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### **ACCEPTED MANUSCRIPT**

# Methane Production and Characteristics of the Microbial Community

#### In a Two-stage Fixed-bed Anaerobic Reactor Using Molasses

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Abstract

Molasses is a typical feedstock for fermentation, but the effluent is hard to treat. In this study, molasses containing a high concentration of organic matter was treated by a two-stage Fix-bed reactor system with an increased organic loading rate (OLR). The results indicated at high molasses loading rate, the two-stage system was more efficient (i.e. organic matter removal, the COD of effluent and biogas production) than the single-stage system. The relative abundance of *Anaerolineaceae* and *W5\_norank* was higher in the first stage (R1), where these organisms digest carbohydrates, while the second stage (R2) had higher relative abundance of *Synergistaceae* and *SB-1\_norank*, which digest VFAs and decomposition-resistant compounds to produce compounds used by hydrogen methanogens. The qPCR analysis demonstrated that the *Methanosaetaceae* dominated the archaeal community in the first stage (R1), while *Methanomicrobiales* and *Methanobacteriales* were predominant in the second stage (R2), where they were involved in hydrogen production.

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