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Microbial bioremediation of produced water under different redox conditions in marine sediments

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ABSTRACT
The discharge of produced water from offshore oil platforms is an emerging concern due to its potential adverse effects on marine ecosystems. In this study, we investigated the feasibility and capability of using marine sediments for the bioremediation of produced water. We utilized a combination of porewater and solid phase analysis in a series of sediment batch incubations amended with produced water and synthetic produced water to determine the biodegradation of hydrocarbons under different redox conditions. Significant removal of benzene, toluene, ethylbenzene and xylene (BTEX) compounds was observed under different redox conditions, with biodegradation efficiencies of 93–97% in oxic incubations and 45–93% in anoxic incubations with nitrate, iron oxide or sulfate as the electron acceptor. Higher biodegradation rates of BTEX were obtained by incubations dominated by nitrate reduction (104–149 nmolC/cm³/d) and oxygen respiration (52–57 nmolC/cm³/d), followed by sulfate reduction (14–76 nmolC/cm³/d) and iron reduction (29–39 nmolC/cm³/d). Chemical fingerprint analysis showed that hydrocarbons were biodegraded to smaller alcohols/acids under oxic conditions compared to anoxic conditions with nitrate, indicating that the presence of oxygen facilitated a more complete biodegradation process. Toxicity of treated produced water to the marine copepod Acartia tonsa was reduced by half after sediment incubations with oxygen and nitrate. Our study emphasizes the possibility to use marine sediment as a biofilter for treating produced water at sea without extending the oil and gas platform or implementing a large-scale construction.

1. Introduction

Significant amounts of produced water are generated from conventional oil and natural gas production (Veil et al., 2004). Produced water is a complex mixture of dissolved and particulate organic and inorganic chemicals, including heavy metals and organic material from geological formations (e.g., polycyclic aromatic hydrocarbons (PAHs) and alkylphenols) as well as polymers and other chemical additives (Bakke et al., 2013; Fakhru’l-Razi et al., 2009; Jiménez et al., 2018). Among these chemicals, monocyclic aromatic hydrocarbons (i.e., benzene, toluene, ethylbenzene, and xylenes (BTEX)), PAHs, and related heterocyclic aromatic compounds are considered major toxicants in produced water (Bakke et al., 2013; Liu et al., 2021; Neff et al., 2011). Compared to other hydrocarbons in produced water, BTEX are relatively water soluble (Ekins et al., 2007; van Agteren et al., 1998) and can therefore be transported far from the original source of discharge. These aromatic hydrocarbons can bioaccumulate in marine organisms and cause acute and chronic toxicities (Neff et al., 2011; Neff, 2002). For instance, aromatic hydrocarbons are found to influence the reproduction of the marine copepod Acartia tonsa, which plays a key role in the marine ecosystem due to a link between primary producers and fish production (Bellas and Thor, 2007; Girling, 1989). Therefore, due to their high toxicity and serious adverse environmental impacts, the removal of these oil contaminants is of great importance. Because of space and weight restrictions on offshore platforms, compact physical equipment (e.g., gravity sedimentation and hydrocyclone) coupled with dosage of various treatment chemicals (e.g., biocides and corrosion inhibitors) are often used to remove the oil and suspended solids during produced

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Degradation of natural organic material in the upper layers of marine sediments occurs under oxic conditions with oxygen (O\textsubscript{2}) as the electron acceptor. O\textsubscript{2} serves both as a high-energy-yielding terminal electron acceptor, both during heterotrophic carbon degradation and oxidation of reduced metabolites from anaerobic processes below the oxic zone (Canfield et al., 2005). During aerobic decomposition, highly reactive oxygen-containing radicals (e.g., superoxide anion (•O\textsubscript{2}^-) and hydroxyl radicals (•OH)), which can break bonds and depolymerize relative refractory organic compounds (Canfield et al., 2005). In coastal sediment, aerobic mineralization of organic material is usually limited to the upper 1–5 mm, while O\textsubscript{2} penetrates deeper in offshore and sandy sediments (3–8 mm) (McGinnis et al., 2014). A large fraction of hydrocarbon biodegradations therefore occurs under anoxic conditions. In the anoxic zone, large and complex organic compounds will be degraded into smaller and more water-soluble molecules under the production of energy by hydrolyzing and fermenting microorganisms (Canfield et al., 2005, 1993b). The smaller organic molecules are then mineralized to inorganic carbon during respiration, using different oxidized inorganic compounds as electron acceptors. These processes generally occur in the following sequence with depth in the sediment: manganese oxides (MnO\textsubscript{2}) ≈ nitrate (NO\textsubscript{3}^-), iron oxides (FeOOH), sulfate (SO\textsubscript{4}^2-) and inorganic carbon respiration, while burrow-dwelling invertebrates, fecal pellets and plant roots create mosaics of oxic and anoxic microenvironments.

Microbial degradations of recalcitrant organic contaminants have been observed in the marine environment, for instance in connection with intensive oil spills (Margaris and Schinner, 1999). Both field after oil spills and laboratory studies have widely demonstrated aerobic and anaerobic biodegradation of aromatic hydrocarbons (e.g., BTEX and phenol) with O\textsubscript{2}, NO\textsubscript{3}^- FeOOH and SO\textsubscript{4}^2- as terminal electron acceptors under both marine and freshwater conditions (Alain et al., 2012; Cunningham et al., 2001; Dou et al., 2008; Head et al., 2006; Miralles et al., 2007; Villatoro-Monzón et al., 2003; Zhu et al., 2020). Strains of aerobic hydrocarbon-degrading bacteria identified in marine sediments after oil spills belong to the genera Acinetobacter, Marinobacter, Pseudomonas, Rhodococcus, etc. (El-Naas et al., 2014; McGinity, 2014). Key anaerobic hydrocarbon-degrading bacteria associated with denitrification, FeOOH and SO\textsubscript{4}^2- reduction have been shown to be phylogenetically affiliated to Azoarcus/Thaïeera, Geobacter and Desulfobacula/Desulfotignum, respectively (Bin et al., 2002; Weelink et al., 2010). Although hydrocarbon-degrading microorganisms are ubiquitous in the marine environment and hydrocarbon biodegradation abilities in natural marine sediments have been previously reported (Head et al., 2006), a quantitative comparison of hydrocarbon biodegradation rates under different redox conditions in marine sediments and contributions of hydrocarbon biodegradation to aerobic and anaerobic organic carbon mineralization have to our knowledge not been investigated.

The principal goal of the present study was to examine the feasibility and capability of using marine sediments for bioremediation of produced water. We designed a sediment incubation experiment to determine the biodegradation rates of BTEX in produced water and synthetic produced water under different redox conditions, and to examine the role of BTEX in aerobic and anaerobic organic carbon mineralization. The organic components of produced water and biodegradation products formed during oxic and anoxic incubations in the presence of NO\textsubscript{3}^- were examined through novel chemical fingerprint analysis. The biodegradation experiments were combined with an evaluation of the impact of untreated and treated produced water on the life cycle of A. tonsa by assessing its growth, reproduction and mortality to provide further insights into the effect of bio-remediated produced water on marine living organisms.

2. Materials and methods

2.1. Study site and sampling

Sediments were sampled at a station south of Knåhagen (55°58′ N, 12°41′ E) in a relatively pristine area in Øresund without any regular oil contaminations. In a recent study, a hydrocarbon content of 1.3 ± 0.32 µg/g (dry wt sediment) was measured in the area of the sampling site (Sánchez-García et al., 2010), which was 2–3 orders of magnitude lower than that reported in heavy oil-contaminated sediments (Kimes et al., 2014; Miralles et al., 2007). Sediments were collected in polycarbonate tubes on board RV Ophelia, equipped with conductivity-temperature-depth (CTD) sensors and HAPS cores. The water depth was 31 m. Salinity, temperature and O\textsubscript{2} in the bottom water were 26.5, 5.4 C and 298 µmol/L, respectively. Seawater was sampled on shore through a direct connection to the bottom water in Øresund. The sediment cores were stored open in the dark at near in situ temperature until further handling within 2 weeks.

Produced water from an oil handling installation in the Danish section of the North Sea was collected at the release point after flotation treatment to remove suspended oil. During the treatment, various production chemicals, including biocides and corrosion inhibitors, were used. A batch portion was stored without headspace at 4 °C until the onset of incubations. Considering previous findings, where hydrocarbon mineralization rates by marine microorganisms appeared to be primarily controlled by the extent of pollutant loading and not by variations in the salinity regime (Kerr and Capone, 1988), produced water was diluted with seawater to mimic the salinity at the sampling site in order to avoid a lag-period in bacteria activity pending adaptation to higher salinity. In the following batch incubations, produced water (PW) refers to the produced water collected from the North Sea oil facility, while synthetic produced water (synthetic PW) refers sterile-filtered seawater from Øresund amended with BTEX, mimicking the concentrations in PW and excluding potential degradation by microorganisms in PW (Supplementary materials Table S1). The PW used for incubations consisted of 14 µmol/L of benzene, 2.1 µmol/L of toluene, < 0.5 µmol/L of ethylbenzene, m-xylene and p-xylene, and 4.5 µmol/L of o-xylene (Table S1).

2.2. Sediment handling and batch incubations

To examine the biodegradation of produced water components under oxic and anoxic conditions, and the role of BTEX degradation in aerobic and anaerobic organic carbon oxidation, the changes in BTEX concentrations and end-products of mineralization (i.e., total dissolved inorganic carbon (ΣCO\textsubscript{2}) and ammonium (NH\textsubscript{4}+)) were monitored in sediment slurry incubations with different electron acceptors, mimicking redox conditions in seafloor (Table S2). Briefly, 18 sediment cores were sliced into the intervals 0–1 cm, 1–4 cm and 4–10 cm at near in situ temperature, and parallel sections were pooled, homogenized and filled in 0.5 and 1 L bluecap bottles. The depth intervals of 0–1 cm, 1–4 cm and 4–10 cm were chosen to represent the zones of O\textsubscript{2}/NO\textsubscript{3}^- consumption, Fe reduction and SO\textsubscript{4}^2- reduction, respectively. Indeed, the dominance of SO\textsubscript{4}^2- reduction below 4 cm of depth was indicated visually by color, going from brownish to black sediment. The sediment from each depth interval was homogenized and mixed with onsite sterile-filtered seawater to obtain slurries. Sediment slurries were filled into glass bottles closed with gastight stoppers and sampling ports, purged with N\textsubscript{2} gas, and left over-night to consume any traces of O\textsubscript{2} and NO\textsubscript{3}-. Incubations were initiated by adding PW, synthetic PW and different electron acceptors to the slurries (Table S2). The incubations were named after the electron acceptor (corresponding to certain depth intervals) and amendment. For example, incubations from 0–1 cm without PW and synthetic PW were named O\textsubscript{2}/Control/NO\textsubscript{3}^-/Control, while slurries with PW or synthetic PW were called O\textsubscript{2}/PW(NO\textsubscript{3}^-)-PW or O\textsubscript{2}/Synthetic PW(NO\textsubscript{3}^-)-Synthetic PW (Table S2). Parallel anoxic...
incubations (NO$_3^-$ Synthetic PW-Acetate, Fe-Synthetic PW-Acetate and SO$_4^{2-}$ Synthetic PW-Acetate) were amended with acetate in a concentration of 1.5 mmol/L to investigate co-metabolic degradations of BTEX (Table S2). For oxic incubations with sediment from 0–1 cm (O$_2$-Control, O$_2$-PW and O$_2$-Synthetic PW), pure O$_2$ gas was added to the headspace at the onset of incubation. O$_2$ concentrations in both oxic and anoxic incubations were monitored with non-invasive optical oxygen sensors (PyroScience, Germany). To stimulate NO$_3^-$ reduction in the anoxic slurries with sediment from 0–1 cm (NO$_3^-$-Control, NO$_3^-$-PW, NO$_3^-$ Synthetic PW, and NO$_3^-$ Synthetic PW-Acetate), NO$_3^-$ was added to an end concentration of ~3 mmol/L. Throughout the incubations, NO$_3^-$ concentrations were measured and maintained above 0.9 mmol/L through additional spikes of NO$_3^-$ Sediment from 1–4 cm was incubated with FeOOH addition (Fe-Control, Fe-Synthetic PW and Fe-Synthetic PW-Acetate) and without FeOOH addition (Fe-Control* and Fe-Synthetic PW*). The concentrations of poorly crystalline Fe (III) and particulate Fe (II) were 2.5 ± 0.8 μmol/cm$^2$ and 12.0 ± 2.5 μmol/cm$^2$, respectively, before the amendment with FeOOH. The concentration of poorly crystalline Fe (III) was increased by a factor of 10–38 (Table S2) to ensure Fe reduction throughout the entire incubations. As the concentration of SO$_4^{2-}$ was ~21 mmol/L at the onset of incubation with pooled sediment from 4–10 cm (SO$_4^{2-}$-Control, SO$_4^{2-}$ Synthetic PW and SO$_4^{2-}$ Synthetic PW-Acetate), there was no need to stimulate SO$_4^{2-}$ reduction with additional spike of SO$_4^{2-}$. Sediment slurries were incubated on shakers.

2.2.2. Sediment characteristics and solid-phase analysis

Separate samples of sediment slurry were taken from each bottle under N$_2$ on several occasions during the incubation for the determination of pH, BTEX, sulfate reduction rates (SRR), solid-phase Fe pools and density/water content/porosity. pH was monitored by a pH probe (WTW GmbH, Weilheim, Germany). Porosity was calculated by multiplying wet density (weight of a known volume) and water content (weight loss after drying at 105°C for 24 h). Subsamples of sediment slurry for BTEX analysis were extracted immediately with hexane (containing fluorobenzene as internal standard) in septum-capped glass vials. After 18 h of mixing in a rotator, the hexane phase was extracted and frozen in 1.8 ml glass vials until analysis. BTEX concentrations were quantified by headspace mass spectrometry coupled to gas chromatography (GC-MS) (Agilent Technologies). Rates of SO$_4^{2-}$ reduction were quantified using $^{35}$SO$_4^{2-}$ tracer method (Jørgensen, 1978). Separate samples of sediment slurry (5 ml) were added to 10 ml serum bottles, which were plugged with gas-tight stoppers and incubated anoxically at 10°C after the addition of $^{35}$SO$_4^{2-}$ tracer. After ~6 h in the dark, the samples were fixed in 5 ml of 20% zinc acetate and stored frozen until distillation. The reduced $^{35}$S pools were recovered by single-step distillation with boiling acidic Cr$_2$O$_7^2-$ solution (Fossing and Barker, 1989). Concentrations of solid phase Fe (III) and Fe (II) pools were determined through cold HCl extraction (Kostka and Luther, 1994). This assay extracts poorly crystalline Fe (III) oxides and particulate Fe (II), such as FeS and FeCoO (Canfield et al., 1993b; Thamdrup et al., 1994). Extractions were performed with 10 ml extractant and ~100 mg wet sediment for 1 h under shaking in the dark. The concentrations of the oxidation stages of Fe were determined colorimetrically using a Ferrozine solution (50 mmol/L HEPES, 0.08% Ferrozine, pH 7), with and without 1% (v/v) hydroxylamine hydrochloride for quantification of total Fe and Fe (II), respectively (Canfield et al., 1993b).

2.3. Calculation of rates

Total carbon oxidation rates and BTEX degradation rates were calculated from the slope of linear regression of porewater $\Sigma$CO$_2$ accumulation and total BTEX concentrations versus time, respectively.

Dissolved Fe$^{2+}$ was quantified colorimetrically using Ferrozine without reducing agent (Stookey, 1970; Thamdrup et al., 1994). An air-segmented continuous-flow analyzer (SKALAR San$^{+}$, Netherlands) was used for colorimetric analysis of NH$_4^+$, nitrite (NO$_2^-$) and NO$_3^-$ (i.e., NO$_2^-$ + NO$_3^-$).

2.2.1. Sediment porewater sampling and analysis

The temporal pattern of BTEX concentrations as well as aerobic and anaerobic biodegradation processes were followed for 35 d (Table S3). Subsampling from the sediment slurry incubations was initiated ~5 h after amendments, where only trace amount of O$_2$ from the amendments was left (~1.7 μM) in anoxic incubations, and repeated on nine following occasions (T0–T9) throughout the incubation period (Table S3). Sampling from the anoxic sediment slurries and handling of porewater were performed under N$_2$ atmosphere in a glove bag. Subsamples of sediment slurry were centrifuged (4000 rpm for 10–15 min) at 4°C, and the supernatant was filtered (0.22 μm-pore-diameter polypropylene filters) under N$_2$ atmosphere. The conservation of porewater samples was described in Supplementary materials Section S1. $\Sigma$CO$_2$ was measured by flow injection with conductivity detection (Hall and Aller, 1992). Dissolved hydrogen sulfide (H$_2$S) was measured colorimetrically using the methylene blue method (Cline, 1969), while SO$_4^{2-}$ was quantified by suppressed anion chromatography (Dionex).
Volumetric rates (nmol C/cm³/d) were calculated instead of mass rates to evaluate the potential of using the seafloor as a biofilter to remove organic compounds in produced water. Sulfate reduction rates were calculated from the fraction of reduced sulfur produced and the concentration of SO₄²⁻, as described in Fossing and Barker (1989), after the correction by subtracting the reversibly recovered from distillation of sediment fixed immediately in zinc acetate after injection of the tracer. Assuming an overall stoichiometry of 2 molar of organic carbon oxidized per 1 molar of SO₄²⁻ reduced, the difference between total carbon oxidation and carbon oxidation coupled to SO₄²⁻ reduction gave a measurement of carbon oxidation coupled to other anaerobic respiration pathways, as O₂ was undetectable in the anoxic incubations (Fig. 1B).

### 2.4. Chemical fingerprint analysis

A chemical fingerprint analysis of naturally occurring and PW organic components was performed on the oxic incubation (O₂-PW-Fingerprint) and anoxic incubation with NO₃ (NO₃-PW-Fingerprint). Due to the high amount of sample needed for this analysis, these two incubations were run in parallel to the aforementioned incubations of O₂-PW and NO₃-PW (Table S2). Slurry samples taken at the beginning (T₀) and end of incubation (T₉) were acidified with 6 mol/L HCl to pH 1–3 to reduce microbial activity and stored at 4 °C until analysis. The sample preparation workflow was designed to target water-soluble and oxygen-containing organics (e.g., carboxylic acids and alcohols). These are the dominant classes of compounds present in produced water after removal of any dispersed oil (Bergfors et al., 2020; Samanipour et al., 2020; Sørensen et al., 2019; Strømgen et al., 1995). The chemical fingerprint of the extracted samples was analyzed using an Agilent 7890B GC coupled to a 7200B QTOF high-resolution mass spectrometer. Common and unique features were evaluated using the Investigator software. The details of sample preparation and QTOF high-resolution mass spectrometer were listed in Supplementary materials Section S12.

### 2.5. Toxicity tests

The focus of the toxicity experiments was to investigate the effects of untreated and treated PW on the mortality and physiology of the coastal copepod A. tonsa, i.e., egg and fecal pellet production. The ingestion was evaluated by the quantification of fecal pellet production, while the egg production rate was used as proxy for reproduction. Experimental details of toxicity tests, including the cultivation of A. tonsa, estimation of lethal concentration 50% (LC₅₀) of untreated PW and synthetic PW, and median effective concentration (EC₅₀) of untreated and treated PW for egg and pellet production, were described in Supplementary materials Section S3 and Tables S4, S5.

### 3. Results and discussions

#### 3.1. Total carbon oxidation

Based on solid-phase and porewater chemistry, the main diagenetic reactions occurring in the sediment incubations can be inferred. Concentrations of ∑CO₂ (H₂CO₃ + HCO₃⁻ + CO₃²⁻) increased linearly during the first 355 h of incubations, indicating constant rates of organic material oxidation (Figs. 1A, S1). Quantification of total carbon oxidation rates from accumulation of ∑CO₂ in porewater over time can be effected by carbonate precipitation or dissolution (CaCO₃, FeCO₃ and MnCO₃) (Jensen et al., 2005; Thamdrup et al., 2000). During anoxic incubations, ∑CO₂ and NH₄⁺ accumulated in parallel with good linearity of ∑CO₂ versus NH₄⁺ concentrations for each incubation (R² > 0.84; data not shown; examples of NH₄⁺ accumulation were shown in Fig. S2). Also, pH values (7.2–8.2) were stable over time in all incubations and within the typical range of marine sediments, excluding substantial carbonate precipitations.

The carbon oxidation rates in the control incubations agreed with the total carbon oxidation rates previously reported in short-term sediment incubations from the Baltic-North Sea transition (10–574 nmol C/cm³/d) (Table 1) (Canfield et al., 1993a, 1993b; Jensen et al., 2005). The highest total carbon oxidation rates were obtained in the oxic control
incubations and the anoxic control incubations with NO$_3^-$, corresponding well with higher rates in the surface of marine sediments (Table 1) (Jensen et al., 2005). The availability of organic material tends to decrease with depth, which is reflected in lower carbon oxidation rates with depth (Table 1) (Jensen et al., 2005). In the incubations with O$_2$ and NO$_3^-$, the amendment with PW enhanced the total carbon oxidation rates by a factor of 1.8 and 3.7, respectively, compared to the controls. There were no significant differences in the total carbon oxidation rates between synthetic PW amended incubations and controls (p > 0.05, unpaired t-test). The addition of acetate stimulated mineralization rates in the anoxic incubations by a factor of 3.4, compared to the unamended incubations (p < 0.05, unpaired t-test) (Table 1).

A previous study of carbon oxidation pathways in sediments of the Baltic-North Sea transition showed that one of the five carbon oxidation pathways (i.e., heterotrophic O$_2$ respiration, denitrification, Mn (IV)-, Fe- or SO$_4^{2-}$ - reduction) can be dominant at a given site, as the different respiration pathways are affected by various different environmental factors, such as water depth, rate of sedimentation and sediment type (Rysgaard et al., 2001). In our experimental setup, we aimed to stimulate one mineralization pathway over the others to look at the potential biodegradation of produced water components under different redox regimes. In the oxic incubations (O$_2$-Control, O$_2$-PW and O$_2$-Synthetic PW), dissolved O$_2$ concentrations gradually decreased from 206 ± 2 μmol/cm$^3$ to 164 ± 12 μmol/cm$^3$ during the initial incubation period of 355 h and to 35 ± 3 μmol/L at the end of incubations (Fig. 1B). The decrease in dissolved O$_2$ concentration with time was partly caused by heterotrophic O$_2$ respiration (organic carbon decomposition), as indicated by the simultaneous $\Sigma$CO$_2$ accumulation and O$_2$ consumption (Figs. 1, S1, Table 1). In addition, O$_2$ was consumed by the reoxidation of reduced inorganic metabolites associated with respiration (e.g., NH$_4^+$ and H$_2$S). In the anoxic incubations amended with NO$_3^-$, plots of $\Sigma$CO$_2$ versus NO$_3^-$ yielded a C:N ratio (0.73 ± 0.13, n = 4; data not shown) close to 0.80, in agreement with the theoretical stoichiometry from the biodegradation of organic carbon through denitrification (Canfield et al., 2005). The addition of FeOOH generally suppressed the carbon oxidation coupled to SO$_4^{2-}$ reduction, compared to unamended incubations without FeOOH addition (Table 1). The divergence of total carbon oxidation and SO$_4^{2-}$ based carbon oxidation in the incubations amended with FeOOH implied that other anaerobic respiration pathways were important. The excess carbon oxidation was assigned to microbial Fe reduction due to: (i) In the anoxic control incubations, O$_2$ remained undetectable, implying there was no contamination with O$_2$ in the anoxic incubations (Fig. 1B); (ii) No NO$_3^-$ was detected throughout the entire incubation period in any of the incubations from 1–4 cm with and without FeOOH (detection limit of ~10 μmol/L; data now shown); (iii) The concentration of reactive MnO$_4^-$ is often low below 1 cm depth (Jensen et al., 2003); (iv) Large pools of poorly crystalline Fe (III) were measured in the incubations with FeOOH amendment (i.e., Fe-Control,}
Fe-Synthetic PW and Fe-Synthetic PW-Acetate), while smaller pools of BTEX were observed in the incubations with unterminated sediment from the same depth (i.e., Fe-Control* and Fe-Synthetic PW*). Fe reduction was partly supported by the accumulation of soluble Fe(II) and biomass using sediment from 1 cm (Fig. 2A, Table 1). Fe (III) was present, yet in low concentrations, and dissolved Fe(II) remained undetectable during the entire period of SO$_4^{2-}$ incubations with sediments from 4–10 cm (Fig. 2). In addition, rates of carbon oxidation and SO$_4^{2-}$ reduction converted to carbon units were similar, implying that SO$_4^{2-}$ reduction was the main anaerobic carbon oxidation pathway in the rest of anoxic incubations with sub-surface sediment (65–100% in Fe-Control*, Fe-Synthetic PW*, SO$_4^{2-}$-Control, SO$_4^{2-}$-Synthetic PW and SO$_4^{2-}$-Synthetic PW-Acetate) (Table 1).

3.2. BTEX biodegradation and the role of BTEX in organic matter mineralization

As BTEX are known human carcinogens and highly toxic to a variety of living organisms, the removal of these contaminants is of great importance (Dean, 1985). The biodegradation of BTEX compounds is feasible under both oxic and anoxic conditions in the presence of various electron acceptors (e.g., O$_2$, NO$_3$-OOH and SO$_4^{2-}$) (Burland and Edwards, 1999; Lovley et al., 1996, 1995). In this study, significant BTEX removals were observed in all incubations (Figs. 3, S3). Biodegradation was considered the main removal process for BTEX in our sediment incubations, while removal through volatilization and/or adsorption was expected to be negligible as BTEX concentrations remained stable after the fast degradation within 284 h (Figs. 3A, S3). Also, the applied hexane extraction on sediment samples detached efficiently any adsorbed BTEX from sediments in confirmed by the high recovery efficiencies of internal standards (99 ± 3%). We observed rapid biodegradations of BTEX compounds with minimum rates of 52–57 nmolC/cm$^2$/d in oxic incubations and 14–149 nmolC/cm$^2$/d in anoxic incubations (Figs. 3, S3, Table 1). The highest biodegradation efficiencies were obtained by incubations with O$_2$ (93–97%) and NO$_3$ (85–93%), followed by Fe reduction (53–56%) and SO$_4^{2-}$ reduction (45–76%) (Figs. 3, S3).

For oxic incubations, the BTEX biodegradation rates were 57 ± 32 nmolC/cm$^2$/d for PW and 52 ± 1.6 nmolC/cm$^2$/d for synthetic PW (Table 1). The fast BTEX biodegradation observed in the oxic incubations was consistent with observations in previous pure culture experiments with aerobic heterotrophic bacteria, where BTEX compounds were readily degraded under oxic conditions with rates ranging from 0.6 to 17 mmolC/L/d (Hocinat et al., 2020; Jin et al., 2013; Morlet-Chávez et al., 2010; Robledo-Ortiz et al., 2011; Xin et al., 2013; Zhang et al., 2013). During aerobic BTEX biodegradation, the compounds are initially converted into catechol intermediates via direct oxidation of the aromatic ring catalyzed by monooxygenases (Kahng et al., 2001) or dioxygenase (Zylstra and Gibson, 1989). Then, the produced catechol intermediates undergo ring cleavage by catechol 1,2-dioxygenase or catechol 3,4-dioxygenase in the β-ketoadipate pathways to Krebs cycle intermediates (El-Naas et al., 2014). In our study, aerobic BTEX biodegradation contributed to 20–35% of total carbon oxidation (Table 1). This is consistent with previous studies on aerobic BTEX biodegradation, where 33% of benzene was converted to CO$_2$ in hypersaline soils (Nicholson and Fathepure, 2005), while 34–89% of the carbon from toluene was recovered as CO$_2$ and biomass using Marinobacter vinifirmus in a pure culture study (Berliendis et al., 2010).

Both NO$_3$- (Burland and Edwards, 1999; Cunningham et al., 2001), Fe(III)- (Lovley et al., 1996; Villatoro-Monzón et al., 2003), and SO$_4^{2-}$-reducing bacteria (Cunningham et al., 2001; Lovley et al., 1995) have been connected to anaerobic BTEX biodegradation. Here, we found the highest BTEX biodegradation rates in the presence of NO$_3$ as terminal electron acceptor, such as 104 and 149 nmolC/cm$^2$/d for NO$_3$-amended incubations with PW and synthetic PW, respectively (Figs. 3, S3, Table 1). This was up to 5 and 10 times faster than rates in incubations dominated by Fe (III) and SO$_4^{2-}$ reduction, respectively (Figs. 3, S3, Table 1). Similarly, more efficient anaerobic BTEX biodegradations (up to 4 times higher rates) have been reported under NO$_3$ compared to under Fe (III)- and SO$_4^{2-}$- reducing conditions using bacterial enrichments obtained from gasoline contaminated soils and sediments (Cunningham et al., 2001; Dou et al., 2008; Phelps and Young, 2010).
According to thermodynamic principles, higher reaction energy can be obtained during BTEX degradation when utilizing NO\textsubscript{3} as electron acceptor, compared to Fe (III) and SO\textsubscript{4}\textsuperscript{2-} (Dou et al., 2008; Ulrich and Edwards, 2003). For instance, standard free energy changes (\(\Delta G^\circ\)) of benzene oxidation with NO\textsubscript{3} as the electron acceptor is \(-2990\) kJ/mol, while it is \(-2660\) kJ/mol and \(-200\) kJ/mol for Fe (III) and SO\textsubscript{4}\textsuperscript{2-}, respectively (Ulrich and Edwards, 2003). Therefore, NO\textsubscript{3} is a more favorable electron acceptor than Fe (III) and SO\textsubscript{4}\textsuperscript{2-} for BTEX biodegradation. Aromatic compounds, like BTEX, can be anaerobically degraded via benzoyl-CoA, resorcinol, phloroglucinol, hydroxyhydroquinone, and possibly other pathways involving reactions of carboxylation, methylation and hydroxylation (Bin et al., 2002; Godin et al., 2020; Harwood et al., 1998). During biodegradation coupled to NO\textsubscript{3} reduction, BTEX compounds were potentially mineralized to CO\textsubscript{2}, accounting for 25–98% of total carbon oxidation (Table 1). The relative contribution to total carbon mineralization estimated for NO\textsubscript{3}-Synthetic PW (98%) in our study was in well agreement with previous experiments with enrichment cultures from soil and groundwater, where approximately 92–95% of benzene was recovered in CO\textsubscript{2} during anaerobic biodegradation linked to NO\textsubscript{3} reduction (Burland and Edwards, 1999). Results in previous studies further suggested that during anaerobic benzene biodegradation, NO\textsubscript{3} could be stoichiometrically reduced to NO\textsubscript{2}, and further to N\textsubscript{2}, with the reduction of NO\textsubscript{2} to N\textsubscript{2} being the rate-limiting steps (Burland and Edwards, 1999; Ulrich and Edwards, 2003).

In our experiments, capacities and rates of BTEX biodegradation in both incubations with PW and synthetic PW were comparable for incubations with O\textsubscript{2} and NO\textsubscript{3} (Figs. 3, S3, Table 1). The addition of acetate increased the total mineralization rates, but did not facilitate any potential co-metabolic degradations of BTEX (Table 1). Possible explanation could be that microorganisms present were able to metabolically degrade BTEX as an energy and/or carbon source (El-Naas et al., 2014; Weelink et al., 2010) or the co-metabolic activities were not limited by organic carbons due to the availability of other easily degradable carbon sources in the sediment (Canfield et al., 2005).

There was likely a decrease in nitrification activity during oxic incubation conditions with PW and synthetic PW, as indicated by NH\textsubscript{4} accumulation and lower NO\textsubscript{3} production over time compared to the control incubation (examples given in Fig. S2). Autotrophic ammonia oxidizers are able to co-metabolically degrade certain organic molecules (including various hydrocarbons) through their key enzyme, ammonia monooxygenase (AMO) (Su et al., 2021). Yet, they are sensitive to the toxicity of hydrocarbons caused by noncompetitive and competitive inhibitions on AMO (Su et al., 2021). In fact, ammonia oxidizing bacteria and archaea were 100 and 1000 times, respectively, more sensitive than typical heterotrophs to hydrocarbon toxicity (Urakawa et al., 2019, 2012). Our results showed that the functional heterotrophic microorganisms were not inhibited by BTEX or other toxic compounds in PW, as indicated by 1.8–3.7 times higher total carbon oxidation rates obtained in PW amended incubations with O\textsubscript{2} and NO\textsubscript{3} compared to the controls (Figs. 3, S3, Table 1).

### 3.3. Chemical fingerprint before and after biodegradations

More than 1000 compounds were detected by non-target screening at the beginning and end of PW incubations with O\textsubscript{2} and NO\textsubscript{3} as electron acceptors. A subset of approximately 100 compounds were tentatively identified via a library search (NIST Library 2017, match factor > 700). Degradation of BTEX-type compounds was assumed to produce oxygenated analogs (e.g., carboxylic acids and alcohols) as intermediates during the mineralization (Chakraborty and Coates, 2004; Gibson et al., 1968; Meckenstock et al., 2004; Yoshikawa et al., 2017). The presence of these intermediates can be utilized as an indicator of the overall degradation progress.

Among the analyzed samples taken at the beginning (T0) and end (T9) of the oxic incubation with PW (O\textsubscript{2}-PW-Fingerprint), the identified compounds were mainly classified as alcohols and acids (Fig. 4A). Some PAHs were detected in low amounts, with no significant differences between the samples taken at T0 and T9 (Fig. 4A). Interestingly, there was a large proportion of phenol and its alkylated analogs at the onset of oxic incubation (T0), which were not detected at the end of oxic incubation (T9) (Fig. 4A). Instead, the slurry sample at T9 contained a larger proportion of C\textsubscript{6} to C\textsubscript{10} linear carboxylic acids and alcohols (Fig. 4A). These linear carboxylic acids and alcohols were likely biodegradation products formed by stepwise oxidation of aromatic hydrocarbons to phenols, followed by ring-opening and reduction (Fig. 4C). The reactions proposed based on chemical fingerprint corresponded to the aerobic biodegradation pathway suggested for BTEX, i.e., stepwise oxidation and cleavage of aromatic ring performed by monooxygenases and/or dioxygenase under oxic conditions. The ratios of long versus short carboxylic acids did not show evident trends. This was in line with the fact that produced water is a highly complex mixture of natural chemicals and biodegradation products (Bergfors et al., 2020). Incomplete biodegradation could be a plausible explanation of the high richness of organics in the treated samples. Another indication of stepwise degradation pathways was the presence of benzenebutanoic acid at the beginning of oxic incubation (T0), which was missing at the end of oxic incubation (T9) (Fig. 4A). In contrast, the abundance of
benzenepropanoic, benzeneacetic and benzoic acids increased at T9. These results indicated a preference for saturated decarboxylation followed by oxidation over ring-opening type reactions (Fig. 4C). However, more research is required to clarify the exact biodegradation mechanisms.

The comparison of biodegradation products formed during oxic and anoxic incubations demonstrated that the chromatogram of the slurry sample after oxic incubation (O₂-PW-Fingerprint at T9) was heavily populated in the low retention time region in both dimensions, while the proportion intensity for anoxic incubation amended with NO₃ (NO₃-PW-Fingerprint at T9) was slightly shifted to higher retention time region (Fig. 4B). In simplified terms, an increase in retention time corresponded to an increase in the boiling point (size) and polarity (Fig. 4B). The region marked in green primarily contained smaller alcohols/acids (e.g., butanoic acid, octanol and cyclohexanol), while the region marked in red was dominated by aromatic acids (e.g., terephthalic acid, toluic acid and 3, 4-dimethylbenzoic acid) (Fig. 4B). These compounds were potential intermediates during the degradation of alkylated phenols (Mei et al., 2019; van Schie and Young, 2000). Thus, a larger number of small molecules were produced during aerobic biodegradation, whereas anaerobic biodegradation resulted in higher concentrations of uncleaved aromatics. O₂ seemed to facilitate a more complete degradation process. The result was in agreement with the previous observation in marine sediments, where aerobic mineralization of complex organic material was 2–3 times faster than that under anoxic conditions (Kristensen et al., 1995). The relatively incomplete decomposition in the anoxic zones of most sediments can be explained by the limited ability of anaerobic microorganisms to hydrolyze certain classes of structurally complex and aromatic organic compounds (Kristensen et al., 1995).

3.4. Toxicity analysis

According to the toxicity tests on A. tonsa, LC₅₀ was 22% for untreated PW (i.e., dilution factor of 4.5) and 8% for untreated and concentrated synthetic PW (i.e., dilution factor of 12.5), which was equal to 4.6 and 2451 µmol/L of BTEX in PW and synthetic PW, respectively (Fig. 5A–B). Furthermore, untreated PW also inhibited egg and pellet production of A. tonsa with EC₅₀ values of 0.4% and 0.7%, respectively (Fig. 5C–D). The lower EC₅₀ values than LC₅₀ values indicated that egg and pellet production of copepods were more sensitive than mortality to untreated PW, which were consistent with previous toxicity tests of PAHs and antifouling biocides on A. tonsa (Bellas and Thor, 2007; Wendt et al., 2016). Compared to untreated PW, higher EC₅₀ values of egg and pellet production were estimated for treated O₂-PW (i.e., 0.8% and 1%, respectively) and NO₃-PW (1% and 1.3%, respectively). Thus, toxic effects of produced water on the physiology of A. tonsa were reduced by half after microbial treatments. Since BTEX-type compounds were efficiently removed in both oxic and anoxic incubations (Figs. 3, S3), the remaining toxicity of treated PW on A. tonsa might be related to other compounds, such as PAHs, which was detected in low amounts with no significant removal during incubations (Fig. 4A) or chemical additives (biocides, corrosion inhibitors, etc.) dosed in drilling to prevent operational problems and enhance oil/water separation (Fakhru’l-Razi et al., 2009; Jiménez et al., 2018). A toxicity identification evaluation is needed in future studies.

3.5. Practical implications

Our study demonstrated the feasibility of using bacteria naturally harboring in marine sediments as an efficient and sustainable option for treating certain components in produced water. On the basis of BTEX removal rates measured in NO₃-amended incubations, we estimated the
The present study examined the feasibility and capability of using marine sediments for the bioremediation of produced water. We obtained significant BTEX removals in a series of sediment batch incubations under different redox conditions, with biodegradation efficiencies of 93–97% in oxic incubations and 45–93% in NO$_3^-$-, Fe$^{2+}$-, and SO$_4^{2-}$-amended anoxic incubations. Higher biodegradation rates of BTEX were obtained by incubations dominated by NO$_3^-$ reduction (104–149 nmolC/cm$^2$/d) and O$_2$ respiration (52–57 nmolC/cm$^2$/d), followed by SO$_4^{2-}$ reduction (14–76 nmolC/cm$^2$/d) and Fe reduction (29–39 nmolC/cm$^2$/d). Chemical fingerprint analysis suggested that the presence of O$_2$ facilitated a more complete biodegradation process, as hydrocarbons were found to be biodegraded to smaller alcohols/ acids under oxic conditions compared to anoxic conditions with NO$_3^-$.

Toxicity of treated produced water to the marine copepod A. tonsa was reduced by half after sediment incubations with O$_2$ and NO$_3^-$. The results advance our understanding of hydrocarbon biodegradation under different redox conditions in marine sediments, and highlight the option to use marine sediment as a biofilter for treating produced water at sea without extending the oil and gas platform or implementing a large-scale construction.

**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.

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**Supplementary materials**

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.simpat.2017.03.014.

**References**


