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Performance of the lysozyme for promoting the waste activated sludge biodegradability

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HIGHLIGHTS

- Lysozyme has a strong ability to disintegrate WAS for biodegradability promotion.
- Soluble protein dominated in SMP contributed to improve sludge biodegradability.
- It is feasible to reclaim the carbon source from sludge by lysozyme digestion.

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ABSTRACT

The fresh waste activated sludge (WAS) from a lab-scale sequencing batch reactor was used to determine the performance of the lysozyme for promoting its biodegradability. The results showed that a strict linear relationship presented between the degree of disintegration (DD_M) of WAS and the lysozyme incubation time from 0 to 240 min (R^2 was 0.992, 0.995 and 0.999 in accordance with the corresponding lysozyme/TS, respectively). Ratio of net SCOD increase augmented significantly by lysozyme digestion for evaluating the sludge biodegradability changes. Moreover, the protein dominated both in the EPS and SMP. In addition, the logarithm of SMP contents in supernatant presented an increasing trend similar with the ascending logarithmic relation with the lysozyme incubation time from 0 to 240 min (R^2 was 0.960, 0.959 and 0.947, respectively). The SMP, especially the soluble protein, had an important contribution to the improvement of WAS biodegradability.

1. Introduction

In recent years, large numbers of residual sludge have been dramatically increasing day by day with the fast development and wide application of the biological wastewater process (Kim et al., 2002). Moreover, the disposal cost of the excess sludge may account for approximately 25–60% of the total wastewater treatment cost (Zhao and Kugel, 1996). Anaerobic biological treatment has been used widely for the excess sludge disposal due to its obvious advantages: (1) the low expenditure, (2) the lower spaces than the traditional process, (3) the biogas production recovery (Ayol, 2005; Romano et al., 2009). Due to the huge disposal cost and the potential risks to the environment of the sludge, it is essential to develop an effective excess sludge pre-disposal technique urgently for the more sludge production in future which can accelerate the sludge hydrolysis process in sludge anaerobic digestion process. A large number of technologies have been used for the sludge pre-treatment to improve the biodegradability, including the physical ones such as the ultrasonic treatment (Khanal et al., 2007), the chemical ones including the ozone treatment (Kameswari et al., 2014), the acid or alkaline disposal (Cassini et al., 2006), and the biological treatment containing adding the commercial enzyme products directly (Yang et al., 2010) or isolation and enrichment the special bacteria from sludge which can synthesis the effective enzymes for sludge digestion (Tang et al., 2012).

Biological enzyme is a kind of effective catalyst in the biochemical reactions which has been applied widely for accelerating the sludge hydrolysis by attacking the sludge flocs and getting the insoluble materials soluble in sludge disposal field (Romano et al., 2009). It could shorten the reaction time and reduce the sludge pre-disposal cost. Meanwhile, its digestion products are friendly to the environment (Ahuja et al., 2004). The previous studies have found that the sludge biodegradability could be improved by adding enzyme products directly. Yang found that the mixed enzymes (protease:amylase = 1:3) had great impact on promoting the sludge solubilisation (Yang et al., 2010). Ayol found that

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utilizing the enzymatic digestion to improve the conditioning of wastewater sludge was significant and effective (Ayol, 2005). The protease and amylase were used widely in the sludge pre-treatment process for improving the biodegradability of excess sludge (Teo and Wong, 2014; Yu et al., 2013). Song found that the lysozyme digestion could reduce the excess sludge production to almost 100% in the sequential batch reactor (SBR) by returning the disintegrated sludge to the reactor in the first 30 days of operation (Song et al., 2013). Compared with the protease and amylase, the lysozyme had more effective and stronger ability to disrupt the microbial cell wall composed of the peptidoglycan. However, studies on lysozyme applied to improving the sludge biodegradability are just at the low level. Also, the variations of the organic substances in sludge caused by the lysozyme digestion, containing the extracellular polymeric substances (EPS) which played a significant role for maintaining the sludge flocs stable (Monique et al., 2008), soluble microbial products (SMP), soluble COD and ammonia, are still in the limitedly cognitive level.

The objective of this study was to elucidate the performance and efficiency of the lysozyme digestion to disrupt the bacteria cell wall and disintegrate or attack the macromolecules in sludge flocs for improving the waste activated sludge (WAS) biodegradability. Besides, relationships between the organic substance (EPS and SMP) and the lysozyme incubation time were also analyzed.

2. Methods

2.1. WAS and the lysozyme

The WAS used in this experiment was taken from a working well lab-scale SBR for treating actual municipal wastewater. Fresh sludge was concentrated by settling for 2 h, then prepared for the following experiment. The traits of the raw sludge were as following: total solids (TS) 8350 mg/L, volatile suspended solids (VSS) 7000 mg/L, soluble COD (SCOD) 50 mg/L, total COD (TCOD) 7500 mg/L. NH_4^+-N 4.8 mg/L, pH 6.7.

The activities of lysozyme (bought from Beijing Biotopped Science and Technology Company, China) were about 20,000 U/mg. Other characteristics of the lysozyme: MW (molecular weight) was about 14,400. pH 3.5–6.5. Moisture content was less than 5%.

2.2. Lysozyme for WAS pre-disposal experiment

Four identical clean flasks were used to determine the effect of different lysozyme concentrations for disintegrating the excess sludge, which were 5%, 10%, 15% (w/w, lysozyme weight/TS weight) and the control one, respectively. Each of them contained with 200 ml WAS and placed in the water-bath at 35 °C. Then the 5%, 10%, 15% lysozyme added to the first three flasks in sequence while the fourth flask without added lysozyme as the control. During the incubation time, the all flasks were kept stirring at proper speed on a shaking bed. The strict anaerobic condition would be kept by using rubber stoppers after injecting the N_2 to the bottles.

2.3. Analytical methods

Protein and polysaccharide are the main components both in EPS and SMP. Also they are the dominant substances used for short-chain fatty acids (SCFAs) production (Luo et al., 2014). The sum of protein and polysaccharide was defined as the EPS or SMP actual contents both in the WAS phase and the liquid phase (Gao et al., 2011). The variations of EPS and SMP concentration could reflect the degree of WAS disintegration by lysozyme digestion as well as the SCOD.

2.3.1. Samples collection and extraction of SMP and EPS

The testing samples were collected from the flasks when the digestion time was 15 min, 30 min, 60 min, 120 min and 240 min, respectively. The collected samples were centrifuged twice at 6000 g for15 min at 4 °C. The extraction of SMP and EPS based on the reference (Malamis and Andreadakis, 2009). Then the supernatant was used for the SMP extraction (the supernatant samples containing SMP was filtered through a 0.45 μm membranes filter and the filtrate represented the total SMP). The ammonium and short-chain fatty acids (SCFAs) in the supernatant also need to be tested. The remaining supernatant samples filtered through a cellulose membrane with a pore size of 0.45 μm for the SCOD (actually it was the sum of the colloidal and soluble COD) analysis (Andreottola and Foladori, 2006). The precipitation was used for EPS extraction and testing the VSS. $EPS_{protein}$ and $EPS_{polysaccharide}$ obtained by the following steps: (1) Re-suspended the precipitation in the buffer solution with the same volume of supernatant; (2) 0.3 mL methanal and 20 mL sodium hydroxide (1 M) were successively added to the suspension, the suspension was sat for 3 h at 4 °C; (3) centrifuged at 15,000*g* for 20 min (4 °C) and the supernatant was filtered through a 0.22 μm membranes filter. The samples for TCOD and TS testing were collected when the fresh WAS settled for 2 h.

2.3.2. Testing samples measurement

TS, VSS, TCOD, ammonium and SCOD were determined according to the standard methods (APHA, 1998). Protein and polysaccharide in EPS,SMP were measured according to the previous publications (Gao et al., 2011, 2013). The standard curve was established for testing the polysaccharide based on the phenol-sulphuric acid method in the previous publication (Michel et al., 1956) used the Glucose (D-glucose, 99.5%, China) as a standard. The protein was tested by the Modified BCA kit (Sangon, China). SCFAs were determined by the gas chromatography according to the method of the previous literature (Tong and Chen, 2007) when the digestion time was 4 h.

2.4. Calculation

2.4.1. DD_M

DD_M was proposed to represent the degree of disintegration in the WAS by Müller (Schmitz et al., 2000).

$$DD_M = \frac{COD_1 - COD_0}{COD_{NaOH} - COD_0} \times 100\% \quad (1)$$

Where COD_1 representing the COD in the supernatant of the treated samples by the lysozyme (mg/L). COD_0 stands for the COD in the supernatant of the original WAS (mg/L). COD_{NaOH} is the maximum COD in the supernatant from the sludge samples which suffered the 1 M NaOH digestion for 10 min at 90 °C in the ratio of 1:2 (the sludge samples volume: 1 M NaOH volume) (mg/L) (Khanal et al., 2007; Schmitz et al., 2000). The centrifugation was done to determine the DD_M in all samples treated by the lysozyme for 10 min with 30,000*g* at 4 °C (Schmitz et al., 2000). All the experiments were done for triple times and the average value was used.

2.4.2. The evaluation of sludge biodegradability improved

SCOD in the liquid phase was the conspicuous index for demonstrating the sludge solubilization and its biodegradability change. Hence, in order to evaluate the promotion of the sludge biodegradability by lysozyme digestion, the concept of net SCOD increase ratio was introduced (Yang et al., 2010) which can be calculated as follows:

$$\text{Ratio of net SCOD increase} = \frac{COD_1 - COD_0}{COD_{NaOH}} \times 100\% \quad (2)$$