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Impact of biochar on anaerobic digestion of piggery wastewater: methane production, performance stability and microbial community structure

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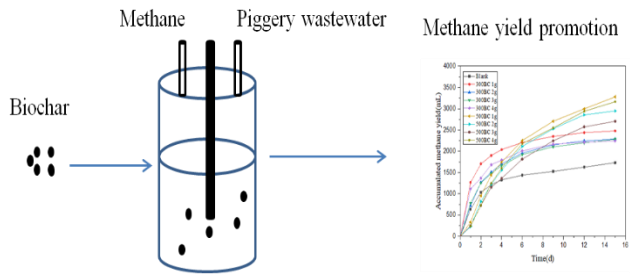
Graphical abstract



Abstract

Anaerobic digestion of piggery wastewater mediated with biochar was evaluated by COD removal rate, methane yield, process stability and microbial community analysis. The results showed that biochar could effectively improve the COD removal rate and methane yield. Compared with blank group, COD removal rate was increased by 2.3%-9.4% for 300BC and by 37.5%-44.0% for 500BC. The maximum methane yield was obtained in 1g 300BC and 500BC, which was increased by 43.20% and 89.52% respectively. Meanwhile, the final H2S concentration was also obviously decreased by BC. Microbial community analysis showed that the addition of BC could increase the abundance of methanogens compared with the blank group. 500BC had a higher positive effect on anaerobic digestion of methanogenic bacteria than 300BC.Meanwhile, the relative abundances of hydrotrophic methanogens in 500BC groups were significantly higher than that of acetoclastic methanogens in 300BC.

Keywords: Anaerobic digestion, biochar, piggery wastewater, methane production

1. Introduction

Climate change is a global problem faced by the world, especially for the greenhouse effect. Piggery wastewater is rich in organic matters, ammonia nitrogen (NH_4^+-N), phosphorus and other contaminants, which will not only cause pollutions to the environment, but also increase the carbon emission if it is not well disposed (Cheng *et al*., 2016; Qunpeng *et al*., 2020).

The usual method for the treatment of piggery wastewater is anaerobic digestion (Sánchez *et al*., 2021; Oladejo *et al*., 2020). Organic matter can be easily degraded accompanied with high value-added products including biogas, biogas slurry and biogas residue during anaerobic digestion. While anaerobic digestion process is a complex biochemical reaction in which a series of reactions occur simultaneously. Meanwhile, due to the complicated composition of piggery wastewater, the treatment efficiency and biogas production are usually relatively low during the anaerobic digestion of piggery wastewater. Reducing the pollution caused by piggery wastewater, increasing the treatment efficiency and cut down the carbon emission during the anaerobic digestion process is very significant.

Recently, the application of carbon materials like biochar, active carbon, carbon nanomaterial in the anaerobic digestion to maintain the stability and increase the biogas production had been attracted much attention (Sunyoto *et al*., 2016; Wang *et al*., 2021., Zhang *et al*., 2020; Tiwari *et al*., 2021; Mostafa *et al*., 2020). Biochar (BC), as an easily obtained material, had been widely used in anaerobic digestion due to the excellent property. It can increase the buffering capacity of anaerobic digestion (Wang *et al*., 2021; Fagbohungbe *et al*., 2016; Wang *et al*., 2017), alleviate the inhibitions caused by NH4+-N, VFAs, H2S and heavy metal (Qunpeng *et al*., 2020; Yang *et al*., 2021; Jun *et al*., 2021; Mumme *et al*., 2014) and promote the interspecies electron transfer (Zhang *et al*., 2020; Lü *et al*., 2019). Shuo Yang *et al*. investigated the Effect of KH2PO4-modified biochar on the immobilization of Cr, Cu, Pb, Zn during anaerobic digestion of swine manure and the results showed that the addition of biochar could improve the passivation of Cr, Cu, Pb and Zn which reduced the ecological risks of these heavy metals (Yang *et al*., 2021). Mumme *et al*. investigated the effect of pyrochar and hydrochar on ammonia inhibition and found that pyrochar could alleviate ammonia inhibition under the initial TAN concentration of 2.1 g/kg (Mumme *et al*.,

2014). Our previous study also showed that biochar could promote biogas production through releasing the ammonium inhibition even under 3500 mg/L NH_4^+-N (Qunpeng *et al*., 2020). Meanwhile, biochar could also immobilize microorganisms and promote the abundance of microorganisms which resulted in an increase of biogas production. Wang Su *et al*., 2018 found that the methane yield was increased by 29% through adding biochar derived from corn stalk to the anaerobic digestion of kitchen waste (Sunyoto *et al*., 2016). Lü *et al*., 2016 found that boichar could enrich Methanosaeta and Methanosarcina and improve the biogas production even under high ammonia nitrogen stress (Lü *et al*., 2016).

The above studies indicated that BC could promote the performance of AD with different mechanisms. However, the effect of biochar on anaerobic digestion could be greatly affected by the kinds of substrates, biochar characteristic and dosage. Hence, in this paper, two kinds of biochar mediated the anaerobic digestion of piggery wastewater were studied. The evolution of microbial community structure mediated by BC as well as the COD removal rate, methane yield and the degradation process has also been analyzed.

2. Materials and methods

2.1. Substrate and inoculums

Piggery wastewater used as the substrate was characteristic of a mean value of COD (11.01 g/L), NH_4^+-N (320.24 mg/L), and pH (7.1). The inoculated sludge was obtained from a sewage treatment plant with a mean value of water content (89.23%), TS (8.28%), VS (6.70%) and C/N (3.0/1). The wastewater and inoculated sludge were mixed at a volume ratio of 7:3.

2.2. Experimental procedures

Two kinds of biochar were prepared by the pyrolysis of rice straw in the lab: (1) 300BC pyrolyzed in 300℃ with a BET surface, pore volume and pore diameter of 44.090 m^2/g, 0.05929 cm^3/g and 5.37927nm respectively;(2) 500BC pyrolyzed in 500 °C with a BET surface, pore volume and pore diameter of 93.3794 m^2/g, 0.053814 cm^3/g and 1.1526 nm respectively. Five groups with three parallels were operated under the same conditions, including four test groups with different dosages of BC (1g, 2g, 3g and 4g) and one control group with no supplementation of BC (Control). The experiments of anaerobic digestion were carried out in a working volume of 0.8 L conical flask with a constant temperature of 35± 1 °C for 15 days. The bioreactor was artificially shaken two times every day to make the sludge and biochar efficiently mixed.

2.3. Analytical methods

Chemical oxygen demand (COD) was determined through potassium dichromate method. Ammonia nitrogen (NH_4^+-N) was measured according to Nessler method by a spectrophotometer (UNIC 7200).TS and VS were tested by gravimetric method. The volatile fatty acids (TVFA) were determined by colorimetric method. pH was measured using a portable pH meter (PHBJ-260). Biogas production was collected by a gas bag and the gas composition was determined by a gas chromatograph (GC Trace 1300), equipped with thermal conductivity detector (TCD) and 5A molecular sieve column. Hydrogen sulfide (H_2S) was obtained by an online gas detector (MIC-500-H2S) with a range of 0-5000 PPM. The detail of microbial community analysis could be seen in our previous work (Chenxi *et al*., 2021).

2.4. Kinetic analyze

Modified Gompertz model (Eq. (1)) was used to evaluate the impact of BC on the methane production process:

$$M_{\text{methane}}=M_{\max}\times\exp\left\{-\exp\left[\frac{R_{\max}\times e}{M_{\max}}(\lambda-t)+1\right]\right\} \quad (1)$$

Where M_{methane} was the cumulative methane yield ($mLCH_4$); $M_{\max}$ was the maximum methane yield of the substrate ($mLCH_4$); t was the time (d); R_{max} was the the maximum methane production rate (mL CH_4), and λ was the lag time (d).

3. Results and discussion

3.1. Effect of BC on COD removal rate and methane production

The COD removal rate related to the BC amount was shown in Figure 1. After 15 days fermentation, the COD removal rates of 300BC group were 62.42%, 58.18%, 64.24% and 66.05%, respectively for 1g, 2g, 3g and 4g. Compared with the blank group (56.85%), it was increased by 2.3%-9.4% for 300BC group. While the COD removal rates of 500BC group were improved obviously which were 80.93%, 78.19%, 80.02% and 81.85%, respectively for 1g, 2g, 3g and 4g. It was increased by37.5%-44.0%. There was not obviously difference of COD removal rate in the same BC group. BC not only can promote the inter-specific electron transfer, but also can enrich the functional microorganisms and promote nutrient metabolism among microorganisms due to the porous structure, which resulted in an increase of COD removal rate. Cooney *et al*. investigated the effect of biochar on the anaerobic digestion of greasy waste-water and found that biochar promoted the colonisation of acidogens, acetogens and methanogens which resulted in a 69% COD removal rate (Cooney *et al*., 2020). Similar results were obtained by Sharma. They found that biochar could accelerate the biofilm formation for the micro-organisms which increased the COD removal rate (Sharma and Melkania, 2018).

The methane production related to the BC addition was shown in Figure 2. It could be seen that methane yield could be obviously increased after BC addition, which was consistent with the COD removal rate. The maximum methane yield was obtained both in 1g 300BC (2477.40 mL) and 500BC (3278.89mL), which was increased by 43.20% and 89.52% respectively compared with the blank group (1730.06 mL). Meanwhile, the methane yields in 500BC groups were higher than that in 300BC groups with was agreed with the results of COD removal rate. Biogas

production process was related to the activity of methanogens. Biochar can immobilize microorganism especially for methanogens which can facilitate electron transfer between interspecies due to the large surface (Fagbohungbe *et al*., 2016), while the properties of biochar are significantly affected by the pyrolytic temperature (Chiappero *et al*., 2020). The higher in the pyrolytic temperature will increase the surface area and distribution of micropores (Masebinu *et al*., 2019). In this study, the BET surface of 500BC was larger than that of 300BC which was more benefit for the immobilization of microorganism leading to an increase of methane yield.

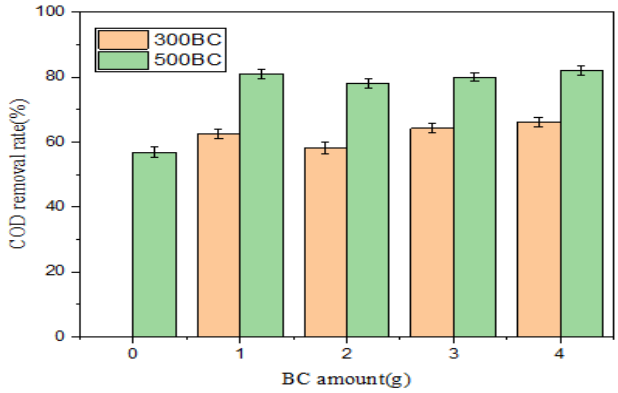


Figure 1. Effect of BC on COD removal rate

Methane production process was analyzed by the Gompertz model to investigate the effect of the biochar on the AD process. As shown in Table.1, the Gompertz model could well fit the data (R^2>0.97). Compared with the blank group, the maximum methane potential (M_{max}) in all the BC groups increased. While the maximum methane production rate (R_{max}) in blank group and 300BC groups were higher than that in 500BC groups. The reason may be caused by high TVFA concentration in 500BC groups at the initial time of anaerobic digestion (Figure.4), which may have inhibited the anaerobic bacteria. Meanwhile, the lag phase (λ) in the blank group was lower than that in the BC groups. The decrease of R_{max} in BC groups led to an increase of longer lag phase.

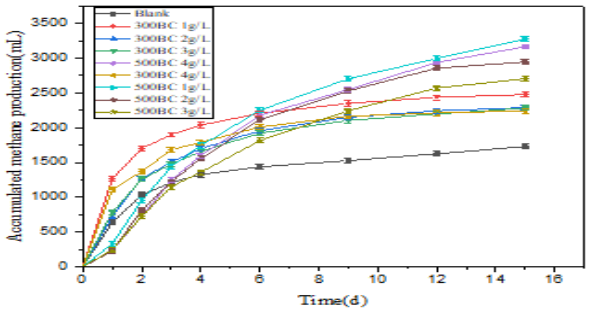


Figure 2. The effect of BC on methane yield

Table 1. Kinetic parameters of modified Gompertz model

Parameter	M_{max}/mL	R_{max}/mL•d^{-1}	λ/d	R^2
Blank	1547.04	480.81	0.03	0.97
1g 300BC	2333.07	855.39	0.09	0.98
2g 300BC	2202.59	540.09	0.28	0.99
3g 300BC	2172.08	522.12	0.26	0.99
4g 300BC	2147.56	622.55	0.08	0.98
1g500BC	3115.51	444.91	0.10	0.98
2g500BC	2886.02	427.22	0.35	0.99
3g500BC	2645.41	351.27	0.15	0.99
4g500BC	3035.52	429.83	0.39	0.99

3.2. Effect of BC on H_2S concentration

In anaerobic digestion, H_2S will cause inhibition on the methane production bacterium and reduce methane yield. Therefore, reducing H_2S content in biogas is very important to maintain the stability of the system. The Effect of BC on H_2S concentration after 15 days fermentation was shown in Figure 3. It could be seen that the H_2S concentration was obviously decreased by BC. The lowest accumulated H2S concentrations appeared at 4g 300BC (0.9 mL) and 500BC (0.8mL), which was decreased by 749% and 961% respectively compared with the blank group (8.49 mL). Kanjanarong *et al*. also found that a removal rate of 98% H2S from biogas with the addition of biochar (Kanjanarong *et al*., 2017). Meanwhile, the H2S concentration was decreased with the increase of BC dosage. For example, the H_2S concentration was decreased from 3.07mL to 0.9 mL with the BC dosage increasing from 1g to 4g. H_2S is mainly produced during the degradation process of sulphate and proteins by sulphate-reducing bacteria. Sulphate-reducing bacteria will compete with methanogens, acetogens or other bacteria for the utilization of acetic acid, hydrogen, propionic acid and butyric acid. When the sulphate-reducing bacteria was in an inferior position during the competition, the available electron donor was reduced which resulted in a decrease of H_2S concentration and then there might be a balance reached between sulphate-reducing bacteria and methanogens. Our previous study had been proven that biochar not only could balance the competition between SRB and MPB for the utilization of substrates, but also could promote methanogenesis (Chenxi *et al.*, 2021).

3.3. Effects of BC on pH, VFAs and ammonia nitrogen

pH can directly affect the activity of microorganisms, and the optimal pH value for anaerobic digestion is 6.8-7.2. The effects of BC on pH were shown in Figure 4(a) and (e).

pH values in 300BC groups were first increased and then decreased. The decrease of TVFA concentration (Figure 4 (b)) and the increase of NH_4^+-N concentration (Figure 4 (c)) resulted in an increase of pH. However, the pH values were maintained at 7.1-7.3 after 4 days. Meanwhile, the variation of pH in 300BC groups was lower than that in blank group. It indicated that BC could improve the buffering capacity of anaerobic digestion. pH in 500BC groups were first decreased and then increased. The increase of TVFA concentration (Figure 4 (b)) resulted in a decrease of pH. However, the decrease of pH inhibited the activity of methane production bacterial which resulted in a relative low biogas production in the first day; the pH was recovered after 4 days which were also maintained at 7.1-7.4. The increase of NH_4^+-N concentration made little influence on the pH of 500BC groups which was also due to the function of BC. Although the mean value of pH in all groups was a little higher than the normal pH range, the biogas production was not inhibited especially in the BC groups. BC not only could increase the buffer capacity of the anaerobic system, but also could reduce the inhibition caused by ammonia through adsorption (Wang *et al*., 2018; Tang *et al*., 2020). The buffering capacity of biochar is dependent on its alkalinity and the alkalinity of biochar is concerned with the pyrolysis temperature. High pyrolysis temperature promoted inorganic carbonate which increased the alkalinity of biochar (Yuan *et al*., 2011).

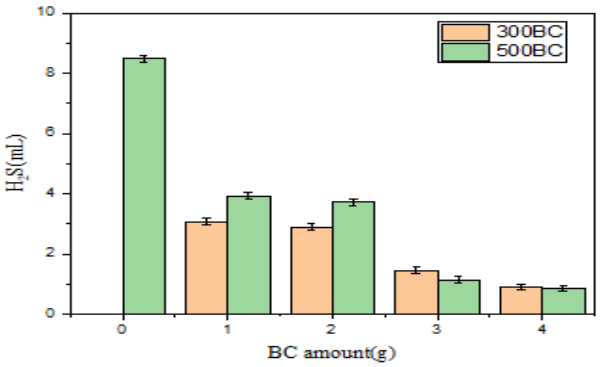


Figure 3. Effect of BC on H2S concentration

VFAs, as the intermediate products, are very important for the stability of anaerobic digestion. Low VFAs concentrations will lead to substrate competition between microorganisms, while the accumulation of VFAs will lead to the acidification of the system. The effects of BC on TVFA were shown in Figure 4 (b) and (f). The TVFA concentrations in 300BC groups were quickly decreased in the first six days which resulted in an increase of biogas production (Figure 1). While the TVFA concentrations in 500BC groups were first increased at initial 3 days and then decreased. That may be due to the promotion of the hydrolysis and acidification process by 500BC leading to a rapid accumulation in a short time. The difference of TVFA between 300BC groups and 500BC groups caused the different increase rate of biogas, which in 500BC groups was lower than that in 300BC groups which was agreed with the R_{max}.

Nitrogen content is rich in piggery wastewater due to urea, protein, cellulose, hemicelluloses and inorganic salts which causes the increase of NH_4^+-N concentration. NH_4^+-N, as a nutrient, not only can provide nitrogen source for microbial growth, but also can alleviate acid inhibition. Previous research showed that the anaerobic digestion could be inhibited when the NH4+-N concentration was exceeding than 3300mg/L (Hobson *et al*., 1976). In this study, though the NH4+-N concentrations in all groups were increased, the NH_4^+-N concentration was relatively low (Figure 4 (c) and (f)). On the one hand, BC could stimulate the activity of microorganisms and accelerate the conversion rate of nitrogen to NH_4^+-N which led to an increase of NH_4^+-N concentration; On the other hand, BC could promote the metabolism of microorganisms including denitrifying bacteria, which resulted in a decrease of NH_4^+-N concentration. Hence, the influence of NH_4^+-N on the anaerobic digestion could be neglect.

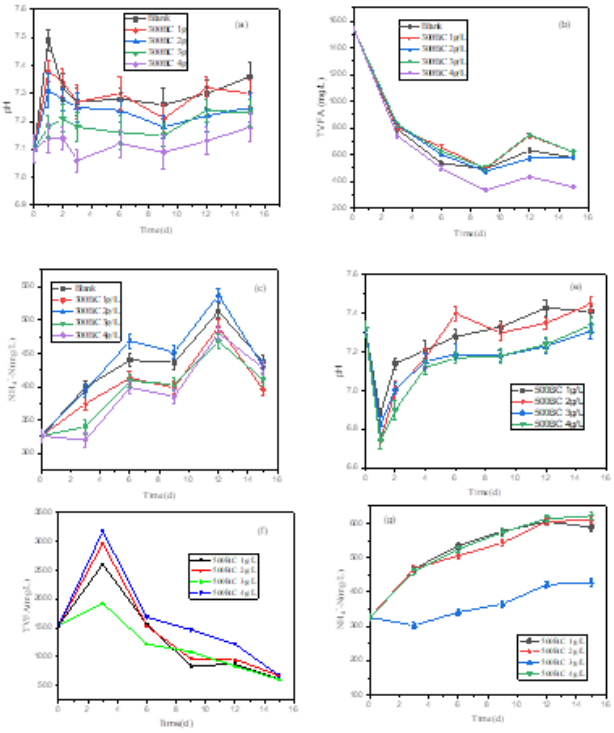


Figure 4. Effect of BC on pH, VFA and NH_4^+-N

3.4. Impact of BC on microbial community structure

The structure of microbial community with 300BC at phylum level, class level and genus level was shown in Figure 5 (a), (b) and (c). At phylum level, Proteobacteria, Bacteroidota, and Euryarchaeota were the dominant phyla in the blank group with a relative abundance of 30%, 14% and 10% respectively. While with the addition of 300BC, the dominant phyla were changed to Desulfobacterota、Bacteroidota and Halobacterota. It indicated that BC promoted the growth of the above three species. The increase of Desulfobacterota was also agreed with the results of H_2S, which can convert sulfate to sulfur or sulfide. At class level, Methanobacteria、Bacteroidia and Gammaproteobacteria were the dominant class in the blank group with a relative abundance of 7.8%, 13.9% and 30.1% respectively. While the uniformity of microbial community was obviously improved after the addition of BC, the dominant classes were Anaerrolineae, Syntrophobacteria, Methanobacteria, Syntrophia,

Syntrophorhabdia and Bacteroidia. The increase of microbial community uniformity could facilitate the electron transfer among different microorganisms and maintain the stability of anaerobic system, which had a positive effect on methane production. At genus level, Aeromonas, Methanobacterium and Methanosaeta were the dominant bacterium in the blank group. Methanolinea and Methanobacterium belong to hydrotrophic methanogens which can utilize H_2/CO_2 as the substrate to produce methane. Methanosaeta is a strict acetoclastic methanogens, which can utilize the acetate for the methane production. However, the abundances of Syntrophobacter and Syntrophorhabdus were increased during the presence of BC. Syntrophobacter can utilize propionic acid for its growth, which effectively reduces the risk of acidification. Syntrophorhabdus is a kind of bacterium which can supply acetate and hydrogen to hydrotrophic methanogens for methane production.

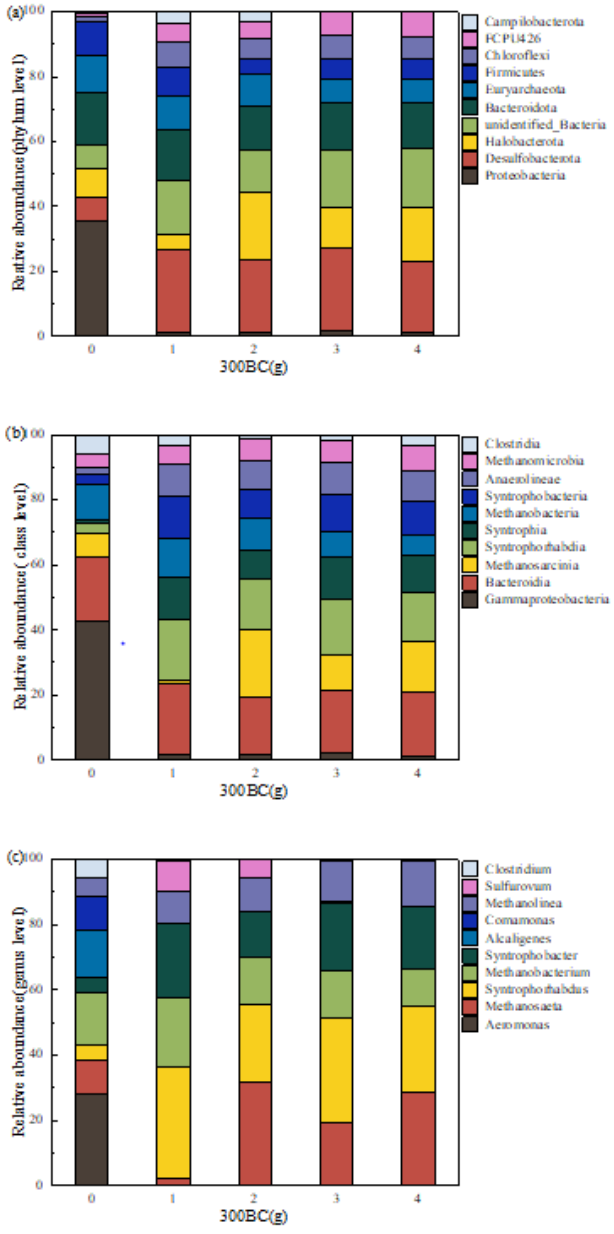


Figure 5. The relative abundances of microbial community with 300BC

The structure of microbial community with 500BC at phylum level, class level and genus level were shown in Figure 6. Compared with the blank group and 300BC groups the relative abundance of Bacteroidota and Euryarchaeota were increased at phylum level; the relative aboundance of Methanobacteria, Bacteroidia were increased at class level; the relative aboundance of Methanofastidiosum and Desulfobacterota were increased at genus level. Bacteroidia belongs to hydrolytic fermentation bacteria, which can decompose macromolecules such as polysaccharides, steroids and proteins into intermediate metabolites such as acetic acid, butyric acid, ethanol, CO_2 and H_2. It indicated that 500BC could further promote the hydrolysis step compared with 300BC which also resulted in an increase of TVFA concentration.

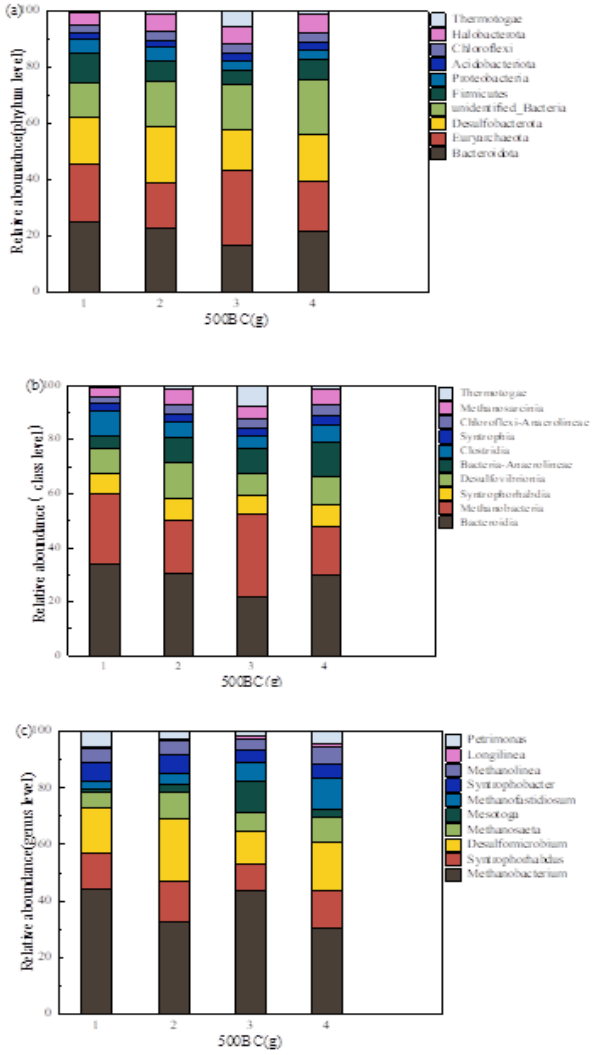


Figure 6. The relative abundances of microbial community with 500BC

There are three main methanogens for methane production: (1) hydrotrophic methanogens using H_2 and CO_2 as substrates (Eq (2)); (2) acetoclastic methanogen using acetate as substrate (Eq (3)); (3) formate nutritive methanogenic using formic acid as substrate (Eq (4)).

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (2)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (3)$$

$$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O \quad (4)$$

Methanobacterium and Methanolinea belong to hydrotrophic methanogens, while Methanosaeta is acetoclastic methanogen. The pathway of methane production was greatly affected by the microbial. In order to further analyze the variation of microbial on the methane production, the content of methane was displayed in Table 2. It could be seen that the content of CH_4 was obviously increased after the addition of BC. Compared with the blank group, the abundance of methanogens was increased significantly with the addition of biochar which resulted in the increase of methane yield. Similar results were obtained by Luo *et al*. (2015) who found that BC could promote the colonization of Methanosarina which resulted in a 86.6% increase of methane production (Luo *et al*., 2015). Meanwhile, the content of CH_4 in 500BC groups was a little higher than that in 300BC groups. Compared with 300℃ biochar, the relative abundances of hydrotrophic methanogens (Methanobacterium and Methanolinea) in 500BC groups were significantly higher than that of acetoclastic methanogen (Methanosaeta) due to higher BET surface. A higher BET surface could provide more active site for the microbial cells' attachment. The increase of relative abundance of hydrotrophic methanogens was benefited to reduce the CO_2 content in biogas which was agreed with the result of Table 2. 500BC had a higher positive effect on anaerobic digestion of methanogenic bacteria than 300BC.

Table 2. The content of methane

	CH_4 (%)	CO_2 (%)
Blank	0.72	0.28
1g 300BC	0.81	0.19
2 g 300BC	0.79	0.21
3g 300BC	0.83	0.17
4g 300BC	0.79	0.21
1g 500BC	0.84	0.16
2g 500BC	0.81	0.19
3g 500BC	0.82	0.18
4g 500BC	0.84	0.16

Note: The other trace gas like H_2S, H_2 was not calculated.

4. Conclusions

The study confirmed that BC could improve the performance of anaerobic digestion of piggery wastewater by increasing the buffering capacity, promoting the hydrolysis and acidification process and enriching the relative abundance of methanogens. It not only could increase COD removal rate, but also could promote biogas production and reduce the H2S content in biogas. Meanwhile, the performance of anaerobic digestion with 500BC was superior to that with 300BC due to high BET surface. However, the effect of biochar on anaerobic digestion of piggery wastewater in a continuous test is further required to investigate.

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References

Cheng Q., Chen Z., Deng F., Liao Y., Xiao B. and Li J. (2016). Kinetic evaluation on the degradation process of anaerobic digestion fed with piggery wastewater at different OLRs. *Biochemical Engineering Journal*, **113**, 123–132.

Chenxi X., Yongyu D., Jiacheng L., Wenwen H., Qunpeng C., Guozhi F., Juntao Y., Shunxi Z., Guangsen S. and Bo X. (2021). Anaerobic digestion of sulfate wastewater mediated by biochar. *Environmental Technology*, **44**, 1667–78.

Chiappero M., Norouzi O., Hu M., Demichelis F. and Fiore S. (2020). Review of biochar role as additive in anaerobic digestion processes. *Renewable and Sustainable Energy Reviews*, **131**.

Cooney M.J., Lewis K., Harris K., Zhang Q. and Yan T. (2020). Start up performance of biochar packed bed anaerobic digesters. *Journal of Water Process Engineering*, **9**, 7–13.

Fagbohungbe M.O, Herbert B.M.J., Hurst L., Ibeto C.N. and Semple KT. (2016). The challenges of anaerobic digestion and the role of biochar in optimizing anaerobic digestion. *Waste Management*, **61**, 236–49.

Hobson P.N. and Shaw B.G. (1976). Inhibition of methane production by Methanobacterium formicicum. *Water Research*, **10**, 849–52.

Jun W., Xiaoxia H., Zile L., Zili G. and Lin B. (2021). Biochar improves heavy metal passivation during wet anaerobic digestion of pig manure. *Environmental Science and Pollution Research*, **28**, 635–44.

Kanjanarong J., Giri B.S., Jaisi D.P., Oliveira F.R. and Khanal S.K. (2017). Removal of hydrogen sulfide generated during anaerobic treatment of sulfate-laden wastewater using biochar: Evaluation of efficiency and mechanisms. *Bioresour Technol*ogy, **234**, 115–21.

Lü F., Liu Y., Shao L. and He P. (2019). Powdered biochar doubled microbial growth in anaerobic digestion of oil. *Applied Energy*, **247**, 605–14.

Lü F., Luo C., Shao L. and He P. (2016). Biochar alleviates combined stress of ammonium and acids by firstly enriching Methanosaeta and then Methanosarcina. *Water Research*, **90**, 34–43.

Luo C., Fan L., Shao L. and He P. (2015). Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes. *Water Research*, **68**, 710–718.

Masebinu S.O., Akinlabi E.T., Muzenda E. and Aboyade A.O. (2019). A review of biochar properties and their roles in mitigating challenges with anaerobic digestion. *Renewable and Sustainable Energy Reviews*, **103**, 291–307.

Mostafa A., Im S., Song Y.C., Kang S. and Kim D.H. (2020). Enhanced Anaerobic Digestion of Long Chain Fatty Acid by

Adding Magnetite and Carbon Nanotubes. *Microorganisms*, **8**, 333.

Mumme J., Srocke F., Heeg K. and Werner M. (2014). Use of biochars in anaerobic digestion. *Bioresour Technol*ogy, **164**, 189–97.

Oladejo O.S., Dahunsi S.O., Adesulu-Dahunsi A.T., Ojo S.O. and Evbuomwan I. (2020). Energy generation from Anaerobic Co-digestion of Food waste, Cow dung and Piggery dung. *Bioresource Technology*, **313**, 123694.

Qunpeng C., Chenxi X., Wenwen H., Meng J. and Guangsen S. (2020). Improving anaerobic digestion of piggery wastewater by alleviating stress of ammonia using biochar derived from rice straw. *Environmental Technology & Innovation*, **19**, 100948.

Sánchez E., Herrmann C.., Maja W. and Borja R. (2021). Effect of organic loading rate on the anaerobic digestion of swine waste with biochar addition. *Environmental Science and Pollution Research,* **28,** 38455-65.

Sharma P. and Melkania U. (2018). Impact of heavy metals on hydrogen production from organic fraction of municipal solid waste using co-culture of Enterobacter aerogenes and E. Coli. *Waste Management*, **75**, 289–96.

Sunyoto N., Zhu M., Zhang Z. and Zhang D. (2016). Effect of biochar addition on hydrogen and methane production in two-phase anaerobic digestion of aqueous carbohydrates food waste. *Bioresource Technology*, **219**, 29–36.

Sunyoto N.M.S., Zhu M., Zhang Z., Zhang D. (2016). Effect of biochar addition on hydrogen and methane production in two-phase anaerobic digestion of aqueous carbohydrates food waste. *Bioresource Technology*, **219**, 29–36.

Tang S., Wang Z., Liu Z., Zhang Y. and Si B. (2020). The Role of Biochar to Enhance Anaerobic Digestion: A Review. *Journal of Renewable Materials*, **8**, 1033–52.

Tiwari S.B., Dubey M., Ahmed B., Gahlot P., Tyagi V.K. (2021). Carbon-based conductive materials facilitated anaerobic co-digestion of agro waste under thermophilic conditions. *Waste Management*, **124**, 17–25.

Wang D., Ai J., Shen F., Yang G. and Zhang Y. (2017). Improving anaerobic digestion of easy-acidification substrates by promoting buffering capacity using biochar derived from vermicompost. *Bioresource Technology*, **227**, 286–96.

Wang G., Li Q., Yuwen C., Gong K., Sheng L., Li Y., Xing Y., Chen R. (2021). Biochar triggers methanogenesis recovery of a severely acidified anaerobic digestion system via hydrogen-based syntrophic pathway inhibition. *International Journal of Hydrogen Energy,* **46,** 9666-77.

Wang T., Zhai Y., Zhu Y., Li C. and Zeng G. (2018). A review of the hydrothermal carbonization of biomass waste for hydrochar formation: Process conditions, fundamentals, and physicochemical properties. *Renewable and Sustainable Energy Reviews*, **90**, 223–47.

Yang S., Wen Q. and Chen Z. (2021). Effect of KH2PO4-modified biochar on immobilization of Cr, Cu, Pb, Zn and as during anaerobic digestion of swine manure. *Bioresource Technology*, **339**, 125570.

Yuan J-H., Xu R-K. and Zhang H. (2011). The forms of alkalis in the biochar produced from crop residues at different temperatures. *Bioresource Technology*, **102**, 3488–97.

Zhang J., Tian H., Wang X. and Tong Y.W. (2020). Effects of activated carbon on mesophilic and thermophilic anaerobic digestion of food waste: process performance and life cycle assessment. *Chemical Engineering Journal,* **399**, 125757.

Zhang L., Lim E.Y., Loh K-C., Ok Y.S., Lee J.T.E., Shen Y., Wang C-H., Dai Y. and Tong Y.W. (2020). Biochar enhanced thermophilic anaerobic digestion of food waste: Focusing on biochar particle size, microbial community analysis and pilot-scale application. *Energy Conversion and Management*, **209**, 112654.