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Fast characterization of soluble organic intermediates and integrity of microbial cells in the process of alkaline anaerobic fermentation of waste activated sludge

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ABSTRACT

Interest in alkaline anaerobic fermentation of waste activated sludge (WAS) is on the rise recently due to its excellent result in sludge reduction and biomass reuse. However, there is still lack of ideal approaches to monitor the fermentation process. The authors present here a fast method for tracing status of microbes and soluble fluorescent organics during alkaline anaerobic fermentation by coupling flow cytometry (FCM) and three-dimensional excitation–emission matrix (3D-EEM) fluorescence spectroscopy. FCM primarily reveals the cell integrity and identifies sources of soluble organics, while 3D-EEM further characterizes them in different fermentation phases by correlation with fluorophores such as soluble protein. High correlation was obtained between fluorescence intensities of three peaks, especially those related to tryptophan-like (Ex/Em: 280/340 nm) and main soluble organics. The proposed method significantly reduced the time by over 90% compared with conventional methods. The method demonstrates great potential for application in efficient controlling and monitoring in practical alkaline fermentation process.

1. Introduction

Increasing production of waste activated sludge (WAS) in wastewater treatment plants brings new environmental problems [1,2]. Microbial cells and extracellular substrates are major components of WAS, and cell wall can prevent intact cells from being directly utilized. Sludge hydrolysis, which plays a rate-limiting role in sludge reduction technology, could be further enhanced by either mechanically [3], thermally [4], chemically [5], biologically [6] or assembly [7]. These methods enable the sludge solids to become soluble, disaggregate the sludge floc, and disrupt the bacterial cells. Substances released during cell lysis could be further degraded in biological wastewater treatment processes through cryptic growth, bringing about a net reduction in the dry mass of WAS.

In recent years, attention has increasingly focused on anaerobic WAS fermentation due to its good performance in sludge reduction and the production of intermediates such as volatile fatty acids (VFAs) which could serve as carbon sources for biological nutrient removal systems [8]. Other investigations have demonstrated [7] that WAS anaerobic fermentation under alkaline conditions can substantially promote growth in soluble chemical oxygen demand (SCOD). Most such studies of alkaline anaerobic WAS fermentation have focused on: (1) the profiles of intermediates such as SCOD, polysaccharide, protein and VFAs, ammonia nitrogen and soluble phosphate under different conditions [9,10]; (2) the utilization of carbon sources obtained from alkaline anaerobic fermentation in biological nutrient removal systems such as A^2O [11] and short-cut nitrification and denitrification [12]; and (3) the sludge reduction performance [13]. Since the aim of WAS alkaline anaerobic fermentation was to recover carbon source and reduce sludge production effectively, efficient monitoring and characterization of the changes of organics and microbial cells in WAS during fermentation process was necessary. However, the dynamic variations in soluble organic substances and the integrity of bacterial cells during the alkaline anaerobic fermentation process have rarely been reported due to inefficiencies in monitoring and characterization.

Abbreviations: WAS, waste activated sludge; FCM, flow cytometry; 3D-EEM, three-dimensional excitation–emission matrix; TVFAs, total volatile fatty acids; SCOD, soluble chemical oxygen demand; EPS, extracellular polymers; AnS, anaerobic sludge; EG, experimental group; CG, control group; S-PS, soluble polysaccharide; S-PN, soluble protein.

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Table 1
Main characteristics of the concentrated WAS.

Items[a]	Concentration
TSS (total suspended solids)	12,228 ± 1007
VSS (volatile suspended solids)	6684 ± 589
TCOD (total chemical oxygen demand)	12,936 ± 1105
SCOD (soluble chemical oxygen demand)	90 ± 15
Total polysaccharide (as COD)	624 ± 96
Total protein (as COD)	2862 ± 156
Total lipid (as COD)	66 ± 16
pH	6.8~7.1

[a] All units were mg/L except pH.

Cultivation, observation, enumeration and gravimetric methods conventionally used for sludge reduction assessment include selective media, microscopic observation of sludge flocs, viable plate count and measurement of mixture liquid volatile suspended solids (MLVSS). In fact, most bacteria in activated sludge were previously considered to be in a "viable-but-not-culturable state" [14] and MLSS measurement would cost much time (more than 2 h) [15]. However, flow cytometry (FCM) has recently been used for rapid quantification of viable and permeabilised cells in activated sludge in different environments [16,17]. In the present paper, we introduce FCM incorporated with fluorescent DNA-staining for instant detection of the variation in bacterial cell integrity during anaerobic WAS fermentation process. This allows much faster cell number counting in WAS, at a rate of over 1000 cells per second no matter the microbes are alive or dead.

Likewise, the deficiency of conventional methods for intermediates analysis could be remedied by introducing three-dimensional excitation–emission matrix (3D-EEM) fluorescence spectroscopy for rapid characterization of fluorescence organics in terms of the relationship between fluorescence intensity and fluorescent functional groups of soluble organics. 3D-EEM has previously been used in characterizations of disinfection byproducts produced by the chlorination of natural organic matters [18] and extracellular polymers (EPS) based on the understanding that fulvic substances and proteins are key components with fluorescence properties in EPS [19]. The integration of FCM and 3D-EEM proposed herein would be of particular use (1) to detect microbial cell integrity, permeabilisation and disruption during alkaline anaerobic fermentation, (2) to roughly identify the source of main soluble organics in fermentation liquid, and (3) to rapidly characterize the soluble organics released in the accompanied process.

2. Materials and methods

2.1. WAS and anaerobic sludge

WAS, collected from the sludge tank of a municipal wastewater treatment plant operated in Langfang, near Beijing, China, was concentrated by settling at 4 °C for 24 h. The main characteristics of the concentrated WAS are presented in Table 1. Mature anaerobic sludge inoculated during fermentation process was obtained from the anaerobic digestion tank of a wastewater treatment plant in Beijing.

2.2. Sludge fermentation treatment

Experiments were carried out in two sealed glass Erlenmeyer flasks containing 1.3 L WAS mixed with 0.2 L mature anaerobic sludge (AnS) to make the WAS: AnS (g/g) = 1:1.5. The pH of experimental group (EG) was 9.0 ± 0.2, whereas the pH of control group (CG) was unadjusted. The changes of pH during CG and EG were monitored every 12 h. The range of pH in CG throughout the process was from 6.2 to 7.1 and had no significant effect on fermentation result in CG. In EG, a constant pH (9.0 ± 0.2) was maintained throughout the process based on results of pH monitoring to avoid the significant dynamic change in pH on alkaline anaerobic fermentation performance. Sludge in these two groups was stirred at 50 rpm under room temperature. Samples were collected daily to determine the main soluble organic components in the fermentation supernate and the bacterial cell integrity in the sludge mixture. All samples were triple tested.

2.3. Analytical methods

Each sludge mixture sample was divided in two portions. One portion was immediately centrifuged at 10,000 rpm and 4 °C for 20 min after being collected. The supernate was then filtered through a 0.45 μm microfiber filter membrane. The filtrate was analyzed for SCOD, soluble polysaccharide (S-PS), soluble protein (S-PN), lipid, VFAs, and 3D-EEM spectra. The other portion was used to detect the variation in bacterial cell integrity after disaggregation.

2.4. Sludge sample disaggregation

Sludge sample used to detect the variation in bacterial cell integrity was diluted in PBS in a ratio of 1:5 and then disaggregated by ultrasound with cooling device. Disaggregated sludge sample was then diluted again in PBS in ratio of 1:25 to obtain a cell suspension of 10^6–10^7 cells/mL, and filtered through a 48 μm membrane in order to avoid blocking the FCM nozzle. The disaggregation treatment was modified based on the method used by Foladori et al. [20].

2.5. Fluorescent staining protocol

The staining procedure and FCM analysis followed the guideline given by Ziglio et al. [21].

2.6. FCM analysis

FCM analysis were performed using a flow cytometry (FACSCalibur, BD) equipped with a 488 nm argon laser and a 635 nm diode red laser. Green and red fluorescence signals were collected by two band pass filters of 530 nm and 610 nm respectively, using logarithmic gain and forward-angle light scatter (which reflects cell size). Non-bacterial particles and debris were excluded during data collection by setting gates. To obtain high quality statistical data, 50,000 cells for each sample were analyzed in a few seconds.

The number of total cells in initial sludge on Day 0 was taken to be the initial cell number (N_0), given by the sum of the number of viable (N_v), dead (N_d) and small sludge flocs (N_f). After applying alkaline anaerobic fermentation the bacterial cell membrane might be damaged, and the amount of disrupted cells (or cell debris) was given by $N_0 - N_t$, where N_t was the number of total cells in the process.

2.7. Soluble organics analysis

Determination of SCOD was according to APHA [15]. Phenol-sulfuric method [22] and Lowry–Folin method [23] were used to analyze S-PS and S-PN, respectively. Lipid was extracted according to Bligh and Dyer [24] and then measured using gravimetric method after the concentrated at 60 °C by blowing nitrogen gas. Polysaccharide, protein and lipid were translated into the form of COD by multiplying coefficients respectively [25]. VFAs was quantified using an Agilent 6890N GC equipped with a flame ionization detector and DB-FFAP column (30 m × 1.0 μm × 0.25 mm)