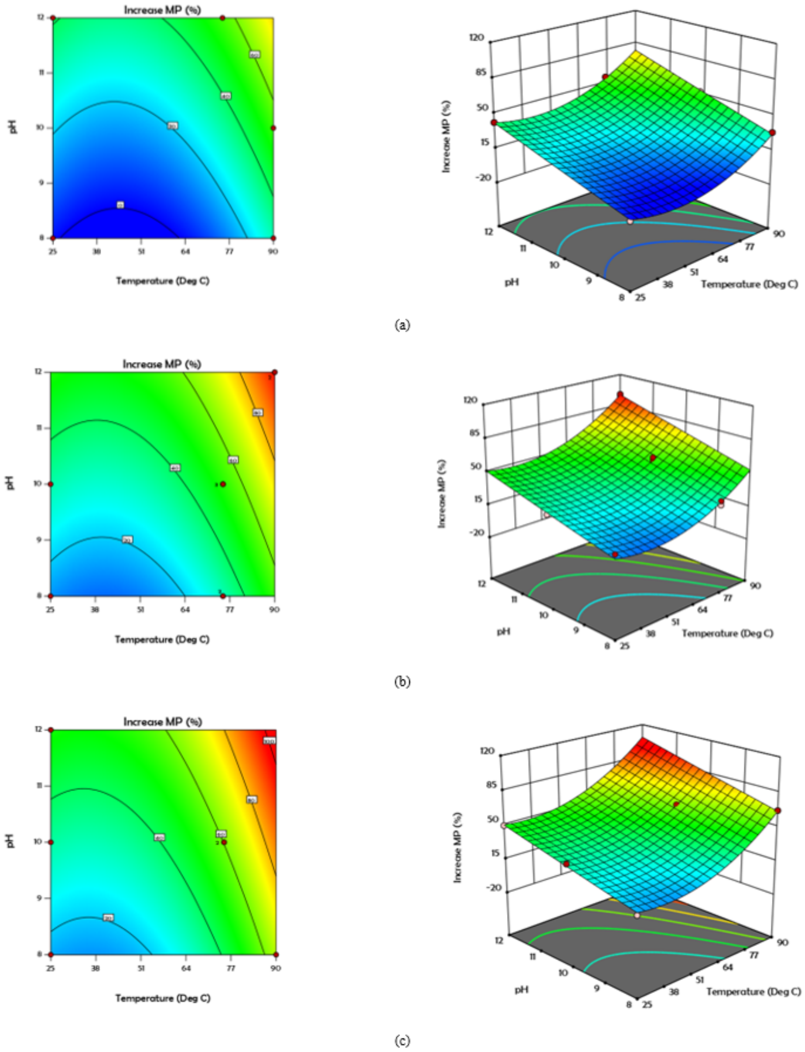

Figure 6

Contour and 3D plots for Increase MP at different concentration of H2O2 (a) 0 (b) 30 and (c) 60 mg/g TS

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Data.docx