An evaluation of lysozyme enzyme and thermal pretreatments on dairy sludge digestion and gas production

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ABSTRACT

Anaerobic digestion is one of the common methods of managing and stabilizing sludge. However, due to the limitations of the biological sludge hydrolysis stage, anaerobic decomposition is slow and requires a long time. This study evaluated the effects of thermal (80 °C) (TH-PRE) and a combination of thermal with the lysozyme enzyme (LTH-PRE) pretreatments on the enhancement of anaerobic activated sludge digestion. Response surface methodology was implemented to optimize enzyme pretreatment conditions (enzyme and mixed liquid suspended solids concentration). The results showed that both pretreatment methods increase soluble chemical oxygen demand (COD) and reduces total and volatile suspended solids (VSS), and phosphate concentration. The COD removal rate in LTH-PRE and TH-PRE was 95% and 81%, respectively. The value of VSS reduction in LTH-PRE and TH-PRE was 41% and 31%, more than the control operation, respectively. The biogas production in LTH-PRE and in TH-PRE also increased by 124% and 96%, respectively.

Key words | biogas, EPS, anaerobic digestion, lysozyme enzyme

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HIGHLIGHTS

- Applying the lysozyme enzyme along with thermal pretreatment has led to the destruction of cell wall and liberation of extracellular polymers into medium culture, resulting in a decrease in the volume of sludge.
- Utilizing lysozyme enzyme and thermal pretreatment due to lysis of Gram-negative bacteria underlying peptidoglycan membrane results in more disintegration of sludge and biodegradability promotion.
- It is feasible to promote biogas generation from sludge by conducting co-thermal-enzyme pretreatment.

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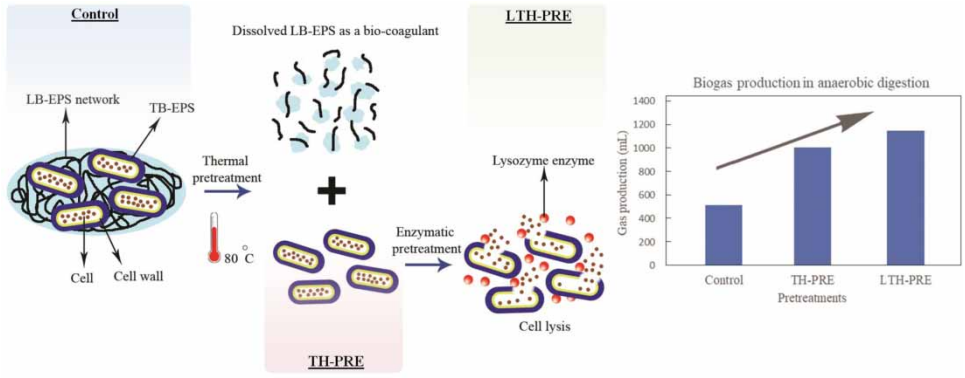
GRAPHICAL ABSTRACT



INTRODUCTION

Activated sludge process is widely used in wastewater treatment plants and, as a result of this process, a significant amount of excess sludge is produced as a byproduct (Christensen *et al.* 2015). Excess sludge treatment involves considerable costs, about 50–60% of the total operating cost of wastewater treatment plants (Campos *et al.* 2009). Nowadays, anaerobic digestion as an environmentally friendly technology is used to convert organic matters into biogas (El Achkar *et al.* 2018), but it has some limitations due to the difficulty of decomposability of the organic matters (Appels *et al.* 2008; Wang *et al.* 2013). The hydrolysis of organic matters is the speed limiting step of anaerobic digestion because the microorganisms participating in the hydrolysis stage have low performance in the decomposition of cellular cell wall components such as cellulose, hemicellulose, and lignin (Batstone *et al.* 2009; Carballa *et al.* 2011). Therefore, increasing the rate of sludge hydrolysis, if inhibitory substances are controlled, leads to an increase in the production of biogas. In order to speed up the hydrolyzing step various methods have been studied, including physical (Sapkaite *et al.* 2017), chemical (Bougrier *et al.* 2007), and biological treatments (Bonilla *et al.* 2018).

Biological treatment methods are always more favorable due to environmental compatibility aspects compared to other methods and have a high efficiency. Hydrolytic enzymes can accelerate the hydrolysis process and break down the cell wall, thereby reducing the time of hydrolysis (Liu, G. *et al.*, 2019). Lysozyme is an antimicrobial enzyme found in animals, plants, and microorganisms (Newman *et al.* 1974). Lysozyme digests bacterial cell walls by cleaving polysaccharide chains that give structural integrity to bacterial cell wall by breaking β-1,4-glycosidic bonds between N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) in the cell wall (Xin *et al.* 2016; Xin *et al.* 2018). It has been alleged that lysozyme could be used for destroying cell walls and releasing the organic intracellular substances into solution (Gill & Holley 2003; Xin *et al.* 2015). Yasunori (1994) reported that the removal of volatile suspended solids (VSS) in a concentrated excess sludge increases from 9.8% to 62% when excess sludge inoculates with slime bacteria that secrete lysozyme. Ogawa (2003) concluded that the average VSS removal of excess sludge improved 18.1%–38.1% with lysozyme enzyme compared with the control. Liu, G. *et al.* (2019) suggested that implementing the lysozyme dosage of 150 mg/g suspended solids within 240 min leads to release of 58.6 mg/L polysaccharide and 662.7 mg/L protein with 236.5 mg/L chemical oxygen demand (COD).

Hydrolyzing enzymes (often amylase, protease, and lysozyme) can be added to sludge as commercial chemicals or can be provided through bacterial strains that produce these enzymes (Xin *et al.* 2016; Xin *et al.* 2018). Since extracellular polymeric substances (EPS) form approximately 60–80% of the secondary sludge content, a large portion of the hydrolyzing enzymes are consumed to separate this layer (Ayol 2005; J-s & Xu 2011) and therefore the efficiency of cell wall disruption will decrease. Generally, the EPS structure consists of three layers: slime (S-EPS), loosely bound (LB-EPS) and tightly bound (TB-EPS) (Lin *et al.* 2014). Polymer substances with

different ratios are distributed in these three layers – the densest layer is TB-EPS and the most dispersed layer is S-EPS. Several studies have examined the effect of temperature on the structure of EPS and the overall result is that the S-EPS layer and the LB-EPS layer are separated from the sludge structure by increasing the temperature (below 80 °C) (Liu, R. *et al.*, 2019; Yang *et al.* 2019). Therefore, pretreatment at low temperature (50–100 °C) has been approved in several studies as an effective way to improve biogas production and organic matter decomposition (Climent *et al.* 2007). Thermal pretreatment disturbs the cell wall and makes organics such as protein and carbohydrate available for biological degradation (Prorot *et al.* 2011).

Gessesse *et al.* (2003) developed a chemical–biological method (ion exchange resin and its composition with the non-ionic detergent Triton X-100), which resulted in the weakening of the EPS structure and increasing the sludge enzymatic hydrolysis efficiency.

Although the effects of thermal and enzymatic pretreatments on sludge hydrolysis have been studied before, no study has yet combined these methods to improve anaerobic digestion of waste activated sludge. The main objective of this research is the development of a hybrid physical–biological approach to improve the sludge enzyme hydrolysis process as an anaerobic digestion pretreatment. First, thermal treatment (80 °C) was performed on the sludge to separate the S-EPS and LB-EPS layers from the floc structure. Then, an optimum dose of lysozyme was added to the sludge that had lost its EPS during the thermal pretreatment. Finally, the anaerobic digestion function fed with hydrolyzed sludge was studied in this research.

METHODS

Sludge pretreatments

The process of LB-EPS extraction by thermal pretreatment (TH-PRE) was such that the sample was heated at 80 °C for 60 min in a water bath and then centrifuged twice at 6,000 rpm for 15 min (Malamis & Andreadakis 2009). The supernatant was removed as the LB-EPS solution, and then distilled water was added to the pellet as much as the separated supernatant in order to create a slurry that could feed nicely into the digester. The protein and carbohydrate contents of the LB-EPS solution were measured.

In the lysozyme-thermal pretreatment (LTH-PRE) method, the lysozyme enzyme was added to the sludge that had lost its EPS with thermal pretreatment, and then the sludge sample was stirred for 10 min. To completely solubilize the enzyme with the sludge, the substrate was placed in an incubator at 28 °C for 3 h during which the sludge sample was stirred for 5 min each hour. The sludge sample was then removed from the incubator and again stirred for 10 min on the stirrer.

The sludge used in this experiment was taken from the sludge return line in the wastewater treatment plant of Gela dairy factory, Iran. The raw sludge characteristics were: COD 6,260 ± 107 mg/L, soluble chemical oxygen demand (SCOD) 206 ± 3 mg/L, total suspended solids (TSS) 5,025 ± 75 mg/L, VSS 3,237 ± 68 mg/L, VSS/TSS 0.64 ± 0.01, $P–PO_4$ 272 ± 4 g/L, total Kjeldahl nitrogen 1,353 ± 70 mg/L, and pH 7.0 ± 0.1. All samples were stored at 4 °C until used (Ennouri *et al.* 2016).

Optimization of LTH-PRE method by response surface methodology

The optimization experiments for LTH-PRE method were conducted for two numerical factors as shown in Table 1. The factors were lysozyme concentration and mixed liquid suspended solids (MLSS) of sludge. Sludge disintegration percentage was considered as the response and the related 12 set of experiments are shown in Table 2. The range of MLSS (between 5,000 and 10,000 mg/L) was determined according to the typical range of MLSS concentration in return line of activated sludge (Scheible *et al.* 1993).

In order to determine the main effects of important factors and interactions in terms of statistics and effectiveness on sludge degradation efficiency, the analysis of variance, second-order polynomial model, and 2FI model were used. The quality of fitness in the polynomial model was expressed by the correlation coefficient (R^2). The optimization process was done using Design-Expert software, version 7.0.0 for mathematical model and statistical analysis.

Pilot setup and operation

In the laboratory-scale study, the experimental setup consisted of a complete mixed digester, which was made up of polyvinyl

Table 1 | Factors level for the process of optimization based on the historical data method

Variable	Unit				
Lysozyme enzyme concentration	mg/L	100	2,550	3,040	5,000
MLSS	mg/L	5,000	7,500	8,000	10,000

Table 2 | Experimental data for the optimization process

No.	Lysozyme enzyme concentration (mg/L)	MLSS (mg/L)
1	2,550	7,500
2	2,550	7,500
3	3,040	7,500
4	2,550	8,000
5	5,000	5,000
6	2,550	7,500
7	100	10,000
8	2,550	7,000
9	100	5,000
10	2,550	7,500
11	2,550	7,500
12	2,550	7,500

chloride with a 15-liter capacity with an impeller (20 rpm) for mixing and circulating heating system (35 ± 1 °C). Figure 1 shows a schematic of the anaerobic digester and test setup apparatus. The digester was connected to a gas measurement setup based on the water displacement principle. The inoculum of digester was taken from a full-scale up-flow anaerobic sludge blanket (UASB) digestion reactor treating dairy wastewater at 37 °C in the Gela dairy factory, Iran. The water and VS content of used sludge were 76.3% and 59.75%, respectively.

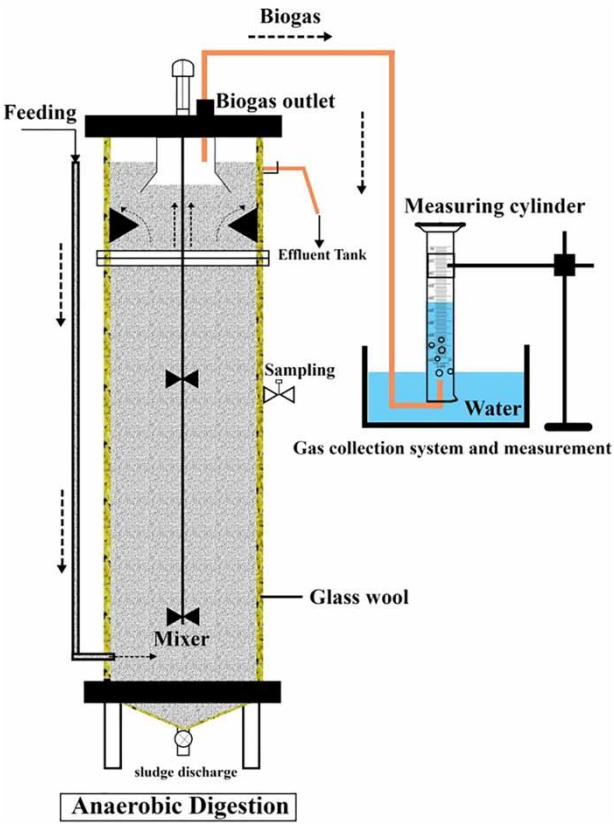


Figure 1 | Schematic of the anaerobic digestion reactor.

After the startup time and stabilizing the bioreactor conditions, the digester was fed with organic loading rate (OLR) of 0.3 kg COD/m^3/d. The operation was kept running until a stable quality of effluent was obtained. Anaerobic biodegradability experiments were carried out for 20 days with a regular hydraulic retention time (HRT) (20 days) of mesophilic anaerobic digestion (Abu-Orf & Goss 2012). Anaerobic biodegradability tests were conducted to determine the biogas yield using raw sludge and pretreated sludge as substrates. All experiments were carried out within 60 days (Figure 2). In the first 20 days, digestion was fed by untreated sludge from the sludge return line in the wastewater treatment plant of the Gela dairy factory, Iran as control. During the second 20 days, digestion was fed by the LTH-PRE sludge, and finally in the third 20 days, digestion was fed by TH-PRE sludge. The initial sludge concentration during control operation and both pretreatments was relatively constant (5,025 ± 75 mg/L) to provide the same conditions for comparing results. All tests were conducted in triplicate to guarantee their producibility.

Analytical methods

Soluble chemical oxygen demand (SCOD) and total COD, solids and P–PO_4 were determined according to standard methods (American Public Health Association *et al.* 1915). The pH of the substrate was measured with a pH meter (multifunction WA-2017SD). In order to evaluate the amount of degradation in the sludge sample through the LTH-PRE method, the Müller concept was used (Müller 2000):

$$\text{Sludge degradation percentage (\%)} = \frac{(SCOD_p - SCOD_0)}{(SCOD_{NaOH} - SCOD_0)} \quad (1)$$

where $SCOD_P$ representing the SCOD in the supernatant of the treated samples by LTH-PRE (mg/L). $SCOD_0$ stands for the SCOD in the supernatant of original sludge (mg/L). $SCOD_{NaOH}$ is the maximum SCOD in the supernatant from the sludge samples that received the 0.5 M NaOH digestion for 10 min at 90 °C (mg/L).

To measure the carbohydrate and protein concentration in LB-EPS solution, the colorimetric method and Bradford method were implemented, respectively (Dubois *et al.* 1956; Bradford 1976).

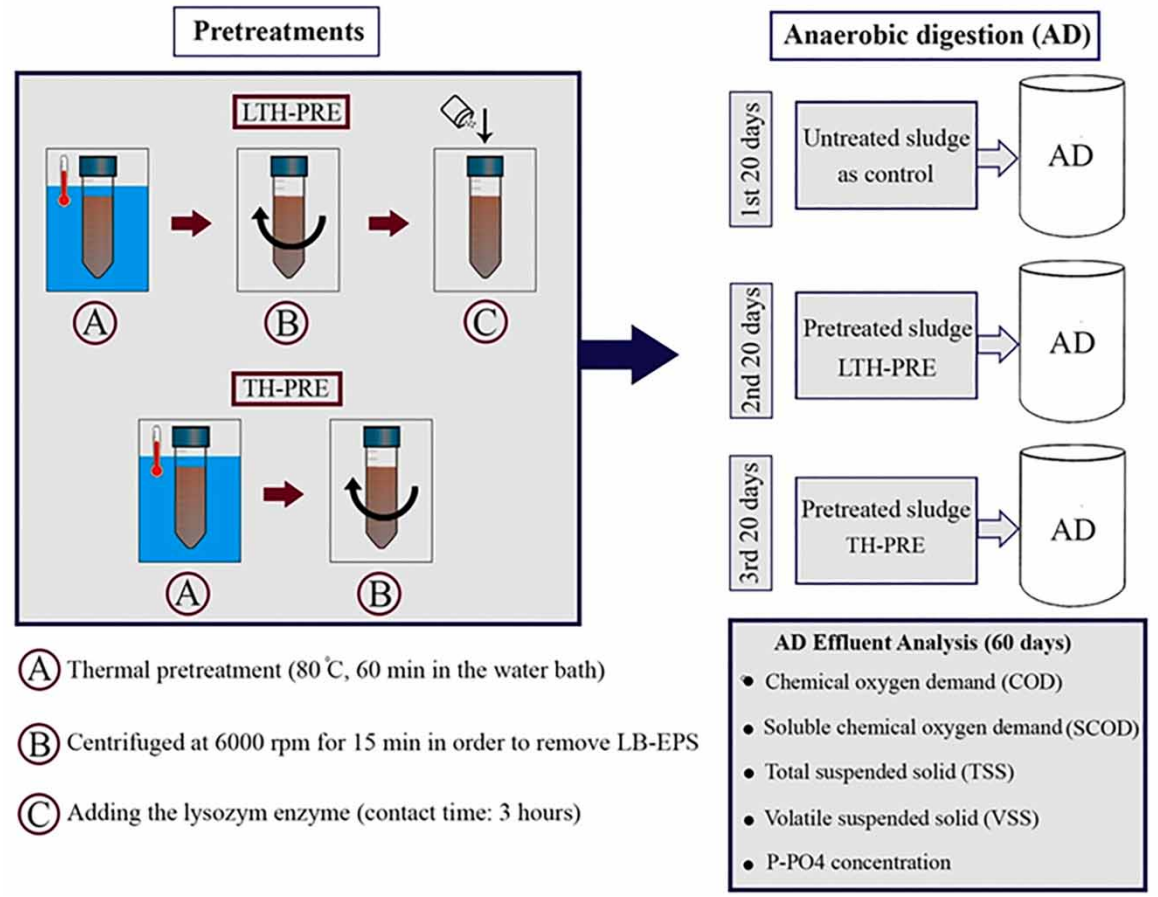


Figure 2 | Experimental setup, operational conditions, and analytical parameters.

RESULTS AND DISCUSSION

Optimum organic loading rate was chosen at an OLR of 0.3 kg COD/m^3/d. Monitoring of COD removal and pH values were performed at the starting point and the COD removal showed good stability at a fixed OLR after almost three weeks. Another indication for the stability of the bio-reactor function was the unchanged pH. Additionally, biogas was produced constantly at stable OLR indicating the termination of the starting time. Operation of the AD process was carried out based on the order of the control operation (sludge without any pretreatment), LTH-PRE, and TH-PRE methods, with an HRT of 20 days.

Optimization of lysozyme enzyme

The optimization process was done using response surface methodology (RSM). The results for 12 runs are shown in Table 3. Based on the sequential model sum of square, the model for lipid yield was selected based on the highest order linear equation. The models were coded *X1* for sludge disintegration percentage and the independent variables in the models were lysozyme concentration (*A*) and MLSS (*B*). The Design-Expert software introduced two models – 2FI and limited-order – as the appropriate model a for obtaining the best equation for estimating sludge degradation efficiency. The R^2 value obtained from the second-order complete model (0.9623) is higher than the 2FI model (0.9182), which indicates that the second-order model is highly accurate and closer to reality. Finally, the proper equation for optimization in this study based on the considered codes is presented in Equation (2). The analysis of variance (ANOVA) statistical analysis data is presented in Table 4.

$$X1 = 66.70 + (76.11*A) + (57.31*B) + (50.28*A*B) + (34.35*B^2) \quad (2)$$

Finally, based on the optimization done in Design-Expert software, the optimal pretreatment rates of LTH-PRE method in this study were considered as the concentration

Table 3 | Results of experiments determining the optimal amount of lysozyme enzyme for hydrolyzing LB-EPS removed sludge

No.	Lysozyme concentration (mg/L)	MLSS (mg/L)	$SCOD_0$ (mg/L)	$SCOD_{NaOH}$ (mg/L)	SCOD lysozyme (mg/L)	Sludge degradation percentage (%)
1	2,550	7,500	154.5	4,583.04	3,205.76	68.9
2	2,550	7,500	154.5	4,583.04	3,052.98	65.45
3	3,040	7,500	154.5	4,583.04	4,140.01	89.996
4	2,550	8,000	178.8	3,847	2,948.66	75.51
5	5,000	5,000	207	5,555.2	2,484.8	68.9
6	2,550	7,500	154.5	4,583.04	3,891.91	62.24
7	100	10,000	205.856	2,941.8	2,910.82	32.66
8	2,550	7,000	154.5	4,263.616	1,099.41	59.24
9	100	5,000	207	5,555.2	2,588.74	17.94
10	2,550	7,500	154.5	4,583.04	1,166.47	61.1
11	2,550	7,500	154.5	4,583.04	2,860.34	67.9
12	2,060	7,500	154.5	4,583.04	3,161.48	52.62

Table 4 | ANOVA for response surface quadratic model

Analysis of variance table [Partial sum of squares – Type III]

Source	Sum of squares	df	Mean square	F value	*p*-value prob > F	
Model	3,818.51	4	954.63	44.66	<0.0001	significant
A-Enzyme	923.1	1	923.10	43.18	0.0003	
B-MLSS	523.40	1	523.40	24.49	0.0017	
AB	387.57	1	387.57	18.13	0.0038	
B^2	175.03	1	175.03	8.19	0.0243	
Residual	149.63	7	21.38			
Cor Total	3,968.14	11				
Std. Dev.	4.62	R^2	0.9623			
Mean	60.20	Adj R^2	0.9407			
C.V. %	7.68	Pred R^2	−1.9223			
PRESS	11,595.91	Adeq Precision	21.447			

of 2,551 mg/L lysozyme enzyme with MLSS equal to 5,000 mg/L.

Anaerobic digestion results

The influent sludge COD was about 6,260 ± 107 mg/L. The results of the control operation were recorded after reaching the stable digestion (steady state). Figure 3 shows parameters of anaerobic digestion of sewage sludge in different steps (COD and SCOD removal, phosphate, TSS, and VSS changes). Regarding Figure 3(a), it can be seen that with anaerobic digestion, COD levels have been reduced by about 77% in the control operation period. After that, on the 21st day, LTH-PRE pretreatment was applied to the system. COD suddenly dropped on day 36, after which the system achieved a steady status along with a 95% COD decrease recorded on day 37. The system received only thermal pretreatment on day 41. From day 50, the COD removal increasingly rose in TH-PRE and reached a steady state in the last days, yielding a COD decrease efficiency of 81%.

LTH-PRE was more effective than TH-PRE as expected due the added lysozyme enzyme. In fact, with extraction of LB-EPS from sludge using thermal method (the

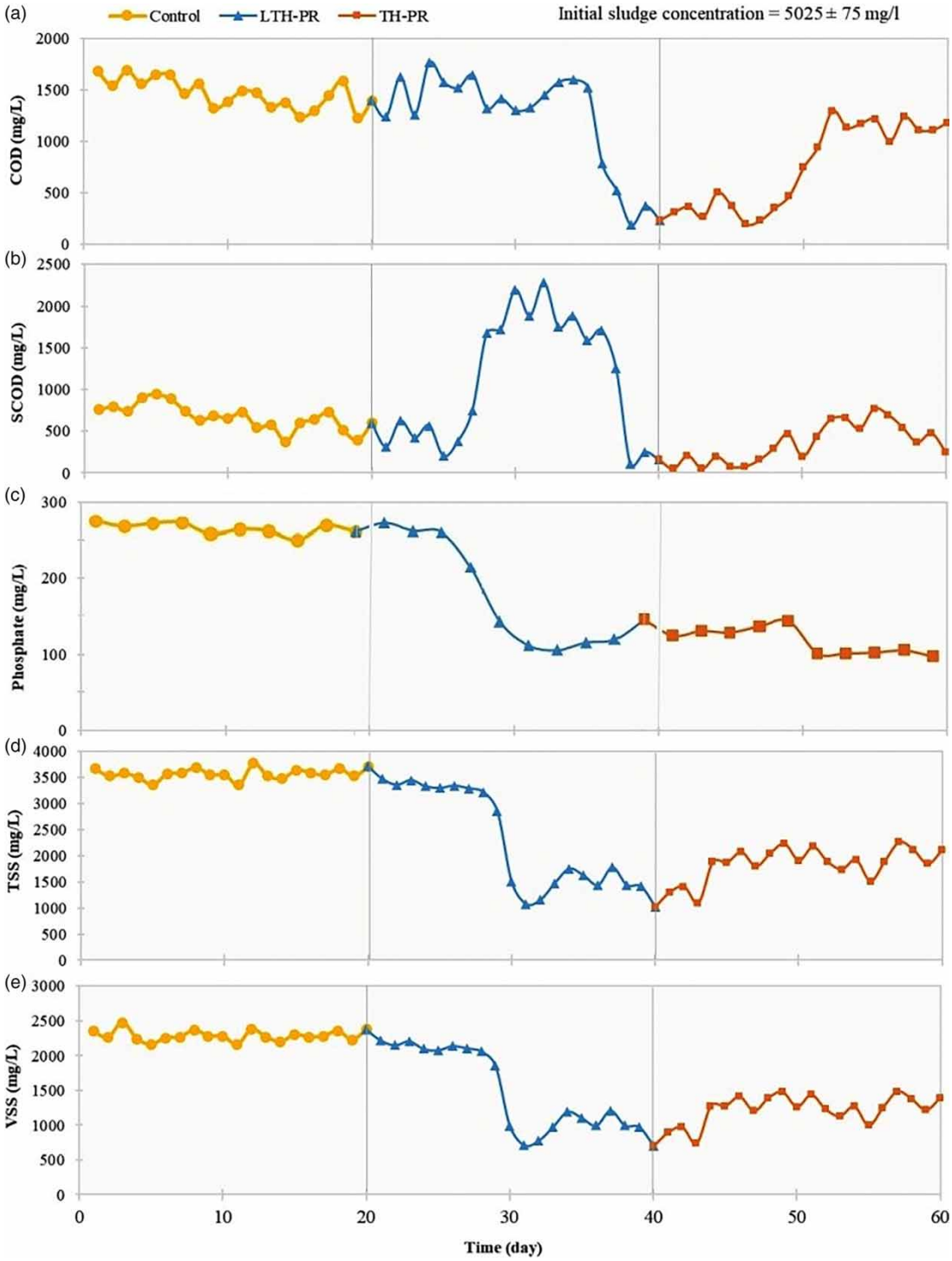


Figure 3 | Parameters of anaerobic digestion of sludge in different steps (COD and SCOD removal, phosphate, TSS, and VSS changes).

concentration of carbohydrate and protein were 87.9 mg/L and 14.53 mg/L, respectively), the protective layer of the microorganisms around them was separated and the intracellular food was released easily and made available to cryptic growth of other anaerobic bacteria. The addition of lysozyme enzymes accelerated this process and led to

the destruction of the cell wall of the bacteria. Therefore digestion takes place easily, more quickly and as a result COD contamination index is reduced (Xu *et al.* 2014).

The incidence of the sludge mass lysis could be explained by the elevation of SCOD as significant indication (Yu *et al.* 2013). The fluctuations in SCOD concentration could characterize the hydrolysis of organic matter (Liao *et al.* 2016). In the first 20 days, the digestion was conducted as a control with an average effluent SCOD of 683.9 ± 98 mg/L. The increase in SCOD represents the creation of readily substrates that can be transformed into biogas during the anaerobic process (Youcai & Guangyin 2016).

The EPS was the dominant component in sludge flocs, which account for about 80% of the weight of sludge (Lin *et al.* 2019). The EPS-protein played an important role in the SCOD rising process, which was caused by the lysozyme rupturing of the bacteria cell wall (Ogawa 2003). Thus, it demonstrates the success in destroying the cell wall and releasing nutrients into the environment using the enzyme (Yang *et al.* 2010; Xin *et al.* 2015).

As can be seen, the SCOD value increased in LTH-PRE compared with the raw sludge. As shown in Figure 3(b), from day 27, the SCOD value was increased gradually, and fluctuations were observed in its value for 10 days. From the 38th day, SCOD declined to a fairly constant level. The SCOD in this period averaged 170 ± 61 mg/L. This sudden increase and the fluctuations of the SCOD value in LTH-PRE can be attributed to the inability of the bacteria to adapt to the secondary metabolite produced by the lysozyme enzyme. In fact, by adding the enzyme to the system, dissolution and cellular degradation increased and also the SCOD. This created SCOD is either consumed by methanogenic bacteria to produce more biogas, or it leaves the system. As shown in Figure 3(b), these fluctuations were completed in the last 4 days of LTH-PRE, and the anaerobic bacteria adapted to the new conditions, and before SCOD decreased.

From day 40, TH-PRE was applied to the system, which did not significantly change the amount of SCOD, but after day 47 SCOD increased slightly and in the last 4 days reached a relatively stable amount. The SCOD in this period averaged 364 ± 98 mg/L.

According to the above, LTH-PRE was more effective than TH-PRE due to the addition of the lysozyme enzyme. Lysozyme is an enzyme (129 amino acid residues) that catalyzes the hydrolysis of the β-1,4-glycosidic linkage of the peptidoglycan in the bacterial cell wall (Imoto *et al.* 1972; Wecke *et al.* 1982). In fact, when the destruction of cell wall occurs using TH-PRE, the underlying structures of the lysozyme-sensitive bacterial cell will collapses and the liberation of the cytoplasmic components into the medium result in the lysis of the bacterial community (Salton 1958).

Adenosine triphosphate (ATP) molecule breaks down in anaerobic digestion to provide energy for metabolism, and as a result, phosphate is released. The concentration of phosphate reduced in the course of each pretreatment as a result of phosphate can be taken up by various phosphor accumulating bacteria for the purpose of energy storage. Figure 3(c) shows phosphate concentration changes during different operations. The concentration of phosphate in the influent sludge was 272 ± 4 mg/L. According to Figure 3(c), phosphate concentration did not change and the average concentration in the control operation was 265.2 ± 7 mg/L. The phosphate concentration gradually decreased since day 26. The concentration of phosphate in this period averaged 123.8 ± 15 mg/L, which was a reduction of 54% in LTH-PRE. With the start of TH-PRE, phosphate concentration decreased to 62%. Comparing LTH-PRE with TH-PRE, it was found that for TH-PRE, phosphate removal was 8% more than for LTH-PRE. This is due to synergism of thermal and lysozyme enzyme pretreatment, which has an adverse effect on phosphate accumulation in gram-negative bacteria, which are responsible for taking the phosphate form substrate (Salton 1958). Therefore, in TH-PRE due to conducting only thermal pretreatment, phosphate reduction reached its maximum amount.

TSS is an indicator for assessing the effectiveness of a process in sludge stabilization (Mohammadi *et al.* 2017). As shown in Figure 3(d), in the first 20 days, the effluent TSS dropped by an average of 29%. After day 20, with the start of LTH-PRE, there was not much change in the value of TSS, but from day 28, there was a decreasing trend in the value of TSS, and this trend continued for 3 days, after which it reached a constant value. On average, in LTH-PRE, effluent TSS decreased by 72% after day 41 when the TH-PRE was applied. In the early days of this pretreatment there was no significant change in the effluent TSS, but after day 44, the TSS value increased slightly and then reached a constant value. On average, in TH-PRE, the TSS value was reduced by 61%. The lysozyme digestion process presented a positive influence on excess sludge reduction. As a result, TSS reduction in LTH-PRE and TH-PRE was 43% and 32% more than the in the control, respectively. Better performance of digestion depends on reducing the volatile matter in the raw sludge (Yi *et al.* 2014). Figure 3(e) shows VSS changes over pretreatment phase in anaerobic digestion. In the first 20 days, a mean drop of 29% was observed in VSS, whereas the average reduction for LTH-

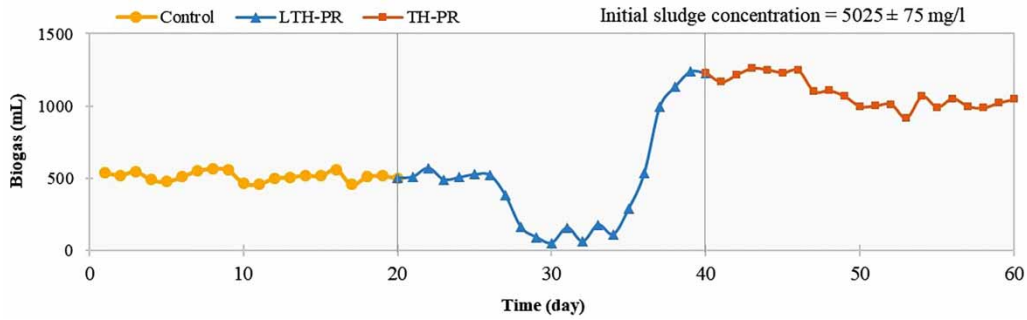


Figure 4 | Biogas production over different phases in anaerobic digestion of sewage sludge.

PRE was 70%. After TH-PRE, the VSS slightly increased and then reached a constant value. On average, VSS dropped by 60% in TH-PRE.

In fact, the pretreatment process facilitated the decomposition of the compounds, which resulted in further degradation of the material and thus reduced VSS (Wang *et al.* 2014).

Biogas production from anaerobic digestion

The volume of biogas produced was measured daily by a column of water. Figure 4 illustrates biogas production over different phases in anaerobic digestion of sewage sludge. In the first 20 days, an average of 513 ± 32 mL of gas was produced. With the start of LTH-PRE, from the day 29 gas production started gradually declining, and the decline continued until day 34. The reason for the reduction of biogas production from the 29th to the 34th day is probably due to the inability of the anaerobic bacteria to decompose and use the new SCOD created by the lysozyme enzyme. From day 34, the anaerobic bacteria gradually adapted to the new conditions and the production of biogas increased. This could have been due to the effect of the enzyme in suppressing the inhibitory substances, which should be discussed further. After that, the production of biogas increased again as the SCOD decreased, and after day 37 it became relatively stable.

It can be seen from Figure 4 that the volume of produced biogas in LTH-PRE period reached an average of 1,150 ± 98 mL/d, which means a 124% increase in gas production compared to the control operation. In the TH-PRE phase, the amount of gas produced in this period gradually decreased after 49 days, and then reached a relatively stable amount. The volume of gas produced in TH-PRE averaged 1,006 ± 39 mL/d, which means a 96% increase in biogas production compared to the control operation.

Therefore, according to the presented results in this research, for explaining the mechanism of suggested methods we can declare that LB-EPS acts as an obstacle toward the hydrolysis of sludge for a couple of reasons. First, LB-EPS traps the lysozyme enzyme and as a result the efficiency of biological pre-treatment will decrease. Second, because microbial EPS covers the cell surface, it plays a deterrent role in the hydrolysis stage of anaerobic digestion, and hence, the present study aimed to withdraw the LB-EPS before the enzymatic pre-treatment, in order to ease the process of interaction between the lysozyme enzyme and the bacteria cell. On the other hand, with this approach the sludge EPS content is reduced, thus the biological performance of anaerobic digestion will improve and consequently the biogas production will increase. From an economic assessment perspective, according to the obtained results for biogas production there is no economic justification for implementing the LTH-PR method in full-scale wastewater treatment plants, rather than the TH-PR method. However, the release of EPS components (i.e. protein and carbohydrate) within the lysozyme treatment (He *et al.* 2014) can provide an abundant source of biocoagulant and carbon from excess sludge. Additionally, some specific features of lysozyme pretreatment, such as improving sludge pre-disposal efficiency and extra energy consumption minimization (He *et al.* 2014), may change this economic judgment in future.

CONCLUSION

In this study, the effects of two pretreatment methods, including TH-PRE and LTH-PRE, on anaerobic digestion of the active sludge were evaluated. The results of the analysis showed that VSS reduction and biogas production for LTH-PRE were 41% and 31%, and for TH-PRE they were

124% and 96%, respectively higher than the control operation. The application of LTH-PRE method also affected the supernatant quality and its results showed that COD removal for LTH-PRE method was 14% higher than thermal pretreatment TH-PRE. The hybrid thermal–enzymatic method presented in this study was able to provide a more efficient condition for the lysozyme enzyme performance of the anaerobic processes of activated sludge digesters.

Even though combined pretreatments of waste activated sludge have been amply investigated, their practical application is still relatively limited. There are significant gaps in our knowledge of changes within bacteria and EPS during different pretreatments, and other factors such as operational costs, pathogen inactivation, and sludge dewaterability should be considered. Other combined pretreatments and their effects on anaerobic digestion efficiency would be interesting and alluring for future studies.

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