Bioresource Technology 175 (2015) 112–119

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Microbial community related to lysozyme digestion process for boosting waste activated sludge biodegradability

Xiao-Dong Xin, Jun-Guo He, Wei Qiu *, Jian Tang, Tian-Tian Liu

School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, China

HIGHLIGHTS

- Microbial community shifted obviously affected by lysozyme digestion.
- Similarity among digested communities diminished with the lysozyme dosage augment.
- Community diversity decreased as sludge suffered lysozyme digestion.
- Microbial population distribution tended to be even affected by lysozyme digestion.
- Sludge with high diversity and poor evenness had huge potential to be solubilized.

ARTICLE INFO

Article history:
Received 5 August 2014
Received in revised form 7 October 2014
Accepted 9 October 2014
Available online 18 October 2014

Keywords:
Microbial community
Biodiversity
Evenness
Lysozyme digestion
Biodegradability promotion

ABSTRACT

Waste activated sludge from a lab-scale sequencing batch reactor was used to investigate the potential relation of microbial community with lysozyme digestion process for sludge solubilization. The results showed the microbial community shifted conspicuously as sludge suffered lysozyme digestion. Soluble protein and polysaccharide kept an increasing trend in solution followed with succession of microbial community. The rise of lysozyme dosage augmented the dissimilarity among communities in various digested sludge. A negative relationship presented between community diversity and lysozyme digestion process under various lysozyme/TS from 0 to 240 min (correlation coefficient R^2 exceeded 0.9). Pareto–Lorenz curves demonstrated that microbial community tended to be even with sludge disintegration process by lysozyme. Finally, with diversity (H) decrease and community distribution getting even, the SCOD/TCOD increased steadily in solution which suggested the sludge with high community diversity and uneven population distribution might have tremendous potential for improving their biodegradability by lysozyme digestion.

1. Introduction

Recent years, the excess sludge production increased greatly with the wide application of wastewater biological treatment process (Kim et al., 2002). The excess sludge problem has been gradually outstanding all over the world. It is needed urgently to develop an advanced technology for excess sludge treatment as the tremendous cost of sludge disposal process (Zhao and Kugel, 1996). Quite numbers of technologies have been applied for sludge pretreatment to improve the biodegradability, including biological method. Currently, one important biological method was utilizing the commercial enzyme products to implement the sludge pretreatment. The previous studies have found that adding enzyme products could not only promote the disintegration of large sludge particles to accelerate hydrolysis, but also improve the soluble organic fraction in solution for further treatment (Ayol, 2005; Yang et al., 2010). The advantages of it were obvious: (1) high digestion efficiency (Wawrzynczyk et al., 2008), (2) shorten hydrolysis time, (3) the low sludge predisposal cost, (4) environment-friendly reaction products (Yang et al., 2010). Another excellence of utilizing the enzyme products directly to digest residual activated sludge was boosting the sludge biodegradability for carbon source recovery (Ahuja et al., 2004; Teo and Wong, 2014). The lysozyme, one of the important commercial individual enzymes, has been used for sludge matrix destruction and excess sludge production minimization widely. It can destruct the β-1, 4 glucoside bond which connected the N-acetylmuramic acid and N-acetylglucosamine in cytoderm and decompose the insoluble mucopolysaccharide into soluble peptide to result in the lysis of bacteria. Song succeeded in reducing sludge by the lysozyme in a lab-scale sequencing batch reactor (SBR) and it performed well for reducing

* Corresponding author. Tel.: +86 0451 862868111.
E-mail address: qiuweihit@126.com (W. Qiu).

http://dx.doi.org/10.1016/j.biortech.2014.10.042
0960-8524/

the excess sludge production to almost 100% in the first 30 days of operation (Song et al., 2013). Also it was reported that lysozyme could be used for the decomposition of sludge microbial cell wall, then release the intracellular substances into solution, such as lipopolysaccharide (Gill and Holley, 2003; Hu and Lu, 2004; Moak and Molineux, 2004). Thus utilizing lysozyme has obvious advantages for sludge predisposal or reuse due to: low expenditure; portable operation; excellent performance for sludge lysis; huge flexibility among the final options for sludge predisposal products. However, the biological response of sludge matrix induced by lysozyme digestion was poorly understood.

Sludge biodegradability promotion by the method of lysozyme digestion depended on the destruction of sludge microbial cell wall. The lysates flowing into the solution, such as soluble protein and polysaccharide, promoted sludge solubilization. Thus rise of lysates in solution by lysozyme digestion was bound to be associated with microbial population variation in biosolids. Moreover, microbial community biodiversity and population evenness had been related to the functional characteristics of sludge ecosystem (Loreau et al., 2001; Saikaly et al., 2005; Stamper et al., 2003; Wittebolle et al., 2009). Community diversity was an essential concept in ecology, and its quantification was basal for investigating phenomena such as community succession, colonization and the response to perturbed shock. The dynamics of microbial communities, especially the dominant microbial population, played an important role in the functionality of an ecosystem in biosolids. For example, the β-*Proteobacteria* could generate and release extracellular polymeric substances (EPS) which had significant effect for maintaining the sludge flocs stable when it was the prior species (Li et al., 2012; Monique et al., 2008). Then the soluble organic fraction of sludge got improved with microbial community varying affected by lysozyme disintegration. On the other hand, it was found that initial community distributive evenness was a key factor in preserving the functional stability of an ecosystem (Wittebolle et al., 2009). And Tao discovered that an evenly distributed community could shorten anammox bioreactor start-up time (Tao et al., 2013). Therefore, exploring the potential relation of community evenness with functional characteristics of sludge under perturbed condition was to be an interesting issue. With the development of molecular microbiological techniques, terminal restriction fragment length polymorphism (T-RFLP) analysis of PCR-amplified 16S rDNA had been used as a useful tool to analyze the diversity and distributive evenness of a microbial community in biosolids and obtained the fingerprints message (Kaplan and Kitts, 2003; Saikaly et al., 2005; Tao et al., 2013). The community diversity was demonstrated by Shannon index in detail (Eichner et al., 1999; Shannon, 1948) while the community evenness evaluated by Equitability index quantitatively (Saikaly et al., 2005; Stamper et al., 2003) or described by the Pareto–Lorenz curve figuratively (Lorenz, 1905).

A better understanding about the relation of microbial population changes with lysozyme incubation process would be beneficial to optimize the operating conditions of lysozyme digestion for elevating sludge biodegradability. However, the cognitive level about the linking of microbial community biodiversity with the sludge lysozyme digestion treatment was still very low. Besides, the development of community structure, biodiversity and distribution evenness in sludge with lysozyme digestion process by using molecular-based approaches had not been investigated. Thus the purpose of this study was to investigate the potential relation of microbial community with lysozyme digestion process from the view points of community ecology. The specific aims of this study were to: (1) investigate the sludge microbial community changes affected by lysozyme digestion and the soluble substance (protein and polysaccharide) variation in solution, (2) elucidate the relationships of communities diversity, similarity and evenness with the sludge lysozyme digestion process, (3) demonstrate the potential correlation between community diversity and population distributive evenness with biodegradation promotion (evaluated by SCOD/TCOD variation in solution).

2. Methods

2.1. Sludge and lysozyme

The waste activated sludge (WAS) was taken from a lab-scale sequencing batch reactor (SBR) used for actual municipal wastewater treatment. The detailed characteristics of the WAS were as follows: total solids (TS) 8000 ± 255 mg/L, volatile suspended solids (VSS) 6850 ± 165 mg/L, soluble COD (SCOD) 80 ± 14 mg/L, total COD (TCOD) 7420 ± 244 mg/L. pH 6.8 ± 0.1.

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

2.2. The experiment design and sampling

A batch of the tests was prepared to detect the effects of the lysozyme digestion on the microbial community in WAS. Three clean 250 ml Erlenmeyer flasks were placed with two hundred milliliters identical and fresh excess sludge, respectively. Then different dosages of lysozyme were added to the Erlenmeyer flasks which were 5%, 10% and 15% (lysozyme weight/TS weight, w/w), respectively. Subsequently the Erlenmeyer flasks were placed in the water-bath at 35 °C when lysozyme digestion began. Meanwhile, the Erlenmeyer flasks kept stirring at prompt speed by a shaking bed. Also all Erlenmeyer flasks were sealed strictly by the rubber stoppers during the lysozyme reaction process.

The testing samples were collected from Erlenmeyer flasks when the reaction time was 0 min (the fresh sludge), 15 min, 30 min, 60 min, 120 min and 240 min, respectively.

2.3. Analytical methods

Parts of each collected sample (about 1 ml) were used to extract DNA for the microbial community analysis. The residual parts of each sample were used to determine some parameters reflected the characteristics of the digested sludge.

Total solids (TS), volatile suspended solids (VSS), soluble chemical oxygen demand (SCOD), total chemical oxygen demand (TCOD) were determined according to the standard methods (APHA, 1998). After the centrifugation at 6000*g* for 15 min (4 °C), then the supernatant samples which containing soluble protein and polysaccharide were filtered through a 0.45 μm membranes filter and the soluble protein and polysaccharide was obtained for next analysis. The standard curve was established for testing the polysaccharides based on the phenol–sulphuric acid method in the previous publication used the Glucose (D-glucose, 99.5%, China) as a standard (Michel et al., 1956). The proteins were tested by the Modified BCA kit (Sangon, China). Then the centrifugation at 12,000*g* for 20 min (4 °C) for the residual samples was conducted. The supernatant in each sample were used to detect the SCOD. DO, pH and temperature were monitored using WTW Handheld Multiparameter Instruments (pH/Oxi 340i, WTW, Germany).

Each collected sample was analyzed in triplicate for reducing the standard deviations of all above analyses and the average value was calculated.