



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

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ARTICLE INFO

Article history:

Received 3 September 2009

Received in revised form 4 November 2009

Accepted 4 November 2009

Available online 4 January 2010

Keywords:

Excess sludge
Additional enzyme
Sludge hydrolysis
First-order kinetics

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 NH₄-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 follows: 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 (mg VSS)⁻¹ for protease treatment, and 0.52–0.73 mg SCOD (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

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