Net Methane Oxidation Performance of Anaerobic Sewage Sludge

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The anaerobic oxidation of methane (AOM) in anaerobic sewage sludge was characterized. The net methane oxidation was observed in samples amended with methane plus sulfate or with methane alone, whereas methane formation was observed in the samples without methane, indicating that methane oxidation and formation occurred simultaneously. The ratio of the net methane oxidation rate to \( \text{H}_2\text{S} \) formation was 100:1, suggesting that the AOM was not closely associated with sulfate reduction in the anaerobic sludge. The net AOM was positively associated with the methane concentration and sludge dilution ratio. However, the rate of AOM was negatively correlated with organic substrate (acetate) concentration. Therefore, the production and oxidation of methane could be controlled by environmental conditions and dissolved organic compounds in the bulk solution.

Keywords: Methane, anaerobic oxidation, sulfate reduction, anaerobic sludge, acetate

The anaerobic oxidation of methane (AOM) is one of the most scientifically intriguing discoveries [2, 14]. The first evidence of AOM within anoxic sediments, enriched with sulfate, came from geochemical observations that methane was generated from deeper sediments and consumed in the anoxic zone [1, 9]. Zehnder and Brock [21] investigated the in vitro AOM by incubating various methanogenic enrichments, and found that the AOM was accompanied by a small percentage of methane production. More recent studies have phylogenetically identified the archaeal groups responsible for AOM: anaerobic methanotrophs (ANME) typically consist of methanogen-related archaea; ANME-1 (distantly related to the *Methanosarcinales* and *Methanomicrobiales*) and ANME-2 (within the *Methanosarcinales*) [14]. It is believed that ANMEs are very closely related to methanogenic archaea owing to methyl coenzyme-M reductase (*mcrA*) [5, 12]. The AOM is likely to proceed via a reversed methanogenic pathway [17]. The AOM has been observed under various conditions; the zone of microbially mediated sulfate reduction in marine sediments [7, 8], the zone of nitrate reduction in fresh water sediments [18], and anaerobic digester [11, 20]. In different situations, the microorganisms associated with the AOM are different. For example, a sulfate reducer coupled with a methanogen is naturally enriched in ocean sediment because the sulfate concentration is high in this type of environment [14], but at sites with high nitrate concentrations, a nitrate reducer is coupled with the AOM [5]. Unlike marine or freshwater sediment samples, however, the net AOM and coupling between the AOM and sulfate reduction have not been observed in anaerobic sludge [6, 21], although methanogens and sulfate reducers are present even in high concentration. Therefore, assessing why no net AOM is observed in anaerobic sludge may contribute to understanding the key parameter determining methane formation or oxidation in anaerobic environments.

This study investigated the AOM and coupling of the AOM to sulfate reduction in anaerobic sludge samples. To assess whether methane oxidation in anaerobic sludge can contribute to sulfate reduction in anaerobic sludge samples. To assess whether methane oxidation in anaerobic sludge can contribute to sulfate reduction, anaerobic sludge was diluted, with the net change of methane in the presence and absence of sulfate quantified. In addition, the effects of the methane concentration, sludge dilution ratio, and acetate concentration on the AOM were evaluated.

**MATERIALS AND METHODS**

**Medium**

The basal medium (BME) consisted of 0.321 g/l \( \text{NH}_4\text{Cl} \) (Duksan Chemical Co. Ltd., Ansan, Korea), 0.489 g/l \( \text{KH}_2\text{PO}_4 \) (Duksan Chemical), 0.074 g/l \( \text{MgSO}_4\cdot7\text{H}_2\text{O} \) (Duksan Chemical), 0.024 g/l \( \text{NaCl} \) (Showa Chemical Co. Ltd., Tokyo, Japan), 0.095 g/l \( \text{CaCl}_2\cdot2\text{H}_2\text{O} \) (Samchun Pure Chemical Co. Ltd., Pyeongtaek, Korea), and 0.005 g/l \( \text{FeSO}_4\cdot7\text{H}_2\text{O} \) (Duksan Chemical), as well as a trace element solution (1 ml/l). The trace element solution contained 0.036 g/l \( \text{Na}_2\text{MoO}_4\cdot2\text{H}_2\text{O} \)
Effects of Methane and Acetate Concentrations on AOM

Effects of Sludge Dilution Ratio on AOM

Effects of Methane and Acetate Concentrations on AOM

Net Methane Oxidation With and Without Sulfate

Results and Discussion

Net Methane Oxidation in Anaerobic Sludge

Analytical Methods
Effects of Sludge Dilution Ratio

Fig. 2 shows the rates of the net AOM and formations of carbon dioxide and H$_2$S in serially diluted anaerobic sludge samples ($10^{-1}$–$10^{-2}$). When the sludge was serially diluted, the microbial community structure remained unchanged. Thus, the only differences between samples were the concentration of biomass, and cell density. At a 1/1 dilution rate, net methane formation was observed, and net methane oxidation per g-dry cell weight (DCW) increased with increasing dilution (Fig. 2A). The net change in the methane concentration was possibly caused by cell density effects only (or possibly cell lysis effects). The net methane formations of the 1/1 and 1/2 diluted samples were possibly due to cell lysis caused by the high cell densities. Meulepas et al. [11] showed that methane formation in anaerobic sludge or sediment samples was caused by the “endogenous” substrate. Moreover, the formation of H$_2$S indirectly indicates the amount of organic matter produced from cell lysis. Evidently, the rate of H$_2$S formation was relatively high with the lower diluted samples, but increasing the dilution decreased the formation of H$_2$S (Fig. 2B). The total formation of H$_2$S was also negatively correlated with dilution (data not shown). At a
1/100 dilution, the rate of H₂S formation was significantly decreased, whereas the net rate of AOM was significantly increased. The rate of AOM coincided positively with the rate of CO₂ formation, and the molar ratio of the AOM to CO₂ formation was 5:1 (Fig. 2C).

**Effects of Methane and Acetate Concentrations**

Fig. 3 shows the net rate of the AOM observed with different methane concentrations in the headspace. The net rate of the AOM was positively affected by the methane concentration, and the rate in the sample containing 15% methane was around 3.5-fold higher at day 8 compared with the samples with 5% methane. Meulepas et al. [11] also observed higher methane oxidation under higher methane pressure conditions, although no net methane oxidation was observed. The rate of H₂S formation in the sample with 15% methane was higher than that with 5% methane (data not shown).

According to Schilov et al. [16], acetoclastic methanogenesis can even be reversed at a methane pressure of 10 MPa in granular sludge consisting of Methanosarcina and Methanosaeta spp.-dominated mixed cultures. To assess the effects of acetate on the AOM, experiments were performed with 1/100 diluted sludge with acetate concentrations of 0, 10, 20, 50, and 100 mg/l (Fig. 4). The change of the methane concentration in the headspace was monitored. The results clearly showed that the AOM was affected by acetate addition. When acetate was not added, the net methane oxidation was highest and the net rate of the AOM decreased with increasing initial acetate concentration. At 50 mg-acetate/l, the oxidation and formation of methane were balanced, whereas net methane formation was observed at 100 mg/l. Although methanogens can generate methane with acetate, methane formation is limited under low acetate conditions, which drive the net oxidation of methane. In marine sediments, the concentrations of substrates, such as H₂ and acetate, are low, which reduces the chance of methane formation. Therefore, low substrate concentrations and high methane pressures stimulate the AOM in marine sediments. In contrast, Treude et al. [19] showed that the addition of acetate did not inhibit the AOM in marine sediment samples. In their experiment, the addition of acetate (under 10 mg-acetate/l) was too low for any inhibition of the AOM to be observed. Fig. 4 shows that no significant inhibition of the AOM was observed with the addition of acetate at between 0 and 20 mg/l. Meulepas et al. [11] reported that higher methane pressures inhibited methane formation; thus, high acetate concentrations could possibly hamper the AOM.

In conclusion, this is the first report observing net methane oxidation in anaerobic sludge. The rate of dilution and acetate concentration were observed to affect the AOM and production of methane, and suggest reasons why previous studies could not observe net methane oxidation in anaerobic sludge samples, and how the cell density and organic matter affect the AOM. Therefore, the presence of an organic substrate or methane in various environments presumably determines the methane oxidation or formation.

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References