Promoting methane production during anaerobic digestion with biochar: Is it influenced by quorum sensing?

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Anaerobic digestion (AD) is a recommended technology to both treat organic waste (e.g., sludge and food waste) and produce bioenergy, due to the advantages of high disposal efficiency and energy recycling (Li et al. 2021). However, the accumulation of essential intermediates, including volatile fatty acids (VFAs), salinity, and long chain fatty acids, are common challenges in long-term semi-continuous AD systems (Zhang et al. 2018). To improve the methane production and process stability of AD systems, the addition of biochar has received comprehensive attention. Biochar can mitigate VFAs toxicity and increase methane yield through selectively immobilizing cells in porous surface, establishing the network of syntrophic metabolism, and promoting direct interspecific electron transfer (DIET) (Kutlar et al. 2022). However, the underlying mechanisms of how microorganisms selectively immobilized in the porous surfaces of biochar by the inter-microbial communication have not been addressed in detail. Therefore, it is essential to further target and expand the entire microbial community in elucidating the mechanism of biochar-enhanced methanogenesis, rather than focusing solely on the DIET species. The addition of biochar could affect the microbial ecology of AD systems through creating abiotic carriers to undertake electron transfer between microorganisms, and enhancing the interaction between suspended cells and coacervate. The social behaviors of dense microflora and interaction of syntrophic metabolism have been demonstrated to be regulated and induced by quorum-sensing (QS) in AD systems, especially N-acyl homoserine lactones (AHLs) (Dang et al. 2022). Based on the previous studies, QS might play a potential role in adjusting and controlling the cooperation between electron-accepting and electron-donating microorganisms (Zhang et al. 2012). However, the function of QS for microbial communities and syntrophic metabolism in AD systems has not been comprehensively investigated, especially with the addition of biochar. Therefore, the internal mechanism of biochar-enhanced methanogenesis through inter-microbial communication in AD were reveal, the concentrations of AHLs and the dynamic of microbial community structures with the biochar addition were analyzed in the AD system.

In this study, semi-continuous experiments were performed in serum bottles of 400 mL working volume without biochar ($AD_{S,\,C}$) and with 10 g/L BC300 ($AD_{S,\,BC300}$) and 10 g/L BC700 ($AD_{S,\,BC700}$), respectively. The semi-continuous experiments lasted for 42 days. The organic loading rate (OLRs) and hydraulic retention time was 2 gVS/(L·d) and 20 days, respectively. During each feeding process, around 0.1 g of biochar was supplemented into the anaerobic digesters to maintain the optimal biochar dosage (10 g/L). After 42 days, the sedimentary cultures were collected to analyze the structure of microbial community, key enzyme activities, and metagenomic sequencing. Partial least squares path modelling (PLS-PM) was adopted to analyze the effects of correlations between QS and methane production.

The methane production in the semi-continuous digesters of FW-AD operated for 42 days was evaluated. The average daily methane production in $AD_{S,BC300}$ and $AD_{S,BC700}$ was significantly higher compared to $AD_{S,C}$ (251 mL/gVS), by 22.1% and 27.4%, respectively (**Fig. 1**). Noteworthily, the $AD_{S,BC700}$ digester exhibited the highest methane production rate (319 mL/gVS) among all the digesters supplemented with biochar. In this study, not all 11 AHLs were detected in the semi-continuous digesters. In particular, the $AD_{S,BC700}$ exhibited a significant concentration of 3OC6-HSL (90.615 μg/L), which accounted for approximately 87% of the total AHLs. Thus, AHL-mediated quorum sensing, particularly 3OC6-HSL, may play a crucial role in the enhancement of anaerobic methanogenesis with the addition of biochar.

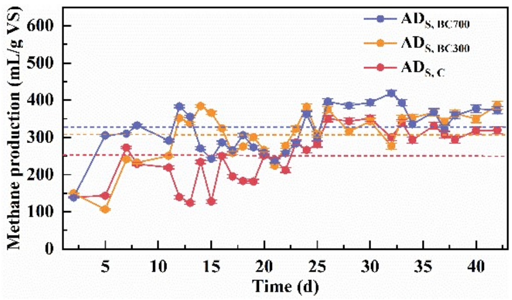

Fig. 1 Methane production in semi-continuous experiments without biochar ($AD_{S,C}$), with BC300 ($AD_{S,BC300}$), and with BC700 ($AD_{S,BC700}$), respectively

Accordingly, the electroactive microorganisms were enriched predominantly such as *DMER64* and *Methanospirillum*. The abundance of key enzyme activities (including acetate kinase and coenzyme F420) increased with BC700 addition, especially coenzyme F420 which was higher by 33.0% compared to without biochar. The total abundance of QS-associated genes in $AD_{S,BC700}$ (0.1839%) and $AD_{S,BC300}$ (0.1516%) was 30.5% and 7.6% higher than that in $AD_{S,C}$ (0.1409%), respectively (**Fig. 2a**). Moreover, various types of QS-related genes were analyzed that the addition of biochar may enhance the synthesis of AHLs, potentially promoting the communication within methanogenic and symbiotic microorganism through AHLs and AIP systems (**Fig. 2b**).

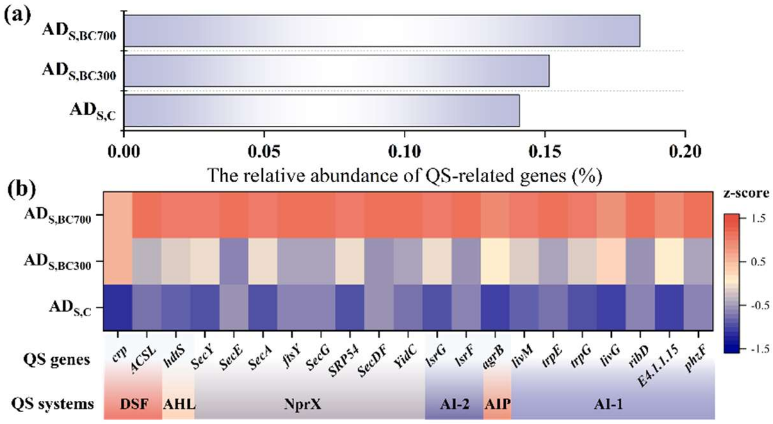

Fig. 2 Relative abundance of QS-related genes in $AD_{S,C}$, $AD_{S,BC300}$, and $AD_{S,BC700}$

Therefore, biochar stimulated gene expression associated with carbon metabolism and methane synthesis, positively enhanced EET and QS-related genes, and facilitated microbial communication based on PLS-PM (**Fig. 3**). This study provided new evidence about the underlying role of QS-mediated inter-microbial communication and extracellular electron transfer in elucidating the mechanism of biochar-enhanced methanogenesis.

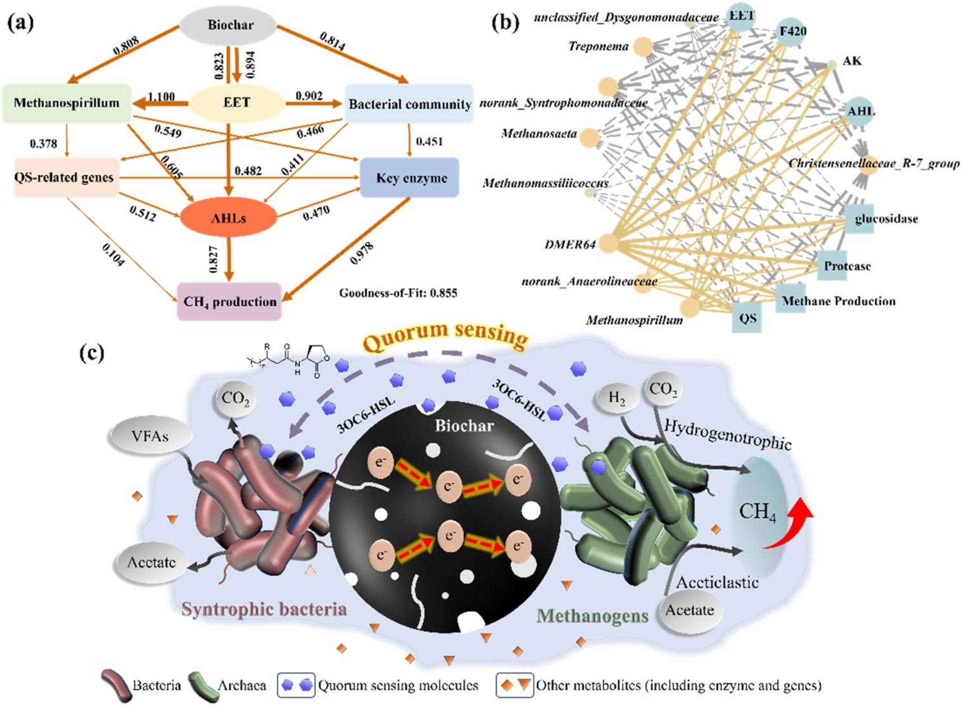

Fig. 3 PLS-PM showing direct and indirect contributions of multi-parameters (including biochar, microbial community, QS, EET, and key enzymes) to methane production in AD of FW (a), correlation network of microbial community and multi-parameters in the digesters with and without biochar (b), and proposed mechanism of quorum sensing and electron transfer for methane production in biochar-amended systems (c).

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