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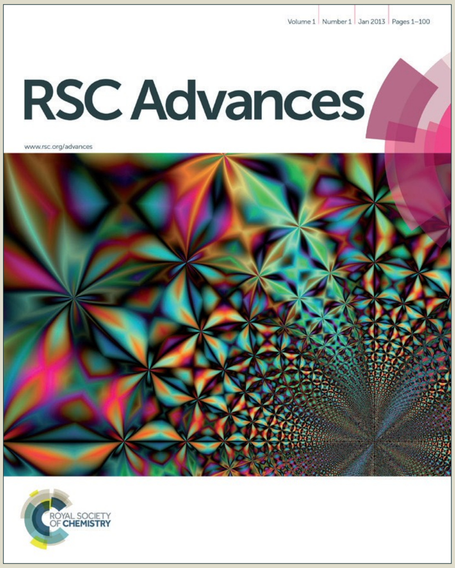

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Comparison of thermophilic microaerobic and alkali pretreatment of sugarcane bagasse for anaerobic digestion

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Abstract

In this study, the effects of thermophilic microaerobic pretreatment (TMP) and alkali pretreatment (AP) on anaerobic digestion (AD) of sugarcane bagasse were investigated. Results showed TMP was efficient at crystallinity disruption and AP was efficient at lignin removal. Maximum methane yield was obtained when the oxygen loads during TMP was10 ml/g $VS_{substrate}$ (TMP2), which was 15.7% and 29.3% higher than those of AP and sample without pretreatment (WP), respectively. Accordingly, the VS removal efficiency of TMP2 was 5.4% and 17.4% higher than those of AP and WP, respectively. In addition, Lag-phase time of TMP2 was 1.55 and 3.82 days shorter than those of AP and WP, respectively. Technical digestion time (T90) of AP was 49 days, which was 10 and 7 days less than those of TMP2 and WP, respectively. In addition to AP, TMP is an alternative and efficient pretreatment method in AD of sugarcane bagasse.

Key word: sugarcane bagasse, anaerobic digestion, alkali pretreatment, thermophilic microaerobic pretreatment

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Abbreviations

AD	Anaerobic Digestion
TMP	Thermophilic Microaerobic Pretreatment
AP	Alkali Pretreatment
WP	Sample without Pretreatment
T90	Technical Digestion Time
TS	Total Solid
VS	Volatile Solid

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1. Introduction

Sugarcane bagasse is mainly generated in the sugar and ethanol industry [1]. The unsuited dispose of sugarcane bagasse is not only waste of resource but also leads to environmental problems[2]. Anaerobic digestion (AD) has been widely employed due to increasing attention to renewable energy, climate change and waste management[3, 4], which is an ideal way for comprehensive utilization of sugarcane bagasse. However, sugarcane bagasse is rich in cellulose (25–47%), hemicelluloses (20–35%) and lignin (15–35%) [1, 5] and the cellulose crystalline structure, hemicellulose hydration and polysaccharide–lignin cross-linking via ester and ether linkages makes the shape and structure of this plant stable. Therefore, the AD of sugarcane bagasse is inefficient. The hydrolysis process is conventionally regarded as the rate-limiting step in AD of lignocellulosic substrate such as sugarcane bagasse[6]. Pretreatment is essential to improve the efficiency of anaerobic digestion[7-9]. Thermal, chemical, biological and mechanical processes, as well as their combinations have been studied as possible pretreatment to accelerate substrate hydrolysis[10, 11].

Among all these pretreatment methods, alkali pretreatment has been studied thoroughly and most used. According to Zhu *et al.*[12], a alkali pretreatment step with the NaOH load of 5% (ambient temperature (20 ±0.5 ℃) for 24h), improved the biogas yield of corn stover for 37.0%. You *et al.*[13] reported a 34.59% higher biogas production from corn stover after pretreatment with 6% NaOH at 35 ℃ for 3 h. Though alkali pretreatment has been considered as efficient pretreatment method for lignocellulosic substrates, there are still some shortages in alkali pretreatment, the

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chemicals required might lead to increasing cost and environmental problems. In addition, the sodium introduced during alkali pretreatment could be an inhibiting factor of anaerobic digestion[14] and a problem for utilization of fermentation residue as fertilizer. These lead to the requirement for an eco-friendly and economically feasible pretreatment of lignocellulosic substrates for anaerobic digestion.

Recent studies have demonstrated that hydrolysis also can be enhanced by introducing limited amounts of oxygen (or air) directly into the anaerobic digester or during a pretreatment step [15]. According to Mshandete *et al.*[16], Nine hours microaerobic pretreatment of sisal pulp prior to anaerobic digestion demonstrated a 26% higher methane yield compared to the sisal pulp without pretreatment. when treating the compound of brown water and food waste, Lim and Wang [17] reported 10-21% higher methane yield with an oxygen load of 37.5 ml O_2 $L^{-1}{}_R$ d^{-1}. According to Fu *et al.* [18], a thermophilic microaerobic pretreatment process at the oxygen loads of 5 ml/g $VS_{substrate}$ improved the methane yield of corn straw for16.24%.

Alkali pretreatment is a traditional pretreatment method for lignocellulosic substrates. However, thermophilic microaerobic pretreatment is a completely new pretreatment. No studies have been carried out to investigate the effect of thermophilic microaerobic pretreatment on the anaerobic digestion of sugarcane bagasse. In this study, the effects of thermophilic microaerobic and alkali pretreatment on the AD of sugarcane bagasse were investigated. In addition, the structure change during pretreatment and the fermentative characteristics (*e.g.* methane yield, T90, lag-phase time and VS removal efficiency *etc*) of alkali and thermophilic

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microaerobic pretreated sugarcane bagasse were compared.

2 Materials and methods

2.1 Substrate and inoculum

Inoculum used in this study was anaerobic sludge, which was obtained from a local wastewater treatment plant (Tuandao Water Treatment Plant, Qingdao, Shandong province, China), and stored in a 4 ℃ refrigerator until further use. The total solid (TS) and volatile solid (VS) of inoculum are 4.67% and 70.60% (based on TS), respectively. Substrate used in this study was sugarcane bagasse, which was collected from a sugar factory in Hainan province of China. The TS and VS of substrate are 29.67% and 96.24% (based on TS), respectively.

2.2 Microaerobic pretreatment of sugarcane bagasse

Thermophilic microaerobic pretreatment of sugarcane bagasse was carried out in 300 ml serum bottles with a working volume of 150 ml in duplicates. In this stage, 22g sugarcane bagasse and 20ml inoculum were mixed in bottles, and then deionized water was added to reach a total volume of 150 ml. Each bottle was flushed with N_2 for 5 min to replace the air, and then the bottles were closed with rubber stoppers. 31.4, 62.8, and 125.6 ml of oxygen at atmospheric pressure was injected to each group with a syringe to reach the oxygen loads of 5, 10, 20 ml/g $VS_{substrate}$ (marked as TMP1, TMP2, TMP3). The bottles were placed in a shaking water bath at 55 ℃ with 120 rpm. The oxygen levels were measured by a gas chromatograph (SP 6890, Shandong Lunan Inc., China) every 4 hours until the oxygen was consumed completely.

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2.3 Alkali pretreatment of sugarcane bagasse

Alkali pretreatment of sugarcane bagasse was conducted in duplicates at ambient temperature for three days. During the alkali pretreatment, the NaOH dose was 2% of substrate (TS) and the loading rate was 65 g/L (TS of sugarcane bagasse loaded per liter effective volume of digester). The alkali pretreatment condition in this study was used in the sugar factory where we collected the sugarcane bagasse, which was also suggested by Zheng *et al.*[19] to be optimal in treating corn stover.

2.4 Batch anaerobic digestion tests

After thermophilic microaerobic pretreatment, the bottles were added with another 20ml anaerobic sludge and 30ml deionized water. The alkali pretreated sugarcane bagasse was transferred to 300 ml serum bottles, then 40ml anaerobic sludge and 138ml deionized water were added to reach a total volume of 200 ml. 22g untreated sugarcane bagasse, 40ml anaerobic sludge and 138ml deionized water were also mixed in bottles to test the biogas production from untreated sugarcane bagasse (marked as WP). Before anaerobic digestion, all the pH values were adjusted to 7.0 with 2 N NaOH and 2 N HCl, and then flushed with N_2 for 5 min to replace the air, after that, the bottles were closed with rubber stoppers. All the bottles were placed in a shaking water bath at 37 ℃ with 110 rpm.

2.5. Structure analysis of solid fraction of sugarcane bagasse

Sugarcane bagasse samples were collected before and after pretreatment for the structure analysis. The structure analyses were conducted by a spectrum One FTIR system (The Nicolet iN10 IR Microscope) with a universal ATR (Attenuated Total

Reflection) accessory and wide angle X-ray diffraction, which was in accordance with the reported methods [18].

2.6 Analytical methods

The biogas yield was measured by water displacement method. Biogas composition was measured by a gas chromatograph (SP 6890, Shandong Lunan Inc., China), equipped with a Porapak Q stainless steel column (180 cm long, 3 mm outer diameter) and a thermal conductivity detector. The temperatures of the injector, detector and oven were 50, 100 and 100 ℃, respectively. The carrier gas was argon. TS, VS were determined according to standard methods [20].

3. Results and discussion

3.1. The optimized oxygen loads during thermophilic microaerobic pretreatment

When thermophilic microaerobic pretreatment is used as the pretreatment method, the oxygen load during TMP is a crucial parameter[17, 21]. Insufficient oxygen will not be strong enough to support the growth of facultative organisms. However, facultative organisms have higher growth rates and would out-compete strict anaerobes under high oxygen levels due to substrate competition. In addition, excessive oxygen may inhibit the activity of methanogens directly. In this study, the oxygen loads during thermophilic microaerobic pretreatment was investigated at the oxygen loads of 5, 10, 20 ml/g $VS_{substrate}$. The methane yields of thermophilic microaerobic pretreated sugarcane bagasse are shown in Fig.1. Daily methane yields of thermophilic microaerobic pretreated sugarcane bagasse increased sharply at the fifth day of

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anaerobic digestion. The maximum daily methane yields of TMP1, TMP2 and TMP3 were obtained at 9th, 9th and 6th day of AD, respectively. The cumulative methane yields of thermophilic microaerobic pretreated sugarcane bagasse were ranged between 196.5 and 229.6 ml/g $VS_{substrate}$, which were obtained at the oxygen loads of 20 and 10 ml/g $VS_{substrate}$, respectively. The maximum cumulative methane yield was obtained at the oxygen loads of 10 ml/g $VS_{substrate}$, which was 29.28% higher than that of WP. However, when the oxygen loads during TMP was 20 ml/g $VS_{substrate}$, the cumulative methane yields decreased to 196.5 ml/g $VS_{substrate}$. This result was quite accordance with what reported by Mshandete *et al.*[16] and Botheju *et al.*[22]. Proper oxygen loads (or the time exposed to oxygen) during microaerobic pretreatment is crucial: microaerobic pretreatment would be beneficial for biogas production in a proper condition, however, would be harmful in an improper condition.

3.2 Comparisons of structural changes of sugarcane bagasse after thermophilic microaerobic and alkali pretreatment

The ultimate purpose of pretreatment is to improve the methane yield or to accelerate the anaerobic digestion process. On this basis, TMP2 was selected to make a comparison with AP and WP.

3.2.1 *FT-IR* analysis of pretreated and untreated sugarcane bagasse

The result of *ATR FT-IR* spectroscopy was shown in Fig. 2. The peak near 3348 cm^{-1} and 2900 cm^{-1} represented wagging vibration in C-H and the O-H stretching of the hydrogen bonds of cellulose [23, 24]. The absorption intensities of this two absorption peaks was in the following order WP＞AP ＞TMP, which means the cellulose of sugarcane bagasse was partly disrupted during pretreatment. Moreover, thermophilic microaerobic pretreatment was more efficient at removal of cellulose. The band at 1595 cm^{-1} is attributed to aromatic ring stretching, which is associated with lignin removal. After alkali pretreatment the intensity of this peak was almost halved, which was quite accordant with what reported by Sambusiti *et al.*[25], alkali pretreatment is effective in altering the structure of lignin. However, thermophilic microaerobic pretreatment almost had no effect on this peak. The band at 1245 cm^{-1} is attributed to C-O adsorption and has been proposed to be associated with the acetyl group in hemicelluloses. The intensity of this absorption peak of TMP decreased slightly. Relatively, the intensity of this absorption peak of AP dropped significantly, which means more hemicelluloses was disrupted during alkali pretreatment. The intensity of the 900 cm^{-1} is very sensitive to the amount of crystalline versus amorphous structure of cellulose [26]. The intensity of this band was in the following order AP ＞WP ＞ TMP, which means the crystalline structure after TMP was partly disrupted.

3.2.2 XRD analysis

The crystallinity of substrate is broadly accepted to be a negative factor for the enzymatic hydrolysis of cellulose [27]. The XRD analysis results were shown in Fig.3 and Table.1. The crystallinity of sugarcane bagasse after TMP was decreased, which was quite accorded with what reported by Fu *et al.*[18]. TMP was efficient in crystallinity disruption. However, the crystallinity increased after AP, the results of XRD analysis were quite accorded with the *FT-IR* analysis results. Increase of crystallinity index after alkali pretreatment was also reported by Kumar *et al.*[24] and

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Yao et al. [28] . The greater hydrolysis of amorphous areas than crystalline areas, the removal of amorphous materials, such as lignin and acetyl groups might be the reason for the increase of crystallinity after NaOH treatment [28].

3.3 Comparisons of fermentative characteristics between thermophilic microaerobic and alkali pretreated sugarcane bagasse

3.3.1 Methane yields of thermophilic microaerobic and alkali pretreated sugarcane bagasse during anaerobic digestion

The methane-producing of sugarcane bagasse with thermophilic microaerobic and alkali pretreatment were shown in Fig.4. The maximum daily methane yield was obtained from TMP, which was 112.5% higher than that of untreated sample. The methane-producing peak of WP was 4 days later compared with those of TMP and AP, which means the methane-producing was accelerated after pretreatment. The maximum cumulative methane yield was obtained from the thermophilic microaerobic pretreated sugarcane bagasse and followed by the alkali pretreated sugarcane bagasse, which were 29.3% and 11.8% higher than that of untreated sample, respectively. As for the parameter of total methane yield, TMP was more efficient than AP. The total cumulative methane yield of TMP2 was 15.7% higher than that of AP. However, daily methane yield during the late stage of AD was tiny and it is not practical and economically feasible if the fermentation lasts too long. Therefore, the methane yield within the initial 40 days was also analyzed. The cumulative methane yields of AP, TMP2 and WP during the initial 40 days of AD were 165.1, 159 and 129.6 ml/g $VS_{substrate}$. The cumulative methane yield of AP during the initial 40 days

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was 3.8% and 27.4% higher than those of TMP2 and WP, respectively, which means the methane-producing rate of AP during the initial 40 days was higher. AP and TMP2 obtained the same cumulative methane yield at the 45th day of AD, after then, the cumulative methane yield of TMP2 exceeded that of AP.

The technical digestion time T90 is defined as the time consumed to achieve 90% of maximum cumulative biogas production[29]. A shorter T90 means the substrate was consumed quickly, therefore, the anaerobic digestion system is more efficient. The T90 of AP, TMP2 and WP were 49, 59 and 56 days, respectively. The T90 of AP was 10 and 7 days less than those of TMP2 and WP, respectively. As for T90, AP was more efficient than TMP, which biogas-producing from alkali pretreated sugarcane bagasse was quicker.

3.3.2 The *modified Gompertz equation* analysis

The *modified Gompertz equation* was usually employed to model the methane-producing process[30-32], which was written as following:

$$P(\mathrm{t}) = P\exp\left[-\exp\left(\frac{R\mathrm{m} * \mathrm{e}}{P}(\lambda - t) + 1\right)\right]$$

Where P (t) is the cumulative total methane yield (ml/g $\mathrm{VS}_{\mathrm{substrate}}$), P is the total methane production potential (ml/g $\mathrm{VS}_{\mathrm{substrate}}$), Rm is the maximum methane-producing rate (ml/d/g $\mathrm{VS}_{\mathrm{substrate}}$), λ is the lag-phase time (d) and t is the elapsed time (d).

The parameters of *modified Gompertz equation* fitting experimental data were shown in table 2. The determination coefficient (R^2) ranged from 0.965 to 0.990, which indicated that methane-producing could well be explained by the *modified Gompertz* *equation*. *P* of alkali pretreated and thermophilic microaerobic pretreated sugarcane bagasse was obviously higher than that of untreated sample, which was quite coincident with the experimental result. The lag-phase time (λ) interpreted as the time elapsed until a significant production of methane was found in the batch assays, A higher λ means a slow startup. The lag-phase time was in order of : WP＞AP＞TMP2, which means the sugarcane bagasse after pretreatment has a higher startup. In addition, the lag-phase time of thermophilic microaerobic pretreated sugarcane bagasse was 1.55 and 3.82 days shorter than those of AP and WP, respectively, which means AD of sugarcane bagasse with TMP2 obtained the quickest startup.

3.3.4 VS removal efficiency

During the digestion process, volatile solids (VS) are degraded to a certain extent and converted into biogas and the degree of stabilization is often expressed as the percent reduction in VS [33]. The VS removal efficiencies of WP, AP and TMP were 54.48±0.35%, 60.65±0.91% and 63.93±0.62%, respectively. The maximum VS removal efficiency was obtained in TMP, which was 5.41% and 17.35% higher than those of AP and WP, respectively. The higher VS removal efficiency means more sugarcane bagasse was digested in TMP, which would be better for the reduction of fermentation residue.

4. Conclusions

The effects of AP and TMP on the AD of sugarcane bagasse were investigated and compared in this study. Both AP and TMP are efficient pretreatment methods in AD of sugarcane bagasse. The oxygen load during TMP is crucial, the maximum cumulative methane yield of sugarcane bagasse was obtained at the oxygen load during TMP was

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10 ml/g $VS_{substrate}$. TMP is efficient in crystallinity disruption, lag-phase time, methane production and VS removal. AP was efficient in lignin removal, the technical digestion time and methane-producing rate. Compared with AP, which needs large amount of chemical reagent during pretreatment, TMP is more eco-friendly and economically feasible pretreatment method in AD of sugarcane bagasse.

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Table 1 crystallinity indices of untreated and pretreated sugarcane bagasse

Groups	Crystallinity index	Relative change (%, relative to WP)
WP	23.0	0
AP	30.4	32.2
TMP2	20.0	-13.0

Table 2 Parameters of *modified Gompertz equation* fitting experimental data

Groups	P (ml/g $VS_{substrate}$)	R_m (ml/d/g $VS_{substrate}$)	λ (d)	R^2
AP	188.4 ±1.7	6.7 ±0.3	1.548 ±0.737	0.983
TMP2	233.5 ±6.4	4.3 ±0.5	0	0.965
WP	174.5 ±2.0	4.4 ±0.2	3.819 ±0.966	0.990

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Figure captions

Fig.1 The methane yields of sugarcane bagasse with thermophilic microaerobic pretreatment (TMP) (A: the daily methane yields of sugarcane bagasse; B: the cumulative methane yields of sugarcane bagasse; C: the relationship between cumulative methane yields and oxygen load)

Fig.2 *FTIR-ATR* patterns of untreated and pretreated sugarcane bagasse

Fig.3 XRD patterns of untreated and pretreated sugarcane bagasse

Fig.4 The methane yields of sugarcane bagasse with thermophilic microaerobic and alkali pretreatment (A: the daily methane yields of sugarcane bagasse; B: the cumulative methane yields of sugarcane bagasse)

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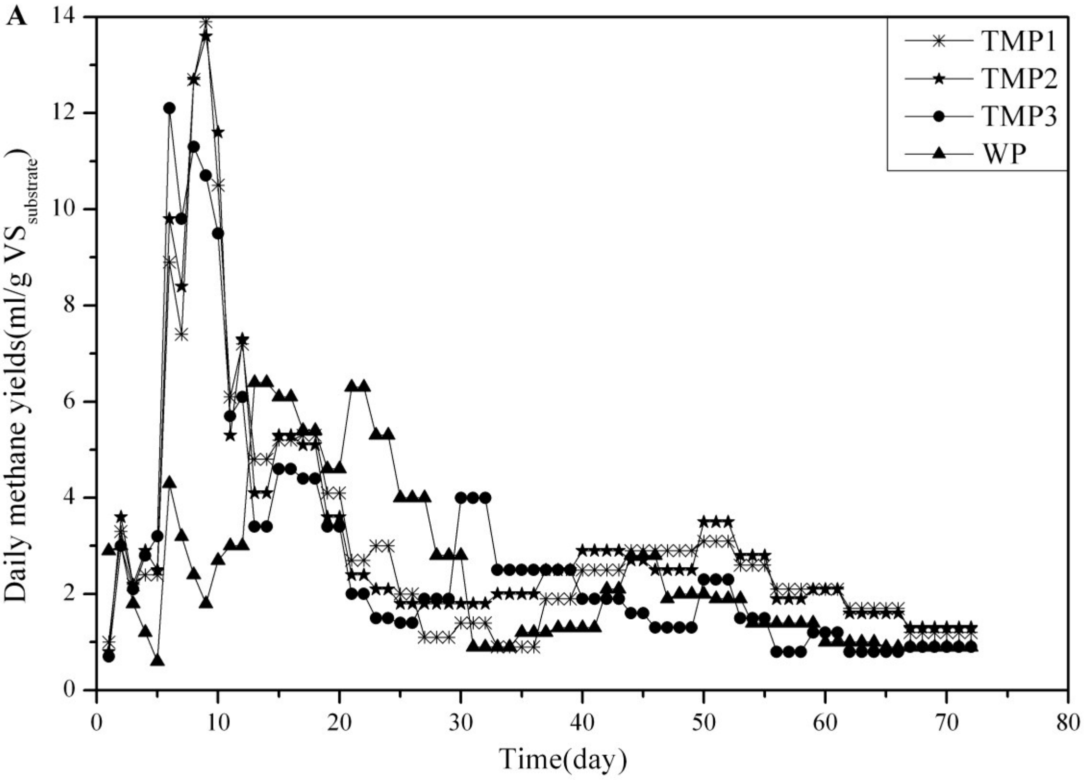

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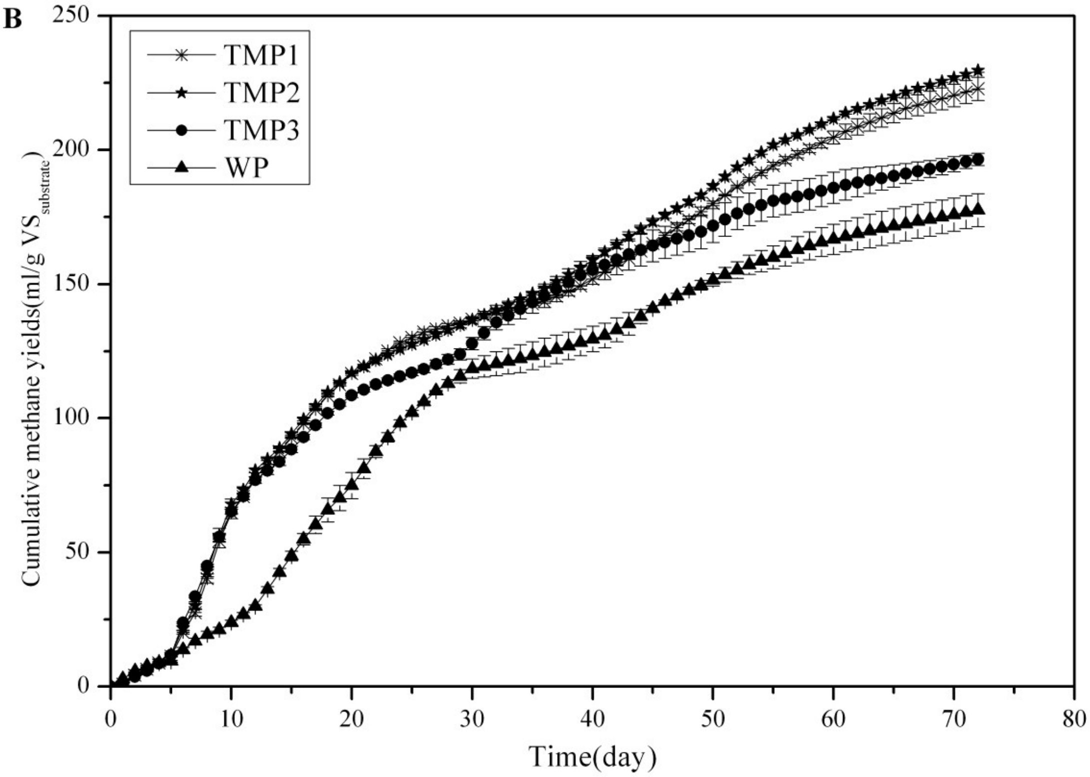

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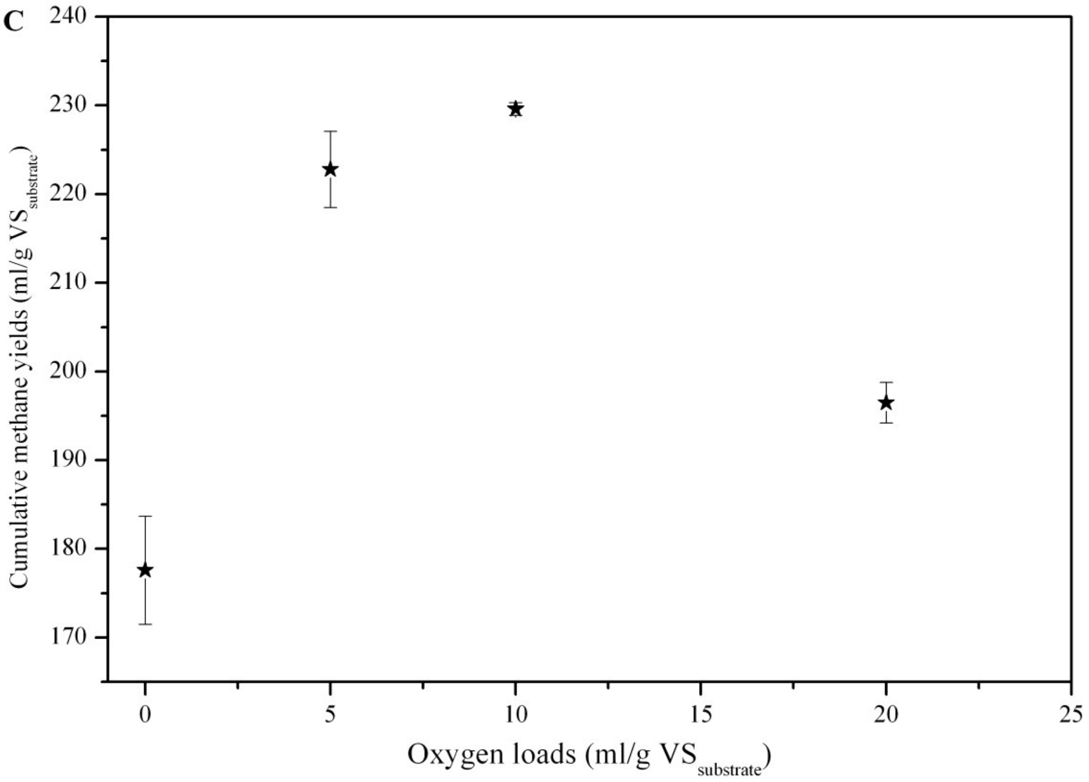

Fig.1

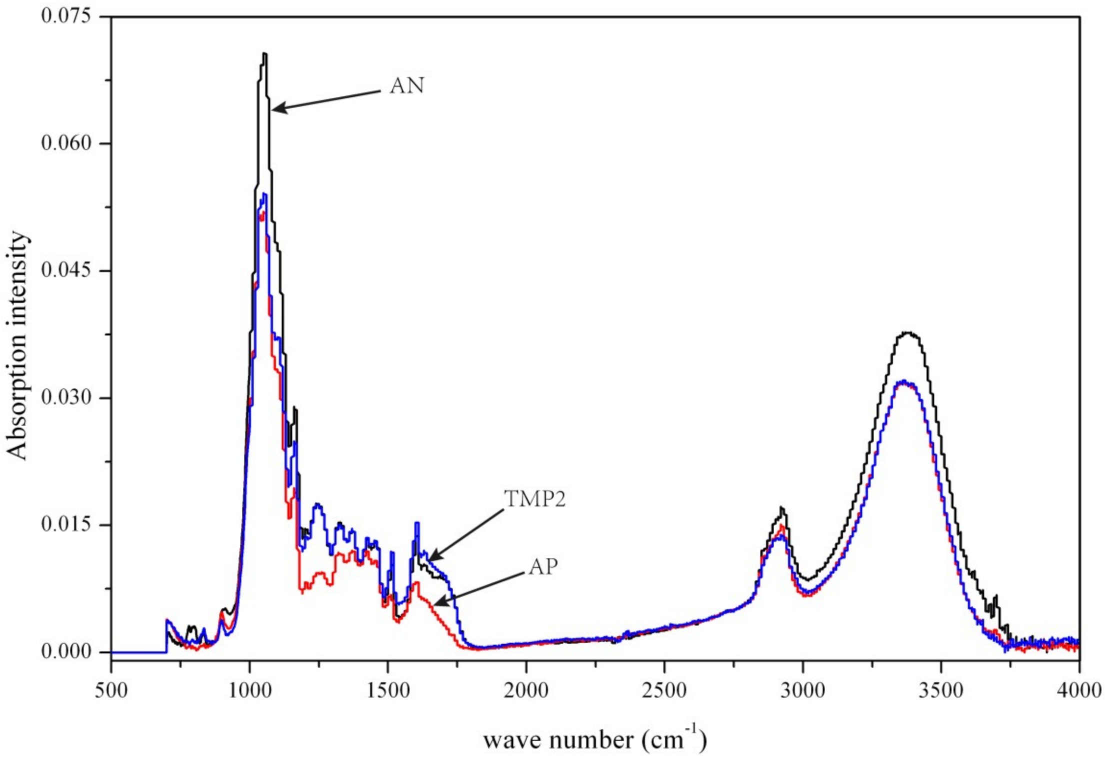

Fig.2

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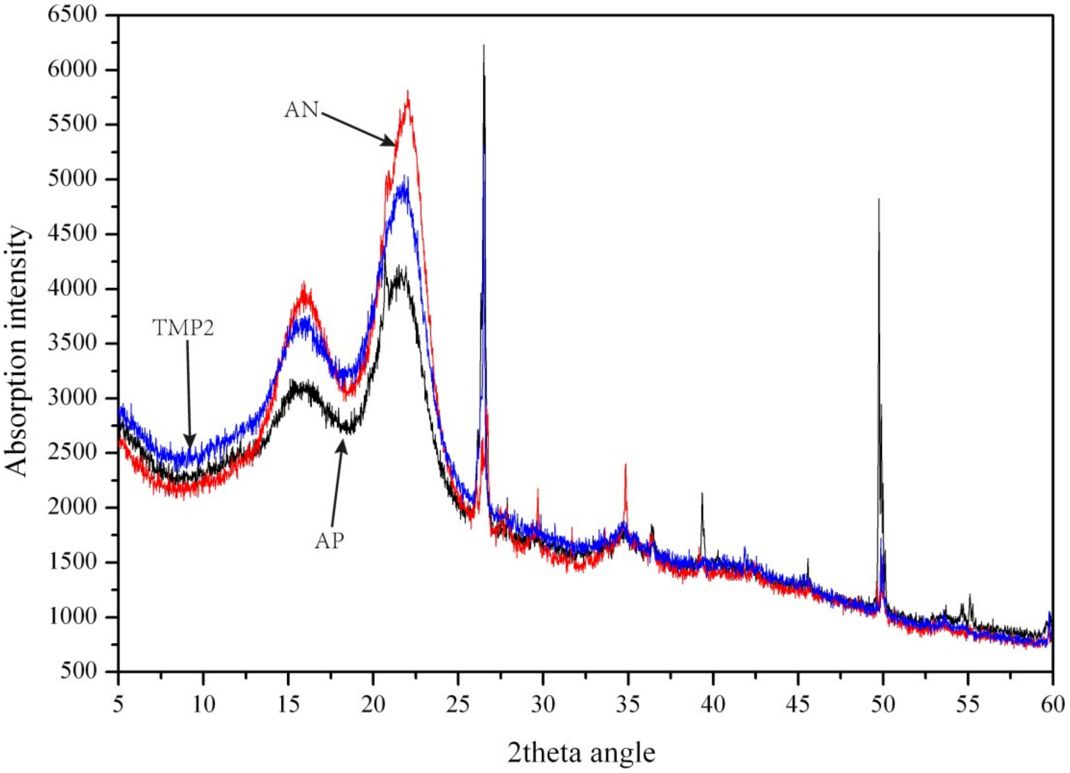

Fig.3

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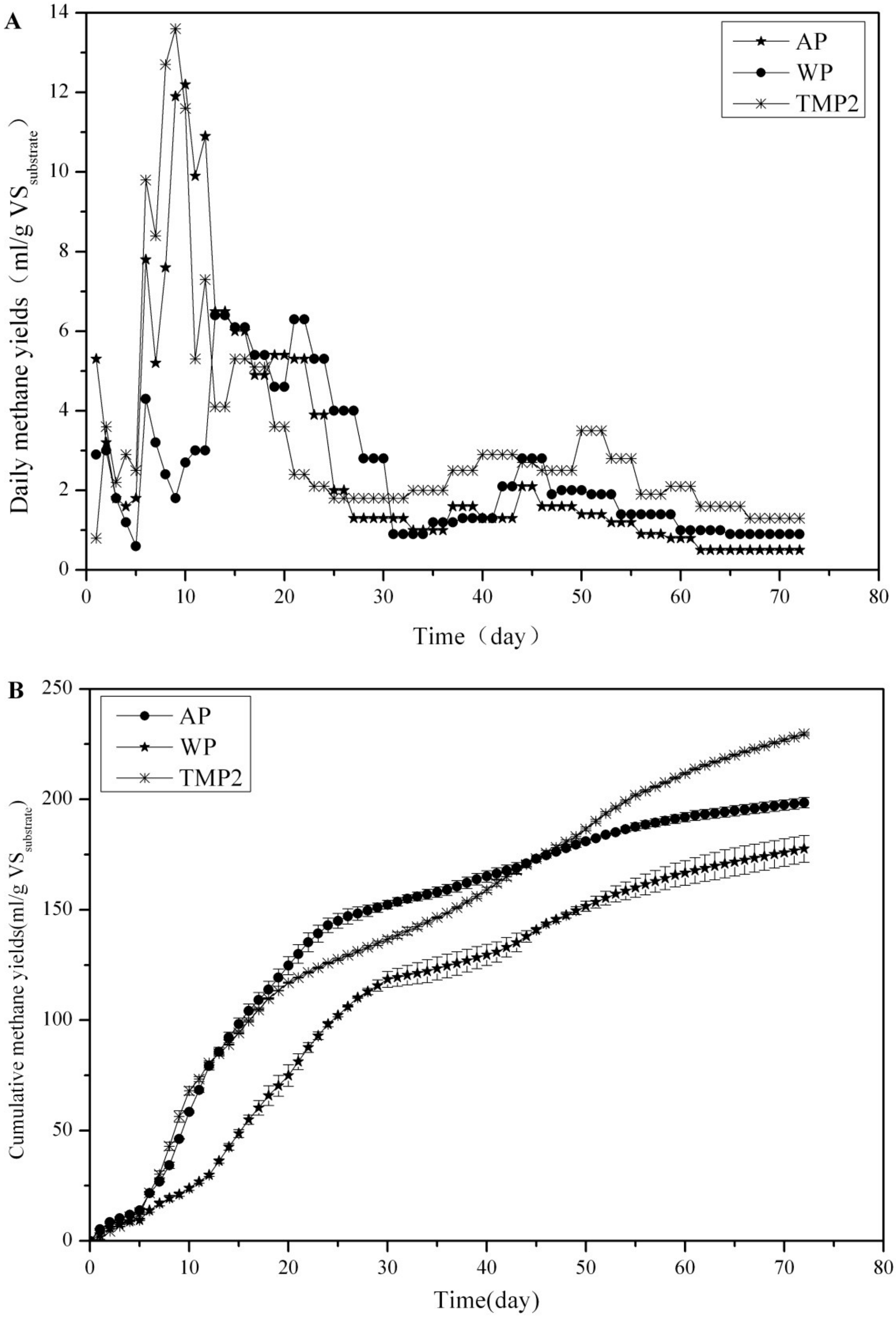

Fig.4

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