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Stepwise hydrolysis to improve carbon releasing efficiency from sludge

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ABSTRACT

Based on thermal alkaline hydrolysis (TAH), a novel strategy of stepwise hydrolysis was developed to improve carbon releasing efficiency from waste activated sludge (WAS). By stepwise increasing hydrolysis intensity, conventional sludge hydrolysis (the control) was divided into four stages for separately recovering sludge carbon sources with different bonding strengths, namely stage 1 (60 °C, pH 6.0–8.0), stage 2 (80 °C, pH 6.0–8.0), stage 3 (80 °C, pH 10.0) and stage 4 (90 °C, pH 12.0). Results indicate stepwise hydrolysis could enhance the amount of released soluble chemical oxygen demand (SCOD) for almost 2 times, from 7200 to 14,693 mg/L, and the released carbon presented better biodegradability, with BOD/COD of 0.47 and volatile fatty acids (VFAs) yield of 0.37 g VFAs/g SCOD via anaerobic fermentation. Moreover, stepwise hydrolysis also improved the dewaterability of hydrolyzed sludge, capillary suction time (CST) reducing from 2500 to 1600 s. Economic assessment indicates stepwise hydrolysis shows less alkali demand and lower thermal energy consumption than those of the control. Furthermore, results of this study help support the concepts of improving carbon recovery in wastewater by manipulating WAS composition and the idea of classifiably recovering the nutrients in WAS.

1. Introduction

In recent years, WAS yield has increased year by year in wastewater plants (WWTPs) of China, and reached 55 million tons with water content of 80% in 2014 (National Bureau of Statistics of China, 2015). WAS disposal has became a critical issue. Recycling carbon source from WAS has gradually been a hot research (Kim et al., 2009; Yan et al., 2013; Liu et al., 2012), and physic-chemical hydrolysis of WAS pretreatment is even listed as one of the 18 most promising technologies in the field of wastewater treatment in future by the International Water Association (IWA) (2016).

Recent development of sludge hydrolysis could be generally divided into two trends: high-strength hydrolysis and mild hydrolysis. The former is often used to direct recovery sludge carbon source, in the process of which, protein and polysaccharide could be directly hydrolyzed into small molecular substances, such as amino acids and simple sugars. For example, Su et al. (2014) obtained amino acids from municipal excess sludge by thermal hydrolysis and used it as the inhibitor for steel corrosion. However, the high energy consumption and harsh equipment requirements seriously hampered its widely application (Koottatep et al., 2016). Mild hydrolysis with low energy consumption and equipment requirements is still widely adopted in the field of sludge pretreatment for enhancing the yields of VFAs or methane in sludge anaerobic fermentation processes (Pei et al., 2016; Yao et al., 2016). There are many kinds of sludge pretreatment methods reported in previous studies, including mechanical, chemical and biological methods, amongst of which, TAH is considered as one of the most effective methods (Hyun et al., 2013; Kim et al., 2013) and was thus implemented for sludge hydrolysis in this test. However, there are also some shortages in mild hydrolysis, such as inadequate release of carbon resource, low biodegradability of the released SCOD, poor dewaterability of hydrolyzed sludge and low recovery of released carbon (Barber, 2016; Tong and Chen, 2009; Xiao et al., 2016).

Carbon source in sludge is mainly composed of organics absorbed on the surface of sludge flocs (OM), loosely bound extracellular polymeric substances (LB-EPS), tightly bound extracellular

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polymeric substances (TB-EPS) and the intracellular matters (IM), which are entrapped in the matrix of sludge flocs at different bonding strengths. In conventional hydrolysis process, uniform hydrolysis strength and reacting time are evenly exposed on those organics distributing at different parts of sludge. Therefore, during mild hydrolysis, those organics with high bonding strengths, such as intracellular matters, could not be complete released, resulting in the low SCOD yields, while other organics with low bonding strengths, mainly extracellular organics, could be easily released and then further reacts to produce non-biodegradable substances. For example, some non-biodegradable matters, such as melanin and melanoidin, could be produced in the process of alkaline thermal hydrolysis by Maillard reactions, or called carbonyl amino reactions, between carbonyl compounds (reducing sugars) and amino compounds (amino acids and proteins), which resulted in the low biodegradability of the released carbon (Neyens et al., 2003; Salsabil et al., 2010). Moreover, during the conventional mild hydrolysis, abundant of dissolved organics would be released and accumulated in the hydrolyzed sludge system. Previous researches indicated the existence of superabundant soluble organics, especially protein and polysaccharide, in the hydrolyzed sludge was the reason to its poor dewaterability (Zhu et al., 2015).

Therefore, based on the distribution and bonding strength of organics in WAS, a novel strategy, stepwise release of carbon source from sludge, was developed in this research to systematically solve those problems mentioned above in mild hydrolysis. By stepwise improving hydrolysis strength and timely recovering sludge carbon in a planned way, (1) the hydrolysis force is directly exposed on the targeted organics in sludge, which could greatly improve hydrolysis efficiency and enhance the amount of released sludge carbon, (2) the released carbon avoids to be excessively hydrolyzed, which could improve the quality of the released carbon, and (3) the accumulations of protein and polysaccharide in hydrolyzed sludge system can be eased, which could alleviate the deterioration of sludge dewaterability. The objective of this study is to provide a mild hydrolysis technology for efficiently recovering sludge carbon. The amount and quality of the released carbon were investigated, and efficiency in improving sludge dewaterability were analyzed. By exploiting the mechanisms of stepwise hydrolysis, the concept of improving carbon recovery in wastewater by manipulating WAS composition and the feasibility of classifiably recovering the nutrients in WAS were discussed.

2. Materials and methods

2.1. Substrates

WAS used as the substrate for hydrolysis was taken from the sludge storage tank of a local WWTP in Wuxi city, China. An intermittent cycle extended aeration/membrane bioreactor process is used in this WWTP. The fresh WAS was pre-concentrated before used. The concentrated WAS had pH of 6.5–7.5, total suspended solids (TSS) concentration of 30.0–31.0 g/L, volatile suspended solids (VSS) concentration of 15.0–16.0 g/L, CST of 40.0–50.0 s, average volume particle diameter of 70.0–80.0 μm, SCOD of 130.0–140.0 mg/L, soluble protein of 13.0–15.0 mg/L and soluble polysaccharides of 45.0–50.0 mg/L. All measurements were conducted in triplicate.

2.2. Seeding sludge for anaerobic fermentation

Anaerobic sludge from an up-flow anaerobic sludge blanket (UASB) for brewery wastewater treatment was collected as the seeding sludge. In order to accumulate acetogenic bacteria, the anaerobic sludge was firstly concentrated by setting for 24.0 h at ambient temperature and the precipitated sludge was then treated at 105 °C for 2.0 h to kill methanogens. To reactivate acetogenic bacteria, the heat-treated sludge was added into a 1000 ml shaking flask holding nutrient solution whose compositions, concluding 3.0 g/L glucose, were referred to previous reports (Jiunn et al., 2003; Ginkel and Logan, 2005). When the mixed-liquor suspended solids (MLSS) was 9.0 g/L, pH was about 6.5, stirring speed was 120.0 rpm and temperature was 35.0 °C, the heat-treated sludge was cultivated for 24.0 h in the completely anaerobic flask. Finally, the seeding sludge was obtained by centrifuging the cultivated sludge at 4800 rpm for 10 min. In cultivation process, pH was adjusted by dilute HCl and NaOH, oxygen in headspace of flask was removed by injecting nitrogen for 10.0 min, dissolved oxygen was removed by adding L-cysteine solution and phosphate was used as the buffer.

2.3. Strategy for WAS stepwise hydrolysis

As shown in Fig. 1, the strategy of WAS stepwise hydrolysis could be divided into 4 stages. In stage 1, the conditions were controlled at temperature of 60 °C and pH of 6.0–8.0. WAS with TSS concentration of about 30.0 g/L was continuously stirred for 3.0 h, aiming to strip the carbon source absorbed on the surface of sludge. Then, the hydrolyzed WAS was centrifuged to separate the liquor and solid. The former contains the carbon source released in stage 1#. The solid fraction was adjusted to the initial TSS concentration of about 30.0 g/L by adding tap water and used for subsequent hydrolysis in stage 2, where the conditions were controlled at temperature of 80 °C and pH of 6.0–8.0. In this stage, the WAS, that is, the solid fraction of stage 1, was also continuously stirred for 3.0 h in order to strip the sludge carbon distributing outside of sludge cell. Then, the liquor and solid in the hydrolyzed sludge was separated by centrifugation. The liquor fraction contains the carbon source released in stage 2#. The solid fraction was adjusted to the initial TSS concentration of about 30.0 g/L by adding tap water for subsequent hydrolysis. Under the same processes, the residual sludge in stage 2 was further hydrolyzed during stages 3 and 4 in sequence. The conditions in stage 3 were controlled at temperature of 80 °C, pH of 10 and stirring time of 6.0 h; and in stage 4 were temperature of 90 °C, pH of 12 and stirring time of 6.0 h. In all stages, the centrifugal intensity was controlled at 7000 × g for 10 min. The liquor fractions were collected separately, and then anaerobic fermented for acids production, respectively. Moreover, as shown in Fig. 1, the conventional TAH was implemented as the control, in which, the conditions were controlled at temperature of 90 °C, pH of 12 and stirring time of 3.0 h.

2.4. Anaerobic fermentation for VFAs production

Six beaker flasks of 500 ml with equal amount of seeding sludge were filled with 250 ml supernatant of the hydrolyzed WAS in stage 1 (L1), stage 2 (L2), stage 3 (L3), stage 4 (L4) and the control (CL), respectively, and adjusted pH of 10.0 by dilute HCl and NaOH. Dissolved oxygen in supernatant and gaseous in the headspace of flasks were removed by sparging gaseous nitrogen for about 30 min to maintain strict anaerobic condition. In the whole process of fermentation, flasks were placed in orbital shaker with rotation speed of 120 rpm, temperature was kept at about 35 °C and pH was kept at 10.0. Samples taken from beaker flasks at certain intervals were analyzed. All the experiments were carried out independently in triplicates.

2.5. Analytical methods

Samples were pretreated by filtering with GF/C glass microfiber of 0.45 μm. Conventional indexes, including COD, BOD, VSS, TSS and

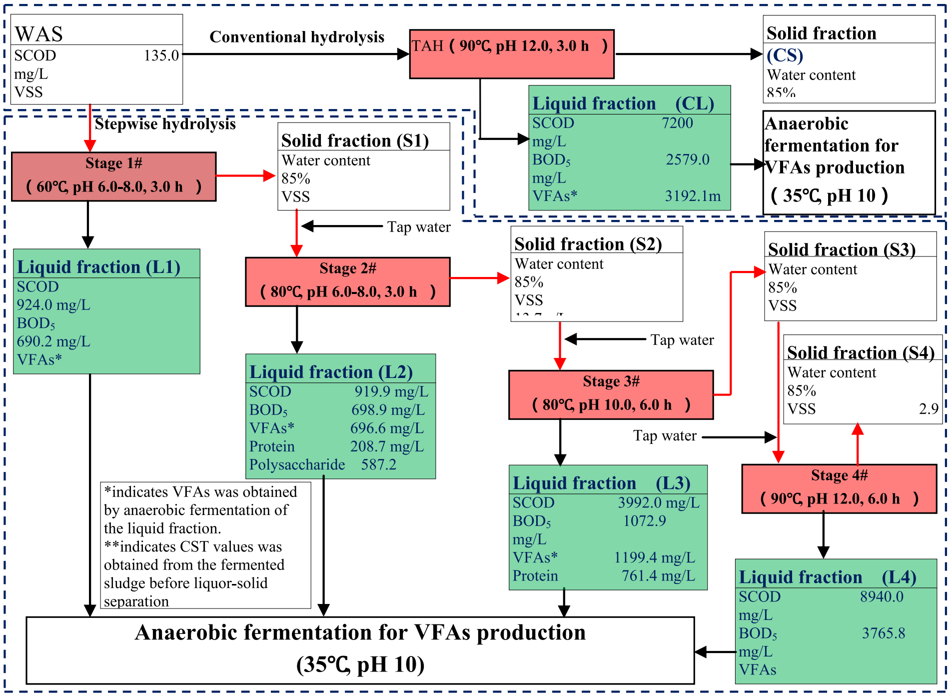

Fig. 1. Scheme of sludge stepwise hydrolysis strategy and its efficiencies in each stage.

sludge moisture content, were analyzed according to the standard methods issued by the State Environmental Protection Administration of China (2002). Particle size of sludge was measured by a BT-2003 laser particle size analyzer, which works on the principle of laser detraction.

The different EPS layers, LB- and TB-EPS, were extracted by centrifugation and ultrasound method (Yuan et al., 2017). Soluble carbohydrate was measured by the phenol-sulfuric method with glucose as standard (Dubois et al., 1956). Soluble protein was determined by the Lowry-Folin method with bovine serum albumin (BSA) as standard (Lowry et al., 1951).

DNA concentrations in the supernatant were measured to analyze the degree of sludge cell rupture in each stage. Firstly, the supernatant of the hydrolyzed sludge in each stage was obtained by centrifuging at 8000r/min for 10 min under temperature of 4°Cand called supernatant 1#. Secondly, the solution of phenol/chloroform/isoamyl alcohol (25:24:1) was added into supernatant 1# at the volume rate of 1:1. After completely mixed, the dosed supernatant 1# was centrifuged at 12000r/min for 10 min under temperature of 4 °C, and the obtained supernatant was called supernatant 2#. Thirdly, the solution of chloroform/isoamyl alcohol (24:1) was added into supernatant 2# at the volume rate of 1:1. After completely mixed, the dosed supernatant 2# was centrifuged at 12000r/min for 10 min under temperature of 4 °C, and the obtained supernatant was called supernatant 3#. Fourthly, isopropyl alcohol was added into supernatant 3# at the volume rate of 0.6:1. After completely mixed, the dosed supernatant 3# was settled for 30 min under temperature of 4°Cand then centrifuged at 13,000 g for 20 min under room temperature. The precipitation, called precipitation 1#, was washed by 70% ethanol with 1 ml and then centrifuged at 13,000 g for 10 min under room temperature. After being dried, TE buffer solution of about 50 μl was added into the precipitation, called precipitation 2#. After completely mixed, the solution was used to analyze DNA concentration by NanoDrop2000.

Excitation-emission matrix (EEM) fluorescence spectra and fluorescence regional integration (FRI) techniques were used to assess the components distributions of organic matters obtained in stage I, II, III and IV, respectively (Sun et al., 2016). Organic matters were divided into five types based on their excitation-emission wavelengths, namely tyrosine-like protein, tryptophan-like protein, fulvic acid-like organics, soluble microbial by-product and humic acid-like organics; and among which, tyrosine-like protein and soluble microbial by-product-like substances were thought of as biodegradable materials; tryptophan-like, fulvic acid-like and humic acid-like substances were regarded as non-biodegradable materials (Jia et al., 2013).

VFAs with pretreatment of filtrating samples through 0.45 μm filter membrane were measured by a gas chromatograph (GC-2010, Shimadzu, Japan) equipped with an auto injector (AOC-20i, Shimadzu, Japan). The detector was a flame ionization and the column was a fused silica capillary (PEG-20M, 30 m × 0.32 mm × 0.51 m, China); 4-methyl-valeric acid was added as an internal standard and the samples were acidized by 3 M phosphoric acid. The initial temperature of the GC column was 80 °C and was held for 3 min, and then increased by 15 °C/min to a final temperature of 210 °C, and then held for 2 min. Both temperatures of the injection port and the detector were set at 250 °C. The total VFAs concentrations were calculated by summing up each individual VFA. Each sample was analyzed in triplicate and the standard deviations of all analyses were always less than 5%.

2.6. Calculation methods

The energy consumptions in each stage of sludge stepwise hydrolysis and the process of conventional hydrolysis (the control) were computed out by Eq. (1).

$$E = \frac{C \times \left[m_{solid} \div W_{solid} \times \left(T_{end} - T_{beginning}\right) + m_{added\ \ liquor} \times (T_{end} - T_{normal})(1 - R)\right]}{m_{SCOD}} \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \tag{1}$$

$$m_{solid} = m_{NSS} + m_{VSS} \times r \tag{2}$$

$$m_{added\ \ liquor} = \frac{m_{solid}}{P_{solid}} - \frac{m_{solid}}{W_{solid}} = m_{discharged\ \ liquor} \tag{3}$$

where C is the specific heat capacity of water (4.2×10^3 J/(kg·°C)). m_{solid} is the dry quantity of the solid fraction in each hydrolysis stage (in stepwise hydrolysis) or process (in the control) (kg). $m_{added\ \ liquor}$ is the quantity of the water added at the beginning of each hydrolysis stage (kg). $m_{dischanged\ \ liquor}$ is the quantity of the water discharged at the end of last hydrolysis stage (kg). m_{NSS} is the quantity of inorganic matters in sludge (kg). m_{VSS} is the quantity of organic matters in sludge (kg). r is the degradation rate of organic matters in sludge by hydrolysis. P_{solid} is mass percentage of solid fraction in sludge (controlled at 3% in this test). W_{solid} is the water content of the solid fraction after dewatering (about 85% in this test). T_{end} is the temperature of the hydrolyzed sludge at the end of each stage or hydrolysis process. $T_{beginning}$ is the temperature of the hydrolyzed sludge at the beginning of each stage or hydrolysis process.T_{normal} is the temperature of the indoor water temperature (20 °C). R is the recovery rate of the waste heat in discharged liquor (30%).

3. Results

3.1. Stepwise releases of organic matters from sludge hydrolysis

Stepwise release of organic matters in sludge was realized by gradually increasing hydrolysis intensity. As shown in Fig. 2(A), SCOD concentrations obtained in stage I, II, III and IV are 924, 1845, 5753 and 14,693 mg/L, respectively, while for the control, SCOD concentration is just about 7200 mg/L after 3 h. Therefore, results indicate most the carbon source is distributed inside of sludge cell and stepwise hydrolysis could enhance the amount of SCOD released from sludge for almost 2 times.

Moreover, this process of sludge stepwise hydrolysis could be well described by the concentration changes of those main components in the supernatant, namely soluble protein, polysaccharide, ammonia and phosphorus. For example, as shown in Fig. 2(B), total phosphorus (TP) in sludge presents obvious stepwise releasing. It is known that phosphorus in activated sludge mainly distributed inside of cells. The hydrolysis intensities of stage I and II have scarcely influence on sludge cells, but in stage III, sludge cell membrane is partly destroyed, resulting in the release of phosphorus from cell membrane and the slight loss of those intracellular matters. In stage VI, sludge cell membrane is completely destroyed and large amount of intracellular matters flow out. The relatively weak increasing trends of soluble protein and polysaccharide concentrations should attribute to molecular decomposition under the gradually increasing hydrolysis intensity. This phenomenon indicates that the composition of the released organics could be adjusted by controlling the intensity of sludge hydrolysis.

3.2. Influence of **s**tepwise hydrolysis on the quality of the released carbon

3.2.1. Biodegradability of the released carbon obtained in each stage

DNA concentrations in the supernatant of each stage were measured to analyze the degree of sludge cell rupture. As shown in Fig. 3 (A), sludge cell rupture mainly happens in stage 3 and 4. Moreover, the total concentration of DNA in sludge was about 38.5 mg/L, which indicates the broken rate of the sludge cell reaches about 94.4% at the end of stage 4. Therefore, results indicate that organics released in stages 1 and 2 are mainly extracellular matters, while organic matters released in stages 3 and 4 are mainly from intracellular matters and partly from the further decomposition of the extracellular carbon.

As shown in Fig. 3 (B), carbon source distributing outside of activated sludge cell has much better biodegradability than that of those intracellular matters. The BOD/COD (B/C) vales of the organics released in stage1 and 2 reach 0.75 and 0.70, respectively, much larger than those of stage 3 and 4, 0.19 and 0.26, respectively. Result indicates, in biological technologies for sludge resourcezation, the conversion efficiency of organics could be greatly improved by increasing the proportion of extracellular carbon source in activated sludge. For example, our previous researches (Liu et al., 2012) indicated more than 60% of the total carbon source in wastewater could be concentrated and separated by the quick adsorption of excess activated sludge, and this carbon source could

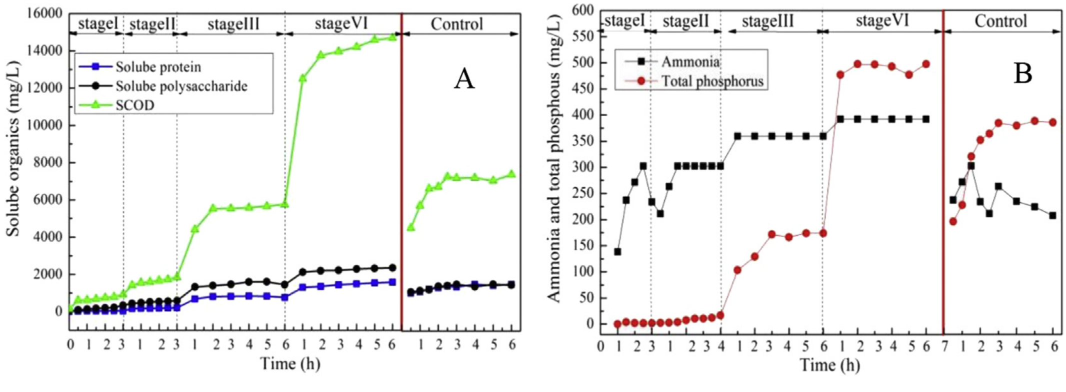

Fig. 2. Stepwise releases of organic matters from sludge hydrolysis, (A) soluble organics release, (B) ammonia and TP releases.

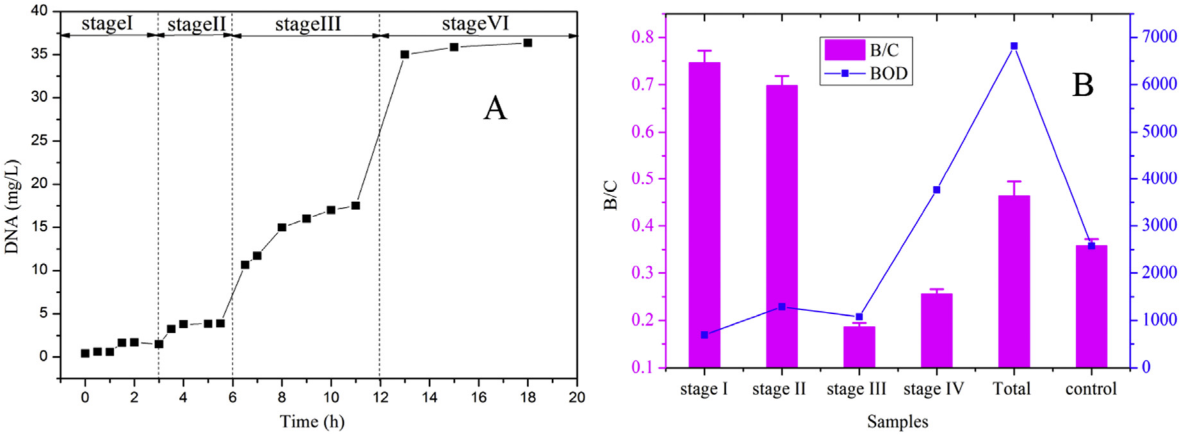

Fig. 3. DNA concentrations in the supernatant of each stage (A) and biodegradability of the released organic matters obtained in each stage (B).

completely substitute methanol as a good quality carbon to support highly efficient biologic denitrification.

Moreover, hydrolysis intensity has obvious influence on the biodegradability of the released carbon. On the one hand, as shown in Fig. 3 (B), the B/C value of the released organics could be increased by strengthening the hydrolysis intensity due to the decomposition of macromolecular organics into biodegradable small molecules, resulting in the larger B/C value of the carbon source obtained in stage 4 than that of stage 3. On the other hand, under high strength hydrolysis conditions, some no-biodegradable matters, such as melanin and melanoidin, could be produced from those released organics via Maillard reactions (Neyens et al., 2003; Salsabil et al., 2010). As shown in Fig. 3 (B), the total carbon source obtained by stepwise hydrolysis has larger B/C value than that of the control. Therefore, results indicate the strategy of stepwise hydrolysis can not only accelerate organics releasing from sludge, but also make the released carbon well biodegradable.

3.2.2. Anaerobic fermentation of the released carbon for VFA production

To further evaluate the promotion of stepwise hydrolysis on the quality of the released carbon, anaerobic fermentation of the organics obtained in stage 1, 2, 3 and 4, as well as the control were implemented for VFA production, respectively. As shown in Fig. 4 (A), the sample with higher SCOD concentration results in higher VFA concentration through anaerobic fermentation. The total VFA concentration from the total SCOD obtained by stepwise hydrolysis reaches approximately 6 g/L, twice as large as that of the control.

Moreover, as shown in Fig. 4 (B), the carbon sources distributing at different parts of activated sludge present different conversion rates in the process of anaerobic fermentation. The VFA/SCOD values of the carbon released in stages 1 and 2 reach as high as more than 0.7 while those are only approximately 0.3 in stages 3 and 4. Results confirm once again that the carbon outside of sludge cell has higher quality than that inside of sludge cell. However, very regretfully, the VFA/SCOD value of the total carbon obtained by stepwise hydrolysis is just slightly higher than that of the control, due to the limited amount of the extracellular carbon in activated sludge.

3.3. Fluorescence EEM spectra and FRI technique assessment

Components distributions in the organic matters obtained in stage I, II, III and IV, were shown in Fig. 5. Humic acid-like substances, a kind of non-biodegradable materials, is the main compounds in all of the four samples, which should be the main reason to the low utilization ratio of the released carbon during fermentation process. Moreover, with the improvement of hydrolysis intensity, the concentrations of humic acid-like substances increase first and then decreases, while soluble microbial by-product-like

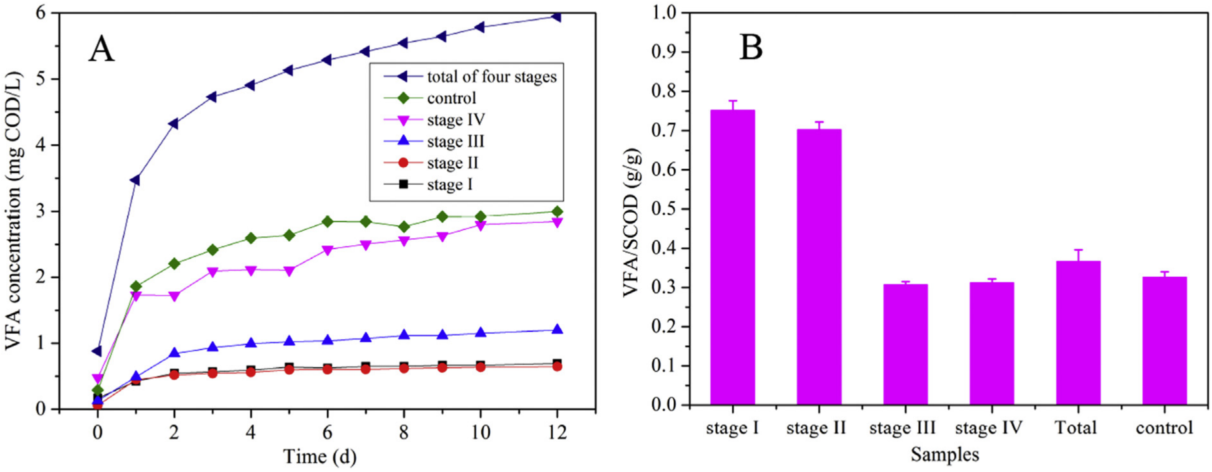

Fig. 4. Anaerobic fermentation of the released carbon for VFA production (A) and conversion rates of SCOD to VFA (B).

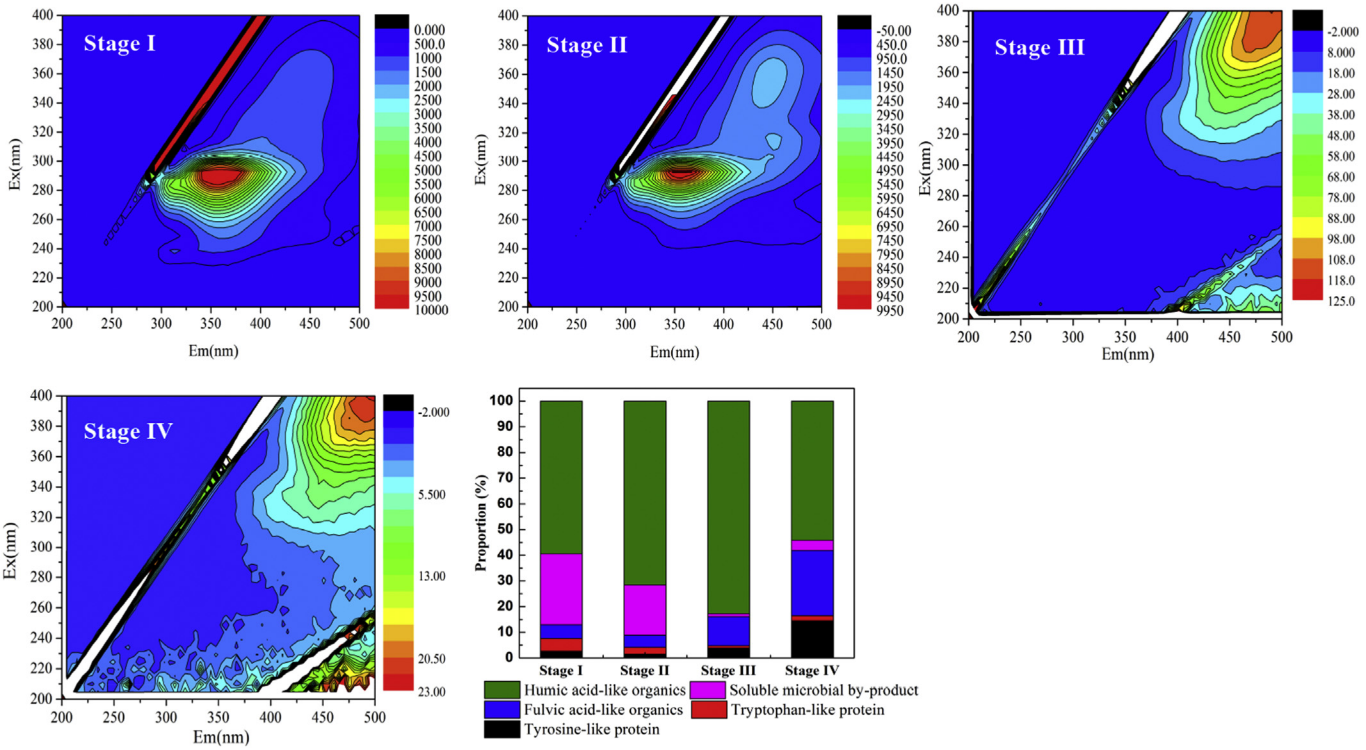

Fig. 5. EEM spectra and FRI distribution of the organic matters released at each stage during stepwise hydrolysis.

substances, a kind of biodegradable materials, decreases first and then slightly increases, which should be the reason to the reduce of released carbon biodegradability in stage I, II and III and increase in stage IV. Furthermore, as shown in Fig. 5, the concentrations of fulvic acid-like substances (non-biodegradable) and tyrosine-like protein (biodegradable) keep increasing, which indicates high-intensity hydrolysis of WAS could simulate their productions.

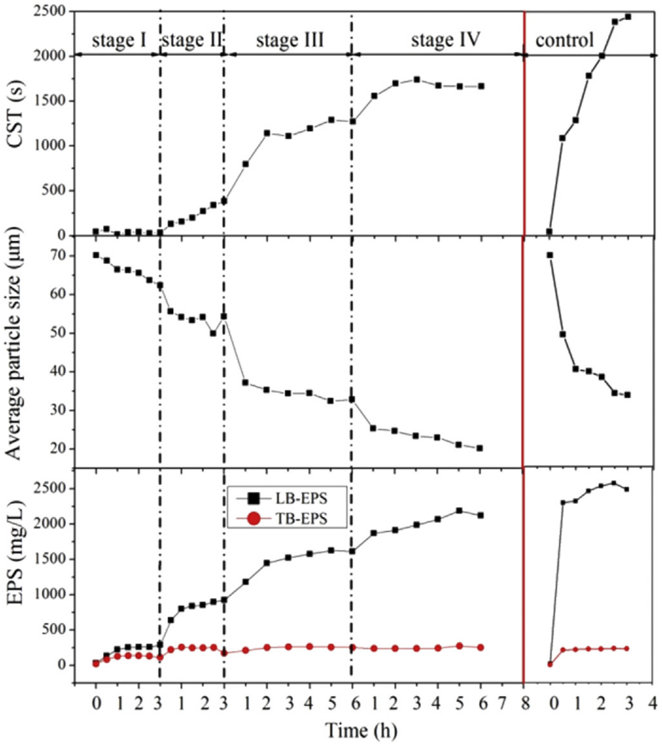

Fig. 6. Influence of stepwise hydrolysis on sludge dewaterability (A), particle size (B) and EPS releases (C).

3.4. Influence of stepwise hydrolysis on sludge dewaterability

Recovery rate of the product from sludge fermentation, such as VFA, is closely dependent on the dewaterability of hydrolyzed sludge. However, in most of the mild hydrolysis, the dewaterability of sludge often becomes very poor due to the destruction of sludge flocs and the release of EPS (Zhu et al., 2015). In the process of stepwise hydrolysis, the released soluble organics, such as EPS, could be recovered in batches. Therefore, theoretically, the dewaterability of hydrolyzed sludge should be improved by the strategy of stepwise hydrolysis.

As shown in Fig. 6 (A), during stepwise hydrolysis, the dewaterability of sludge become more and more poor with the increase of hydrolysis intense, while the sludge after stepwise hydrolysis presents much better dewaterability than that of the conventional hydrolysis (the control). Although the sludge in stage 4 has the largest CST value in stepwise hydrolysis, about 1600 s, it is much smaller than that of the control, about 2500 s.

The reduce of sludge particle and increase of soluble EPS maybe the main reasons to the deterioration of sludge dewaterability. As shown in Fig. 6, the curves of sludge particles and soluble EPS concentrations show good correlations with the that of CST, respectively. Moreover, the influence of soluble EPS concentration on sludge dewaterability seems greater than that of sludge particle. Comparing with the stepwise hydrolyzed sludge, although the common hydrolyzed sludge (the control) has larger particle size, its CST value still higher due to its greater EPS concentration.

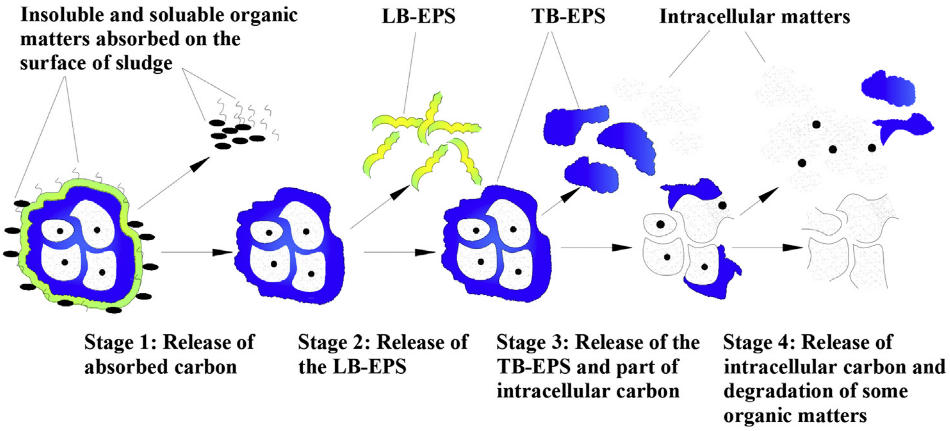

Fig. 7. Mechanism of sludge stepwise hydrolysis.

4. Discussion

4.1. Mechanism of the stepwise hydrolysis process

Obtained results indicate that, by orderly releasing and recovering the carbon source from WAS, the strategy of sludge stepwise hydrolysis could simultaneously accelerate carbon release from sludge, improve the quality of the recovering carbon and enhance the dewaterability of hydrolyzed sludge. As shown in Fig. 7, based on the difference of bonding strengths, the carbon sources in WAS are divided into 4 groups, namely OM, LB-EPS, TB-EPS and IM. In stage 1, most of the OM could be released under the conditions of continuously stirring and intermediate temperate. In stage 2, hydrolysis intensity was improved by increasing the temperature, aiming to strip the LB-EPS from sludge. In stage 3, the exposed EPS, mainly TB-EPS, is released by the further enhancement of hydrolysis intensity, accompanying with some ruptures of sludge cells. In stage 4, sludge cells are completely destroyed under the high hydrolysis intensity and almost all of the intracellular matters is released, accompanying with the further decomposition of some extracellular matters.

4.2. Improving carbon recovery in wastewater by manipulating WAS composition

In conventional technologies, carbon source in wastewater is mainly translated into CO_2 and sludge cell. A lot of energy is required to provide enough dissolved oxygen by aeration. Moreover, most of carbon source in sludge is in the form of biologic cells and difficult to be released. As shown in Fig S2, more than 60% of the carbon source in sludge distributes inside of biologic cells. Furthermore, as shown in Figs. 3 and 4, the bioavailability of intracellular carbon is much lower than that of extracellular carbon. Therefore, the efficiency of carbon recovery is very low in traditional wastewater treatment technologies.

Increasing the proportion of extracellular carbon source in WAS seems to be a feasible way to accelerate nutrients recovery in wastewater. Previous results of our researches (Liu et al., 2012) indicated more than 60% of COD in wastewater could be directly removed by the adsorption of aerobic activated sludge and then recovered via sludge hydrolysis. Therefore, before hydrolyzed for carbon release and recovery, waste activated sludge could be used to adsorb the carbon source in wastewater, which could increase the proportion of extracellular carbon source in WAS, reduce the amount of carbon source in the form of biologic cells and decrease the demand of energy in wastewater treatment.

4.3. Feasibility of classifiably recovering the nutrients in WAS

The quality of the released carbon from WAS could be seriously influenced by the co-existing of carbon, nitrogen and phosphorus in it. According to the distribution difference of carbon, nitrogen and phosphorus in WAS, classifiably recovering the nutrients in sludge via accurately controlling hydrolysis intensity could greatly improve the quality of the released carbon, which is also a hot and interesting topic.

As shown in Fig. S2, during stage 1and 2 of stepwise hydrolysis, the values of SCOD/TP changed greatly in the released carbon source. It is about 150 in the supernatants of initial WAS and quickly climbs to 400 with the releasing of organic matter from solid sludge. Moreover, at the beginning of stage 3, the values of SCOD/TP rapidly drop to 50, mainly resulting from the ruptures of crackly sludge cells. Results indicate the contents of phosphorus is very low in extracellular matters. However, during stage 3 and 4, the releasing processes of SCOD and TP present highly consistent and the values of SCOD/TP could stably maintain at about 50. That is, it is difficult to classifiably recovery carbon and phosphorus from intercellular matters by manipulating hydrolysis process. But, it is feasible to control the content of phosphorus in the reclaimed sludge carbon by separately recovering intercellular and extracellular carbon sources.

4.4. Economic assessment

Based on experiment results, the economic expenses of stepwise and conventional hydrolysis are compared in the aspects of required reaction time, material demand and thermal energy consumptions. In each hydrolysis process, the period for SCOD releasing to reach the equilibrium is regarded as the required reaction time. As shown in Table 1, the stepwise hydrolysis needs reaction time of about 5.0 h, longer than that of conventional hydrolysis, about 3.5 h. However, when the reduce of VSS in sludge is considered, especially in stage 3 and 4, the demanded reactor volumes have not obvious difference between stepwise and conventional hydrolysis. Because TSS is controlled at about 30 g/L in each stage and the volume of the added tap water would be reduced with the decrease of VSS. Moreover, as shown in Table 1, the stepwise hydrolysis presents less alkali demand than that of conventional hydrolysis, because the demands of alkali for carbon release in stage 1, 2 and 3 are very small under the condition of relatively low pH and the efficiency of carbon release is greatly improved by stepwise hydrolysis. Finally, stepwise hydrolysis also shows low energy demand for heating when thermal energy is

Table 1
Comparison of stepwise hydrolysis and conventional hydrolysis in required reacting time, material demand and energy consumptions.

Samples	Items	Required reaction time (hours)	Alkali demand (g/g SCOD)	Energy demand for heating (kJ/g SCOD)
Stepwise hydrolysis	Stage 1	0.50	0.00	137.26
	Stage 2	0.50	0.00	164.21
	Stage 3	2.00	0.44	27.46
	Stage 4	2.00	0.11	11.60
	Total	**5.00**	**0.26**	**33.30**
Conventional hydrolysis	**The control**	**3.50**	**1.34**	**40.83**

considered to be partly recovered. As shown in Table 1, in stepwise hydrolysis, carbon release in stage 1 and 2 need a lot of energy for heating. Although the temperatures in stage 1 and 2 are just about 60 and 80 °C, respectively, the quantity of WAS is large and the amount of released SCOD is limited. In stage 3 and 4, the demand of energy for per unit SCOD is very few, mainly resulting from the large amount of released SCOD and the reduce of sludge volume.

Economic assessment includes investment evaluation and operation cost analysis. Firstly, comparing with the conventional hydrolysis, stepwise hydrolysis requires higher investment expense, since more equipments and reactors would be used in the stepwise hydrolysis due to its complex process. However, the total volume of all the reactors needed in stepwise hydrolysis is nearly equal to that of conventional hydrolysis due to their similar reaction time. Moreover, considering the proportion of pretreatment expense is often very low in the whole investment for sludge fermentation process, investment expense of stepwise hydrolysis would thus be economically acceptable. Secondly, stepwise hydrolysis seems to require similar operation cost as that of conventional hydrolysis, since the former has slightly lower reagent cost and heating energy consumption. Operation cost is mainly composed of reagent cost and energy consumption, while energy consumption further includes energy demands for heating, pumping and stirring, amongst of which, energy demand for heating often occupies more than 80% of the total energy consumption in sludge anaerobic digestion process. Therefore, results indicate that stepwise hydrolysis is economically feasible.

5. Conclusions

A novel strategy for stepwise releasing of sludge carbon was introduced to improve the efficiency of mild hydrolysis. Results of this study also presented an insight into the distribution mechanism of carbon source in WAS, a new concept of improving carbon recovery in wastewater by manipulating WAS composition and the idea of classifiably recovering the nutrients in WAS. Conclusions are commented below:

(1) Stepwise release of organic matters in sludge could be realized by gradually increasing hydrolysis intensity, and stepwise hydrolysis could enhance the amount of SCOD released for almost 2 times of the control, from 7200 to 14,693 mg/L.
(2) Obvious difference of biodegradability exists between carbon sources distributed at different parts of the sludge and stepwise hydrolysis could improve the quality of the released carbon. Extracellular carbon has much higher B/C of more than 0.7 than that of the intracellular carbon, and presents higher conversion rate during anaerobic fermentation for acids production, about 0.7 g VFA/g SCOD.
(3) Humic acid-like substances are the main compounds in the supernatants of hydrolyzed sludge. Improving hydrolysis intensity, fulvic acid-like substances and tyrosine-like protein would be simulated to be produced, the proportion of humic acid-like substances increase first and then decreases, and the concentrations of soluble microbial by-product-like substances decrease first and then slightly increase.
(4) Stepwise release and recovery of organic matters in sludge could greatly alleviate the deterioration of sludge dewaterability during mild hydrolysis.
(5) Comparing with conventional hydrolysis, stepwise hydrolysis has slightly higher investment expense and similar operation cost.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.04.055.

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