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Microbial nitrate reduction in propane- or butane-based membrane biofilm reactors under oxygen-limiting conditions

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ABSTRACT

Nitrate contamination has been commonly detected in water environments and poses serious hazards to human health. Previously methane was proposed as a promising electron donor to remove nitrate from contaminated water. Compared with pure methane, natural gas, which not only contains methane but also other short chain gaseous alkanes (SCGAs), is less expensive and more widely available, representing a more attractive electron source for removing oxidized contaminants. However, it remains unknown if these SCGAs can be utilized as electron donors for nitrate reduction. Here, two lab-scale membrane biofilm reactors (MBfRs) separately supplied with propane and butane were operated under oxygen-limiting conditions to test its feasibility of microbial nitrate reduction. Long-term performance suggested nitrate could be continuously removed at a rate of ~40–50 mg N/L/d using propane/butane as electron donors. In the absence of propane/butane, nitrate removal rates significantly decreased both in the long-term operation (~2–10 and ~4–9 mg N/L/d for propane- and butane-based MBfRs, respectively) and batch tests, indicating nitrate bio-reduction was driven by propane/butane. The consumption rates of nitrate and propane/butane dramatically decreased under anaerobic conditions, but recovered after resupplying limited oxygen, suggesting oxygen was an essential triggering factor for propane/butane-based nitrate reduction. High-throughput sequencing targeting 16S rRNA, bmoX and narG genes indicated Mycobacterium/Rhodococcus/Thauera were the potential microorganisms oxidizing propane/butane, while various denitrifiers (e.g. Dechloromonas, Denitratisoma, Zoogloea, Acidovorax, Variovorax, Pseudogulbenkiania and Rhodanobacter) might perform nitrate reduction in the biofilms. Our findings provide evidence to link SCGA oxidation with nitrate reduction under oxygen-limiting conditions and may ultimately facilitate the design of cost-effective techniques for ex-situ groundwater remediation using natural gas.

1. Introduction

Nitrate (NO$_3^-$) contamination has been widely detected in surface water (Zhang et al., 2014) and groundwater (Wick et al., 2012) due to the excessive use of nitrogen fertilizers and improper use of animal manures in agriculture (Power and Schepers, 1989). Exposure to nitrate-contaminated groundwater could potentially pose serious health threats to human beings, such as methemoglobinemia, gastric cancer and goitre (Majumdar and Gupta, 2000; Wolfe and Patz, 2002). As such, a maximum contaminant level of 10 mg/L NO$_3^-$-N has been established by the US environmental protection agency (US EPA) (Doudrick et al., 2015).

Multiple techniques have been developed to remove nitrate from contaminated water, including physical (reverse osmosis or electrolysis), physicochemical (ion exchange) and biological methods (Ao et al., 2019; Duan et al., 2020; Rezvani et al., 2019). Compared to physical or physicochemical strategies, biological reduction of nitrate has received more attention in recent years as it is more sustainable and environmentally friendly (Rezvani et al., 2019). Methane (CH$_4$), which is an easily available carbon source and generates less residue electrons, has been recently proposed as a promising electron donor for nitrate removal (Luo et al., 2018; Modin et al., 2016; Shi et al., 2021). However, pure or 95% CH$_4$ used in these studies is relatively expensive (~$1–1.5/m$) (Global Petrol Prices, 2020) for practical application in contaminated water remediation, therefore requiring the utilization of an inexpensive source of methane, such as biogas and natural gas (<$0.1/m$) (Markets Insider, 2022). In comparison to biogas which would acidify bioreactors by carbon dioxide (CO$_2$, 30–50% in biogas)
and thus deteriorate microbial activities (Ghafari et al., 2009; Liu et al., 2019), natural gas generally contains very low CO$_2$ (~0.9–9%) (Shimelik and Mukhtar, 2012), making it an ideal source of CH$_4$. However, in addition to CH$_4$ as the primary component, natural gas also contains considerable amount of non-methane short chain gaseous alkanes (SCGAs, up to 20%), such as ethane, propane and butane (Tissot and Welte, 1984). Although these gaseous alkanes are potent carbon and energy sources for microorganisms, it remains unknown whether they can be utilized as electron donors to remove nitrate from contaminated water.

Microbial oxidation of non-methane SCGAs could occur under both aerobic and anaerobic conditions. Under aerobic conditions, various microbes able to utilize propane and butane have been isolated from soil and oilfields, including Arthrobacter, Corynebacterium, Mycobacterium, Nocardiia and Rhodococcus (Shenman, 2006). Similar with aerobic methane oxidation pathway, aerobic degradation of propane and butane are also catalyzed by monoxygenases, with reactive oxygen species from molecular oxygen incorporated into the terminal or sub-terminal carbon atom in propane or butane, yileding propanol or butanol (Musat, 2015; Shenman, 2006). Electrons generated from aerobic propane and butane oxidation processes are presumably available for reducing electron acceptors. Recently, propane and butane were supplied as electron donors in membrane biofilm reactors (MBfRs) to drive seelenate or perchlorate reduction under oxygen-limiting conditions (Lai et al., 2020; 2021a; 2021b). Trichloroethylene (TCE), which is a suspected carcinogen and persistent in the environment, could also be degraded by several aerobic bacteria using monoxygenases, such as propane-grown R. rhodochrous and Mycobacterium vaccae JOBS (Malachowsky et al., 1994; Wackett et al., 1989) and butane-grown Pseudomonas butanovora (Hamamura et al., 1997). Under anaerobic conditions, sulfate has been identified as the electron acceptor to be coupled to the oxidation of non-methane SCGAs. The pure culture Strain BuS5, a deltaproteobacterium, was proved to anaerobically oxidize propane and butane to CO$_2$ with sulfate reduced to sulfide (Kniemeyer et al., 2007). Recently, anaerobic enriched archaea, Candidatus „Syntrophoarchaeum” and Candidatus „Argoarchaeum”, was shown to activate butane and ethane via buty1-coenzyme M and ethyl-coenzyme M formation, respectively, and reducing equivalents are channelled to the partner sulfate reducing bacteria (Laso-Perez et al., 2016, Chen et al., 2019). Given that nitrate is a thermodynamically more favourable electron acceptor than sulfate (Marietou et al., 2009), nitrate reduction coupled to propane or butane oxidation would be energetically feasible. Recently, a bacterial lineage within the class Symbiodiaria, Candidatus Alkanivorans nitratireducens, was proved to mediate nitrate-dependent anaerobic propane oxidation (Wu et al., 2022). Compared to the limited nitrate reduction rate under anaerobic conditions (~0.5-1.2 mg N/L/d) (Wu et al., 2022), biological nitrate reduction driven by propane or butane under oxygen-limiting conditions would be more favorable to achieve a quick nitrate removal from contaminated water.

Overall, the objective of this study was to test the feasibility of microbial nitrate reduction using propane or butane as electron donors under oxygen-limiting conditions. In order to achieve this aim, two independent MBfRs were set up, in which hollow fibre membranes were employed to supply propane or butane and to provide surfaces for biofilm attachment. Nitrate was continuously fed into the reactors as the sole electron acceptor to evaluate the long-term nitrate removal performance. Multiple batch tests were conducted to study the mechanisms of microbial nitrate reduction driven by propane or butane. The key microbes involved in propane or butane oxidation and nitrate reduction were identified by high-throughput sequencing for targeting both 16S rRNA gene and functional genes (bmaX encoding alpha hydroxylase subunit of propane/butane monoxygenase and narG encoding nitrate reductase). It is expected that the findings will improve our understanding of propane or butane-based nitrate reduction processes and may ultimately help us develop techniques to remove nitrate from contaminated water.

2. Materials and methods

2.1. MBfR setup and operation

Two MBfRs which have similar configurations with that used in our previous study (Lai et al., 2020) were set up in this study (Fig. S1). Each MBfR was equipped with two glass tubes and two bundles of hollow fiber membranes which contained 84 fibers (model MHR-200T, Mitsubishi, Ltd., Japan). The MBfR has a total volume of 200 mL and total membrane surface area of 123 cm$^2$. Pure propane or n-butane (hereafter butane) gas (99.99%, Coregas, Australia) was supplied to each MBfR with the gas pressure inside the hollow fibers controlled at 10 psi (165 kpa) by gas-pressure regulators (Coregas, Australia). The bulk liquor in the MBfRs was mixed and recirculated at a flow rate of 78 mL/min using a peristaltic pump (Masterflex, USA). In order to trigger propane and butane oxidation, limited oxygen was delivered at 150 mg/L/d to the systems through an oxygen-permeable tubing for the recirculation (Silicone tubing, Longerpump, China). The MBfRs were covered with aluminium foil to avoid alga growth and operated in a temperature-controlled lab (22 ± 2 °C).

In order to achieve biofilm attachment on the membrane surfaces, 20 mL of activated sludge from a full-scale wastewater treatment plant (Brisbane, Australia) was used as the inoculum for each MBfR, and then the sludge was mixed with fresh medium containing 20 mg N/L NO$_3^-$ via recirculation for 48 h. The synthetic medium was prepared as follows (unit: g/L if not specified): CaCl$_2$ 1 mg, MgSO$_4$·7H$_2$O 5 mg, K$_2$HPO$_4$ 0.20 g, and Na$_2$HPO$_4$ 0.16 g, acidic trace elements 0.5 mL/L, and alkaline trace elements 0.2 mL/L (Lai et al., 2020). Afterwards, fresh medium with nitrate concentration of ~10 and ~20 mg N/L (correspond to the surface NO$_3^-$ loading rate of 0.53 and 1.05 g N/m$^2$/d) was continuously fed into the MBfR at HRT of 6.5 h in stages 1 and 2, respectively. In stage 3, influent nitrate concentration was maintained at ~20 mg N/L and HRT was increased to 10 h to improve nitrate removal efficiency. In order to test if nitrate could be consumed without propane/butane, the gas supply of propane/butane to each MBfR was intentionally stopped for 10 days after stage 3 and then recovered. During the entire operational period, liquid samples were collected from the reactor 2–3 times per week to monitor the concentrations of nitrate and nitrite.

2.2. Batch tests

Three batch tests (each bath test was run in triplicate) were conducted for each MBfR at the end of long-term operation (Table S1). Bath test A was carried out in situ MBfR to study the dynamic changes of propane/butane and nitrate under oxygen-limiting conditions. The pumps feeding influent medium was stopped and the supply of propane/butane to the hollow fibers was cut off. Fresh medium containing ~20 mg N/L NO$_3^-$ was sparged with propane/butane for 30 min and then introduced into the MBfRs to eliminate any headspace. Liquid samples were collected every 1–2 h to monitor the dissolved propane/butane and nitrate concentration. Fresh medium saturated with helium was injected into the MBfR after each sampling to compensate for liquid losses due to sampling, and the dilution effect of the extra liquid brought to the reactor was considered during data analysis. Bath test B was conducted to evaluate the nitrate reduction rates in the absence of propane/butane. The MBfR was disconnected from the gas cylinder to stop propane/butane supply and the residual gas in the gas tubes and hollow fibers was removed by flushing with N$_2$ for 30 min. Oxygen supply was controlled at the same rate (150 mg/L/d) with Batch test A. Fresh medium containing ~20 mg N/L NO$_3^-$ was fed into the MBfR to replace the medium containing dissolved propane/butane. Liquid samples were regularly taken to measure the concentrations of nitrate. Batch test C was carried out to study the pattern of propane/butane oxidation and nitrate reduction under anaerobic conditions. Biofilms were scrapped off from the hollow fibers and mixed with fresh synthetic medium containing
3. Results

3.1. Long-term performance of nitrate reduction in propane/butane-based MBfRs

Initially in stage 1, synthetic influent containing ~ 10 mg N/L NO\textsubscript{3} was continuously fed into the two MBfRs supplied with propane/butane. Nitrate was completely removed without nitrite accumulation at HRT of 6.5 h for both MBfRs, giving rise to a nitrate removal rate of ~37 mg N/L/d (Fig. 1a and b). With nitrate concentration in influent increased to ~20 mg N/L in stage 2, nitrate removal efficiency decreased to 25–40% and 40–65% initially but finally recovered to 50–65% and 70–80% for propane and butane fed MBfRs, respectively. With HRT increased from 6.5 h to 10 h at stage 3, the propane and butane-based MBfRs achieved a nitrate removal efficiency of 75–85% and 85–95%, respectively, and the effluent nitrate concentration was below 5 mg N/L, which could meet the groundwater standard (<10 mg N/L) established by US EPA (Doudrick et al., 2013). However, when propane/butane gas supply was stopped for the reactors, the nitrate removal rates significantly decreased from above 32 to ~2–10 mg N/L/d for propane, and above 38 to ~4–9 mg N/L/d for butane. Correspondingly, nitrate concentration increased to 13–19 and 13–15 mg N/L in propane-based and butane-based MBfRs, respectively. Once gases were re-supplied to each MBfR, nitrate removal efficiency was recovered to ~75–80%.

3.2. Dynamic changes of nitrate, propane/butane in batch tests

In order to further understand the mechanisms of propane/butane-based microbial nitrate reduction processes, multiple batch tests were conducted at the end of long-term operation for each MBfR. In Batch test A, nitrate was consistently decreased without nitrite accumulation for both MBfRs (Fig. 2a and b) at similar reduction rates (1.23 ± 0.10 and 1.18 ± 0.15 mg N/L/h for propane and butane, respectively). Simultaneously, propane and butane were also decreased with time, at consumption rates of 4.48 ± 0.81 mg/L/h for propane and 5.73 ± 0.75 mg/L/h for butane (Fig. 2a and b). The electrons generated by propane and butane oxidation was 2.4-fold and 3.1-fold of electrons required for nitrate and oxygen reduction, respectively (Table S2). However, nitrate reduction rates (0.39 ± 0.06 mg N/L/h for propane and 0.42 ± 0.03 mg N/L/h for butane) in Batch test B without the supply of propane/butane were significantly lower than that in Batch test A when propane/butane was provided (p < 0.01, Fig. 2c and d, Fig. S2).

For Batch test C, the biofilms were incubated under anaerobic conditions in order to explore the role of oxygen in propane and butane-based nitrate reduction. It was found that the oxidation rates of propane and butane under anaerobic conditions were less than 0.1 mg/L/h, significantly lower (p <0.01) than that in Batch test A when limited oxygen was supplied (Fig. 2e and f, Fig. S2). In addition, nitrate reduction rates were also dramatically decreased to 0.07 ± 0.01 mg N/L/h for propane and 0.05 ± 0.00 mg N/L/h for butane (p < 0.01, Fig. 2e and f, Fig. S2). However, with oxygen gas injected into the serum bottles used in Batch test C (oxygen: propane or butane, 2:1), nitrate reduction rates were quickly recovered to 0.46 ± 0.07 mg N/L/h for propane and 0.48 ± 0.02 mg N/L/h for butane (Fig. 2e and f). Concurrently, propane and butane consumption rates were increased to 6.62 ± 0.07 mg/L/h and 4.20 ± 0.37 mg/L/h, respectively.

3.3. Microbial community structure

Microbial communities of the inoculum and propane/butane-shaped biofilms were first investigated using 16S rRNA gene amplicon sequencing to track which microorganisms were enriched in the biofilms. Results (Fig. 3a and b) showed that Mycobacterium, which is negligible in the inoculum (with a relative abundance of ~0.1% of total microorganisms), was obviously enriched in the propane/butane-shaped biofilms (~3.0–6.1% for propane and ~1.1–2.1% for butane). Genus
affiliated to *Rhodococcus* was undetectable in the inoculum, but it became dominant in the butane fed biofilms (3.3–5.7%). Differently, *Rhodococcus* was not found in any biofilms fed by propane. In addition, the relative abundances of *Dechloromonas, Denitratisoma* and *Zoogloea*, which all affiliated to the *Rhodocyclaceae* family, were increased from 0.5%, 1.9% and being undetected in the inoculum to 2.5–9.4%, 2.8–6.0% and 0.4–4.3% in the propane-shaped biofilms, and 6.5–17.6%, 2.4–9.0% and 0.8–7.2% in the butane fed biofilms, respectively. Propane fed biofilms were also dominated by *Holophagaceae* (7.1–10.1%) and *Fimbriimonadaceae* (1.4–11.4%), while both reactors boosted the growth of *Anaerolineaceae* (3.7–11.4%) and *Flavobacterium* (0.9–5.8%) that were suggested to be able to utilize extracellular polysaccharides and proteins as carbon sources (Bernardet and Bowman, 2006; Sun et al., 2016).

In order to further identify major functional microbes performing propane/butane oxidation and nitrate reduction, high-throughput sequencing targeting *bmx* and *narG* genes was applied. Results suggested that the genus of *Mycobacterium* dominated in the microbial groups containing *bmx* gene in both propane (22.4–54.8%) and butane (2.6–15.7%) shaped biofilms (Fig. 4a), which is in accordance with the 16S rRNA gene sequencing results that propane/butane supply significantly promoted the growth of *Mycobacterium*. In addition, another genus of *Thauera* was also found to be predominant in the propane-fed biofilms (13.3–58.2%) and its relative abundance varied from 0.2% to 33.3% in the biofilms supplied with butane. However, *Thauera* was very minor (<0.1%) in the biofilms according to 16S rRNA gene sequencing. *Acidovorax* was the major genus in the *narG* gene containing community for both propane and butane fed biofilms (3.6–52.3%, Fig. 4b), followed by *Variovorax* (0.7–12.5%) and *Pseudogulbenkiania* (0.5–8.0%). *Rhodanobacter* accounted for 3.0–6.3% in the microbial groups containing *narG* gene for the butane shaped biofilms but was undetected for propane. The relative abundances of these four genera detected by *narG* gene sequencing were consistently low (<0.1%) for all biofilms based on the 16S rRNA gene sequencing.

## 4. Discussion

### 4.1. Potential mechanisms of microbial nitrate reduction coupled to propane/butane oxidation

Although nitrate removal driven by aerobic or anaerobic methane oxidation has been intensively studied in previous work (Cai et al., 2015; Luo et al., 2018; Sun et al., 2013), the feasibility of nitrate reduction using propane or butane as electron donors has not been investigated so far. This study demonstrated that nitrate removal could be achieved in two independent MBRs supplied with propane/butane under oxygen-limiting conditions. Considering these gaseous alkanes were the sole electron donors for each MBR, the continuous nitrate removal with the presence of propane/butane and the dramatically decreased reduction rates without these alkanes collectively suggested that nitrate reduction was associated with propane/butane oxidation. In addition, simultaneous propane/butane and nitrate consumption were observed in Batch test A, while nitrate reduction rates were also significantly lower in the absence of propane/butane (Batch test B) than that with the presence of propane/butane (Batch test A), further confirming that nitrate bio-reduction was driven by propane/butane oxidation. The observed nitrate reduction without propane/butane was likely driven by organic components such as extracellular polymeric substances and polyhydroxyalkanoates generated in the biofilms, which have been shown to support selenate, perchlorate or vanadate reduction in ethane/propane/methane-based MBRs (Lai et al., 2018; Luo et al., 2020; Sun et al., 2021).

It should be noted that limited oxygen (150 mg/L/d) was intentionally supplied to the reactors to boost the aerobic oxidation of propane/butane. Indeed, limited oxygen was found to play an important role in propane/butane-based nitrate reduction. Propane/butane and nitrate consumption rates were obvious with oxygen provided in Batch test A, while their consumption rates were significantly decreased in Batch test C when the biofilms were incubated under anaerobic conditions. Moreover, after limited oxygen was re-provided to the biofilm...
cultures, the consumption rates of propane/butane and nitrate were significantly increased, further confirming that oxygen supply was essential for triggering propane/butane-based nitrate reduction. It has been suggested that limited oxygen supply was necessary for ethane/propane-driven microbial selenate and perchlorate reduction (Lai et al., 2020; 2021a). The perchlorate removal rate in a methane-based MBfR was also increased from 4 mg Cl/L/d under anoxic conditions to 16 mg Cl/L/d when 10 mg/L/d of oxygen was externally supplied (Wu et al., 2019). In addition, the \( \text{O}_2:\text{CH}_4 \) ratio was suggested to have a significant impact on nitrate removal rates of the aerobic methane oxidation coupled to denitrification processes, in which 0.25 was identified as the optimal ratio for denitrification (Zhu et al., 2017). Nevertheless, the role of oxygen in propane/butane driven nitrate reduction requires systematical investigations in future studies, which will also ultimately improve nitrate removal capacities for bioremediation.

In terms of microbial community analysis using 16S rRNA gene and functional gene sequencing, microbial nitrate reduction driven by propane/butane appears to be completed via a partnership between two functional groups (aerobic propane/butane oxidation bacteria and nitrate reducers). Compared to microbial communities in the inoculum, Mycobacterium was significantly enriched in both MBfRs supplied with propane and butane. Moreover, high-throughput sequencing targeting the \( \text{bmoX} \) gene also showed that microbial communities were dominated by Mycobacterium for both biofilms, indicating that Mycobacterium played an important role in propane/butane oxidation. In addition, the butane fed biofilms were also dominated by Rhodococcus. Although Rhodococcus was not identified by \( \text{bmoX} \) gene sequencing, it should be noted that the primer sets used in this study might not be specific for amplifying butane oxidation genes in Rhodococcus, and thus we cannot exclude the possibility that Rhodococcus also performed butane oxidation in the butane-fed MBfR. Indeed, many strains affiliated to the genera of Mycobacterium and Rhodococcus have been reported to be able to grow on propane and butane (Shennan, 2006). For example, Mycobacterium strain NBB4 harbours complete gene clusters encoding multiple monooxygenases (MO) including propane MO and butane MO, and was shown to grow on propane and butane (Coleman et al., 2011). Rhodococcus sp. strain BCP1 could oxidize butane and generate 1-butanol as the terminal oxidation product (Cappelletti et al., 2015). Another potential propane/butane oxidation genus Thauera, which has been reported to oxidize \( \text{C}_3-\text{C}_5 \) alkanes via the expression of soluble
butane monooxygenase (Cooley et al., 2009), was also detected in the biofilms by the bmoX gene sequencing (0.2-58.2%), although they were negligible (<0.1%) in the 16S rRNA gene sequencing results. Regarding nitrate reduction, diverse nitrate reducers were enriched in the propane/butane fed biofilms, including the genera of Dechloromonas, Denitratisoma and Zoogloea based on 16S rRNA gene sequencing, and Acidovorax, Variovorax, Pseudogulbenkiania and Rhodanobacter according to narG gene sequencing. Various strains affiliated to these genera have been well recognized as denitrifiers (Chen et al., 2019; Coates et al., 2001; Fahrbach et al., 2006; Huang et al., 2015; Im et al., 2010; Maintinguer et al., 2013; Prakash et al., 2012), indicating they may have performed nitrate reduction in the biofilms, using the electrons or intermediates generated from aerobic propane/butane oxidation.

In order to provide insights into the mechanisms of microbial nitrate reduction driven by propane/butane under oxygen-limiting conditions, long-term operation, batch tests together with 16S rRNA and functional gene sequencing were combined in this study. It is proposed that propane/butane was aerobically oxidized by Mycobacterium/Rhodococcus/Thauera, in which unknown intermediates might be generated and simultaneously utilized to reduce nitrate by denitrifiers (e.g., Dechloromonas, Denitratisoma, Zoogloea, Acidovorax, Variovorax, Pseudogulbenkiania and Rhodanobacter, Fig. S3). Potential intermediates such as acids and alcohols were monitored in this study but found to be negligible (<0.5 mg/L), possibly because they were completely consumed by denitrifiers. Indeed, acetate was not detected in a methane-based MBR while the external addition of acetate immediately boosts up perchlorate reduction rates, suggesting it may be a key syntrophic linkage between aerobic methanotrophs and perchlorate reducers (Wang et al., 2022). However, it cannot be completely excluded that Mycobacterium/Rhodococcus might independently carry out the propane/butane-driven nitrate reduction, since a few strains of these bacteria were capable of performing denitrification (Chen et al., 2012; Sohaskey and Modesti, 2009) and Mycobacterium was also detected in the biofilms by narG gene sequencing (0.1-2.1%). Further studies are required to reveal the detailed pathways by using isotope tracing and meta-omics techniques.

4.2. Implications of this work

This work was undertaken to evaluate the feasibility of removing nitrate from contaminated water using propane and butane as electron donors and give insights into the mechanisms. Given natural gas is composed of methane, ethane, propane and butane, further studies are required to investigate the feasibility of using mixtures of these gases for nitrate removal in MBfRs, which will ultimately help the development of cost-effective techniques for groundwater remediation using natural gas. Groundwater constitutes a primary drinking water source for ~33% and ~70% of the population in the US and China (Kenny et al., 2009; Qiu, 2011), respectively, while it suffers from serious contamination and nitrate is one of the dominant pollutants (Burow et al., 2010). Although a multitude of technologies have been developed for nitrate remediation, such as catalytic reduction of nitrate (Reddy and Lin, 2000) and adding extra carbon sources (ethanol or methanol) to stimulate nitrate bio-reduction (Gomez et al., 2000), nitrate removal using natural gas
based MBfRs has a few advantages. Firstly, natural gas (~$0.019-0.100 per mol electrons) is much cheaper than organic carbon sources such as ethanol (~$0.17-0.29 per mol electrons) (Markets Insider., 2022), which suggests it is more economically attractive to utilize natural gas for bio-reduction compared to ethanol. Secondly, natural gas is less soluble than organic matters and thus will not remain in effluent, avoiding post-treatment. Therefore, it might be feasible to use natural gas based MBfRs to treat nitrate-contaminated groundwater. In addition, SCGAs and nitrate are found to co-exist in shale gas extraction sites, where leakage of shale gas causes SCGA emissions and hydraulic fracturing process leads to generation of high volumes of wastewater containing nitrate (Umukoro and Ismail, 2017; Sun et al., 2019). It is potentially feasible to utilize propane and butane produced in-situ shale gas exploitation sites to treat nitrate contamination.

Nevertheless, compared to the reported nitrate removal rates driven by aerobic methane oxidation processes (~24-260 mg N/L/d) (Modin et al., 2007), the rates achieved in this study (~40-50 mg/L/d) were relatively lower, while it could be potentially further optimized via the optimization of oxygen supply. Oxygen was found to be an essential triggering factor for propane/butane oxidation in this study, since it is required to incorporate O atom into the C-H bond of SCGAs to activate them. However, it should be noticed that given the high redox potential of oxygen, it can also be a strong electron acceptor outcompeting nitrate for electrons. Indeed, a proper oxygen supply (184 mg/L/d) was suggested to enhance the selenate reduction rate in a methane-based MBfR while an excessive oxygen supply (626 mg/L/d) suppressed selenate reduction, indicating oxygen plays a dual role in methane-based selenate reduction (Wang et al., 2021). Therefore, it requires further investigation how oxygen supply rate could be precisely controlled to maintain the balance between propane/butane oxidation and nitrate reduction and eventually maximize nitrate removal rates.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data availability**

Data will be made available on request.

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Supplementary materials


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