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Enhanced efficiency of biological excess sludge hydrolysis under anaerobic digestion by additional enzymes

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ABSTRACT

In this investigation, the effects of commercial enzyme preparation containing alpha amylase and neutral protease on hydrolysis of excess sludge and the kinetic analysis of hydrolysis process were evaluated. The results indicated that amylase treatment displayed higher hydrolysis efficiency than that of protease. VSS reduction greatly increased to 39.70% for protease and 54.24% for amylase at the enzyme dosage of 6% (w/w), respectively. The hydrolysis rate of sludge improved with temperature increasing from 40 to 50 °C, which could be well described by the amended Arrhenius equation. Mixed-enzyme had great impact on sludge solubilisation than single enzyme. The mixture of two enzymes (protease:amylase = 1:3) resulted in optimum hydrolysis efficiency, the efficiency of solids hydrolysis increased from 10% (control test) to 68.43% at the temperature of 50 °C. Correspondingly, the concentration of reducing sugar and NH_4^+-N improved about 377% and 201%, respectively. According to the kinetic analysis of enzymatic hydrolysis process, VSS solubilisation process within prior 4 h followed first-order kinetics. Compared with control test, the hydrolysis rate improved significantly at 50 °C when either single enzyme or mixed-enzyme was added.

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1. Introduction

Due to the quantitative and qualitative expansion of wastewater treatment, sludge produced from biological wastewater treatment processes has dramatically increased in recent decades (Kim et al., 2002). Annual sludge production is about 25 million tons in China presently (calculated by 80% of water content), which will pose a significant threat to the ecology system if it is not properly disposed. Costs for traditional treatment and disposal of excess sludge are quite expensive and can account for up to 60% of the total operation costs of a wastewater treatment plant (Low et al., 2000; Wei et al., 2003). The sludge problem has been gradually outstanding all over the world. Therefore, an advanced and environmentally friendly way of reducing sludge is required.

Anaerobic digestion is an attractive technology for the treatment of organic waste (Romano et al., 2009).The enzymatic hydrolysis of sludge had been investigated for the last three decades and a number of enzymes were reported to play an important role in a range of waste treatment applications. Previous studies have reported that adding enzymes into anaerobic digestion process could not only cut down digesting time, improve sludge digestibility (Wawrzynczyk et al., 2008), and reduce disposal costs (Ronja, 2008), but also could be easily controlled, and its products were harmless to environment (Ahuja et al., 2004). Dey et al. (2006) demonstrated that enzymes were useful both for releasing extracellular polymeric substances (EPS) and identifying polysaccharides and glycoconjugates together with lectins panel. Hydrolytic enzymes can break down polymeric substances through multi-step processes, during which compounds can be transformed from a recalcitrant state to one that is more biodegradable (Gianfreda and Rao, 2004). In other cases, enzymes are able to increase the degradation rate of biodegradable substances, such as activated sludge, allowing for more efficient treatment processes (Whiteley et al., 2002). Kim et al. (2006) investigated the effects of enzymatic pretreatment on solubilisation of food waste with commercial enzymes, and obtained good reduction efficiency. A number of researches had shown the benefit of additional enzymes on the conditioning of wastewater solids and enhancement of the degree of dewaterability of anaerobically digested biosolids (Ayol, 2005; Roman et al., 2006).

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EPS were important integral components of the matrices of the sludge flocs, and composed of a variety of organic substances: polysaccharides, proteins, humic acids, uronic acids, lipid compounds and other non determined molecules (Monique et al. 2008). The hydrolysis of complex organic structures in the degradation of biodegradable particulate organic matter heavily depended on the hydrolytic enzymes, like glucosidases, lipases, and proteases. It was demonstrated that a combination of protease, lipase and endo-glycanases could accelerate solubilisation of municipal sludge (Roman et al., 2006; Wawrzynczyk et al., 2003). However, previous studies reported mainly focused on the effects of enzymes on the hydrolysis of particulate organic waste, enzymatic hydrolysis of sewage sludge has not been conclusively determined, especially the kinetic models has hardly been used to describe the enzymatic hydrolytic kinetics of sewage sludge. Therefore, the objective of this study was to investigate the enhanced efficiency of excess sludge hydrolysis process by additional enzymes and analyze the kinetics parameters during this process. The kinetic equations were tested for their fitness of the hydrolysis data, in order to demonstrate the enhancement of enzymes on the sludge hydrolysis.

2. Methods

2.1. Sludge and enzymes

Excess sludge was obtained from the secondary sedimentation tank of the second municipal wastewater treatment plant in Changsha, China. Fresh sludge was concentrated by settling for 4 h, further filtered through a 0.71-mm metal sieve and then stored at 4 °C for later use. The characteristics of sludge were as followings: pH 6.74, TCOD 7783 mg/L, SCOD 90 mg/L, TSS 8270 mg/L, VSS 5650 mg/L, NH_4^+-N 39.5 mg/L, reduction sugar 32.65 mg/L.

The enzymes were preparations of commercial Neuter protease and α-amylase (both from Jiehui biotechnology Ltd. in Shanghai, China). The activities of protease and α-amylase were 5000 U/g and 6000 U/g, respectively.

2.2. Enzymatic hydrolysis tests

Two batches of hydrolysis tests with single enzyme and mixed-enzyme treatment were conducted. One-hundred milliliters of well-mixed sludge samples were placed in 250 mL Erlenmeyer flasks. After that, different dosages of single enzyme, protease or amylase, were respectively added to flasks in batch I tests, while the batch II tests were conducted using mixed-enzyme with different mixture ratios of protease to amylase. For batch I, the dosages of single enzyme were 3%, 6%, 12%, 18% (w/w, enzyme/TS). For batch II, the mixture ratios of protease and amylase were 1:1, 1:2, 2:1, 1:3, 3:1 (w/w).

In each test, flasks were sealed with rubber stoppers after injecting N_2 for 4 min to maintain strict anaerobic condition. The flasks were shaken at 200 rpm, and the temperature was adjusted by temperature controller according to the demands of different tests.

All tests were conducted in duplicate. The hydrolysis tests without added enzyme were defined as the control test where other corresponding reagents were similar to the enzymatic hydrolysis tests.

2.3. Analytical methods

All samples were filtered through a 450 nm filter and maintained at 4 °C until analyze. Reducing sugar was detected by the phenol sulfuric acid method (Dubois et al., 1956). Other sludge parameters, including ammonia nitrogen (NH_4^+-N), total suspended solids (TSS), volatile suspended solids (VSS), soluble chemical oxygen demand (SCOD), total chemical oxygen demand (TCOD) were determined according to the Standard Methods (APHA, AWWA, WEF, 2005).

Each treatment sample was analyzed in triplicate and the standard deviations of all analyses were always less than 5%, unless noted in the text.

3. Results and discussion

3.1. Effects of single enzyme on sludge hydrolysis

The SCOD/TCOD ratio was measured after 4 h of hydrolysis to evaluate the release of soluble organics from sludge flocs, the results were outlined in Fig. 1a. The SCOD/TCOD ratio of the raw excess sludge was only 1.2% in the beginning. After 4 h of hydrolysis, the ratios respectively increased to 4.7% and 6.3% for the control tests of protease and amylase. However, they increased greatly to 16.3% for protease and 22.3% for amylase at the dosage of 6% (w/w). Furthermore, when the dosage of enzyme reached 18% (w/w), the ratios achieved 22.1% and 26.2%, respectively. The SCOD/TCOD ratios after enzymatic hydrolysis were greatly improved, suggesting that a large amount of particulate organic in sludge flocs was transferred into soluble organic. The increase of SCOD might result from the destruction of flocs structure after enzymatic hydrolysis, promoting the release of colloidal and soluble organics into the solution. But the SCOD/TCOD ratios alone couldn't adequately reflect the optimal dosage of enzyme applied, so further experiments should be performed.

In this study, VSS reduction was used as a critical parameter responsible for sludge hydrolysis. The changes of VSS reduction under single enzyme treatment were displayed in Fig. 1b, and the variation trend of this curve was similar to the curve of Michaelis–Menten equation. VSS reduction linearly increased as the increase of enzyme dosage at low concentration. At the dosage of 6% (w/w), VSS reduction greatly increased to 39.70% for protease and 54.24% for amylase, respectively. With a further increase of dosage, no significant increase of VSS reduction was observed.

As shown in Fig. 2, the ratio of net SCOD increase to net VSS reduction was estimated as 0.50–0.67 mg SCOD $(\text{mg VSS})^{-1}$ for protease treatment, and 0.52–0.73 mg SCOD $(\text{mg VSS})^{-1}$ for amylase treatment. The results suggested that 0.50–0.73 mg SCOD would be generated when 1 mg VSS of excess sludge was destroyed under enzymes treatment.

Linking the high ratios of SCOD/TCOD (Fig. 1a) to the high VSS reduction (Fig. 1b), the addition of enzymes could significantly enhance excess sludge hydrolysis. However, the enzymes failed to enhance VSS disintegration efficiently when the dosage was more than 6% (w/w). Therefore, 6% (w/w) appeared to be the optimum dosage in terms of economy and efficiency.

3.2. Effects of mixed-enzyme on sludge hydrolysis

Single enzyme has limited hydrolytic activity while mixed-enzyme can exhibit a synergistic effect in sludge hydrolysis where the hydrolytic activity of the mixed-enzyme is greater than the sum of the hydrolytic activity of the single enzyme (Roman et al., 2006). By optimizing operational factors such as mixture ratio and temperature to fit bacterial systems and enzymes, it could be possible to attain a higher rate and more effective hydrolysis of excess sludge. Based on the results of single enzymes hydrolysis, the parabolic effects of mixed-enzyme on hydrolysis should be further explored. Since particulate organic matters were changed into soluble organic matters after enzymatic hydrolysis, VSS

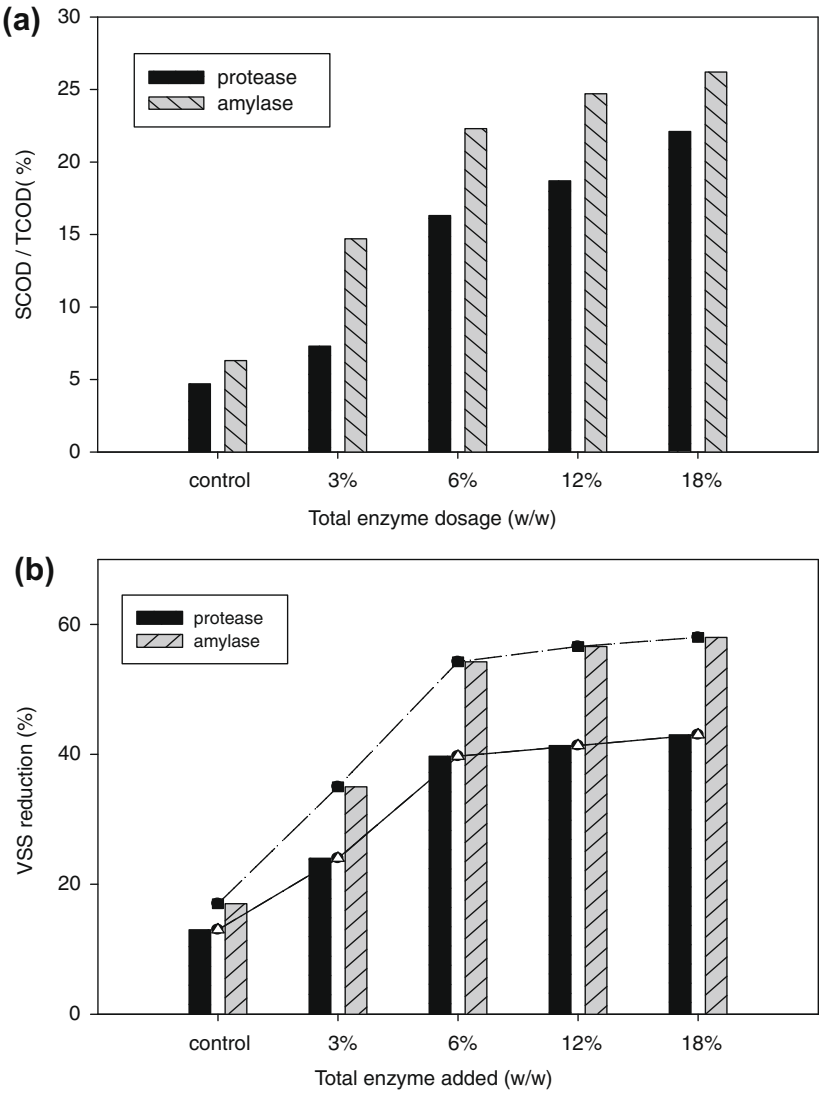

Fig. 1. Changes of SCOD/TCOD and VSS reduction in single enzymes treatment (a) SCOD/TCOD; (b) VSS reduction.

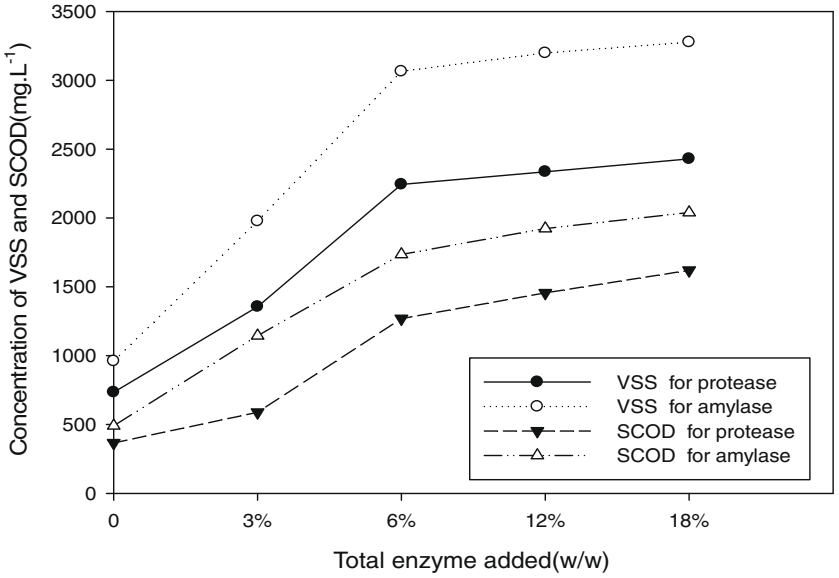

Fig. 2. Effect of single enzyme treatment on solubilisation of excess sludge.

concentration of sludge samples was lower than control test (Fig. 3). The observations confirmed that there was virtually no significant relationship between temperature and VSS reduction for the control test, while it had dramatically effect on enzyme-treated samples within the temperature range of 40–50 °C. Apparently, the VSS concentration of control test only decreased from 5650 mg/L (40 °C) to 5350 mg/L (50 °C). However, the obvious decrease of VSS concentration to 2420 mg/L was observed at the mass ratio, approximately 1:3 for protease and amylase at the temperature of 50 °C, which seemed to improve VSS reduction effectively.

Proteins and carbohydrates are two of the most predominant organic matters in EPS and compose a large proportion of SCOD or COD in the sludge. After enzymatic hydrolysis, the cells autolyzed or be hydrolyzed: proteins converted into polypeptides, two peptides, amino acid, and amino acid further transformed into low molecule organic acid, ammonia and carbon dioxide; carbohydrates were hydrolyzed into polysaccharides, or even reducing sugar (Ji and Brune, 2005). Therefore, the changes of reducing sugar and NH_4^+-N concentration during sludge hydrolysis could provide a more thorough understanding of the influence of enzymatic hydrolysis. Fig. 4 depicted the influence of enzymatic hydrolysis on proteins and carbohydrates. It can be seen that the results were in accordance with previous observations of VSS reduction (Fig. 3). With the increase of temperature from 40 to 50 °C, the concentration of reducing sugar and NH_4^+-N increased obviously under the same mixture ratio. When treated with the mixture ratio of 1:3 for protease and amylase at the temperature of 50 °C, the two indicators achieved the maximal value, 177.8 and 143.43 mg/L, respectively.

According to the experiment results obtained above, it can be noted that temperature played a vital role in sludge hydrolysis. Under the same mixture ratio within the chosen temperatures, the higher the temperature, the higher the solubilisation of VSS and the concentrations of dissolving reducing sugar, NH_4^+-N. Recalcitrant organic in sludge is broken into dissolving organic by the combination effects of heat and enzymatic, and is further decomposed. Firstly, it is quite evident that almost all cell reactions are influenced by temperature, thus high temperature can accelerate destroying rate of flocs structure. According to the amended Arrhenius equation $\gamma(T) = \gamma(20\ ^\circ C)\ \theta^{(T-20)}$, reaction rate is correlated with temperature. Within the range of optimal temperature, the reaction rate improves 1–2 times every elevated 10 °C. On the other hand, the enzyme activity can be increased by improving temperature, thus resulting in improved sludge solubilisation. As discussed above, VSS reduction increased from 25.6% to 58% at the mixture ratio of 1:3 within the temperature range of 40–50 °C. Correspondingly, the concentration of reducing sugar and NH_4^+-N improved about 377% and 201%, respectively. Consequently, the mixture ratio of 1:3 for protease and amylase and the temperature of 50 °C appeared to be the optimum condition for enhancing excess sludge hydrolysis.

3.3. Kinetic analysis of sludge hydrolysis

During sludge hydrolysis, the changes of VSS and SCOD with hydrolysis time under different enzymes were illustrated in Fig. 5. Clearly, the slope of the curve indicated the changes of VSS and SCOD per unit time. Therefore, any slope of points on the curve represented the reaction rate of corresponding time. It seemed that the slopes for each test were all nearly invariable during the initial 4 h of hydrolysis either for the changes of VSS or SCOD. 42%, 56.32% and 68.43% of VSS reduction for protease, amylase and mixed-enzyme treatment were respectively obtained compared with 10% for the control test after hydrolysis, the concentration of SCOD also improved correspondingly.

Enzymatic hydrolysis of sludge can disrupt EPS matrix, which resulted in enhanced solubilisation of the sludge flocs. As the flocs disintegrated, macromolecular substances that were previously protected from enzyme attack are exposed and may be degraded by hydrolytic enzymes. Subsequently, the curve gradually approached stationary value after about 4 h of hydrolysis, and observed only a slight VSS reduction beyond that time, which was perhaps caused by depletion and deactivation of enzymes. It was also observed that most of the VSS reduction process occurred within prior 4 h of hydrolysis, which adequately proved the reaction time of 4 h chosen above was reasonable.

It is quite evident from Fig. 5 that amylase displayed higher hydrolysis efficiency than protease. According to Feng et al. (2009), the hydrolysis of particulate COD (mainly protein) to soluble substances is the rate limiting step of SCFAs production. Therefore, the hydrolysis efficiency of protein was poorer than carbohydrates. Furthermore, the reduction efficiency of amylase might be higher than that of protease used in the experiments. In

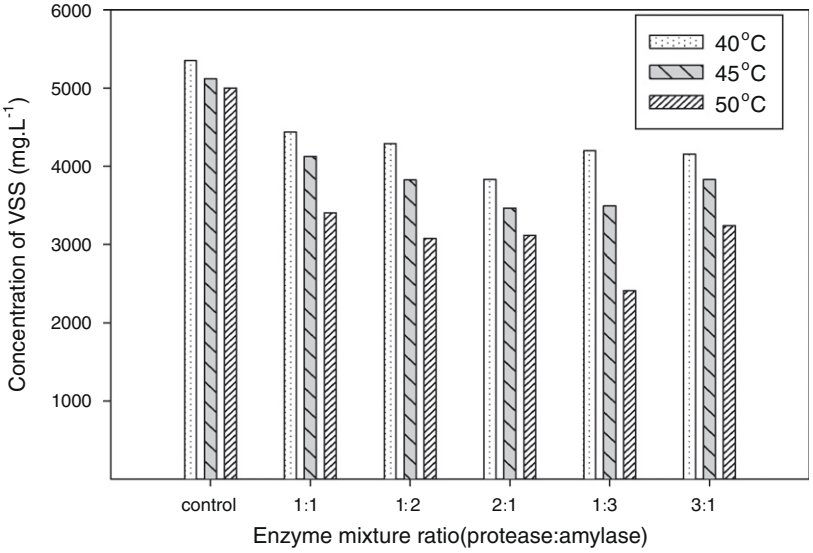

Fig. 3. Effect of temperature and enzyme mixture ratio on enzymatic hydrolysis.

addition, mixed-enzyme treatment showed higher hydrolysis efficiency than that of single enzymes. The reason is that a specific type of enzyme can only be effective to one definite substrate (Zhou et al., 2009). Due to the complexity of the composition of sludge, it is quite likely that a wide range of enzymes are essential for the hydrolysis of different substrates.

Sludge mainly contains carbonaceous and is rich in organic matter. Considering the high percentage of organic matter, VSS dissolving plays an essential role in sludge hydrolysis. VSS reduction was directly proportional to hydrolysis time at the initial stage (Fig. 5a). The slope of regression line for mixed-enzyme versus hydrolysis time was higher than that for protease and amylase, indicating mixed-enzyme treatment showed higher hydrolysis efficiency than single enzyme treatment.

Feng et al. (2009) analyzed waste activated sludge (WAS) hydrolysis and short-chain fatty acids production at pH 10, and observed that both the hydrolysis of WAS particulate COD and the accumulation of SCFAs followed first-order kinetics. As discussed above, the hydrolysis of VSS and SCOD in our study could also be assumed by the first-order kinetics. The first-order kinetic equation of hydrolysis (VSS reduction) can be described as:

$$-\frac{dX}{dt} = K_h X \tag{1}$$

$$\ln X = -K_h t + b \tag{2}$$

where, K_h is the solubilisation rate constant and b is the constant of integration. By plotting $\ln X$ ($\ln c_{vss}/c_{vss0}$) versus t, the slope and the intercept can be obtained, which corresponded respectively to the value of K_h and b. The $\ln(c_{vss}/c_{vss0}) - t$ regression curves of different enzymes were illustrated in Fig. 6 and the value of VSS hydrolysis rate constants were summarized in Table 1.

As Fig. 6 and Table 1 shown, it was clear the goodness of fit values for different enzymes were generally good in the range of 0.95–0.99, which further indicated that the model fitted the experimental data adequately. Therefore, VSS solubilisation within prior 4 h followed the first-order kinetics sufficiently.

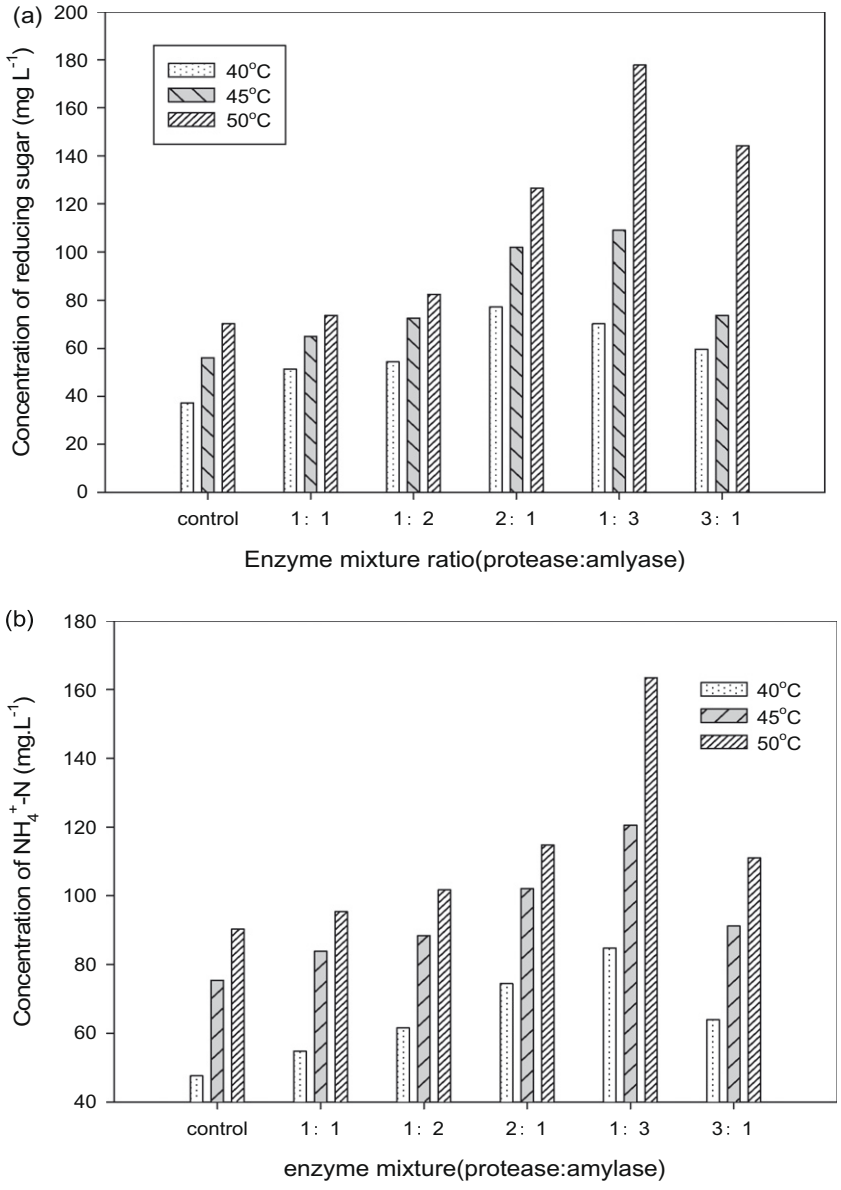

Fig. 4. Variation of reducing sugar and NH_4^+-N concentrations under various enzyme mixture ratio and temperatures: (a) reducing sugar concentration; and (b) NH_4^+-N concentration.

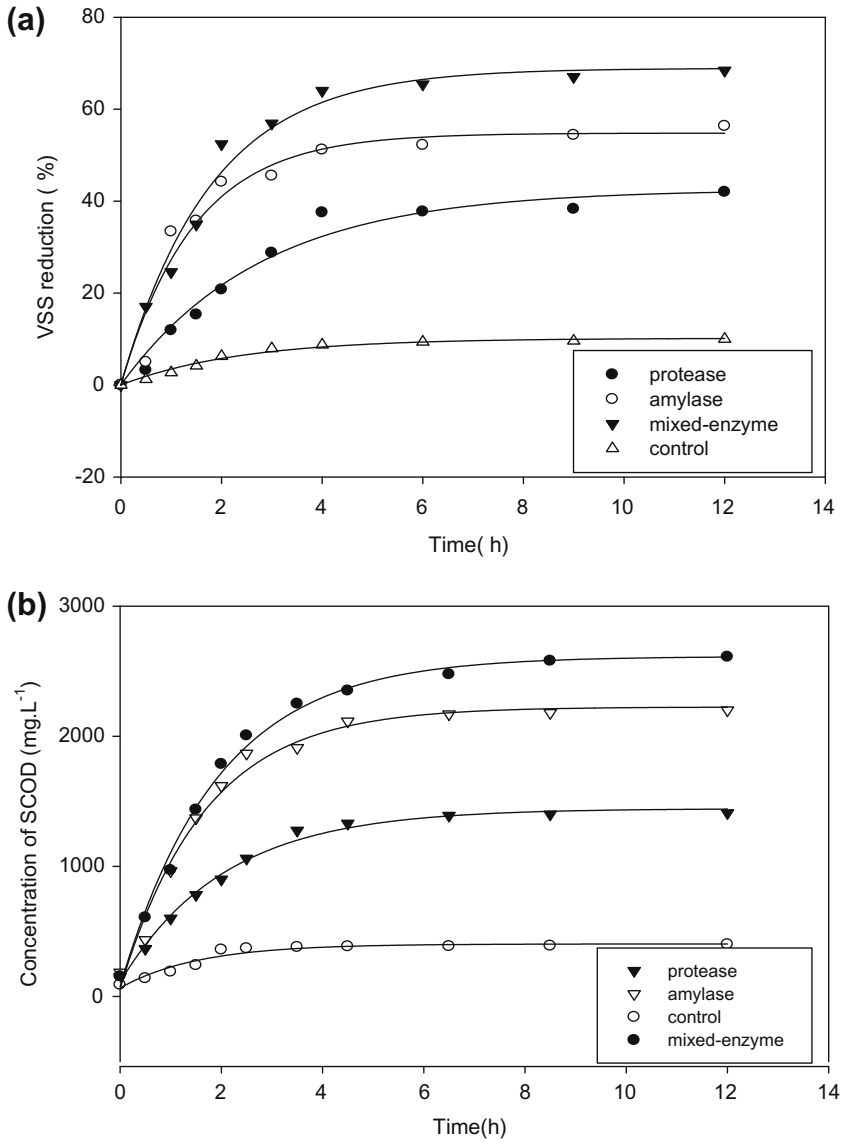

Fig. 5. Variation of VSS reduction and SCOD generation in different enzymes hydrolysis: (a) VSS reduction; and (b) SCOD generation (dosage of enzymes = 6%; protease:amylase = 1:3; hydrolysis temperature = 50 °C).

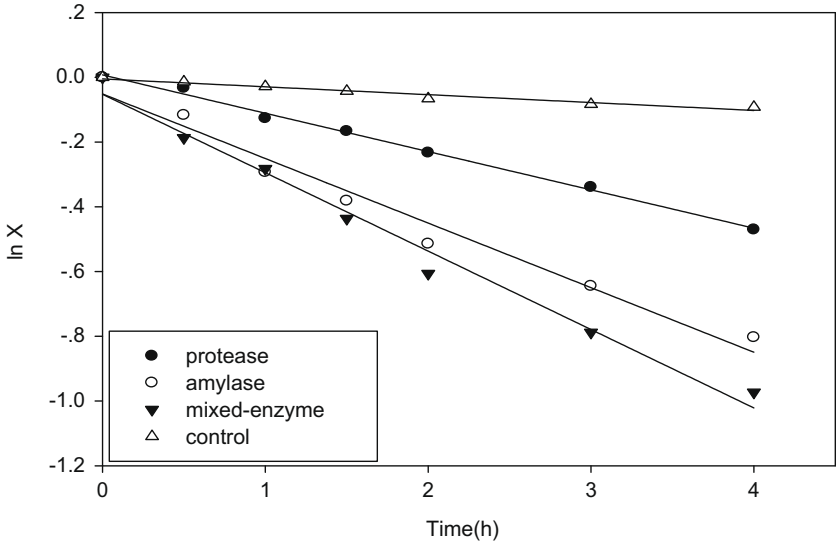

Fig. 6. Relationship between lnX and t during sludge hydrolysis.

Table 1
Hydrolysis rate constants under different enzymes hydrolysis.

Enzymes	Dynamic equation	Rate constants K_h (h^{-1})	Coefficients R^2
Control	$y = -0.024x - 0.005$	0.024	0.95
Protease	$y = -0.118x + 0.007$	0.12	0.99
Amylase	$y = -0.199x - 0.051$	0.2	0.97
Mixed-enzyme	$y = -0.242x - 0.052$	0.24	0.98

As the hydrolysis process of sludge is affected by several factors, such as pH, temperature, enzyme types and hydrolytic substance, different K_h were reported. For example, the K_h at 35 °C was 0.169 d^{-1} in the study of Ferreiro and Soto (2003). In order to understand the effect of enzyme on the K_h of sludge hydrolysis, the enzymatic hydrolysis of sludge was compared with control test. It was observed that the hydrolysis of sludge (VSS reduction) in the control test followed the first-order kinetics (K_h was 0.024 h^{-1}). As summarized in Table 1, the hydrolysis rate constants K_h for protease, amylase and mixed-enzyme treatment were 0.12, 0.20 and 0.24 h^{-1}, respectively, which were 5, 8 and 10 times that of the control test, indicating that amylase had higher sludge reduction efficiency than protease, and mixed-enzyme treatment displayed the highest hydrolysis efficiency.

4. Conclusions

The study shows that the biological excess sludge hydrolysis can be enhanced by additional enzymes. Sludge solubilisation was enhanced by 39.70% for protease and 54.24% for amylase. The efficiency of sludge hydrolysis didn't linearly improve with the addition of enzyme and reached to the balance at the dose 6% (w/w, enzyme/ TS). The highest VSS reduction (68.43%) was observed with the mixture ratio of 1:3 for protease and amylase at 50 °C. Stable hydrolysates can be obtained within prior 4 h of hydrolysis. VSS solubilisation followed first-order kinetic, and the hydrolysis rate constants K_h for protease, amylase, mixed-enzyme treatment were 0.12, 0.20 and 0.24 h^{-1}, respectively.

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