Application of zeolite adsorption and biological anaerobic digestion technology on hydrothermal liquefaction wastewater

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Abstract: The post-hydrothermal liquefaction wastewater (PHWW) was obtained from a conversion process of *Spirulina* sp. to bio-crude oil via hydrothermal liquefaction (HTL) technology that was rich in organic matter and toxicant. In this study, zeolite was applied to overcome the inhibitions and improve anaerobic digestion (AD) efficiency. Three zeolite adding ways were evaluated: PHWW pretreated with zeolite for 5 h with/without solid-liquid separation before AD process and zeolite added to PHWW AD process directly, in order to find the most ideal way to add zeolite. Results indicated that zeolite had a positive effect on the AD process of the PHWW. The gas potential of PHWW after adding zeolite, as well as the chemical oxygen demand (COD) removal rate, was much higher than those of its initial state. Especially when the PHWW was pretreated by zeolite for 5 h without separation, the biogas yield increased by 73.96% (6170 μmol/g $COD_{removed}$) compared with the initial PHWW (3546.7 μmol/g $COD_{removed}$).

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1 Introduction

Hydrothermal liquefaction (HTL) is an attractive thermochemical process as pyrolysis and gasification to convert various wet feedstock such as algae into biofuels. It could reduce energy consuming steps like drying and dewatering for feedstock[1]. The main products of HTL are bio-crude oil, gases (CH_4, CO_2, etc.), solid residue, and a considerable amount of aqueous phase, hereafter called post-hydrothermal liquefaction wastewater (PHWW)[2-4]. It has been reported that the PHWW represented about 82% in mass of the total obtained products after the HTL process and >25% of the organics from the raw materials[5]. Moreover, it contained almost all the nitrogen and phosphorus. Thus, the PHWW has been considered to be a high value-added wastewater with complex components and classified as petrochemical refineries wastewater. If it was treated correctly before directly being discharged, it will not only be harmless to the environment but could also produce energy and clear water, which agreed with Marcilla et al.[6]

The excessive nitrogen, phosphorus and other possible inhibitors made PHWW difficult for aerobic or anaerobic treatment. Although Tommaso et al.[7] and Zhou et al.[8] had confirmed that AD of PHWW was available, there still exist problems that need to be improved such as the relative long reaction time and

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high dilution multiples. Co-digestion with other wastes, acclimating microorganisms to adapt inhibitory substance, and incorporation of methods to remove or counteract toxicants before AD can significantly improve the waste treatment efficiency[9]. Adding adsorbent is also an alternative improving the AD process by decreasing the inhibition concentration through adsorption[10]. According to Tommaso et al.[11], a faster CH_4 production rate as well as a shorter lag phase appeared when the PHWW of *Spirulina* sp. was pretreated with activated carbon. Zhou et al.[8] also found AD efficiency could be improved when the PHWW of swine manure was absorbed with the aid of activated carbon before AD.

Zeolite, as one of the common adsorbents, could treat ammonia-rich wastewater. For its peculiar cage structure of zeolite, water molecules and positive ions like K^+, Na^+, Ca^{2+} were with a weak capacity of fixation to the framework, leading to easy ion-exchange with other cations in the aqueous phase[12]. Due to the small particle size and cations distribution, significant selectivity of ion-exchange was exhibited. NH_4^+ was smaller than the zeolite particle in size and easier to adhere to its surface, resulting in the possible exchange with unstable cations inside the zeolite. Yang et al.[13] mentioned that the decrease of NH_4^+ through zeolite was the incorporation of ion-exchange and adsorption, with the former as the major factor. Zeolite was first used to pretreat the ammonia-rich PHWW in this study. The effects of adding zeolite on the AD process were analyzed. Three addition ways were tested: PHWW absorbed for 5 h with/ without solid-liquid separation before AD process and zeolite added to PHWW AD process directly, attempting to find the optimal zeolite utilization way. Our hypothesis is that zeolite could overcome inhibitions for AD process of PHWW and improve its AD efficiency.

2 Materials and methods

2.1 HTL process

The PHWW in this study was a by-product from the *Spirulina* sp. conversion after the HTL reaction (220 ℃, 60 min, 35 MPa and 25% of the total solids) and obtained through vacuum filtration, which resulted in a chemical oxygen demand (COD) of 185 g/L. The reaction was carried out in a stainless steel cylinder with 1.8 L capacity (Parr 4578, Parr Instrument Co., Moline, IL, USA) in batch mode.

2.2 Biogas potential assays

The batch AD test was conducted in four treatments: the initial PHWW, zeolite absorbed PHWW with solid-liquid separation before AD process (AS-PHWW), zeolite absorbed PHWW without solid-liquid separation before AD process (A-PHWW) and PHWW with zeolite absorption-AD process carrying out simultaneously (ZA-PHWW). Inoculum was collected from a continuous stirred tank reactor (CSTR) during normal operation. Both the PHWW and inoculum were added to 100 mL flasks with a reaction volume of 50 mL. Equal inoculated sludge was set as the control to find the background of inoculum to produce biogas. All reactors were sealed with rubber plugs and incubated in a (35±1) ℃ time controlled water bath. The batch test was conducted in triplicates. An air-pocket was created in each reactor to collect biogas generated during the AD process. This batch experiment was conducted until there was no biogas production. After the AD process, samples were centrifuged at 10 000× for 5 min and filtered using 0.22 μm filters (ANOW Microfiltration Co., Ltd., Hangzhou, China). Then the total ammonia nitrogen (TAN), total nitrogen (TN), total phosphorus (TP), total organic carbon (TOC) and COD were quantitatively analyzed.

2.3 Characteristics of PHWW and inoculums

TP was measured through the molybdenum-antimony anti-spectrophotometric method. TAN was analyzed using salicylic acid - hypochlorite spectrophotometric method. TN content was obtained via the ultraviolet photometric method. Table 1 shows the characteristics of the PHWW (before and after zeolite absorption for 5 h) and inoculums. TOC and COD were both indices for organics. They were obtained through the TOC analyzer (TOC-Cvpn, Shimadzu, Japan) and the COD analyzer (DR-2800, HACHI, USA) respectively.

Table 1 Characteristics of PHWW, AS-PHWW and the inoculum

Parameters	PHWW	AS-PHWW	Inoculum
pH	8.24±0.55	7.63±0.27	7.58±0.33
TP/mg L^{-1}	1138±94	62±12	162±38
TN/mg L^{-1}	21525±738	18197±495	4003±677
TAN/mg L^{-1}	6549±749	3534±332	679±57
TOC/mg L^{-1}	78960±5453	76891±4345	NA
COD/mg L^{-1}	185053±3455	181643±6583	5.4±0.4
Biodegradability/%	32	48	ND

2.4 GC-MS analysis

The composition of the PHWW extracts (Vaqueous phase: Vdiethyl ether = 1:9) was analyzed using a gas chromatograph-mass spectrometer (GC-MS) (Shimadzu QP2010, Kyoto, Japan).

Gas chromatograph was performed on a 30 m Varian DB-5 column, with 0.25 mm inner diameter and 0.25 μm film thickness. Helium gas was used as the carrier gas at a flow rate of 1.64 mL/min. The injection, interface and ion source temperatures were 250℃, 250℃ and 200℃, respectively. The oven temperature was (1) 35℃ for 5 min, (2) 130℃ for 4 min, 240℃ for 4 min and 280℃ for 7 min sequentially, all with an increasing speed of 25℃/min. The voltage of the mass spectrometer was set at 70 eV and the range of scan was 35-500 m/z.

2.5 Biogas analysis

CH_4 concentration in the biogas was analyzed using a gas chromatograph (GC 1490, Agilent Technologies, USA) equipped with a thermal conductivity detector (TCD) and nitrogen gas as the carrier gas at a flow rate of 50 mL/min. The injector, oven and detector temperatures were 150℃, 120℃ and 150℃, respectively. CH_4 and CO_2 could be measured sequentially. Gas compositions and volumes were monitored within a day after sampling.

2.6 Data analysis

The cumulative biogas production was fitted by the modified Gompertz equation (Equation (1)). Specific biogas production potential ($P_{\max}$), specific biogas production rate (R) and lag phase (λ) were predicted.

$$P(t) = P_{\max} \times \exp\{-\exp\frac{R \times e}{P_{\max}}(\lambda - t) + 1]\} \qquad (1)$$

where, $P(t)$ is the cumulative specific biogas production (μmol/g $COD_{removed}$) for a given time t; $P_{\max}$ is the specific biogas production potential, μmol/g $COD_{removed}$; R is the specific biogas production rate, μmol/g $COD_{removed}$/d; λ is lag phase (days) and e is the constant 2.71828.

All statistics were conducted in triplicate and expressed in means ± standard deviation. All means and standard deviations were analyzed using Microsoft Excel 2010 and figures were processed through Origin 9.0.

3 Results and discussion

3.1 Effect of zeolite absorption on PHWW

Table 1 shows the effect of the zeolite on PHWW. Compared with the initial PHWW, TAN, TN, TP, TOC and COD contents decreased to a relatively low level after zeolite absorption for 5 h, which made the PHWW more feasible for the AD process. TAN was nitrogen source for microorganisms growth, while excess TAN would cause inhibition. The toxicity of ammonia was related to changes in the intracellular pH caused by the non-ionized form NH_3 that easily penetrates the microbial cell membrane[14]. The zeolite adsorption decreased the TAN content by 46% to a more suitable content (3534±332) mg/L for AD process.

Biodegradability was the ratio between biochemical oxygen demand (BOD) and COD, indicating the portion of biodegradable COD. After zeolite absorption, the biodegradability increased from 32% to 48%, which meant that zeolite could adsorb certain inhibitive matters for microbial.

The main classes of molecules in PHWW before and after the zeolite adsorption were identified by GC-MS (Table 2). It should be noted that major molecules in the initial PHWW were complex. The highest percentage of the relative peak area (RPA%) was N-heterocyclic compounds of 46.51%. In addition, there were other benzene homologs and derivatives such as hydrocarbons, ketones and esters. The average molecules length was larger than 5C. Considering the influence of the zeolite adsorption, the nitrogenous compounds, for example, aziridine and pyrazine, decreased in the AS-PHWW. The average appearance time was delayed as well. The above observation indicated that zeolite could absorb nitrogenous organic matters from PHWW, which helped to decrease nitrogenous compounds, adjust the ratio of C/N and was benefit for the AD process.

Table 2 Major organic compounds of initial PHWW and AS-PHWW by GC-MS analysis

Molecules	PHWW/%	AS-PHWW/%
Amino acid	7.92	5.62
Ketones	18.62	15.28
Straight amides	6.30	4.87
N-heterocyclic cmpds	46.51	34.42
Straight hydrocarbons	5.49	1.61
Esters	5.84	19.08
Alcohols	1.91	7.88
Actinomycin	3.32	1.76
Dihydroergotamine	4.09	4.74
Phenylacetic acid	ND	3.01
Heptadecyl trifluoroacetate	ND	1.73

3.2 PHWW characteristics after AD process

After the 15 d AD process, TP concentrations of the four treatments present a decrease regardless of zeolite addition ways, indicating improvement of the aqueous quality of PHWW. However, anabolism does little in TAN reduction at the end of AD, which consisted with Wang et al.[15] ZA-PHWW presented the highest COD removal rate (Table 3). In addition to ion exchange, zeolite could act as an adsorbent[13]. Some organic matters could be absorbed during the zeolite treatment. At the same time, microbial was immobilized to the zeolite attributed to its porous structure. Previous study has reported stable immobilization of zeolite for *Bacillus*[16]. This may account for the higher COD removal rate in the ZA-PHWW with the longest contact time between the zeolite and substances in the PHWW.

Table 3 Effects of different zeolite adding ways on aqueous parameters before and after AD process

Type	pH		TAN content/g·L^{-1}		TN content/g·L^{-1}		TP content/g·L^{-1}		Initial COD /g·L^{-1}	COD removal rate /%
	Start	End	Start	End	Start	End	Start	End		
PHWW	7.32	7.75	0.59	1.63	3.34	3.33	0.96	0.82	48.78	15.53
AS-PHWW	6.98	7.65	0.34	1.05	2.43	2.68	0.62	0.58	45.48	24.84
A-PHWW	7.39	7.67	0.44	1.08	2.35	2.62	0.65	0.61	43.15	22.43
ZA-PHWW	7.50	7.67	0.53	1.11	2.87	2.67	0.84	0.67	46.75	34.98

3.3 Biogas analysis

Figure 1 depicts the influences of the zeolite addition ways on cumulative biogas production. Compared with the PHWW before (3546.7 μmol/g $COD_{removed}$ with CH_4 content 26.63%) and after zeolite treatment, all the latters exhibited higher cumulative biogas yields and CH_4 contents, which could reach 6170.2 μmol/g $COD_{removed}$ and 54.69% respectively for A-PHWW. This may be attributed to large amounts of toxicants, such as excessive TAN, being absorbed by zeolite, which may have inhibitory effect on anaerobic micoorganisms as mentioned earlier in this research (Table 1). The highest accumulated biogas yield occurred in the trials where the PHWW was absorbed by zeolite for 5 h and then digested without solid-liquid separation (A-PHWW). For A-PHWW and ZA-PHWW, zeolite was the part of the fermenting process, they both achieved higher biogas yield as well as CH_4 content than AS-PHWW. It indicated that the small particle size and large specific surface area made the zeolite possible for the uniform mixture with aqueous phase and easier formation of colloidal structure, which could promote enrichment of microbial. However, for ZA-PHWW, the zeolite adsorption and the AD process of PHWW carried out simultaneously. It was inferred that NH_3 and N_2 released during the ion-exchange process, which diluted the CH_4 content compared with A-PHWW. In addition, microbes were more sensitive for NH_3 than NH_4^+, which was proved by the deceased biogas production in this study. Thus the zeolite absorption and AD process simultaneously was not advisable for PHWW treatment. The highest biogas yield for A-PHWW did not necessarily imply that the NH_3 generation from zeolite ion-exchange during AD had no influence for AD process. Indeed it is suggested that the impact of NH_3 has been masked by the high biogas accumulation.

Table 4 presents the parameters evaluated through the nonlinear Gompertz fitting model (Equation (1)) with $R^2 > 0.98$. The regression result presented in Table 4 showed that P_{max}, R and λ depended on the different zeolite addition ways. It is possible that when the reactors were fed with A-PHWW, P_{max} and R reached the highest level of 6136.97 μmol/g $COD_{removed}$ and 1599.33 μmol/g $COD_{removed}$/d, demonstrating the optimal zeolite addition way for AD. Interestingly λ was consistent with P_{max} and R, with the highest value of 1.12 d in the A-PHWW. This occurrence was also observed by Miao et al.[17] This may be related to the adaptive phase microorganisms needed to adapt to the new environment when they were transferred to a new condition.

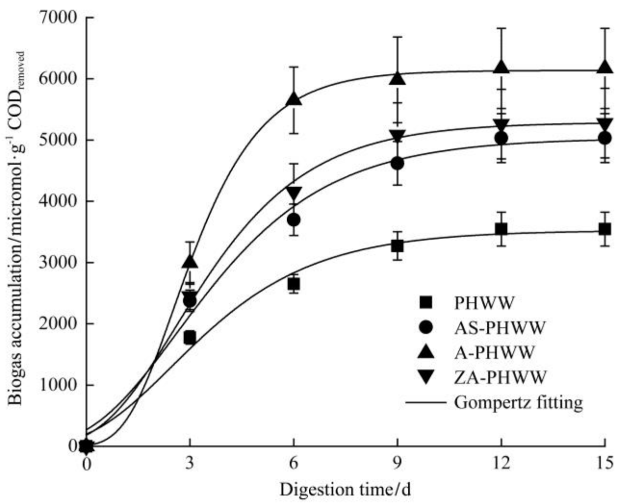

Figure 1 Effects of different zeolite addition ways on accumulated biogas production.

Table 4 Values predicted by the nolinear Gompertz model to biogas production potential (P_{max}), specific biogas production rate (R) and duration of the lag phase (λ)

Groups	P_{max} /μmol $gCOD_{removed}^{-1}$	R /μmol $(gCOD_{removed}\ d)^{-1}$	λ/d	R^2
PHWW	3524.22	557.11	0.13	0.9800
AS-PHWW	5032.73	757.74	0.18	0.9834
A-PHWW	6136.97	1599.33	1.12	0.9994
ZA-PHWW	5289.30	913.65	0.45	0.9939

AD of swine manure-based PHWW previously studied by Zhou et al.[8] reported 0.5 mL/mg $COD_{removed}$ (about 22321 μmol/g $COD_{removed}$) biogas just at relatively low concentrations of PHWW (<6.7%). Higher concentrations (>13.3%) of PHWW had a significant inhibitory effect on the AD process, as indicated by delayed, slower, or no biogas production at all. The CH_4 content at low PHWW concentration (<13.3%) was no less than 70%, which was higher than that measured in this study, while the PHWW was diluted into the supernatant of anaerobic inoculum sludge, which provided a growth medium suitable for the anaerobic culture. It is inferred that a considerable part of the biogas probably generated from the dilution. Similar study has been reported by Tommaso et al.[7], who diluted the microalgae-based PHWW with basal medium to relative low concentrations and achieved high CH_4 yields (from 8935 μmol/g VSS to 16304 μmol/g VSS) with contribution of the basal medium.

Neither the biogas yields (6170.2 μmol/g $COD_{removed}$ for A-PHWW) nor the CH_4 content (54.69% for A-PHWW) in this study seem advantageous compared with previous studies. This is because the raw PHWW contained a variety of polycyclic aromatic hydrocarbons and nitrogen heterocyclic compounds, which were toxic for anaerobic microbes. While the zeolite added could absorb these toxic compounds in PHWW to some extent and make the AD process of PHWW without dilution at relative high COD concentration feasible. For the further study, measures on how to improve biogas yield and COD removal rate should be taken into account, such as cultivated inoculum with strong toxic tolerance, increasing zeolite dosage, etc.

4 Conclusions

The utilization of zeolite as an additive represented an optimal operating performance in the AD process of PHWW from *Spirulina* sp. due to its ion-exchange and absorption properties. The gas potential of PHWW after being pretreated by zeolite was much higher than that of its initial state. The largest accumulative biogas yield and highest biogas production rate was obtained when the PHWW was previously absorbed for 5 h using zeolite and then digested without solid-liquid separation, indicating the optimal zeolite addition way. Measures on how to improve biogas yield and COD removal rate would be further studied, such as cultivated inoculum with strong toxic tolerance, increasing zeolite dosage, etc.

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