

# Electron Acceptor Addition to Stimulate Anaerobic Methanotrophy in Oil Sands End Pit Lakes

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## Abstract

A major question for the Alberta oilsands extraction industry is how to return tailings ponds to their original ecosystem functions at the end of their lifespan. This question is one of the most pressing in the oil industry, and while many potential solutions are being trialed, none are without issue. One solution is the creation of end pit lakes (EPLs), formed by filling a mine pit or tailings pond with fluid tailings and covering this with a freshwater cap. Over time, tailings settle to the bottom of the lake and become sequestered. As of 2018, there were 23 plans to pursue EPLs in Albertan oilsands mining operations (COSIA 2021), so research in oilsands reclamation via EPLs is essential.

Alberta has only one full-scale EPL formed using tailings, Base Mine Lake (BML). It has been under reclamation since 2011. Although BML water quality is gradually improving over time, one persistent problem is the production of methane bubbles from the entrenched hydrocarbons. Ebullition of these bubbles carries contaminants into the water column. The potential to limit methane production biologically by stimulating methanotroph metabolism, is therefore of interest. Methanotrophs are microbes that consume methane. These methanotrophs can be aerobic, in the water column, or anaerobic below the sediment interface, where oxygen is depleted. Anaerobic oxidizers of methane, or AOM, are of particular interest due to methane only being produced in anaerobic conditions. However, AOM are not well-studied and there are many gaps in our knowledge about them. Base Mine Lake presents an important opportunity to document the presence of AOM in oilsands ecosystems, and identify if AOM can remove methane before it enters the water column.

Methane is probably not the limiting factor for AOM in BML- instead, the electron acceptors used in the absence of oxygen are scarce. If microbiological methane removal were to increase after supplementing electron acceptors such as nitrate, sulfate, or iron, then this could be applied to the reclamation of artificial end pit lake systems. Our research aims to illustrate if AOM are present in BML, and if we can stimulate their metabolism via electron acceptor amendment.

We performed amplicon and metagenomic sequencing across 5 sediment cores from BML. These cores penetrate over two meters below the water-sediment interface, up to 14 meters below the water's surface. We designed anaerobic,  $^{13}\text{C}$ -isotope-spiked incubations for this sediment to track if any  $\text{CH}_4$  to  $\text{CO}_2$  conversion was underway. By tagging methane molecules with  $^{13}\text{C}$ , we are able to follow the biogeochemical transformations and demonstrate if any  $^{13}\text{CH}_4$  is converted to  $^{13}\text{CO}_2$ , which would indicate methanotrophic activity. Additionally, microbes who take up  $^{13}\text{C}$  produce heavier DNA, possibly allowing us to identify the DNA of the players at work. Additionally, Illumina sequencing was done on all incubations to compare how electron acceptor addition prompted community shifts.

In the incubation experiments,  $^{13}\text{CO}_2$  is being produced in several core samples incubated without  $\text{O}_2$ . This finding indicates that methanotrophs are, in some capacity, active in anoxic conditions. When comparing different amendments of electron acceptors, there did appear to be a significant amount ( $p = 0.027$ ) of  $^{13}\text{CO}_2$  produced in the sulfate-added  $^{13}\text{CH}_4$  treatment (Fig. 1). Other amendments had encouraging results, but were not statistically significant. Ongoing trials will provide more illuminating results via larger sample sizes, which will be completed by the end of the summer. Next we will characterize communities in the post-incubation sediment, using both amplicon sequencing and DNA stable isotope probing. This will suggest not only the identity of the organisms at play, but will also illuminate which electron acceptors were most beneficial to which groups. This analysis is complex and underway.

Overall, the study of methanotrophy in artificial ecosystems with high emissions is an area of great interest, due to the high potential for amendment and improvement of the ecosystems using methanotrophs, as well as the ongoing discovery on AOM in artificial ecosystems. Oilsands end pit lakes are of interest, as an issue of cultural and political emphasis.

## **Keywords**

oilsands, reclamation, end pit lake, methane, microbiology, tailings, bacteria, archaea, sediment

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## Hosting institution

University of Calgary

## Conflicts of interest

The authors have declared that no competing interests exist.

## References

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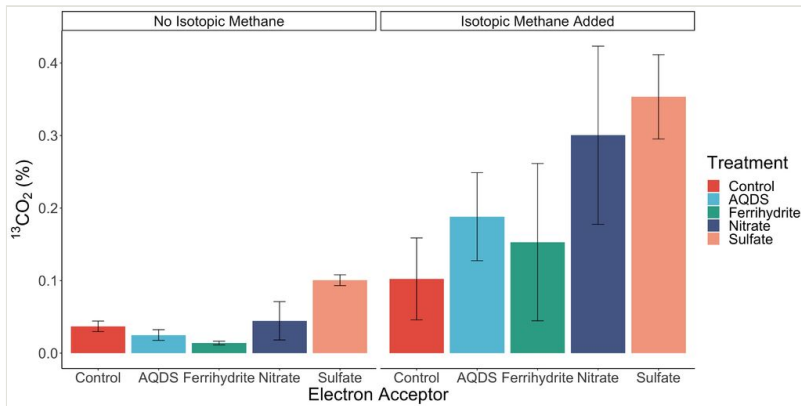


Figure 1.

Mean  $^{13}\text{CO}_2$  production at 50 days across 59 incubations, as measured by GC-IRMS, where  $^{13}\text{CO}_2$  represents the specific product of AOM. Incubations contained 50 mL of BML sediment and 1 mL of the electron acceptor indicated. Incubations either had no methane added (left) or 50 mL of  $^{13}\text{CH}_4$  added (right). All samples contained 0%  $\text{CO}_2$  at Day 0. Error bars represent standard error of the mean. Control treatments contained 4 replicates, and experimental treatments contained 8-12 replicates.