Chemical Engineering Journal 284 (2016) 979–988

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

# Solubilization augmentation and bacterial community responses triggered by co-digestion of a hydrolytic enzymes blend for facilitating waste activated sludge hydrolysis process

Xiaodong Xin, Junguo He, Jinghan Feng, Lin Li, Zhidan Wen, Qi Hu, Wei Qiu *, Jie Zhang

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

## HIGHLIGHTS

- WAS solubilization could be boosted distinctly by the enzymatic co-digestion.
- The high compressibility of digested WAS might be conducive to a further treatment.
- Bacterial community shifted obviously triggered by the enzymatic co-digestion.
- WAS with high diversity had huge potential to be solubilized by the enzymolysis.
- Rising co-digestion abated community dominance and caused the WAS further lysis.

## ARTICLE INFO

*Article history:*
Received 5 July 2015
Received in revised form 8 September 2015
Accepted 12 September 2015
Available online 25 September 2015

*Keywords:*
Enzymatic co-digestion
Waste activated sludge solubilization
Microbial community structure
Diversity decrease
Community distribution evenness

## ABSTRACT

Performance of a hydrolytic enzymes blend for WAS solubilization augmentation and bacterial community characteristics responses were investigated in this study. The hydrolytic enzymes blend (lysozyme, α-amylase, protease and cellulase) was used to digest WAS with the adding dosage of 0 (control test), 5%, 10% and 15% (enzymes/TSS, w/w, the adding proportion of each enzyme in the mixture was maintained at 1:1:1:1), respectively. Results showed that soluble COD, protein and carbohydrate in solution presented a substantial increase up to about 6000–9000 mg/L, 1500–3000 mg/L and 550–700 mg/L approximately just in 180 min by the enzymatic co-digestion. Moreover, slight augmentation of VSS/TSS inferred that the stubborn substances were solubilized partially in solid phase. Tryptophan protein-like and simple aromatic proteins-like substances were the main digestive compositions in WAS lysis which promoted the biodegradability largely. Increase of particle size and the broad distribution boosted the compressibility of digested WAS. Community traits changes in digested WAS phase contained: (1) bacterial community shifted conspicuously with the enzymes mixture dosage rising which exaggerated the communities' dissimilarity; (2) community diversity diminished obviously during the enzymatic incubation process, which might decrease the initial community relative stability to cause serious disintegration; (3) community distribution tending to be even with the enhancing co-digestion effect. The observations above sparked an inspiration that the sludge community with high diversity and poor initial evenness might have great potential for carbon resource release by the enzymolysis.



## 1. Introduction

In past years, production of waste activated sludge (WAS) from municipal wastewater treatment plants (WWTPs) had increased sharply with lots of hazardous risks to the environment. Anaerobic digestion (AD), one of the most efficient measurement for reducing and stabilizing WAS with biogas (methane) generation [1], was considered to be the most energy efficient method for WAS treatment, meanwhile, the drawbacks of WAS accumulation could be diminished [2]. However, the WAS hydrolysis process, as the rate-limiting stage in AD process, needed a relatively long time (even days) which restricted the WAS treatment efficiency and increased operational cost [3] .

To boost the WAS hydrolysis efficiency, many pre-treatment methods had been implemented and developed, including the

* Corresponding author at: No. 73 Huanghe Road, Nangang District, Harbin, PR China. Tel.: +86 0451 862868111.
*E-mail addresses:* xinxiaodong1988@126.com (X. Xin), qiuweihit@126.com (W. Qiu).

http://dx.doi.org/10.1016/j.cej.2015.09.060
1385-8947/

chemical [4], physical [5,6], thermal [7] and biological ones [8,9]. By contrast, biological pre-treatment method showed an incomparable superiority in accelerating the hydrolysis process of refractory solids in the aspect of time and efficiency, especially the hydrolytic enzymes co-digestion [10]. Biological enzymes, a kind of effective catalyst in biochemical reactions, had a strong ability to realize sludge solubilization efficiently in a brief time (hours). The commonly used enzymes for wastes digestion among current publications mainly contained lysozyme [11,12], proteases, amylases, cellulase and lipases [10,13]. Lysozyme could destruct the β-1,4 glucoside bond which connected the N-acetylmuramic acid and N-acetylglucosamine in cytoderm and resulted in bacteria lysis in biosolids phase. A previous study had corroborated that the solubilization of WAS could be promoted rapidly (within 4 h) by lysozyme incubation process with the net soluble chemical oxygen demand (SCOD) increase ratio up to nearly 30% [12]. Proteases, amylases and cellulase played a significant role on sludge macromolecular disruption to increase the biodegradable fraction in solution. The mixture of two enzymes (protease: amylase = 1:3) resulted in an optimum biosolids hydrolysis efficiency, which increased from 10% (control test) to 68.43% at the temperature of 50 °C [13]. The co-digestion of cellulase and pronase *E* could result in an 80% reduction in solids, 93% removal of particulate COD and 97% total COD removal [14]. Kinetics analysis of α-amylase for sludge hydrolysis indicated that enzymatic hydrolysis process well fitted the first-order kinetics model at 50 °C and the conversion coefficient of volatile suspended solids (VSS) to soluble COD was 0.266 [8] .

Furthermore, the microorganism in WAS ecosystem was linked closely to sludge functionality and stability. As two important indicators for describing ecosystem community dynamics traits, community diversity and distributive evenness were investigated deeply in the previous studies. Shifts of ecosystem traits in sludge, such as microbial community succession, colonization and response to disturbances, were related fundamentally to community diversity dynamics in macroecology [15]. Bacterial community with high diversity in ecosystem was associated closely with sludge functional stability which could facilitate the resilience (rate of recovery after disturbance) and resistance to disturbance [16–18]. Previous study found that adequate dynamics (high diversity) of the bacterial community (flexibility to adapt in response to changes in environments) were of importance for the stable performance of bioreactors [19]. Moreover, community shifts changed the diversity inevitably by bacterial cell disintegration, especially the splitting effect from enzymatic digestion, which was related to sludge lysis process [20]. The second estimator was community evenness, which could be evaluated by equitability index (*E*) quantitatively or the Pareto–Lorenz evenness curve figuratively [18,21,22]. Previous publications reported that community evenness was associated with the functional stability in a given ecosystem closely. Balvanera et al. proposed that even without species loss, changes in species abundances that reduced functional evenness might also diminish the stability of a certain ecosystem function [23]. Wittebolle et al. proposed that a community must have an even distribution among its functional redundant members of a given community if it was to respond rapidly to selective stress, which hinted the community evenness was inseparably linked with the ecosystem stability [24]. Tao also obtained a similar conclusion: the inoculated sludge with evenly distributed community could benefit the start-up with less time, which implied the initial evenness played a critical role in shortening the anammox start-up process [25]. Based on the aforesaid background, it was essential to understand the potential temporal and spatial variation of individuals in WAS ecosystem during the enzymes blend incubation process by grasping the bacterial community dynamics of community diversity and evenness, which would be beneficial for enhancing the WAS solubilization by using the enzyme engineering method.

Although some literatures have elucidated the performance of particle solubilization or sludge flocs disintegration by using individual hydrolytic enzyme or combined enzymes, few of them focused on the bacterial community responses from the perspective of community traits variation (bacterial community shifts, diversity and community evenness) during the biosolids hydrolysis process. This paper proposed a new investigating view to obtain the potential relationship of the WAS hydrolysis process with community characteristics changes triggered by enzymatic co-digestion to demonstrate the hydrolysis essence in terms of bacterial community dynamics. Thus both the performance of enzymes mixture (lysozyme, protease, α-amylase and cellulase) for boosting WAS hydrolysis and the corresponding responses of bacterial community dynamics were investigated in this study. The specific objectives were to (a) illustrate the co-digestion performance of the enzymes blend for facilitating WAS solubilization, (b) demonstrate the sludge bacterial community dynamics affected by the co-digestion, (c) trace the changing traits of community diversity and distributive evenness in WAS phase caused by enzymatic co-digestion. It would benefit the actual application of biological enzymes engineering for resource recovery from discharged WAS by illustrating the bacterial community responses in WAS ecosystem during the sludge hydrolysis promoting process by enzymatic co-digestion.

## 2. Materials and methods

### 2.1. Source of WAS and hydrolytic enzymes blend

The WAS used in this study was taken from the secondary sedimentation tank of a full-scale municipal wastewater treatment plant in Harbin, China. The corresponding traits were as follows: total suspended solids (TSS) 16,450 ± 285 mg/L, VSS 12,550 ± 2 10 mg/L, soluble chemical oxygen demand (SCOD) 210± 40mg/L, total chemical oxygen demand (TCOD) 16,322 ± 3 51 mg/L, total carbohydrate (in initial supernatant) 53 ± 8.5 mg/L, total protein (in initial supernatant) 210 ± 32 mg/L, pH 6.7 ± 0.1.

Hydrolytic enzymes combination contained lysozyme, α-amylases, protease and cellulase, all of which were extracted and purified from natural sources (purchased from Biotopped Science and Technology Company, China). The corresponding activities of lysozyme, α-amylases, protease and cellulase exceeded 20,000 U/mg, 6000 U/mg, 60,000 U/mg and 30 U/mg, respectively.

### 2.2. Experiment setup and operation

A batch of the tests was prepared to test the digestion effects of the enzymes blend on sludge solubilization and bacterial population changes in biosolids phase. Four clean 1000 mL Erlenmeyer flasks separately loaded with 500 mL identical fresh WAS (labeled as test A, B, C and D) were placed in water-bath at 35 ± 2 °C.

Test A was conducted with operation 1 with the enzyme mixture dosage of 5% (enzymes mixture/TSS, w/w); Test B was conducted with operation 2 (enzymes dosage was 10%). Similarly, operation 3 (enzymes dosage was 15%) was taken on test C. Test D was the control one with adding nothing. The adding ratio of individual enzyme (lysozyme, α-amylases, protease and cellulase) in the mixture maintained at 1:1:1:1 in above experiments. All Erlenmeyer flasks were stirred at a proper speed (60 r/min) by a shaking bed and sealed strictly by rubber stoppers during the enzymes blends co-digestion process. All of above operations were conducted triply for reducing the experimental deviation. The mean value and standard deviation was used in this paper.