TITLE: MICROBIAL COMMUNITY ANALYSIS OF AN ANAEROBIC METHANE OXIDIZING ENRICHMENT CULTURE REVEALED COEXISTENCE OF AEROBIC AND ANAEROBIC METHANOTROPHS

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ABSTRACT

Methane is produced in anoxic environments, such as anaerobic reactors used to treat wastewaters, and can be consumed by methanotrophs, that are widely distributed in various environments: such as fresh and salt water samples, paddy field soil, upland forest soils, pastures, landfill soils, wetlands and wastewater sludges. In addition, previous studies have used mixed samples containing wastewater sludge and freshwater sediments and industrial wastewater sludge. The composition and structure of a microbial community enriched from anaerobic sewage sludge were investigated, since little is known about methanotrophs in anaerobic reactors treating domestic wastewater. IlluminaMiSeq sequencing of the16S rRNA geneswere used to investigate changes in the composition of the microbial community developed over long-term enrichment (417 days). Deep sequencing analysis revealed a complex community that changed over time and was affected by methane concentration. *Methylocaldum* (8.2%), *Methylosinus* (2.3%), *Methylomonas* (0.02%), *Methylacidiphilales* (0.45%), *Nitrospirales* (0.18%) and *Methanosarcinales* (0.3%) were detected. Despite denitrifying conditions provided, *Nitrospirales* and *Methanosarcinales*, known to performan aerobic methane oxidation coupled to denitrification (DAMO) process were in very low abundance. Results demonstrated that aerobic and anaerobic methanotrophs coexisted in the reactor together with heterotrophic microorganisms, suggesting that a diverse microbial community was important to sustain methanotrophic activity. The methanogenic sludge was a good

inoculum to enrich methanotrophs and cultivation conditions play a selective role in determining community composition.

Keywords: anaerobic sewage sludge, methane oxidation, methanotrophic, *Methylocaldum*.

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