

Inhibition of Methanogenesis through Redox Processes in Oil Sands Tailings

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Abstract

Bitumen extraction from oil sands ore in Alberta has generated >1.3 billion m³ of tailings, a slurry of fine silt and clay, residual bitumen and diluent hydrocarbons, deposited in ponds. Methane emissions are one of the environmental issues associated with tailings. Methane produced during anaerobic microbial metabolism of hydrocarbons is emitted from tailings ponds and end-pit lakes where tailings are reclaimed. We investigate hydrocarbon biodegradation under alternative redox conditions to inhibit methanogenesis. Our results reveal the potential of indigenous microbes to biodegrade hydrocarbons in the tailings under iron- and sulfate-reducing conditions channelling carbon flow from hydrocarbons to carbon dioxide.

Keywords: Oil sands tailings, Hydrocarbon biodegradation, Methane emissions, Nitrate-, Sulfate- and iron-reducing conditions

Introduction

Alberta, Canada has the third largest oil reserves in the world. Current hot water technology for bitumen extraction from surface mined oil sands ore generates huge volumes of fluid fine tailings (FFT), which are temporarily stored in tailings ponds, but eventually must be reclaimed and integrated into mine closure landforms. FFT are a water suspension of mineral particles (silt and clay), unrecovered bitumen, and residual diluent hydrocarbons used to aid bitumen recovery. The growing inventory of FFT is currently estimated at >1.3 billion m³, and tailing ponds have covered over 100 km² (<http://osip.alberta.ca/map/>) in the Athabasca oil sands region of Alberta. The reclamation of FFT is an environmental challenge for oil sands companies and a prospective reclamation strategy is to construct end-pit lakes (EPLs) where FFT are covered by a layer of oil-sand processed water (OSPW) mixed with fresh water. With time, EPLs are supposed to become a self-sustained wetland ecosystem (Kabwe *et al.*, 2017) with adequate water quality to connect to the surrounding watershed and other water bodies. For the reclamation of legacy FFT at massive scale,

more than 30 EPLs are planned for the Athabasca oil sands region.

Both tailings ponds and EPLs are biologically active and harbour microbial communities (both Bacteria and Archaea) that degrade residual hydrocarbons in FFT to produce greenhouse gases (GHG), primarily methane (CH₄) (Siddique and Kuznetsova, 2020). Total fugitive emission from oil sands tailings ponds, estimated using floating flux chambers, is 2.8 million tonnes CO₂ eq. y⁻¹ (Burkus *et al.*, 2014). The main substrates for methanogenesis are the labile hydrocarbons that are constituents of diluents used in bitumen extraction process. For example, Syncrude Canada Ltd., Suncor Energy, and Canadian Natural Resources Ltd. use naphtha (primarily C₅-C₁₀ hydrocarbons), whereas paraffinic solvent (mainly C₅-C₆) is employed by Canadian Natural Upgrading Ltd. and Imperial Oil to aid in bitumen recovery from oil sands ore. Metabolism of n-alkanes, iso-alkanes and monoaromatics (toluene and isomers of xylenes) fractions of these diluents under methanogenic conditions supports CH₄ emissions from tailings ponds (Mohamad Shahimin and Siddique, 2017a, 2017b; Siddique *et al.*, 2006, 2007, 2015,

2020). Methanogenesis in these EPL is not only a source of fugitive gas emissions, but may also induce: (1) turbidity in cap water, (2) flux of inorganic constituents, and (3) bitumen transport from underlying FFT to cap water, which may affect EPL water quality and effectiveness and sustainability of this reclamation strategy (manuscript, Kuznetsov *et al.* in preparation).

To prevent CH₄ production in oil sands tailings, we investigate the biodegradation of labile hydrocarbons in FFT using alternative terminal electron accepting conditions such as nitrate-, iron-, and sulfate-reducing conditions. We report here some of our preliminary results that reveal potential of indigenous microbial communities to metabolize hydrocarbons in FFT under alternative reducing conditions. The results will help develop a technology using *in-situ* or *ex-situ* amendments to exploit different redox pathways for mitigating GHG emissions.

Methods

We established a series of laboratory microcosm experiments to investigate biodegradation of labile fractions of diluent petroleum hydrocarbons under different redox (nitrate-, iron- and sulfate-reducing) conditions by adding either naphtha or individual groups of hydrocarbons (*n*-alkanes, *iso*-alkanes and monoaromatics) separately, as these labile hydrocarbons are the primary source of methane emissions from oil sands tailings ponds. The FFT were collected from Mildred Lake Settling Basin (MLSB; oldest tailing pond) for our first iron-reduction experiment, whereas OSPW and FFT were collected from Base Mine Lake (BML; end-pit lake) at 1 m and 9-11 m below the water surface, respectively, by Syncrude Canada for our subsequent experiments for nitrate-, iron- and sulfate-reducing conditions. Upper layer of OSPW represents suboxic conditions where nitrate-reducing conditions may prevail and we can expect more abundance of nitrate-reducing bacteria (NRB). In FFT, mostly iron- and sulfate-reducing bacteria (SRB) are expected. The anaerobic microcosms were prepared using 50 mL of media mixed with 50 mL of either OSPW or FFT in 158-mL serum

bottles with a headspace of 30% CO₂ balance N₂ as previously described (Siddique *et al.*, 2006). Microcosms were amended with 10 mM of separate groups of hydrocarbons or 0.2 wt% Syncrude naphtha. Triplicate autoclaved microcosms (abiotic controls) were prepared in parallel by autoclaving them for four consecutive days prior to hydrocarbon amendment to account for abiotic degradation. Triplicate unamended microcosms (viable baseline controls) were also prepared to account for terminal electron acceptor (TEA) reduction from any residual endogenous substrates in the substrate. In all microcosms, specific TEAs were added to maintain redox conditions: first iron reduction experiment contained 10mM ferrihydrite (fig. 1) or hematite (fig. 2) as TEAs, and subsequent experiments had 10 mM NaNO₃ in nitrate-reducing, 90 mM Fe^{III} as ferrihydrite for iron-reducing, and 10 mM NaSO₄ for sulfate-reducing conditions (tab. 1). Additionally, in half of the microcosms under Fe-reducing conditions, 2mM nitrilotriacetic acid (NTA, chelating agent) concentration was attained to increase Fe availability for microbes. Role of crystalline iron mineral (magnetite) in hydrocarbon biodegradation was also tested in this experiment. The microcosms were incubated statically in dark at room temperature.

We monitor the biodegradation of spiked hydrocarbons by analyzing the headspace gas composition, injecting 100uL directly into a gas chromatograph equipped with a mass-spectrometer. The depletion of electron acceptors and formation of reduced species are measured using spectrometric methods for nitrate/nitrite (Schnetger and Lehnert, 2014), sulfate/sulfide (Trüper and Schlegel, 1964) and reduced Fe (ferrozine method (Siddique *et al.*, 2014b)). We also characterize the indigenous microbial communities involved in hydrocarbon biodegradation under different redox conditions performing 16S rRNA gene sequence analysis using Illumina MiSeq 2 (Gadol *et al.*, 2022). CH₄ in the headspace of each microcosm is measured periodically using a gas chromatograph equipped with a flame ionization detector to confirm that microcosms have not switched

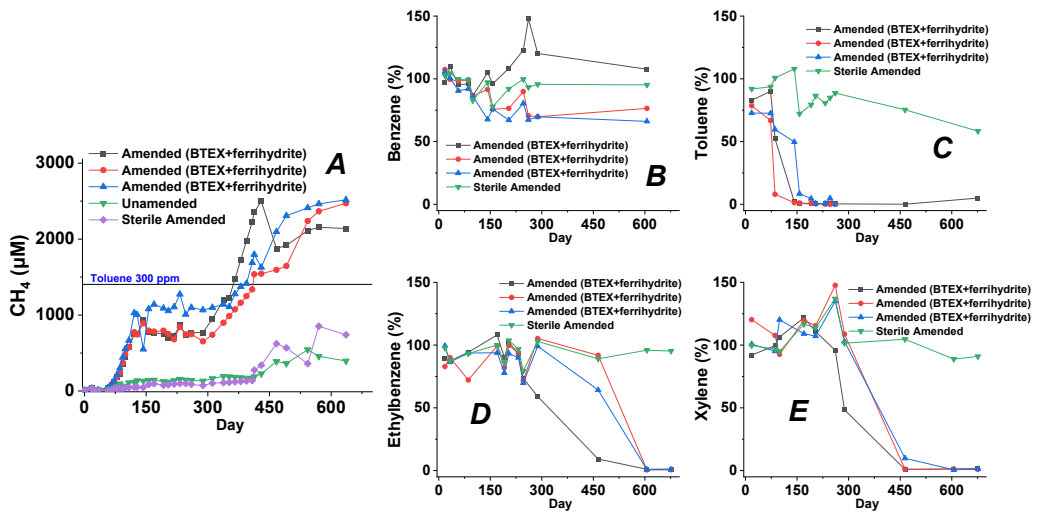


Figure 1 Methane production (panel A) and biodegradation of benzene (panel B), toluene (panel C), ethylbenzene (panel D) and xylene (panel E) in FFT microcosms with amorphous Fe, ferrihydrite. Biodegradation is present as per cent of initial amount in amended microcosms compared to abiotic sterile control.

to methanogenic conditions and to analyze the positive baseline controls.

Results and Discussion

Microcosms containing FFT collected from MLSB were amended with BTEX (benzene, toluene, ethylbenzene and xylenes) and incubated under iron-reducing conditions using amorphous (ferrihydrite) or crystalline (hematite) iron minerals as TEAs for hydrocarbon biodegradation. Among BTEX, biodegradation of toluene occurred by ~75 days primarily under iron-reducing condition (fig. 1C) with no visible CH₄ production (fig. 1A). Afterwards, redox conditions became methanogenic due to insufficient supply of ferrihydrite (10mM). Subsequently, remaining toluene, ethylbenzene and xylenes were completely metabolized under methanogenic conditions by ~600 days (fig. 1C-E) with corresponding increase in CH₄. Crystalline Fe^{III} (hematite) did not produce iron-reducing conditions as we noticed CH₄ production right at the outset in one of the replicates (fig. 2A) without any hydrocarbon biodegradation (fig. 2B-E). We speculate that crystalline Fe enhanced methanogenic degradation of unknown endogenous substrates. Biodegradation of hydrocarbons with concomitant CH₄ production suggests

little role of crystalline Fe^{III} in CH₄ inhibition. Though experimental designed lacked positive control (methanogenic treatment without crystalline Fe), we did observe faster biodegradation of ethylbenzene in the presence of crystalline Fe (fig. 2D). There are several recent reports that support enhanced methanogenesis in the presence of crystalline Fe^{III} (Gadol *et al.*, 2022; Vigderovich *et al.*, 2019).

Since Fe-reducing conditions lasted for a very short period of time in ferrihydrite amended microcosms, analysis of the microbial communities by 16S rRNA gene sequencing at the end of the experiment indicated development of methanogenic community in all the microcosms. The most abundant bacterial taxa were δ -Proteobacteria and Clostridia in all ferrihydrite and hematite-amended cultures. Both Clostridiales and δ -Proteobacteria enriched in most of our methanogenic cultures during hydrocarbon biodegradation (Siddique *et al.*, 2018) and usually ferment organic carbon to simple compounds such as alcohols, fatty acids, H₂ and CO₂. Clostridiales has also been reported as a fermentative iron-reducing bacteria that use Fe^{III} as a sink of electrons without conserving any energy for growth (Dong *et al.*, 2022). The archaeal communities were

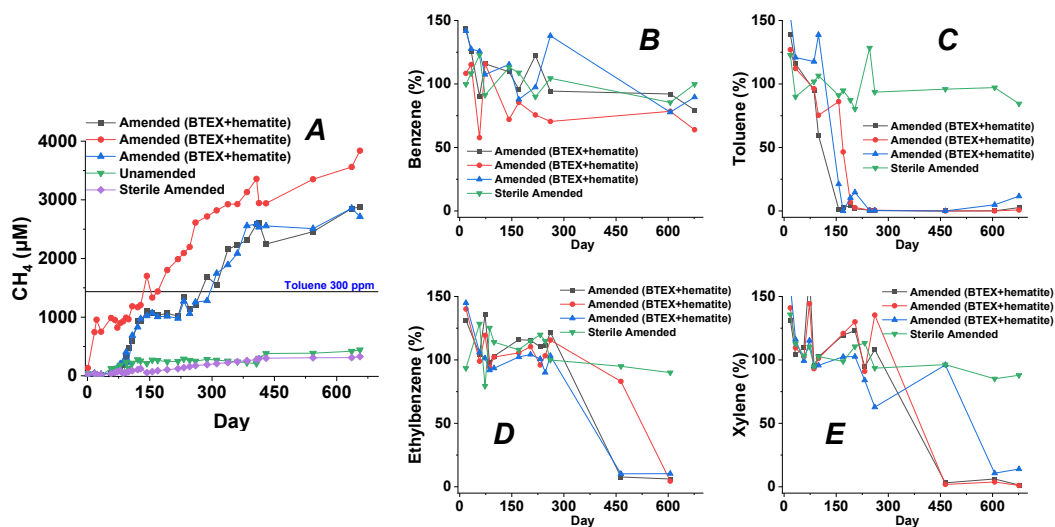


Figure 2 Methane production (panel A) and biodegradation of benzene (panel B), toluene (panel C), ethylbenzene (panel D) and xylene (panel E) in FFT microcosms with crystalline Fe, hematite. Biodegradation is present as per cent of initial amount in amended microcosms compared to abiotic sterile control.

dominated by methanogens - both acetoclastic *Methanosaetaceae* and hydrogenotrophic *Methanoregulaceae* indicating both routes of CH_4 production. We observed the enrichment of these archaeal taxa in most of our previous studies investigating methanogenic degradation of hydrocarbon (Mohamad Shahimin and Siddique, 2017b; Siddique *et al.*, 2015, 2018).

Based on our findings and issues, such as insufficient Fe amendment used in first experiment using amorphous Fe^{III} for creating iron-reducing conditions and lack of positive control in crystalline Fe^{III} experiment, a set of new experiments was established using OSPW and FFT from BML to continue to study the possibility of suppressing the methanogenesis in the FFT amended with naphtha (source of hydrocarbons) using other TEAs. Microcosms with only OSPW representing suboxic environment did not show any microbial metabolism under nitrate- or sulfate-reducing conditions (tab. 1). No degradation of any hydrocarbons was detected regardless of amendment – naphtha or separate groups of hydrocarbons (n-alkanes, iso-alkanes, BTEX). Low microbial activity is likely related to a low abundance of microorganisms (DNA yield was much lower compared to FFT samples)

as well as with suboxic conditions of OSPW in tailing ponds where presumably NRB and SRB consortia are not well-developed (Foght *et al.*, 2017).

Under iron-reducing conditions using sufficient amorphous Fe^{III} (90 mM ferrihydrite), only toluene has degraded so far in the microcosms amended with naphtha in 50-100 days (tab. 1); all other hydrocarbons (we monitor ~30 labile naphtha compounds) remained undegraded during this short period (on anaerobic microbiology time-scale). Microcosms with amorphous Fe did not produce CH_4 indicating sole prevailed Fe-reducing conditions. Biodegradation of toluene started almost immediately in the microcosms where NTA (chelating agent) was added (compared to day 50th without NTA; tab. 1) to increase Fe^{III} (ferrihydrite) bioavailability. However, complete toluene degradation took ~70 days with or without NTA. In the microcosms with crystalline Fe^{III} (magnetite), methanogenic conditions prevailed at day 25-30th of incubation; earlier than in the positive control (methanogenic condition without magnetite) and complete toluene degradation was faster (80 vs 125 days) compared to positive control (tab. 1). This finding substantiates our earlier Fe experiment where we observed faster CH_4



Table 1 Biodegradation of hydrocarbons in the microcosms amended with 0.2% naphtha under nitrate, iron- and sulfate-reducing conditions.

Redox conditions	Matrix	Degraded compound	Time frame (per cent of biodegradation)
Nitrate-reducing	OSPW	No degradation	No degradation
	OSPW+FFT	Toluene	50-350 days (100%)
Iron-reducing*	FFT+ferrhydrite	Toluene	50-75 days (100%)
	FFT+magnetite	Toluene	80 days (100%)
	FFT without magnetite	Toluene	125 days (100%)
Sulfate-reducing	OSPW	No degradation	No degradation
	OSPW+FFT	Toluene	20-120 (80%)
		Heptane	30-120 (50-74%)
		Octane	30-120 (43-62%)
		Decane	300-400 days (44-69%)

* Iron-reducing conditions could only be achieved in the microcosms amended with amorphous Fe^{II} mineral (ferrhydrite). Adding crystalline mixed-valent Fe^{II,III} mineral (magnetite) did not produce iron-reducing conditions. FFT without magnetite served as a methanogenic positive control and included in the experiment to infer role of magnetite in the study.

production and hydrocarbon biodegradation (fig. 2). In conclusion, our results comparing amorphous and crystalline Fe minerals reveal that (1) amorphous Fe^{III} mineral creates Fe-reducing conditions preventing redox conditions to fall into methanogenic conditions, and (2) crystallinity of Fe minerals (irrespective of valence because we compared Fe^{III} hematite in first experiment and Fe^{II,III} magnetite in second experiment), enhances methanogenic conditions probably mediating electron transfer among microbial community members (Zhuang *et al.*, 2015).

Regarding other redox conditions, unlike OSPW, microbes active under-sulfate-reducing conditions seemed more abundant in FFT. Among BTEX, only toluene degradation was observed by FFT microbes after 20 days of incubation under sulfate-reducing conditions in microcosms filled with FFT collected from methanogenic BML. In addition, biodegradation of heptane, octane, and decane, three hydrocarbons from a broad suite of labile hydrocarbons, has been achieved so far during 400 days of incubation. Experiments are in progress and we expect a long incubation period as anaerobic microorganisms take years to develop catabolic abilities to degrade a variety of structurally different hydrocarbons. For example, it took 700-2200 days to degrade n-alkanes and 1200-2500 days for iso-alkanes in FFT under methanogenic conditions. (Siddique *et al.*,

2015). We will keep monitoring depletion of all BTEX and major n-alkanes, iso-alkanes and cycloalkanes under nitrate-, iron- and sulfate-reducing conditions. We will also explore metabolic pathways of hydrocarbon degradation by indigenous microorganisms using 'Omics' approaches. We are also analysing FFT solid phase to determine transformation and distribution of nitrogen, sulphur and iron species/compounds under anaerobic environment. This comprehensive approach/model will establish linkages between methanogenesis and nitrogen, iron, and sulfur cycling in the FFT. The results will help develop strategies suggesting *in situ* amendments of redox compounds or *ex situ* application of redox processes to inhibit methanogenesis in oil sands tailings ponds and EPLs.

Conclusions

Our study uses a combined geochemical and microbial approach to investigate hydrocarbon biodegradation and microbial metabolic pathways under different redox conditions to mitigate GHG emission. The data retrieved so far suggests that consuming labile hydrocarbons under iron- and sulfate-reducing conditions is potentially a promising strategy for GHG mitigation in oil sands tailings. Tailings themselves can serve as a source of Fe as oxide minerals that are present in FFT, but it is necessary to increase the availability of

Fe for microbes (e.g. adding chelating agents and inhibiting ferrihydrite crystallization). Fe transformation in tailings also carries additional benefits, such as accelerating water recovery and tailing consolidation (Siddique *et al.*, 2014a). Developing Fe- and sulfate reducing cultures may serve as inoculants for scale-up for mitigating GHG emissions from tailings ponds and future EPLs. The successful management of tailings ponds and EPLs through the mitigation of GHG emissions will support sustainable oil sands development in Alberta, aligning with the Alberta government's mandate of a 45% reduction in methane emissions by 2025.

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References

- Burkus, Z., Wheler, J., Pletcher, S., 2014. GHG emissions from oil sands tailings ponds: overview and modelling based on fermentable substrates. Part I: review of the tailings ponds facts and practices . Alberta Environ. Sustain. Resour. Dev. November 2, 54pp.-doi.org/10.7939/R3F188 (accessed 22 Oct 2019).
- Dong, H., Zhang, F., Xu, T., Liu, Y., Du, Y., Wang, C., Liu, T., Gao, J., He, Y., Wang, X., Sun, S., She, Y., 2022. Culture-dependent and culture-independent methods reveal microbe-clay mineral interactions by dissimilatory iron-reducing bacteria in an integral oilfield. *Sci. Total Environ.* 840. <https://doi.org/10.1016/j.scitotenv.2022.156577>
- Foght, J.M., Gieg, L.M., Siddique, T., 2017. The microbiology of oil sands tailings: Past, present, future. *FEMS Microbiol. Ecol.* 93, fix034, doi: 10.1093/femsec/fix034. <https://doi.org/10.1093/femsec/fix034>
- Gadol, H.J., Elsherbini, J., Kocar, B.D., 2022. Methanogen Productivity and Microbial Community Composition Varies With Iron Oxide Mineralogy. *Front. Microbiol.* 12. <https://doi.org/10.3389/fmicb.2021.705501>
- Kabwe, L.K., Scott, J.D., Beier, N.A., Wilson, G.W., Jeeravipoolvarn, S., 2017. Environmental implications of end pit lakes at oil sand mines in Alberta, Canada. *Environ. Geotech.* 6, 67–74. <https://doi.org/10.1680/jenge.17.00110>
- Mohamad Shahimin, M.F., Siddique, T., 2017a. Sequential biodegradation of complex naphtha hydrocarbons under methanogenic conditions in two different oil sands tailings. *Environ. Pollut.* 221, 398–406. <https://doi.org/10.1016/j.envpol.2016.12.002>
- Mohamad Shahimin, M.F., Siddique, T., 2017b. Methanogenic biodegradation of paraffinic solvent hydrocarbons in two different oil sands tailings. *Sci. Total Environ.* 583, 115–122. <https://doi.org/10.1016/j.scitotenv.2017.01.038>
- Schnetger, B., Lehnert, C., 2014. Determination of nitrate plus nitrite in small volume marine water samples using vanadium(III)chloride as a reduction agent. *Mar. Chem.* 160, 91–98. <https://doi.org/10.1016/J.MARCHEM.2014.01.010>
- Siddique, T., Fedorak, P.M., Foght, J.M., 2006. Biodegradation of short-chain n-alkanes in oil sands tailings under methanogenic conditions. *Environ. Sci. Technol.* 40, 5459–5464. <https://doi.org/10.1021/es060993m>
- Siddique, T., Fedorak, P.M., Mackinnon, M.D., Foght, J.M., 2007. Metabolism of BTEX and naphtha compounds to methane in oil sands tailings. *Environ. Sci. Technol.* 41, 2350–2356. <https://doi.org/10.1021/es062852q>
- Siddique, T., Kuznetsov, P., Kuznetsova, A., Arkell, N., Young, R., Li, C., Guigard, S., Underwood, E., Foght, J.M., 2014a. Microbially-accelerated consolidation of oil sands tailings. Pathway I: Changes in porewater chemistry. *Front. Microbiol.* 5. <https://doi.org/10.3389/fmicb.2014.00106>
- Siddique, T., Kuznetsov, P., Kuznetsova, A., Li, C., Young, R., Arocena, J.M., Foght, J.M., 2014b. Microbially-accelerated consolidation of oil sands tailings. Pathway II: Solid phase biogeochemistry. *Front. Microbiol.* 5. <https://doi.org/10.3389/fmicb.2014.00107>
- Siddique, T., Kuznetsova, A., 2020. Linking hydrocarbon biodegradation to greenhouse gas emissions from oil sands tailings and its impact on tailings management1. *Can. J. Soil Sci.* 100, 537–545. <https://doi.org/10.1139/cjss-2019-0125>
- Siddique, T., Semple, K., Li, C., Foght, J.M., 2020. Methanogenic biodegradation of iso-alkanes and cycloalkanes during long-term incubation



- with oil sands tailings. *Environ. Pollut.* <https://doi.org/10.1016/j.envpol.2019.113768>
- Siddique, T., Shahimin, F., Zamir, S., Semple, K., Li, C., Foght, J., 2015. Long-Term Incubation Reveals Methanogenic Biodegradation of C5 and C6 iso-Alkanes in Oil Sands Tailings. *Environ. Sci. Technol.* 49 (24), 14732–14739.
- Siddique, T., Stasik, S., Mohamad Shahimin, F., and Wendt-Potthoff, K., 2018. Microbial Communities in Oil Sands Tailings: Their Implications in Biogeochemical Processes and Tailings Management, in: McGenity, T.J. (Ed.), *Microbial Communities Utilizing Hydrocarbons and Lipids: Members, Metagenomics and Ecophysiology*, *Handbook of Hydrocarbon and Lipid Microbiology*. Springer Nature, Switzerland, https://doi.org/10.1007/978-3-030-14785-3_10, pp. 1–33.
- Trüper, H.G., Schlegel, H.G., 1964. Sulphur metabolism in Thiorhodaceae I. Quantitative measurements on growing cells of *Chromatium okenii*. *Antonie Van Leeuwenhoek* 30, 225–238. <https://doi.org/10.1007/BF02046728>
- Vigderovich, H., Liang, L., Herut, B., Wang, F., Wurgaft, E., Rubin-Blum, M., Sivan, O., 2019. Evidence for microbial iron reduction in the methanic sediments of the oligotrophic southeastern Mediterranean continental shelf. *Biogeosciences* 16, 3165–3181. <https://doi.org/10.5194/bg-16-3165-2019>
- Zhuang, L., Tang, J., Wang, Y., Hu, M., Zhou, S., 2015. Conductive iron oxide minerals accelerate syntrophic cooperation in methanogenic benzoate degradation. *J. Hazard. Mater.* 293, 37–45. <https://doi.org/10.1016/j.jhazmat.2015.03.039>