

INSIGHT INTO MICROBIAL COMMUNITY DYNAMIC AND ENERGY RECOVERY FROM FOOD WASTES: THE EFFECTS OF TEMPERATURE AND OLR STRESSES ON THE LONG-TERM HYDROGEN PRODUCTION SYSTEMS

Yong Wang¹, Beidou Xi², Xuan Jia^{1*}, Mingxiao Li^{2*}, Lianhai Ren¹, Xue Wang³, Jiaqi Hou², Meiyong Ye²

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

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

3 Key Laboratory of Aquatic Science and Technology, Tianjin Chengjian University, Tianjin 300384, China

ABSTRACT

The effects of temperature and organic load rate (OLR) stress on dark fermentative hydrogen production from food wastes (FW) were investigated through analysis of shaping metabolic pathways, microbial communities and energy balance in the long-term hydrogen production systems. The gas production rate (GPR) of 9.32 mL/g VS·h and hydrogen proportion of 58.19% were reached with 6 g VS/L·d under thermophilic condition. In depth analysis of metabolite profiles and microbial communities showed that thermophilic fermentation favored a stable community dominated by *Thermoanaerobacterium*, which was in the ascendant with increasing of OLR. In contrast, mesophilic fermentation led to ever changing microbial community comprised *Lactobacillus*, *Olsenella*, *Caproiciproducens*, *Roseburia*, *Streptococcus*, performed low hydrogen production, high butyric and acetic acids concentrations in long-term system. The energy assessment showed the higher energy recovery rate was obtained in thermophilic fermentation than that of the mesophilic system. Results suggested that stability and shock resistance capability of microbial community are crucial elements related hydrogen production and metabolic pathways.

Keywords: hydrogen production; metabolic pathways; microbial community; dark fermentation; long-term; food wastes

NONMENCLATURE

Abbreviations

OLR	organic load rate
FW	food wastes
GPR	gas production rate
DF	dark fermentative
LAB	lactic acid bacteria
CCA	canonical correspondence analysis
MR	mesophilic bioreactors
TR	thermophilic bioreactors
VFAs	volatile fatty acids
TVFA	total volatile fatty acids

Symbols

ρ	mass concentration
E_i	input electricity for biomass pumping and reactor mixing
E_h	energy consumption of the hydrothermal pretreatment
E_f	energy consumption of the fermentation process
E_{H_2}	energy generation of hydrogen
η	energy recovery rate

1. INTRODUCTION

In view of depletion of fossil fuel resources, we are facing serious environmental and demand for energy challenges. Hydrogen is considered an attractive future fuel on account of its clean product and high energy density[1]. FW has been considered as the substrate for fermentative hydrogen production, due to high energy content of organic wastes, rich in carbohydrates[2].

However, system instability of hydrogen production is the main problems. Hydrogen yields is far below the maximum theoretical hydrogen yield of 4 mol H₂/mol glucose due to complex metabolic pathway[3]. There are correlations of hydrogen production and metabolic pathways with the complexity of microbial communities presented in DF. Previous studies showed that Lactic acid bacteria (LAB) constitutes a microbial group commonly found in DF, which may be outcompete hydrogenogenic microorganisms for the carbon source, and also capable to produce growth-inhibitory compounds[4]. However, there is still knowledge scarcity about their effects on hydrogen production, metabolic pathways and microbial community roles in long-term systems of FW.

Based on the above considerations, the long-term hydrogen production performances were analyzed in continuous DF reactors. Canonical correspondence analysis (CCA) was reflected the correlation between hydrogen yield, metabolites and microbial communities under temperature and OLR stresses. Furthermore, the energy balance provided basis information of hydrogen production efficiency of FW.

2. MATERIALS AND METHODS

2.1 Materials

FW was periodically collected from a canteen and smashed into small particles (<2 mm) after manually removed the impurities such as bones and plastics and stored at 4 °C. Hydrothermal pretreatment for 30 min at 90°C was applied and then separated the oil slick from the pretreated FW [5]. The seed sludge was enriched from an anaerobic reactor used for treating pig manure.

Table 1 The basic characteristics of FW and seed sludge.

Item	pH	TS/%	VS/%	$\rho(\text{SCOD})/(\text{g/L})$	$\rho(\text{TOC})/(\text{g/L})$
FW	6.07	20.38	17.65	103.49	46.63
Sludge	7.41	11.45	5.67	1.34	—

2.2 Bioreactors setup

Continuous systems were established in 2 L mesophilic bioreactors (MR, 35 ± 1°C) and thermophilic bioreactors (TR, 55 ± 1°C) conditions fed with hydrothermal pretreated FW. 50 g of seed sludge was loaded into each bioreactor and the working volume was 1.5 L using distilled water. The bioreactors were passed over nitrogen for 10 min. The OLR stress experiments were run over a period of 44 days with the OLR increasing from 3.0 to 9.0 g VS /L·d for TR, and 56 days with the OLR increasing from 3.0 to 10.0 g VS /L·d

for MR. The pH value was controlled at 6.0. Samples were collected daily before being fed.

2.3 Analytical methods

The total gas production was measured with wet type gas flowmeter (LMF-2, China). The biogas composition was measured by a gas chromatograph (Perkin Elmer Clarus 500, USA) with a thermal conductivity detector and 2m high-porosity polymer bead-packed column. The volatile fatty acids (VFAs) and ethanol concentration were determined using a GC (GC7980, Fu Li, Zhejiang) equipped with a flame ionization detector and a 30 m×0.25 mm×0.25 mm fused-silica capillary column (KB-Wax) after pretreatment with 0.45 µm membrane filter.

2.4 Microbial community analysis

Samples were collected from different OLR levels which were named H-OLR3, H-OLR6, H-OLR9, M-OLR3, M-OLR6, M-OLR9. PCR products were sequenced on the Illumina Hiseq2500 PE250 pyrosequencing platform. The taxonomy of each 16S rRNA gene sequence was analyzed by the RDP Classifier algorithm (<http://rdp.cme.msu.edu/>) against the Silva (SSU123). CCA was conducted using a web platform (<http://www.i-sanger.com/>) to quantify differences in community composition and operational parameters.

2.5 Energy calculation

Energy calculation includes the value of consumption and generation. The consumption energy includes the electricity demand for mixing and pumping, energy for heating the substrate to hydrothermal, and compensating for the heat losses of fermentation. The energy generate from the DF was calculated from the hydrogen yield. Fermentation tank using single continuous stirred reactor[6]. Daily flow rate is 100m³/d, and hydrothermal pretreatment for 30 min at 90°C was applied.

3. Results and discussion

3.1 Bioreactor performance

As shown in Fig1a, the gas production rate and hydrogen proportion stabilized at around 8.0 mL/g VS·h and 40% in TR after the bioreactor startup stage (0-3 days). The best hydrogen production of food wastes was acquired, with highest observed hydrogen proportion was 67.11% during 18-24 days (OLR of 6 g VS/L·d). With OLR increasing to 9 g VS/L·d, GPR and hydrogen proportion were reduced to 4.02 mL/g VS·h and 33.24%,

respectively. Compared with TR, the performance of hydrogen production was significant reduction under mesophilic condition. During 16-25 days (OLR of 6 g VS/L-d), GPRs were maintained at around 7.56 mL/g VS-h. Afterwards, the GPR decreased drastically below 20% with OLR of 9 g VS/L-d, and GPRs fluctuated within 2-8 mL/g VS-h during 38-47 days. Based on these results, it was possible to predict that the desirable OLR was 6 g VS/L-d from FW at 55°C.

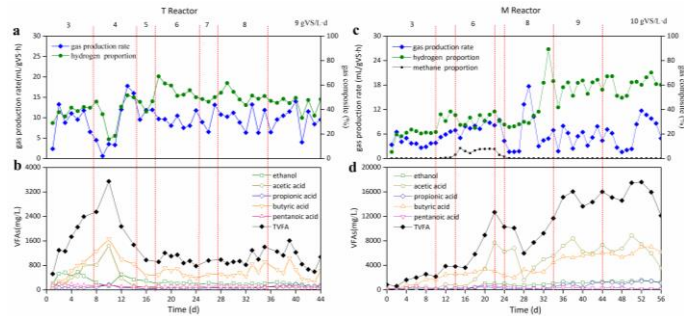


Fig.1 Hydrogen production and metabolite performance with time course in thermophilic and mesophilic fermentation from food wastes under OLR stress.

3.2 VFAs and ethanol distribution

The concentrations of VFAs and ethanol of fermentation are shown in Fig.1b and d. It is worthy to note that higher production of hydrogen and lower production of VFAs were obtained in TR than that of the MR. With OLR increasing, Total volatile fatty acids (TVFA) reached a peak value of 16042.23 mg/L at 9 g VS/L-d, which was more than 15 times of TR. Interestingly, butyrate production followed a similar trend to TVFA, there was no correlation with hydrogen yield. The result suggested that butyrate was produced also through other pathways with no hydrogen production in MR reactor. Moreover, the much lower hydrogen yield and relatively higher concentration of acetate observed in MR might be attributed to homoacetogenesis[7].

3.3 Microbial community dynamics and difference analysis

Distribution of sequences at the class and genus level in each samples are shown in Figs. 3 and 4. The increasing of *Clostridia* with high relative frequency accounted for 83.91% of T-OLR6 sample. The abundance of *Clostridia* was the lowest at 9 g VS/L-d, but the abundance of *Clostridia* in MR was about 17%-25% less than TR. As shown in Fig. 4b. *Thermoanaerobacterium* was the predominant genera in microbial communities under thermophilic condition. The highest relative frequency of the genus accounted for 82.13% with best performance of hydrogen production under 6 g VS/L-d.

Thermoanaerobacterium produce hydrogen from carbohydrates and grow at pH 5-6 and an optimum temperature of 60°C[8]. Furthermore, many genera had significant changes with OLR stress, such as *Lactobacillus* and *Weissella* which were especially notable in T-OLR9 with relative frequencies of 15.02% and 9.78%, respectively. The analysis of mesophilic fermentation showed that *Streptococcus* was the main microorganism under 3 g VS/L-d with the relative abundances of 32.72%, it dropped rapidly when OLR had gone up to 9 g VS/L-d. *Streptococcus* was reported that play an essential role in symbiosis and mutualism with the hydrogenogens[9].

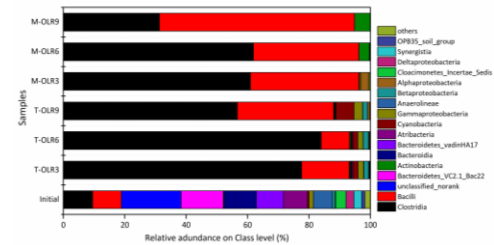


Fig. 2 Major bacterial class (the abundant >1%) found in OLR stress and temperature groups.

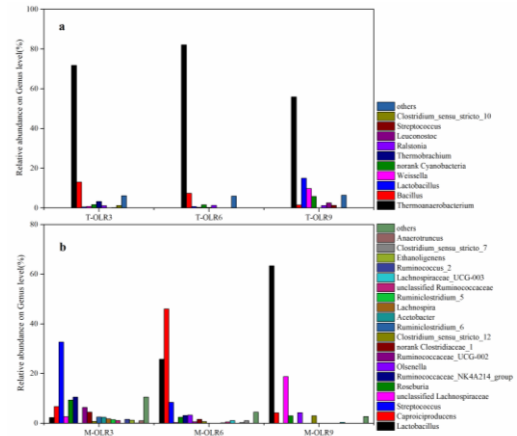


Fig. 3 Major bacterial genera (the abundant >1%) found in OLR stress and temperature. Distributions in (a) and (b) are at the level of mesophilic and thermophilic fermentation under OLR stress, respectively.

The results of CCA showed that the first and second axes explained 46.17% and 30.48% of the total variation for microbial communities. Obviously, the bacteria were significantly related to samples from TR including *Thermoanaerobacterium*, *Bacillus* and *Weissella*, while *Lactobacillus*, *Olsenella*, *Caproiciproducens*, *Roseburia*, *Streptococcus* distributed among samples from MR. Acetic and butyric acid concentrations showed passive correlation with bacteria related to TR. In contrast, the metabolites showed positive correlation in MR. Based on the above, thermophilic fermentation has higher hydrogen

production potential, lower acid accumulation inhibition and more stable system.

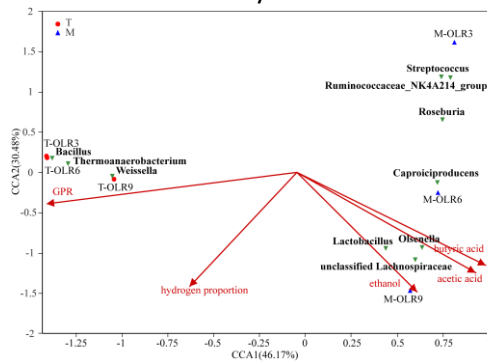


Fig. 4 Relationship between microbial community dynamics and process parameters analyzed by CCA.

3.4 Energy assessment

The energy assessment of long-term hydrogen production system was based on the experimental results in continuous reactors. The table 2 shown that energy balance of MR was much less effective than TR, the recovery rate is only 18.23%. It is worth taking into consideration that thermophilic fermentation system which obtained the recovery rate of 41.03%. Stable microbial communities of hydrogen production and higher GPR were crucial factors for superior energy balance. It should be noted that energy balance can be adjusted with Increasing of hydrogen yield, and system optimization, including environmental factors and microbial communities.

Table 2 Energy assessment of food waste DF for hydrogen production

Fermentation reactors	consumption(GJ/d)			generation(GJ/d)	η (%)
	E_i	E_h	E_f	E_{H_2}	
TR	1.08	29.26	0.83	12.79	41.03
MR	1.08	29.26	1.93	5.88	18.23

E_i : input electricity for biomass pumping and reactor mixing.

E_h : energy consumption of the hydrothermal pretreatment.

E_f : energy consumption of the fermentation process.

E_{H_2} : energy generate of hydrogen.

η : energy recovery rate.

4. Conclusions

Take into consideration energy balance, thermophilic fermentation system was obtained two times recovery rate than mesophilic fermentation. It could be attributed to *Thermoanaerobacterium* was in the ascendant with GPR of 9.32 mL/g VS·h corresponding to hydrogen proportion of 58.19% at thermophilic. On the contrary, the microbial community changed significantly at mesophilic with increasing of OLR. Overall, stability and shock resistance capability of

microbial community are critical elements that determine the shape of hydrogen production and metabolism in long-term hydrogen production systems.

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