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Metaproteomic analysis of the relationship between microbial community phylogeny, function and metabolic activity during biohydrogen-methane coproduction under short-term hydrothermal pretreatment from food waste

Xuan Jia[a], Beidou Xi[b], Mingxiao Li[b,*], Dongming Liu[b], Jiaqi Hou[b], Yan Hao[b], Fanhua Meng[b]

[a] Key Laboratory of Cleaner Production and Integrated Resource Utilization of China National Light Industry, Beijing Technology and Business University, Beijing 100048, China

[b] State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China

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ABSTRACT

Short-term hydrothermal pretreatment (SHP) is an attractive method for food waste anaerobic digestion, which facilitates the solubilisation of recalcitrant particles. This study employed metaproteomic method to evaluate the relationships among microbial community phylogeny, function, and metabolic activity during two-stage anaerobic digestion under SHP (SHPT) from food waste. The presence of 651 bacterial proteins and 477 archaeal protein has been detected by liquid chromatography online linked to mass spectrometry, revealing a high metabolic heterogeneity during SHPT. The different stages of SHPT highlighted important roles for the bacterial proteins from *Gammaproteobacteria*, *Bacilli*, and *Clostridia* and the archaeal proteins from *Methanosarcinales*. The identified proteins related to biohydrogen production come from pyruvic acid decarboxylase and formic acid decomposition pathway in carbohydrate metabolism and methanogenesis from acetate, CO_2 and a methylotrophic pathway during energy metabolism. This could provide functional evidence of the metabolic activities and biogas production during SHPT.

1. Introduction

In China, more than 30 million tons of food waste (FW) is generated each year (Jia et al., 2017). FW is a major component of municipal solid waste which has accounted for 20–54% of the total produced and causes critical environmental and social problems. Due to the high content of carbohydrate, protein, and a small quantity of cellulose and hemicellulose, FW is considered a good candidate for use as a substrate for anaerobic digestion (AD; De Clercq et al., 2017). Until now, more than 100 pilot projects have been nominated, which have chosen AD as their main waste disposal technique in China. Compared with traditional single-stage AD, two-stage AD for biohydrogen and methane production provides a promising method of FW energy utilisation, which can effectively get rid of the feedback inhibition of intermediate metabolite and produces two clean energy (Jia et al., 2014). However, a high lipid content, and complicated composition are primary characteristics of Chinese FW and these affect AD performance (Ding et al., 2017). In our previous study, short-term hydrothermal pretreatment (SHP) is an attractive method, which could cause the degradation of complex molecules and the solubilisation of recalcitrant particles. Moreover, SHP can recycle more lipids and these may be reused for biodiesel production (Li et al., 2014). The analysis of the biogasification potential and economic feasibility of combining SHP with different AD processes revealed that SHP at 90 °C for 30 min, when coupled with two-stage AD (SHPT) achieved a satisfactory biogasification performance from FW (Jia et al., 2017).

Complex consortia of microorganisms are responsible for biogas production; however, changes in the microbial community structure and the functional capacities of individual members in different stages pose severe challenges to the regulation of operational stability and enhanced biological biogasification with SHPT. Recently, the taxonomic structure and enzymatic potential of such microbial communities have been analysed (in detail) by gene-based approaches (Jonge et al., 2017; Lee et al., 2017; Sun et al., 2017; Vanwonterghem et al., 2014), while failing to identify, and explain the correlation of functional proteins and metabolic activity throughout the AD process of biohydrogen and methane generation. Stolze et al. (2016) revealed the taxonomically profiled and genetic potential on different production-scale biogas plants using high-throughput 16S rRNA gene amplicon sequencing. A temporal genome-centric metagenomic analysis was performed that hosted complex microbial communities fulfilling a series of interlinked metabolic processes to enable the conversion of cellulose to

* Corresponding author.
E-mail address: limingxiao8122@163.com (M. Li).

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methane (Vanwonterghem et al., 2016). A metaproteomic method provides information about functional capacities that goes beyond a community census and is informative for predictive functional proteins interactions in complex biological systems (Bastida and Jehmlich, 2016; Siggins et al., 2012; Vanwonterghem et al., 2014). Nowadays, the metaproteomic analysis method has been used in complex natural environments, such as activated sludge, soils, oceans, and groundwater, and is a powerful tool for analysing function and metabolic activities of microbial populations (Bastida and Jehmlich, 2016; Grzymski et al., 2012; Gunnigle et al., 2015; Lin et al., 2016; Wu et al., 2017). The functional evidence of key metabolic pathways in AD was provided by the highly expressed proteins in a mixed anaerobic microbial consortium (Abram et al., 2011; Gunnigle et al., 2015). However, such techniques have rarely been applied in biohydrogen and methane coproduction from FW by two-stage AD.

This study is the first to use metaproteomic to evaluate the membership phylogeny and functional response of a complex community to biohydrogen and methane coproduction from FW in the SHPT based on previous research. The response relationship between microbial community structure, protein functions, and metabolic activity at the organismal level were also investigated. The efficiency of biohydrogen and methane coproduction was regulated by their proteins levels, which provides functional evidence of key metabolic pathways for energy generation using FW subjected to two-stage AD.

2. Materials and methods

2.1. Reactor startup and operation

The FW was collected from a dining hall and pulverised into 1–3 mm pieces. The 200 g raw FW with distilled water (50% m/m) was added to the 500-mL stirred tank reactor and heated to 90 °C and reacted for a short 30 min. Fermentation experiments were conducted using pretreated FW as substrates (organic loading of 5.6 g VS/L) and performed in 2-L reactors. FW, of a mass of 50 g with 400 mL anaerobically digested sludge was added to the reactors, which were adjusted to a working volume of 1.6 L using distilled water and placed on a magnetic stirrer at 100 rpm. The reactor headspace was filled with nitrogen gas for 10 min. The initial pH value was 6.3 and this was changed to 7.5 using 2 M NaOH or HCl in the later stages of hydrogen production. The reactors were operated under mesophilic conditions (35 ± 1 °C) and the biogas production measured at 6 hourly intervals. Each experimental condition was performed in triplicate. Control bottles were also prepared using the FW without any pre-treatment at the same time.

2.2. Analytical methods

The cumulative biogas production was measured with a gas counter (Ritter MGC-1, Germany). The headspace biogas composition and proportions (i.e., H_2, CH_4, and CO_2) were examined by using a gas chromatograph (Perkin Elmer Clarus 500, USA) provided with a thermal conductivity detector and a 2-m high-porosity polymer bead-packed column. The operating temperatures of the injection port, oven, and detector were set to 50, 150, and 150 °C, respectively. Argon was used as the carrier gas at a flow rate of 40 mL/min.

2.3. Sample collection and protein extraction

The mixed liquor were collected from reactors in three different stages of SHPT for metaproteome analysis, including the peak stage of hydrogen production (stage I, 16–24 h), peak methanogenic stage (stage II, 276–324 h), and late methanogenic stage (stage III, 420–450 h). Proteins were extracted from 50 mL samples using 20 mL extraction buffer [1: 1 (v: v) SDS–phenol buffer: 50 mM Tris, 1% SDS pH 7.5 + phenol (pH 8.0)], and subsequently separated by one-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Liu et al. (2015). For identification of proteins from effluent samples, each lane of the SDS-PAGE was cut into seven slices. The gel slices were further cut into 1 mm^3 gel pieces and subjected to immediate in-gel tryptic digestion (Promega, Madison, WI, USA).

2.4. Mass spectrometric analyses

Digested peptides were separated by nano-LC (Ultimate 3000, Dionex, Sunnyvale, CA, USA; trap column: Acclaim PePmap 100, C18, 3.0 μm, 75 μm × 2 cm, 100 A, Thermo Scientific, Pittsburgh PA, USA; column: Venusil × BPC, C18, 5.0 μm, 150 A, Agela Technologies, Wilmington, DE, USA) and analysed by MS/MS (Q Exactive, Thermo Scientific, Pittsburgh PA, USA). Peptides were eluted using a linear gradient of H_2O:CH_3CN (98:2, 0.1% formic acid) to H_2O:CH_3CN (64:36, 0.1% formic acid) at 250 nL/min over 30 min. Database searches were carried out with MS/MS ion search (MASCOT, http://www.matrixscience.com) against a non-redundant protein database, SwissProt2013 xyzzy (539 616 sequences; 191 569 459 residues). The following search parameters were applied: (a) trypsin was chosen as the protein-digesting enzyme and one missed cleavage was tolerated, (b) carbamidomethyl (C) was chosen for fixed modification, and (c) Gln- > pyro-Glu (N-term Q) and Oxidation (M) were chosen as variable modifications. Searches were performed with a peptide mass tolerance of ± 15 ppm and a fragment mass tolerance of ± 20 mmu. Mascot searches with a false discovery rate > 5% were rejected. Protein matches were only accepted if they were identified by a minimum of one unique peptide.

2.5. Metaproteomic analysis

All proteins were manually annotated with the aid of BLASTP against the Swiss-Prot databases, and the protein hit that showed the highest sequence identity was recorded, including the organism name thereof. KEGG Orthology and Links Annotation (KOALA) was used to analyze the function of identified proteins (Kanehisa et al., 2016). Higher protein abundance is represented by a higher number of MS/MS spectra acquired from peptides of the respective protein. Thus, protein abundances were calculated based on the normalized spectral counts (SpCn; Piersma et al., 2010). All phylogenetic group abundances presented in the metaproteome are based on SpCns.

3. Results and discussion

3.1. Biogasification performance

Based on the previous experiment, the FW under SHP and combined production in the two-stage AD can reach its optimum biogasification performance and net energy gain (Jia et al., 2017). The biohydrogen and methane production and proportions, in the SHPT are shown in Fig. 1. The maximum cumulative biogas production and rate were 6.43 L and 714.44 mL/g VS, respectively. In this process, the daily biogas production increased to 300 mL/d at 20 h in the Stage I, and then decreased at 70 h by the end of biohydrogen production. After the change of the pH value to 7.5, the daily biogas production increased with methane production. The maximum daily biogas production and methane proportion reached 750 mL/d and 76.75% at 350 h, respectively, in Stage II. The relationship between microbial community phylogeny, protein function and metabolic activity were evaluated by metaproteomic in three typical stages of SHPT.

3.2. Phylogenetic analysis based on the metaproteomic analysis

Proteins were subjected to nanoHPLC coupled to tandem mass spectrometry for characterisation. A total of 1337 proteins were