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Characterization of tobermolite as a bed material for selective growth of methanotrophs in biofiltration

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ABSTRACT

Tobermolite was characterized as a bed material for methanotrophic biofiltration. A lab-scale biofilter packed with tobermolite was operated for different operation times under identical conditions. The three different runs showed similar acclimation patterns of methane oxidation, with methane removal efficiency increasing rapidly for the first few days and peaking within three weeks, after which the efficiency remained stable. The mean methane removal capacities ranged from 766 g m^{-3} d^{-1} to 974 g m^{-3} d^{-1} after acclimation. Pyrosequencing indicated that the methanotrophic proportion (methanotroph/bacteria) increased to 71–94% within three weeks. Type I methanotrophs *Methylocaldum* and *Methylosarcina* were dominant during the initial growth period, then *Methylocaldum* alone dominated the methanotrophic community. A community comparison showed that total bacterial and methanotrophic communities were temporally stable after the initial growth period. Quantitative PCR showed that methanotrophic density increased during the first 3–4 weeks, then remained stable over 120 days. Tobermolite can provide a special habitat for the selective growth of methanotrophs, resulting in rapid acclimation. Tobermolite also allows the microbial community and methanotrophic density to remain stable, resulting in stable methane biofiltration.

1. Introduction

Biological filtration is used for methane mitigation from landfills (Scheutz et al., 2009) and animal husbandries (Melse and van der Werf, 2005). Methanotrophs (aerobic methane-oxidizing bacteria), which utilize methane as a sole carbon and energy source, are used as a catalyst for methane in filtration (Delhoménie and Heitz, 2005; Semrau et al., 2010). Therefore, methanotrophs should become well established and active for successful methane removal. Filter beds are the most important component of biofiltration since they immobilize microorganisms that degrade the filtered compounds and provide support for the growth of microorganisms (Delhoménie and Heitz, 2005). Methanotrophic biofilter bed materials must be supportive for the growth of methanotrophs.

Bed materials used in methane biofiltration can be classified into two major groups: biologically active and inactive materials. Biologically active materials including soil, peat, wood chip and compost were common in prior studies on methane biofiltration (Nikiema et al., 2007). In general, they have significant biological complexity (i.e., trophic interaction and microbial competition) and/or chemical complexity (i.e., various nutrients and chemicals), in addition to supplying nutrients for microbial growth. Biologically inactive materials play a role in immobilizing microorganisms to form a biofilm on the material surface. Inactive materials, such as gravel, perlite, granular activated carbon and glass tubes, have also been used to prevent biological and chemical complexities originating from bed materials (Kim et al., 2012a; Melse and van der Werf, 2005; Nikiema et al., 2007; Sly et al., 1993). Selective growth of the desirable microorganisms on filter bed materials can provide many advantages, e.g., easy use, operation and maintenance. Selective growth of methanotrophs is more likely achieved using biologically inactive materials due to its low biological complexity. For instance, Plessis et al. (2003) reported that known methanotrophs were not retrieved from a biofilter packed with a mixture of compost and perlite, since the intrinsic complexity of the compost may have provided low selectivity for the growth of methanotrophs. To the best of our knowledge, the greatest degree of selectivity (75% of the total bacteria) was observed from an inorganic material by Nikiema et al. (2005).

Tobermolite has been found to be a potential inorganic filter bed material for methane filtration, with selectivity for methanotrophic growth (Jeong et al., 2013). Tobermolite is amorphous and porous, with characteristics such as high specific surface area, porosity, durability and moisture retention capacity (Jeong et al., 2013). In addition, tobermolite ($5CaO{\cdot}6SiO_2{\cdot}5H_2O$), which is synthesized with CaO and SiO_2, is also a building material for heat insulation. Thus, it is inexpensive and readily available. Tobermolite has also been used in the removal of ions due to its high

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sorption capacity (Ichiba and Ohne, 1995). The above characteristics satisfy the majority of the criteria needed for biofilter bed materials (Nikiema et al., 2007). The main purpose of this study was to evaluate tobermolite as a bed material for methanotrophic biofiltration in terms of methanotrophic performance and growth. In addition, to evaluate the start-up of tobermolite biofilter, the acclimation pattern of methane oxidation was determined. For these, there were three independent biofilter runs using fresh tobermolite as a bed material. Firstly, a 21-day run was used to evaluate the selectivity of methanotrophs on tobermolite. A 68-day run was used to determine the methanotrophic performance and microbial community dynamics. A 145-day run was used to determine the population dynamics of methanotrophs.

2. Materials and methods

2.1. Tobermolite

Tobermolite (with a diameter of 4–8 mm) was obtained from JawooBio (Daejeon, Korea) and was thoroughly washed with tap water and air-dried. The water holding capacity, pH, bulk density, skeletal density, porosity, surface area and intrusion volume of the tobermolite are $109.5 \pm 5.1\%$, 7.0 ± 0.1, $0.276 \pm 0.000\,g\,cm^{-3}$, $2.281 \pm 0.030\,g\,cm^{-3}$, $72.1 \pm 0.7\%$, $119.1 \pm 5.3\,m^2\,g^{-1}$ and $1.131 \pm 0.038\,mL\,g^{-1}$, respectively. Noticeably, the surface area of tobermolite is comparable to those of clay minerals, such as illite and montmorillonite (Macht et al., 2011). It was confirmed using a batch experiment that tobermolite is unable to absorb methane.

2.2. Preparation of a lab-scale biofilter

A laboratory-scale biofilter was made using cylindrical acrylic resin (Kim et al., 2013b). The biofilter consisted of three parts: a packing section, watering system and drainage container. The height and inner diameter of the packing section were 100 and 8 cm (approximately 5 L), respectively. The packing section contained a perforated plate at the bottom in order to allow gases to evenly spread. The filter material was packed into the packing section, and the working volume was 5 L. The watering system, which was 20 cm in height with an 8 cm inner diameter, contained a water inlet port at the top and a perforated plate 5 cm away from the inlet. The volume of the drainage container, which was 20 cm in height with a 15 cm inner diameter, was approximately 3.5 L. The three parts were assembled (for the watering system-packing section-drainage container) using rubber packing and six bolts/nuts. After set-up, the gas tightness of the reactors was verified with water and compressed air. A synthetic gas composed of methane (99.9%, Seoul Gas Inc., Seoul, Korea) and compressed air was humidified by passing through a 50-cm-long humidifier. Gas flows were controlled with commercial flow meters (Dwyer, Michigan City, USA and Kofloc, Kyoto, Japan).

2.3. Runs of the tobermolite methane biofilter

There were three individual runs with different time periods (21, 68 and 145 days). For each run, fresh tobermolite was used as a packing material.

A 21-day run was used to determine the selectivity of methanotrophs on tobermolite. Inoculum was prepared from the packing material (perlite and granular activated carbon, 10:1, w:w) of a lab-scale methanotrophic biofilter (Kim et al., 2012a), which had been overwintered without a methane supply. The packing material was thoroughly mixed with nitrate mineral salts (NMS) medium (1:10, w:w), which contained 1 g of $MgSO_4{\cdot}7H_2O$, 0.295 g of $CaCl_2{\cdot}2H_2O$, 1 g of KNO_3, 0.26 g of KH_2PO_4 and 0.41 g of $Na_2HPO_4{\cdot}2H_2O$ in 1 L (Whittenbury et al., 1970). $CuSO_4$ was added to a final concentration of 30 μM. After mixing, a static condition was maintained for 30 min. The supernatant was used as the inoculum for the biofilters. The inoculum (2.5 L) was added into the drainage container and circulated 4 times per day for 7 days to allow cell attachment to the packing materials. The synthetic gas (air and CH_4) was continuously introduced into the bottom of the biofilters at flow rates of 250 mL min^{-1} (corresponding to retention times of 20 min). Methane inlet concentrations were maintained at 50,000 ppm. The biofilter was operated at $20 \pm 5\,^{\circ}C$ with the modified NMS medium containing 1 g of $MgSO_4{\cdot}7H_2O$, 0.295 g of $CaCl_2{\cdot}2H_2O$, 10 g of KNO_3, 2.6 g of KH_2PO_4 and 4.1 g of $Na_2HPO_4{\cdot}2H_2O$ in 1 L (30 μM $CuSO_4$). During operation, the medium was circulated 4 times per day and replaced every week. Tobermolite samples were collected from the biofilter at days 14 and 21.

A 68-day run was used to determine the methanotrophic performance and microbial community dynamics of the tobermolite biofilter. Inoculum was a fresh drain from the perlite methane biofilter. The drain (2.5 L) was added into the drainage container and circulated 4 times per day for 7 days to allow cell attachment to the packing material. The operation conditions were identical to those of the 21-day run. For methane mineralization rate, CH_4 and CO_2 concentrations were measured at the inlet and outlet ports. Tobermolite samples were collected from the biofilter at days 21, 28 and 68.

A 145-day run was used to determine the population dynamics of methanotrophs in the tobermolite biofilter. The inoculum was the same as that of the 21-day run. Tobermolite samples were collected from the biofilter at days 26, 56, 75, 97, 107, 121 and 145.

2.4. Gas analysis

Gas was sampled from the inlet and outlet of the biofilter using a 0.5-mL gastight syringe (Hamilton, Reno, USA) equipped with Teflon minnert fitting. Methane concentration was monitored by gas chromatography (GC, 6850N, Agilent Technologies, Santa Clara, USA), which was equipped with a flame ionization detector and a wax column (30 m × 0.32 mm × 0.25 μm, Supelco, Bellefonte, USA). N_2 was used as the carrier gas. The temperatures of the oven, injector and detector were fixed at 100, 250 and 250 °C, respectively. CO_2 concentration was monitored by GC (6890N, Agilent), which was equipped with an HP-PLOT/Q column (30 m × 0.53 mm × 40 μm) and a thermal conductivity detector. The oven, injector and detector temperatures were set at 60, 100 and 250 °C, respectively.

2.5. DNA extraction for pyrosequencing analysis

A 10-g tobermolite sample was added to a sterile conical tube (50 mL) containing 20 mL of sterile saline solution (0.9%). Ultrasonication was performed for 5 min using a Q500 ultrasonic processor (Qsonica, Newton, USA). The tube was placed in a bucket containing ice to prevent any thermal effects caused by the ultrasound, and the horn tip (6 mm diameter) of the processor was immersed into the solution at a depth of 1 cm. The actual power delivered to the suspension was 10 W at a fixed frequency of 20 kHz. A 1.5-mL suspension was transferred to a 1.5-mL microtube and centrifuged at 16,000 × *g* for 10 min. The supernatant was discarded from the tube. For DNA extraction from inoculums, 1.5-mL suspensions were collected and transferred to 1.5-mL microtubes. They were centrifuged at 16,000 × *g* for 5 min, and the supernatant was discarded from the tubes. DNA was extracted using the NucleoSpin Soil kit (Macherey-Nagel GmbH, Düren, Germany), with a modification that the samples were disrupted using a BeadBeater-8 system (BioSpec, Bartlesville, USA) at 5,000 rpm for 30 s. DNA was eluted in 100 μL of the elution buffer and stored at −20 °C prior to use. DNA