Efficient and convenient pretreatment to enhance waste activated sludge anaerobic hydrolysis by iron porphyrin biomimetic enzyme

Min Ruan¹, Jian Zhang¹, Cheng-gang Niu², Jing Huang³*, Wei Zhang¹, Zhi-hao Zeng¹, Kang Zhou¹ and Xue Li⁴

¹School of Energy and Power Engineering, Changsha University of Science and Technology, Changsha, Hunan, 410114, P.R. China
²College of Environmental Science and Engineering, Hunan University, Changsha, Hunan, 410082, P.R. China
³Institute of Biological and Environmental Engineering, Hunan Academy of Forestry, Changsha, Hunan, 410004, P.R. China
⁴Department of Biological and Environmental Engineering, Changsha University, Changsha, Hunan, 410022, P.R. China

*Corresponding author’s e-mail: gavinhj@163.com

Abstract. A novel biomimetic enzyme was applied in the pretreatment of waste activated sludge (WAS) for enhancing the hydrolysis properties. An iron porphyrin complex was efficiently synthesized and purified. In comparison with the blank test, iron porphyrin complex used as biomimetic enzyme could significantly improve the sludge disintegration. Hydrogen peroxide was not the main influencing factor. During the pretreatment process, concentrations of the ammonia nitrogen (NH₄⁺-N) and reducing sugar (RS) both almost increased, which indicated that the protein and polysaccharide in sludge were effectively decomposed. Results demonstrated that the optimum treatment time and temperature of this biomimetic enzyme was at 7 hours and 40 ℃, respectively. The efficient and economical dosage of the biomimetic enzyme used in treatment sludge digestion was 1%. Under the optimal treatment condition, the SCOD/TCOD ratio, the rate of VSS reduction, the concentrations of NH₄⁺-N and RS reached the values of 65.3%, 38.4%, 215.9 mg/L and 65.9 mg/L, respectively. And the possible mechanism of iron porphyrin biomimetic enzyme to promote anaerobic hydrolysis of WAS had been proposed. Therefore, the application of biomimetic enzyme was a desirable method in the WAS pretreatment.

1. Introduction

As the rate of municipal wastewater treatment going up, large amounts of waste activated sludge (WAS) is generated during wastewater treatment process. It will pose a significant threat to the ecology environment if the WAS is not timely and properly disposed[1]. A part of sludge has been currently used for co-firing feedstocks, industrial application, and composting as a fertilizer, but a large amount of WAS is still disposed in landfills and by incineration[2]. The cost of properly
disposing and treating of WAS is quite expensive and account for nearly 40-60% of the total wastewater treatment plants (WWTPs) operating cost[3]. Therefore, much attention is focus on methods of minimizing sludge production[4, 5] and reducing the excess sludge[6, 7], which has proven to be an efficient approach to alleviate this problem.

Anaerobic digestion is usually perceived as a cost-efficient approach for the WAS treatment. During sludge digestion process, a large proportion of the organic matter could be decomposed into biogas (e.g. methane and hydrogen)[8] and valuable products (e.g. organic acid)[9]. Hydrolysis is recognized as the major rate-limiting step in the complex digestion process[10]. For this reason, many types of pretreatment have been developed to enhance the sludge solubilization, including microwave[11], thermal[12], chemical[13], biological[14], and ultrasonic treatments[15].

The enzymatic hydrolysis of sludge has been employed for the last three decades and a number of enzymes were reported to play a significant role in a range of waste treatment application. Pretreatment of WAS with enzymes prior to the anaerobic digestion was shown to improve the degradation of the sludge and led to enhance methane production[16, 17]. Comparison to other sludge pretreatment methods, the enzymatic treatment has the unique advantages, such as convenient process, non-secondary pollution, and friendly environment.

Nonetheless, due to the chemical components, most of enzymes are poor in chemical stability, especially in complicated environment. With the development of biomimetic technology, there is a growing interest for biomimetic enzyme. Metalloporphyrin complexes are similar to the nuclear structure of cytochrome P450 enzymes and the complexes have been successfully used as the bionic enzyme in many research fields such as metal organic catalysis reaction[18], non-degradable organics degradation[19, 20]. Published studies show that the metalloporphyrin complexes mimic cytochrome P450 enzymes which could efficiently catalyze hydrogen peroxide to degrade the lignin compounds and carbohydrate[21, 22].

In this study, the treatment object was WAS, in which major compositions were cells, cells’ fragment and extracellular polymeric substances (EPS). It’s not difficult to recognize that the common chemical constituents of those compositions were the carbohydrate, which has been reported of degradation by metalloporphyrin complexes. Inspired by biomimetic technology and mimic function of metalloporphyrin complexes, the authors attempted to apply the iron porphyrin complex as biomimetic enzymes to pretreatment process of sludge digestion. To the authors’ knowledge, little information is available on biomimetic enzyme applying to the sludge pretreatment. This work aims at using a biomimetic enzyme to improve the adaptive performance in complicated sludge environment and meanwhile to get an efficient and economical approach in sludge digestion process.

2. Methods

2.1. Waste activated sludge characteristics

The waste activated sludge used in this study was obtained from a sludge return well of the secondary sedimentation tank of a municipal wastewater treatment plant in Changsha, China. Large particles in sludge samples were removed by a metal filter with a mesh size of 0.9 mm. Then, the sludge samples were concentrated by settling at 4 °C prior to use. Table 1 shows the characteristics of sludge samples.

| Parameter                                | Value               |
|------------------------------------------|---------------------|
| pH                                       | 6.82 ± 0.14         |
| TCOD (total chemical oxygen demand) (mg/L)| 16, 032.25 ± 153.06 |
| SCOD (soluble chemical oxygen demand) (mg/L)| 426.40 ± 5.71     |
| TSS (total suspended solids) (g/L)       | 15.26 ± 0.78        |
| VSS (volatile suspended solids) (g/L)    | 8.16 ± 0.12         |
| NH₄⁺-N (ammonia nitrogen) (mg/L)         | 50.36 ± 1.03        |
| RS (reduction sugar) (mg/L)              | 28.13 ± 1.45        |
2.2. Preparation of iron porphyrin complex

In this work, the iron porphyrin complex plays a role of mimetic enzymatic activity and catalyzes chemical reactions in the process of WAS anaerobic digestion. And its synthetic method was according to the literature with some modifications[23].

Firstly, 100 mg of the porphyrin, 5,10,15,20-tetra(4-aminophenyl)-porphyrin (abbreviated from here on in as TAPP) synthesized by our group, was dissolved in 100 ml of dimethylformamide (DMF) and the solution was heated at about 100 °C. When the solution in the neck flask was boiling, 500 mg of FeCl$_2$·4H$_2$O was added into the solution. Then, the reaction temperature was increased to 150 °C, and the mixture was refluxed at this temperature for 4 to 5 hours. The completion of reaction was checked by Thin-Layer Chromatography (TLC) method. After removal of about 30 ml of DMF by a rotary evaporator, a few milliliters of 6 mol/L HCl was slowly added to the reaction mixture and then kept on evaporated DMF in it. Finally, the mixture was washed with some anhydrous methanol and filtered for three times. The filtrate was concentrated to dryness in a flash evaporator and the product was obtained from a vacuum drying apparatus at 70 °C for 24 h. The schematic diagram of synthesis reaction is shown in figure 1.

![Figure 1 Schematic diagram of iron porphyrin complex synthesis reaction](image)

2.3. Biomimetic enzyme sludge digestion tests

Sludge digestion experiments were carried out in 250 mL Erlenmeyer flasks, each containing 100 mL of WAS. One milliliter of iron porphyrin complex ethanol solution was mixed into the sludge. Then, the flasks were placed in an incubator with roundabout shaker and treated at 200 rpm and at 35 °C. After reacted for 8 h, the flasks were taken out to detect of the key parameters of digestion sludge. During the whole procedure of treatment, the pH of sludge samples was not adjusted and also the influence factor of metal ion was neglected in this study.

In the optimization experiments of treatment condition, the injected volume of iron porphyrin complex, the treated time and temperature were changed in terms of the investigated condition.

2.4. Samples analysis

The sludge samples from digestion reaction were centrifuged at 5000 rpm for 30 min and then filtered through a 0.45 μm membrane. The filtrate was collected to measure SCOD, Reducing sugar (RS) and Ammonia nitrogen (NH$_4^+$-N). All of the samples were evaluated using chemicals of analytical grade. Parameters of SCOD, TCOD, TSS and VSS were determined by the Standard Methods[24]. NH$_4^+$-N was measured with the Nessler’s reagent spectrophotometric method. RS was tested by the phenol sulfuric acid method. The value of pH was detected using a pH meter.

3. Results and Discussion

3.1. Characteristics of the iron porphyrin complexe
For the sake of verification the result of synthesis, both of the TAPP and iron porphyrin complex were determined by ultraviolet spectrophotometry method. From the scan spectrogram, the peak width of iron porphyrin complex was wider than that of TAPP. Table 2 shows the detection results of the porphyrins. In the Soret region, both the porphyrins present an absorption peak, but the peak of iron porphyrin complex is red-shifted compared to TAPP. Furthermore, in the Q bank region, the iron porphyrin complex has only two maximum absorption peaks, which is less than that of TAPP. The phenomena of redshift and two absorption peak disappearance are in accord with the results presented in previous studies. According to the detection results, it could be concluded that the iron porphyrin complex is successfully synthesized and therefore it can be used as a biomimetic enzyme in the further investigations.

| Porphyrins   | \( \lambda_{\text{max}} \) Soret / nm | \( \lambda_{\text{max}} \) Q₁ / nm | \( \lambda_{\text{max}} \) Q₂ / nm | \( \lambda_{\text{max}} \) Q₃ / nm | \( \lambda_{\text{max}} \) Q₄ / nm |
|--------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| TAPP         | 425                             | 521                             | 568                             | 609                             | 662                             |
| Iron porphyrin| 430                             | 540                             | 593                             | -                               | -                               |

From the test of solubility property of the iron porphyrin complex, it was found that the iron porphyrin complex was slightly soluble in water but completely soluble in ethanol, trichloromethane and DMF. For the performance of safety and economy, the ethanol was chosen as dissolvent in the following experiments.

3.2. Effects of hydrogen peroxide on sludge digestion by catalytic oxidation

In the literature of metalporphyrins used as catalysts, it seemed that the hydrogen peroxide was employed as an oxygen source and took part in the oxidizing reaction. Consequently, the hydrogen peroxide was the primary element in this sludge digestion investigation catalyzed by the iron porphyrin complex.

A batch of tests was conducted and four kinds of different components were added to sludge samples respectively. Table 3 shows the contents and results of sludge digestion treated by different components. From the table, the following results were obtained. (1) Sample B versus sample A, concentrations of VSS, SCOD, \( \text{NH}_4^+ \)-N and RS between the two samples were insignificant different respectively. In another word, the hydrogen peroxide in the digestion reaction did not effectively promote the catalysis oxidation reaction. (2) Sample B versus sample C, the comparison could be considered as separate investigation between iron porphyrin complex and hydrogen peroxide in the role of digestion. Results show apparent distinction between them and also illustrate that the single hydrogen peroxide has no significant effect on the digestion reaction. Thus, it could be preliminarily demonstrated that iron porphyrin complex was the crucial factor in the sludge anaerobic digestion process, as expected of the author’s assumption. (3) Sample B versus sample D, it also presents that the iron porphyrin complex has great advantage in digestion reaction according to the testing indices. The sample D, adding with one milliliter water, was equivalent to a blank control and the comparison results further illustrated the availability of this biomimetic enzyme using in sludge anaerobic digestion.

| Samples | Components | VSS (g/L) | SCOD (mg/L) | \( \text{NH}_4^+ \)-N (mg/L) | RS (mg/L) |
|---------|------------|-----------|-------------|-----------------------------|-----------|
| A       | 1 mL iron porphyrin + 500µL \( \text{H}_2\text{O}_2 \) | 6.84      | 9466.08     | 128.65                     | 44.28     |
| B       | 1 mL iron porphyrin | 6.90      | 9210.25     | 122.87                     | 43.91     |
| C       | 500 µL \( \text{H}_2\text{O}_2 \) | 7.83      | 710.47      | 83.13                      | 30.64     |
| D       | 1 mL distilled water | 7.98      | 445.26      | 75.49                      | 28.89     |

In addition, for the purpose of investigating treatment efficiency, the rate of VSS removal, the ratio of SCOD and TCOD, the concentration of \( \text{NH}_4^+ \)-N and RS were also examined. Figure 2 shows the results of sludge samples treated by different components. Obviously, the SCOD/TCOD ratio of Sample A (iron porphyrin complex and \( \text{H}_2\text{O}_2 \)) and B (iron porphyrin complex) were both up to 45%,
which were higher than that results reported in the literature of sludge digestion [21]. The VSS removal rates of the two samples were up to 25%, the NH$_4^+$-N concentration were increased to 122mg/L and the RS concentration were reached to 43mg/L, which could be considered as an efficient treatment effect [18]. Therefore, the above results further demonstrate that the method of iron porphyrin complex using as biomimetic enzyme in sludge digestion treatment is feasible.

![Figure 2 Results of SCOD/TCOD, VSS reduction rates, ammonia nitrogen (NH$_4^+$-N) and reducing sugar (RS) treated by four test samples](image)

3.3. Effect of treatment time on sludge hydrolysis

For further understanding the treatment efficiency by the biomimetic enzyme, the authors firstly investigated the effect of treatment time in the digestion process. In the evaluation index of sludge, the SCOD/TCOD ratio and the rate of VSS reduction are used to evaluate the degree of solubilization, the concentration of NH$_4^+$-N and RS are used to evaluate the degree of macromolecular degradation, which could reflect the degree of sludge digestion to some extent.

As shown in figure 3, when the treatment time increased from 3 h to 12 h, the SCOD/TCOD ratio, the rate of VSS reduction, the concentration of NH$_4^+$-N and RS of experimental group showed an increasing trend firstly, then gradually stabilized near its maximums. It was noteworthy that both the SCOD/TCOD ratio and the rate of VSS reduction reached its maximums at 7 h. when continued to increase the treatment time after 7 h, there was no significant change in the ratio. One possible explanation for this phenomenon could be that it related to the characteristics of catalysis and oxidation of the biomimetic enzyme. In the initial stage of the enzymatic reaction (from 3 h to 7 h), the substrate concentration was dense enough to participate in the chemical reaction and the reaction rate increased straightly. During this period, some solid substance transferred to liquid phase, such as macromolecular organic substance, non-straight chain organic compounds, microorganism cells. More precisely, macromolecular substances were oxidized and decomposed to other small molecular substances, which were easier soluble in aqueous phase. Besides, VSS including some volatile organics, microorganism cells, EPS, and other decomposable material might also be oxidative decomposition and transferred to aqueous phase. Thus, the concentration of SCOD presented in an ascending trend and VSS presented in a descending trend in this stage. However, with the increasing of enzymatic reaction, the concentration of substrates was decreased and the reaction rate was weakened from 7 h to 12 h. And there would be some inhibitors formed in the products, which might as well be the decline cause of reaction rate. Therefore, there was no significant change in the SCOD and VSS concentration after 7 h treatment. It could be seen that the trend of SCOD/TCOD and VSS removal rates were approximately same, and there might be a certain correlation between them. But researches of this relevance would be conducted in our next stage studies. Additionally, from the result of figure 3, the blank control group of SCOD/TCOD ratio and the rate of VSS reduction were no obvious different in the whole treatment process.

Unexpectedly, the concentration of NH4+–N and RS reached its maximums at 9 h, which was lagged two hours compared with the SCOD/TCOD ratio and the rate of VSS reduction. The reason
could be ascribed that most of the organic matter in the sludge is mainly solid phase, and there was few organic matter in the water phase. Iron porphyrin biomimetic enzyme needed to transfer the solid organic matter to the liquid phase before it could achieve the hydrolysis of the macromolecular organic matter. With the increasing of treatment time, proteins were oxidized and deaminized by the added biomimetic enzyme and transformed into the free ammonia. Therefore, the concentration of free ammonia increased gradually till the proteins were completely decomposed. The biomimetic enzyme apparently accelerated the formation of free ammonia. And polysaccharide were oxidized and decomposed to various reducing sugars and some small molecular polysaccharide. So the concentration of RS was also increased to its maximum. However, the increase rate of NH$_4^+$-N is significantly greater than that of RS, which may due to this WAS contained more protein and few polysaccharides, and some of the degradation products of sugars are also converted to free ammonia. Therefore, the concentration of NH$_4^+$-N and RS in experimental group were larger than that of blank control group and increased till the treatment time arrived to 12 h.

3.4. Effect of reaction temperature on sludge hydrolysis
As is known, it is very significant that temperature affects the reaction rate of enzyme-catalyzed. Generally, the enzymatic activity could reach the best effect in its optimum temperature range, and also the reaction rate reaches the max. In this temperature range, the reaction rate of enzyme could improve 1 to 2 times when the reaction temperature increases per 10 °C. According to this rule, a series of temperatures were employed in studies, and they were 25, 30, 35, 40, 45 and 50 °C, respectively. In this section, the treatment time was 7 hours, and results were shown in figure 4.

Figure 3 Effect of treatment time on SCOD/TCOD (a), VSS reduction (b), concentration of ammonia nitrogen (NH$_4^+$-N) (c) and reducing sugar (RS) (d)
Figure 4 Effect of reaction temperature on SCOD/TCOD, VSS reduction, ammonia nitrogen (NH$_4^+$-N) and reducing sugar (RS)

From the results of figure 4, it was at 40 ℃ that the SCOD/TCOD ratio and VSS removal rate almost both reached the maximum. Particularly, it had a very significant increase from 25 to 35 ℃ in the SCOD/TCOD ratio, which could effectively indicate that the enzymatic activity was promoted at 35 ℃ and most of the solids in sludge were catalyzed and oxidized and transferred to liquids. The change trend of NH$_4^+$-N concentration was similar to that of SCOD/TCOD ratio, but the reduce sugar concentration did not have the same trend. At 50 ℃, only the reduce sugar (RS) concentration reached its maximum, the three other parameters nearly stopped increasing. Therefore, based on the main factors of sludge digestion, it could be considered that the optimum temperature of this biomimetic enzyme was at 40 ℃.

3.5. Effect of biomimetic enzyme dosage on sludge hydrolysis

The dosage of biomimetic enzyme used in sludge digestion process was an important evaluation index, because it was related to the economy and applicability of this biomimetic enzyme and might be determine whether the enzyme could popularize in practical usage. In the following experiments, six groups of dosage were examined in 7 hours at 40 ℃. The results were shown in figure 5.

As presented in figure 5, when the dosage reached to 1%, the SCOD/TCOD ratio, VSS removal rate and reduce sugar (RS) concentration nearly reached the each maximum. And the NH$_4^+$-N concentration was shown its maximum in the dosage of 1.5%. It was obvious that in from 1.5% to 2.5% of the biomimetic enzyme dosage there were no significant changes in the four tested indexes. Thus, the efficient and economical dosage of the biomimetic enzyme used in treatment sludge digestion was 1%. It was noteworthy that under the optimal treatment condition (7 hours, 40 ℃, 1% dosage) the SCOD/TCOD ratio and the VSS removal rate reached the values of 65.8% and 37.5%, respectively.

Figure 5 Effect of biomimetic enzyme dosage on SCOD/TCOD, VSS reduction, ammonia nitrogen (NH$_4^+$-N) and reducing sugar (RS)
3.6. Possible mechanism in biomimetic enzymatic hydrolysis of sludge

The possible mechanism of iron porphyrin biomimetic enzyme promoting sludge hydrolysis was hypothesized by referring to relevant literatures and combining with experimental data. As shown in figure 6, when the iron porphyrin complex with strong redox ability was added to the sludge system, the biomimetic enzyme would first adsorb and aggregate in the EPS, which could destroy the flocs structure of the EPS. The outermost layers of EPS, such as slime and loosely bound extracellular polymeric substance (LB-EPS), were hydrolyzed. Protein and humic acid in these EPS were released into aqueous solution, accompanied by the dissolution of tightly bound extracellular polymeric substance (TB-EPS) during this period. As the flocs disintegrated, zoogloea bacteria were exposed and hydrolysis by biomimetic enzyme. Iron porphyrin complex which were adsorbed on the surface of zoogloea would change the permeability of cell membrane, and transmit the NADPH which produced in anaerobic respiration from intracellular into the environment. As a result, partial microorganisms died and cracked due to metabolic obstruction, while EPS was further dissolved and dissolved organic matter (DOM) was further increased. This is consistent with the experimental results, in which SCOD/TCOD increased from 2.78% to 65.3%, and VSS removal rate increased from 1.21% to 38.4%. Meanwhile, in order to maintain the structure and function of EPS, zoogloea bacteria would synthesis new EPS by absorb nutrients from extracellular environment. However, it was difficult for macromolecular organics to enter the cell to participate in metabolism through mass transfer, so more hydrolases were released into the extracellular environment to degrade macromolecular organics. The hydrolysis function of biomimetic enzymes further enhanced the conversion of macromolecular DOM into small-molecule organics. This was reflected in the significant increase in NH$_4^+$-N and RS, which increased from 75.49 and 28.89 to 215.9 mg/L and 65.9 mg/L, respectively. Carbohydrates and proteins are two of most predominant DOM of WAS. After biomimetic enzyme hydrolysis, carbohydrates could degrade into polysaccharides and RS, while proteins could hydrolyze to polypeptides and amino acid. Besides, iron porphyrin complex could further transform RS into carbon dioxide, water and ammonia, while amino acid could convert into pyruvic acid and ammonia.

![Figure 6 The possible mechanism of biomimetic enzyme hydrolysis of sludge](image)

4. Conclusions

The experiments demonstrated that the iron porphyrin complex could be successfully synthesized and used as a biomimetic enzyme in improving the hydrolysis properties during the pretreatment of WAS. In addition, the hydrogen peroxide did not effectively promote the catalysis oxidation reaction. The SCOD/TCOD ratio and VSS removal rate of experimental groups both reached the maximums at 7 h. The optimum temperature of this biomimetic enzyme was at 40 °C. The efficient and economical dosage of the biomimetic enzyme was 1%. And the possible mechanism of iron porphyrin biomimetic
enzyme to promote anaerobic hydrolysis of WAS had been proposed. Therefore, the above results further demonstrate that iron porphyrin complex using as biomimetic enzyme in sludge digestion treatment is promising and feasible.

**Acknowledgements**

The authors gratefully acknowledge the financial support provided by the National Natural Science Foundation of China (Grant Nos.51608052, 51808216), Natural Science Foundation of Hunan Province, China (Grant No.2019JJ50665), Science and Technology Planning Project of Hunan Province, China (Grant No. 2017SK2383), Forestry Science and Technology Planning Project of Hunan Province, China (XLK201803), Research Foundation of Education Department of Hunan Province, China(Grant No. 18B406), the Key Laboratory of Efficient & Clean Energy Utilization, College of Hunan Province (Changsha University of Science & Technology, NO. 2013NGQ008), and the Key Laboratory of Renewable Energy Electric-Technology of Hunan Province (Changsha University of Science & Technology, NO. 2014ZNDL004) and Innovative Team of Key Technologies of Energy Conservation, Emission Reduction and Intelligent Control for Power-Generating Equipment and System, CSUST.

**References**

[1] Khursheed, A., Kazmi, A.A. (2011) Retrospective of ecological approaches to excess sludge reduction. Water Research., 45(15): 4287-4310.

[2] Keffala, C., Harerimana, C., Vasel, J.L. (2013) A review of the sustainable value and disposal techniques, wastewater stabilisation ponds sludge characteristics and accumulation. Environmental Monitoring and Assessment., 185(1): 45-58.

[3] Yu, S.Y., Zhang, G. M., Li, J. Z., Zhao, Z. W., Kang, X. R. (2013) Effect of endogenous hydrolytic enzymes pretreatment on the anaerobic digestion of sludge. Bioresource Technology., 146: 758-761.

[4] Gu, J., Liu, H., Wang, S. Y., Zhang, M., Liu, Y. (2019) An innovative anaerobic MBR-reverse osmosis-ion exchange process for energy-efficient reclamation of municipal wastewater to NEWater-like product water. Journal of Cleaner Production., 230: 1287-1293.

[5] Collivignarelli, M.C., Abba, A., Miino, M. C., Torretta, V. (2019) What Advanced Treatments Can Be Used to Minimize the Production of Sewage Sludge in WWTPs? Applied Sciences-Basel., 9(13): 24.

[6] Huang, J., Zhou, Z., Zheng, Y., Sun, X., Yu, S. Q., Zhao, X. D., Yang, A. M., Wu, C. H., Wang, Z. W. (2020) Biological nutrient removal in the anaerobic side-stream reactor coupled membrane bioreactors for sludge reduction. Bioresource Technology., 295: 10.

[7] Yang, C.X., Liu, W. Z., He, Z. W., Thangavel, S., Wang, L., Zhou, A. J., Wang, A. J. (2015) Freezing/thawing pretreatment coupled with biological process of thermostophilic Geobacillus sp G1: Acceleration on waste activated sludge hydrolysis and acidification. Bioresource Technology., 175: 509-516.

[8] Zhang, Z.S., Guo, L., Wang, Y., Zhao, Y. G., She, Z. L., Gao, M. C., Guo, Y. D. (2020) Application of iron oxide (Fe3O4) nanoparticles during the two-stage anaerobic digestion with waste sludge: Impact on the biogas production and the substrate metabolism. Renewable Energy., 146: 2724-2735.

[9] Astals, S., Esteban-Gutierrez, M., Fernandez-Arevalo, T., Aymerich, E., Garcia-Heras, J. L., Mata-Ahiarez, J. (2013) Anaerobic digestion of seven different sewage sludges: A biodegradability and modelling study. Water Research., 47(16): 6033-6043.

[10] Yang, S.S., Guo, W. Q., Chen, Y. D., Wu, Q. L., Luo, H. C., Peng, S. M., Zheng, H. S., Feng, X. C., Zhou, X., Ren, N. Q. (2015) Economical evaluation of sludge reduction and characterization of effluent organic matter in an alternating aeration activated sludge system combining ozone/ultrasound pretreatment. Bioresource Technology., 177: 194-203.

[11] Cosgun, S., Semerci N. (2019) Combined and individual applications of ozonation and
microwave treatment for waste activated sludge solubilization and nutrient release. Journal of Environmental Management., 241: 76-83.

[12] Kuglarz, M., Karakashev D., Angelidaki I. (2013) Microwave and thermal pretreatment as methods for increasing the biogas potential of secondary sludge from municipal wastewater treatment plants. Bioresource Technology., 134: 290-297.

[13] Fang, W., Zhang, P. Y., Zhang, G. M., Jin, S. G., Li, D. Y., Zhang, M. X., Xu, X. Z. (2014) Effect of alkaline addition on anaerobic sludge digestion with combined pretreatment of alkaline and high pressure homogenization. Bioresource Technology., 168: 167-172.

[14] Hendrickx, T.L.G., Temmink, H., Eisma, H. J. H., Buisman, C. J. N. (2010) Design parameters for sludge reduction in an aquatic worm reactor. Water Research., 44(3): 1017-1023.

[15] Rumky, J., Ncibi, M. C., Burgos-Castillo, R. C., Deb, A., Sillanpaa, M. (2018) Optimization of integrated ultrasonic-Fenton system for metal removal and dewatering of anaerobically digested sludge by Box-Behnken design. Science of the Total Environment., 645: 573-584.

[16] Donoso-Bravo, A., Fdz-Polanco M. (2013) Anaerobic co-digestion of sewage sludge and grease trap: Assessment of enzyme addition. Process Biochemistry., 48(5-6): 936-940.

[17] Yang, Q., Luo, K., Li, X. M., Wang, D. B., Zheng, W., Zeng, G. M., Liu, J. J. (2010) Enhanced efficiency of biological excess sludge hydrolysis under anaerobic digestion by additional enzymes. Bioresource Technology., 101(9): 2924-2930.

[18] Gao, L.C., Deng, K. J., Zheng, J. D., Liu, B., Zhang, Z. H. (2015) Efficient oxidation of biomass derived 5-hydroxymethylfurfural into 2,5-furandicarboxylic acid catalyzed by Merrifield resin supported cobalt porphyrin. Chemical Engineering Journal., 270: 444-449.

[19] Pires, S.M.G., Simoes, M. M. Q., Santos, Icms., Rebelo, S. L. H., Pereira, M. M., Neves, Mgpms., Cavaleiro, J. A. S. (2012) Biomimetic oxidation of organosulfur compounds with hydrogen peroxide catalyzed by manganese porphyrins. Applied Catalysis a-General., 439: 51-56.

[20] Linhares, M., Rebelo, S. L. H., Simoes, M. M. Q., Silva, A. M. S., Neves, Mgpms., Cavaleiro, J. A. S., Freire, C. (2014) Biomimetic oxidation of indole by Mn(III)porphyrins. Applied Catalysis a-General., 470: 427-433.

[21] Zucca, P., Rescigno, A., Rinaldi, A. C., Sanjust, E. (2014) Biomimetic metalloporphines and metalloporphyrins as potential tools for delignification: Molecular mechanisms and application perspectives. Journal of Molecular Catalysis A: Chemical., 388-389: 2-34.

[22] Liu, Q., Bai, X. H., Feng, G. X., Tan, Z., Jiang, Q., Guo, C. C. (2016) Biomimetic Conversion of Glucose to Organic Acid Facilitated by Metalloporphyrin under Mild Conditions. Bioresources., 11(4): 10251-10260.

[23] Serra, A.C., Docal C., Rocha Gonsalves A.M.d.A. (2005) Efficient azo dye degradation by hydrogen peroxide oxidation with metalloporphyrins as catalysts. Journal of Molecular Catalysis A: Chemical., 238(1-2): 192-198.

[24] APHA, (2005) Standard Methods for the Examination of Water and Wastewater. Washington DC, USA., American Public Health Association.