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Effect of alkyl polyglycosides on the performance of thermophilic bacteria pretreatment for saline waste sludge hydrolysis

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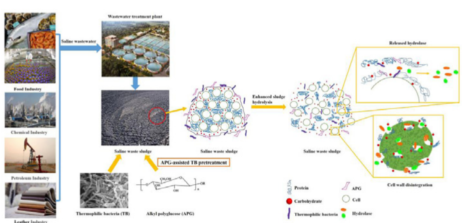
GRAPHICAL ABSTRACT



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ABSTRACT

In this study, alkyl polyglycosides (APG) was used to further accelerate the hydrolysis of saline waste sludge with thermophilic bacteria (TB) pretreatment. In the presence of 0.4 g/g TSS APG, the concentrations of soluble chemical oxygen demand (SCOD), soluble carbohydrate and soluble protein in dissolved organic matters (DOM) were 0.4, 2.4 and 1.3 times of that without APG addition, respectively. Excitation emission matrix (EEM) fluorescence spectroscopy revealed that the addition of APG led to the increase of soluble microbial materials and the decrease of fulvic acid-like substances in DOM, which was beneficial for the subsequent process of anaerobic digestion. Using APG promoted the releasing of enzymes trapped in saline waste sludge and improved the activity of enzymes during hydrolysis. The activities of α-glucosidase and protease increased by 8.8% and 21.3% respectively in the presence of 0.4 g/g TSS APG comparing no APG addition.

1. Introduction

Nowadays, more saline wastewater streams are generated by several industries such as the fish processing (Rio et al., 2018), chemical, agro-food, petroleum, and leather industry (Shi et al., 2015). On the other hand, to alleviate freshwater shortage, seawater has been increasingly used to substitute freshwater for domestic purposes, such as lavatories flushing in Hong Kong (Hulsen et al., 2019). In recent decades, the inflow of saline and hypersaline wastewater to treatment plants has increased considerably, representing as much as 5% of the worldwide wastewater treatment streams (Ismail et al., 2010). Consequently, large quantities of waste activated sludge with salinity was produced, which is a challenge for waste sludge disposal (Zhang et al., 2017a).

Anaerobic digestion of waste activated sludge could reduce environmental pollution and recover energy, and the hydrolysis step is generally considered as the rate-limiting step (Wei et al., 2018; Zhen

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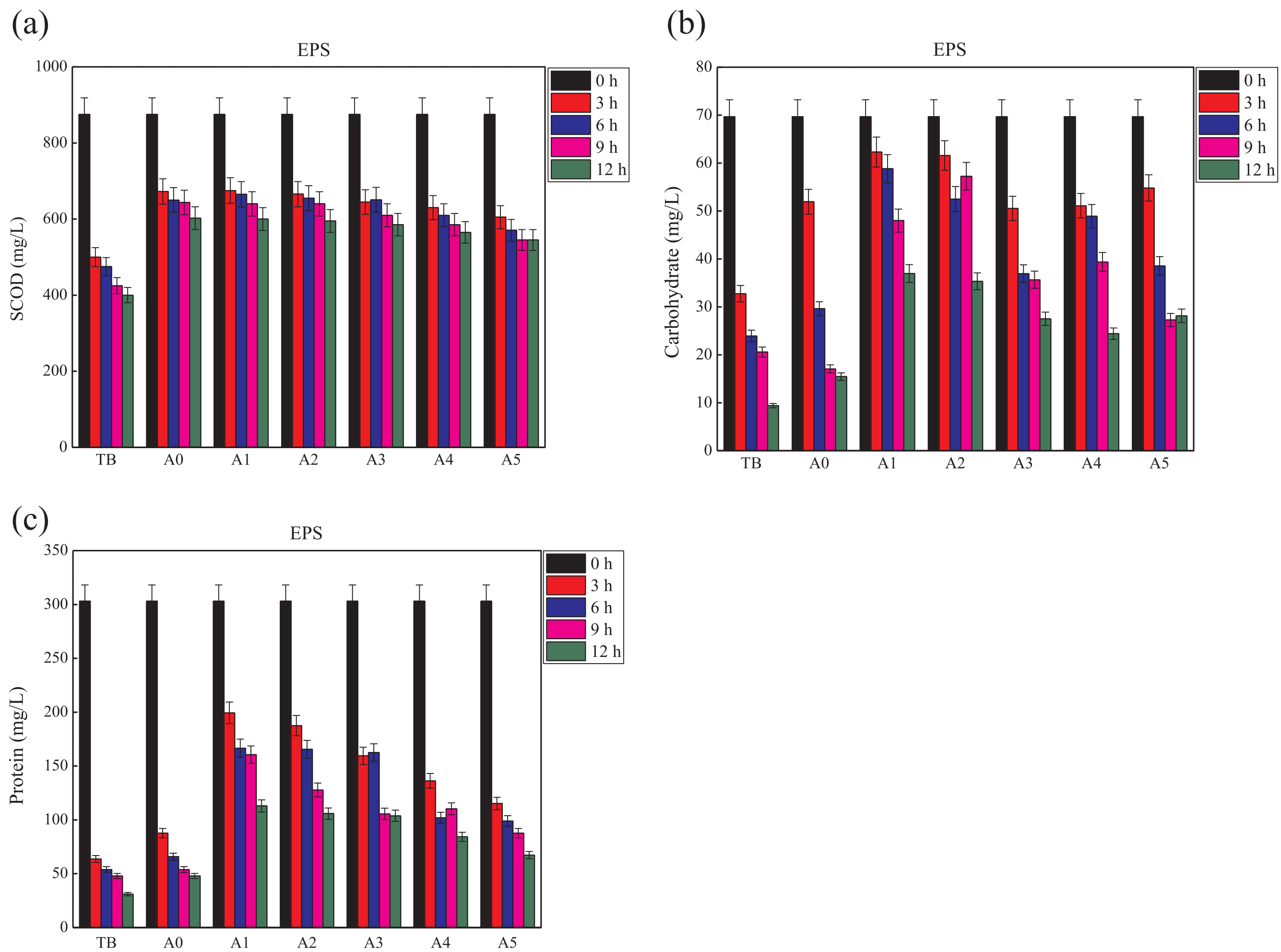

Fig. 1. The effect of APG on SCOD (a), carbohydrate (b), and protein (c) release in EPS with TB pretreatment. The error bar represents the standard errors.

et al., 2017). Recently, the disposal of saline waste sludge using anaerobic digestion has drawn growing attention, and the effect of salinity on the anaerobic digestion of waste sludge has been investigated extensively (Jin et al., 2016; Zhang et al., 2017a; Zhao et al., 2016). However, few studies were conducted to accelerate the hydrolysis of saline waste sludge. To release sufficient cellular organic materials into liquid, various pretreatment methods, such as mechanical, thermal, chemical, biological processes or integrations of these, have been studied to accelerate the hydrolysis and enhance subsequent biogas productivity (Lin and Li, 2018; Sun et al., 2016; Yang et al., 2015a). Among these approaches, thermophilic bacteria (TB) is regarded as an economical and promising pretreatment method having the advantages of highly efficient enhancement of solid reduction, simple control requirements and better prospect of application (Zheng et al., 2014). Moreover, TB can excrete exoenzyme to lyse the cells of other microorganisms, break the polymeric substances, hydrolyses large complicated molecules to simpler ones (Kavitha et al., 2016; Yang et al., 2015b). In our previous study, TB pretreatment was proved to be an efficient method to promote the saline waste sludge hydrolysis, but the performance of TB pretreatment was limited with high salinity ($>2.0\%$) (Gao et al., 2019).

Recently, alkyl polyglycosides (APG) has been applied to improve the short-chain fatty acid production and organic matter degradation of waste sludge (Luo et al., 2015; Zhao et al., 2015a). However, the application of APG to enhance the hydrolysis of saline waste sludge has never been reported, and the substrate metabolism process of saline waste sludge assisted by APG was still unclear. As an emerging nonionic biosurfactant, APG possesses the advantage of low toxicity, high biodegradability and good ecological compatibility (Zhang et al., 2011). It was reported that APG can promote the processes of solubilization and hydrolysis during organic matter anaerobic digestion, thereby reducing the time of the anaerobic digestion process (Xiao et al., 2017). APG has the ability to liquefy a larger amount of soluble organics since it has the feature of solubilization (Zhao et al., 2015b), and therefore hasten the liquefaction rate of solid substances into the liquid phase. Moreover, APG is able to weaken the immobilization of floc matrix, set free the trapped enzyme (within the floc matrix and on the cell surface), and also release more organics from the floc matrix (Xiao et al., 2017). In view of the unique properties of APG, APG pretreatment is a feasible and promising strategy to promote the sludge hydrolysis.

In this study, APG was applied to promote the hydrolysis of saline waste sludge with TB pretreatment. The mechanism of sludge hydrolysis accelerated by APG coupled with TB was explored from the variation of soluble protein, soluble carbohydrate and soluble chemical oxygen demand (SCOD) in extracellular polymeric substances (EPS) and dissolved organic matters (DOM). Meanwhile, the activities of protease and α-glucosidase with different pretreatments were evaluated to reveal the microbial activities. The characteristics of EPS and DOM were also investigated using excitation-emission matrix (EEM) fluorescence spectroscopy with fluorescence regional integration, which was important to understand the compositional and structural characteristics of EPS and DOM. The results obtained in this work could provide valuable information for the disposal of saline waste sludge and the application of APG and TB pretreatment.

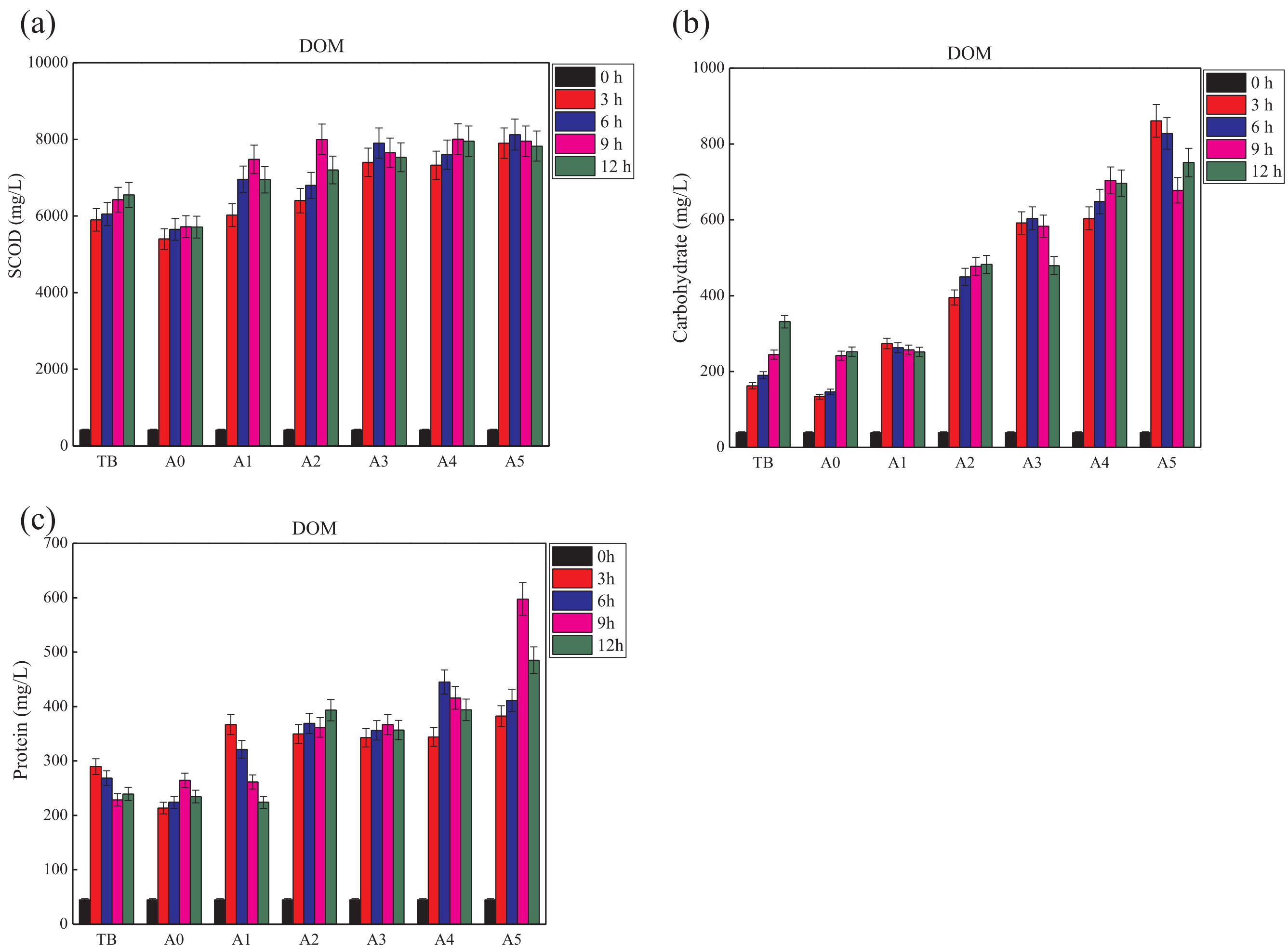

Fig. 2. The effect of APG on the variation of SCOD (a), carbohydrate (b), and protein (c) in DOM with TB pretreatment. The error bar represents the standard errors.

2. Material and methods

2.1. Sludge source, inoculum and APG

The waste sludge was obtained from the secondary sedimentation tank of the Tuandao municipal wastewater treatment plant (Qingdao, China), whose operation mode was anaerobic-anoxic-oxic (A^2O). The precipitate in sludge was firstly removed by screening through a 2.0 mm sieve and then stored at 4 °C for later use. The raw sludge had a pH of 7.0 ± 0.4, suspended solids (SS) of 12.1 ± 0.6 g/L, volatile suspended solids (VSS) of 10.1 ± 0.5 g/L, SCOD of 412.5 ± 20.6 mg/L, soluble protein of 44.8 ± 2.2 mg/L, and soluble carbohydrate of 39.1 ± 2.0 mg/L.

The thermophilic bacterial strain used for accelerating sludge hydrolysis was *Bacillus* sp. AT07-1, which was isolated according to our previous work (Guo et al., 2010). The strain was cultivated in 250 mL flask with 100 mL of Luria-Bertani liquid medium at 65 °C, with shaking in an orbital shaker at 140 r/min for 48 h. After cultivation, the culture was harvested and was used as the inoculum for sludge hydrolysis. The cell concentration of the bacterial suspension is 5.3×10^7 cells/mL. The Luria-Bertani liquid medium contain: yeast extract 5.0 g; tryptone 10.0 g; NaCl 10.0 g; distilled water 1000 mL.

The biosurfactant APG was obtained from Lusen Chemical Incorporation Ltd. (Shandong Province, China). The main characteristics of APG were as follows: solid content 50%, density 1.10 g/cm^3.

2.2. Experimental design

Batch experiments were conducted to investigate the effect of APG on the hydrolysis of saline waste sludge with TB pretreatment. Prior to use, the salinity of saline waste sludge was adjusted to 2.0% by adding seawater crystal into raw sludge. 200 mL of saline waste sludge was taken in six identical serum bottles with a working volume of 250 mL each. After 5 mL of bacteria suspension (strain AT07-1) was inoculated into saline waste sludge, APG was dosed to each serum bottle with its dosage of 0.05, 0.1, 0.2, 0.3 and 0.4 g/g TSS, respectively. The blank tests were conducted without APG simultaneously. To investigated the effect of salinity (2.0%) on the sludge hydrolysis with TB pretreatment, the blank tests without seawater crystal and APG were conducted, which was named as TB. The 250 mL serum bottles containing 0, 0.05, 0.1, 0.2, 0.3 and 0.4 g/g TSS were named as A0 (TB + 0 g/g TSS APG), A1 (TB + 0.05 g/g TSS APG), A2 (TB + 0.1 g/g TSS APG, A3 (TB + 0.2 g/g TSS APG), A4 (TB + 0.3 g/g TSS APG), A5 (TB + 0.4 g/g TSS APG), respectively. The waste sludge was hydrolyzed in a water bath shaker for 12 h at a constant temperature of 65 °C and an agitation rate of 140 r/min. The samples were extracted every 3 h to measure the SCOD, soluble carbohydrate, soluble protein and the EEM fluorescence spectroscopy in EPS and DOM. All the experiments were performed in triplicate.

2.3. Analytical methods

2.3.1. Chemical analysis

The pH value was measured by a pH meter (PHB-5, Aolilong,

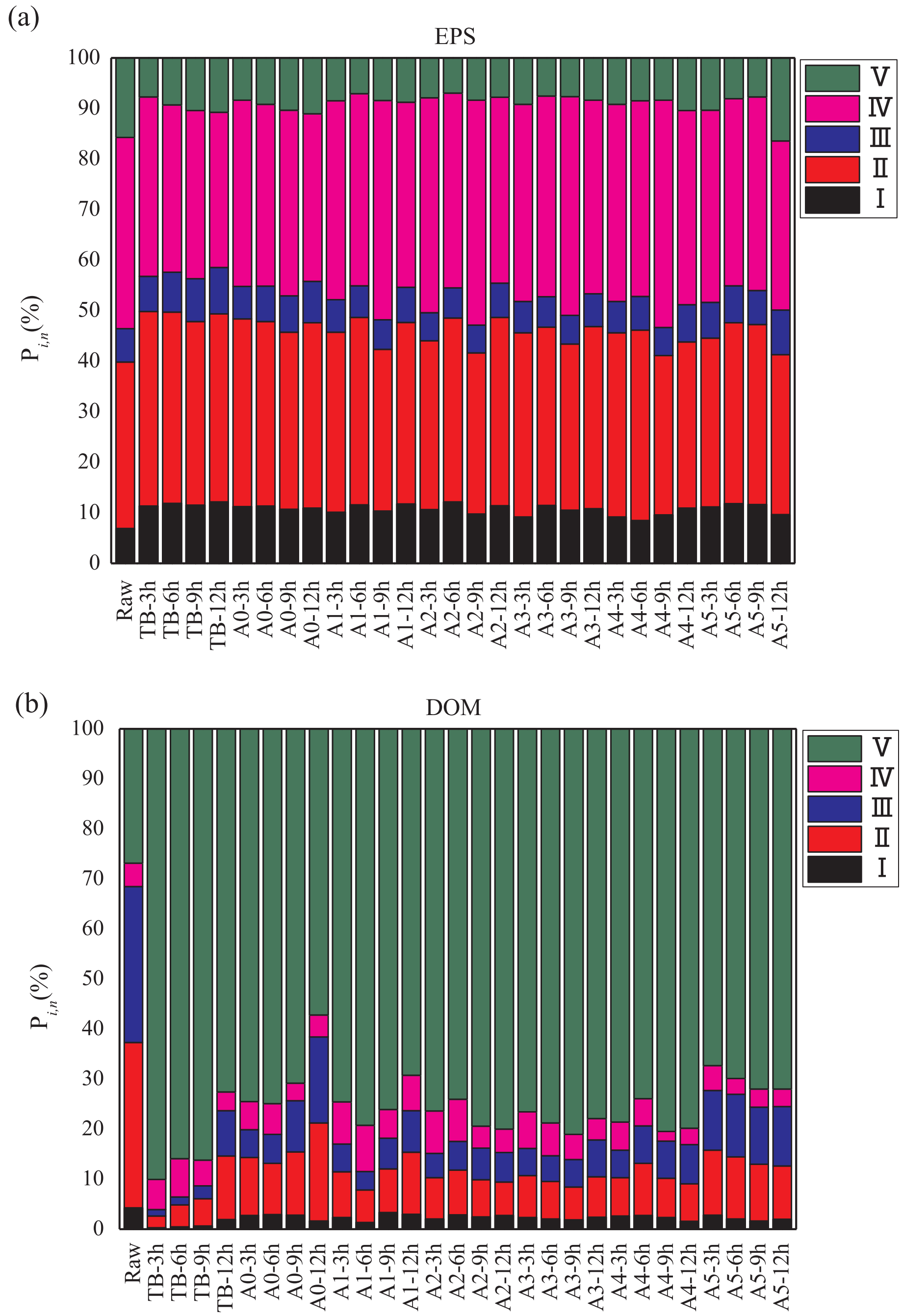

Fig. 3. The effect of APG on the distribution of fluorescence regional integration in EPS (a) and DOM (b) with TB pretreatment.

Hangzhou). SS, VSS, and SCOD were measured by the procedure of Standard Methods (APHA et al., 2005). The concentrations of soluble carbohydrate and soluble protein were quantified using the Phenol-sulfuric acid and Lowry's method (Yi et al., 2013). The activities of α-glucosidase and protease were determined according to previous work (Lin and Li, 2018), using 0.1% *p*-nitrophenyl-α-D-glucopyranoside and 0.5% azocasein, respectively, as the substrates for the reaction.

2.3.2. The extraction of EPS and DOM

EPS extraction: The EPS of saline waste sludge were extracted using a heat extraction method as described by our previous study (Guo et al., 2014). The sludge samples were first centrifuged (8000 r/min, 10 min) to remove the bulk solution. After discarding the supernatant, the sediment was washed thrice with a 2.0% NaCl solution. The remaining solids were re-suspended to the original volume using a 2.0% NaCl solution and then were subjected to water bath for 10 min at 80 °C. After centrifuging (8000 r/min, 10 min), the supernatants were filtered with a 0.45 μm cellulose acetate membrane and collected as soluble EPS (Guo et al., 2018).

DOM extraction: The DOM was obtained as described by our previous study (Guo et al., 2014). The sludge samples were centrifuged at 8000 r/min for 10 min at first. Then the centrifuged supernatant passed through a 0.45 μm cellulose acetate membrane and the filtrates were used for further analysis.

2.3.3. EEM fluorescence spectroscopy and fluorescence regional integration analysis

Fluorescence measurements were conducted using a Hitachi F-4500 spectrofluorometer (Tokyo, Japan) at 24 °C. The EEM spectra were collected with subsequent scanning emission spectra from 200 to

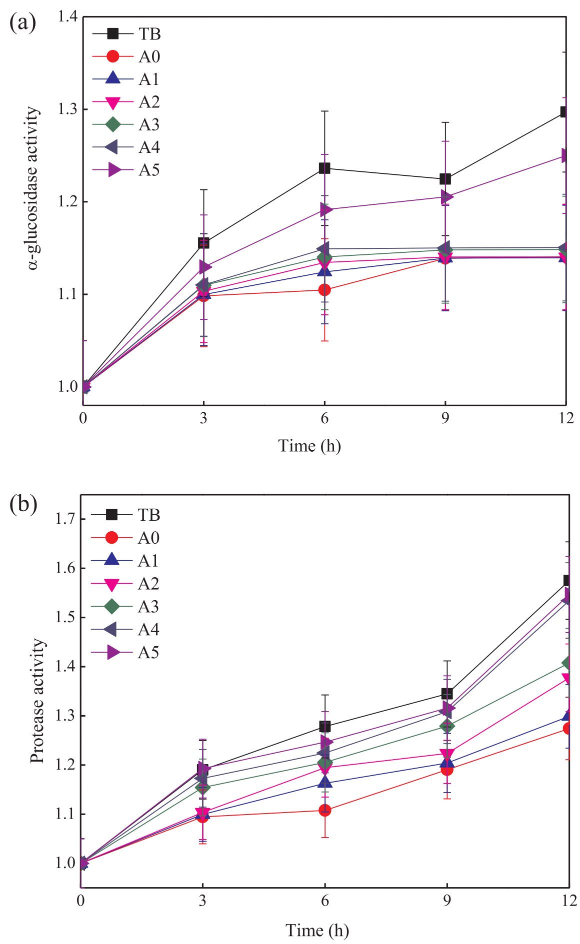

Fig. 4. The effect of APG on the activities of α-glucosidase (a) and protease (b) with TB pretreatment. The error bar represents the standard errors.

500 nm at 5 nm increments by varying the excitation wavelength from 200 to 400 nm in 5 nm increments. A 5 nm band-pass of excitation and emission slits and 1200 nm/min of scanning speed were set in this study. The Raman scattering was removed by subtracting the blank spectrum (pure distilled water) from the samples, and the Rayleigh scattering was overcome by interpolation. The EEM spectrum was delineated into five regions, i.e. tyrosine-like proteins (Region I, E_x/E_m wavelengths: 200–250/200–330), tryptophan-like proteins (Region II, E_x/E_m wavelengths: 200–250/330–380), fluvic acid-like materials (Region III, E_x/E_m wavelengths: 200–250/380–500), soluble microbial by-product materials (Region IV, E_x/E_m wavelengths: 250–280/200–380 nm), and humic acid-like substances (Region V, E_x/E_m wavelengths: 250–400 nm/380–250 nm nm). The EEM data were further analyzed using fluorescence regional integration technique. The calculation of percent fluorescence response ($P_{i,n}$, %) was in accordance with our previous study (Zhang et al., 2017b).

3. Results and discussion

3.1. The releasing of EPS

EPS were complex high-molecular-weight mixture of polymers excreted by microorganisms, which could be hydrolyzed to serve as carbon and energy source for the gas production in anaerobic digestion process (Ye et al., 2018). For the raw sludge, the concentrations of SCOD, soluble carbohydrate and soluble protein in EPS were 875.0 mg/L, 69.7 mg/L and 303.1 mg/L, respectively. As shown in Fig. 1, compared with the no saline sludge, increased content of SCOD, soluble carbohydrate, and soluble protein in EPS was observed in saline waste sludge with TB pretreatment. This may attribute to the osmotic pressure created by salinity could stimulate the microorganisms to produce EPS for self-protection (Abbasi and Amiri, 2008). For the saline waste sludge without APG addition, the concentration of SCOD in EPS reached 602.5 mg/L with TB pretreatment. In comparison, the addition of APG led to a decrease in SCOD content in EPS. After 12 h, the concentration of SCOD reached 545 mg/L in the presence of 0.4 g/g TSS APG. Protein and carbohydrate are the main components of EPS and the main substrates in the sludge digestion system (Lu et al., 2018). For the saline waste sludge without APG addition, the concentrations of soluble carbohydrate and soluble protein in EPS decreased by 77.8% and 84.2% with TB pretreatment after 12 h. As shown in Fig. 1(b) and (c), the addition of APG was not effective in accelerating the releasing of protein and carbohydrate in EPS. This may attribute to the toxic effects created by surfactants, which stimulates the microorganisms to produce exopolymers to keep them safe from harsh environmental conditions (Guan et al., 2017). It can be seen that the soluble carbohydrate and soluble protein content in EPS decreased with the increasing APG. With the addition of 0.4 g/g TSS APG, the concentrations of soluble carbohydrate and soluble protein reached 28.1 mg/L and 67.3 mg/L, which decreased by 23.9% and 40.4% than that obtained with 0.05 g/g TSS APG, respectively. Therefore, it implied that the addition of APG had a positive effect on the releasing of SCOD in EPS, and it was not an effective way of releasing soluble carbohydrate and soluble protein in EPS.

3.2. The variation of substrates in DOM

The releasing of EPS resulted in the increase of SCOD, carbohydrate, and protein in DOM, which are considered as the direct assimilable carbon source to microorganisms in anaerobic digestion process (Lu et al., 2018). The changing of SCOD, soluble carbohydrate and soluble protein in DOM during the hydrolysis of saline waste sludge is shown in Fig. 2. In the raw sludge, the concentrations of SCOD, soluble carbohydrate and soluble protein in DOM were 412.5 mg/L, 39.1 mg/L and 44.8 mg/L, respectively. As shown in Fig. 2, the increase of SCOD, soluble carbohydrate and soluble protein in DOM with TB pretreatment was inhibited by salinity. This may because the activity of TB was inhibited, and an adaptation period was required for TB to adapt to the salinity environment (Corsino et al., 2016). For the hydrolysis of saline waste sludge, the addition of APG had a positive impact on the increase of SCOD, soluble carbohydrate and soluble protein in DOM with TB pretreatment (Fig. 2a). Surfactants can not only improve the solubilization and dispersion of organic matters, but also change the affinity between the microbial cells and the organic matters through increasing cell surface hydrophobicity, thus accelerating the dissolution rate of non-aqueous phase substance (Luo et al., 2013; Wang et al., 2018). It was found that the releasing of SCOD was enhanced with rising APG dosage in the range of 0.05–0.4 g/g TSS. The maximum concentration of SCOD (8125.0 mg/L) in DOM was obtained in the presence of 0.4 g/g TSS APG, which enabled 42.0% increase over that without APG addition.

For the saline waste sludge without APG addition, the concentration of soluble carbohydrate in DOM increased to 252.0 mg/L with TB pretreatment. As shown in Fig. 2b, the releasing of carbohydrate was enhanced in the presence of APG. Surfactants possess good solubilization ability, so the additional surfactants can enhance the solubilization of EPS and break the sludge matrix, resulting in more inner carbohydrate and protein release (Yuan et al., 2011). Moreover, surfactants could reduce the repelling action between bacteria and sludge particulate, resulting in the enhancement of the contact between TB and sludge flocs (Luo et al., 2011). Compared with TB pretreatment without APG addition, the addition of APG increased the yield of soluble carbohydrate and shortened the reaction time. For example, the concentration of soluble carbohydrate increased to 482.4 mg/L in the

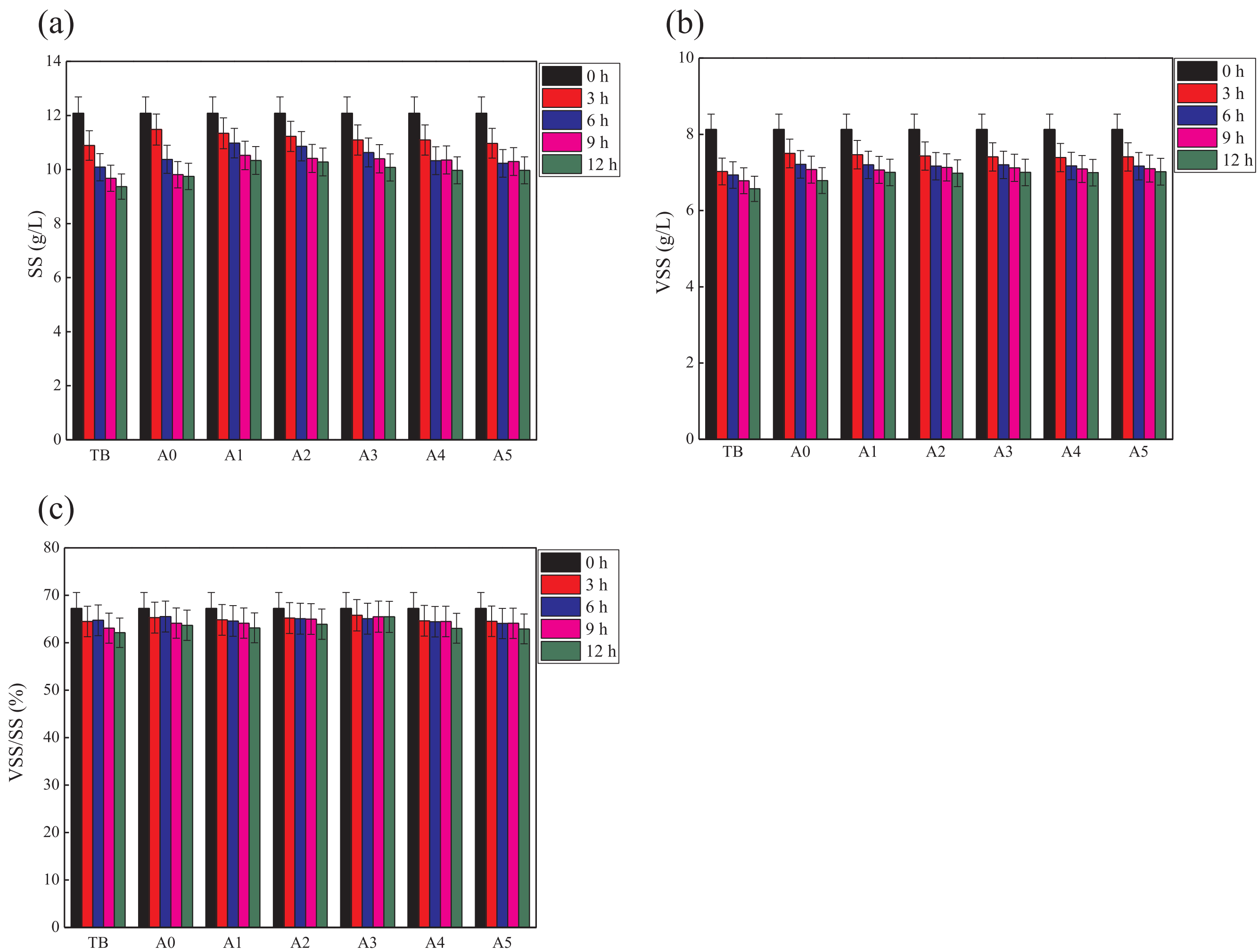

Fig. 5. The effect of APG on the sludge reduction and sludge stabilization with TB pretreatment. The error bar represents the standard errors.

presence of 0.1 g/g TSS APG after 3 h, which increased by 91.4% than that without APG addition. When the dosage of APG was above 0.2 g/g TSS, the soluble carbohydrate content in DOM increased gradually at the initial stage of hydrolysis and then decreased. After 3 h, the concentration of soluble carbohydrate in DOM reached 860.9 mg/L with 0.4 g/g TSS APG, which increased by 2.4 folds than that without APG addition.

The variation of soluble protein in DOM shows a similar trend with soluble carbohydrate. It can be seen that the concentration of soluble protein in DOM first increased and then decreased during the hydrolysis process (Fig. 2c). For the saline waste sludge without APG addition, the concentration of soluble protein in DOM increased to 264.4 mg/L after 9 h with TB pretreatment. As shown in Fig. 2c, the addition of APG was beneficial for the releasing of protein with TB pretreatment. After 3 h, the soluble protein concentration was 366.9 mg/L at APG dosage 0.05 g/g TSS, and 445.0 mg/L at APG dosage 0.3 g/g TSS. The results indicated the optimum hydrolysis time was shortened in the presence of APG, which means that the volume of the fermentation reactor can be reduced and the residence time of the solid can be shortened (Xiao et al., 2017). The maximum concentration (597.5 mg/L) of soluble protein in DOM was obtained in the presence of 0.4 g/g TSS APG, which enhanced by 1.3 folds than that obtained without APG addition. Moreover, it can be seen that the soluble protein content in DOM increased more than 89.0% with APG in the range of 0.3–0.4 g/g TSS, but it kept almost the same at APG dosage of 0.05–0.2 g/g TSS. Similar results were also observed in the previous study, which found that the effect of APG on hydrolysis and acidification was obvious when the APG dosage exceeded 0.3 g/g TSS (Luo et al., 2015). Based on these results, it implied that the addition of APG was beneficial for the releasing of organic matters such as protein and carbohydrate from sludge solids into sludge supernatant. However, the variation of specific organic matter fractions in EPS and DOM is still unclear. In order to get insights into the changes in the chemical composition of EPS and DOM, EEM and fluorescence regional integration analysis were further investigated.

3.3. Fluorescence EEM spectra

As a highly sensitive and selective method, EEM spectroscopy had been extensively utilized to characterize organic matter (Wei et al., 2017). The percent fluorescence responses of EPS are shown in Fig. 3a. In the raw sludge, the highest contribution proportion of $P_{i,n}$ in EPS was 37.8% (Region IV, soluble microbial by-product) and 32.9% (Region II, tryptophan-like protein), followed by the Region V (humic acid-like organics) of 15.8%, Region I (tyrosine-like protein) of 6.9%, and Region III (fulvic acid-like substances) of 6.6%. For the saline waste sludge without APG addition, the fluorescence intensity of Region I, Region II and Region IV in EPS of saline waste sludge decreased to 1068.1 (au), 1380.7 (au), 2701.1 (au), respectively. In comparison, the addition of APG did not lead to a decrease in the fluorescence intensity of Region I, Region II and Region IV in EPS. In the presence of 0.4 g/g TSS APG, the fluorescence intensity of Region I, Region II and Region IV in EPS reached 1328.6 (au), 1680.0 (au), 3838.5 (au), respectively. These results were in accordance with the releasing of SCOD, carbohydrate and protein from EPS, which might attribute to the increased secretion of organic matters from microorganisms for self-protection induced by the surfactant (Muthukumar et al., 2007). The salinity had a negative effect on TB pretreatment for releasing humic acid-like substances in EPS, and the $P_{i,n}$ of Region V increased to 11.0% with TB pretreatment. The releasing of fulvic acid- and humic acid-like substances in EPS was promoted in the presence of APG. The minimum $P_{i,n}$ of Region III (5.5%) and Region V (8.4%) were obtained at APG dosage 0.3 g/g TSS after 9 h, and the corresponding fluorescence intensity were 842.9 (au) and 3758.4 (au), respectively. Therefore, for the hydrolysis of saline waste sludge, the variation of EEM fluorescence spectra suggested that the addition of APG had a positive effect on the releasing of fulvic acid- and humic acid-like substances in EPS with TB pretreatment. The releasing of protein-like substances and soluble microbial by-product in EPS was inhibited in the presence of APG.

It was reported that EEM may offer indications of the biodegradability of DOM because of its capability to distinguish biodegradable substances (e.g. tyrosine protein-like and microbial byproduct-like compounds) and non-biodegradable materials (e.g. humic acid-, fulvic acid- and tryptophan protein-like compounds) (Jia et al., 2013). The distribution of fluorescence regional integration in DOM is shown in Fig. 3b. In the raw sludge, the highest contribution proportion of $P_{i,n}$ in DOM was 33.1% (Region II, tryptophan-like protein) and 31.2% (Region III, fulvic acid-like substances), followed by Region V (humic acid-like organics) of 26.9%, Region IV (soluble microbial materials) of 4.7%, Region I (tyrosine-like protein) of 4.3%. Comparing with the no saline sludge, the $P_{i,n}$ of Region IV in DOM increased in saline waste sludge with TB pretreatment, which might be attributed to the cell lysis induced by salinity (Li et al., 2013). For the saline waste sludge without APG addition, the fluorescence intensity of Region IV in DOM increased to 238.9 (au) with TB pretreatment, and the corresponding $P_{i,n}$ value was 4.4%. In comparison, the addition of APG was effective in releasing soluble microbial materials, and the maximum fluorescence intensity (402.3 (au)) of Region IV in DOM was obtained in the presence of 0.05 g/g TSS APG. The salinity was disadvantageous to the decrease of fulvic acid-like substances in DOM with TB pretreatment. With TB pretreatment, the fulvic acid-like substances were accumulated in saline waste sludge, and the fluorescence intensity in Region III reached 1040.7 (au) in saline waste sludge. The decrease of fulvic acid-like substances in DOM was promoted by APG addition, and the minimum fluorescence intensity (307.2 (au)) in Region III was obtained in the presence of 0.1 g/g TSS APG. As shown in Fig. 3b, the humic acid-like organics were accumulated in the hydrolysis process. It was reported that the humic acid-like substances mainly existed in the slime and EPS (Yu et al., 2010). With the degradation of waste sludge, the outer layer of the EPS gradually released to the supernatant, leading to an increase of humic acid-like organics in DOM. The fluorescence intensity of Region V in DOM increased from 4424.0 (au) to 10390.2 (au) without APG addition, and it increased to 9452.2 (au) in the presence of 0.4 g/g TSS APG. It can be seen that the accumulation of humic acid-like organics was reduced at 0.4 g/g TSS APG. The protein-like substances were degraded in the hydrolysis process of saline waste sludge. Compared with the raw sludge, the fluorescence intensity of Region I and Region II decreased by 58.2% and 67.1% without APG addition, and decreased by 69.9% and 81.8% in the presence of 0.3 g/g TSS APG, respectively. For the hydrolysis of saline waste sludge, it implied that the addition of APG was advantageous for the increase of soluble microbial materials and the decrease of fulvic acid-like substances in DOM. In addition, the accumulation of humic acid-like organics in DOM was reduced in the presence of APG, which was beneficial for the subsequent biochemical processes of sludge fermentation (Liu et al., 2015).

3.4. The activities of hydrolytic enzymes

The effect of APG on the relative activities of two types of hydrolytic enzymes (protease and α-glucosidase) in saline waste sludge with TB pretreatment is shown in Fig. 4. Protease and α-glucosidase have been reported to play crucial roles in sludge hydrolysis by breaking the peptide bonds of protein and α-1,4-glucosidic linkage in maltose (Yuan et al., 2016). Since proteins and carbohydrates represented the major organic fraction of waste sludge (Chen et al., 2013), the activities of α-glucosidase and protease are important in the hydrolysis process. As shown in Fig. 4, the salinity had a negative effect on TB pretreatment for increasing the activities of protease and α-glucosidase. Under salinity conditions, more energy was required for the production of osmolytes, hence less energy was usable for TB to releasing extracellular enzymes, resulting in a decrease in enzymatic activity (Cortes-Lorenzo et al., 2012). Comparing with TB pretreatment, higher activities of protease and α-glucosidase in saline waste sludge were observed in the presence of APG. This indicated that the addition of APG was beneficial for the increase in the activities of hydrolytic enzymes. More hydrolytic enzymes in the EPS were released into the supernatant, owing to the solubilization of EPS promoted by APG (Wang and Li, 2016). The maximum increasing (25.0%) of the activity of α-glucosidase was observed at 0.4 g/g TSS APG, which was consistent with the solubilization of saline waste sludge. Compared with that without APG addition, the increase in the activity of α-glucosidase was less than 0.9% with a low dosage of APG ($<$ 0.3 g/g TSS). The activity of α-glucosidase increased to 1.14 without APG addition, and increased to 1.15 in the presence of 0.3 g/g TSS APG. For the activity of protease, it can be seen that the activity of protease was positively related to the APG dosages. The maximum activity of protease (1.55) was observed at 0.4 g/g TSS APG, leading to an additional 18.1% improvement relative to that without APG addition. These results indicated that the addition of APG was beneficial for the enhancement of the activities of hydrolytic enzymes with TB pretreatment, which was advantageous for the sludge hydrolysis (Jin et al., 2018).

3.5. The reduction of SS and VSS

The variations of SS, VSS and VSS/SS ratio are shown in Fig. 5. It can be seen that the salinity had a negative effect on the sludge reduction with TB pretreatment. Compared with TB pretreatment without APG addition, the decrease of SS and VSS in saline waste sludge was not observed in the presence of APG. For the hydrolysis of saline waste sludge, the highest reductions of SS (19.8%) and VSS (16.0%) were obtained without APG addition. In response to 0.4 g/g TSS APG, the SS and VSS decreased by 17.4% and 13.6%. The degree of sludge stabilization can be expressed by the VSS/SS ratio, and the lower VSS/SS indicated the high content of inorganic sludge, lack of activities and effective stabilization (Erden et al., 2010). Compared with that without APG addition, the addition of APG did not lead to a decrease in the VSS/SS ratio. The minimum VSS/SS ratio (62.1%) occurred without APG addition, and the VSS/SS ratio reached 63.0% in the presence of 0.4 g/g TSS APG. The results indicated that APG was not efficient in the reduction and stabilization of saline waste sludge with TB pretreatment.

4. Conclusions

APG addition could effectively promote the hydrolysis of saline waste sludge with TB pretreatment. The increase of organic compounds in DOM was accelerated with APG, which was beneficial for the subsequent biochemical process. The fluorescent properties of DOM characterizing by EEM indicated that the biodegradability of saline waste sludge was enhanced in the presence of APG. The addition of APG was advantageous for the enhancement of the activities of hydrolytic enzymes in saline waste sludge, and 0.4 g/g TSS was determined to be the optimal dosage of APG for the maximum activities of α-glucosidase (1.25) and protease (1.55).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.122307.

References

Abbasi, A., Amiri, S., 2008. Emulsifying behavior of an exopolysaccharide produced by Enterobacter cloacae. Afr. J. Biotechnol. 7 (10), 1574–1576.
APHA, AWWA, WEF. 2005. Standard Methods, 19th ed. Washington, DC, USA.
Chen, Y., Liu, K., Su, Y., Zheng, X., Wang, Q., 2013. Continuous bioproduction of short-chain fatty acids from sludge enhanced by the combined use of surfactant and alkaline pH. Bioresour. Technol. 140 (3), 97–102.
Corsino, S.F., Capodici, M., Morici, C., Torregrossa, M., Viviani, G., 2016. Simultaneous nitritation-denitritation for the treatment of high-strength nitrogen in hypersaline wastewater by aerobic granular sludge. Water Res. 88, 329–336.
Cortes-Lorenzo, C., Rodríguez-Diaz, M., Lopez-Lopez, C., Sanchez-Peinado, M., Rodelas, B., Gonzalez-Lopez, J., 2012. Effect of salinity on enzymatic activities in a submerged fixed bed biofilm reactor for municipal sewage. Bioresour. Technol. 121 (7), 312–319.
Erden, G., Demir, O., Filibeli, A., 2010. Disintegration of biological sludge: effect of ozone oxidation and ultrasonic treatment on aerobic digestibility. Bioresour. Technol. 101 (21), 8093–8098.
Gao, P., Guo, L., Sun, J., Wang, Y., She, Z., Gao, M., Zhao, Y., 2019. Enhancing the hydrolysis of saline waste sludge with thermophilic bacteria pretreatment: new insights through the evolution of extracellular polymeric substances and dissolved organic matters transformation. Sci. Total Environ. 670, 31–40.
Guan, R., Yuan, X., Wu, Z., Wang, H., Jiang, L., Li, Y., Zeng, G., 2017. Functionality of surfactants in waste-activated sludge treatment: a review. Sci. Total Environ. 609, 1433–1442.
Guo, L., Li, X.M., Zeng, G.M., Zhou, Y., 2010. Effective hydrogen production using waste sludge and its filtrate. Energy 35 (9), 3557–3562.
Guo, L., Lu, M., Li, Q., Zhang, J., Zong, Y., She, Z., 2014. Three-dimensional fluorescence excitation-emission matrix (EEM) spectroscopy with regional integration analysis for assessing waste sludge hydrolysis treated with multi-enzyme and thermophilic bacteria. Bioresour. Technol. 171, 22–28.
Guo, L., Zhang, Z., Gao, M., She, Z., Zhao, Y., Guo, Y., Sun, J., 2018. Comparison of thermophilic bacteria and alkyl polyglucose pretreatment on two-stage anaerobic digestion with waste sludge: biogas production potential and substrate metabolism process. Bioresour. Technol. 249, 694–703.
Hulsen, T., Hsieh, K., Batstone, D.J., 2019. Saline wastewater treatment with purple phototrophic bacteria. Water Res. 160, 259–267.
Ismail, S.B., Parra, C.J.D.L., Temmink, H., Lier, J.B.V., 2010. Extracellular polymeric substances (EPS) in upflow anaerobic sludge blanket (UASB) reactors operated under high salinity conditions. Water Res. 44 (6), 1909–1917.
Jia, X., Zhu, C., Li, M., Xi, B., Wang, L., Yang, X., Xia, X., Su, J., 2013. A comparison of treatment techniques to enhance fermentative hydrogen production from piggery anaerobic digested residues. Int. J. Hydrog. Energy 38 (21), 8691–8698.
Jin, B., Niu, J., Dai, J., Li, N., Zhou, P., Niu, J., Zhang, J., Tao, H., Ma, Z., Zhang, Z., 2018. New insights into the enhancement of biochemical degradation potential from waste activated sludge with low organic content by Potassium Monopersulfate treatment. Bioresour. Technol. 265, 8–16.
Jin, B., Wang, S., Xing, L., Li, B., Peng, Y., 2016. The effect of salinity on waste activated sludge alkaline fermentation and kinetic analysis. J. Environ. Sci. 43 (5), 80–90.
Kavitha, S., Stella, P.B.C., Kaliappan, S., Yeom, I.T., Banu, J.R., 2016. Enhancement of anaerobic degradation of sludge biomass through surfactant assisted bacterial hydrolysis. Process Saf. Environ. Protect. 99, 207–215.
Li, Y., Li, A., Xu, J., Li, W., Yu, H., 2013. Formation of soluble microbial products (SMP) by activated sludge at;various salinities. Biodegradation 24 (1), 69–78.
Lin, L., Li, X.Y., 2018. Effects of pH adjustment on the hydrolysis of Al-enhanced primary sedimentation sludge for volatile fatty acid production. Chem. Eng. J. 346, 50–56.
Liu, K., Chen, Y., Xiao, N., Zheng, X., Li, M., 2015. Effect of humic acids with different characteristics on fermentative short-chain fatty acids production from waste activated sludge. Environ. Sci. Technol. 49 (8), 4929–4936.
Lu, D., Xiao, K., Yun, C., Soh, Y.N.A., Yan, Z., 2018. Transformation of dissolved organic matters produced from alkaline-ultrasonic sludge pretreatment in anaerobic digestion: from macro to micro. Water Res. 142, 138–146.
Luo, J., Feng, L., Chen, Y., Sun, H., Shen, Q., Li, X., Chen, H., 2015. Alkyl polyglucose enhancing propionic acid enriched short-chain fatty acids production during anaerobic treatment of waste activated sludge and mechanisms. Water Res. 73, 332–341.
Luo, K., Yang, Q., Yu, J., Li, X.M., Yang, G.J., Xie, B.X., Yang, F., Zheng, W., Zeng, G.M., 2011. Combined effect of sodium dodecyl sulfate and enzyme on waste activated sludge hydrolysis and acidification. Bioresour. Technol. 102 (14), 7103–7110.
Luo, K., Ye, Q., Yi, X., Yang, Q., Li, X., Chen, H., Liu, X., Zeng, G., 2013. Hydrolysis and acidification of waste-activated sludge in the presence of biosurfactant rhamnolipid: effect of pH. Appl. Microbiol. Biotechnol. 97 (12), 5597–5604.
Muthukumar, N., Maruthamuthu, S., Palaniswamy, N., 2007. Role of cationic and nonionic surfactants on biocidal efficiency in diesel-water interface. Colloid Surf. B-Biointerfaces 57 (2), 152–160.
Rio, A.V.D., Pichel, A., Fernandezgonzalez, N., Pedrouso, A., Fravázquez, A., Morales, N., Mendez, R., Campos, J.L., Mosqueracorral, A., 2018. Performance and microbial features of the partial nitritation-anammox process treating fish canning wastewater with variable salt concentrations. J. Environ. Manage. 208, 112–121.
Shi, X., Ng, K.K., Li, X.-R., Ng, H.Y., 2015. Investigation of intertidal wetland sediment as a novel inoculation source for anaerobic saline wastewater treatment. Environ. Sci. Technol. 49 (10), 6231–6239.
Sun, J., Guo, L., Li, Q., Zhao, Y., Gao, M., She, Z., Wang, G., 2016. Structural and functional properties of organic matters in extracellular polymeric substances (EPS) and dissolved organic matters (DOM) after heat pretreatment with waste sludge. Bioresour. Technol. 219, 614–623.
Wang, J., Li, Y., 2016. Synergistic pretreatment of waste activated sludge using CaO2 in combination with microwave irradiation to enhance methane production during anaerobic digestion. Appl. Energy 183, 1123–1132.
Wang, L.F., Huang, B.C., Wang, L.L., Min, Y., Yu, H.Q., 2018. Experimental and theoretical analyses on the impacts of ionic surfactants on sludge properties. Sci. Total Environ. 633, 198–205.
Wei, J., Hao, X., Loosdrecht, M.C.M.V., Li, J., 2018. Feasibility analysis of anaerobic digestion of excess sludge enhanced by iron: a review. Renew. Sust. Energy Rev. 89, 16–26.
Wei, L., Li, Y., Noguera, D.R., Zhao, N., Song, Y., Ding, J., Zhao, Q., Cui, F., 2017. Adsorption of Cu^{2+} and Zn^{2+} by extracellular polymeric substances (EPS) in different sludges: effect of EPS fractional polarity on binding mechanism. J. Hazard. Mater. 321, 473–483.
Xiao, J., Zhao, L., Shen, Z., 2017. Enhanced sludge anaerobic fermentation using microwave pretreatment combined with biosurfactant alkyl polyglycoside. RSC Adv. 7 (69), 43772–43779.
Yang, C., Zhou, A., He, Z., Jiang, L., Guo, Z., Wang, A., Liu, W., 2015a. Effects of ultrasonic-assisted thermophilic bacteria pretreatment on hydrolysis, acidification, and microbial communities in waste-activated sludge fermentation process. Environ. Sci. Pollut. Res. 22 (12), 9100–9109.
Yang, Q., Luo, K., Li, X.M., Zhong, Y., Chen, H.B., Yang, G.J., Shi, Y.W., Zeng, G.M., 2015b. Solubilization of waste activated sludge and nitrogenous compounds transformation during solubilization by thermophilic enzyme (S-TE) process. Appl. Biochem. Biotechnol. 176 (3), 700–711.
Ye, J., Hu, A., Ren, G., Chen, M., Tang, J., Zhang, P., Zhou, S., He, Z., 2018. Enhancing sludge methanogenesis with improved redox activity of extracellular polymeric substances by hematite in red mud. Water Res. 134, 54–62.
Yi, H., Han, Y., Zhuo, Y., 2013. Effect of combined pretreatment of waste activated sludge for anaerobic digestion process. Procedia Environ. Sci. 18 (99), 716–721.
Yu, G., He, P., Shao, L., 2010. Novel insights into sludge dewaterability by fluorescence excitation-emission matrix combined with parallel factor analysis. Water Res. 44 (3), 797–806.
Yuan, H., Zhu, N., Song, F., 2011. Dewaterability characteristics of sludge conditioned with surfactants pretreatment by electrolysis. Bioresour. Technol. 102 (3), 2308–2315.
Yuan, Y., Liu, Y., Li, B., Wang, B., Wang, S., Peng, Y., 2016. Short-chain fatty acids production and microbial community in sludge alkaline fermentation: long-term effect of temperature. Bioresour. Technol. 211, 685–690.
Zhang, F., Gu, W., Xu, P., Tang, S., Xie, K., Huang, X., Huang, Q., 2011. Effects of alkyl polyglycoside (APG) on composting of agricultural wastes. Waste Manage. 31 (6), 1333–1338.
Zhang, Z., Guo, L., Li, Q., Zhao, Y., Gao, M., She, Z., 2017a. Study on substrate metabolism process of saline waste sludge and its biological hydrogen production potential. Environ. Sci. Pollut. Res. 24 (19), 16383–16395.
Zhang, Z., Guo, L., Wang, Y., Li, F., Zhao, Y., Gao, M., She, Z., 2017b. Degradation and transformation of extracellular polymeric substances (EPS) and dissolved organic matters (DOM) during two-stage anaerobic digestion with waste sludge. Int. J. Hydrog. Energy 42 (15), 9619–9629.
Zhao, J., Yang, Q., Li, X., Wang, D., An, H., Xie, T., Xu, Q., Deng, Y., Zeng, G., 2015a. Effect of initial pH on short chain fatty acid production during the anaerobic fermentation of membrane bioreactor sludge enhanced by alkyl polyglcoside. Int. Biodeterior. Biodegrad. 104, 283–289.
Zhao, J., Yang, Q., Li, X., Wang, D., Luo, K., Zhong, Y., Xu, Q., Zeng, G., 2015b. Enhanced production of short-chain fatty acid from food waste stimulated by alkyl polyglycosides and its mechanism. Waste Manage. 46, 133–139.
Zhao, J., Zhang, C., Wang, D., Li, X., Yang, Q., 2016. Revealing the underlying mechanisms of how sodium chloride affects short-chain fatty acid production from the co-fermentation of waste activated sludge and food waste. ACS Sustain. Chem. Eng. 4 (9), 4675–4684.
Zhen, G., Lu, X., Kato, H., Zhao, Y., Li, Y.Y., 2017. Overview of pretreatment strategies for enhancing sewage sludge disintegration and subsequent anaerobic digestion: current advances, full-scale application and future perspectives. Renew. Sust. Energy Rev. 69, 559–577.
Zheng, H.S., Guo, W.Q., Yang, S.S., Feng, X.C., Du, J.S., Zhou, X.J., Chang, J.S., Ren, N.Q., 2014. Thermophilic hydrogen production from sludge pretreated by thermophilic bacteria: analysis of the advantages of microbial community and metabolism. Bioresour. Technol. 172 (4), 433–437.