Anaerobic Propane Oxidation for Biological Sulphate and Thiosulphate Reductions

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ABSTRACT

The existence of microbial populations that use short chain alkanes (ethane, propane and butane) as potential electron donors for the reduction of sulphate has been recently reported. The use of sulphur compounds in many chemical processing leads wastewaters containing high concentration of sulphate and thiosulphate. Batch experiments were studied to determine the ability of mixed sediment cultures from Aarhus and Eckernförde Baytoanaerobically reduce sulphate and thiosulphatecoupled to propane as electron donor.In the presence of propane, sulphide production from all sulphate and thiosulphate bottles was higher than the sulphide production when the propane was not added.

***Keywords*:** anaerobic oxidation, sulphur compounds, sulphide production

INTRODUCTION

The occurrence of anaerobic methane oxidation (Amethox) in anaerobic marine waters and sediments has been reported during geochemical in situ studies (Barnes and Goldberg, 1976; Reeburgh, 1976 and 1980; Alperin*et al.,* 1988). An obligate syntrophic interaction between a reversed methanogenicarchaeon and a sulfate-reducing bacterium (SRB) has been considered as responsible process for Amethox (Valentine and Reeburgh, 2000; StrousandJetten, 2004). Studies have reported that Amethox conversion rates in different marine sediments are between 0.001 µmol g dry weight^{-1} day^{-1} (North Sea) and 20.9 µmol g dry weight^{-1} day^{-1} (Black Sea) (Kruger *et al.,* 2005; Treude*et al.,* 2007).

However, some biogeochemical studies at hydrocarbon seep sites have recently detected that sulphate reduction rate (SRR) exceeds Amethox rates (Joye*et al.,* 2004; Niemann*et al.,* 2006; Orcutt*et al.,* 2010). This indicates that sulphate reduction (SR) at marine sediments might also be potentially influenced with anaerobic biodegradation of non-methane hydrocarbons. Furthermore, the existence of microbial populations that use short chain alkanes (SCA) (ethane, propane and butane) as potential electron donors for the reduction of sulphate has been recently studied (Kniemeyer, 2007; Savage *et al.,* 2010; Jaekel*et al.,* 2012). Moreover, strain BuS5 has been isolated, and is reported to be capable of using propane as electron donors coupled to SR (Kniemeyer et al., 2007; Jaekel*et al*., 2012).

Besides sulphate, the majority of the sulphate reducers can also utilize thiosulphateas substrate (Widdel*et al.,* 2007). The widely use of sulphuric acid in many industrial processes, generates wastewaters containing high levels of sulphate (Zub*et al.,* 2008). Besides, in chemo-thermomechanical pulping (CTMP) process, thiosulphate is also present in pulp bleaching wastewater (Lens *et al.,* 1998). In addition, the capability of anaerobic propane oxidation coupled to sulphate and thiosulphate is still very limited and has not received much attention.

Economically, with the application of propane as electron donor for biological different sulphur compounds instead of hydrogen would allow to reduce the operational costs of wastewater treatment due to a four times cheaper price of propane when compared to hydrogen (www.fsec.ucf.edu). This study assessed the feasibility of propane as electron donor for biological sulphate and thiosulphate reductions.

MATERIALS AND METHODS

The origin of biomass

The biomass used for inoculation was originated from sediment of Aarhus and Eckernförde Bay. The sampling site and sampling method in Eckernförde Bay have been described by Treude*et al.* (2005) and Meulepas*et al*. (2010) and in Aarhus Bay have been reported by Jensen and Laier (2003). The sediment from Aarhus and Eckernförde Bay was stored with the medium of sulphate
nately. The sediment was mixed with medium
erobic conditions.

Standard incubation procedure

The batch experiments were done in serum bottles of 250 ml for sulphate and of 125 ml for thiosulphateunder an anoxic condition. The bottles were closed with butyl rubber stoppers and caps. Before medium and biomass stock were injected to the bottles by syringe, the exact weight and volume of all bottles were first determined and the oxygen gas in the bottles was removed by flushing for ten times with helium gas. The total liquid volume (medium and biomass) for each bottle is half of bottle volume. The headspaces were made vacuum and filled with 1.5 to 1.9 bar of propane. The experiments were conducted in triplicate. In addition, two bottles without propane gas for each sulphur compound were made and observed as controls. Cultures were grown anaerobically with the pH between 7.2 and 7.8. The bottles were incubated at temperature 15^{o}C and shaken at 80 rpm.

Medium

The batch bottles were fed with synthetic medium consisted of :NaCl (26.4 g L^{-1}), $MgCl_2$. $6H_2O$ (5.6 g L^{-1}), $CaCl_2$. $2H_2O$ (1.47 g L^{-1}), KCl (0.66 g L^{-1}), KBr (0.09 g L^{-1}), NH_4Cl (0.2 g L^{-1}), a vitamin solution (1 mL L^{-1}), a thiamin solution (1mL L^{-1}), a riboflavin solution (1 mL L^{-1}), a trace element solution (3 mL L^{-1}), a selenite-tungstate solution (1 mL L^{-1}), a KH_2PO_4 solution (1 mL L^{-1}), a $NaHCO_3$ solution (30 mL L^{-1}), a Na_2S solution (1 mL L^{-1}) and demineralized water. Prior to the addition of additional solutions, synthetic medium was deoxygenised with nitrogen. The concentration of sulphate and thiosulphate used in the medium is 28 and 14 mM respectively.

ANALYTICAL METHODOLOGIES

Sulphate and thiosulphate

The sulphate and thiosulphate concentration were measured with on DIONEX ICS2100 ionic chromatography (IC) system with the injection volume was 25 µl. The analytical and guard columns were IonPac AS19 and AG19 respectively with inner diameter is 4 mm. The columns were operated at a temperature of 30°C with a flow rate of 1.0 ml min^{-1}. The eluent generation cartridge was performed on-line equipped with a KOH cartridge (Dionex P/N 058900) and deionized water as the carrier. Samples were diluted to appropriate concentration ranges before injection into the chromatograph. A 0.2 ml of sample was first mixed with a 0.2 ml ZnAcetate solution 1 M and centrifuged to separate the liquid from the solids. A 1.62 ml of manitol (1 M) was then added to a 0.18 ml of centrifuged sample in an IC-flask. Manitol stabilizes partly oxidized sulphur compounds. The retention time of sulphate was 20.3 min and for thiosulphate was 24.13 min.

Hydrogen sulphide

Hydrogen sulphide was quantified colorimetrically in a reaction yielding methylene blue using DR Lange kits (LCK 653) and a photo spectrometer (XION 500). This method measures all the dissolved sulphide compounds (H2S, HS^- and S^{2-}). Liquid samples were taken with syringe and needle, which were washed with helium gas to prevent the oxygen in the syringe and needle to enter the batch bottles.

Carbon dioxide

Carbon dioxide was quantified using a gas chromatograph (GC-2010A). This GC uses two columns, which are connected parallel (Parabond Q (50m x 0.53mm x 10µl) and Molsieve 5A (25m x 0.53mm x 50µl)). The detector was operated at temperature 175°C. A 50 µl of gas from the headspace of batch bottle was directly injected to the GC with syringe. Syringe was washed with helium gas to prevent the interference of oxygen from the air. The samples were analysed with a longer retention time to avoid the accumulation of gas in GC column. The peak for carbo dioxide is detected at a retention time of 2.51 min.

pH and pressure

The pH was determined with pH paper test strip (DosatestProlabo). The range measured with this pH paper is 6.0 – 8.1. The pressure in the headspace of the bottles was checked using a digital pressure meter (GMH 3150 – Greisinger electrode, Germany).

Calculations and estimation conversion rates

To estimate the sulphide and carbon dioxide production rates; and the sulphur compounds reduction rates, the following equations are used:

Sulphide production rate = $\frac{\Delta H_2S_{tt} - \Delta H_2S_{to}}{\Delta t}$ (1)

Sulphate reduction/production rate = $\frac{[SO_4]_{tt} - [SO_4]_{tt}}{\Delta t}$ (2)

Carbon dioxide production rate = $\frac{\{\Delta CO_2 + \Delta HCO_3^- + \Delta H_2CO_3\}/\Delta t}{V_{liquid}}$ (3)

RESULTS

Sulphate reduction with propane as electron donor

Batch bottle studies were used to determine the ability of mixed sediment cultures to anaerobically reduce sulphate coupled to propane as electron donor. Sulphide, sulphate and carbon dioxide were measured in regular intervals, and based on the measured concentrations; the sulphide production, sulphate reduction andcarbon dioxide production rates were estimated. The sulphide concentrations as function of time in sulphate bottles with propane are shown in Figure 1. As can be observed in the figure, in earlier incubations, from 0 to 15 incubation days, the sulphide concentration increased rapidly in which the total amount of sulphide produced is 0.3 – 0.6mM. This sulphide could feasibly be originated from the presence of organic matter in the mixed sediment. Besides, different microorganisms present in the sediment from Aarhus and Eckernförde Bay could also be possibly responsible to produce sulphide in early incubations. However, after the initial organic compounds were consumed, only propane degrading-sulphate reducing microorganisms could still convert sulphate to sulphide since propane present as solely available electron donor for sulphate. The high sulphide concentration during early incubation was also found in two control bottles.

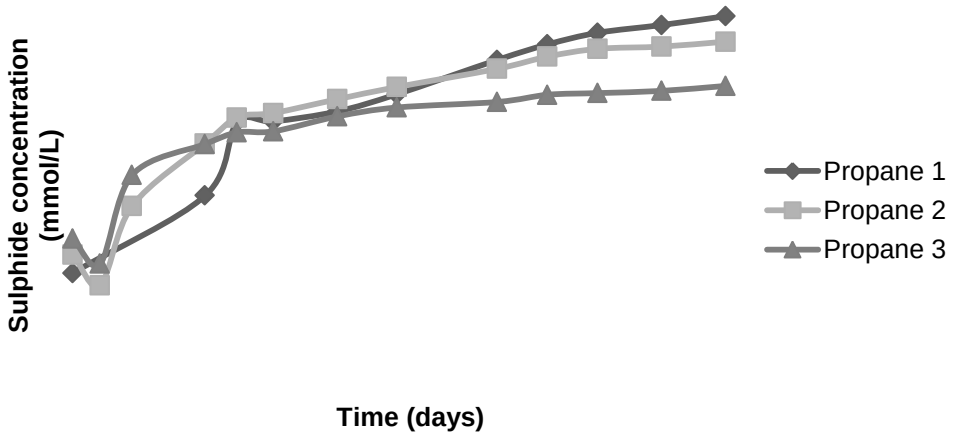

Figure 1. Sulphide concentration in sulphate bottles with propane as electron donor

The sulphide production and sulphate reduction rates using propane were calculated and are depicted in Figure 2. The sulphate was reduced by Aarhus and Eckernförde Bay cultures in the presence of propane in which the sulphate reduction rate is approximately 10 times higher than the amount of sulphate reduced without electron donors. In general, the ratio of sulphide production and sulphate reduction is comparable in 1:1 indicating that the increasing of sulphide in the bottles was solely resulted from the reduction of sulphate.

In order to analyse the reaction balance, oxidation of electron donor was measured. The carbon dioxide production, which is arguably as representative of electron donor oxidation is used to evaluate whether sulphate in bottles was reduced using propane as electron donor. As depicted in Figure 2, the carbon dioxide production in bottles incubated with different alkanes exceeded that of substrate-free controls. In general, the production of carbon dioxide is comparable with the reduction of sulphate according to equation 4 (Jaekel*et al*., 2012).

$2C_3H_8 + 5SO_4^{2-} + 4H^+ \rightarrow 6HCO_3^- + 5H_2S + 2H_2O$ ΔG^o_{pH7} = -41.0 kJ.mol^{-1} sulphate (4)

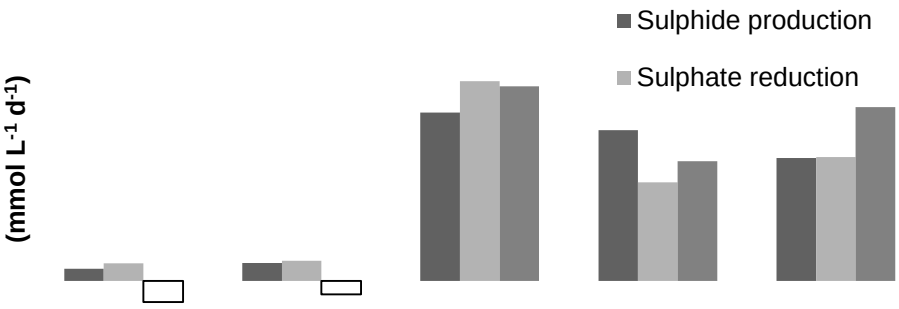

Figure 2. Sulphide and carbon dioxide productions and sulphate reduction in sulphate bottles with and without propane

Thiosulphate reduction with propane as electron donor

Enrichment of propane degrading – thiosulphate reducing bacteria was attempted with sediments from Aarhus and EckernfördeBay. As can be seen in Figure 3, sulphide was produced exponentially after 45 days of incubation. In general, most of cultures produced up to 6.2mM sulphide during a 120 incubation days. In the incubations, sulphide was allowed to accumulate and 5.8 (±0.1) mM of total sulphide was reached as maximum concentrations. After that, the sulphide production declined briefly from 0.1 to 0.02 mmol L^{-1} day^{-1}. On day 105, sulphide was removed by flushing the liquid with helium gas and as result the sulphide started again to be produced.

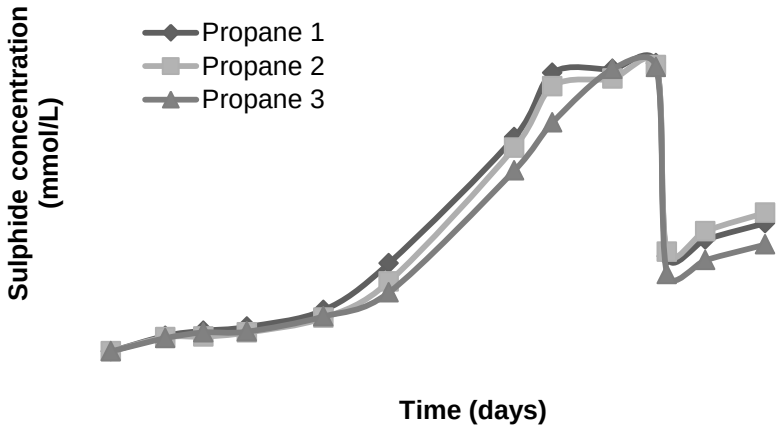

Figure 3. Sulphide concentration in thiosulphate bottles with propane as electron donor.

The sulphide production rate in thiosulphate bottles with propane was calculated during exponential phase with the production rate was 0.08 – 0.12 mmol L^{-1} day^{-1}. In the presence of propane, sulphide was produced faster than that of control bottles (Figure 4). However, in the negative bottles where electron donor was absent, up to 3.2 mM of sulphide was also produced. This can be explained with equation 5, which shows that sulphide can be produced from the disproportional reaction of thiosulphate. In addition, sulphate was also produced in the control bottles confirming the disproportionation. This sulphate production could not be found in the bottles where propane was present as electron donor for thiosulphate reduction. In the bottles with propane, the sulphide production rate is generally 1.5 times higher than thiosulphate reduction. Meanwhile, in control bottles, the sulphide production is lower than thiosulphate reduction. This indicates that the use of propane as electron donor could potentially enhanced thiosulphate reduction.

$$S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \quad (5)$$

sing the measurements before flushing. The ttles could not be measured after flushing to

remove sulphide. As depicted in Figure 4, there is no carbon dioxide production in bottles when the electron donor was absence. In general, the production of carbon dioxide is comparable with the sulphide production in accordance with the anaerobic thiosulphate reaction with propane as electron donor with a ratio 1:2.

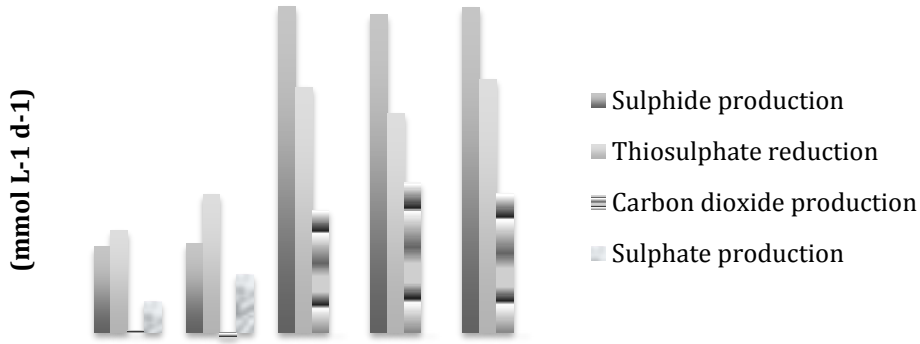

Figure 4. Sulphide carbon dioxide and sulphate productions and thiosulphate reduction in thiosulphate bottles with and without propane

DISCUSSION

The estimated rates

Compared with previous studies of the anaerobic oxidation of methane, ethane and propane oxidation for sulphate reduction, the Eckernförde and Aarhus Bay cultures from this study exhibited a relatively low adaptability to the propane as electron donor, with the average sulphide production rate was 0.006, 0.005 and 0.006 mmol.L^{-1}.day^{-1} respectively. Kniemeyer*et al.* (2007) reported that BuS5 strain was successfully isolated for sulphate reduction using propane and butane obtaining a maximum sulphide production rate was 1.12 mmol.L^{-1}.day^{-1}. Besides, a study from Jaekel*et al.* (2012) succeeded to enrich a new strain (Prop 12-GMe) capable of using propane for sulphate reduction with the maximum sulphide production rate of 0.62 mmol.L^{-1}.day^{-1}at temperatures between 16-20^{o}C. Meulepas*et al.* (2010) revealed that the sulphide production rate was 0.06 mmol.L^{-1}.day^{-1}, of which the biomass used by Meulepas*et al.* (2010) was taken on day 884 from a 1-L submerged-membrane bioreactor. When compared with a bioreactor system, the volumetric rate that was obtained in this study is even much lower. For instance, 298 mmol.L^{-1}.day^{-1} was reached as maximum sulphate removal rate in an expanded granular sludge bed (EGSB) reactor with acetate as electron donor (de Smul and Verstraete, 1999).

However, the sulphide production rate in the thiosulphate conditions from this study is comparable with other studies. The sulphide production rate from mixed Eckernförde and Aarhus Bay cultures in the presence of propane was 0.11 mmol.L^{-1}.day^{-1}. The study from Khelifi*et al.* (2010), a 0.1 mmol.L^{-1}.day^{-1} was estimated as sulphide production rate from thiosulphate reduction using fatty acids and alkenes as electron donors by the hyperthermophilicsulfate-reducing archaeon*Archaeoglobusfulgidus*. Meulepas*et al.* (2010) reported that the sulphide production rate for thiosulphate reduction using methane as electron donor was 0.11 mmol.L^{-1}.day^{-1}. Besides, a study from Mohn and Tiedje(1990) reported that DCB-1 strain was capable of using formate for thiosulphate reduction with the sulphide production rate of 0.09 mmol.L^{-1}.day^{-1}.

Significance of results

In the present study, mixed sediment cultures from Eckernförde and Aarhus Bay were able to use propane as electron donor for sulphate andthiosulphate. In the presence of propane, sulphide production from all sulphate andthiosulphate bottles was higher than the sulphide production when the propane was not added. The propane degrading – thiosulphate reducing microorganisms could [illegible] our knowledge this is the first study reporting [illegible] n rates of mixed sediments using propane.

Besides, it is also possible that thiosulphate was not utilized directly using propane, but that sulphate produced by the disproportional reaction was used by microbial community (Meulepas*et al.,* 2010). It is also confirmed by Widdel*et al.* (2007) that most sulphate reducing bacteria are able to use thiosulfate as substrate.

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