Biochemical Engineering Journal 105 (2016) 281–287

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Promotion effects of ultrasound on sludge biodegradation by thermophilic bacteria *Geobacillus stearothermophilus* TP-12

Wan-Qian Guo[a,*], He-Shan Zheng[a], Shuo Li[a], Shih-Hsin Ho[a], Shan-Shan Yang[a], Xiao-Chi Feng[a], Jo-Shu Chang[a,b,*], Xiang-Jing Wang[c], Nan-Qi Ren[a]

[a] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China
[b] Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan
[c] Research Center of Life Science and Biotechnology, Northeast Agricultural University, Harbin 150030, China

ARTICLE INFO

Article history:
Received 5 February 2015
Received in revised form
25 September 2015
Accepted 1 October 2015
Available online 9 October 2015

Keywords:
Sludge
Waste treatment
Biodegradation
Thermophiles
Protease
Low-frequency ultrasound

ABSTRACT

Sludge biodegradation using thermophilic bacteria is a promising method for sludge treatment. In order to further enhance the efficiency of sludge reduction and hydrolysis, low-frequency ultrasound was used to promote this process. We isolated a thermophilic strain that is effective in secreting extracellular protease to hydrolyze sludge. Then the key ultrasound parameters were selected using the response surface methodology method. After 12 h treatment using thermophilic bacteria with short-time ultrasound promotion, volatile suspended solids (VSS) reduction ratio was achieved 32.8%, which is 41.4% higher than that without ultrasound promotion. Meanwhile the contents of soluble chemical oxygen demand (SCOD), protein and carbohydrate were increased by 20.2%, 16.8% and 15.9%, respectively. The composition of dissolved organic matter of sludge products evaluated by excitation-emission matrix spectroscopy demonstrated the promotion effect and eliminated the possibility of the direct sludge degradation caused by ultrasound treatment. Low-frequency ultrasound could effectively promote the thermophilic bacteria hydrolysis to achieve higher sludge biodegradation ratio without directly degrading the raw sludge. The promoted process of sludge biodegradation can further reduce the environmental risk and make sludge to be more readily usable.

1. Introduction

Increasing volumes of sludge generated from sewage treatment plants severely threatens environmental safety and human health [1]. The existing methods of sludge disposal, such as landfill internment, incineration, land application, etc., have potential environmental risks and obvious cost issues [2,3]. Thus, finding the more cost-effective and environmentally-friendly alternatives for sludge degradation has become a research direction that is receiving more attention [2,4].

Due to the special complex organism structure and particular microbial characteristic of sludge, main organic matters are wrapped in its extracellular polymeric substances (EPS) [5]. Thus, the essence and difficulty of sludge degradation is floc fragmentation and hydrolysis. To release wrapped organic matter and increase dissolved organic matter (DOM) content for further anaerobic digestion and utilization, currently, many sludge degradation methods have been developed to hydrolyze EPS and inner microorganism, such as ozonation [6], peracetic acid oxidation [7], sonication [8], alkali addition [9], mechanical and sludge thickening [10], and ozonation combined with sonication [11]. Due to high costs and serious secondary pollution caused by these physical and chemical approaches, biodegradation has aroused increasing attention, especially in sludge hydrolysis using thermophilic bacteria [12]. Sludge biodegradation by thermophilic bacteria is a technology of adding thermophilic bacteria solution which contains secreted thermophilic hydrolytic enzymes into sludge for hydrolysis at a thermophilic condition (ranging from 40 to 80 °C) [13]. Sludge pretreated with thermophilic bacteria has drawn more interests for volatile fatty acids (VFAs) and hydrogen production [14,15]. Although this method seems superior to sludge biodegradation, it has not been extensively introduced to sludge treatment because of its relatively longer processing period and low degradation ratio compared to the physical-chemistry methods which is not feasible for large-scale commercial process [16]. To date, few

* Corresponding authors at: State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China.
E-mail address: guowanqian@hit.edu.cn (W.-Q. Guo).

http://dx.doi.org/10.1016/j.bej.2015.10.002
1369-703X/

Table 1
Characteristics of raw sludge and mixure of sludge with bacteria solution.

Parameter	Raw sludge	Mixure of sludge with bacteria solution (9:1)
TSS ($mg\,L^{-1}$)	14137.0	14371.0
VSS ($mg\,L^{-1}$)	9872.0	10388.0
TCOD ($mg\,L^{-1}$)	14934.0	15326.0
SCOD ($mg\,L^{-1}$)	73.0	1414.3
Soluble protein ($mg\,L^{-1}$)	58.7	480.2
Soluble carbohydrate ($mg\,L^{-1}$)	15.2	77.6
pH	7.0	7.2

studies have been conducted to explore the promotion methods for enhancing sludge hydrolysis. A pretreatment method combining freezing/thawing with thermophilic bacteria was employed in pretreating sludge in order to achieve a higher degradation ratio [17]. However, the freezing step will also result in huge economic costs and practical resistance in commercial scale.

Therefore, developing a cost-effective and convenient method to accelerate the thermophilic enzyme secretion and improve the enzyme hydrolysis efficiency is vital and urgent towards practical application. Low-frequency ultrasound (20–100 kHz) has been reported to have positive effects on microbial productivity and enzyme hydrolysis [18,19]. For instance, Song et al. [20] reported that enzymatic hydrolysis of alkaline protease from *Bacillus licheniformis* was greatly accelerated by ultrasound irradiation, and the final conversion ratio was significantly improved. Through the studies on stability of enzymes, including a-amylase [21], lactase [22], horse radish peroxidise and alkaline phosphatase [23], the active sites of enzyme were demonstrated not destroyed during the sonication process. In order to investigate the promotion effect of low-frequency ultrasound on bacterial activation and sludge solubilization products, the distributions of DOM was also analyzed using three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy. As a time-saving and accurate tool, EEM was extensively used to determine DOM which exhibits fluorescent emission characteristics [24]. This method can be regarded as an overall "fingerprint" of DOM that covers vitamins, NADH, protein-like and humic acid-like substances [25].

In this study, a strain that secretes high-efficiency thermophilic extracellular protease was first isolated under the specific growing conditions using selective culture mediums. This thermophilic strain was cultured as bacteria solution and then added into the raw sludge. Meanwhile the promotion effect of low-frequency ultrasound on sludge degradation process was comprehensively studied. The optimal parameters of ultrasound application were determined via the response surface methodology (RSM). Finally, three-dimensional EEM fluorescence spectroscopy was used to characterize the DOM distribution to evaluate the promotion effect of low-frequency ultrasound on sludge reduction and hydrolysis efficiency.

2. Materials and methods

2.1. Thermophilic strain isolation and identification

For isolation of thermophilic bacteria which produce protease, the sludge was cultured in a shaking bath at 60 °C for 36 h. The remainder liquor containing thermophilic bacteria was diluted using distilled water at a ratio of 10:1. A volume of 0.1 mL^{-1} sample of every level was screened on skim milk agar medium. After 24 h of cultivation at 60 °C, the strains with transparent circles appeared were determined as protease producing ones. The colony with the biggest transparent hydrolytic zone on skim milk agar medium was selected and cultured in a soluble starch agar medium for 24 h. This procedure was repeated at least ten times to ensure a pure strain. The isolated colony was cultured on a standard LB agar medium, and then stored at 4 °C. The isolated pure strain of the thermophilic bacteria was designated as strain TP-12.

Medium composition ($g\,L^{-1}$): The LB agar medium contain: yeast extact 5; peptone 10; NaCl 10; agar 20. The skimmed milk agar medium contained: NaCl 5; peptone 3; skimmed milk 3; agar 20. The composition of fluid nutrient medium: yeast extact 5; peptone 10; NaCl 10.

The DNA was extracted from the 1 mL pure culture solution (LB medium, 60 °C, 24 h) using DNA Isolation Kit (Watson, Shanghai, China). The resultant DNA was subjected to polymerase chain reaction (PCR) with primers of 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′). The 16S rRNA gene obtained from isolated bacteria was cloned, purified, amplified and sequenced by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The sequencing was done in triplicate in order to get the correct sequence. In the phylogenetic analysis, the nucleotide sequence was compared with the sequences in the GenBank/EMBL/DDBJ nucleotide sequence databases by the Basic Local Alignment Search Tool (BLAST) program (http://www.ncbi.nlm.nih.gov/BLAST/).

2.2. Source and preparation of WAS

Sewage sludge was obtained from the secondary sedimentation tank of Harbin Wenchang Sewage Treatment Plant (Harbin, China). The raw sludge was first filtrated through a 20-mesh sieve to separate large debris, then settled for 24 h and stored at 4 °C. The characteristics of the sludge used in this study are shown in Table 1.

2.3. Single-factor experiments

To study the promotion effect of different ultrasound density and exposure time on thermophilic bacteria, and select ranges of independent variables for optimizing parameters by RSM, single-factor experiments were carried out. Different ultrasound densities (0.04 W mL^{-1}, 0.08 W mL^{-1}, 0.12 W mL^{-1}, 0.16 W mL^{-1}, 0.20 W mL^{-1}) at the same exposure times of 20 s and different ultrasound exposure times (5 s, 10 s, 15 s, 20 s, 25 s) at the same ultrasound density of 0.12 W mL^{-1} were performed. The low-frequency ultrasound radiation was conducted using an ultrasound probe system (CY-5D, Ningbo Scientz Biotechnology Co., Zhejiang, China) that emitted 20 kHz ultrasound wave, and had a variable power from 0 to 200 W. The diameter of the horn used was 20 mm. For each sonication experiment, thermophilic bacteria solution (cultured for 48 h) was inoculated in sludge in a ratio of 1:9 (the mixture of bacteria solution with sludge was shown in Table 1). 700 mL mixture sample was seeded into the reactor, heating at 60 °C in a solubilization for 12 h at 140 rpm, timing supplement the evaporation of water, and the ventilation was about 0.16 L min^{-1}. Simultaneously, an ultrasonic instrument equipped with a sonoprobe was dipped into sludge treatment samples for 2 mm at three hours intervals. Liu et al. [26] found that enzyme remained its high activity even some hours after irradiation was stopped and the enhancement effects would be disappeared after 24 h. So the ultrasound interval is three hours in this experiment.