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# Enhanced methane production and syntrophic connection between microorganisms during semi-continuous anaerobic digestion of chicken manure by adding biochar



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### ABSTRACT

The synergetic inhibition of ammonia and organic acids during anaerobic digestion with high organic loading rate limits the methane yield and endangers process stability. Here, the anaerobic digestion of chicken manure was tested under three different experimental conditions (corresponding to organic loading rates of 0.625, 3.125, and 6.25 g VS/L•d) with and without biochar in a semi-continuously stirred tank reactor, operating within a mesophilic range  $(35 \pm 1 \,^{\circ}\text{C})$ . The results showed that methane yields were increased by 33%, 36%, and 32% with the addition of biochar at the three different organic loading rates, respectively. Investigating ammonia nitrogen concentrations, organic carbon transformation, and the 16S rRNA gene sequences together demonstrated that by streamlining the metabolic capability of methanogens for the most efficient utilization strategy, biochar facilitates the direct interspecies electron ransfer between fermentative bacteria and electro-trophic methanogens. Results also revealed that biochar supplement can enhance the conversion efficiency of macromolecule organic matter into dissolved organic acids and to methane for energy recovery, and stimulate the denitrification process for nitrogen removal by activating *Epsilonproteobacteria*. This study offers a microbiological addition to the body of work focusing on the function and mechanism of biochar in AD.

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## 1. Introduction

Anaerobic digestion (AD) has been used for biogas production worldwide for decades as a promising technology for producing an energy carrier from renewable resources and achieving multiple environmental benefits (Alexander et al., 2019), and it is capable of utilising nearly all biological feedstocks. Of these, the major resources are animal manure and slurries from livestock husbandry (Li et al., 2018a). Even though it presents numerous benefits, such as odour control, greenhouse gas reduction, and nutrient preservation for use as high-quality fertiliser (Moraes et al., 2017), the AD process is often associated with a poor methane yield (Mata-Alvarez et al., 2014) caused by the accumulation of inhibitory intermediate products (Siddique and Ab Wahid, 2018). Among these products, ammonia nitrogen (Yenigun and Demirel, 2013) and organic acids

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(Zhang et al., 2014) are considered as the most significant inhibitors. With the increasing benefits expected to be obtained from the AD of organic materials, solutions are required to counteract intermediate products inhibition during the AD to enhance process stability and increase methane yield. During AD, organic material is anaerobically decomposed by a complex consortium of microorganisms. Hence, the efficiency and stability of the process mainly depend on the concerted and syntrophic activity of microbial communities belonging to different functional guilds that perform hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Vanwonterghem et al., 2014). Ammonia is reported to directly inhibit microbial activity at concentrations exceeding the critical value, which was documented in the range of 1.7-14 NH<sub>3</sub>-N g/L (Chen et al., 2014a). Methanogens are believed to be the least tolerant and most likely to cease growth due to ammonia inhibition (Yuan and Zhu, 2016). Characterised by a high content of organic nitrogen, chicken manure is more likely to encounter anaerobic digester upset caused by inhibitory substances (Lee et al., 2017). The addition of ionic exchangers or adsorbents that can remove inhibitors is supposed to mitigate ammonia inhibition during AD and increase the stability of the process (Ma et al., 2018).

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