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Persistence and carbon isotopic fingerprints of alkylotrophic methanogenesis under sulfate-amended condition

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Methanogenic degradation of petroleum is a prevalent process in subsurface reservoirs, leading to significant methane accumulation (*1*). In sulfate-rich environments, sulfate reduction commonly outcompetes canonical methanogenesis for shared electron donors, reshaping carbon flux and isotopic signatures (*2-3*). However, the response of alkylotrophic methanogenesis, a widespread novel methanogenic pathway in oil reservoirs enabling direct methanogenic degradation of *n*-alkanes within single cells (4), to sulfate remains enigmatic. In this study, we incubated an enrichment culture of alkylotrophic methanogens, initially containing a low abundance of sulfate-reducing bacteria (SRB), under sulfate-amended conditions (20 mM). Results showed that sulfate declined 3.3 mmol on average. Notably, the production of CH_4 (average 1.9 mmol) and CO_2 (average 1.3 mmol) showed a concurrent decrease. Coupled with the enrichment of *Desulfoglaeba* (SRB), this indicates that a portion of the degraded petroleum carbon was potentially diverted toward SRB biomass synthesis. Methane under sulfate-amended conditions was more ^{13}C-depleted (–48.7 ± 0.7‰) compared to sulfate-free conditions (–45.6 ± 1.1‰). The $\delta^{13}C$-CO_2 values reached +5.6 ± 0.7‰ with sulfate and +10.4 ± 0.7‰ without sulfate incubation. The 16S gene analysis suggested that hydrogenotrophic methanogenesis was suppressed, while alkylotrophic methanogenesis was not significantly impacted. The relative depletion of ^{13}C in CO_2 (by 5.6‰) likely stemmed from competition for petroleum hydrocarbons degradation by *Desulfoglaeba*. This ^{13}C-depleted CO_2 subsequently served as a source for the CO_2 reduction step in alkylotrophic methanogenesis, ultimately resulting in overall ^{13}C-depleted CH_4. These findings reveal that alkylotrophic methanogens can persist under sulfate conditions, serving as a persistent methane source in sulfate-rich oil environments. This study provides isotopic constraints for fingerprinting methane sources in complex anaerobic ecosystems.

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