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A comparison of different pretreatments on hydrogen fermentation from waste sludge by fluorescence excitation-emission matrix with regional integration analysis

Liang Guo [a,b,*], *Mingmin Lu* [b], *Qianqian Li* [b], *Jiawen Zhang* [b], *Zonglian She* [b]

[a] *Key Laboratory of Marine Environment and Ecology, Ministry of Education, Ocean University of China, Qingdao 266100, China*

[b] *College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China*

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ABSTRACT

To gain a better understanding of the influence on hydrogen fermentation with different pretreatments, the compositional and structural characteristics of extracellular polymeric substances (EPS) and dissolved organic matters (DOM) were analyzed during hydrogen fermentation using excitation-emission matrix (EEM) with fluorescence regional integration (FRI). In order to accelerate the hydrogen production with waste sludge, multi-enzyme, thermophilic bacteria, heat and microwave were performed for sludge pretreatments. The best method was found to be heating with the maximum hydrogen yield of 15.3 ml H_2/g VSS (Volatile suspended solid). EPS and DOM component of soluble chemical oxygen demand (SCOD), carbohydrate and protein, the microbial community structure evolutions, soluble metabolite of volatile fatty acids (VFAs) and ethanol distribution characteristics and pH were also evaluated. Different pretreatments could change the microbial community structure in waste sludge, which led to the diversity of substrates degradation and metabolite with hydrogen bacteria.

Introduction

The waste activated sludge (WAS) produced from the biological waste water treatment process has dramatically increased in recent decades due to quantitative and qualitative expansion of waste water treatment [1]. Recently, high sewage sludge production has become a common environmental and cost problem for waste water treatment plants, so there has been increasing researches focused on sludge reduction and utilization [2]. Hydrogen produced in a biological way is energy-efficient and zero emission, providing a new alternative to environmental and energy issues as well as a platform for the hydrogen economy [3]. Biohydrogen

* *Corresponding author*. Key Laboratory of Marine Environment and Ecology, Ministry of Education, Ocean University of China, Qingdao 266100, China. Tel.: +86 532 66781020; fax: +86 532 66782810.
E-mail address: geletu@ouc.edu.cn (L. Guo).

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Table 1 – Characteristics of waste sludge.

TCOD (mg/L)	SCOD (mg/L)	Protein (mg/L)	Carbohydrate (mg/L)	TSS (g/L)	VSS (g/L)	pH
19495	1632.5	20.2	32.1	13.4	8.4	7.9

production through the use of WAS represents a promising aspect of sustainable development [4]. To improve the biohydrogen production efficiency and stability of a continuous hydrogen production system, it is important to develop treatment methods that inhibit the activity of hydrogen-consuming bacteria and enrich hydrogen producing bacteria [5]. Therefore, the first step must be its physical, chemical and biological pretreatment for breaking complex molecules into simple monomers, to increase solubilization of organic material and improve the efficiency of the anaerobic treatment in the second step [6].

During hydrogen production, the activity of microorganisms plays a critical role [7]. Extracellular polymeric substances (EPS), which consists of a variety of organic substances, such as exopolysaccharide, exoprotein, DNA, and humic acid, and high-molecular substances in EPS were then hydrolyzed to low-molecular substances and then utilized by the hydrogen producing sludge [8,9]. EPS may play a role in autoaggregation of hydrogen producing Ethanoligenens [10]. Usually, hydrogen formation is accompanied by the generation of VFAs and ethanol during anaerobic digestion processes. Hence, the composition and concentration of the soluble metabolites produced were useful indicators for monitoring the hydrogen process [11]. Dissolved organic matters (DOM) could be released from WAS after different pretreatments [12] or produced from microbial metabolism, which are composed mainly of carbohydrates, proteins, humic substances and soluble microbial products. Main organic components of the DOM for treated sludge sample were protein, polysaccharide and lipid [2]. The main soluble microbial products after hydrogen production were ethanol, acetate and butyrate with small quantities of propionate [13].

Spectroscopy method is a powerful tool in environmental analyses to study the structure of the complex organic compounds [14]. Compared with other available approaches, three-dimensional excitation–emission matrix (EEM) fluorescence spectroscopy gives more fluorescent information with high sensitivity and component selectivity, and has already become an important method for quantifying and characterizing the DOM and EPS in WAS during the anaerobic hydrogen fermentation process [4]. EEM can provide an overall view of the fluorescent properties of DOM over a selected spectral range by characterizing the locations and intensities of fluorescence peaks [15]. The good correlation relationships among the degradation rate of the substrate, production rate of hydrogen, and aqueous production also demonstrate that it is feasible to use the EEM fluorescence spectroscopy to monitor the microbial metabolic state as well as the anaerobic reactor performance [16].

Until recently, the transformation process of organic matters using multi-enzyme, thermophilic bacteria, heat and microwave pretreated sludge for hydrogen fermentation were not clear. Therefore, the present aim was using EEM fluorescence spectra combined with regional integration analysis to quantitative assessment the changing of EPS and DOM in waste sludge during hydrogen fermentation process, as well as the investigation of EPS and DOM component by soluble chemical oxygen demand (SCOD), carbohydrate and protein. The microbial community structure evolutions, soluble metabolite of volatile fatty acids (VFAs) and ethanol distribution characteristics and pH were also evaluated.

Materials and methods

Characteristic of sewage sludge and pretreated hydrolysis

Waste sludge was obtained from the secondary sedimentation tank of the Tuandao municipal waste water treatment plant in Qingdao, China. Prior to use, sludge was sieved by grid size 2.0 mm to remove coarse matter and stored in 4 °C refrigerator. The thickened sludge which settled about one week was used for this study. The characteristics of excess sludge are given in Table 1.

Four pretreatments were applied to hydrolyze the sludge solid and inhibit methanogenic bacteria. The pretreated sludge was the substrate used for hydrogen fermentation.

(1) Microwave: the sludge was pretreated in the microwave (Kedibo, KDB-III, China) for 2 min at a power of 900 W.
(2) Heating: the sludge was heated at 100 °C for 30 min using water bath shaker (Yuecheng, SHY-2, Chian).
(3) Multi-enzyme: The multi-enzyme was provided by Guangzhou Taoxin environment, Science and Technology Inc. The main components of multi-enzyme comprise American composite enzyme (Seattle Environment Science and Technology Inc., UAS), bromelain (activity is 3 × 106 U/g) which was made by pineapple peel, and lysozyme (activity is 2 × 104 U/g) which was extracted from egg, according to certain proportion. Multi-enzyme was incubated in waste sludge in the ratio of 1:50 (v/v) at 30 °C, 140 r/min for 5 h.
(4) Thermophilic bacteria: Thermophilic bacteria were separated in our previous study [17], and bacteria suspension was inoculated in the waste sludge in the ratio of 1:50 (v/v) at 65 °C for 12 h. The lysis enzyme secreted by thermopile bacteria was the principal theory for sludge hydrolyzing.

Experimental design and hydrogen fermentation

Pretreated sludge was anaerobically (blow N_2 for 10 min) incubated at 35 °C in 250 ml serum bottles with stirring of 125 r/min and was not added other nutrients. The bottles were capped with silica gel stoppers. Under identical condition three fermentation bottles were measured and their average of data was reported to prevent any possible errors introduced