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Methane oxidation linked to chlorite dismutation

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INTRODUCTION

In-situ production of CO2 by microbial activity is encouraged during enhanced oil recovery as a means of reducing oil viscosity and improving flow characteristics (Lazar et al., 2007; Youseff et al., 2009). Further, targeted growth of microbes and intentional precipitation of solid phase minerals can be applied to selectively decrease permeability and direct flow to enhance oil recovery (Jenneman et al., 1984; Zhu et al., 2013). These enhancements rely on the availability of appropriate electron acceptors to supply oxidant to microbes utilizing hydrocarbons or other reduced compounds as electron donors. Considerable attention has been paid thus far to the use of sulfate, nitrate or nitrite as electron acceptors in these applications (Youseff et al., 2009) with sulfate being more favored because its reduction results in H2S and leads to oil souring (Gieg et al., 2011). There has been recent interest in the use of perchlorate or chlorate, together known as (per)chlorate as electron acceptors. However, little is known about the fate of (per)chlorate in anoxic environments like oil reservoirs. Here we examine the potential reaction of (per)chlorate and chlorite with the low molecular weight hydrocarbon methane.

Chemical reduction of perchlorate is generally quite slow (Urbansky, 2002). However, under anoxic conditions dissimilatory perchlorate reducing bacteria (DPRB) rapidly reduce (per)chlorate to form chlorite. Chlorite thus formed is further degraded by these bacteria using chlorite dismutase to produce Cl− and O2 (Rikken et al., 1996; Kostan et al., 2010; Mlynek et al., 2011). These microbial processes reduce (per)chlorate from both natural (Rao et al., 2007; Kounaves et al., 2010) and anthropogenic (Coates and Achenbach, 2004) sources. Degradation of intentionally added (per)chlorate is therefore likely in the proximity of hydrocarbon reservoirs given the abundance of suitable electron donors. There is a potential for O2 liberation during this process (see reaction 1 below) that may be used by aerobic bacteria to oxidize aromatic compounds (benzene, naphthalene, catechol) via oxygenase-dependent pathways in otherwise anoxic soils and sediments (Coates et al., 1998, 1999a; Coates and Achenbach, 2004; Weelink et al., 2007; Carlström et al., 2013). A similar phenomenon was noted that could link biological oxidation of arsenite to the reduction of chlorate ions, presumably also by liberation of O2 (Sun et al., 2010). This type of interaction has not been extended to the oxidation of low molecular weight hydrocarbons such as methane (CH4).

Methane is produced by geothermal and microbial processes in the Earth's crust (Martini et al., 1996) and in marine and terrestrial sediments (Cicerone and Oremland, 1988). Methane is removed photochemically in the atmosphere by reaction with hydroxyl radicals but the most important removal mechanism in aqueous and terrestrial environments is by the action of anaerobic and aerobic methane oxidizing microbes (Cicerone and Oremland, 1988; Boetius et al., 2000). Significant quantities of methane are associated with oil reservoirs (Jones et al., 2007; Gieg et al., 2008) hence we hypothesize that ClO2− disproportionation, and by extension dissimilatory reduction of the upstream ClO2−
or $\text{ClO}_2^-$ ions, could be linked to aerobic CH$_4$ oxidation by a biochemical release of O$_2$:

$$\text{ClO}_2^- \rightarrow \text{Cl}^- + \text{O}_2 \quad \Delta G^{\circ} = -135 \text{ kJ/mol} \text{ ClO}_2^- \quad (1)$$

$$\text{CH}_4 + 2\text{O}_2 \rightarrow \text{CO}_2 + 2\text{H}_2\text{O} \quad \Delta G^{\circ} = -842 \text{ kJ/mol} \text{ CH}_4 \quad (2)$$

$$\text{Net } \text{CH}_4 + 2\text{ClO}_2^- \rightarrow \text{CO}_2 + 2\text{Cl}^- + 2\text{H}_2\text{O} \quad \Delta G^{\circ} = -1114 \text{ kJ/mol} \text{ CH}_4 \quad (3)$$

Two other well-studied microbiological processes can achieve a net oxidation of CH$_4$ under prevailing anaerobic conditions, (1) a reverse process of methanogenesis involving “ANME” archaea in syntrophy with bacterial sulfate- or sulfur-reduction (Hinrichs et al., 1999; Boetius et al., 2000; Milucka et al., 2012) and (2) nitrite-linked CH$_4$ oxidation that putatively liberates O$_2$ via NO dismutation as achieved by *Methylomirabilis oxyfera* (Ettwig et al., 2010). In our study we explored the potential for aerobic CH$_4$ oxidizing bacteria to utilize oxygen produced by DPRB during (per)chlorate reduction and chlorite dismutation.

**MATERIALS AND METHODS**

**PREPARATION OF CULTURES**

The DPRB *Dechloromonas agitata* CKB was grown at 30°C under N$_2$ on 20 mM sodium acetate and 10 mM NaClO$_4$ using phosphate buffer media (pH 7.0) consisting of the following salts in solution (g/liter): Na$_2$HPO$_4$ (0.971), NaH$_2$PO$_4$ (0.379), NH$_4$Cl (0.25) plus 10 ml/l vitamins and 10 ml/l mineral stock solution (Sun et al., 2009). *Methylomonas capsulatus* Bath, *Methylosinus trichosporium* OB3b, and *Methylocystis album* BG8, were grown and maintained at 30°C on air + 30 kPa CH$_4$ using nitrate mineral salts media (NMS; Whittenbury et al., 1970). Cultures (1 l) for washed cell suspensions were harvested during late exponential phase, centrifuged (7000 × g), and washed twice with medium lacking substrates and vitamins. Final suspension volumes ranged from 5 to 150 ml. Cell concentrations at the start of incubations ranged from 1.8 × 10$^8$ cells ml$^{-1}$ to 6.9 × 10$^8$ cells ml$^{-1}$.

**PREPARATION OF SOILS AND SLURRIES**

Soil from the seasonally exposed shoreline of Searsville Lake previously shown to harbor methanotrophic activity (Orem and Culbertson, 1992) was air dried for two days at room temperature before sieving (<1 mm) to assure uniformity of soil particle size. Dried soil was stored for several weeks in stoppered 1 l glass flasks with air headspace and periodically augmented with 0.2 kPa CH$_4$ after consumption had removed all of the previously added CH$_4$ (4–6 days). Soil with thusly enhanced methanotrophic activity was used to determine CH$_4$ uptake in studies with added ClO$_2^-$ and ClO$_2^-$ in the absence of O$_2$.

Sediment slurries were prepared by adding 100 ml SeFr$_2$ freshwater media (flushed with 20 kPa CO$_2$/80 kPa N$_2$; Miller et al., 2013) to 10 g Searsville Lake soil in N$_2$ flushed serum bottles (160 ml). Slurry pH was adjusted to 7.1 using 1 ml of 1 M NaHCO$_3$. Slurries were incubated under N$_2$ headspace following periodic amendments with 1 to 2 mmoles acetate and 0.5 to 1 mmole ClO$_2^-$. Slurries were periodically sampled by syringe using 22 g needles and filtered through a 0.2 um Spin-X centrifuge tube. Acetate and ClO$_2^-$ amendments were made after both were depleted (usually several days to weeks) during which time copious quantities of CH$_4$ were produced. Slurries with enhanced perchlorate reducing activity were used to determine methane uptake activity in the presence or absence of added O$_2$.

**MEASUREMENT OF (PER)CHLORATE REDUCTION AND CHLORITE DISMUTASE ACTIVITY**

Aliquots of washed cell suspension of *D. agitata* CKB were distributed into stoppered and N$_2$ flushed 25 ml Balch tubes containing 10 ml PBM amended with 5 mM acetate and 10 mM NaClO$_4$, NaClO$_3$, or NaClO$_2$. Initial cell densities were 1.8 × 10$^8$ cells per ml. The headspace was sampled over 7 days by syringe for CO$_2$ and O$_2$. Aqueous samples (0.3 ml) were collected by syringe for analysis of dissolved acetate and anions (Cl$^-$, ClO$_2^-$, ClO$_3^-$, ClO$_4^-$). A short-term (10 min) experiment was conducted to follow ClO$_2^-$ disproportionation. In this study, triplicate samples were sacrificed at pre-determined times. Activity was stopped by addition of 0.1 ml 4N NaOH before measurement of headspace O$_2$. Aqueous samples were subsequently collected for analysis of dissolved anions (Cl$^-$ and ClO$_2^-$).

**INCUBATIONS WITH MIXED CULTURES**

Mixtures (10 ml) of *M. capsulatus* Bath and *D. agitata* CKB were prepared by adding washed cell suspensions of the cultures together in N$_2$ flushed Balch tubes (25 ml) sealed with butyl rubber stoppers. Methane (0.2 kPa) was introduced by syringe to all tubes and NaClO$_2$ (5 mM) was added to 3 tubes at the start of the incubation which was conducted at 37°C. Headspace CH$_4$ was monitored over 1 day. Single tubes were prepared without addition of ClO$_2^-$ or without one of the cultures (i.e., no *D. agitata* CKB or no *M. capsulatus* Bath) to act as negative controls. A tube containing only *M. capsulatus* Bath under an air headspace acted as a positive control.

Additional microcosms were prepared in serum bottles (37 or 67 ml) using washed cell suspensions of *D. agitata* CKB and *M. trichosporium* OB3b or *M. album* BG8. Inocula were either combined in bottles (5 ml each) in one aqueous phase or kept separate by placing methanotrophs (1 ml) inside an open-topped glass tube contained within the bottles before flushing with N$_2$ and later adding *D. agitata* CKB (5 ml) by syringe to the bottom of the bottles. In this manner, the cultures were segregated but shared a common headspace. Methane (0.2 kPa) was introduced by syringe to all bottles and NaClO$_4$, NaClO$_3$ or NaClO$_2$ (5 or 10 mM) was added aseptically to start the incubations which were conducted at 28°C. Headspace CH$_4$ and aqueous anions were monitored over time. Controls were prepared without additions of ClO$_2^-$. 

**INCUBATIONS WITH 14C-LABELED CH$_4$**

Washed cell suspensions (5 ml each) of *D. agitata* CKB and *M. trichosporium* OB3b were added together to N$_2$ flushed serum bottles (13 ml). Radiolabeled $^{14}$CH$_4$ (5 μCi; specific activity = 21 μCi/μmole) was added along with 1 kPa CH$_4$ to the headspace of each bottle. Perchlorate (5 mM) was added to triplicate bottles and ClO$_2^-$(5 mM) was added to a single bottle by syringe.
to start the incubation which was conducted at 30°C. Gas samples for analysis of \(^{14}\text{CH}_4\) and \(^{14}\text{CO}_2\) were collected by syringe. At the end of the incubation, samples were acidified using 0.5 ml of 1.2N HCl to cause dissolved inorganic carbon (DIC = HCO\(_3^-\) + CO\(_3^{2-}\)) to react to form CO\(_2\) gas which was partitioned into the headspace. The headspace was again sampled by syringe. Control incubations consisted of single bottles of \(M.\) trichosporium OB3b alone under an air headspace and \(D.\) agitata CKB alone under N\(_2\).

**INCUBATIONS WITH SOIL SLURRIES**

Slurry microcosms were prepared in N\(_2\) flushed serum bottles (57 ml) containing 5 g of dried Searsville Lake soil with enhanced methanotrophic activity to which 10 ml Searsville Lake sediment slurry with enhanced perchlorate reducing activity (above) was added. Half the bottles were maintained under N\(_2\) while half were flushed with air. Substrate (5 mM ClO\(_3^-\) or ClO\(_4^-\)) was added by syringe followed by 0.5 ml CH\(_4\) (1 kPa). Incubations were conducted at 22°C. Headspace and liquid samples were collected by syringe over 9 days.

**INCUBATIONS WITH CULTURES PLUS SOIL**

Soil microcosms were prepared in serum bottles (67 ml) using washed cell suspensions of \(D.\) agitata CKB and dried Searsville Lake soil which was enhanced in methanotrophic activity (above). Soil (2 g) was placed inside open-topped glass tubes contained within the bottles prior to sealing and flushing with N\(_2\). Subsequently, \(D.\) agitata CKB (10 ml) was added by syringe to the bottom of the bottles followed by aseptic addition of 5 mM acetate. The culture and the soil were thus segregated under a common headspace. Methane (0.1 kPa) was introduced by syringe to all bottles and 10 mM NaClO\(_4\), NaClO\(_3\), or NaClO\(_2\) was added to \(D.\) agitata CKB to start the incubations. Incubations were conducted at 22°C. Headspace CH\(_4\) and CO\(_2\) and aqueous acetate and anions were monitored over 7 days.

**ANALYTICAL**

Headspace O\(_2\) was determined by ECD-GC using a molecular sieve 5A column (3.2 mm O.D. × 2.4 m) operated at 75°C using hydrocarbon-free UHP N\(_2\) carrier. Background O\(_2\) was minimized by flushing syringes and needles with O\(_2\)-free N\(_2\) prior to sampling. The detection limit was 0.05 mmol O\(_2\)/L. Headspace CH\(_4\) and CO\(_2\) were determined by FID- and TCD-GC, respectively (Miller et al., 2013). Cell densities were determined by direct cell counting of liquid samples using acridine orange epi-fluorescence microscopy (Hobbie et al., 1977). Additional aqueous samples, including slurries, were filtered using Spin-X centrifuge filter tubes (0.2 µm; Corning Inc., Corning, NY) before determination of dissolved acetate by HPLC (Hoeft et al., 2004) or anions by IC (Miller et al., 2003). Dissolved ClO\(_4^-\) was analyzed separately by suppressed conductivity IC using a Dionex ISC 1100 containing an AS16 analytical column (4 × 250 mm) and an AG16 guard column (4 × 50 mm) with 0.035 M NaOH eluent. Measurements of headspace \(^{14}\text{CH}_4\) and \(^{14}\text{CO}_2\) were made by gas proportional counting (Culbertson et al., 1981) following TCD-GC analysis of CH\(_4\) and CO\(_2\) with separation on a Hayesep D column (100/120; 3.2 mm O.D. × 4.8 m) using UHP He carrier.

**CALCULATIONS**

The total amount of gas in each bottle or tube was calculated from the headspace concentration using Henry’s Law and the volumes of gas and liquid present. The dimensionless Henry’s Law constants (\(K_H = C_G/C_L\)) used were 31.43 for O\(_2\), 29.46 for CH\(_4\) and 1.20 for CO\(_2\) and were not corrected for ionic strength.

**RESULTS**

**PER|CHLORATE REDUCTASE AND CHLORITE DISMUTATION ACTIVITY**

Dissimilatory (per)chlorate reduction by \(D.\) agitata CKB resulted in conversion of 85–100 μmoles added ClO\(_4^-\) or ClO\(_3^-\) to Cl\(^-\) in the presence of 50 μmoles added acetate (Figures 1A,B). Much less ClO\(_3^-\) or ClO\(_4^-\) (<15 μmoles) was consumed without added acetate and a corresponding lesser amount of Cl\(^-\) was produced. These observations suggest endogenous metabolism of intrinsic electron donors such as glycogen or polyhydroxybutyrate (PHB). No activity was observed in killed controls or in incubations with media and chloroxyanions alone (data not shown). Biological reduction of ClO\(_3^-\) or ClO\(_4^-\) and consumption of acetate occurred over approximately 2 days. Chlorite dismutation was much more rapid. More than half of the 100 μmoles ClO\(_4^-\) added was consumed and converted to Cl\(^-\) before the initial sampling at \(T = 2\) min (Figure 1C). An additional 20 μmoles ClO\(_3^-\) were consumed over 7 days, however more than 20 μmoles ClO\(_3^-\) remained unreacted at the end.

Carbon dioxide (CO\(_2\)) was the dominant gaseous product of dissimilatory reduction of ClO\(_4^-\) or ClO\(_3^-\) in the presence of acetate (Figures 2A,B). Slightly more CO\(_2\) was produced than could be accounted for by the added acetate. Little CO\(_2\) was produced without added acetate. Small amounts of O\(_2\) (up to 15 μmoles) were produced during incubations with ClO\(_4^-\) or ClO\(_3^-\). In contrast, ClO\(_3^-\) dismutation resulted in substantial and rapid O\(_2\) production (Figure 2C) corresponding to release of >35% of the added ClO\(_4^-\) within the first day. As expected, there was no effect of added acetate on disproportionation of ClO\(_3^-\); however details of the early evolution of O\(_2\) were obscured by the coarse sampling schedule.

The pattern of early O\(_2\) production during ClO\(_4^-\) dismutation was made clear in a subsequent short-term (10 min) experiment where 56 μmoles of both O\(_2\) and Cl\(^-\) were produced during the consumption of 56 μmoles of ClO\(_4^-\) (Figures 3A,B). A minor amount of CO\(_2\) (<1 μ mole) was produced (Figure 3C). Nearly half (40 μmoles) of the added ClO\(_2^-\) remained unreacted at the end of the experiment.

**MIXED CULTURES OXIDIZED CH\(_4\)**

Methane was oxidized by methanotrophic bacteria \(M.\) capsulatus Bath (Figure 4A) and \(M.\) album BG8 (Figure 4B) during the reaction of \(D.\) agitata CKB with ClO\(_4^-\). No removal of CH\(_4\) was observed in mixed cell suspensions amended with ClO\(_4^-\) or ClO\(_3^-\) (i.e., no added ClO\(_2^-\)). Methane consumption occurred while the methanotrophs were in direct contact with up to 10 mM ClO\(_3^-\). Methane removal during incubations of co-cultures of \(D.\) agitata CKB with \(M.\) capsulatus Bath at 37°C occurred within 1 day while removal of CH\(_4\) by \(M.\) album BG8 at 28°C occurred over 4 days. Similar rates of CH\(_4\) consumption were observed for these mixed cultures whether they were segregated or co-mingled.
METHANE OXIDATION LINKED TO CHLORITE DISMUTATION

MIXED CULTURES OXIDIZE $^{14}$CH$_4$ TO $^{14}$CO$_2$

Strain *M. trichosporium* OB3b co-cultured with *D. agitata* CKB containing 5 mM acetate oxidized $^{14}$CH$_4$ directly to $^{14}$CO$_2$ (*Figure 5*). The rate of $^{14}$CH$_4$ loss under anaerobic conditions with 5 mM added ClO$_2^−$ was similar to that under aerobic conditions. No loss of $^{14}$CH$_4$ occurred in mixed cell suspensions amended with 5 mM ClO$_4^−$ in lieu of ClO$_2^−$ or in controls without methanotrophs. During the incubation, the product of methanotrophy ($^{14}$CO$_2$) was distributed about equally between liquid and gas phases. Roughly 20% of the added $^{14}$CH$_4$ appeared as $^{14}$CO$_2$ in the headspace after 20 h (0.8 days). Most of the $^{14}$CH$_4$ added was recovered as $^{14}$CO$_2$ (60–90% recovery after acidification).

OXIDATION OF CH$_4$ BY SOILS

Searsville Lake sediment slurries removed repeated pulses of added ClO$_4^−$ during anaerobic incubations using freshwater...
media with added acetate. Over a period of 1 month, 4 additions of 10 mM ClO$_2^-$ were removed in bottles with commensurate consumption of 4 additions of 5 mM added acetate (data not shown). These slurries, thus enhanced in ClO$_2^-$ reducing capacity, were used in separate incubations with added CH$_4$ (Figure 6). Under aerobic conditions, incubations with or without additions of ClO$_4^-$ or ClO$_2^-$ (5 mM) completely consumed 30 μmoles CH$_4$ within 5 days. However, no oxidation of CH$_4$ occurred during anaerobic incubations of Searsville Lake sediment slurries either with or without additions of ClO$_4^-$ or ClO$_2^-$.

Methane oxidation was observed when Searsville Lake soil (previously enhanced in methanotrophic activity) was segregated from liquid cultures of D. agitata CKB. Methane was completely consumed over the next 5 days by soil methanotrophs during the reaction of DPRB with 10 mM ClO$_2^-$ (Figure 7). No CH$_4$ loss was observed when DPRB were provided with either 10 mM ClO$_4^-$ or ClO$_3^-$. 

**DISCUSSION**

Few enzymes outside of photosystem II are capable of generating dioxygen in anoxic settings. In addition to chlorite dismutase (Cld), these include superoxide dismutase and catalase (McCord et al., 1971), and a putative nitric oxide dismutase (Ettwig et al., 2012). Chlorite dismutase has been purified from at least four DPRB (Mehboob et al., 2009) and is well studied (Coates et al., 1999b; Lee et al., 2008; Goblirsch et al., 2010, 2011). It is a heme enzyme which operates hyperselectively; its assumed sole function is to detoxify ClO$_2^-$. The present study is the first report of anaerobic methane oxidation linked to the combined presence of DPRB and ClO$_2^-$. We emphasize that this is a “cryptic” aerobic methane oxidation in that the organisms oxidizing...
methane are aerobic methanotrophs as opposed to anaerobic methanogenic archaea utilizing reverse methanogenesis to consume methane (Hinrichs et al., 1999). As such, this process is somewhat analogous to nitrite-dependent anaerobic methane oxidation as purportedly carried out by the mixed culture containing Methylosinus oxysphaerica (Ettwig et al., 2010). In our study, methanotrophs use O2 derived from disproportionation of ClO2− by DPRB (reaction 1) to oxidize CH4 (reaction 3). This is in contrast to the mechanism proposed for M. oxyfera in which a single organism may use O2 from disproportionation of NO derived from NO2− (reaction 4) to oxidize CH4 (reaction 5):

\[
2\text{NO} \rightarrow \text{N}_2 + \text{O}_2 \quad \Delta G^\circ = -173 \text{ kJ/mol O}_2 \quad (4)
\]

\[
3\text{CH}_4 + 8\text{NO}_2^- + 8\text{H}^+ \rightarrow 3\text{CO}_2 + 4\text{N}_2 + 10\text{H}_2\text{O} \quad \Delta G^\circ = -928 \text{ kJ/mol CH}_4 \quad (5)
\]

Previous work by Coates et al. (1998, 1999a); Coates and Achenbach (2006) demonstrated a link between Cld activity and other aerobic hydrocarbon oxidizing bacteria during the degradation of benzene and naphthalene. In their experiments with pristine soil and hydrocarbon contaminated sediment, 14C-benzene was oxidized to 14CO2 over several days when provided with ClO2− in the presence of washed cells of D. agitata CKB under anaerobic conditions. Similarly, 14C-naphthalene was rapidly oxidized to 14CO2 in the presence of washed cells of D. agitata CKB and Pseudomonas sp. strain JS150 (an aerobic hydrocarbon oxidizer) when provided with ClO2−.

Here we demonstrated that O2 released by the reaction of ClO2− with pure cultures of DPRB could be utilized by a variety of methane oxidizing bacteria, including γ-Proteobacteria (M. capsulatus Bath and M. album BG8) and α-Proteobacteria (M. trichosporium OB3b) methanotrophs. Addition of 10 mM ClO2− to DPRB resulted in only 40–60% recovery as O2 and Cl− (Figure 3). This may be attributed to a toxic effect of elevated ClO2− or bleaching of the Cld enzyme (Strait and DuBois, 2008). Nonetheless, much of the available O2 was freely released during the reaction of ClO2− with D. agitata CKB, consistent with localization of Cld in the periplasm of DPRB (O’Connor and Coates, 2002). We demonstrated that direct addition of 5 or 10 mM ClO2− to mixed cultures of DPRB and methanotrophs did not inhibit the methanotrophs. We also showed that direct contact between the cells was not required as CH4 oxidation also occurred when the cells were contained in separate compartments under a common headspace. We further showed that 14CH4 was quantitatively oxidized to 14CO2 by the methanotrophs in culture. Our conclusion is that methane oxidizers utilized O2 provided by the dismutation of ClO2− by DPRB.

We were unable to link methane oxidation to perchlorate or chlorate reduction. Small amounts of oxygen were produced when cultures of D. agitata CKB were amended with ClO4− or
CO
\textsubscript{3}O
\textsuperscript{−} (Figure 2), however methane was not consumed during co-culturing with methanotrophs (Figures 4, 6). This was previously observed for benzene and naphthalene (Coates et al., 1999b; Coates and Achenbach, 2006) and may be explained by O\textsubscript{2} scavenging attributable to other processes, including activation of a terminal oxidase during (per)chlorate reduction (Rikken et al., 1996). It is also possible that slower kinetics of CO
\textsubscript{3}O
\textsuperscript{−} and ClO
\textsubscript{3}− reduction limits the production and subsequent dismutation of ClO
\textsubscript{3}− and therefore release of O\textsubscript{2}. Relief of this bottleneck could lead to more O\textsubscript{2} being available to aerobic methanotrophs and stimulation of the unique process described herein.

\textit{In-situ} oxidation of CH\textsubscript{4} using O\textsubscript{2} derived from chlorite dismutation may be useful in removing elevated levels of CH\textsubscript{4} in subsurface environments. Chlorite is 10\textsuperscript{4} times more soluble in water than O\textsubscript{2} and could be easily and safely directed to the anaerobic zone (where methane may be present) during bioremediation. One example is enhanced oxidation of landfill methane without the use of forced air, reducing the risk of fire and explosion. The ability of methanotrophs and DPRB to function in separate compartments under a common headspace could be exploited at distal stages of oil development, for instance at well heads where unusable CH\textsubscript{4} is typically flared off to reduce transportation costs or risk. In addition, production of CO\textsubscript{2} formed during oxidation of CH\textsubscript{4} may be viewed similarly to injected CO\textsubscript{2} in efforts to dissolve and flush oil from developed petroleum reservoirs (Blunt et al., 1993). Further, bioflocculation by cells and biocementation resulting from carbonate precipitation may be enhanced by the growth and activity of microbes capable of linking methane oxidation with (per)chlorate reduction. Bioflocculation and biocementation are features of microbial enhanced hydrocarbon recovery most likely to be exploited by geotechnologists to direct hydrocarbon flow into more permeable substrates in order to enhance recovery. However, it must be noted that we observed no methane oxidation in water saturated soils exposed to both CH\textsubscript{4} and ClO
\textsubscript{3}− (Figure 6) indicating that O\textsubscript{2} derived from chlorite dismutation may face transport limitations in saturated anaerobic environments and may be consumed before reaching nearby CH\textsubscript{4}. Even under our unsaturated experimental conditions where liquid cultures and soil were segregated under a common headspace (Figure 7) the amount of soil present in each microcosm had to be optimized in order to balance methanotrophy with other soil O\textsubscript{2} utilizing processes.

Oxidation of CH\textsubscript{4} by the mechanism identified here may reduce the greenhouse impact of fugitive gases during hydrocarbon reservoir development and recovery because the global warming potential of CO\textsubscript{2} is 25 times lower than CH\textsubscript{4} on a 100 year time scale (IPCC 5th assessment, 2013). In addition, intermediates along the pathway of aerobic methane oxidation (e.g., methanol, formaldehyde, and formate) are themselves quite useful as chemical feedstocks for a myriad of industrial applications including biofuel production.

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