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Research article

Evaluation of the influence of methane and copper concentration and methane mass transport on the community structure and biodegradation kinetics of methanotrophic cultures

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ABSTRACT

The environmental conditions during culture enrichment, which ultimately determine its maximum specific biodegradation rate (q_{max}) and affinity for the target pollutant (K_s), play a key role in the performance of bioreactors devoted to the treatment of methane emissions. This study assessed the influence of Cu^{2+} and CH_4 concentration and the effective CH_4 supply rate during culture enrichment on the structure and biodegradation kinetics of methanotrophic communities. The results obtained demonstrated that an increase in Cu^{2+} concentration from 0.05 to 25 μM increased the q_{max} and K_s of the communities enriched by a factor of ≈3, even if the Cu^{2+} concentration did not seem to have an effect on the enzymatic "copper switch" and only pMMO was detected. In addition, high Cu^{2+} concentrations supported lower diversity coefficients (Hs ≈ 1.5× lower) and apparently promoted the growth of more adapted methanotrophs such as *Methylomonas*. Despite no clear effect of CH_4 concentration on the population structure or on the biodegradation kinetics of the communities enriched was recorded at the two low CH_4 concentrations studied (1 and 8%), a higher agitation rate increased the q_{max} by a factor of ≈2.3 and Ks by a factor of ≈3.1.

1. Introduction

Methane (CH_4) is the second most relevant greenhouse gas (GHG) emitted by anthropogenic activities, representing more than 20% of the total worldwide GHG emissions. CH_4 atmospheric concentration increases yearly at 0.2–1% its current tropospheric level exceeding preindustrial concentrations by ≈150%. Anthropogenic emissions are mainly attributed to agriculture, livestock farming, waste management and energy production, which combined represent over 60% of the total CH_4 emissions worldwide (European Environmental Agency, 2013; IPCC, 2013; United States Environmental Protection Agency, 2013). In addition, the impact of CH_4 on climate change is ≈34 times more detrimental than that of carbon dioxide (CO_2) in a 100-y horizon (European Environmental Agency, 2013; IPCC, 2013; Scheutz et al., 2009). This scenario has caused a raised governmental and public awareness, which has promoted both the enforcement of multidisciplinary political initiatives and an increased research on CH_4 abatement technologies (European Environmental Agency, 2013; IPCC, 2013; Scheutz et al., 2009). Although emissions with CH_4 concentrations above 20% are suitable for energy recovery by incineration or low-cost treatment by flaring, more than 50% of anthropogenic emissions contain CH_4 at concentrations below 3% (old landfills (0–20%), ventilated coal mines (0.1–1%), covered liquid manure storage tanks (0–3%), etc.) (Nikiema et al., 2007; Scheutz et al., 2009). CH_4 abatement at such low concentrations using conventional physical/chemical technologies is either inefficient or too costly, and often entails a large CO_2 footprint (Estrada et al., 2014; Nikiema et al., 2007). In this context, biological treatment technologies can become, if appropriately optimized, a low-cost and environmentally friendly alternative for the treatment of CH_4 due to their already proven effectiveness and low operating costs during the abatement of malodors and volatile organic compound emissions (López et al., 2013).

However, the cost-effective implementation of current biotechnologies for the treatment of CH_4 is still limited by the understanding of the communities underlying CH_4 biodegradation (Li et al., 2014; López et al., 2013). In this regard, there is still a need for studies assessing the effect of environmental factors during culture

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enrichment on the performance and characteristics of methanotrophic communities. Among them, CH_4 gas concentration, O_2 gas concentration and Cu^{2+} concentration in the aqueous media have been identified as the main parameters influencing microbial CH_4 abatement due to their key role on the nature and level of expression of CH_4 monooxygenases (MMO), and therefore on the population structure of methane oxidizing bacteria (MB) (Li et al., 2014; Murrel et al., 2000; Semrau et al., 2010). However, most studies assessing the role of CH_4 and Cu^{2+} concentrations have focused only on the physiological and enzymatic aspects of CH_4 biodegradation (Murrel et al., 2000). On the contrary, studies on the macroscopic performance and characteristics of the microbial communities have not found a clear effect of these parameters on CH_4 abatement (Estrada et al., 2014; Ho et al., 2013; Li et al., 2014). Likewise, the influence of the effective CH_4 supply rate to the microbial communities during culture enrichment on the kinetics of CH_4 biodegradation, which can differ at similar gas CH_4 concentrations depending on the bioreactor configuration, has often been disregarded (López et al., 2014, 2013; Yoon et al., 2009). In this context, there is a limited understanding of the effect of CH_4 and Cu^{2+} concentration and of the effective CH_4 supply rate during culture enrichment on the characteristics and structure of methanotrophic communities (Semrau et al., 2010), which could shed light on the optimal operating conditions leading to an enhanced performance of CH_4 abatement biotechnologies.

The present study aims at systematically elucidating the influence of CH_4 and Cu^{2+} concentrations and of the effective CH_4 supply rate (governed by the CH_4 mass transfer to the microbial community) during the enrichment of MB communities on CH_4 biodegradation kinetic parameters (q_{max} and Ks) and community structure in order to identify the optimum environmental factors supporting an efficient CH_4 abatement and high biodiversity in full scale bioreactors.

2. Materials and methods

2.1. Chemicals and mineral salt medium

The mineral salt medium (MSM) utilized during the enrichment of the methanotrophic communities and in the kinetic assays was a modified Brunner medium (Lopez et al., 2014) containing (g L^{-1}): $Na_2HPO_4 \cdot 2H_2O$, 3.17; KH_2PO_4, 1.50; $NaNO_3$, 5.28 (used instead of $(NH_4)_2SO_4$ to avoid the inhibition of methanotrophs by NH_4 (Carlsen et al., 1991)); $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.05; EDTA, 0.005; $FeSO_4 \cdot 7H_2O$, 0.002; H_3BO_3, 0.0003; $CoCl_2 \cdot 6H_2O$, 0.00011; $ZnSO_4 \cdot 7H_2O$, 0.0001; $Na_2Mo_4 \cdot 2H_2O$, 0.00003; $MnCl_2 \cdot 4H_2O$, 0.00003; $NiCl_2 \cdot 6H_2O$, 0.00002. Cu^{2+} was added to the MSM as $CuCl_2 \cdot 2H_2O$ at two Cu^{2+} concentrations (0.05 μM and 25 μM). The final pH of the MSM was 7. All chemicals and reagents were procured from Panreac (Barcelona, Spain) with a purity higher than 99.0%. CH_4 was purchased from Abello-Linde, S.A. (Barcelona, Spain) with a purity of at least 99.5%. Silicone oil 200 cSt was obtained from Sigma-Aldrich (Madrid, Spain).

2.2. Inoculum

The inoculum used for the enrichment of the methanotrophic communities was a mixture (50/50%/% on a volume basis) of aerobic activated sludge ($\approx$6 g L^{-1}) from a denitrification-nitrification wastewater treatment plant (Valladolid, Spain) and fresh cow manure from a dairy farm (Cantabria, Spain) 10× diluted in MSM.

2.3. Microbial community enrichments

Eight enrichment series (45 days/enrichment) were carried out at two different mixing ratios of CH_4 (1% and 8%) and Cu^{2+} (0.05 μM and 25 μM) under two different magnetic stirring rates (300 rpm and 650 rpm). The series were performed in duplicate (Table 1).

In each enrichment series, 1.2 L batch gas-tight reactors containing 100 mL of MSM at its corresponding Cu^{2+} concentration and 50 mL of silicone oil (in order to support a high CH_4 mass transfer from the headspace to the microbial community), were inoculated with 10 mL of the inoculum previously described. The reactors were closed with gas-tight butyl septa and plastic screw caps, and monitored daily for O_2, CO_2 and CH_4 concentrations in the headspace. O_2 was daily supplied via air flushing of the reactor headspace prior injection of pure CH_4 using a calibrated 100 mL gas tight syringe to obtain the desired headspace concentration (before methane injection the same volume of air was removed from the reactors to avoid overpressure). The enrichment batch reactors were maintained under agitation at 25 °C. A dilution rate of 0.25 ± 0.05 day^{-1} was set in order to maintain the pH at ≈7.0, to replenish essential nutrients and, to remove any potential inhibitory metabolite accumulated in the medium. Silicone oil was not removed from the batch reactors during the enrichment series. Biomass samples from the aqueous phase were drawn with a liquid sampling syringe at the end of each enrichment series to determine the microbial population structure by denaturing gradient gel electrophoresis (DGGE)-sequencing, the presence of soluble or particulate methane monoxygenases (sMMO or pMMO, respectively), and the CH_4 biodegradation kinetics constants (q_{max} and Ks). The concentration of CH_4 and CO_2 in the headspace of the enrichment bottles was periodically measured by GC-TCD.

2.4. Structure of the enriched communities

The 16 samples from the 8 duplicate enrichment series stored at −20 °C were thawed and centrifuged at 5000 rpm for 15 min. The biomass pellets were resuspended in 10 mL of phosphate buffered saline (PBS) medium at pH 7 by vortexing.

The total DNA of these samples was extracted by the Fast® DNA Spin Kit for Soil (MP Biomedicals, LLC) and its quantity and quality were evaluated by spectrophotometry using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) (English et al., 2006). The DNA was amplified with polymerase chain reaction (PCR) universal bacterial primers for 16S rRNA gene amplification (968-F-GC and 1401-R (10 μM)) (Sigma- Aldrich, St. Louis, MO, USA) according to Lopez et al. (2014). The PCR products of the bacterial 16S rRNA fragments from the samples were separated by DGGE according to Lopez et al. (2014). The gels were stained for 60 min with GelRed Nucleic Acid Gel Stain (biotium). Specific PCR-DGGE bands were detected by a transilluminator, UV wavelength, 254–312 nm (Sigma-Aldrich) and carefully cut from the gel. The DNA contained in each band was extracted by incubation in 50 mL of sterile water at 63 °C for 70 min. The last PCR cycle was accomplished without the GC-clamp attached to the primer 968-F. The PCR products obtained were sequenced by

Table 1
Cultivation conditions during microbial community enrichments.

Test series (TS)	CH_4 headspace (%)	Cu^{2+} (μM)	Agitation (rpm)
TS 1	8	25	300
	8	25	650
TS 2	8	0.05	300
	8	0.05	650
TS 3	1	0.05	300
	1	0.05	650
TS 4	1	25	300
	1	25	650