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# Nitrate enhances TCE removal by enriching bifunctional denitrifying/aerobic co-metabolizing microbes in the joint $H_2/O_2$ system†

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Trichloroethylene (TCE) is a pervasive groundwater contaminant that poses significant health and ecological safety risks. The coexistence of hydrogen ($H_2$) and oxygen ($O_2$), resulting from electrochemical remediation technologies, has been shown to promote the degradation of TCE by fostering specific microbial communities. However, the role of nitrate ($NO_3^-$), a common co-contaminant with TCE in groundwater, in this $H_2/O_2$ system remains poorly understood. This study revealed that increasing the nitrate concentrations (0–240 mg $L^{-1}$) significantly enhanced TCE removal rate and efficiency, with the maximum removal rate increased by 1.84 times and removal efficiency by 19.79%. The variation in nitrate concentration could influence microbial diversity. In particular, denitrifying microorganisms, such as *Pseudomonas*, *Hydrogenophaga*, and *Methyloversatilis*, were enriched, which could reduce nitrate and nitrite using $H_2$ as an electron donor and degrade TCE through aerobic co-metabolism. The increased presence of these microorganisms led to an abundance of the functional gene *phe*, which is responsible for TCE aerobic co-metabolic degradation, thereby enhancing TCE removal in the joint $H_2/O_2$ system. However, a high nitrate concentration of up to 240 mg $L^{-1}$ inhibited the accumulation of *Pseudomonas* and *Methyloversatilis*. Meanwhile, it increased the abundance of *Hydrogenophaga*, which could use $H_2$ and $O_2$ to fix $CO_2$ and provide organics for bacterial growth. These insights provide valuable technical guidance for the remediation of TCE and nitrate composites in groundwater environments, highlighting the potential of leveraging the interactive effects of $H_2$, $O_2$, and nitrate on microbial communities for effective bioremediation strategies.

### Water impact

Trichloroethylene (TCE) is widely utilized in the chemical industry. Electrochemical synergistic microbial technology is an effective method to enhance the bioremediation of TCE in underground water. Electrochemistry generates hydrogen ($H_2$) and oxygen ($O_2$), which create a joint hydrogen–oxygen ($H_2/O_2$) region that is conducive to carbon cycling and the removal of TCE. This study explored the effects of different concentrations of nitrate on the degradation of TCE by *in situ* microorganisms in a combined $H_2/O_2$ system through an indoor batch experiment. This study elucidates the mechanisms by which nitrate influences microbial diversity, enriching denitrifying bacteria using $H_2$ as an electron donor to degrade TCE through co-metabolism under aerobic conditions. These insights provide valuable technical guidance for the remediation of TCE and nitrate composite contaminants in groundwater environments, highlighting the potential of leveraging the interactive effects of $H_2$, $O_2$, and nitrate on microbial communities for effective bioremediation strategies.

## 1 Introduction

Trichloroethylene (TCE) is extensively employed as an essential organic solvent and chemical raw material.[1] Owing to challenges in handling and disposing during its production, use and transportation, TCE is one of the most critical organic pollutants in the groundwater environment, posing severe ecological risks.[2,3] Additionally, TCE is biotoxic and can enter the human body through inhalation and dermal absorption, potentially causing poisoning and damage to internal organs as well as neurological impairment.[4] Given the global emphasis on groundwater safety, remediation of TCE contamination has emerged as a pivotal area of scientific inquiry.

Currently, microbial remediation is one of the predominant TCE remediation technologies, operating

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primarily through three distinct biological processes, namely, microbial aerobic co-metabolism, aerobic direct metabolism, and anaerobic reductive dichlorination.[5] Aerobic co-metabolism of TCE involves a process where oxygen ($O_2$) acts as an electron acceptor under aerobic conditions and microorganisms utilize TCE as an electron donor or an externally supplied carbon and energy source, facilitating the conversion of substrates that are not typically metabolized when they are growing.[6] Direct aerobic metabolism of TCE involves microorganisms that utilize TCE as their exclusive carbon and energy source, leading to its direct degradation and metabolism under aerobic conditions.[7] Anaerobic reductive dechlorination involves the degradation of TCE by microorganisms *via* respiration in an anaerobic environment, where TCE acts as an electron acceptor and hydrogen ($H_2$) acts as an electron donor.[8]

In recent years, electrochemical technology has emerged as a novel approach for efficient remediation of organic pollutants in groundwater; it supplies $H_2$ and $O_2$ to the aquifer through water electrolysis, thereby promoting TCE degradation.[9] Research works indicate that combining electrochemical and microbial remediation technologies can effectively promote the *in situ* microbial degradation of chlorinated hydrocarbons (CHCs) in groundwater.[9–11] The cathodic electrode produces $H_2$, enriching genes associated with the anaerobic degradation of pollutants. In contrast, the anode produces $O_2$, which enhances the electron transfer activity within the microbial community and enriches functional genes related to phenol monooxygenase.[12,13] Furthermore, the cathode-produced $H_2$ and anode-produced $O_2$ mix in the aquifer through groundwater flow, establishing a joint hydrogen–oxygen ($H_2/O_2$) zone. Compared to solely an $H_2$ or $O_2$ environment, the joint $H_2/O_2$ system effectively enhances denitrification and sulfate oxidation.[12] The joint $H_2/O_2$ environment will promote the formation of microbial communities with specific structures and functions, increasing the relative abundance of the aerobic functional gene *phe*,[14] encoding phenol monooxygenase and the TCE anaerobic dechlorination functional gene *tceA*.[15,16] The joint $H_2/O_2$ system enables aerobic and anaerobic degradation of CHCs, thereby effectively enhancing the removal of CHCs like trichloroethane.[15,17] However, the subsurface environment is inherently complex, and groundwater constituents such as $SO_4^{2-}$, $NO_3^-$, and Fe(III) can potentially impact the degradation of TCE.[18,19] Consequently, additional research is required to elucidate the impact of these groundwater components on TCE biodegradation under the joint $H_2/O_2$ system.

Nitrate ($NO_3^-$) has become a prevalent inorganic contaminant in groundwater. The nitrate concentration in groundwater ranges from 2 to 776 mg $L^{-1}$.[20,21] $NO_3^-$ contaminant frequently co-exists with organochlorine pollutants in the subsurface environment.[22] For instance, at a site in the U.S.A., groundwater was found to contain nitrate (up to 230 mg $L^{-1}$) mixed with TCE (up to 300 μg $L^{-1}$).[23] The presence of $NO_3^-$ can impact the degradation process of CHCs through denitrification.[24,25] Studies have confirmed that $NO_3^-$ inhibits reductive dechlorination under anaerobic conditions, with the inhibition effect intensifying as $NO_3^-$ concentrations increase.[26] The redox potentials for CHCs reductive dechlorination and denitrification are similar, leading to competition for electron donors such as $H_2$ (ref. 24) and $NO_3^-$ typically precedes TCE in reductive processes under $H_2$-limited conditions.[19] Furthermore, in the process of aerobic denitrification aimed at $NO_3^-$ removal under aerobic conditions, microorganisms may simultaneously utilize either $NO_3^-$ or $O_2$ as an electron acceptor, followed by organic compounds serving as a carbon source and electron donor for reducing $NO_3^-$.[27] Leahy[28] discovered that under hypoxic conditions, $NO_3^-$ could act as an alternative electron acceptor, promoting the degradation of TCE. While numerous studies have explored the impact of $NO_3^-$ on TCE degradation under anaerobic and aerobic conditions, the regulatory mechanism of $NO_3^-$ on TCE degradation in environments where $H_2$ and $O_2$ coexist remains unclear. Therefore, it is crucial to investigate the influence of $NO_3^-$ on TCE degradation within the joint $H_2/O_2$ system to understand the role of denitrification in this process.

This study was conducted on an indoor batch experiment to explore the impact and underlying mechanisms of different $NO_3^-$ concentrations on the degradation of TCE by *in situ* microorganisms within the joint $H_2/O_2$ system. 16S rRNA gene sequencing coupled with quantitative PCR (qPCR) were utilized to monitor changes in microbial community structure and functional genes following the introduction of $NO_3^-$, and to investigate the mechanism by which the denitrification process either enhances or impedes the oxidation or reduction of TCE. The results of this study are expected to offer valuable technical insights for the remediation of TCE and $NO_3^-$ co-contamination in the joint $H_2/O_2$ system.

## 2 Materials and methods

### 2.1 Sediment characterization

The sediment samples for the experiment were collected from a chemical plant site in Tianjin (China) at a depth of 3–5 m, which was contaminated by high concentrations of CHCs. The TCE concentration in the sediments was 171.41 mg $kg^{-1}$, with $NO_3$ concentrations varying from 17.48–23.16 mg $kg^{-1}$ and total organic carbon (TOC) concentrations ranging from 650–940 mg $kg^{-1}$.

### 2.2 TCE degradation experiment

The experimental device consisted of a 250 mL screw glass vial and a 300 mL Teflon bag connected by a Viton hose, as depicted in Fig. S1.†[17] The preparation was conducted in an anaerobic chamber (Coy Laboratory Products Inc., Michigan). Each experimental unit added 40 g of wet sediment and 200 mL of sterilized ultrapure water, along with 80 μM TCE. $NO_3^-$ was introduced at 0, 30, 60, and 240 mg $L^{-1}$ concentrations across different experimental groups. The gas bag was filled

 

with 100 mL of $H_2$ and 50 mL of $O_2$ ($H_2$ and $O_2$ were obtained by water electrolysis). Sterilized sediments without additional $NO_3^-$ supplementation served as controls. Microcosms were prepared in duplicate, and all experimental systems were cultured at 25 °C and 150 rpm. Soil samples were collected for subsequent microbial analysis on the 0th and 5th days of the reaction.

### 2.3 Analytical methods

The determination of TCE and possible intermediates in the samples was performed on an automatic purge and trap-gas chromatography-mass spectrometry (PT-GC-MS) (PT: Atomx, Teledyne Tekmar, USA; GC-MS: Thermo Fisher Scientific Inc., USA) with an Agilent DB-624 capillary column (30 m × 0.25 mm × 1.4 μm). The oven temperature was held at 35 °C for 2 min, heated at a rate of 5 °C $min^{-1}$ to 120 °C, then heated at a rate of 10 °C $min^{-1}$ to 220 °C and held for 2 min. The EI ion source was used with a temperature of 230 °C and the full scanning range was 35–270 amu. The quantification was done using the internal standard method.

Immediately after sampling, the oxidation–reduction potential (ORP) was measured using a redox potential tester (PHS-3C, Rex of Shanghai Co., Ltd. China). Dissolved hydrogen (DH) was measured with a hydrometer (DH200, CLEAN, USA) and dissolved oxygen (DO) was measured directly using a noninvasive oxygen sensor. The pH was determined using a pH meter. The concentrations of $NO_3^-$ and $NO_2^-$ in solution were determined by ultraviolet spectrophotometry.

### 2.4 Taxonomic and functional microbial composition analyses

Total DNA from sediments was extracted using a soil DNA extraction kit (Power Soil® DNA). Microbial gene sequencing was performed using an Illumina MiSeq instrument (MiSeq, Illumina, USA) at the Personal Biotechnology Company (Shanghai, China). Microbial community analysis was performed using QIIME 2 (2019.4), and microbial diversity and abundance were estimated using Mothur software (version 1.35.1, USA).

### 2.5 Quantification of functional genes by qPCR

The real-time quantitative polymerase chain reaction (qPCR) was performed on an ABI QuantStudio 3 (Version 1.4.1 software, Applied Biosystems, USA) to quantify microbial numbers and functional genes in the samples. The 16S rRNA gene was amplified using primers 341F and 515R. The abundance of genes related to bio-dechlorination (*tceA*), aerobic co-metabolic degradation (*pmoA*, *phe*), and $NO_3^-$ reduction (*narG*, *nirS*, *nosZ*) were quantified by qPCR. Detailed information on the primer sequences is shown in Table S1.† The 16S rRNA genes were normalized for each sample. The relative abundances of functional genes were calculated using the $2^{-\Delta\Delta CT}$ method, and the absolute abundances of functional genes were calculated by multiplying the number of 16S rRNA gene copies.[29,30]

## 3 Results and discussion

### 3.1 TCE removal under different $NO_3^-$ concentrations in the joint $H_2/O_2$ system

The changes in TCE concentration under the joint $H_2/O_2$ system with different nitrate concentrations and sterilized control are shown in Fig. 1a. In the sterilized control, TCE concentrations gradually decreased over 15 days, likely due to volatilization and adsorption. In the unsterilized sediments, TCE concentrations dropped quickly during the initial 7 days and almost stopped declining afterwards. Nitrate concentration showed a significant impact on the TCE removal. Higher nitrate concentrations, such as 60 and 240 mg $L^{-1}$, caused faster and more TCE removal.

To quantitatively compare the effect of different nitrate concentrations on the TCE removal rate, the first-order kinetic model in eqn (1) was used to fit the TCE removal curves, as shown in Fig. 1b.

$$\ln(C_0/C_t) = kt \quad (1)$$

where $C_0$ and $C_t$ denote the concentration of TCE at initial and time $t$, and $k$ is the kinetic constant of the primary reaction.

As shown in Fig. 1b and c, the $k$ values increased from 0.1115 to 0.3167, with nitrate concentrations elevating from 0 to 240 mg $L^{-1}$, which followed a nonlinear eqn (2).

$$k = 0.3843C/(48.2138 + C) \quad (2)$$

where $C$ denotes different nitrate concentrations in the different groups.

This trend indicated that the TCE removal rate was more affected by lower nitrate concentrations. After 15-day experiments under different nitrate concentrations, the final TCE removal efficiencies were calculated using eqn (3) and shown in Fig. 1d.

$$\text{TCE removal efficiency} = [(C_0 - C_t)/C_0] \times 100\% \quad (3)$$

where $C_0$ and $C_t$ denote the concentration of TCE at initial and time $t$.

During the 15-day experimental period, the TCE removal efficiencies under 0–30 mg $L^{-1}$ nitrate were 64.60% to 67.25%, and those with 60 and 240 mg $L^{-1}$ nitrate increased to 74.16% and 87.04%, respectively, indicating that a higher concentration of nitrate significantly promoted TCE removal efficiency in enough reaction time.

Prior research has found that $NO_3^-$ inhibits the degradation of chlorinated olefins under anaerobic conditions.[31] However, Yu[32] demonstrated that low concentrations of $NO_3^-$ could serve as a nitrogen source to enhance the biological reductive dechlorination of organic

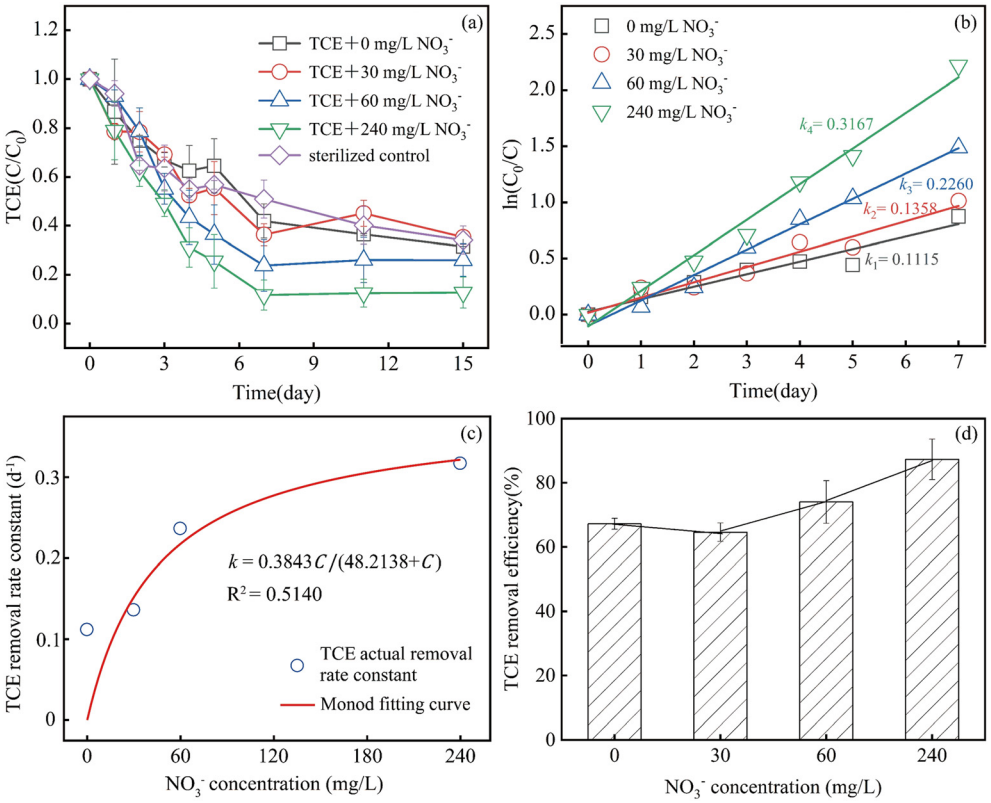


**Fig. 1** TCE removal kinetics (a), fitting of TCE removal curves (b), nonlinear regression analysis of the relationship between TCE removal rate constants and nitrate concentrations (c), and TCE removal efficiencies (d) under different nitrate concentrations.

compounds. Our study is the first to report that nitrate enhances TCE removal in a joint $H_2/O_2$ system, which is common in the electrochemical remediation sites.

### 3.2 Denitrification during TCE removal in the joint $H_2/O_2$ system

Denitrification reactions were observed by detecting $NO_3^-$, $NO_2^-$, and $NH_4^+$ variations during 15-day experiments (Fig. 2a and b) to understand the TCE removal kinetics impacted by nitrate concentrations in the joint $H_2/O_2$ system. No $NH_4^+$ was detected in all the experiments. During the initial 7 days when TCE was quickly removed, nitrate concentrations dropped dramatically, accompanied by nitrite generation. Under 30 and 60 mg $L^{-1}$ nitrate concentrations, nitrates were exhausted in 5 days, while the formed nitrite reached a maximum and declined afterwards, indicating further nitrite reductions. In contrast, under 240 mg $L^{-1}$ nitrate, the nitrate removal efficiency reached 53.86% within 7 days, and then the nitrate reduction stopped. Meanwhile, nitrite was gradually produced in 7 days and then kept stable, suggesting that nitrate and nitrite reductions were limited after 7 days. This limitation should be attributed to the accumulation of toxic nitrite. It was discovered that nitrite accumulation inhibited the growth of *Pseudomonas fluorescens*.[33] The free nitrite concentrations of 0.01–0.025 mg $L^{-1}$ can reduce 40% nitrate reduction activity.[34]

To further elucidate the relationship between nitrate denitrification and TCE removal in the joint $H_2/O_2$ system, the quantitative relationship was determined by calculating the molar ratio of consumed nitrate to removed TCE (Δnitrate/ΔTCE) in 15 days (Fig. 2c). It showed that the Δnitrate/ΔTCE varied with time and nitrate concentrations. Under lower nitrate concentrations such as 30 mg $L^{-1}$, the Δnitrate/ΔTCE values were about 20 on the first day, increased to 35 on the second day, and then decreased to and kept stable around 10 after 3 days. The Δnitrate/ΔTCE values when the nitrate concentration was 60 mg $L^{-1}$ showed a similar trend with 30 mg $L^{-1}$ nitrate. In contrast, the high nitrate concentration of 240 mg $L^{-1}$ nitrate showed a high Δnitrate/ΔTCE value of 87 and gradually decreased to around 22. These results indicated that increased nitrate concentration enhanced denitrification more than TCE removal, especially during the first several days of the fast reaction stage.

### 3.3 Variation of environmental factors during TCE removal in the joint $H_2/O_2$ system

DH, DO, ORP, and pH are crucial parameters affecting CHC degradation.[30,35,36] Their variations were monitored during the experiment further to analyze the mechanism of nitrate effects on TCE removal. The results are shown in Fig. 3. DH and DO values were 0–0.6 mg $L^{-1}$ and 0–6 mg $L^{-1}$,

 

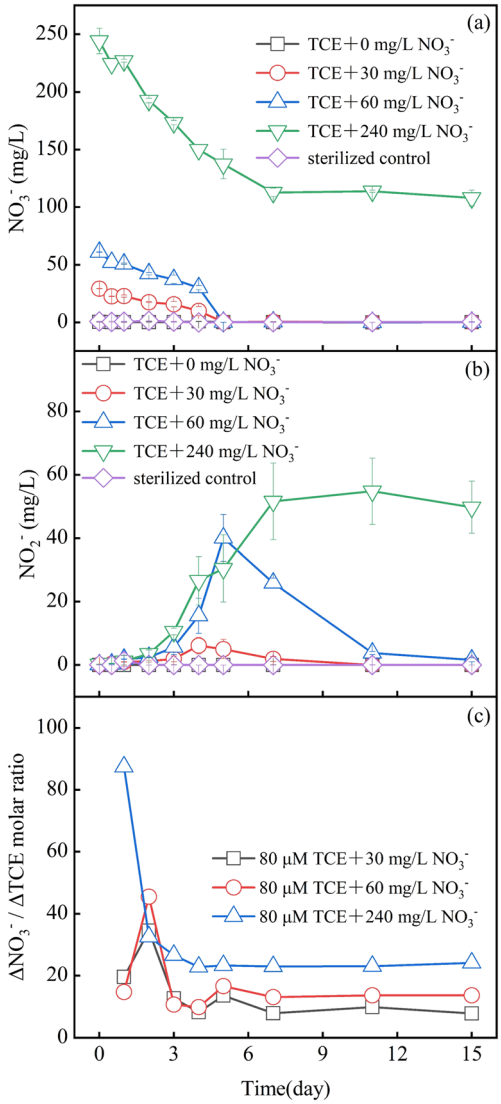


Fig. 2 Variations in aqueous-nitrate (a) and -nitrite (b) and Δnitrate/ΔTCE molar ratios (c) during the experiments at different nitrate concentrations.

respectively, in all the treatments, while ORP values fluctuated from −300 mV to 30 mV in the pH range of 8 to 9.

The significant variations of these parameters happened in 7 days. The increased DH and DO in two days was due to mixing the gases in the experimental solutions. The decrease in DH and DO should be attributed to their consumption in the systems. DH showed more vigorous fluctuations than DO, which also caused significant variations in ORP. Compared to other treatments, the sterilized control and nitrate-free microcosms consumed less DH and DO, while their ORP values stayed relatively stable between −200 mV and −300 mV. The presence of 30 and 60 mg $L^{-1}$ nitrate significantly increased the consumption of DH and DO, causing an increased ORP. The high nitrate concentration of 240 mg $L^{-1}$ consumed the most DH, but less DO than lower nitrate concentrations, which induced the highest ORP above zero. Overall, ORP in the sterilized control group was maintained between −300 mV and −200 mV, indicating an anaerobic state. In contrast, the ORP in the nitrate-free group rapidly decreased from −100 mV to −250 mV, also reflecting an anaerobic condition. In the 30 and 60 mg $L^{-1}$ nitrate groups, the ORP fluctuated between anaerobic and aerobic states. In the 240 mg $L^{-1}$ nitrate group, the ORP gradually increased from −300 mV to 10 mV within 4 days, indicating a transition from an anaerobic to an aerobic state. It has been demonstrated that the rate of anaerobic dechlorination of TCE is optimal at a redox potential of −260 mV,[37] while aerobic metabolism of TCE occurs at potentials above 50 mV.[38] As shown in Fig. 1a, the ORP in the experimental group varied from −300 mV to 30 mV, indicating that the alternation between anaerobic and aerobic states in the system is more favourable for TCE removal. In particular, the removal of TCE was more significant during the transition from anaerobic to aerobic conditions. The pH of all experimental groups was slightly alkaline, and nitrate addition caused a slight increase in pH.

The variations of DH and DO in the nitrate-containing groups demonstrated that DH and DO were simultaneously consumed in the joint $H_2/O_2$ system. Nitrate concentrations adjusted the consumption balance between DH and DO. Consequently, ORP and pH were changed by the DH and DO consumption. These critical environmental parameters can change the microbial compositions reversibly, especially some functional microbes, thereby impacting TCE removal.

### 3.4 Microbial community evolution under different nitrate concentrations in the joint $H_2/O_2$ system

16S rRNA high-throughput sequencing was used to analyze the microbial community structure impacted by nitrate concentrations in the joint $H_2/O_2$ system. In all the experimental microcosms, the total microorganism counts ranged from $10^9$–$10^{10}$ copies per g (Fig. 4a). A total of 4765 amplicon sequence variants (ASVs) were obtained based on gene sequencing analysis. The core ASVs were analyzed and demonstrated using a Venn diagram in Fig. 5a. There were 539 core ASVs in the original sediment before the experiment, which increased to 705 upon introducing the joint $H_2/O_2$ system. However, adding nitrate at 30, 60, and 240 mg $L^{-1}$ concentrations decreased the core ASV to 316, 380, and 281, respectively. Only 133 ASVs were common across all groups, indicating that adding different nitrate concentrations altered the microbial community structure in the joint $H_2/O_2$ system.

Chao1 and observed species indices were utilized to indicate microbial community richness, while Shannon and Simpson's indices were employed to characterize microbial community diversity, as detailed in Table S2.† Following a 5-day experimentation period in the joint $H_2/O_2$ system, richness and diversity were highest in the nitrate-free group compared to the start point. However, the addition of nitrate

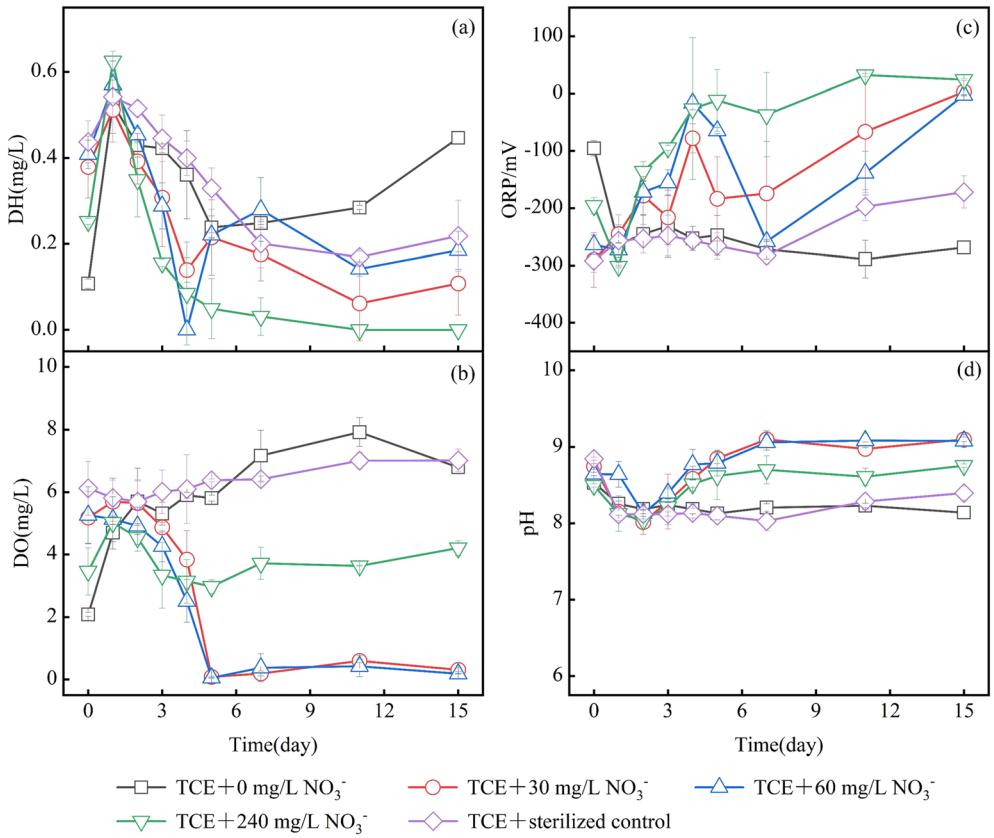


Fig. 3 Variations in DH (a), DO (b), ORP (c) and pH (d) during the experiments at different nitrate concentrations.

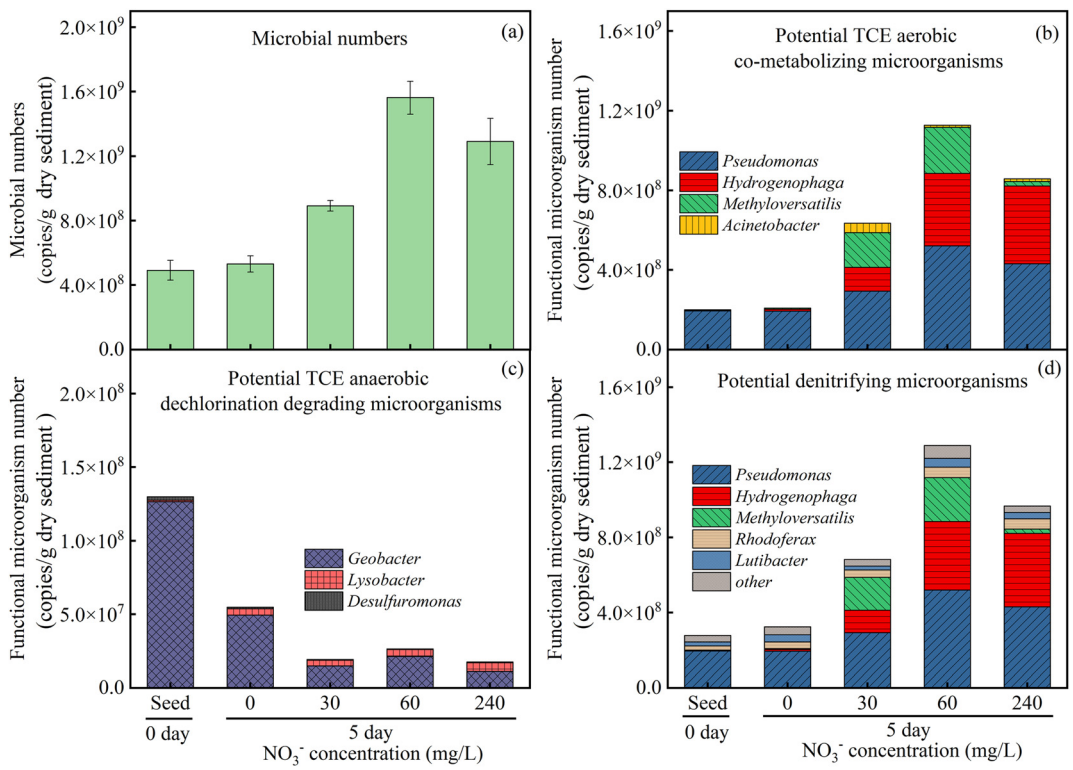


Fig. 4 Variation in the number of microorganisms (a), TCE aerobic co-metabolizing (b), TCE anaerobic dechlorinating (c) and denitrifying microbes (d) in the sediments before and after 5th day of experiment at different nitrate concentrations.

 

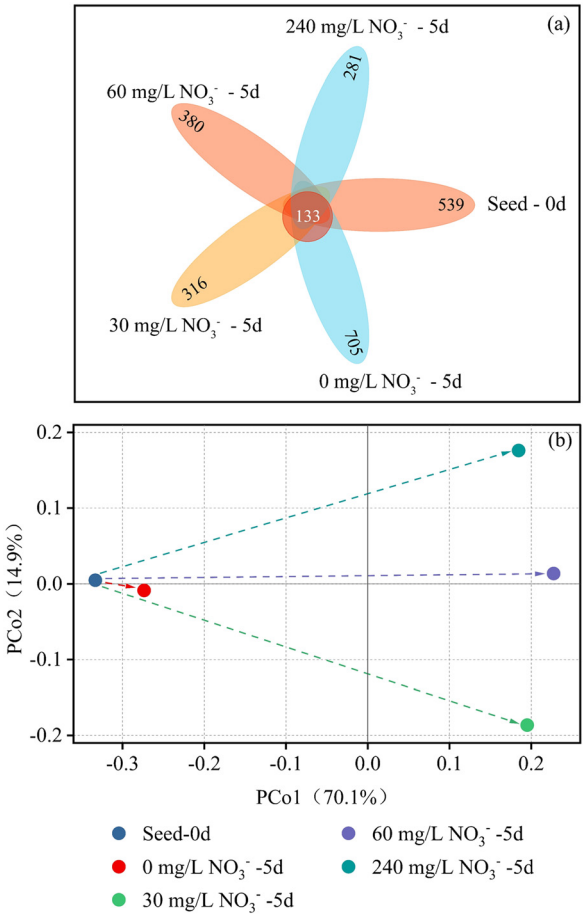


Fig. 5 Venn diagram of microbial community core ASV (a) and PCoA changes of microbial communities in microcosms (b) at different nitrate concentrations.

led to a decrease in richness and diversity across all supplemented groups. Notably, as nitrate concentration increased, there was a corresponding decline in community richness and diversity. These results further demonstrate that nitrate concentration is a key determinant for changes in microbial community structure in the joint $H_2/O_2$ system.

Clustering and principal coordinate analysis (PCoA) using Bray–Curtis distance revealed the evolutionary pathways of microbial communities between the different nitrate concentrations, as depicted in Fig. 5b. The horizontal and vertical axes accounted for 70.1% and 14.9% of the variance in microbial community structure, respectively. The microbial communities within each group exhibited variation in the direction opposite to PCo1. In contrast to the nitrate-free group, introducing nitrate induced more pronounced changes in community structure, with more significant divergence observed in the direction opposite to PCo2.

The microbial community composition focusing on the top 50 abundances in each nitrate concentration is depicted in Fig. 6. In the original sediments, *Pseudomonas* (39.63%) was the dominant genus, which reduced to 36.46% after the 5-day introduction of the joint $H_2/O_2$ system, and to 32.79–33.21% after the introduction of nitrate. *Geobacter* (21.75%) was the second dominant genus; its relative abundance was reduced to 7.96% after introducing $H_2/O_2$ and decreased to 0.69–1.32% after adding 30–240 mg $L^{-1}$ nitrate. *Hydrogenophaga* gradually increased from 0.99% to 30.31% in relative abundance and emerged as the dominant genus in the mixed $H_2/O_2$ and nitrate systems. *Methyloversatilis* had a low relative abundance in the original sediments, which rapidly increased to 19.46% upon adding 30 mg $L^{-1}$ nitrate, after which its abundance decreased with increasing nitrate concentrations.

*Pseudomonas* is frequently recognized as a fundamental component of the bacterial community capable of degrading TCE through co-metabolism, utilizing substrates such as phenol and toluene.[5,16] *Pseudomonas* is also capable of aerobic denitrification, with certain *Pseudomonas* strains

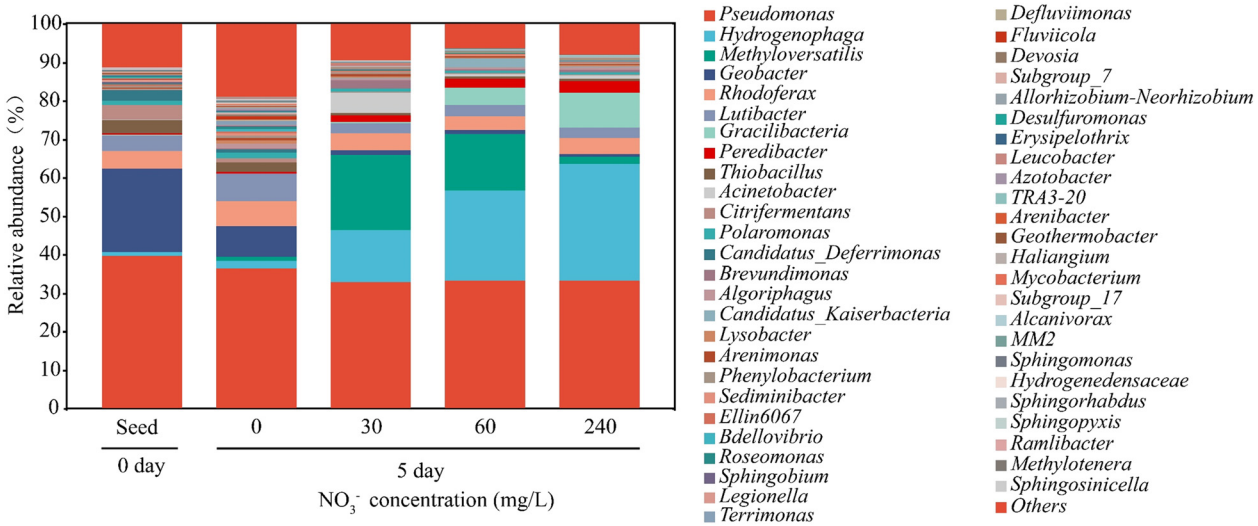


Fig. 6 Variations in relative abundance of microbial community composition in the sediments before and after 5th day of experiment at different nitrate concentrations.

carrying the *narG* gene, which encodes an enzyme that reduces $NO_3^-$ to $NO_2^-$.[5,39,40] *Hydrogenophaga* has been identified as a hydrogen-oxidizing bacteria,[41] capable of fixing $CO_2$ using $H_2$ acting as an electron donor and $O_2$ as an electron acceptor.[42,43] *Hydrogenophaga* was found to synthesize enzymes for the aerobic metabolism of TCE, contributing to its biodegradation.[17,44,45] Additionally, the genus can utilize nitrate as the sole nitrogen source to support its growth and remove nitrate under aerobic conditions,[46] and it is also capable of denitrifying nitrate using $H_2$ as an electron donor.[47] *Methyloversatilis* can produce enzymes that facilitate aerobic co-metabolic degradation of TCE.[9] It has also been identified as a denitrifying bacterium, with *Methyloversatilis universalis* FAM5 being able to reduce nitrate to nitrite.[48,49] *Geobacter* is an organohalide-respiring bacterium that mediates TCE reductive dechlorination to *cis*-dichloroethylene (cDCE), playing a significant role in anaerobic dechlorination processes.[30,50,51] No reports show that *Geobacter* is related to denitrification.

The variations in the numbers of aerobic co-metabolizing-, anaerobic dechlorinating-, and denitrifying functional bacteria are shown in Fig. 4(b–d). The bacteria with bi-functions of TCE aerobic co-metabolic degradation and denitrification, *i.e.*, *Pseudomonas*, *Hydrogenophaga*, and *Methyloversatilis*, were dominant in the potential TCE aerobic co-metabolizing and denitrifying microbial communities. Among them, under a high nitrate concentration of 240 mg $L^{-1}$, *Pseudomonas* and *Hydrogenophaga* declined, and *Hydrogenophaga* remained high due to the different resistance to nitrite. *Geobacter* numbers decreased significantly with the addition of $H_2/O_2$ and nitrate due to the negative effect of $O_2$.[52]

### 3.5 Changes in functional genes under different nitrate concentrations in the joint $H_2/O_2$ system

The functional genes associated with biological dechlorination (*tceA*),[16] aerobic co-metabolism (*pmoA*, *phe*)[45,53] and denitrification (*narG*, *nirS*, *nosZ*)[54] were quantified using qPCR to verify the effect of denitrification on the enrichment of anaerobic dechlorination and aerobic co-metabolism genes in the joint $H_2/O_2$ system. Fig. 7a illustrates the relative changes in three TCE degradation functional genes across different nitrate concentrations. The abundances of *tceA* and *pmoA* genes were lower than $2.4 \times 10^4$ copies per g, whereas that of *phe* was high up to $10^7$ copies per g. Compared with the initial sediment group, the expression of *tceA* and *pmoA* genes decreased in joint $H_2/O_2$ in the presence of nitrate. In contrast, the *phe* genes in the nitrate-free group with joint $H_2/O_2$ ($4.12 \times 10^7$ copies per g) increased relative to the original sediment ($2.83 \times 10^7$ copies per g). This number significantly rose to $8.95 \times 10^7$ copies per g when nitrate increased to 240 mg $L^{-1}$. These results proved that nitrate enhanced aerobic co-metabolism in the joint $H_2/O_2$ system.

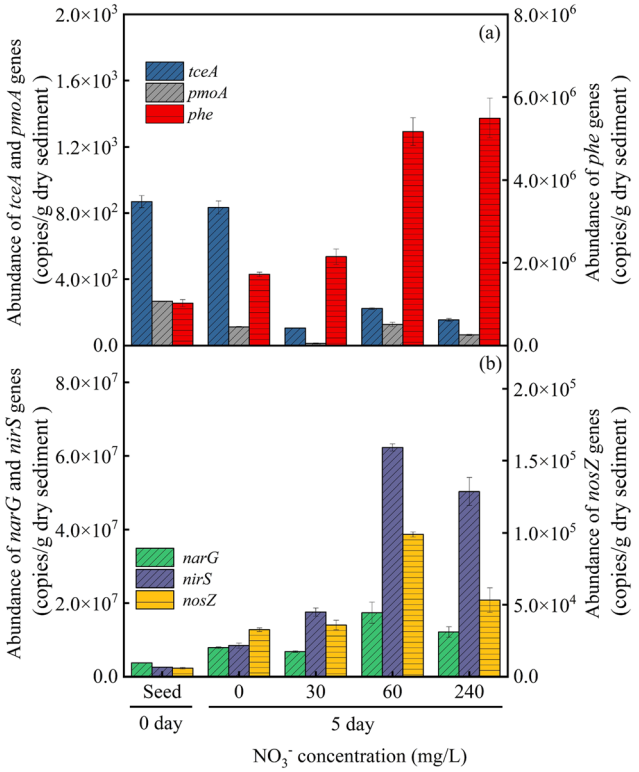


Fig. 7 Variation in gene abundances of *tceA*, *pmoA*, and *phe* (a) and *narG*, *nirS*, and *nosZ* (b) in the sediments before and after 5th day of experiment at different $NO_3^-$ concentrations.

The denitrification functional gene changes are shown in Fig. 7b. Introducing $H_2/O_2$ increased the abundance of *narG*, *nirS*, and *nosZ* compared to the original sediment. The *narG*, *nirS* and *nosZ* genes encode enzymes that drives the conversion of nitrate to nitrite, nitrite to nitrous oxide, and nitrous oxide to nitrogen, respectively.[55] Our previous research indicated that the abundance of functional genes related to denitrification could increase under the joint $H_2/O_2$ system.[12] This study demonstrated that introducing nitrate increased the abundance of *narG*, *nirS*, and *nosZ* genes, favoring the denitrification process to the nitrite and nitrous oxide conversion stages. However, the high nitrate concentration of 240 mg $L^{-1}$ showed lower denitrification functional genes than 60 mg $L^{-1}$, consistent with the trend of denitrification functional microbes due to nitrite accumulation.

### 3.6 Mechanism of the nitrate effect on TCE removal in the joint $H_2/O_2$ system

The experimental results showed that increased nitrate concentration from 0 mg $L^{-1}$ to 240 mg $L^{-1}$ enhanced the TCE removal rate, efficiency, and denitrification, while more DH and DO were consumed, which induced a higher ORP. It also caused evolution of microbial communities to accumulate microbes such as *Pseudomonas*, *Hydrogenophaga*, and *Methyloversatilis* with bi-functions of denitrifying and

aerobic co-metabolizing TCE, which was further demonstrated by the increased gene abundance of *phe* and *narG*, *nirS*, and *nosZ*. The highest nitrate concentration of 240 mg $L^{-1}$ inhibited denitrification.

The denitrification process necessitates reducing highly oxidized nitrate using organic or inorganic electrons; for instance, organic matter commonly serves as an electron donor in natural environments.[56] Studies have shown that aerobic denitrifying microorganisms (*Pseudomonas*, *Hydrogenophaga*, *etc.*) can use organic matter as a carbon and energy source to perform aerobic denitrification, a process in which oxygen and nitrate act as the electron acceptors and the carbon source is the electron donor.[57,58] *Hydrogenophaga* can fix $CO_2$ using $H_2$ as an electron donor and $O_2$ as an electron acceptor.[42,43] Additionally, it can reduce nitrate using $H_2$ as an electron donor.[47] Heterotrophic denitrifying bacteria such as *Methyloversatilis* can also perform denitrification using carbon compounds as carbon sources.[58] Certain autotrophic denitrifying bacteria within *Pseudomonas* can employ $H_2$ as an electron donor for denitrification.[59] Meanwhile, *Pseudomonas*, *Hydrogenophaga*, and *Methyloversatilis* can synthesize co-metabolizing degradative enzymes that facilitate the aerobic metabolism of TCE under aerobic conditions.[9,16,17] It is also reported that in the presence of $O_2$, TCE can serve as a growth substrate, providing a carbon and energy source for microbial cells.[7]

Therefore, the mechanism for the effect of nitrate on TCE removal in the joint $H_2/O_2$ system is proposed. Nitrate induces accumulation of denitrifying bacteria, such as *Pseudomonas*, *Hydrogenophaga* and *Methyloversatilis*, which possess denitrifying genes of *narG*, *nirS*, and *nosZ*. These bacteria reduce nitrate and nitrite using $H_2$ as an electron donor and aerobically co-metabolize TCE or use TCE as a carbon source. *Hydrogenophaga* uses $H_2$ as an electron donor and $O_2$ as an electron acceptor to fix $CO_2$ and produce organic compounds for the growth of the bacteria. However, a nitrate concentration of 240 mg $L^{-1}$ can inhibit the accumulation of *Pseudomonas* and *Methyloversatilis* due to the remaining nitrite.

## 4 Conclusion

This study proves that introducing nitrate into the joint $H_2/O_2$ system significantly enhances TCE removal. The enhancement effect is pronounced with increasing nitrate concentrations, indicating a positive correlation between nitrate levels and TCE removal efficiency. Adding nitrate enriches denitrifying bacteria, such as *Pseudomonas*, *Hydrogenophaga*, and *Methyloversatilis*, capable of denitrification using $H_2$ as an electron donor and aerobic TCE co-metabolism. This dual functionality of the microbes and the increased abundance of the aerobic co-metabolic gene *phe* suggest a synergistic mechanism by which nitrate promotes TCE removal in the joint $H_2/O_2$ system. However, a high nitrate concentration of up to 240 mg $L^{-1}$ inhibits the accumulation of *Pseudomonas* and *Methyloversatilis*. Alternatively, it increases the abundance of *Hydrogenophaga*, which could use $H_2$ and $O_2$ to fix $CO_2$ and provide organics for bacterial growth. The coexistence of $H_2$ and $O_2$ altered the redox state of the system, favouring aerobic co-metabolizing microorganisms and thereby enhancing aerobic co-metabolism processes. This observation suggests that organic pollutants amenable to aerobic co-metabolism may also undergo more efficient degradation in systems where nitrate, $H_2$ and $O_2$ co-exist.

The findings of this research underscore the importance of nitrate, along with $H_2$ and $O_2$, in influencing the microbial-mediated degradation of TCE. The results suggest that manipulating these electron donors and acceptors could be a viable strategy to enhance bioremediation outcomes. In areas with high concentrations of nitrate and TCE contamination, remediation can be achieved by introducing $H_2$ and $O_2$ systems. Conversely, remediation can be done in TCE-contaminated sites with low nitrate concentrations by supplementing the joint $H_2/O_2$ system with up to 60 mg $L^{-1}$ of nitrate. This study provides technical guidance for the remediation of TCE and nitrate co-contamination in groundwater environments, offering a promising avenue for developing more effective environmental remediation technologies that leverage the interactive effects of $H_2$, $O_2$, and nitrate on microbial communities.

## Data availability

Microbiological data for this article are available from the NCBI website at **https://dataview.ncbi.nlm.nih.gov/object/PRJNA1206323?reviewer=jhknm0tverjns8r9vv6iv5orel**.

## Author contributions

Wenyi Huang: conceptualization, methodology, validation, formal analysis, software, writing – original draft and editing. Weiwei Ouyang: writing – review & editing. Haonan Bian: writing – review & editing. Hui Liu: supervision, project administration, funding acquisition, conceptualization, writing – review & editing.

## Conflicts of interest

All authors declare no conflict of interest.

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## References

1 B. T. Oba, X. H. Zheng, M. A. Aborisade, J. S. Liu, A. Yohannes, S. Kavwenje, P. Z. Sun, Y. K. Yang and L. Zhao, Remediation of trichloroethylene contaminated soil by

unactivated peroxymonosulfate: Implication on selected soil characteristics, *J. Environ. Manage.*, 2021, **285**, 8.
2 L. Hermon, J. Denonfoux, J. Hellal, C. Joulian, S. Ferreira, S. Vuilleumier and G. Imfeld, Dichloromethane biodegradation in multi-contaminated groundwater: Insights from biomolecular and compound-specific isotope analyses, *Water Res.*, 2018, **142**, 217–226.
3 S. Y. Zhao and J. Z. He, Reductive dechlorination of high concentrations of chloroethenes by a *Dehalococcoides mccartyi* strain 11G, *FEMS Microbiol. Ecol.*, 2019, **95**, 9.
4 Z. Q. Zhou, Q. Zeng, G. Y. Li, D. F. Hu, Q. Y. Xia and H. L. Dong, Oxidative degradation of commingled trichloroethylene and 1,4-dioxane by hydroxyl radicals produced upon oxygenation of a reduced clay mineral, *Chemosphere*, 2022, **290**, 10.
5 Z. Wu, Q. Man, H. Niu, H. Lyu, H. Song, R. Li, G. Ren, F. Zhu, C. Peng and B. Li, Recent advances and trends of trichloroethylene biodegradation: A critical review, *Front. Microbiol.*, 2022, **13**, 1053169.
6 D. Frascari, G. Zanaroli and A. S. Danko, In situ aerobic co-metabolism of chlorinated solvents: A review, *J. Hazard. Mater.*, 2015, **283**, 382–399.
7 Z. L. Xing, X. Su, X. P. Zhang, L. J. Zhang and T. T. Zhao, Direct aerobic oxidation (DAO) of chlorinated aliphatic hydrocarbons: A review of key DAO bacteria, biometabolic pathways and in-situ bioremediation potential, *Environ. Int.*, 2022, **162**, 19.
8 J. Hellal, C. Joulian, C. Urien, S. Ferreira, J. Denonfoux, L. Hermon, S. Vuilleumier and G. Imfeld, Chlorinated ethene biodegradation and associated bacterial taxa in multi-polluted groundwater: Insights from biomolecular markers and stable isotope analysis, *Sci. Total Environ.*, 2021, **763**, 10.
9 S. H. Yuan, Y. Liu, P. Zhang, M. Tong and H. Liu, Electrolytic groundwater circulation well for trichloroethylene degradation in a simulated aquifer, *Sci. China: Technol. Sci.*, 2021, **64**, 251–260.
10 C. H. Chang, H. Y. Yang, S. K. Chen, J. M. Hung, C. J. Lu and M. H. Liu, Electrokinetic-enhanced bioremediation of tetrachloroethylene, *Int. Biodeterior. Biodegrad.*, 2018, **132**, 251–258.
11 O. Modin and F. Aulenta, Three promising applications of microbial electrochemistry for the water sector, *Environ. Sci.: Water Res. Technol.*, 2017, **3**, 391–402.
12 C. Li, R. Chen, W. Ouyang, C. Xue, M. Liu and H. Liu, The response of C/N/S cycling functional microbial communities to redox conditions in shallow aquifers using in-situ sediment as bio-trap matrix, *Environ. Technol.*, 2024, **45**, 3666–3678.
13 S. T. Lohner, D. Becker, K.-M. Mangold and A. Tiehm, Sequential Reductive and Oxidative Biodegradation of Chloroethenes Stimulated in a Coupled Bioelectro-Process, *Environ. Sci. Technol.*, 2011, **45**, 6491–6497.
14 A. M. Costello and M. E. Lidstrom, Molecular characterization of functional and phylogenetic genes from natural populations of methanotrophs in lake sediments, *Appl. Environ. Microbiol.*, 1999, **65**, 5066–5074.
15 C. Li, R. Chen, H. Liu, Y. Huang, J. Yu, W. Ouyang and C. Xue, Response of chlorinated hydrocarbon transformation and microbial community structure in an aquifer to joint $H_2$ and $O_2$, *RSC Adv.*, 2022, **12**, 23252–23262.
16 I. Dolinová, M. Strojsová, M. Cerník, J. Nemecek, J. Macháčková and A. Sevcu, Microbial degradation of chloroethenes: a review, *Environ. Sci. Pollut. Res.*, 2017, **24**, 13262–13283.
17 C. Li, C. Xue, W. W. Ouyang, M. H. Liu, Y. T. Sun and H. Liu, Identification and synergetic mechanism of TCE, $H_2$ and $O_2$ metabolic microorganisms in the joint $H_2/O_2$ system, *Sci. Total Environ.*, 2023, **879**, 9.
18 X. H. Wang, J. Xin, M. J. Yuan and F. Zhao, Electron competition and electron selectivity in abiotic, biotic, and coupled systems for dechlorinating chlorinated aliphatic hydrocarbons in groundwater: A review, *Water Res.*, 2020, **183**, 17.
19 J. J. Weatherill, S. Atashgahi, U. Schneidewind, S. Krause, S. Ullah, N. Cassidy and M. O. Rivett, Natural attenuation of chlorinated ethenes in hyporheic zones: A review of key biogeochemical processes and in-situ transformation potential, *Water Res.*, 2018, **128**, 362–382.
20 E. Abascal, L. Gómez-Coma, I. Ortiz and A. Ortiz, Global diagnosis of nitrate pollution in groundwater and review of removal technologies, *Sci. Total Environ.*, 2022, **810**, 152233.
21 Q. Zhang, A. Wei, J. Ren, H. Qian and K. Hou, Multi-isotope tracer for identifying nitrate sources in shallow groundwater in a large irrigation area, China, *J. Environ. Manage.*, 2025, **376**, 124424.
22 P. J. Squillace, J. C. Scott, M. J. Moran, B. T. Nolan and D. W. Kolpin, VOCs, pesticides, nitrate, and their mixtures in groundwater used for drinking water in the United States, *Environ. Sci. Technol.*, 2002, **36**, 1923–1930.
23 P. Bennett, D. Gandhi, S. Warner and J. Bussey, In situ reductive dechlorination of chlorinated ethenes in high nitrate groundwater, *J. Hazard. Mater.*, 2007, **149**, 568–573.
24 L. F. Cao, W. H. Sun, Y. T. Zhang, S. M. Feng, J. Y. Dong, Y. M. Zhang and B. E. Rittmann, Competition for electrons between reductive dechlorination and denitrification, *Front. Environ. Sci. Eng.*, 2017, **11**, 10.
25 A. Lai, R. Verdini, F. Aulenta and M. Majone, Influence of nitrate and sulfate reduction in the bioelectrochemically assisted dechlorination of cis-DCE, *Chemosphere*, 2015, **125**, 147–154.
26 X. Y. Zhang, Z. L. Li, F. Chen, S. P. Wang, J. Nan, C. Huang, X. Q. Chen, D. Cao, C. H. Bai, H. C. Wang, J. L. Han, B. Liang and A. J. Wang, Influence of nitrate concentration on trichloroethylene reductive dechlorination in weak electric stimulation system, *Chemosphere*, 2022, **295**, 9.
27 C. N. R. Simón, A. Pedetta, J. P. Busalmen and S. Bonanni, Aerobic denitrification with an electrode as the sole electron and energy source, *Environ. Sci.: Water Res. Technol.*, 2024, **10**, 498–509.
28 J. G. Leahy, A. M. Byrne and R. H. Olsen, Comparison of factors influencing trichloroethylene degradation by toluene-

 

oxidizing bacteria, *Appl. Environ. Microbiol.*, 1996, **62**, 825–833.

29 J. Ma, H. Liu, C. Zhang, K. Ding, R. Chen and S. Liu, Joint response of chemistry and functional microbial community to oxygenation of the reductive confined aquifer, *Sci. Total Environ.*, 2020, **720**, 137587.

30 W. W. Ouyang, Y. Huang, C. Li, C. Xue, M. H. Liu, J. Ma, S. H. Yuan and H. Liu, Response of TCE biodegradation to elevated $H_2$ and $O_2$: Implication for electrokinetic-enhanced bioremediation, *Environ. Res.*, 2024, **248**, 11.

31 D. Nelson, R. Hozalski, L. Clapp, M. Semmens and P. Novak, Effect of nitrate and sulfate on dechlorination by a mixed hydrogen-fed culture, *Biorem. J.*, 2002, **6**, 225–236.

32 H.-Y. Yu, Y.-k. Wang, P.-c. Chen, F.-b. Li, M.-j. Chen, M. Hu and X. Ouyang, Effect of nitrate addition on reductive transformation of pentachlorophenol in paddy soil in relation to iron (III) reduction, *J. Environ. Manage.*, 2014, **132**, 42–48.

33 J. Almeida, S. Julio, M. Reis and M. Carrondo, Nitrite inhibition of denitrification by *Pseudomonas fluorescens*, *Biotechnol. Bioeng.*, 1995, **46**, 194–201.

34 J. Ma, Q. Yang, S. Wang, L. Wang, A. Takigawa and Y. Peng, Effect of free nitrous acid as inhibitors on nitrate reduction by a biological nutrient removal sludge, *J. Hazard. Mater.*, 2010, **175**, 518–523.

35 B. Wanga, Z. Liub, Y. Huangc, Q. Jianga, D. Yand, C. Xud, K. Wanga, K. Shia and J. Xuea, Evaluation of the response of dynamic changes in the natural attenuation of chloride-polluted groundwater via high-throughput sequencing: degradation characteristics and environmental changes, *Global NEST J.*, 2024, **26**, 05124.

36 S. Niu, C. Li, S. Gao, J. Tian, C. Zhang, L. Li, Y. Huang and H. Lyu, Biochar, microbes, and biochar-microbe synergistic treatment of chlorinated hydrocarbons in groundwater: a review, *Front. Microbiol.*, 2024, **15**, 1443682.

37 F. Chen, Z.-L. Li, B. Liang, J.-Q. Yang, H.-Y. Cheng, C. Huang, J. Nan and A.-J. Wang, Electrostimulated bio-dechlorination of trichloroethene by potential regulation: kinetics, microbial community structure and function, *Chem. Eng. J.*, 2019, **357**, 633–640.

38 S. K. Chen, Y. L. Chin, H. Y. Yang, C. J. Lu and M. H. Liu, Cometabolic biodegradation of chlorinated ethenes with methanotrophs in anaerobic/aerobic simulated aquifer, *J. Environ. Biol.*, 2021, **42**, 1033–1045.

39 X. Chen, C. M. Liu, B. L. Zhu, W. X. Wei and R. Sheng, The Contribution of Nitrate Dissimilation to Nitrate Consumption in *narG* and *napA* Containing Nitrate Reducers with Various Oxygen and Nitrate Supplies, *Microbiol. Spectrum*, 2022, **10**, 12.

40 L. Girard, C. Lood, M. Höfte, P. Vandamme, H. Rokni-Zadeh, V. van Noort, R. Lavigne and R. De Mot, The ever-expanding *Pseudomonas* genus: description of 43 new species and partition of the *Pseudomonas putida* group, *Microorganisms*, 2021, **9**, 1766.

41 X. Feng, S. J. He, T. Sato, T. Kondo, K. Uema, K. Sato and H. Kobayashi, Enrichment of hydrogen-oxidizing bacteria using a hybrid biological-inorganic system, *J. Biosci. Bioeng.*, 2023, **135**, 250–257.

42 E. Ehsani, C. Dumolin, J. B. A. Arends, F. M. Kerckhof, X. N. Hu, P. Vandamme and N. Boon, Enriched hydrogen-oxidizing microbiomes show a high diversity of co-existing hydrogen-oxidizing bacteria, *Appl. Microbiol. Biotechnol.*, 2019, **103**, 8241–8253.

43 L. Lin, H. Huang, X. Zhang, L. Dong and Y. Chen, Hydrogen-oxidizing bacteria and their applications in resource recovery and pollutant removal, *Sci. Total Environ.*, 2022, **835**, 155559.

44 M. R. Fries, L. J. Forney and J. M. Tiedje, Phenol- and toluene-degrading microbial populations from an aquifer in which successful trichloroethene co-metabolism occurred, *Appl. Environ. Microbiol.*, 1997, **63**, 1523–1530.

45 S. H. Liang, J. K. Liu, K. H. Lee, Y. C. Kuo and C. M. Kao, Use of specific gene analysis to assess the effectiveness of surfactant-enhanced trichloroethylene co-metabolism, *J. Hazard. Mater.*, 2011, **198**, 323–330.

46 X. Fan, L. Nie, Z. J. Chen, Y. L. Zheng, G. J. Wang and K. X. Shi, Simultaneous removal of nitrogen and arsenite by heterotrophic nitrification and aerobic denitrification bacterium *Hydrogenophaga* sp. H7, *Front. Microbiol.*, 2023, **13**, 12.

47 M. M. Jiang, Y. Y. Zhang, Y. H. Yuan, Y. C. Chen, H. Lin, J. J. Zheng, H. X. Li and X. H. Zhang, Nitrate removal and dynamics of microbial community of a hydrogen-based membrane biofilm reactor at diverse nitrate loadings and distances from hydrogen supply end, *Water*, 2020, **12**, 13.

48 Y. Liu, C. P. Feng, N. Chen, Y. Z. Sheng, S. S. Dong, C. B. Hao and K. Lei, Bioremediation of nitrate and Fe(II) combined contamination in groundwater by heterotrophic denitrifying bacteria and microbial community analysis, *RSC Adv.*, 2016, **6**, 108375–108383.

49 V. Baytshtok, H. J. Lu, H. Park, S. Kim, R. Yu and K. Chandran, Impact of Varying Electron Donors on the Molecular Microbial Ecology and Biokinetics of Methylotrophic Denitrifying Bacteria, *Biotechnol. Bioeng.*, 2009, **102**, 1527–1536.

50 G. L. W. Heavner, C. B. Mansfeldt, M. J. Wilkins, C. D. Nicora, G. E. Debs, E. A. Edwards and R. E. Richardson, Detection of Organohalide-Respiring Enzyme Biomarkers at a Bioaugmented TCE-Contaminated Field Site, *Front. Microbiol.*, 2019, **10**, 12.

51 S. H. Chen, Z. T. Li, C. Y. Lai and H. P. Zhao, Enhancing reductive dechlorination of trichloroethylene in bioelectrochemical systems with conductive materials, *Environ. Res.*, 2024, **261**, 9.

52 V. D. Appanna, C. E. A. Engel, D. Vorländer, R. Biedendieck, R. Krull and K. Dohnt, Quantification of microaerobic growth of *Geobacter sulfurreducens*, *PLoS One*, 2020, **15**, e0215341.

53 Y. Liang, X. K. Liu, M. A. Singletary, K. Wang and T. E. Mattes, Relationships between the Abundance and Expression of Functional Genes from Vinyl Chloride (VC)-Degrading Bacteria and Geochemical Parameters at VC-Contaminated Sites, *Environ. Sci. Technol.*, 2017, **51**, 12164–12174.

54 Z. L. Li, R. Cheng, F. Chen, X. Q. Lin, X. J. Yao, B. Liang, C. Huang, K. Sun and A. J. Wang, Selective stress of antibiotics on microbial denitrification: Inhibitory effects, dynamics of microbial community structure and function, *J. Hazard. Mater.*, 2021, **405**, 13.

55 X. Y. Zhang, Z. L. Li, F. Chen, S. P. Wang, J. Nan, C. Huang, X. Q. Chen, D. Cao, C. H. Bai, H. C. Wang, J. L. Han, B. Liang and A. J. Wang, Influence of nitrate concentration on trichloroethylene reductive dechlorination in weak electric stimulation system, *Chemosphere*, 2022, **295**, 133935.

56 K. Fan, F. Wang, X. Xu, J. Shi, W. Wang, D. Xing, N. Ren, D.-J. Lee and C. Chen, *Enterobacter* sp. HIT-SHJ4 isolated from wetland with carbon, nitrogen and sulfur co-metabolism and its implication for bioremediation, *Environ. Res.*, 2024, **260**, 119593.

57 C. Huang, Q. Liu, Z. L. Li, X. D. Ma, Y. N. Hou, N. Q. Ren and A. J. Wang, Relationship between functional bacteria in a denitrification desulfurization system under autotrophic, heterotrophic, and mixotrophic conditions, *Water Res.*, 2021, **188**, 116526.

58 M. Kim, S.-Y. Jeong, S. J. Yoon, S. J. Cho, Y. H. Kim, M. J. Kim, E. Y. Ryu and S.-J. Lee, Aerobic denitrification of *Pseudomonas putida* AD-21 at different C/N ratios, *J. Biosci. Bioeng.*, 2008, **106**, 498–502.

59 Y. Ma, X. Zheng, Y. Fang, K. Xu, S. He and M. Zhao, Autotrophic denitrification in constructed wetlands: Achievements and challenges, *Bioresour. Technol.*, 2020, **318**, 123778.

 