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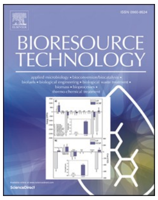

Insight into sludge anaerobic digestion with granular activated carbon addition: Methanogenic acceleration and methane reduction relief

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HIGHLIGHTS

- The methanogenesis of pretreated sludge was accelerated by GAC.
- The toxin disinhibition is the reason for the methanogenic acceleration.
- The methane yield was inhibited by GAC simultaneously.
- The GAC inhibition could be mitigated by pretreatment and elevated temperature.

GRAPHICAL ABSTRACT

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ABSTRACT

In this study, the multiple effects of granular activated carbon (GAC) on sludge anaerobic digestion at ambient (16–24 °C), mesophilic (35 °C) and thermophilic (55 °C) temperature were investigated. After GAC addition, although the methane yields of raw sludge were reduced by 6.5%–36.9%, the lag phases of methanogenesis were shortened by 19.3%–30.6% and the reductions of methane yields were declined to only 5.9%–8.1% simultaneously for pretreated sludge. The inhibitory substances like phenols that generated by thermal pretreatment were reduced after GAC addition, which were demonstrated to be responsible for the methanogenic acceleration. Meanwhile, the methane reduction due to the non-selective adsorption by GAC could be mitigated by pretreatment and elevated temperature. Thus, a strategy coupling thermal pretreatment with detoxification by GAC was proposed to improve the methane production rate and avoid the negative effects during sludge anaerobic digestion with GAC addition.

1. Introduction

Anaerobic digestion has been considered as a mature treatment for the reduction and recycling of waste activated sludge (WAS) (Zhang et al., 2016). However, low conversion rate of organic matters and time-consuming start-up limit the sludge reduction and efficiency of energy recovery (Appels et al., 2011; Zhen et al., 2017). Generally, hydrolysis was considered as the rate-limiting step for sludge anaerobic digestion, and methods including mechanical, chemical, ultrasonic and biological pretreatments were demonstrated to improve the hydrolysis and solubilization of organic components in WAS. Among them, thermal technologies like thermo-chemical and thermal hydrolysis pretreatment have gained significant consideration during sludge anaerobic digestion (Zhen et al., 2017).

Along with the advantages thermal pretreatment brings, however, there is also increasing concern over the inhibitory by-products that generated by pretreatment. For example, thermal pretreatment results in the generation of carbohydrate-derived furanic and phenolic

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compounds (Park et al., 2012; Sambusiti et al., 2013), recalcitrant dissolved nitrogen and phosphorus species (Gu et al., 2018; Zhang et al., 2020). These toxic substances generated by pretreatment may inhibit the subsequent acidogenic and methanogenic processes, which bring new limitations on anaerobic digestion (Khadem et al., 2017; Yang et al., 2016).

Recently, investigators have found that the addition of carbon materials, such as activated carbon, biochar, carbon cloth, graphite and graphene, improved the efficiency of anaerobic digestion (Dang et al., 2017; Lin et al., 2018; Luo et al., 2015; Pan et al., 2020; Zhang et al., 2019; Zhao et al., 2017). The mechanisms like process stability, buffering capacity, microbial immobilization and syntrophic communities were often involved in the carbon-based enhancements (Masebinu et al., 2019; Zhang et al., 2018). However, owning to their intricate physicochemical characteristics, the influences of carbon materials on anaerobic digestion remain controversial and the inhibition effects caused by carbon materials are also noteworthy. For example, Bueno-Lopez et al. (2018) demonstrated that graphene-based adsorption could impede the dissolution and hydrolysis of starch particles, which might further inhibit the substrate metabolism during digestion process. Moreover, the inhibitory effects of GAC and overdosed biochar were also proposed by other investigators (Florentino et al., 2019; Li et al., 2019). Notably, the negative impacts of carbon materials during sludge anaerobic digestion were often overlooked.

After thermal pretreatment, both the solubilization and transformation of organic matters in WAS are enhanced and the chemical composition of soluble organic matters was also changed, which inevitably bring significant influences to the sludge anaerobic digestion (Ma et al., 2018). Due to the carbon-based adsorption, the efficiency of sludge anaerobic digestion would be influenced by the changed concentration of substrates and inhibitory substances for the anaerobic microorganisms. However, the multiple effects and mechanisms induced by the non-selective adsorption with the addition of carbon materials during sludge anaerobic digestion have yet to be fully investigated.

As a model carbon-based functional material, granular activated carbon (GAC) has been added in the anaerobic digestion systems for enhancement of methane production (Pan et al., 2020; Xu et al., 2018). Therefore, using GAC as the representative of the carbon materials, the objectives of this study included (1) to explore the multiple effects of GAC addition in the anaerobic digestion of raw sludge and thermal pretreated sludge, (2) to clarify the mechanisms of GAC adsorption on anaerobic digestion of raw sludge and thermal pretreated sludge, (3) to develop a novel strategy combined the pretreatment and GAC addition to enhance the anaerobic digestion of WAS. The results of this study provided a more comprehensive understanding and novel insight into the role of carbon materials in sludge anaerobic digestion.

2. Materials and methods

2.1. Sludge and inoculum

Waste activated sludge was collected from the sludge thickening department of Xincheng wastewater treatment plant (Wuxi, China) and stored at 4 °C before use. Main characteristics of the WAS were as follows: pH 7.25 ± 0.17, total solids (TS) of 41.35 ± 2.86 g/L and volatile solids/total solids (VS/TS, w/w) of 47.4 ± 1.0%. The WAS was thermo-alkaline pretreated under 105 ± 2 °C for 24 h (Li et al., 2012). The inoculum was collected from a laboratory scale anaerobic digestion reactor with the effective volume of 1.6 L. Feeding with the same sludge from Xincheng, the reactor has been operated semi-continuously at 35 ± 2 °C and the sludge retention time was set to 30 days. After more than 300 days' operation, the daily methane production has been maintained at 140–180 mL/d. Coal-based GAC with size of 0.2–0.5 cm diameter was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), then grounded and passed through a 12-mesh sieve before use.

2.2. Effects of granular activated carbon on anaerobic digestion of raw and pretreated sludge

Batch experiments were conducted in 500 mL serum bottles with the effective volume of 400 mL, and the inoculum and substrate ratio based on volume was set to 1:9. To investigate the impact of GAC on sludge anaerobic digestion at ambient (AAD, 16–24 °C), mesophilic (MAD, 35 ± 1 °C) and thermophilic temperature (TAD, 55 ± 2 °C), GAC was added to each bottle at the dosage of 10 g/L and both raw and pretreated sludge were used as the substrates for anaerobic digestion. Besides, only the inoculum and substrate sludge were added to the control group. Before sealing with rubber plugs, all vials were flushed with high purity nitrogen gas for more than 20 min to maintain anaerobic condition. Then all the serum bottles were shaken at 130 rpm and maintained at the settled temperatures. Biogas samples were collected by gasbags and liquid samples were taken from vials following reaction for analysis. All the batch experiments were performed in duplicated.

2.3. Evaluating the role of granular activated carbon during the batch digestion of pretreated sludge

In order to evaluate the role of GAC-based adsorption during anaerobic digestion of the pretreated sludge, batch digesters M1 to M4 were arranged according to Table 1. In brief, only the inoculum and pretreated sludge were added to the control group M1, the GAC addition group M2 was settled as the digestion of pretreated sludge with GAC addition (10 g/L). For the solid–liquid separation groups M3, sludge hydrolysate (SH) that obtained through centrifugation (8000 rpm, 10 min) of the pretreated sludge was used as the digestion substrate. For the adsorbed groups M4, GAC at the dosage of 10 g/L was added to the pretreated sludge for adsorption treatment (37 °C, 130 rpm, 2 h). Then the adsorbed sludge hydrolysate after centrifugation was used as the substrate for M4. Notably, M3 was set as the other control for the reason that both substrate solid–liquid separation and GAC adsorption were involved in M4. Then results from M1 to M4 were compared to evaluate the role of GAC-mediated adsorption process during sludge anaerobic digestion. All the digestions were performed under 35 ± 1 °C with proper agitation and methane productions were analyzed as mentioned previously. Batch experiments were performed in duplicated.

2.4. Impacts of granular activated carbon on model intermediates and typical substances in pretreated sludge

The GAC impacts on model intermediates for anaerobic digestion were tested by adding known concentrations of glucose (10 g/L), VFAs (5 g/L, acetic acid: propionic acid = 3:2, w/w) and ammonium (1 g/L, calculated by ammonium chloride) with GAC (10 g/L), and then monitoring them periodically in suspension for up to 24 h, the adsorption temperature was settled as 37 °C with agitation speed of 130 rpm. Besides, considering the pH variations in the anaerobic digesters, acidic (pH = 3.5 ± 0.2), neutral (pH = 7.2 ± 0.3) and alkaline (pH = 10.2 ± 0.2) conditions were involved during the adsorption process.

For the GAC impacts on pretreated sludge, WAS was thermally pretreated at two pH conditions (pH = 10 and 12) under 105 ± 2 °C for 24

Table 1
Experimental setting for evaluation the role of GAC adsorption during sludge anaerobic digestion at mesophilic temperature.

No.	Setting for anaerobic digestion
M1	Pretreated sludge + inoculum
M2	Pretreated sludge + inoculum + GAC
M3	Sludge hydrolysate + inoculum
M4	Sludge hydrolysate after GAC adsorption* + inoculum

* Adsorption parameters: GAC (10 g/L), 37 °C, agitation speed 130 rpm for 2 h. GAC was separated by centrifugation (8000 rpm, 10 min) after adsorption

h. Then the adsorption treatment of pretreated sludge was conducted under the same conditions as the model intermediates above, except that the adsorption time and pH were changed to 2 h and 7.0, respectively. Negative control experiments were performed in bottles only with the selected substrates. All the adsorption experiments were carried out in duplicated. Soluble proteins and polysaccharides, soluble chemical oxygen demand (sCOD), ammonium and extracted organic compounds in the pretreated sludge were analyzed before and after GAC addition.

2.5. *Evaluating the role of granular activated carbon during the continuous batch digestion of pretreated sludge*

In order to further evaluate the role of GAC-based disinhibition in methanogenic

acceleration during the pretreated sludge digestion, a reversed validation experiment that based on microbial acclimation was performed in the batch operation continuously. Specifically speaking, during the batch 1 digestion, digesters M1 to M4 were settled according to Table 1. After 35 days' digestion, the acclimated sludge from M1 of the batch 1 digestion was used as seed sludge for all the digesters in batch 2, other parameters remained the same as the batch 1. Likewise, pH variation, methane production and other parameters were measured for the digestion evaluation, and all the digestions were conducted in glass reactors with the effective volume of 1.0 L.

2.6. *Methane production modeling analysis*

The modified Gompertz model was used to simulate the methane production performance in the batch digestion assays (Nopharatana et al., 2007):

$$P_{(t)} = P_m \bullet \exp\left\{ - exp\left[\frac{R_{m\bullet e}}{P_m}(\lambda - t) + 1\right]\right\} \tag{1}$$

where: $P_{(t)}$ is the cumulative methane production at time t, mL $gVS_{substrate}^{-1}$; P_m is the maximum methane production potential, mL $gVS_{substrate}^{-1}$; R_m is the maximum methane production rate, mL $gVS_{substrate}^{-1}$; λ is the lag phase time, d; t is the duration time, d; e is constant (2.71). The methane production curve and the relevant parameters were simulated using Origin Pro 9.0 (Origin Lab Corporation, MA, US).

2.7. *Chemical analysis*

The chemical oxygen demand (COD), TS, VS, the concentrations of ammonium, proteins and polysaccharides were conducted according to the methods described in our previous study (Ma et al., 2016). The volatile fatty acids (VFAs) concentration in the filtrate samples was detected by a gas chromatography (GC-2010, Japan) equipped with a flame ionization detector and a fused-silica capillary column (PEG-20M, China). Gas component was analyzed by a gas chromatography (GC9790II, FuLi, China) with a thermal conductivity detector and a stainless steel packed column (AE.TDX-01, China). The volume of the produced biogas was measured by displacement of saturated aqueous NaCl in a graduated measuring cylinder. The gas volume was calibrated to standard conditions (273 K,1 atm) after measurement.

The organic compounds of raw, pretreated and adsorbed sludge were identified by gas chromatography-mass spectrometer (GC–MS). In brief, sludge and sludge hydrolysate samples were firstly extracted using n-hexane (V_{SH}:$V_{n\text{-}hexane}$ = 2:1) and then analyzed by a GC–MS (Thermo scientific, TRACE1300-ISQ, USA). The GC–MS analysis was conducted under injection temperature 300 °C, interface 250 °C and ion source 250 °C with a 30 m DB-5 MS column. The oven temperature programming was set at 40 °C for 1 min, 180 °C for 2 min, 260 °C for 5 min and 300 °C for 2 min. All the temperatures increased at a rate of 10 °C/min. Helium gas was used as the carrier gas with the flow rate of 1.0 mL/min. Data reports were analyzed and compounds were verified only if the probability was over 60.0%.

2.8. *Statistical analysis*

The one-way analysis of variance was used to test the significance of the results, and $p < 0.05$ was considered as a statistical criterion.

3. Results and discussion

3.1. *Methane yields of sludge anaerobic digestion with granular activated carbon*

The influences of GAC addition on methane production during anaerobic sludge digestion under three temperatures were shown in Fig. 1. pH variations during the digestions of raw and pretreated sludge at all three temperatures were in consistent with the common batch anaerobic digestion process (shown in supplementary material).

For raw sludge digestions (Fig. 1a, b, c), the cumulative methane yields of AAD, MAD and TAD with GAC addition were reduced by 36.9%, 25.3% and 6.5% respectively, comparing to the control group. But for pretreated sludge (Fig. 1d, e, f), the cumulative methane reduction rates were declined to 8.1%, 6.9% and 5.9%, respectively. Although methane yields were reduced by GAC during the digestions of raw sludge at three temperatures, it should be noted that the GAC-based inhibition on methane yields were observed to be diminished by the elevated temperature, as the methane reduction rate of raw sludge TAD was almost 6 times lower than that of AAD. The same trends were also observed during the pretreated sludge digestions.

Moreover, the methanogenic inhibition by GAC were also observed to be mitigated by sludge pretreatment. Specifically, comparing to the raw sludge digestion, methane reduction rates of pretreated sludge digestion were all declined significantly ($p < 0.05$), especially for AAD and MAD, almost 4 times lower than that of raw sludge digestions. Obviously, these results suggested that the methane productions under all three temperatures were inhibited by GAC, but the methanogenic inhibitions by GAC were significantly diminished by the elevated temperature and sludge pretreatment.

It has been widely reported that carbon materials could promote methanogenic process. However, recent literature has emerged that offers contradictory findings about methanogenic inhibitions with the participation of carbon nano-tube (Li et al., 2015), graphene (Bueno-Lopez et al., 2018) and carbon black (Fujinawa et al., 2019). The redox potential interference and cytotoxicity, mostly in the case of nano-sized carbon materials, were involved for the explanation. Besides, overdosed biochar (50 g/L) has been proposed to have an inhibitory effect on methane production as well (Li et al., 2019). While GAC addition with the same dosage (10 g/L) has been proved to be benefit for anaerobic digestion by a series of investigators (Capson-Tojo et al., 2018; Lee et al., 2016; Yan et al., 2017). Yang et al. (2017) even confirmed the improved effects at the GAC dosage of 33 g/L during anaerobic sludge digestion. So neither cytotoxicity nor excessive addition was responsible for the methanogenic reduction in our study.

Otherwise, the significant decline in the methane reduction rates were obtained due to the elevated temperature as well as the sludge pretreatment (Fig. 1), which reminds us that in addition to the adding dosage of GAC, parameters like process kinetics and substrate states that related to digestion operations might affect the GAC-based inhibition on the methanogenic process during anaerobic sludge digestion, which has been seldom mentioned before.

3.2. *Methane production accelerations by granular activated carbon*

It was noteworthy that methane production accelerations in terms of lag phase reduction were observed simultaneously during the digestion of pretreated sludge. The kinetic parameters were simulated by the modified Gompertz model and showed in Table 2. Comparing to the

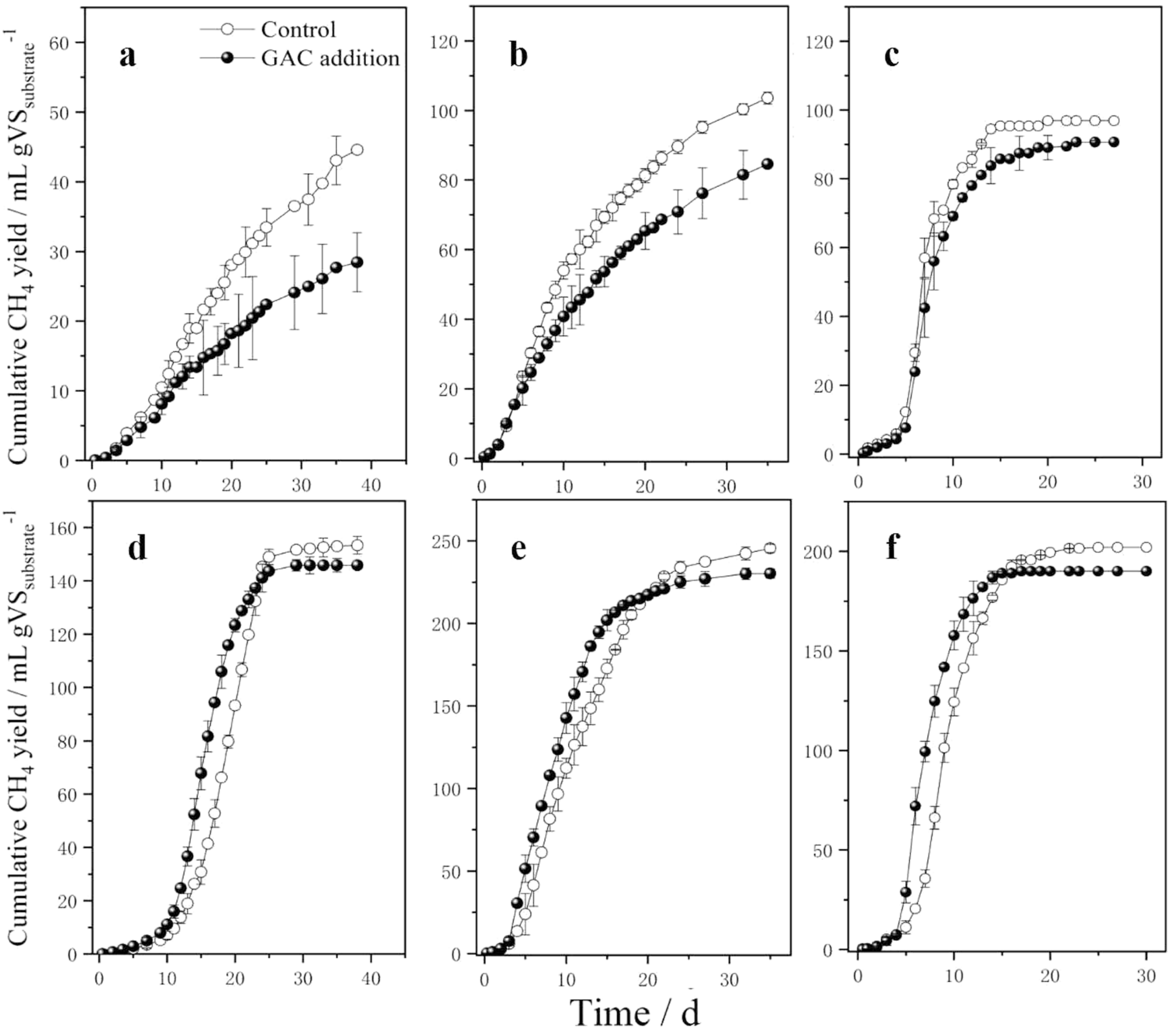


Fig. 1. Influences of GAC addition on anaerobic digestion of raw sludge (a, b, c) and pretreated sludge (d, e, f) at ambient (a, d), mesophilic (b, d) and thermophilic temperature (c, f).

Table 2
The constants obtained from mathematical model analysis.

Sample		Modified Gompertz model constants				Experimental yield (mL CH_4/g VS)
		P_m (mL CH_4/ gVS)	R_m (mL CH_4/ gVSd)	λ (d)	R^2	
AAD	Control	159.69 ± 3.24	14.21 ± 0.81	12.88 ± 0.31	0.9913	152.98
	GAC	141.94 ± 0.89	14.90 ± 0.31	10.39 ± 0.11	0.9989	140.54
MAD	Control	245.37 ± 2.23	19.05 ± 0.47	4.05 ± 0.25	0.9977	245.43
	GAC	229.86 ± 1.49	20.76 ± 0.51	2.81 ± 0.56	0.9982	228.56
TAD	Control	195.25 ± 4.34	29.76 ± 1.79	5.48 ± 0.19	0.9948	202.01
	GAC	194.03 ± 1.11	31.10 ± 1.03	3.91 ± 0.11	0.9978	190.11

control, the lag phase time of GAC-amended groups in AAD, MAD and TAD were reduced by 19.3%, 30.6% and 28.7%, respectively. Moreover, results in Fig. 2 showed that methane peak times of the GAC-amended groups in AAD, MAD and TAD were 14 d, 4 d and 6 d, while that of control groups were 19 d, 8 d and 9 d, respectively. It was apparent that GAC addition accelerated methane production by shortening the lag phases and methane peak times at all three temperatures. Furthermore, the total VFAs concentrations of AAD, MAD and TAD with GAC addition were all found to decline faster than the control groups, suggesting that the GAC addition have promoted the VFAs consumption and might help to accelerate the methane production during the digestion process (shown in supplementary material).

As the major improvements of methanogenic acceleration, the shortened lag phase is of great importance for practical applications of anaerobic digestion treatment. Methanogenic accelerations have been confirmed during the anaerobic digestion processes with kinds of carbon materials, like GAC (Zhang et al., 2017), biochar (Wang et al., 2018b), carbon nano-tube (Yan et al., 2017) and graphene (Lin et al., 2018). It is believed that higher electron transfer efficiency could be achieved through direct interspecies electron transfer (DIET) relationships between bacteria and archaea (Zhao et al., 2020). As a matter of experience, methane production acceleration was often attributed to the DIET-based syntrophic enhancements that induced by carbon materials (Lee et al., 2016; Wang et al., 2018a; Wu et al., 2019).

However, DIET alone does not explain the anaerobic digestion performance with carbon materials addition in many cases (Martins et al., 2018; Salvador et al., 2017), question remains since it is challenging to confirm the occurrence of DIET in anaerobic digestion systems (Van

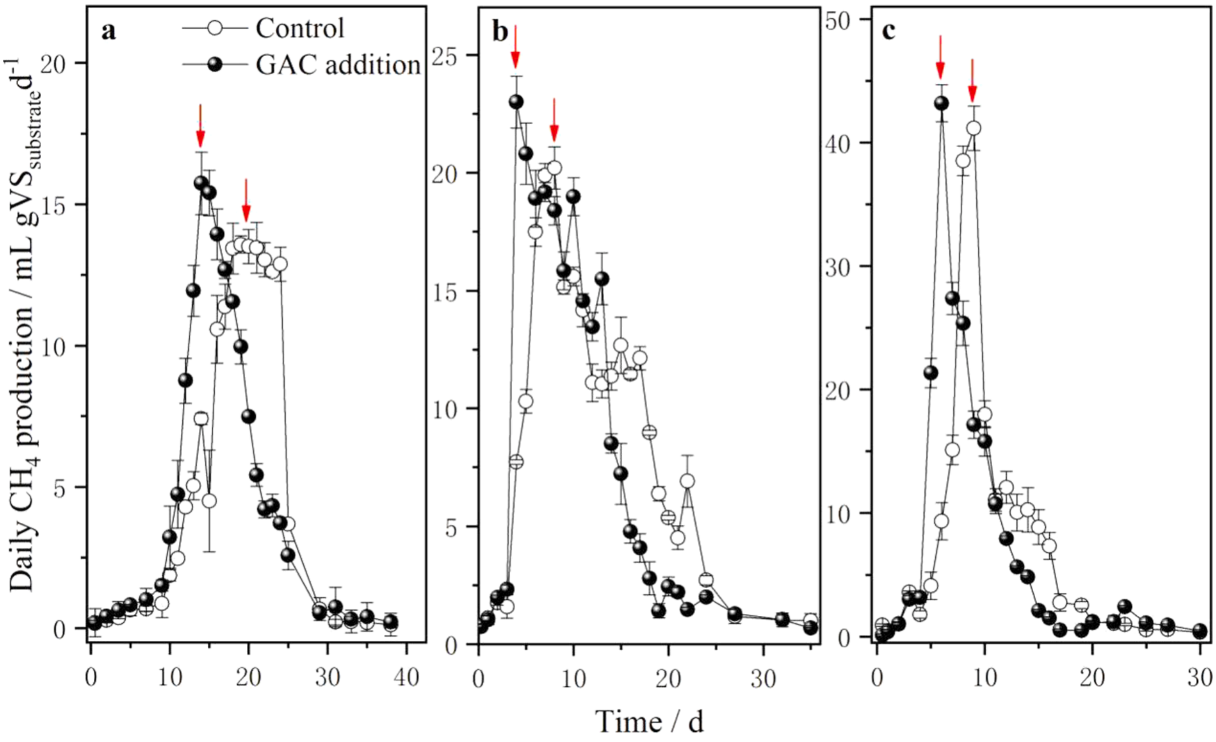
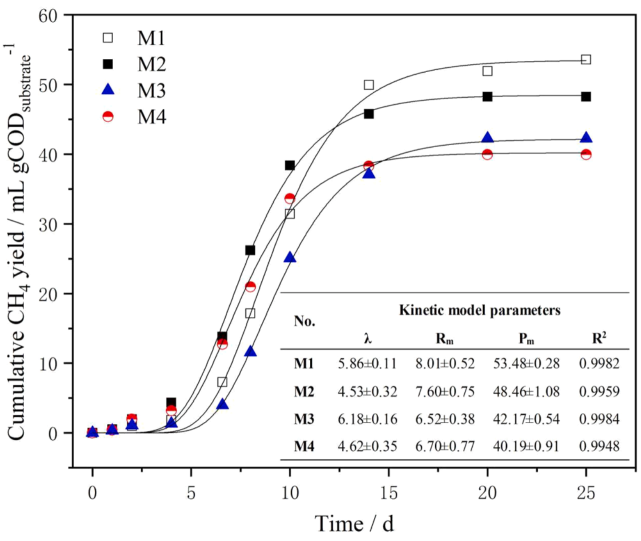


Fig. 2. Influences of GAC addition on daily methane production during anaerobic digestion of pretreated sludge at ambient (a), mesophilic (b) and thermophilic temperature (c). The methane peak time for each digestion was marked with the red arrow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Steendam et al., 2019). According to our results, both the reduction of methane yields and the acceleration of methanogenic process were induced by GAC simultaneously during sludge anaerobic digestion, which were failed to be explained by the emerging DIET mechanism. And this combined phenomenon warrants further investigation.

3.3. The role of granular activated carbon during sludge anaerobic digestion

Batch experiments were arranged according to Table 1, and methanogenic results of the GAC addition and adsorption groups were compared for evaluating the role of GAC during sludge anaerobic digestion. As shown in Fig. 3, comparing to the control M1, the simultaneous reduction and acceleration of methane production were observed in the GAC addition groups M2. Notably, the lag phase of the GAC adsorption groups M4 was reduced by 25.2% (compared to M3), which was close to the lag phase reduction (22.7%) in the GAC addition group M2. While a prolonged lag phase (6.18 d) was observed in the solid–liquid separation group M3, which indicated that methane production could not be accelerated by the substrate solid–liquid separation. Obviously, these results confirmed the simultaneous reduction and acceleration of methane production were triggered during the digestion of pretreated sludge, and the main reason could be attributed to the adsorption effects that mediated by GAC.



No.	Kinetic model parameters			
	λ	R$_m$	P$_m$	R^2
M1	5.86±0.11	8.01±0.52	53.48±0.28	0.9982
M2	4.53±0.32	7.60±0.75	48.46±1.08	0.9959
M3	6.18±0.16	6.52±0.38	42.17±0.54	0.9984
M4	4.62±0.35	6.70±0.77	40.19±0.91	0.9948

Fig. 3. Cumulative methane yield and kinetic analysis of batch digestions at mesophilic temperature.

3.3.1. The relief of non-selective adsorption effects from granular activated carbon

In order to elucidate methane reduction during the sludge anaerobic digestion with GAC addition, the main components of pretreated sludge before and after GAC adsorption were analyzed. As shown in Table 3, after GAC adsorption, sCOD concentration of the pretreated sludge at two pH conditions were reduced by 15.5% and 15.1%, respectively. Correspondingly, the contents of soluble polysaccharides and proteins were all declined significantly ($p < 0.05$), which might be the reason for sCOD decrements. As to VFAs and ammonium, two kinds of common intermediates in anaerobic digestion process, their concentrations were found to remain stable after adsorption. This result was confirmed by the model-based adsorption assays (shown in supplementary material). Model intermediate substrates like glucose, acetic acid/propionic acid and ammonium were applied for the GAC adsorptions under acidic, neutral and alkaline pH conditions. Likewise, there were no significant decline in the concentration of three small molecular substances after adsorption processes at all three pH conditions. Results indicating that large molecular weight substances (e.g. proteins, polysaccharides) rather than small molecules (e.g. glucose, VFAs) were preferably adsorbed by GAC, which was responsible for the sCOD decrements after adsorption.

Table 3
Main characteristics of pretreated sludge before and after GAC adsorption.

	TA1 (pH = 10)	TA1 + GAC (pH = 10)	TA2 (pH = 12)	TA2 + GAC (pH = 12)
sCOD (mg/L)	23450.78 ± 236	19823.29 ± 358	22316.38 ± 352	18950.29 ± 684
Soluble polysaccharide (mg/L)	1430.08 ± 78	1298.59 ± 145	1219.28 ± 99	1092.18 ± 39
Soluble protein (mg/L)	4493.38 ± 560	3556.08 ± 284	5127.49 ± 496	3949.29 ± 368
VFAs (mg/L)	668.67 ± 25	572.29 ± 16	781.59 ± 154	740.53 ± 97
NH_4^+-N (mg/L)	82.09 ± 11	77.85 ± 19	79.08 ± 26	84.23 ± 7

The substrate adsorption assays confirmed that sCOD, soluble polysaccharides and proteins were reduced after GAC adsorption, which were highly related to the methane reduction during anaerobic sludge digestion. In addition to its high specific surface area, surface functional groups were proposed to be responsible for this substrate adsorption (Bueno-Lopez et al., 2018). Moreover, mass transfer efficiencies of small molecular weight substances (e.g. glucose, VFAs) were unaffected, indicating that the GAC-based inhibition on anaerobic sludge digestion mainly attributed to the adsorption of large molecular weight substrates (e.g. soluble polysaccharides and proteins) during the sludge disintegration and hydrolysis process. As a result, GAC adsorption further aggravated the limiting effects of rate-limiting step (e.g. disintegration and hydrolysis) during the anaerobic digestion of raw sludge at ambient temperature. This also explained the strongest inhibition by GAC during the raw sludge digestions at room temperature (Fig. 1a). And it reminds us that the enhanced process kinetics at the elevated temperature, as well as after the pretreatment, would mitigate the GAC-based inhibition on methanogenic process by the rate-limiting step elimination during sludge anaerobic digestion.

3.3.2. Toxin disinhibition by granular activated carbon

Furthermore, the organic compounds distribution in raw and pretreated sludge, as well as the GAC adsorbed sludge hydrolysate, were analyzed by GC–MS assay. Both the visual photos and GC–MS chromatograms could be found in Fig. 4. From this data, we can see that the composition of sludge hydrolysate from pretreatment was complicated, phenols and heterocyclic substances might be the major inhibitory substances in the hexane extracted sludge hydrolysates. Especially for phenols, which accounting for nearly 36.0% with respect to the relative peak area (Table 4).

It is believed that pretreatment process is essential for enhancing the anaerobic digestion of refractory substrates (e.g. WAS, lignin waste), and thermal technologies like thermo-alkaline and thermal hydrolysis

Table 4
Organic compounds in pretreated sludge that identified by GC/MS.

No.	Retention time	Formula	Compound	Relative area [a] (%)	Removal efficiency [b] (%)
1	10.98	$C_{14}H_{22}O$	2,4-Di-*tert*-butyl-phenol	3.42	76.66
2	23.02	$C_{23}H_{32}O_2$	2,2′-methylenebis [6-(1,1-dimethylethyl)-4-methyl-phenol	32.56	66.90
3	25.05	$C_{24}H_{38}O_4$	Diisooctyl phthalate	7.86	100.00

[a] The relative area of organic compounds in sludge hydrolysate.
[b] With respect to peak area.

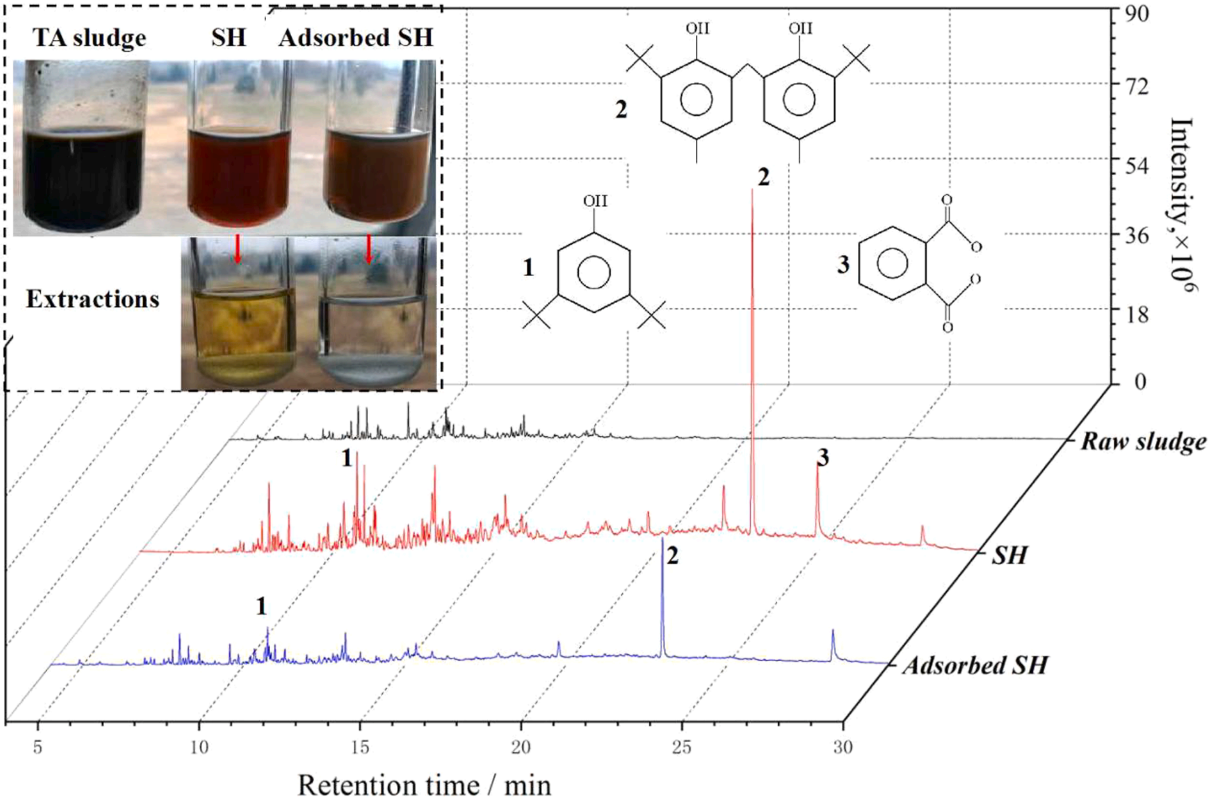


Fig. 4. Gas chromatography-mass spectrometer chromatograms for the extractions of raw sludge, sludge hydrolysate (SH) and adsorbed sludge hydrolysate.

pretreatment have been widely applied (Zhen et al., 2017). However, several drawbacks were also identified with the formation of by-products like furanic, phenolic compounds (Monlau et al., 2014), brown and ultraviolet-quenching compounds (Zhang et al., 2020), and this was also confirmed in the sludge pretreatment by our study. As shown in the GC–MS assays (Fig. 4), toxic inhibitors like phenols were generated during the TA pretreatment process, and the inhibitory effects of phenolic compounds have been widely described in microbial fermentation processes (Monlau et al., 2014). Moreover, according to the removal efficiency (Table 4) with respect to the peak area in the GC–MS reports, the concentrations of phenols were reduced by 66.9% to 76.7% after adsorption, and this adsorption effects on sludge hydrolysate also could be confirmed visually by the photos in Fig. 4. So it was possible that the toxin inhibitions of sludge hydrolysate were alleviated after GAC adsorption, and the methanogenic acceleration in the batch digestions could be explained by the substrate disinhibition by GAC. That is the GAC-based adsorption of toxic organic compounds reduced their bio-availability for inhibiting anaerobic digestions (Wang & Han, 2012), thereby shortening the adaptation time of microorganisms during the digestions of pretreated sludge. Moreover, the influence of other substrate inhibitor, like heavy metals, has been reported to be mitigated by carbon materials as well (Komnitsas et al., 2015), which is also benefit for the enhancement of anaerobic digestion.

3.4. Verification of the toxin disinhibition effect on methanogenic acceleration

It was reported that microbial acclimation to toxic organic compounds (e.g. phenols), as well as the removal efficiency, could be enhanced through the continuous operation during anaerobic digestion (Wang et al., 2011). Hence, in order to confirm the GAC-based disinhibition was the main reason for methanogenic acceleration during the digestion of pretreated sludge, the reversed validation experiments that based on continuous operation were performed and the simulation results were shown in Fig. 5 and Table 5.

As described, methanogenic accelerations that in terms of lag phase reductions were observed in the GAC addition and the GAC adsorption groups during the batch 1 operation (Fig. 5a). When it came to the batch 2 operation, the lag phases of methanogenic processes were all declined to nearly 2 days, which suggested that the anaerobic microbial adaptability to pretreated sludge was improved through the continuous operation. Meanwhile, it was notable that there were no significant differences between the GAC amended groups (M2 and M4) and the control groups (M1 and M3), suggesting that the methane production processes were not accelerated with or without GAC addition during the batch 2 digestions (Fig. 5b, Table 5).

Table 5
Mathematical model analysis of continuous batch operations at mesophilic temperature.

Sample		Modified Gompertz model constants			
		P_m (mL CH_4/gVS)	R_m (mL CH_4/gVSd)	λ (d)	R^2
M1	Batch 1	110.4 ± 2.25	16.64 ± 1.81	6.63 ± 0.28	0.9938
	Batch 2	110.1 ± 2.57	12.57 ± 1.01	2.13 ± 0.31	0.9912
M2	Batch 1	99.52 ± 1.25	14.40 ± 1.29	5.29 ± 0.11	0.9955
	Batch 2	84.96 ± 1.76	12.88 ± 1.16	1.96 ± 0.27	0.9911
M3	Batch 1	83.17 ± 1.89	10.56 ± 0.98	7.26 ± 0.57	0.9966
	Batch 2	70.84 ± 1.55	10.09 ± 0.92	1.90 ± 0.28	0.9904
M4	Batch 1	76.79 ± 1.52	12.31 ± 1.58	5.10 ± 0.59	0.9943
	Batch 2	61.51 ± 1.08	9.86 ± 0.77	1.84 ± 0.22	0.9936

During the batch 1 digestion, inhibitory phenolic compounds in the pretreated sludge were adsorbed by GAC and thus providing a more favorable environment for the anaerobes. So it was possible that methanogenic processes could be accelerated through this toxin disinhibition. While in batch 2 operation, the inhibitions that caused by phenolic compounds was weakened by the microbial acclimation after 35 days' operation in batch 1, as a result, the methanogenic accelerations that related to GAC-based disinhibition were vanished compared to the control. Results from this reversing verification methods further validated that the toxin disinhibitions by GAC adsorption were responsible for the methanogenic acceleration during the first batch digestion of pretreated sludge.

3.5. Implications

Although methane production was found to be inhibited by GAC during the anaerobic digestion of raw sludge, the reductions of methane yields could be alleviated by the sludge pretreatment and elevated temperature for pretreated sludge. Moreover, methanogenesis was

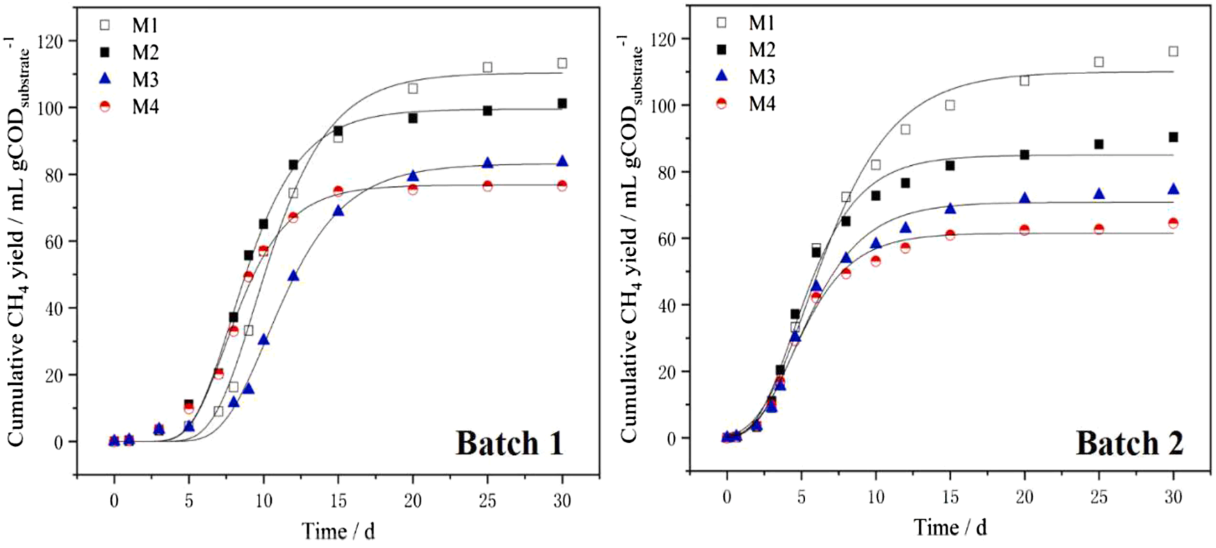


Fig. 5. Methanogenic analysis of continuous batch operations at mesophilic temperature.

accelerated by GAC for the pretreated sludge digestions. From the point of multiple effects, the GAC-based adsorption of inhibitory substances (e.g. phenols) and typical substrates (e.g. polysaccharides, proteins) were proposed to be responsible for this combined phenomenon. In light of these discoveries, attentions should be paid to the long-held view that the methane production improvement via GAC addition during sludge anaerobic digestion.

On one hand, although methane reduction that caused by the GAC adsorption of soluble substrates is undesirable, it should be noted that the GAC inhibition could be mitigated by the enhanced process kinetics, which reminds us that the inhibitory effects by GAC could be weakened and even eliminated by pretreatment during sludge anaerobic digestion. Moreover, it is worth expecting that the selective adsorption of toxic inhibitors could be achieved through carbon modification methods. And application of GAC to anaerobic digestion, especially for the organic waste that contained inhibitory substances (e.g. limonene, furanic and phenolic compounds), will be further developed.

On the other hand, as the major improvements of methanogenic acceleration, the shortened lag phase is of great importance for achieving energy-efficient recycling of organic wastes by anaerobic digestion treatment. Inhibitory substances like phenolic compounds that generated by thermal pretreatment could be effectively removed by GAC. Therefore, a method coupling thermal pretreatment with carbon-based detoxification was proposed in this study, which was proved to be beneficial to shorten the start-up during sludge anaerobic digestion at all three temperatures. Overall, results in this study could provide a more comprehensive understanding about the effectiveness of GAC in sludge anaerobic digestion, which is crucial for the simultaneous achievement of process efficiency and product quality in anaerobic digestion treatment.

4. Conclusion

Although the methane yields of raw sludge were reduced by GAC, the reductions of methane yields were declined significantly by pretreatment and elevated temperature. Meanwhile, the methanogenesis was accelerated by GAC during the pretreated sludge digestion. The toxins dis-inhibition by GAC were demonstrated to be responsible for the methanogenic acceleration. Moreover, the methane reduction caused by the non-selective adsorption of GAC could be mitigated by pretreatment and elevated temperature. Thus, a strategy coupling thermal pretreatment with carbon-based detoxification by GAC addition was proposed to improve the methane production rate and avoid the negative effects during sludge anaerobic digestion.

CRediT authorship contribution statement

Qian Jiang: Methodology, Data curation, Writing - original draft, Investigation. **He Liu:** Conceptualization, Writing - review & editing. **Yan Zhang:** Supervision, Writing - review & editing. **Min-hua Cui:** Methodology, Writing - review & editing. **Bo Fu:** Supervision, Writing - review & editing. **Hong-bo Liu:** Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2020.124131.

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