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New insights into the evaluation of anaerobic properties of sludge: Biodegradability and stabilization

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ABSTRACT

Evaluating anaerobic biodegradability of sludge and then identifying the stabilization of digestate is necessary in sludge treatment and disposal. 48 sludge samples from 24 typical waste water treatment plants (WWTPs) in 11 provinces in China were selected to investigate the relationship between Biochemical Acidogenic Potential (BAP) test and Biochemical Methane Potential (BMP) test. The volatile fatty acid (VFA) production obtained from BAP tests was found linearly related to the ultimate methane production from corresponding BMP tests. Satisfying results were obtained with Pearson correlation coefficient as 0.929 and R^2 value as 0.76. Furthermore, the physio-chemical characteristics (FCI, $SUVA_{254}$, E_4/E_6) of supernatant, which were associated with humic-like substances (HS), were investigated before and after BMP tests. Through which a new criterion (FCI>1.50, $SUVA_{254}$>1.10, E_4/E_6<4.0) was proposed to evaluate the stabilization level of anaerobic digested sludge.

Introduction

Sewage sludge contains large amounts of substances including perishable organics, heavy metals, pathogenic bacteria and parasite eggs, etc., which would bring about secondary pollution (Gantzer et al., 2001). Meanwhile, sewage sludge also contains nutrients such as carbon, nitrogen, phosphorus and so on (Carrère et al., 2010). With the shortage of global resources nowadays, the safety treatment and energy recovery of sewage sludge has received growing attention (Mao et al., 2015). Anaerobic digestion (AD) which can recover biomass energy (biogas) while removing perishable organic matters from sludge, has been reported to be one of the most popular technologies for sludge reduction, resource utilization and stabilization (Appels et al., 2008). Affected by the factors such as wastewater treatment processes, organic and inorganic contents and components, the anaerobic biodegradability of sludge fluctuates significantly (Bougrier et al., 2006; Khalid et al., 2011; Kim et al., 2003). To evaluate the biodegradability of sludge is necessary for the utilization of anaerobic digestion technology and the improvement of efficiency.

Biochemical Methane Potential (BMP) test is one of the most widely used and effective methods to evaluate anaerobic biodegradability (Jeong et al., 2005; Shelton and Tiedje, 1984). And it is usually carried out in a culture flask at proper

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temperature, in which substances and inoculation are added, and biogas production is recorded daily until no more gas. Meaningful results can be obtained from BMP test by the maximum methane production under anaerobic conditions, as well as the gas production kinetics. However, BMP test was reported to be limited by long-term operation (50 to 70 days) and complex gas phase measurements, and it also required a proper balance maintained between acidification and methanogenesis processes (Kianmehr et al., 2012a). An imbalance would cause the accumulation of intermediates such as volatile fatty acids (VFAs), which would inhibit methanogenesis process and then affected the results of BMP tests (Kianmehr et al., 2012b, 2010).

Considering the limitations of BMP test, Pigeon firstly put forward the concept of Biochemical Acidogenic Potential (BAP) in 1990s, which was roughly described as the stable maximum concentration of volatile fatty acids (VFAs) produced from sewage sludge by spontaneously fermentation under anaerobic conditions (Pigeon, 1993; Lie and Welander, 1997). BAP test was characterized by the significant advantage of being completed in a relatively short time (6−10 days), and only supernatant was required to be determined (Ahn et al., 2009). At the same time, unlike BMP test, BAP test did not depend on the activity of methanogenic bacteria, un-conspicuous negative effects would be caused by the accumulation of intermediates. It has been widely reported that the appropriate operating conditions such as the inhibitor of methanogenic process, temperature, testing time et al. were directly related to the efficient conduct of BAP tests. While there is no unified standard for the determination of BAP till now.

According to the four-steps theory in anerobic digestion, the VFAs produced in BAP test are intermediate products and will eventually be converted into methane produced in BMP test (Gewa, 2009). VFAs production is not only the terminal index of hydrolysis and acidogenesis stage, but also the initial index of methane production stage, therefore there should be a certain correlation between the results of BAP and BMP test (Feng et al., 2009). However, the results of most previous studies were lack in representativeness owing to the neglect of different sludge characteristics in different areas. A more systematic study on correlation of BAP value and BMP value of sludge in different organic content and properties is in urgent need to be conducted.

Referring to the anaerobic properties of sludge, except for the evaluation of anaerobic biodegradability, to rapidly and accurately identify whether the stable state of sludge has been reached is also of great significance for the subsequent sludge disposal after AD in practical application. At present, the stabilization criterions for treated sludge differed among countries (Mei et al., 2020). According to European Union (EU) regulation, no less than 50% of volatile suspended solids are controlled to be degraded during stabilization and/or 1.5 mg O_2/(g VSS hr) is the highest oxygen utilization rate allowed for the end-product (Council Directive, 1999; The Council of the European Communities, 1986). It was reported that a well-digested product could be indicated in the case that VFAs were no more than 300 mg/L or the bio-carbonate alkalinity was above 2000 mg/L (Nguyen et al., 2015). As for China, the reduction rate of organic matters is required to reach 40% for AD and aerobic composting, respectively (Ministry of Construction, PRC, 2006; Ministry of Construction, PRC, 2017). However, the above standards in China failed to take different characteristics including sludge age, micro-sized grits content, metal irons content and organic matter content of sludge from different wastewater treatment plants (WWTP) into consideration. Thus, most of these criteria are not suitable for application in reality.

Furthermore, degradation conditions of most organic matters can be determined by the content of Humic-like substances (HS) as they are too complex to be microbially degraded. Ultraviolet-visible (UV–Vis) absorbance has been widely used for characterizing HS. Specific UV absorbance at 254 nm ($SUVA_{254}$) mainly represents aromatic functional groups and double-bonded C groups in the humic macromolecule. It is a good indicator of the humic fraction of dissolved organic carbon (DOC) (Corvasce et al., 2006; Shao et al., 2009). Similarly, the ratio of absorbance of dissolved organic matter under 465 nm and 665 nm (E_4/E_6) can reflect the degree of polymerization of benzene ring skeleton (Zbytniewski and Buszewski, 2005). It is a common index of composting stabilization and a decrease of E_4/E_6 value indicated an increase of organic matter polymerization, characterizing the formation of more humic substances (Hautala et al., 2000; Thomsen et al., 2002). Three-dimensional excitation-emission matrix fluorescence spectra (3D-EEM) can reflect the information of proteins, humus and melanin in dissolved organic matter (DOM) during AD by fluorescence complexity index (FCI) (He et al., 2013; Muller et al., 2014). In previous studies, only individual method was mostly applied to investigate the stabilization of organic matters. And the combination of various physiochemical characteristics would characterize the degree of humification and stability of organic matter in a more comprehensive and systematic way.

Therefore, the objectives of this study were: (1) to investigate the correlation between BAP value and BMP value of sludge; (2) to investigate the feasibility for systematic physiochemical indexes to characterize the stabilization of digestate, with widely sludge samples of different organic content and properties from different area. As a result, the evaluation of biodegradability and stabilization of sludge for the sequencing treatment and disposal would be furthered.

1. Materials and methods

1.1. *Samples*

Sludge samples used to determine optimal operating conditions of BAP were recorded as Sludge A, which were obtained from a sequencing batch anaerobic reactor under mesophilic condition running for 62 days. Samples were taken at intervals of 0d, 5d, 11d, 16d, 22d, 32d, 42d, 52d, 52d and 62d, respectively, to obtain the sludge with differed (volatile solids)VS/(total solid)TS (%) and biodegradability. All the Sludge A were heated under 121 °C for 30 min before BAP, in order to eliminate the microbial activities which might affect the VFAs production. The sludge samples used to investigate the relationship between BAP and BMP, as well as to analyze liquid phase physiochemical parameters before and after AD were recorded as Sludge B, which were obtained from 24 typical WWTPs in 11

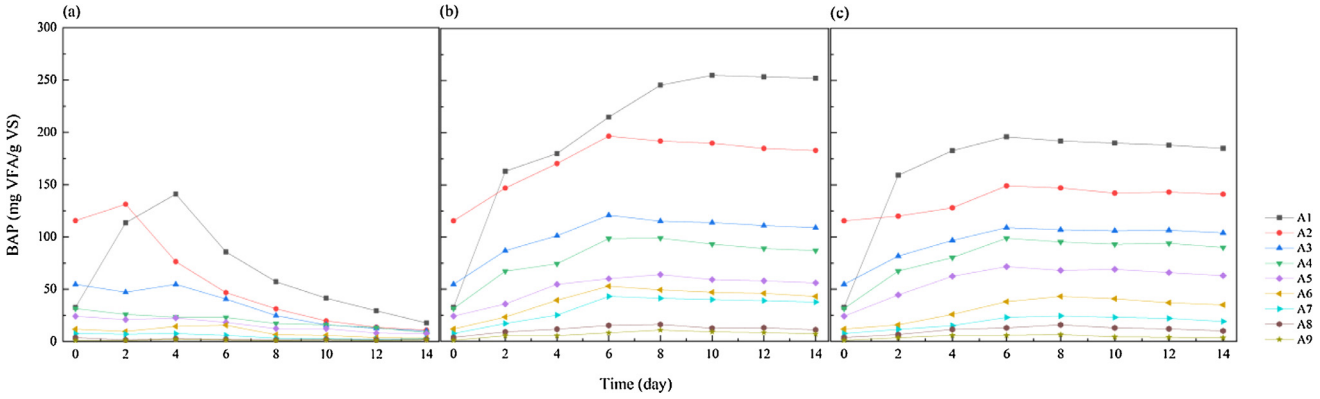

Fig. 1 – Production of VFAs per VS under three operating conditions: (a) UC, (b) pH_0=10, (c) +BES.

provinces in China. The inoculum was obtained from a long-term stable semi-continuous reactor with high solid content. The specific information of sludge A and inoculum, Sludge B and the corresponding WWTPs were described in Appendix A **Table S1**.

1.2. *Characterization of the samples*

1.2.1. *BAP test*

The BAP tests were conducted in conical bottles. Sludge sample was added in each bottle, the ratio of inoculum to substrate was 1:1 (calculated as VS) and the total VS was set to 1.5 g in each test. After being flushed with N_2 for 2 min to drive out the air, all bottles were sealed and operated at 35 ± 1 °C, and inoculated sludge was taken as a blank group. The test time was 14 days, and the composition and concentration of VFAs, as well as the biogas production were determined every 2 days. When examining the optimal operating conditions of BAP test, pH and methanogenesis inhibitors were used as influencing factors, which were commonly chosen in previous studies as (1) methanogenic process was not inhibited; (2) adjusting initial pH to 10; (3) adding methanogenic inhibitor BES (0.06 mol/L) (Kianmehr et al., 2011; Ruel et al., 2005, 2002).

1.2.2. *BMP test*

The BMP tests were conducted in 500 mL bottles with sludge samples and inoculation sludge added, the ratio of inoculum to substrate was 1:1 (calculated as VS), and the total VS was set to 1.5 g in each test. Other conditions were the same as those in BAP test. At the beginning of experiment, gas production was recorded and methane content was determined every 2 days. After gas accumulation was stable, it was recorded and measured every 5 days, and the whole testing time was 60 days.

1.2.3. *3D-EEM*

The concentration of sample in liquid phase was adjusted to 30 mg/L with deionized water, and the 3D-EEM spectrum was recorded using a fluorescence spectrometer (HORIBA Fluoro MAX-4, France) with scanning emission spectra from 280 to 650 nm at 10 nm increments by varying excitation wavelengths from 240 to 500 nm at 5 nm increments.

The qualitative analysis of 3D-EEM spectrum was carried out by Fluorescence Regionalization Integration (FRI) (Menassé et al., 1975). Referring to the method of (Muller et al., 2014), semi-quantitative analysis of 3D-EEM was carried out on the basis of FRI method.

1.2.4. *UV–Vis spectroscopy*

TOC concentration of sample was adjusted to about 10 mg/L with deionized water in liquid phase. Then UV–Vis spectroscopy was performed with a UV-755B spectrophotometer (Shanghai Yitian Precision Instrument, China). Specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) was calculated as the absorbance divided by the TOC concentration. The E_4/E_6 ratio was calculated as the ratio of absorbance at 465 and 665 nm.

1.2.5. *Analytical methods*

All analyses were duplicated, and the results are given as mean values with standard deviations. TS and VS of sludge samples were determined with the standard method (American Public Health Association (APHA), 2005). Sludge pH value was recorded by a pH meter (Mettler FE20-K, China).

To analyze VFAs, formic acid was added to the filtrate to adjust the pH to approximately 4.0. A GC2010-plus with flame ionization detector and equipped with a DBWAXTRE column was utilized to analyze the composition of VFAs. Nitrogen was the carrier gas and the flux was 50 mL/min, split ratio is 10:1, air flow rate is 400 mL/min, hydrogen flow rate is 40 mL/min. The injection port and the detector were maintained at 200 and 250 °C, respectively. The oven of GC was programmed to begin at 110 °C and to remain there 2 min, then to increase at a rate of 10 °C/min to 250 °C, and to hold at 250 °C for an additional 2 min. The sample injection volume was 1.0 mL, running time is 15 min (Chen et al., 2007).

Methane was measured by a GC112A (Shanghai Yitian Precision Instrument, China) equipped with a thermal conductivity detector (TCD). Nitrogen was used as the carrier gas and the pre-column partial pressure is 0. 1 kPa. The temperature of the injection, column, and detector was set at 40, 40 and 80 °C, respectively.

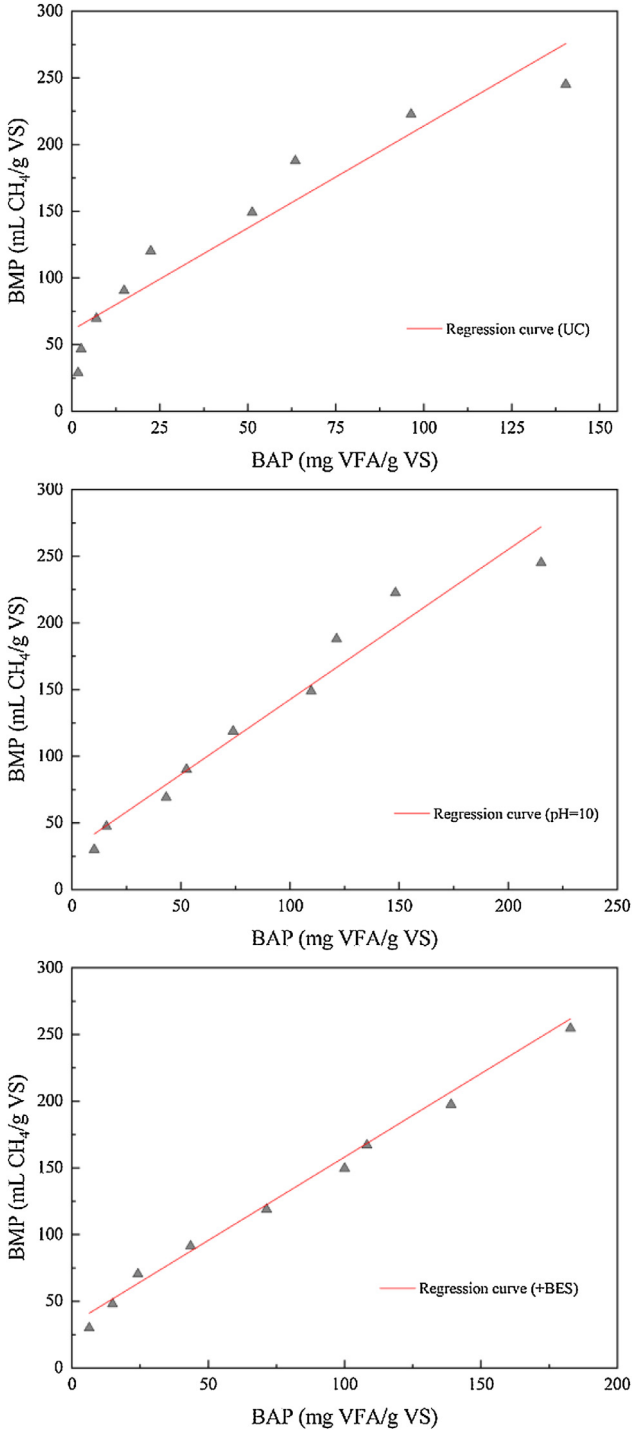

Fig. 2 – Analysis of BAP–BMP linear regression under three operating conditions: (a) UC, (b) pH_0=10, (c) +BES.

1.3. *Statistical analysis*

Statistical analysis was carried out using software OriginPro2020 64bit (OriginPro Lab Corp., Northampton, MA). Pearson's correlation coefficient was used to evaluate the linear correlation between two parameters. The indexes used to evaluate the prediction accuracy of the model were Root Mean Square Error of Prediction (RMSEP) and relative Root Mean Square Error of Prediction (rRMSEP). The RMSEP of the test group and the independent test group were called RMSEPt and RMSEPiv, respectively.

$$\mathrm{RMSEP} = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\hat{y}_i - y_i\right)^2} \quad (1)$$

$$\mathrm{rRMSEP}(\%) = \frac{\mathrm{RMSEP}}{\bar{y}} \times 100 \quad (2)$$

where $\hat{y}_i$ (mL CH_4/g VS) is the predicted BMP value of sludge sample; y_i (mL CH_4/g VS) is the actual measured BMP value; n is the number of sludge samples in the calibration group; $\bar{y}$ (mL CH_4/g VS) is the average value of predicted BMP values in the test group or independent test group.

Semi-quantitative analysis of excitation-emission three-dimensional fluorescence spectra was performed by MATLAB and ImageJ software.

2. Results and discussion

2.1. *Optimal operating conditions of BAP test*

Sludge samples of different anaerobic digestion stages (0d, 5d, 11d, 16d, 22d, 32d, 42d, 52d, 62d) in the laboratory reactors were named A1 to A9. During the BAP tests, VFA evolution was monitored for 14 days. BAP value under the three operating conditions and required time were shown in Fig. 1 and Appendix A **Table S2**. Under the condition that methanogenesis process was not inhibited (Fig. 1a, Appendix A **Table S2**), acid production of all sludge samples reached the maximum within 4 days, and sludge A2 even reached the maximum on the 2nd day, which was a very short time. And then as digestion time increased, VFA content of all sludge samples decreased, indicating the great impact of methanogenesis process on the VFA accumulation in this condition.

As shown in Fig. 1b and Appendix A **Table S2**, under $pH_0 = 10$, the cumulative VFA production increased to a maximum value in 6–10 days. Compared with the control group (UC), the methanogenesis process was obviously inhibited and its effect on accumulation of VFA was avoided. And it can be seen that all the BAP values were much higher than those of the control groups. It has been reported that the production of VFA would be significantly improved by alkaline treatment (Xu et al., 2018; Yuan et al., 2006). Therefore, the BAP values under alkaline conditions would be higher than actual value, then it would be unsuitable and inaccurate for reflecting the BMP performance. Under the condition that BES was added, the maximum production of VFA could been obtained in 6–8 days. After reaching the peak value, the VFA concentrations of all sludge were maintained at a relatively stable level, indicating the inhibitory effect on methanogenesis by BES (Fig. 1c, Appendix A **Table S2**).

Furtherly, the BAP values and BMP values obtained under the three operating conditions were linearly fitted respectively, results were shown in Fig. 2. The Pearson correlation coefficient in control group (UC) was 0.954 with R^2 as 0.897,

Table. 1 – Ranges of FCI, $SUVA_{254}$ and E_4/E_6 value in supernatant of sludge before and after AD.

	FCI	$SUVA_{254}$	E_4/E_6
Before AD	0.99 - 1.51	0.13 - 0.37	5.21 - 9.51
After AD	1.54 - 1.87	1.17 - 2.03	4.22 - 2.53

and the low BAP values of A7, A8, and A9 leaded to the poor fitting results. After adjusting the initial pH or adding BES, the linear fitting results were better. Under these two conditions, the Pearson correlation coefficient was 0.978 (pH_0=10) and 0.996 (+BES), and R^2 was 0.949 (pH_0=10) and 0.993 (+BES), respectively. In addition, from the perspective of practical operation, adding BES was easier than adjusting initial pH. Therefore, adding BES was selected as the optimal operating condition for BAP test under comprehensive consideration. The specific operating conditions of BAP test in the sequencing investigation were selected as medium temperature (35 $\pm$ 1 °C) with shaking at 100 r/min, I: S = 1:1 (based on VS), and with 0.06 mol/L BES addition.

2.2. *Correlation analysis of BMP and BAP values*

Among the 48 sludge B samples, the BMP values ranged from 66.2 mL CH_4/g VS to 299.5 mL CH_4/g VS, with the average value as 162.6 mL CH_4/g VS, with the reproducible standard deviation as 13.4. These could be considered as a reasonable range after comparing with data in the literatures (Abelleira-Pereira et al., 2015; Riau et al., 2015; Strömberg et al., 2015).

The BAP value of sludge B was calculated by the peak value of VFAs concentration based on VS added. The time to achieve maximum value was regarded as the time required to obtain the BAP value, namely the time required for BAP test. The time required for BAP test of 48 sludge B samples fluctuated from 4 to 9 days. There were 26 sludge samples that obtained BAP values at 5th or 6th day, accounting for 46.4% of the total samples. The smallest and largest BAP value was 6.5 mg VFAs/g VS and 228.0 mg VFAs/g VS, with others more evenly distributed within this range, many were 50–80 mg VFAs/g VS and 150–200 mg VFAs/g VS. The average value was 127.1 mg VFAs/g VS, with the reproducible standard deviation as 8.7.

The BAP value and corresponding BMP value of sludge B were randomly divided into two groups. One group was a calibration set, which contained 39 samples, used to build a BAP-BMP prediction model. The other group was a test set, which contained 9 samples, used to evaluate the prediction accuracy of the model for internal testing. 39 sludge samples from the calibration set were used to get the scatter plot of BAP values and BMP values (Fig. 3), and a more obvious linear relationship (P <0.01) were found between them. Univariate linear regression analysis was performed on the BAP and BMP values in the calibration group to obtain the prediction model (Eq. (3)).

$$y = 0.87\ x + 48.69 \quad (3)$$

where y (mL CH_4/VS) is the biochemical methane potential; x (mL VFAs/VS) is the biochemical acidogenic potential, and the regression model was simulated using OriginPro 2020 64 bit (OriginPro Lab Corp., Northampton, MA).

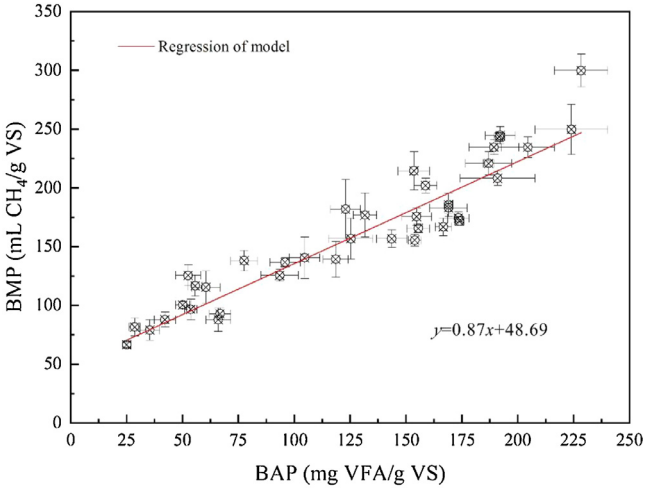

Fig. 3 – Analysis of BAP–BMP correlation.

The Pearson correlation coefficient was 0.929 and the determination coefficient R^2 was 0.884, minimum and maximum predicted residuals were 0.6 mL CH_4/g VS and 45.5 mL CH_4/g VS. The strong correlation between BAP and BMP values confirmed that BAP test was an proper alternative to BMP test, which was consistent with that reported by Liu et al. (2014). Test group was applied to evaluate the prediction accuracy of Model A, R^2 between measured BMP value and predicted BMP value was 0.909, RMSEPt was 15.5 mL CH_4/g VS, and rRMSEPt was 9.8%, which was acceptable comparing to previous studies (Giraud et al., 2010; Kianmehr et al., 2010). It was worth mentioning that even though there were studies having explored the correlation, they mostly focused on the sludge with similar sources, which limited the representativeness of results (Pigeon, 1993; Lie and Welander, 1997; Feng et al., 2009). In this study, the sludge samples with different original characteristics from various areas were investigated and the correlation results would be more reasonable.

2.3. *Physio-chemical characteristics of supernatants*

FCI, $SUVA_{254}$ and E_4/E_6 were chosen as typical liquid phase physio-chemical characteristics as they were effective to reflect sludge properties and stable performance (Hautala et al., 2000; Muller et al., 2014; Zheng et al., 2014). The FCI values of initial liquid phase in sludge B samples ranged from 0.99 to 1.51, and 85.7% of them ranged from 1.20 to 1.50 (Fig. 4a, Table 1). With the progress of anaerobic digestion, FCI values increased and were found to roughly separate at the value of 1.50 before and after AD. Mei et al. (2020) proposed the application of FCI (up to 5.0) to evaluate the stabilization level of anaerobic digested sludge. Considering the differences between laboratory experiment and practical AD process, it is normal for the parameter value to differ. However, in this study it should be noticed that there were three samples with initial FCI values slightly above 1.50. In addition, the samples with initial FCI values above 1.45 accounted for 22.4 %, and after AD, there were also 8.2 % of the samples with FCI below 1.55. Thus, it was hard to evaluate the stabilization level

Table. 2 – Characteristics of sludge B after anaerobic digestion.

NO.	VS Reduction rate	FCI	$SUVA_{254}$	E_4/E_6	NO.	VS Reduction rate	FCI	$SUVA_{254}$	E_4/E_6
B1	40.77%*	1.68	1.51	3.58	B25	34.39%	1.87	1.47	3.00
B2	39.52%	1.77	1.18	3.00	B26	20.28%	1.64	1.51	3.94
B3	26.03%	1.60	1.66	3.97	B27	42.90%*	1.59	1.38	3.72
B4	20.49%	1.58	1.27	3.97	B28	34.92%	1.79	1.35	3.51
B5	35.04%	1.64	1.41	3.08	B29	25.01%	1.63	1.27	3.79
B6	48.42%*	1.61	1.38	3.25	B30	28.12%	1.56	1.39	3.72
B7	28.75%	1.56	1.34	3.86	B31	49.93%*	1.68	1.72	3.22
B8	34.29%	1.77	2.03	3.22	B32	51.68%*	1.82	1.67	2.75
B9	46.50%*	1.76	1.51	3.25	B33	40.67%*	1.64	1.22	3.22
B10	39.73%	1.76	1.39	3.58	B34	47.67%*	1.76	1.58	3.51
B11	26.24%	1.74	1.24	3.36	B35	41.71%*	1.73	1.48	3.22
B12	16.62%	1.49#	1.16	4.22#	B36	45.41%*	1.54	1.52	3.68
B13	41.15%*	1.72	1.75	3.61	B37	41.29%*	1.69	1.40	3.72
B14	19.65%	1.53	1.27	4.11#	B38	22.89%	1.56	1.33	3.90
B15	45.16%*	1.62	1.43	3.58	B39	37.38%	1.74	1.38	2.54
B16	34.46%	1.64	1.48	3.40	B40	41.65%*	1.63	1.67	3.04
B17	59.96%*	1.63	1.92	3.47	B41	47.84%*	1.69	1.44	3.33
B18	39.73%	1.62	1.25	3.54	B42	48.17%*	1.76	1.59	3.68
B19	48.76%*	1.64	1.42	3.54	B43	45.47%*	1.59	1.36	3.29
B20	42.23%*	1.73	1.68	3.36	B44	34.60%	1.55	1.51	3.47
B21	22.16%	1.54	1.71	3.83	B45	44.22%*	1.58	1.45	3.54
B22	34.50%	1.61	1.65	3.08	B46	23.73%	1.54	1.51	3.86
B23	43.59%*	1.66	1.59	3.72	B47	38.99%	1.69	1.65	3.40
B24	17.48%	1.59	1.04#	4.13#	B48	31.26%	1.64	1.55	3.04

Note: * represents the VS reduction rate was above 40%. # represents the value failed to meet the criterion.

only with FCI values and other physio-chemical characteristics need to be combined for further analysis.

The distribution and change of $SUVA_{254}$ values before and after AD are shown in Fig. 4b and Table 1. The initial $SUVA_{254}$ values ranged from 0.13 to 0.37, after anaerobic stabilization, the range of $SUVA_{254}$ values was 1.17 - 2.03 except for a single point (1.048). 1.10 was found to be the dividing point, considering the boundary was relatively clear. And it was suggested that sludge with $SUVA_{254}$ >1.10 obtained better anaerobic stability, which was in accord with the research that investigated the characteristics of a series of biodegradable solid wastes at different stages of anaerobic digestion and found the $SUVA_{254}$ value peak was above 1.0 at the end (Zheng et al., 2014).

As shown in Fig. 4c and Table 1, after AD the E_4/E_6 values decreased for all the samples, indicating the increase of polymerization degree of benzene ring skeleton in liquid organic matter and the increase of humification. And most of the E_4/E_6 values after AD were below 4.0, except for three data points (4.115, 4.218, 4.114). And this results were in accord with Liu' study in which few VFAs were found to produce after 46 days and the E_4/E_6 decreased to 4.0 after 37 days during anaerobic digestion(Liu, 2014). Thus, E_4/E_6<4.0 was also suggested to be a criterion for comprehensive consideration.

Table 2 shows the VS reduction rate, FCI, $SUVA_{254}$ and E_4/E_6 value of sludge B after AD. It can be seen that samples with FCI, $SUVA_{254}$ and E_4/E_6 values within the criteria (FCI>1.50, SUVA254>1.10, E4/E6<4.0) recommended above almost reached a relatively higher VS reduction rate (B1, B6, B9 etc.). And the VS reduction rate of samples with abnormal FCI, $SUVA_{254}$ and E_4/E_6 values were lower correspondingly (B12, B14, B24 etc.). Thus, the feasibility of the criteria was further verified. In previous study, it was reported that FCI > 5.0 could be set as the new criterion to evaluate the anaerobic stabilization of sludge and was consistent with the current criterion

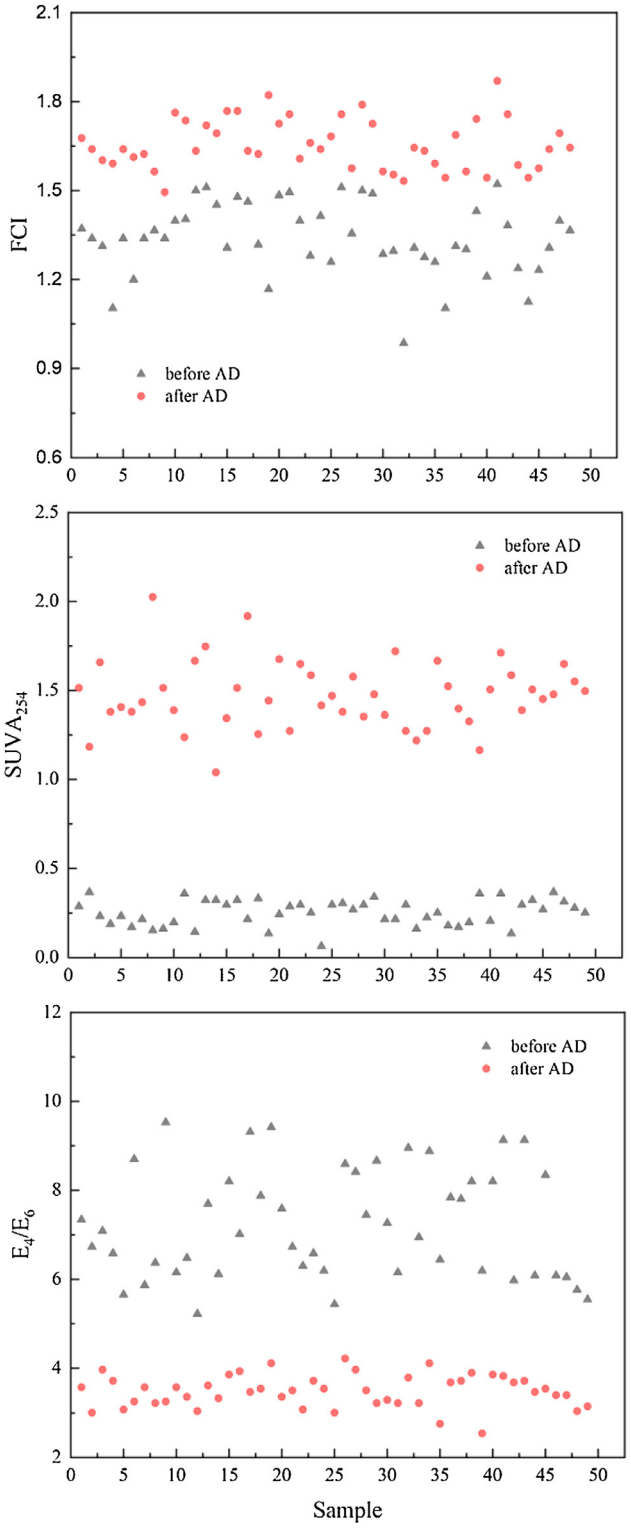

Fig. 4 – Variations of FCI (a), $SUVA_{254}$ (b) and E_4/E_6 (c) values before and after AD.

(VS reduction rate of 40%) applied in China (Mei et al., 2020). In their study, the sludge was obtained from effluent sludge from various commercial anaerobic digesters, which were operated in different anaerobic conditions (e.g. SRT, pre-treatment, etc.). Thus, it might be unsure whether the VS reduction rate could represent the maximum biodegradability performance of the sludge and might improper to further evaluate the stable state of sludge. In addition, we also found that with all the three index values meeting the criteria, there were still many samples presented low VS reduction rate (lower than 40%). Considering all the sludge have been anaerobic digested with no more methane production and VS reduction, the digestate obtained in this study could almost be regarded as reaching the stabilization. Therefore, the new criteria for the evaluation of sludge stabilization (FCI>1.50, $SUVA_{254}$>1.10, E_4/E_6<4.0) was suggested in this study, and setting the VS reduction rate of 40% as the standard of sludge anaerobic stabilization might be limited in representativeness. In addition, most researchers used only one method to investigate organic matter composition and stable performance of sludge (He et al., 2011; Lie and Welander, 1997), the combination of these three methodologies in this study was better sufficient for charactering the stabilization of digestate from different sludge.

3. Conclusion

According to the investigation of 48 sludge samples from 24 wastewater treatment plants, volatile fatty acid (VFA) production obtained from BAP tests was found linearly related to the ultimate methane production from corresponding BMP tests, with Pearson correlation coefficient as 0.929 and R^2 value as 0.76. And physio-chemical characteristics (FCI, $SUVA_{254}$, E_4/E_6) of supernatant were investigated and a new criterion (FCI>1.50, $SUVA_{254}$>1.10, E_4/E_6<4.0) was proposed to evaluate the stabilization level of anaerobic digested sludge. The results provided new insights into the evaluation of anaerobic biodegradability of sludge and the stabilization of digestate.

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Appendix A Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.07.021.

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