Journal of Hazardous Materials 235–236 (2012) 286–290

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

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

# Proteomic profiling of the acid tolerance response (ATR) during the enhanced biomethanation process from Taihu Blue Algae with butyrate stress on anaerobic sludge

Qun Yan[a,b,d,*], Yongcan Li[a], Bin Huang[c], Aijie Wang[b,**], Hua Zou[a], Hengfeng Miao[a], Rongqing Li[d]

[a] School of Environmental and Civil Engineering, Jiangnan University, Wuxi 214122, China
[b] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China
[c] Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, CAS, Shenyang 110016, China
[d] Jiangsu Key Laboratory for Biomass-based Energy and Enzyme Technology, Huaian 223300, China

## HIGHLIGHTS

- Along with the enhanced anaerobic digestion process, both the activity of dehydrogenase and the protein expression were increased accordingly.
- Proteomic profiling was investigated, and proteins associated with the bioenergy metabolism were identified by MALDI-TOF MS.
- pIs of the identified proteins were found to be acidic, which strongly indicated a microbial ATR with acid stress on anaerobic sludge.

## ARTICLE INFO

*Article history:*
Received 7 March 2012
Received in revised form 30 July 2012
Accepted 31 July 2012
Available online 10 August 2012

*Keywords:*
Anaerobic digestion
Biomethanation
Acid stress
ATR
Proteomic profiling

## ABSTRACT

Enhanced biomethanation with acid stress on anaerobic sludge, dehydrogenase activity, protein expression, and the primary proteomic profiling of microbial communities during the enhanced anaerobic digestion process from Taihu Blue Algae were investigated. It was found that the accumulation of organic acids and the specific biogas accumulation rate were 1.8 and 1.3 times of the control, when 10 g/L and 7.5 g/L of butyrate were selected for acid stress, respectively. Meanwhile, dehydrogenase activity of the 7.5 g/L acid stress group exhibited an increase of 1.6 times of the control, and protein expression was also found to be enhanced sharply as revealed by 1D-PAGE. Finally, twenty of the matched protein spots through 2D-PAGE from both the control and the 7.5 g/L stress groups were identified by MALDI-TOF MS, and five of which were proved to be involved in bioenergy metabolism. Significantly, ATR related proteins might be induced as the pIs of which were acidic as 5.92, 5.51 and 5.54, respectively.



## 1. Introduction

Facing an increasing shortage of fossil energy, investigation, pilot and even large scale of the production of bioenergy, such as methane, hydrogen and biodiesel from biomass via anaerobic digestion are now overwhelming all over the world [1]. Resulted from the eutrophication and poorly flushing of water bodies, the annual blooming of cyanobacteria (also called Blue Algae) across the Taihu Lake, China, always poses a major threat to the water supply of the lakeside city Wuxi [2]. To alleviate the eutrophication of Taihu Lake, it was more competitive to refloat and then to make bioenergy from the annually blue algae through anaerobic digestion [1,3]. However, efficiency of the anaerobic digestion would be inhibited in turn, along with the accumulation of excess organic acids during the anaerobic digestion process. To help microorganisms within the anaerobic sludge overcome the acidified circumstance, it was viable to transfer the excess organic acids into another reactor for the production of polyhydroxyalkanoates (PHA), and other valuable chemicals as well [3]. Nevertheless, it would be more substantial to stimulate the microbial acid tolerance response (ATR) during the anaerobic digestion process, in order to achieve higher bioenergy productivity. Previously, we reported that activities of glutamate decarboxylase (GAD), the key ATR of nearly all types of microbes, and activities of other three different types of hydrolytic enzymes could all be improved, with appropriate acid stress on anaerobic sludge [4]. However, few publications could be tracked about the further biochemical influence of acid stress on anaerobic populations.

* Corresponding author at: School of Environmental and Civil Engineering, Jiangnan University, Wuxi 214122, China. Tel.: +86 510 85197872; fax: +86 510 85197872.
** Co-corresponding author.
*E-mail addresses:* yanqun@jiangnan.edu.cn (Q. Yan), waj0578@hit.edu.cn (A. Wang).

0304-3894/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2012.07.062

Due to the complicated microbial population dynamics and physiological responses under variable environmental circumstances, traditional molecular enrichment technique seemed not be sufficient to obtain useful information during the anaerobic digestion process [5]. However, proteomic analysis might reveal instant physiological responses, since proteins would be synthesized and then folded immediately after being exposed under different situations [6]. Benefited from the fast development of environmental proteomics, such as the protein recovery strategy from environmental samples, and the 2D-PAGE electrolysis combined with matrix-assisted laser desorption/ionization time of light mass spectrometry (MALDI-TOF MS), more and more groups are applying this technique to gain bio-information on microbial cooperation and competition within complex natural ecosystems [7]. It was reported that the up- or down-regulation of protein expression pattern could be observed, when bacterial community was exposed to short-, medium- and long-term stress of cadmium, indicating that the microbial response shift from resistance to sustained tolerance, and finally to adaptation under such environmental stress [8]. To date, proteomic profiling of microbial ATR under acid stress during the anaerobic digestion process has not been reported yet.

In this study, the enhanced methane production during anaerobic digestion with acid stress on anaerobic sludge, the activity of dehydrogenase, the performance of protein expression, and firstly, the primary proteomic profiling of the microbial populations during the biomethanation process were all investigated.

## 2. Experimental

### 2.1. Taihu blue algae and anaerobic sludge

Blue algae used in this study were freshly collected from the blue algae refloating sites alongside Taihu Lake and then cryopreserved for future use. For alkaline pretreatment, blue algae were diluted to the TS (total solids) as about 1%, and then adjusted to pH 13 with 6 mol/L of NaOH, finally the well blended blue algae were kept for 12 h at room temperature to maintain the pretreatment effect [3]. Anaerobic granular sludge used in this study was from DSM Citric Acid (Wuxi) Ltd. Prior to inoculating for methane production, the anaerobic sludge was conducted for acid stress in a 1 L tightly sealed reaction bottle for 48 h, with the stress medium the same as previously described, except that the butyrate was adopted for stress acid in this study [4]. After acid stress, the sludge was washed twice with tap water to remove residual acids, and was then kept for biomethanation production.

### 2.2. Experimental apparatus and operating procedures

The biomenthanation process was conducted in a 2 L digester, the mass ratio of Taihu blue algae and anaerobic granular sludge was 6:1, pH was adjusted to about 7.5, and the reaction temperature was maintained at 35 °C with a water bath. To maintain an anaerobic environment, the digester was flushed with nitrogen, then the anaerobic sludge with/without acid stress was inoculated to start the anaerobic digestion process.

### 2.3. Protein preparation from anaerobic fermentation system

50 mL of fermentation sample was collected and then centrifuged at 2000 rpm, 4 °C for 15 min. Centrifuged deposit was diluted with PBS buffer to 50 mL and was re-centrifuged at 2000 rpm, 4 °C for 15 min. Afterwards, the centrifuged deposit was re-diluted with PBS buffer to 50 mL, and was washed three times using ultrasonic disruptor (Sonics VCX130PB, USA) for 20 min at 20 kHz. After being centrifuged at 40,000 rpm for 30 min, the supernatant was left for protein quantification using PlusOne 2-D Quant Kit (GE, USA). Finally, 800 μg of the protein aliquots were stored at −80 °C for further investigation.

### 2.4. Analytic methods

Dehydrogenase was determined spectrophotometrically at 492 nm (Unico 2100, USA) based on the amounts of triphenyl formazan released from the triphenyltetrazolium chloride (TTC). In detail, 1.5 mL of Tris–HCl, 0.5 mL of 0.1 M glucose, 0.4% of TTC and 0.36% of $Na_2SO_3$ were added into 2 mL of the sample, respectively. After the mixture was incubated at 37 °C for 2 h, the reaction was stopped by 0.5 mL of formaldehyde, then the mixture was centrifuged for 5 min at 3000 rpm with the addition of 5 mL of acetone. Finally, the supernatant was left for the colorimetric analysis.

*Biogas* composition was assayed with a Gas Chromatograph (GC910, Kechuang, China) equipped with a stainless packed column (with Porapak N 60–80 as carrier) connected to a thermal conductivity detector. The column temperature was isothermic at 90 °C. The carrier gas was argon and the flow rate was 15 mL/min [9].

*2-D PAGE and in-gel protein digestion*. Samples containing 800 μg proteins were used to rehydrate an 18-cm immobilized gradient strip for 12 h at 20 °C. Focusing was started at 300 V for 1 h, and was increased to 1000 V for 1 h and then 8000 V for 12 h, respectively. After focusing, the strips were equilibrated for 15 min in equilibrium buffer (2% SDS, 50 mM Tris–HCl, pH 8.8, 6 M urea, 30% glycerol, 0.002% bromphenol blue, 1% DTT). The strips were then overlaid onto 12.5% SDS–polyacrylamide gels for the second dimensional separation. The protein spots were carefully excised from the Coomassie-stained 2-DE gel, destained, washed, and then digested for 13 h with sequencing grade modified trypsin (Roche Applied Science). Image analysis and abundance calculation was performed with Image Master 2D Elite Platinum (GC Healthcare, USA). Peptides from digested proteins were used for MALDI-TOF/TOF mass analysis [10,11].

*MS protein identification and protein identification*. All mass spectrum measurements were performed on a Bruker Reflex III MALDI-TOF-MS (Bremen, Germany) operating in the reflectron mode [11]. Database searching was performed by using the Mascot software (Matrix Science Ltd., London, UK) against the database of NCBInr, with other parameters for searching as: trypsin digestion with one missed cleavage; carbamidomethyl modification of cysteine as a fixed modification and oxidation of methionine as a variable modification; peptide tolerance maximum of 0.2 Da; MS/MS tolerance maximum of 0.6 Da; peptide charge of 1+; scores over 81 were significant ($p < 0.05$) for a local Peptide mass finger (PMF) search.

## 3. Results and discussion

### 3.1. Enhanced methane production process with acid stress on anaerobic sludge

It would be beneficial to obtain adequate organic acids during the anaerobic digestion process, in order to achieve higher methane production, as more than 70% of the methane accumulation was originated from organic acids, mainly as acetate [12]. As shown by Fig. 1A, accumulation of organic acids was increased sharply with butyrate stress on microbial communities within the anaerobic sludge. In comparison with other acid components, amounts of the butyrate of almost all groups were found to be the largest. Interestingly, the larger the concentration of acid for stress, the higher the amount of butyrate was obtained until the 10 g/L stress group. Additionally, total amount of organic acids reached the maximum of 13.7 g/L in the case of the 10 g/L stress group, which was about 1.8 times of the control. However, further increase of the butyrate