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Methanotroph-heterotroph community resilience towards Cu2+/Fe2+ ratios

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Methane (CH4) is a greenhouse gas (GHG) with a Global Warming Potential of 28 - 36 over 100 years. Mining activities (e.g., coal mining) account for 11% of global methane emissions from anthropogenic activities, and CH4 that continues to be emitted from abandoned mines has higher CH4 content (˜50 percent higher) than previously estimated. Advancement of the biological method for coal mine oxidation based on the methanotrophy combined with biopolymer (PHB - Polyhydroxybutyrate) production is a more feasible and sustainable approach for reducing CH4-associated climate change impacts. Divalent copper (Cu2+) and iron (Fe2+) play a vital role in CH4 oxidation and are critical for the expression of methane monooxygenase (pMMO or sMMO) enzymes. However, understanding their effects on methanotrophic growth and resilience is of paramount importance for improved CH4 oxidation and subsequent carbon storage as PHB. This study, as a first of its kind, therefore quantified the combined effects of variable Cu2+ and Fe2+ (5:5, 5:25 and 5:50 µM) ratios on a mixed methanotroph-heterotroph (and stable) consortium enriched from landfill top cover (LB) and compost soil (CB) over 100 days. Two identical 10 L continuous stirred tank reactors (CSTRs, Bioflo® & Celligen® 310 Fermentor/Bioreactor; John Morris Scientific, Chatswood, NSW, Australia) were used and the reactors were purged with CH4:CO2:air at the percentage ratio of 30:10:60 at the flow rate of 0.25 L min−1 (30% CH4). Specifically, we stressed the consortiums with the increasing molar concentration of Fe2+ under semi-continuous fed-batch operations and compared the microbial community shifts and PHB accumulation potentials. Cu2+/Fe2+ ratios had no significant impact on methane oxidation capacity for the first ten days of fed-batch operations, although there were significant differences in the microbial community structures in both LB and CB. Surprisingly increase in Cu2+/Fe2+ ratios favored the abundance of Sphingopyxis growth in both systems. High Fe2+ concentration also favored the growth of the type -II methanotroph population (Methylosinus sp.) in the CB-CSTR. In contrast, methanotroph abundances decreased in LB-CSTR, but increased the growth of Azospirllum. Fatty acid-profiles also changed significantly with the increasing Cu2+/Fe2+ ratios, whilst PHB content was similar in the LB- and CB-CSTR, decreasing with increasing Cu2+/Fe2+ ratios, while biomass growth was unaffected. After 13 days, methane oxidation capacities and PHB content decreased by ~50% and more in response to increasing Fe2+ concentrations. Despite similar methanotroph community structure and controlled environmental variables, increasing Cu2+/Fe2+ ratios significantly altered the microbial community distributions in the LB- and CB-CSTR, indicative of complex microbial interactions largely driven by unexplored allelopathic interactions within the mixed consortia, which might be affecting the CH4 to PHB accumulations under fed-batch operations. The dominance of certain non-methanotrophs indicates Cu2+/Fe2+ positively affected the overall resilience of community structure in both systems. Further studies will help to develop simulative community models to investigate the potential for CH4 emission abatement a priori and commercialize the PHB production.

Keywords: Methane, methanotrophs, copper, methane monooxygenase, biopolymer, CSTR

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