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# Anaerobic oxidation of methane coupled to thiosulfate reduction in a biotrickling filter

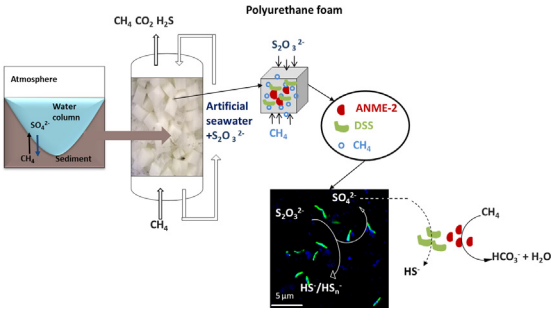
Chiara Cassarini [a], Eldon R. Rene [a,*], Susma Bhattarai [a], Giovanni Esposito [b], Piet N.L. Lens [a]

[a] UNESCO-IHE, Institute for Water Education, PO Box 3015, 2601 DA Delft, The Netherlands
[b] University of Cassino and Southern Lazio, Department of Civil and Mechanical Engineering, via Di Biasio 43, 03043 Cassino, FR, Italy

## HIGHLIGHTS

- A BTF was tested for anaerobic methane oxidation in the presence of thiosulfate.
- Thiosulfate disproportionation was observed in the BTF.
- The DSS population was enriched after long term (213 days) BTF operation.
- Thiosulfate reduced the reactor start-up time and increased the sulfate reduction rate.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Microorganisms from an anaerobic methane oxidizing sediment were enriched with methane gas as the substrate in a biotrickling filter (BTF) using thiosulfate as electron acceptor for 213 days. Thiosulfate disproportionation to sulfate and sulfide were the dominating sulfur conversion process in the BTF and the sulfide production rate was 0.5 mmol $l^{-1}$ $day^{-1}$. A specific group of sulfate reducing bacteria (SRB), belonging to the *Desulforsarcina/Desulfococcus* group, was enriched in the BTF. The BTF biomass showed maximum sulfate reduction rate (0.38 mmol $l^{-1}$ $day^{-1}$) with methane as sole electron donor, measured in the absence of thiosulfate in the BTF. Therefore, a BTF fed with thiosulfate as electron acceptor can be used to enrich SRB of the DSS group and activate the inoculum for anaerobic oxidation of methane coupled to sulfate reduction.



## 1. Introduction

Sulfate and other sulfur oxyanions, such as thiosulfate, sulfite or dithionite, are contaminants discharged in fresh water due to industrial activities such as food processing, fermentation, coal mining, tannery and paper processing. Biological treatment of these wastewaters has been successfully applied wherein the sulfur oxyanions are anaerobically reduced to sulfide, which is then either oxidized to elemental sulfur or precipitated as metal sulfide (Liamleam and Annachhatre, 2007; Weijma et al., 2006). Many sulfate rich wastewaters are deficient in electron donor and the addition of an external carbon source is often required to achieve complete sulfate reduction by sulfate reducing bacteria (SRB). Electron donors such as ethanol, methanol, hydrogen, acetate, lactate and propionate are usually supplied, but these increase the operational and investment costs (Bhattarai et al., 2017; Meulepas et al., 2010). Therefore, the use of easily accessible and low-priced electron donors such as methane is appealing (Gonzalez-Gil et al., 2011). Moreover, methane is also a well-known green house gas (GHG) and its increase in atmospheric concentration could have large implications for future climate change (Forster et al., 2007).

* Corresponding author.
*E-mail address:* e.raj@unesco-ihe.org (E.R. Rene).

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Besides, the surface layers of wetlands, sediments, paddy fields and several other terrestrial and aquatic surfaces are known to produce methane and hence, reducing its concentration in the atmosphere is thus important (Kirschke et al., 2013).

Anaerobic oxidation of methane (AOM) coupled to sulfate reduction is a naturally occurring process in anaerobic environments, such as in marine sediments. This process is mediated by a special group of slow growing and so far uncultured anaerobic methanotrophs (ANME) and SRB that can thrive in harsh environments by using the abundance of methane and $H_2S$ present in such habitats. ANME are grouped into three distinct clades, i.e. ANME-1, ANME-2 and ANME-3. The common SRB associated with ANME are *Desulfosarcina/Desulfococcus* (DSS) and *Desulfobulbaceae* (Schreiber et al., 2010).

The main challenge of using AOM coupled to sulfate reduction (AOM-SR) as a process for methane removal and desulfurization of wastewater is the slow growth rate of the microorganisms involved (Meulepas et al., 2009; Zhang et al., 2010). The highest AOM-SR rates reported so far in the literature (0.6 mmol $l^{-1}$ $day^{-1}$, Meulepas et al., 2009) are too low (~100 times lower) to economically compete with the electron donors hydrogen or ethanol (Meulepas et al., 2009; Suarez-Zuluaga et al., 2014). The AOM-SR rates could be increased by using more thermodynamically favorable sulfur compounds, such as thiosulfate (Eq. (1)) or by growing them in a bioreactor with high biomass retention capability, such as membrane bioreactors (Meulepas et al., 2009).

$$S_2O_3^{2-} + CH_4 \rightarrow 2HS^- + HCO_3^- + H^+ \qquad \Delta G^{0\prime} = -39\ kJ/mol \quad (1)$$

In this study, the biotrickling filter (BTF), a reactor type commonly used in waste gas treatment but so far never been tested in AOM studies, was used to enrich the microorganisms involved in AOM coupled to thiosulfate reduction and to increase the rates of sulfide production and methane oxidation. The inoculum used was collected from an active AOM site (Alpha Mound, Gulf of Cadiz). However, the *in situ* or *ex situ* AOM-SR rate of the Alpha Mound sediment has not yet been estimated and the specific group of microorganisms involved has not yet been investigated.

The polyurethane foam was used as the packing material of the BTF because of its high porosity, good biomass retention capacity and its ability to enhance gas to liquid mass transfer of the poorly soluble methane by increasing gas-liquid mixing and retaining methane in the pores (Aoki et al., 2014; Estrada et al., 2014). The carbon and sulfur bioconversions of the consortia growing on the polyurethane foam and the possible abiotic processes were assessed with the help of batch tests and the microorganisms enriched in the BTF after long term operation (213 days) were visualized and identified.

## 2. Material and methods

### 2.1. Source of sediment biomass

Sediment samples were obtained from the Alpha Mound (35°17.48′N; 6°47.05′W, water depth ca. 528 m), Gulf of Cadiz (Spain), during R/V Marion Dufresne Cruise MD 169 MICROSYSTEMS to the Gulf of Cadiz in July 2008. The Gulf of Cadiz is located in the eastern Atlantic ocean, North West of the Strait of Gibraltar, along the Spanish and Portuguese continental margin (Niemann et al., 2006). This is an area of mud volcanism and gas venting through the seafloor. Moreover, cold-water coral carbonate mounds, such as the Alpha Mound, have been discovered at the Pen Duick escarpment on the Moroccan margin (Maignien et al., 2010). In previous studies, the Alpha Mound showed evidence for the presence of a shallow sulfate-methane transition zone at ~300 cm sediment depth with increased sulfate reduction rates indicating the presence of microbial mediated AOM (Templer et al., 2011).

Sediment samples were recovered by gravity coring from Alpha Mound, retrieving up to 4.3 m of sediment. Gravity cores were sectioned into 1 m sections and immediately stored at 4 °C. The cores were then opened, subsampled (the sampling interval for all parameters was 10 to 20 cm) (Templer et al., 2011; Wehrmann et al., 2011), capped and stored at 4 °C in trilaminate polyetherimide coated aluminum bags (KENOSHA C.V., Amstelveen, The Netherlands) under nitrogen rich atmosphere (Zhang et al., 2010). The sediment used in this study was retrieved from 250 to 270 cm below the sea floor and was stored at 4 °C with a headspace of methane for five years before it was inoculated into the BTF.

### 2.2. Composition of the artificial seawater medium

The artificial seawater based liquid medium used in the BTF had the following composition per liter of demineralised water (Zhang et al., 2010): NaCl (26 g), KCl (0.5 g), $MgCl_2{\cdot}6H_2O$ (5 g), $NH_4Cl$ (0.3 g), $CaCl_2{\cdot}2H_2O$ (1.4 g), $Na_2S_2O_3$ (1.6 g), $KH_2PO_4$ (0.1 g), trace element solution (1 ml), 1 M $NaHCO_3$ (30 ml), vitamin solution (1 ml), thiamin solution (1 ml), vitamin $B_{12}$ solution (1 ml), 0.5 g $L^{-1}$ resazurin solution as a redox indicator (1 ml) and 0.5 M $Na_2S$ solution (1 ml). The vitamins and trace element solution were prepared according to the protocol outlined by Widdel and Bak (1992). The pH was adjusted to 7.0 with sterile 1 M $Na_2CO_3$ or $H_2SO_4$ solutions, which was stored under nitrogen atmosphere. All chemicals were purchased as lab grade in anhydrous form from Fisher Scientific (Sheepsbouwersweg, the Netherlands). The medium was kept anoxic with the help of nitrogen purging until it was recirculated within the BTF.

### 2.3. BTF set-up and operation

The BTF (Fig. 1) consisted of an acrylic pipe (height 32 cm and diameter 55 mm), sealed air-tight to prevent leakage or air intrusion during its operation. The filter bed volume of the reactor was 0.4 L, which was packed with polyurethane foam cubes of 1 $cm^3$ (98% porosity and a density of 28 kg $m^{-3}$) and 20 ml of the sampled Alpha Mound sediment (0.03 ± 0.01 g volatile suspended solids). Two circular acrylic sieve plates (pore size of 3.5 mm) were placed at the bottom and top of the BTF to hold the polyurethane foam pieces (Fig. 1).

The BTF was operated in sequential fed-batch mode for the influent (artificial seawater), while the methane gas (99.5% methane, Linde gas, Schiedam, the Netherlands) stored in Tedlar bags was continuously supplied to the bioreactor using a peristaltic pump (Verder International BV, Utrecht, the Netherlands) at a flow rate of 2 ml $min^{-1}$. The estimated empty bed residence time of methane was 200 min. The BTF was operated in a counter-current mode: the gas was passed from the bottom of the BTF to the top, while the seawater medium was recirculated from the top to the bottom. The medium trickled uniformly over the entire cross sectional area of the packing through a spray head having a pore size of 4.0 mm. The trickled medium flowed into the nutrient holding tank (1.5 L), which was then continuously recirculated to the BTF with the help of a Masterflex S/L peristaltic pump (Metrohm Netherlands B.V., Schiedam, the Netherlands) operating at a flow rate of 10 ml $min^{-1}$ (Fig. 1).

The BTF was operated for 213 days and it was maintained in the dark and at room temperature (~20 ± 2 °C). During BTF operation, the seawater medium containing 10 mM thiosulfate was replaced periodically (days 38, 104, 139, 189 and 206, Fig. 2), on day 91 thiosulfate was added to the not refreshed medium (Fig. 2) and from days 46 to 88 the BTF was operated in the absence of thiosulfate