Use of anaerobic hydrolysis pretreatment to enhance ultrasonic disintegration of excess sludge

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ABSTRACT

To improve the excess sludge disintegration efficiency, reduce the sludge disintegration cost, and increase sludge biodegradability, a combined pretreatment of anaerobic hydrolysis (AH) and ultrasonic treatment (UT) was proposed for excess sludge. Results showed that AH had an advantage in dissolving flocs, modifying sludge characteristics, and reducing the difficulty of sludge disintegration, whereas UT was advantageous in damaging cell walls, releasing intracellular substances, and decomposing macromolecular material. The combined AH–UT process was an efficient method for excess sludge pretreatment. The optimized solution involved AH for 3 days, followed by UT for 10 min. After treatment, chemical oxygen demand, protein, and peptidoglycan concentrations reached 3,949.5 mg O_2/L, 752.5 mg/L and 619.1 mg/L, respectively. This work has great significance for further engineering applications, namely, reducing energy consumption, increasing the sludge disintegration rate, and improving the biochemical properties of sludge.

Key words | anaerobic hydrolysis, excess sludge, response surface methodology, ultrasonic treatment

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INTRODUCTION

Excess sludge, a by-product of the wastewater treatment process, is attracting increasing concern (Zhou *et al.* 2015). Excess sludge is agglomerated in a polymeric network formed by microbial extracellular polymeric substances (EPS) and cations (Frølund *et al.* 1996). Its major constituents are microbial cells and organic matter. Currently,1 resource reclamation from sludge is a common sludge disposal method, and sludge disintegration is an important pretreatment process used to completely release resources from sludge and increase the resource recovery efficiency.

The mechanical methods are commonly used for pretreatment. These methods are highly effective in solubilizing EPS and disintegrating microbial cells. However, the disadvantages of these methods during their practical application have caused great concern. For example, the high pressure homogenization method (Zhang *et al.* 2012) is easily blocked because sludge contains gravel. Meanwhile, the disperser (Devi *et al.* 2014) and deflaker (Kampas *et al.* 2007) do not easily affect microscopic bacteria by fluid shear stress. Finally, the energy utilization rate is low because of energy dissipation in the form of heat.

Alkaline pretreatment can induce the swelling of particulate organics at high pH, making the cellular substances more susceptible to enzymatic reactions (Baccay & Hashimoto 1984). Recently, this treatment has been combined with other disintegration methods, such as ultrasonic–alkaline (Jin *et al.* 2009; Kim *et al.* 2010; Li *et al.* 2010), high pressure homogenization–alkaline (Fang *et al.* 2014), and microwave–alkaline (Doğan & Sanin 2009) treatments. The aforementioned methods can be applied as a pretreatment method to disintegrate sludge flocs and disrupt bacterial cell walls.

Sludge hydrolysis is a well-known critical and time-consuming step of anaerobic digestion. In the hydrolysis step, insoluble organic material and high-molecular-weight compounds, such as lipids, polysaccharides, proteins, and nucleic acids, are transformed into soluble organic materials (Fang *et al.* 2014).

This study proposed to replace alkaline pretreatment with anaerobic hydrolysis (AH) for a short time period, followed by sludge disintegration with ultrasonic treatment (UT), because AH can dissolve microbial EPS and weaken

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the strength of bacterial cell walls in sludge and produce ethanol and organic acids, enhancing the effect of ultrasonic cavitation, whereas UT can damage bacterial cell walls and release cytoplasmic materials into the aquatic phase.

MATERIALS AND METHODS

Excess sludge

The seed sludge in this study was collected from a sewage plant. The active sludge was artificially cultivated in the laboratory. The characteristics of the raw (active) sludge in this study are presented in Table 1.

Experimental methods

A batch of AH experiments was carried out in a container with an effective volume of 5 L, stable temperature of 20 °C, and stirring speed of 120 rpm. Sludge degradation was performed by an ultrasonic cell disruption system with the frequency of 20 kHz and a probe of Φ20 mm (GM1200D, Shunmatech Ltd, China; Figure 1). The ultrasonic probe with a power of 60 W (max. 1,200 W, duty ratio is 1:1) was immersed 10 mm into the sludge with a volume of 800 mL (max. 2,000 mL).

Table 1 | Characteristics of the raw sludge

Parameters	Value	Units
TS	6,735 ± 300	mg/L
SCOD	127.5 ± 27.5	mg O_2/L
TCOD	6,680 ± 127.5	mg O_2/L
PR	35 ± 10	mg/L
PGN	0 + 1.8	mg/L

TS: total solids; SCOD: soluble chemical oxygen demand; TCOD: total chemical oxygen demand; PR: protein; PGN: peptidoglycan.

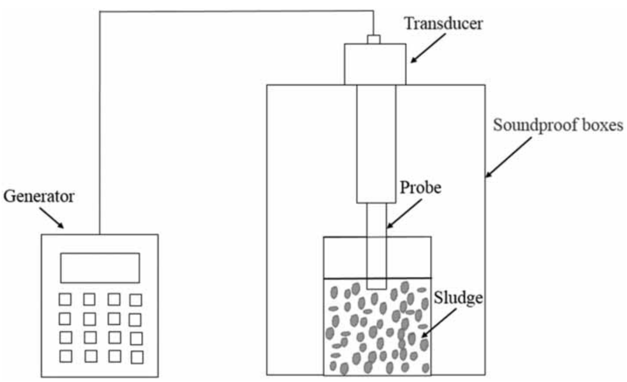

Figure 1 | Schematics of ultrasonic cell disruption system.

First, raw sludge was treated with AH for 0 (as control) or 3 days. A series of UTs were applied at different working times from 0 to 15 min. The influence of AH on UT for sludge disintegration was measured by changes in the soluble chemical oxygen demand (SCOD), protein (PR) and peptidoglycan (PGN). Subsequently, according to the above-mentioned results, the combined method at different intensity levels of AH and UT was investigated by response surface methodology (RSM). The relations between the time of AH and that of UT and optimum values of design variables were found by changes in the SCOD, PR and PGN. Finally, microscopic features of sludge disintegrated by the AH–UT process were contrasted.

In this study, all experiments were conducted in duplicates, and the average values were determined in triplicate for each set of parameters.

Disintegration degree

Sludge disintegration efficiency was represented by disintegration degree (DD_{COD}), which was calculated as in Equation (1) (Bougrier *et al.* 2005).

$$DD_{COD} = \frac{SCOD - SCOD_0}{TCOD - SCOD_0} \times 100\% \quad (1)$$

where $SCOD_0$: the COD of raw sludge; TCOD: total COD.

Specific energy

Optimization of energy consumption for efficient sludge disintegration is significantly important, so specific energy for the combined process was considered as an important parameter in analysis (Bougrier *et al.* 2005) and was defined in accordance with (Equation (2)).

$$E_S = \frac{P_1 t_1 + P_2 t_2}{v \cdot TS} \quad (2)$$

where E_S: specific energy (J/g·TS); P_1: power of ultrasonic equipment (W); P_2: power of stirrer (W); t_1: time of UT (s); t_2: time of AH (s); v: sample volume (L); TS: initial total solid concentration (g/L).

Analytical methods

The microbial cell walls protect the cells from direct physical and chemical attack to limit the rate and extent of organic degradation in anaerobic sludge digestion (Rajagopal & Béline 2011). The predominant component of active sludge is Gram-negative bacteria (Lu *et al.* 2012), and cell walls of Gram-negative bacteria are 5–20% PGN. Thus, PGN can be used to evaluate the degree of cell wall degradation in sludge.

The sludge sample was centrifuged at 9,000 rpm for 10 min with a centrifuge (3H16RI, Hersey Ltd, China). SCOD in the supernatant was measured according to the American Public Health Association closed reflux method (Clesceri *et al.* 1998). The sludge was treated by alkali with 0.5 mol/L NaOH for 24 h, and the supernatant was sampled to determine TCOD (Fang *et al.* 2014). PR in the supernatant was measured according to the Folin–phenol method (Lowry *et al.* 1951). PGN in the supernatant was measured by colorimetry (Elson & Morgan 1933). The microscopic features of sludge disintegration were measured using a transmission electron microscope (TEM; JEM-1200EX, Japan).

RESULTS AND DISCUSSION

Effects of AH–UT on the chemical composition

SCOD is a key parameter to evaluate soluble organics in the liquid phase. PR was estimated to account for approximately 50% of the dry weight of bacterial cells (Shier & Purwono 1994), and PGN is the main part of cell wall. All of these can be used to evaluate the degree of cell degradation in sludge. As Figure 2 shows, after applying UT for 15 min (control), SCOD, PR and PGN increased to 2,348 mg O_2/L, 564.7 mg/L and 387.2 mg/L, respectively. UT can release soluble organics from the solids to the liquid phase (Jiang *et al.* 2014).

The SCOD was 1,027.5 mg O_2/L in the sludge after applying AH for 3 days, which was 8.1 times that of raw sludge (127.5 mg O_2/L). This change indicated that organic matter in the solid phase of sludge was dissolved in the liquid phase, and the weak and sensitive cells were selectively disrupted (Doğan & Sanin 2009) by AH. Meanwhile, PR reached 6.5 times that of the raw sludge (35 mg/L), but PGN only had a small increase. This difference was probably because AH significantly affected bacterial EPS in sludge but did not destroy the bacterial cell walls.

The changes in PR and PGN were consistent with that in SCOD; that is, SCOD significantly increased with increasing UT time. After AH–UT for 15 min, SCOD, PR and PGN were 5,202.5 mg O_2/L, 954.2 mg/L and 682.9 mg/L, which were 2.1, 1.7 and 1.8 times, respectively, that of the control for the same treatment time. These results suggest that AH reduced the difficulty of cell wall disintegration in sludge. But the energy consumption of UT in this process is very large. In the AH–UT method, AH can reduce the difficulty of solids disintegration and enhance the effect of ultrasonic cavitation (Nie *et al.* 2013), thus providing a basis for the large-scale application of UT in sludge degradation.

The AH–UT process dissolved flocs, broke the bacterial cell walls, caused the outflow of cytoplasm from sludge bacteria into the liquid phase, and made them more susceptible to attack by enzymes to enhance the rate of hydrolysis before fermentation (Xu *et al.* 2012), thereby improving anaerobic digestion. The combined AH–UT process is an efficient sludge pretreatment method.

Optimized analysis by RSM

RSM is a series of statistical techniques for designing experiments, building empirical models, and evaluating the

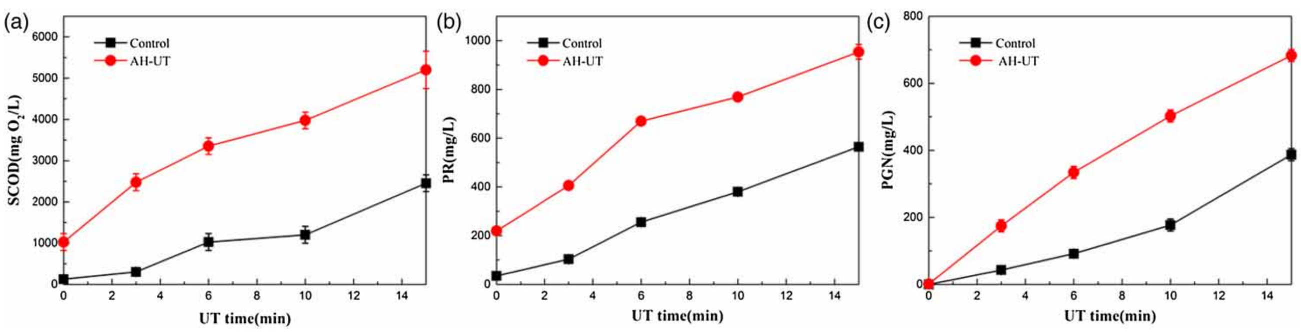

Figure 2 | Effects of AH–UT on SCOD, PR, and PGN.

combined effects of factors. A central composite design (CCD) of Design-Expert (Stat-Ease, Inc., USA), one of the most popular second-order designs, was used for RSM in the present study. Based on the previous experiment (in which a shorter time period for AH could not produce the desired effects, whereas UT after a longer period of AH was meaningless), CCD was used to analyze sludge degradation under different time periods of AH and UT in this research (Table 2).

The experimental results were analyzed by RSM to obtain empirical models for the responses. To obtain the optimized sludge degradation, the quadratic polynomial equations (Equations (3)–(5)) for the responses related to the varied parameters were established:

$$\mathrm{SCOD} = -503.3 + 1313.2X_1 + 83.6X_2 + 7.2X_1X_2 - 118.8{X_1}^2 + 5.3{X_2}^2 \quad (3)$$

$$\mathrm{PR} = 229.9 + 49.0X_1 + 17.9X_2 + 6.5X_1X_2 \quad (4)$$

$$\mathrm{PGN} = 27.3 - 39.6X_1 + 10.7X_2 + 20.1X_1X_2 \quad (5)$$

Table 2 | Experimental range and levels of the independent test variables

Variables	Factor	Units	Low	High	−α	+α
AH time	X_1	day	1	5	0.1716	5.828
UT time	X_2	min	5	15	2.929	17.071

The summary of an analysis of variance (ANOVA) is important in determining the adequacy and significance of a predictive model (Table 3). The *F*-values of the three models implied the significance of each. Values of '$P > F$' less than 0.05 indicated that the model terms were significant. In this case, X_1, X_2, and X_1^2 were significant model terms because their values were smaller than 0.1. The 'lack of fit *F*-values' implied that the lack of fit was significant with only 0.04, 1.54, and 0.01% chances that a 'Lack of Fit *F*-value' this large could be attributed to noise.

For SCOD and PR, the predicted R^2 values were in reasonable agreement with the adjusted R^2 values. However, the predicted R^2 value for PGN was not as close to its adjusted R^2 value. The value for 'adequate precision indicated an adequate signal. Therefore, this model could be used to navigate the design space.

The 2D contour plots describing the trend of the chemical index with respect to AH and UT levels are shown in Figure 3 When the SCOD, PR and PGN were constant, with the increase of the time of AH, the time needed for UT gradually reduced, and during the first 3 days of AH, the time needed for UT reduced quickly, then gradually slowed down. An interaction between AH and UT was observed. AH–UT reduced energy consumption and increased sludge degradation compared with UT alone. The combined method increased the required time (by a few days of AH) to reduce the energy consumption of degradation and greatly improved the degradation efficiency.

Table 3 | ANOVA for sludge degradation of AH and UT

Source	SCOD		PR		PGN	
	F-value	$P > F$	*F*-value	$P > F$	*F*-value	$P > F$
Model	52.13	<0.0001	79.73	<0.0001	22.61	0.0002
X_1	149.17	<0.0001	139.39	<0.0001	28.23	0.0005
X_2	92.26	<0.0001	94.10	<0.0001	34.12	0.0002
X_1X_2	0.21	0.6582	5.68	0.0410	5.47	0.0441
X_1^2	16.23	0.0050	–	–	–	–
X_2^2	1.27	0.2977	–	–	–	–
Lack of fit	86.29	0.0004	12.29	0.0154	253.84	<0.0001
Standard deviation	311.23		54.75		172.04	
R^2	0.9738		0.9637		0.8828	
Adjusted R^2	0.9552		0.9516		0.8438	
Predicted R^2	0.8162		0.8870		0.5705	
Adequate precision	23.560		27.416		14.483	
Coefficient of variation %	8.32		7.27		27.79	

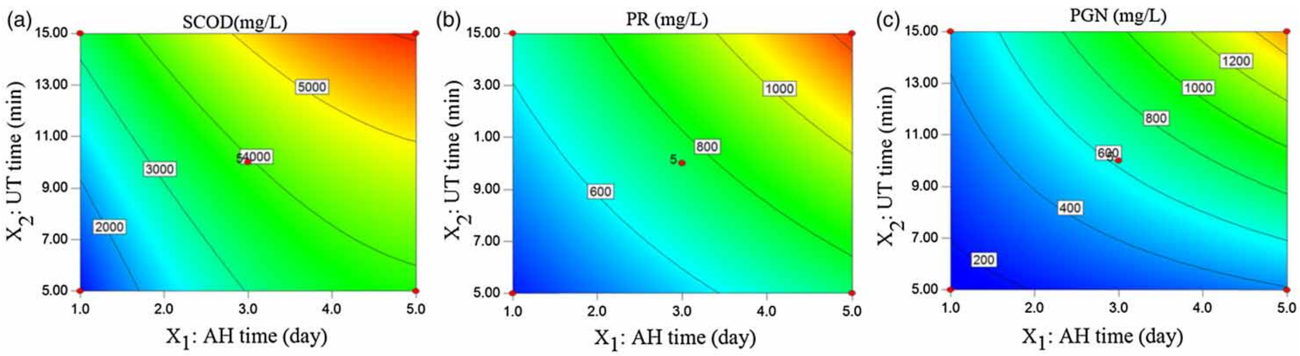

Figure 3 | Contour plots of interactive effects of AH and UT on RSM.

Experimental results showed that the optimized pretreatment was AH for 3 days, followed by UT for 10 min. At this point, AH was the most influential on UT: the time of AH and UT was relatively small, but the sludge disintegration efficiency was relatively high. To confirm the validity of the statistical experimental strategy, a batch of experiments was conducted under the optimal conditions, in which SCOD, PR, and PGN reached 3,949.5 mg O_2/L, 752.5 mg/L, and 619.1 mg/L, respectively. Therefore, the optimization model was correct.

The effects of AH alone compared with those of alkaline treatment with ultrasonic degradation (Kim *et al.* 2010) are shown in Table 4. The DD_{COD} values of sludge treated with AH for 3 days and that of sludge treated with alkali at pH 10 were similar. The alkaline condition of the sludge enhanced the disintegration efficiency of the subsequent UT. However, the degradation efficiency of AH–UT was higher than that of alkaline–UT. This difference might be attributed to the fact that anaerobic sludge digestion produced ethanol, organic acids, and CO_2, thereby enhancing the effect of ultrasonic cavitation (Nie *et al.* 2013). The E_S indicated that the energy consumption of AH–UT was larger than that of alkaline–UT to achieve the same DD_{COD}. However when DD_{COD} was 58.3%, UT consumed 6,716 J/g·TS and the stirrer consumed 7,737 J/g·TS in the AH-UT process. In addition, the alkaline–UT method can cause secondary pollution, increase input costs, etch ultrasonic equipment and seriously affect sludge biodegradability because of the high pH levels. Therefore, AH–UT is a highly effective technology for further engineering applications.

Table 4 | Effects of AH–UT and alkaline–UT

Treatment	DD_{COD} by alkaline or AH	DD_{COD} by UT	DD_{COD} by combined treatment	E_s (J/g TS)
Alkaline at pH 12*	21.4	19.5	59.8	7,500
Alkaline at pH 11*	17.1	41.1	66.0	30,000
Alkaline at pH 10*	12.3	19.5	50.5	7,500
AH for 3 d	13.5	16.4	58.3	14,453

*Results are from Kim *et al.* (2010).

Effects of AH–UT on physical changes

In this study, the morphological characteristics of sludge degradation were observed by TEM. The morphological changes can further indicate the response to sludge degradation.

A large amount of EPS was present on the bacterial cellular walls in raw sludge. Bacteria had aggregated to form flocs bound by EPS (Figure 4(a)). After AH for different time periods, the bacterial EPS was dissolved, and the bacteria became more evenly and uniformly dispersed with the increase in AH time (Figure 4(b) and 4(c)). AH significantly affected bacterial cells and EPS in sludge, which increased SCOD and PR in the sludge supernatant (Figure 2(a) and 2(b)). However, the effect of AH on the dissolution of PGN in sludge was not significant (Figure 2(c)). Therefore, AH affected sludge degradation by mainly affecting EPS degradation.

Ultrasonic cavitation could disperse the sludge floc structure, damage bacterial cells, and release intracellular substances. However, ultrasonic cavitation caused the formation of numerous holes and did not fully break down flocs and the bacterial cell structure. A large number of ultrasonic cavitation bubbles were consumed because the flocs and bacteria in the sludge were heterogeneous (Figure 5(a) and 5(b)). The combined AH–UT process had the combined advantages of AH (dissolving flocs, modifying sludge characteristics, and reducing sludge

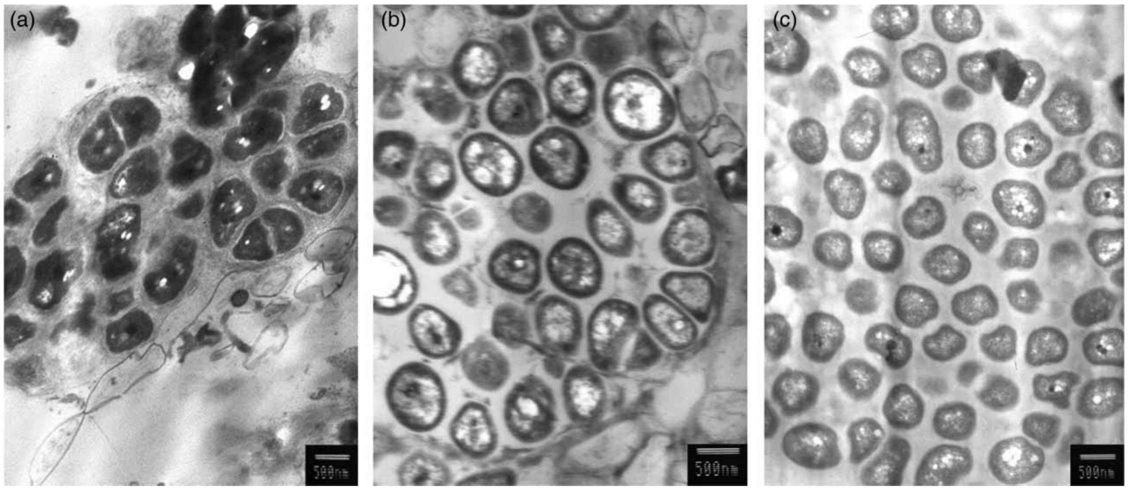

Figure 4 | TEM micrographs after AH: (a) raw sludge, (b) after AH for 3 days, (c) after AH for 5 days.

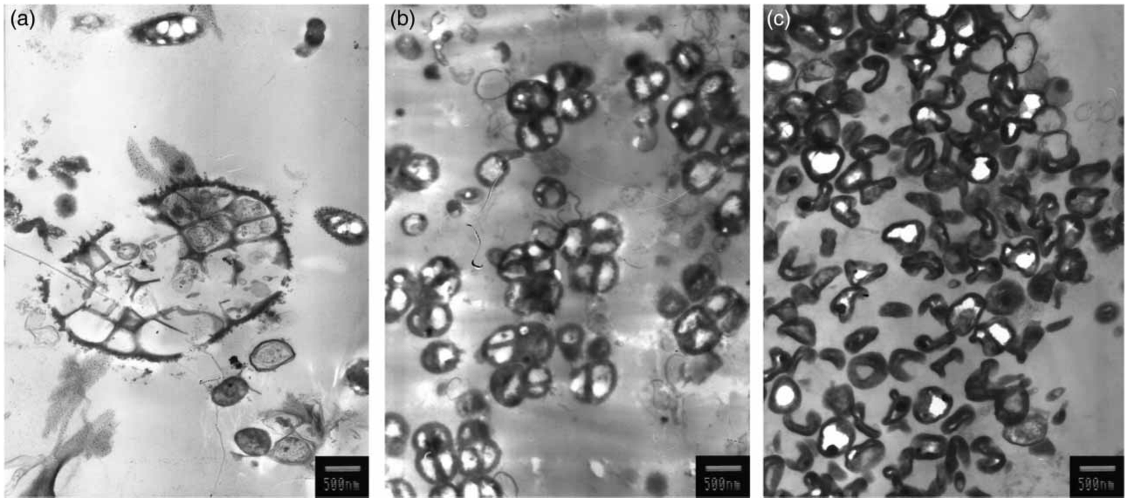

Figure 5 | TEM micrographs after treatments: (a) flocs after UT for 7 min, (b) bacteria after UT for 7 min, (c) bacteria after AH for 3 days followed by UT for 7 min.

disintegration difficulty) and UT (damaging cell walls, releasing intracellular substances, and degrading macromolecular materials; Figure 5(c)). The combined AH–UT process is an efficient method for excess sludge pretreatment.

CONCLUSIONS

The advantages of AH are its ability to dissolve flocs, modify sludge characteristics, and reduce sludge disintegration difficulty. The advantages of UT are its ability to damage cell walls, release intracellular substances, and decompose macromolecular materials. Applying AH for 3 days followed by UT for 15 min, resulted in SCOD, PR and PGN of 5,202.5 mg O_2/L, 954.2 mg/L and 682.9 mg/L, which were 2.1, 1.7 and 1.8 times, respectively, those of only UT for 15 min. Thus, the combined AH–UT process is an efficient sludge pretreatment method accompanied by changes in the chemical, physical and biological characteristics of sludge. The optimum degradation condition derived via RSM was AH for 3 days, followed by UT for 10 min. At this point, SCOD, PR, and PGN reached 3,949.5 mg O_2/L, 752.5 mg /L, and 619.1 mg/L, respectively. This method has great significance for further engineering applications by reducing energy consumption, increasing disintegration rate, and improving the biochemical properties of sludge.

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REFERENCES

Baccay, R. A. & Hashimoto, A. G. 1984 Acidogenic and methanogenic fermentation of causticized straw. *Biotechnol. Bioeng.* **26** (8), 885–891.

Bougrier, C., Carrere, H. & Delgenes, J. P. 2005 Solubilisation of waste-activated sludge by ultrasonic treatment. *Chem. Eng. J.* **106** (2), 163–169.

Clesceri, L. S., Greenberg, A. E. & Eaton, A. D. 1998 *Standard Methods for the Examination of Water and Wastewater*, 20th edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

Devi, T. P., Ebenezer, A. V., Kumar, S. A., Kaliappan, S. & Banu, J. R. 2014 Effect of deflocculation on the efficiency of disperser induced dairy waste activated sludge disintegration and treatment cost. *Bioresour. Technol.* **167** (8), 151–158.

Doğan, I. & Sanin, F. D. 2009 Alkaline solubilization and microwave irradiation as a combined sludge disintegration and minimization method. *Water Res.* **43** (8), 2139–2148.

Elson, L. A. & Morgan, W. T. J. 1933 A colorimetric method for the determination of glucosamine and chondrosamine. *Biochem. J.* **27** (6), 1824–1828.

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. *Bioresour. Technol.* **168** (6), 167–172.

Frølund, B., Palmgren, R., Keiding, K. & Nielsen, P. H. 1996 Extraction of extracellular polymers from activated sludge using a cation exchange resin. *Water Res.* **30** (8), 1749–1758.

Jiang, J. G., Gong, C. X., Wang, J. M., Tian, S. C. & Zhang, Y. J. 2014 Effects of ultrasound pre-treatment on the amount of dissolved organic matter extracted from food waste. *Bioresour. Technol.* **155**, 266–271.

Jin, Y. Y., Li, H., Mahar, R. B., Wang, Z. Y. & Nie, Y. F. 2009 Combined alkaline and ultrasonic pretreatment of sludge before aerobic digestion. *J. Environ. Sci. (China).* **21** (3), 279–284.

Kampas, P., Parsons, S. A., Pearce, P., Ledoux, S., Vale, P., Churchley, J. & Cartmell, E. 2007 Mechanical sludge disintegration for the production of carbon source for biological nutrient removal. *Water Res.* **41** (8), 1734–1742.

Kim, D. H., Jeong, E., Oh, S. E. & Shin, H. S. 2010 Combined (alkaline + ultrasonic) pretreatment effect on sewage sludge disintegration. *Water Res.* **44** (10), 3093–3100.

Li, C. L., Liu, G. F., Jin, R. F., Zhou, J. T. & Wang, J. 2010 Kinetics model for combined (alkaline + ultrasonic) sludge disintegration. *Bioresour. Technol.* **101** (22), 8555–8557.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. 1951 Randall protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193** (1), 265–275.

Lu, L., Xing, D. F. & Ren, N. Q. 2012 Pyrosequencing reveals highly diverse microbial communities in microbial electrolysis cells involved in enhanced H_2 production from waste activated sludge. *Water Res.* **46** (7), 2425–2434.

Nie, Y., Huang, Y. H., Li, B., Yu, Z. J. & Zhu, C. X. 2013 Surfactant–ultrasonic synergy to extract total flavonoids also technology research. *Chin. Tradit. Pat. Med.* **35** (9), 2040–2042 (in Chinese).

Rajagopal, R. & Béline, F. 2011 Anaerobic hydrolysis and acidification of organic substrates: determination of anaerobic hydrolytic potential. *Bioresour. Technol.* **102** (10), 5653–5658.

Shier, W. T. & Purwono, S. K. 1994 Extraction of single-cell protein from activated sewage sludge: thermal solubilization of protein. *Bioresour. Technol.* **49** (2), 157–162.

Xu, S. Y., Karthikeyan, O. P., Selvam, A. & Wong, J. W. C. 2012 Effect of inoculum to substrate ratio on the hydrolysis and acidification of food waste in leach bed reactor. *Bioresour. Technol.* **126**, 425–430.

Zhang, S., Zhang, P. Y., Zhang, G. M., Fan, J. & Zhang, Y. X. 2012 Enhancement of anaerobic sludge digestion by high-pressure homogenization. *Bioresour. Technol.* **118** (6), 496–501.

Zhou, A. J., Luo, H. C., Varrone, C., Wang, Y. Z., Liu, W. Z., Wang, A. J. & Yue, X. P. 2015 Enhanced anaerobic digestibility of waste activated sludge by plant-derived biosurfactant. *Process Biochem.* **50** (9), 1413–1421.

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