Iron Oxide-Bearing Wastes as Media for Supporting Biodegradation of BTEX

Safaa A. Al-Obaidi [1,†] **, Pallavee Srivastava** [1] **, Gordon Webster** [2] **, Andrew J. Weightman** [2] **and Devin Sapsford** [1,*]

1 School of Engineering, Cardiff University, Queen's Building, The Parade, Cardiff CF24 3AA, UK
2 School of Biosciences, Cardiff University, Sir Martin Evans Building, Museum Avenue, Cardiff CF10 3AX, UK
* Correspondence: sapsforddj@cardiff.ac.uk
† Current address: Institute of Genetic Engineering and Biotechnology for Post Studies, Baghdad University, Baghdad 10071, Iraq.

Abstract: Two common iron oxide-bearing wastes—a drinking water treatment residual and a passive mine water treatment sludge (MWTS)—were utilised with and without modification as media in microcosm experiments to treat artificial benzene, toluene, ethylbenzene, and xylene (BTEX)-contaminated wastewater. In all cases, the removal of BTEX was observed over the 160-day experiments, with benzene being the most recalcitrant. The solubilisation of iron was observed, which, alongside the syntropic relationship between the methanogens and firmicutes, allowed several anaerobic processes to occur, including iron reduction in concert with the biodegradation of BTEX. Nitrogen sparging prior to microcosm establishment, compared to aeration, was seen to lead to the greater subsequent removal of BTEX, indicating that anaerobic conditions favoured removal. The rates of BTEX removal indicated that these iron oxide-bearing wastes, an abundant waste stream, may be an interesting candidate for cost-effective media for BTEX remediation in applications such as permeable reactive barriers.

Keywords: bioremediation; benzene; iron-reducing bacteria; circular economy; drinking water treatment residuals; mine water treatment sludge; industrial symbiosis

1. Introduction

Hydrous ferric oxide (HFO)-containing sludges are a common waste resulting from water treatment processes treating ferruginous water or where ferric iron-based coagulants have been used. The present study focuses on passive mine water treatment sludge (MWTS) and potable water treatment sludge (WTS), also known as drinking water treatment residuals. Estimates of national levels are typically in the range of thousands of tonnes per annum depending on water content (e.g., [1–3]). Research is increasingly investigating reuse/recycling opportunities for these sludges, particularly in remediation applications, promoting a circular economy and avoiding landfilling [4]. Recent examples include a soil amendment to boost carbon retention [5] and hormone removal from water [6]. This study investigates a novel application for the remediation of benzene, toluene, ethylbenzene, and xylene (BTEX).

Volatile organic pollutants like BTEX have attracted significant attention due to their widespread presence and recalcitrant nature [7]. The primary sources of these monoaromatic hydrocarbons include the leakage of petroleum and its products, vehicle exhaust, petrochemical industries and products, processing industries, and industrial raw materials [8,9]. These compounds are detrimental to humans as they are known mutagens, carcinogens, and endocrine disruptors [10]. BTEX are easily transported to deep soil and groundwater due to their high solubility [11], thus affecting the groundwater quality and aquifer ecosystem. Therefore, it has become imperative to mitigate the risk associated with BTEX contamination.

Several physical (soil vapour extraction, air sparging), chemical (oxidation, flushing), and biological (bioventing, phytoremediation) methods have been developed for mitigating the BTEX contamination of subsurface and groundwater systems [12,13]. Amongst these, bioremediation is the preferred technique due to its green nature and cost-effectiveness [14–17]. A plethora of microorganisms are capable of mitigating the hazards of aromatic compounds [18] by breaking down the aromatic hydrocarbon as a sole carbon source [19]. The anaerobic biodegradation of BTEX is of particular interest as these contaminants often occur in anoxic zones of the environment [19]. Many studies have reported the degradation of benzene and toluene under iron-reducing conditions in microcosm studies amended with organic ligands [20–22] and enriched with microorganisms of the *Geobacteraceae* family [23,24]. The complete mineralisation of benzene to CO_2 using a ferric iron-reducing enrichment culture has been reported [25]. The degradation of xylene and ethylbenzene, on the other hand, is more common under sulphate-reducing conditions [26–28] or denitrifying conditions [29–31]. Several studies in the recent past have exhibited the efficient degradation of xylene and ethylbenzene under iron-reducing conditions in sediment-free enrichment cultures [32,33]. Chelators and humic substrates that are found in soil in abundance accelerate the rate of Fe(III) reduction and eliminate the need for direct contact between microorganisms and Fe(III) oxides [22,34]. Benzene is more recalcitrant than toluene under iron-reducing anoxic conditions, with the biodegradation of benzene occurring only after the anaerobic biodegradation of toluene [35]. There is a lack of studies on the use of hydrous ferric oxide-containing wastes for the remediation of BTEX-contaminated water. However, several studies have reported the application of iron-bearing-activated sludge for the treatment of wastewater contaminated with hydrocarbons [36–38].

The present study investigates the ability of the indigenous microbiota present in hydrous ferric oxide (HFO)-containing sludges/wastes from drinking water treatment and mine water treatment in the bioremediation of BTEX-containing simulated wastewater. The physicochemical characteristics of the substrate, the chemistry of the contacting water, and the evolution of the microbial community were used to evaluate the effectiveness of the media and microbial community to degrade BTEX.

2. Methods

2.1. HFO Sludges and Their Characterisation

WTS (water treatment sludge), MWTS (mine water treatment sludge), and MWTS mixed with autoclaved wastewater treatment sludge (WWS) in a mass ratio of 9:1 (MX) were used for this study. WWS was autoclaved at 121 °C and 15 psi for 30 min prior to use. WWS was added to enhance the nutritive value of MWTS. MWTS was collected at a mine water treatment system located at the site of the former Lindsay colliery in Capel Hendre, Carmarthenshire, South Wales, UK, while WTS was collected from Hirwaun water treatment works in South Wales, UK. WWS was collected from the digestor unit of the wastewater treatment plant at the Cardiff wastewater treatment works. The sludges were homogenised and dewatered and subsamples were preserved at −80 °C for microbial community analysis. Subsamples were subjected to various physicochemical analyses, as described in [39].

2.2. Artificial BTEX Wastewater and Sludge Sorption Studies

Artificial BTEX (benzene, toluene, ethylbenzene, xylene) wastewater was prepared according to Shariati et al. [40], with slight modifications. In brief, the wastewater contained benzene (45 mg/L), toluene (45 mg/L), ethylbenzene (45 mg/L), xylene (45 mg/L), NH_4Cl (46 mg/L), KH_2PO_4 (10 mg/L), $NaHCO_3$ (200 mg/L), $CaCl_2.2H_2O$ (0.01 mg/L), $MgSO_4.7H_2O$ (0.05 mg/L), and $FeCl_3.6H_2O$ (0.07 mg/L).

Batch sorption studies were carried out to determine the adsorption behaviour capacity of the sludge for the same initial BTEX concentrations used in the microcosm studies. In brief, 100 g wet sludge (MWTS, WTS, and WWS) was sterilised twice by autoclaving (121 °C at 15 psi for 30 min) and then dried at 40 °C for 48 h [41]. Initially, open-lid

experiments were conducted, but the loss of BTEX by volatilisation was high and, therefore, sealed experiments were developed. For these, 0.25 g sterilised dry sludge was added to 20 mL GC-MS (gas chromatography–mass spectrometry) sealed glass vials with PTFE gas-tight aluminium silicone crimp lids. BTEX wastewater (21 mL) was added, and the vials were incubated in the dark on a shaker (150 rpm) for 25 days. A gas-tight syringe was used to withdraw samples for GC-MS analysis to determine the BTEX concentrations. The experiments were carried out in duplicate with appropriate controls. The adsorption capacity of MX was calculated by taking 10% of the adsorption capacity of WWS and combining it with 90% of the adsorption capacity of MWTS.

2.3. Batch/Microcosm Study

Headspace vials with PTFE gas-tight aluminium silicone crimp lids were used as batch reactors. In brief, 15.0 g wet MTWS/WTS/MX along with 10.4 mL BTEX wastewater were added to these vials. The biodegradation of BTEX was studied under initially aerobic or anoxic conditions. For initial aerobic conditions, wastewater was aerated for 30 min before the addition of BTEX. The vials were sealed with no headspace immediately after decanting BTEX wastewater. These vials were labelled as A̲erobic MWTS (AMWTS)/A̲erobic WTS (AWTS)/A̲erobic MX (AMX). For anoxic conditions, all the components including the microcosms and wastewater were placed in a nitrogen glove box. The wastewater was de-oxygenated by bubbling nitrogen through the solution for 30 min before the addition of BTEX. Following this, the BTEX wastewater was immediately decanted into vials containing MWTS/WTS/MX in a nitrogen chamber and labelled as A̲noxic MWTS (NMWTS)/A̲noxic WTS (NWTS)/A̲noxic MX (NMX). The vials were incubated on a shaker (150 rpm) for 160 days. Sacrificial sampling was employed for batch studies, where 20 replicates were prepared for each sludge sample, and samples were sacrificed in duplicates at each sampling point (every 20 days). These samples were subjected to GC-MS analysis to determine the BTEX concentration (as described in Section 2.4) and other parameters like pH, DO, EC, total iron, and Fe(II) concentration (as described in Section 2.5).

2.4. GC-MS Analysis

A PerkinElmer Clarus 500 GC-MS equipped with an HS Turbo Matrix 40 was used for headspace analysis to determine the concentration of BTEX. All glassware was washed with ethanol prior to analysis. A gas-tight glass syringe was used to withdraw 0.01 mL sample and injected into a glass container with a PTFE gas-tight silicone crimp lid (GC-MS vials). BTEX solution (Sigma) containing benzene, toluene, ethylbenzene, p-xylene, m-xylene, and o-xylene at 1 mg/mL (in dimethyl sulfoxide) was used as the GC-MS standard. The headspace system was maintained at a temperature of $90 \pm 1\ ^{\circ}$C, with the needle and transfer line set at $120 \pm 1\ ^{\circ}$C. The sample was heated and agitated for 10 min at $80 \pm 1\ ^{\circ}$C, collected with a needle, and transferred to the column. Helium was used as the carrier gas for the analysis, with a flow rate of 2 mL/min, a 50:1 split ratio, and an inlet temperature of $180\ ^{\circ}$C. The analysis employed a Chrom-624 column (30 m $\times$ 0.25 mm $\times$ 1.4 μm), with the oven initially set at $45\ ^{\circ}$C for 4 min, then increased to $145\ ^{\circ}$C at a rate of $17\ ^{\circ}$C/min, and held for 2 min. BTEX compounds were detected using a mass spectrometry (MS) detector in scan mode (40–150 *m/z*). A standard curve was generated using calibration standards of 250, 500, and 1000 ppb BTEX to determine the compound concentrations from the peak areas; the coefficient of determination was 0.999.

2.5. Physicochemical Analysis

A Mettler Toledo Seven Excellence™ multi-parameter system was used to measure pH, electrical conductivity (EC), dissolved oxygen (DO), and redox potential (ORP). Elemental analyses on acidified samples (stored at $4\ ^{\circ}$C) were performed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Perkin Elmer Optima 2100DV). Ferrous ion (Fe(II)) was determined using the HACH DR900 colourimeter and the phenan-

throline method. A HANNA temperature data logger was used to record the ambient lab temperature during the study.

2.6. Microbial Community Analysis

DNA was extracted from pre- and post-experimental aerobic and anoxic HFO sludges using the Fast DNA® SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) as per the instruction manual, with slight modifications according to Webster et al. [42]. A Qubit dsDNA assay kit (Invitrogen, Carlsbad, CA, USA) was used to quantify the extracted DNA. Next-generation sequencing (NGS) and community analysis of the sludges for both pre- and post-experiments was carried out on the Illumina MiSeq™ sequencer (Illumina, San Diego, CA, USA), following the protocol described in [43]. 16S rRNA gene V4 region amplification primers with suitable Illumina adaptors were used (515F: 5′–AATGATACGGCGACCACCGAGATCTACAC NNNNNNNNNTATGGTAATTGTGT-GCCAGCMGCCGCGGTAA, 806R: 5′–CAAGCAGAAGACGGCATACGAGAT NNNNNN-NNAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT). Sequence data were simultaneously demultiplexed and quality-filtered using default parameters of QIIME v.1.8 [44]. UCLUST was used to generate OTUs (operational taxonomic units) with a 97% sequence similarity threshold. BLAST and SILVA 128 were used to generate taxonomy [45].

3. Result and Discussions

3.1. Sorption of BTEX by Sludges

The detailed physicochemical properties of the sludges used for this study were presented by Srivastava et al. [39] and are briefly described here. MWTS had a dry density of 2.87 g/cm^3, lower than ferrihydrite (3.96 g/cm^3), likely due to clay mineral fines [46], and similar to other ochres in the South Wales coalfield [47]. WTS and MX had dry densities of 1.17 and 1.03 g/cm^3, respectively. Fe was the most abundant element in MWTS (41.4% DW), followed by Ca (2.37% DW), while WTS had lower Fe (263.13 mg/g) and Ca (0.64 mg/g). XRD data showed goethite and a background peak at 35° 2θ, indicating amorphous ferrihydrite. A small undulation at 62.5° 2θ was also observed, attributed to 2-line ferrihydrite [48]. The adsorption capacity of sludges used as media for the mineralisation of BTEX was determined in sealed experiments. Figure 1 shows the concentration of BTEX (mg/L) over 30 days, illustrating the decrease in BTEX levels from an initial concentration of 45 mg/L for each compound as the contact time with different adsorbent sludges increased. The concentration of BTEX in the control (without any substrate) remained almost constant till the end of the experiment, with minimal loss, which could be attributed to volatilisation during sample handling (Figure 1a). In general, MWTS and MX exhibited similar adsorption capacities for BTEX. MWTS exhibited ~0.41 mg/g, ~0.67 mg/g, ~0.28 mg/g, and ~1.76 mg/g adsorption capacities for benzene, toluene, ethylbenzene, and xylene, respectively. The adsorption capacity of MX for BTEX was determined to be 0.479 mg/g for benzene, 0.692 mg/g for toluene, 0.448 mg/g for ethylbenzene, and 1.838 mg/g for xylene, based on adsorption calculated from the mass-weighted contributions (1:9) of adsorption to WWS and MWTS. The adsorption process likely involved multiple mechanisms, including surface adsorption, pore-filling, and chemical bonding with the active sites on the sludge surface. The mass transfer process, driven by concentration gradients, likely governed the diffusion of BTEX molecules from the bulk solution to the sludge surface, followed by intraparticle diffusion within the porous structure [49–51]. The rate of sorption was relatively slow, indicating that mass transfer limitations, such as film diffusion and pore diffusion, played a significant role. The maximum adsorption was observed for xylene, where approximately 50% was adsorbed. WWS showed the highest adsorption capacity, achieving 1.10 mg/g for benzene, 0.89 mg/g for toluene, 1.96 mg/g for ethylbenzene, and 2.54 mg/g for xylene. The sorption data indicated a relatively low affinity for all but xylene to the sludges, and, even then, sorption occurred slowly over days rather than rapidly over minutes to hours.
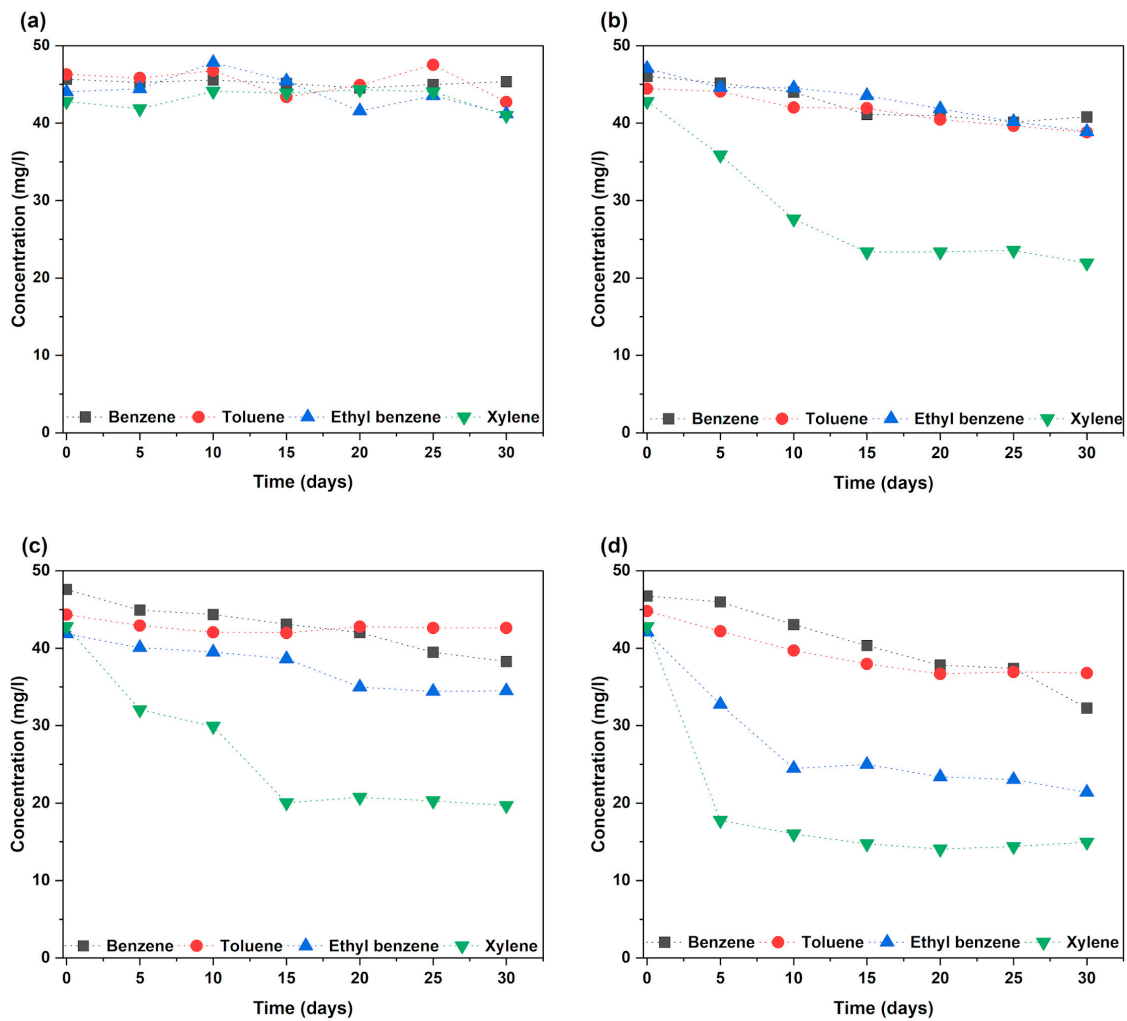
Figure 1. Concentration versus time of sorption experiments: (**a**) control, (**b**) mine water treatment sludge (MWTS), (**c**) water treatment sludge (WTS), and (**d**) wastewater treatment sludge (WWS) for BTEX.

3.2. Biodegradation of BTEX

Figure 2 shows that in all cases, the microcosm experiments led to large decreases in BTEX concentrations. Given the limited sorption indicated by the batch adsorption tests (Figure 1), biodegradation was likely to be the mechanism through which BTEX was degraded. Microcosms with MWTS, WTS, and MX as media fed with aerobically prepared BTEX wastewater were analysed for the degradation of BTEX. A sharp decline in ethylbenzene and xylene was observed from day 20 onwards in AMWTS compared to benzene and toluene (Figure 2a). Although AWTS exhibited a decrease in BTEX from day 20 onwards, ethylbenzene and xylene decreased at a higher rate and remained at ~2.5 mg/L from day 60 onwards (Figure 2b). Benzene, toluene, and ethylbenzene exhibited a drastic decline from day 20 onwards when AMX was used as media, while toluene exhibited a sharp decrease from day 40 onwards (Figure 2c). AMX was the most effective in degrading TEX, with only 0.11 mg/L, 2.98 mg/L, and 0.04 mg/L left remaining by the end of the experiment, respectively. AMX degraded 99.8% toluene by the end of the experiment, while AMWTS and AWTS degraded ~60% toluene. Both AWTS and AMX exhibited a ~93% degradation of ethylbenzene. Under aerobic conditions, biodegradation primarily occured through enzymatic oxidation, where monooxygenase or dioxygenase enzymes introduced oxygen into the aromatic ring, leading to ring cleavage and further breakdown into non-toxic metabolites [52]. The biodegradation observed when using aerobically prepared BTEX-contaminated wastewater could be attributed to these phenomena. Benzene was the

most recalcitrant amongst BTEX, followed by toluene, ethylbenzene, and xylene. This is in keeping with various previous studies that reported benzene to be the least amenable to degradation, while the R-group present in toluene, ethylbenzene, and xylene accelerated biodegradation [17,53]. Only 21%, 64%, and 56% benzene degraded when AMWTS, AWTS, and AMX were used as media, respectively. AWTS was better at degrading benzene compared to AMWTS and AMX (Figure 2). The initial lag observed in degradation could be attributed to the time required by the indigenous microbial community to acclimatise.
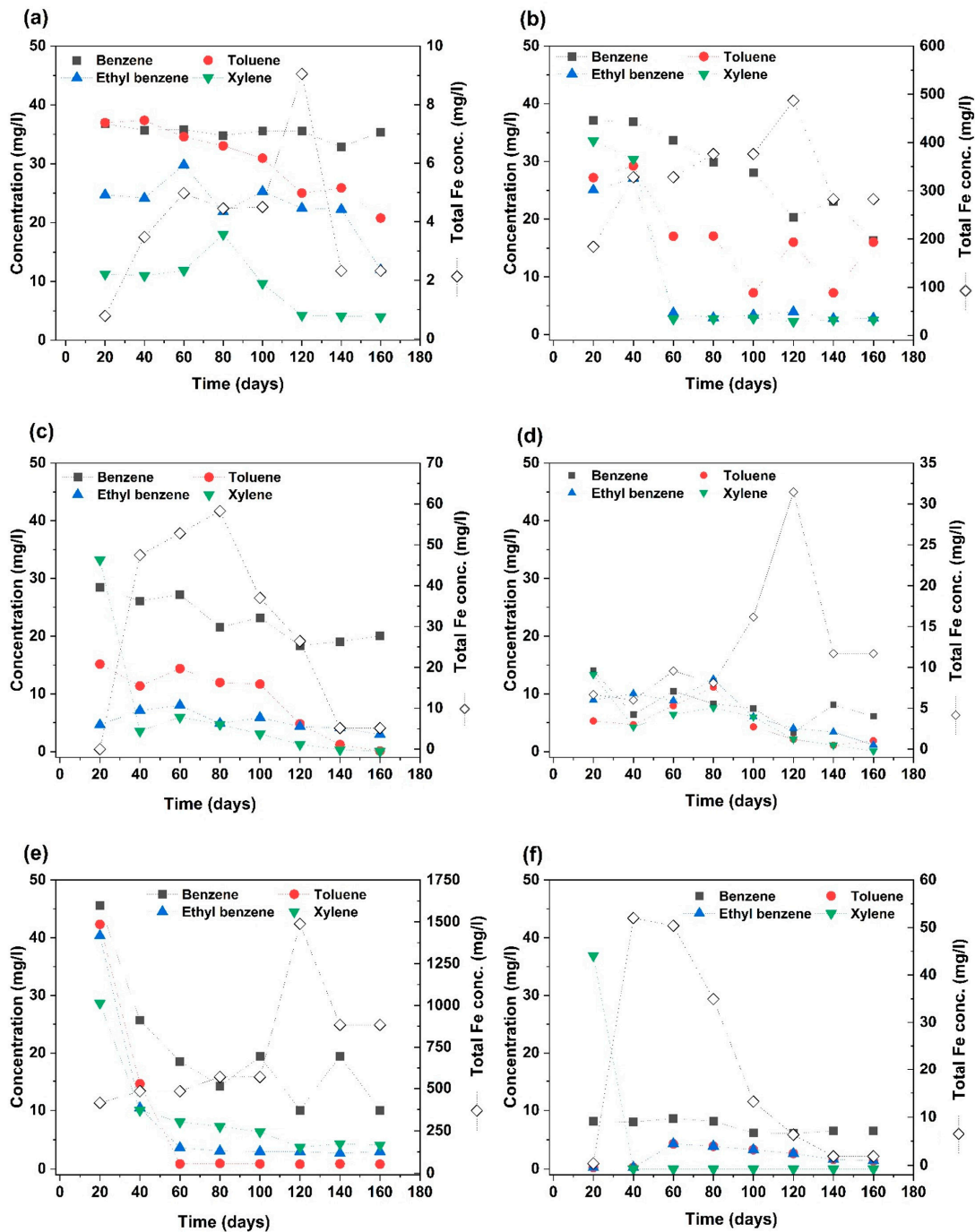

Figure 2. BTEX degradation evident with the decrease in BTEX concentration when treated with (**a**) AMWTS, (**b**) AWTS, (**c**) AMX, (**d**) NMWTS, (**e**) NWTS, and (**f**) NMX with respect to changes in total iron concentration. (AMWTS/AWTS/AMX—aerobically prepared BTEX exposed to MWTS, WTS, and MX, respectively; NMWTS/NWTS/NMX—anaerobically prepared BTEX exposed to MWTS, WTS, and MX, respectively).

The media used for this study were rich in iron oxides [39] and, therefore, iron reduction can reasonably be hypothesised to be a mechanism for BTEX degradation or mineralisation, which requires predominantly anaerobic or anoxic conditions [54,55]. BTEX compounds are often found in anoxic zones of the environment [18]. For the second set of experiments, the BTEX in the wastewater prepared anoxically was found to have been more effectively degraded (Figure 2d–f). NMWTS and NMX exhibited a similar trend in the decline of BTEX concentration, while NWTS exhibited a distinct and greater lag period before the onset of BTEX degradation. The anaerobic degradation of benzene is known to be the most difficult, while toluene has been shown to be the most biodegradable [18]. Benzene was still the most recalcitrant, with 6.12 mg/L, 10.05 mg/L, and 6.51 mg/L residual benzene found in NMWTS, NWTS, and NMX, respectively. Both NMWTS and NMX exhibited ~86%, ~96%, 97%, and ~100% efficiency in the degradation of BTEX, respectively. This was much higher than the efficiency exhibited when the sludges were in contact with aerobically prepared BTEX wastewater. Indicative degradation rates of BTEX were determined by linear regression. The data (Table 1) exhibited by the media used for this study were comparable to those reported in [56] and [57] (and the references therein).

Table 1. Decay rates of BTEX based on linear regression during microcosm studies on biodegradation of BTEX by iron (oxy)hydroxide media.

Media	Degradation Rate (µM/day)			
	Benzene	Toluene	Ethylbenzene	Xylene
NMWTS	0.9	0.6	0.8	0.7
NWTS	2.7	1.9	1.7	1.3
NMX	0.2	0.2	0.2	1.4
AMWTS	0.09	1.4	0.7	0.7
AWTS	1.8	1.6	1.9	1.9
AMX	0.9	1.2	0.2	1.3

The indigenous microbes present within the iron (oxy)hydroxide-rich waste were mostly anaerobic and, therefore, under anoxic conditions, BTEX was the sole carbon source [58] and Fe(III) was the electron acceptor [38]. Additional nutrients have been shown to further enhance the biodegradation of BTEX by acting as electron shuttles [59]. Wastewater treatment sludge, rich in nutrients and humic substances, was added to MWTS (MX). Interestingly, however, in this case, both NMWTS and NMX were equally effective in the degradation of BTEX.

3.3. Parameters During BTEX Degradation

The pH during BTEX degradation remained between 6.0 and 8.0, except for AWTS, which exhibited a pH of 4.8 (Figure 3). Aerobically prepared BTEX-bearing wastewater exhibited a pH of 7.0, while that of anoxically prepared BTEX wastewater was 4.70. AMWTS and AWTS exhibited a decrease in pH with time, while AMX exhibited an increase. For degradation studies using anoxic BTEX wastewater, the pH increased over time, with NMX (7.79) exhibiting the highest increase. Except for AMX, which exhibited a negative correlation between BTEX degradation and pH, AMWTS and AWTS microcosms exhibited a positive correlation. On the other hand, a significant negative correlation was obtained between BTEX degradation and pH for NMWTS, NWTS, and NMX microcosms. Although most studies report efficient BTEX biodegradation between pH 6.0 and 8.0 [14,60,61], a few studies have also exhibited efficient BTEX degradation between pH 3.5 and 7.0, i.e., under acidic conditions [62–64]. The electrical conductivity (EC) of the aerobically prepared BTEX wastewater was 115.7 µS/cm, while anoxically prepared BTEX wastewater exhibited EC of 98.8 µS/cm. All the samples exhibited an increase in EC with the onset of BTEX degradation, where AMX and NMX exhibited the highest EC of 2566 µS/cm and 2615 µS/cm on day

20, respectively (Figure 3). An increase in electrical conductivity has been reported during dissimilatory iron reduction [65,66] and was indicative of biodegradation in this study.
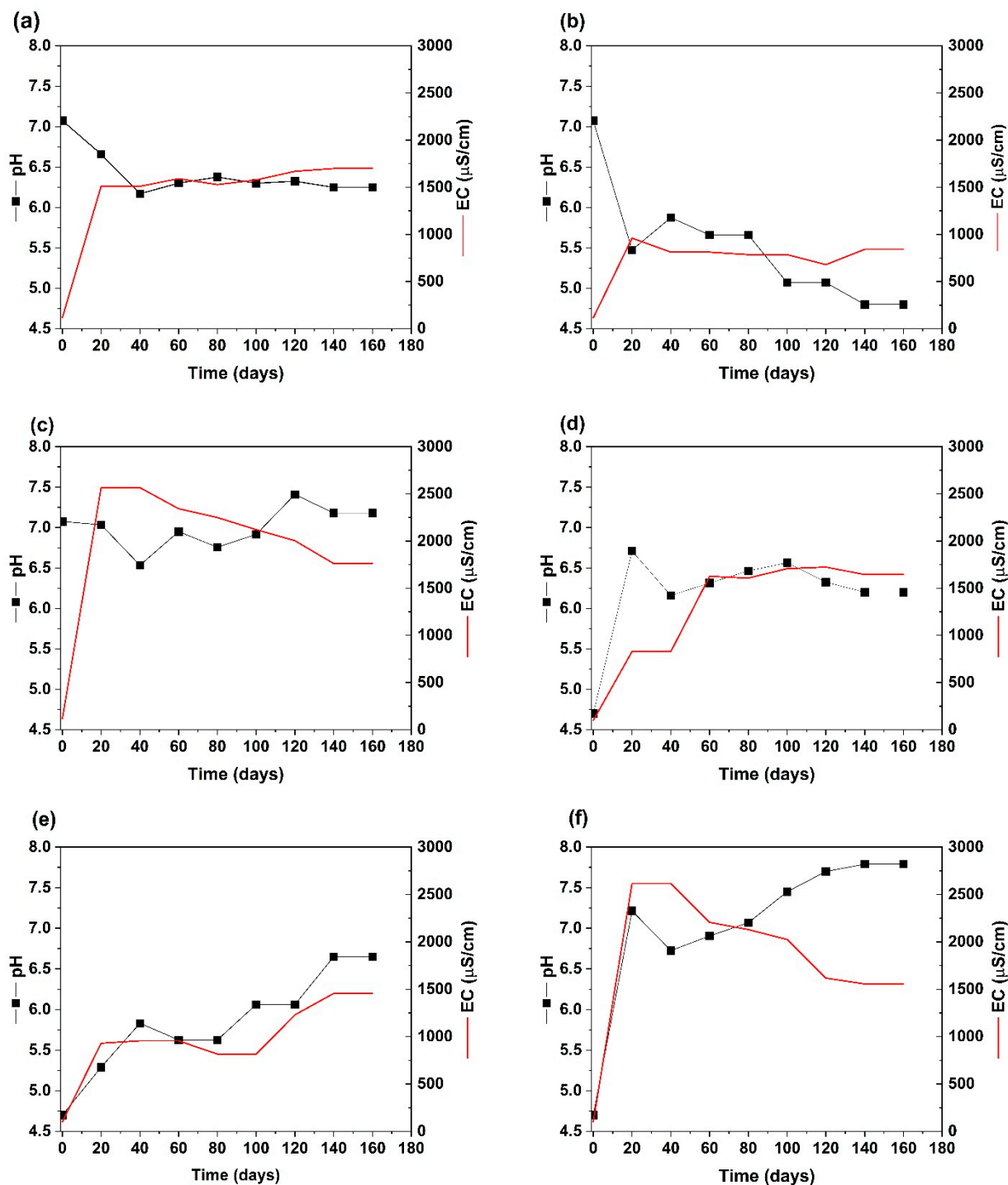

Figure 3. Changes in pH and electrical conductivity (EC) during degradation of BTEX when treated with (**a**) AMWTS, (**b**) AWTS, (**c**) AMX, (**d**) NMWTS, (**e**) NWTS, and (**f**) NMX. (AMWTS/AWTS/AMX—aerobically prepared BTEX exposed to MWTS, WTS, and MX, respectively; NMWTS/NWTS/NMX—anaerobically prepared BTEX exposed to MWTS, WTS, and MX, respectively).

The experiment was conducted under ambient temperature conditions with no temperature control; however, the changes in the ambient temperature were recorded during the course of the study using a HANNA temperature logger. The temperature during the

experiment varied between 1 °C and 24.5 °C, with the initial 20 days exhibiting temperatures lower than 9.5 °C. The temperature from day 20 onwards exhibited an increase, with the highest observed at the end of the experiment. Most studies report the efficient biodegradation of BTEX between 25 °C and 33 °C [60,61,67,68]; however, in this study, BTEX biodegradation was observed at lower temperatures between 9.5 °C and 24.5 °C. No clear correlation was observed between temperature and BTEX degradation, but it is likely that rates are affected by temperature (e.g., [60,61]). This is an important aspect that future studies should examine.

Fe(III) present in MWTS, WTS, and MX is a potential electron acceptor during BTEX biodegradation. Total dissolved iron and Fe(II) were quantified during the course of the biodegradation of BTEX by HFO-bearing wastes (Figure 4 and alternative axes). Although iron release was observed in all the microcosms, the behaviour was noticeably different. Irrespective of the type of BTEX wastewater being remediated, the total iron released by MWTS and MX was much lower than WTS, despite MWTS exhibiting a higher Fe content (36.5%). MWTS and WTS exhibited a significant negative correlation between BTEX degradation and Fe(II) release, while MX did not exhibit any correlation between the two. The iron release was observed with the onset of degradation. AWTS microcosms exhibited a gradual increase to 486 mg/L on day 120, after which, it declined to 283 mg/L by day 160. AMWTS and AMX microcosms exhibited the highest total iron concentrations of 9 mg/L (day 120) and 58 mg/L (day 80), respectively. Compared to MWTS and MX microcosms, both AWTS and NWTS microcosms exhibited the highest release of Fe(II) before the maximum release of total dissolved iron was obtained. The Fe(II) release was coeval with the total iron release and indicative of the mobilisation of Fe by iron-reducing conditions within the microcosm, microbially driven either directly or indirectly by the generation of other reductants, e.g., HS⁻. Under iron-reducing conditions, Fe(III) acts as the terminal electron acceptor and can result in the oxidation of BTEX (e.g., [32,69]). However, under experimental conditions, during anaerobic growth, some Fe(III) may also be used for making cellular components, with less Fe(III) being used for BTEX degradation [69].

3.4. Microbial Community Changes

3.4.1. Changes in Microbial Community Composition of MWTS

Next-generation sequencing was conducted on the substrates before and after the experiment to assess the role of microbes in BTEX degradation. Metataxonomic analysis revealed significant changes in community structure at both phylum and genus levels (Figure 5). The microbial diversity in pre-test wastes varied distinctly from post-test wastes, with phylum-level diversity dropping from 16 to 7 after treatment. *Proteobacteria* remained the dominant phylum, decreasing from 58.35% in pre-test to 51.51% and 39.67% in post-test samples. Conversely, *Firmicutes* and *Bacteroidetes* showed increases in abundance, with *Firmicutes* rising from 0.42% to 30.32% in AMWTS and 17.98% in NMWTS and *Bacteroidetes* rising from 5.65% to 21.80% in AMWTS and 30.32% in NMWTS. Other genera, including *Planctomycetes*, *Actinobacteria*, and *Spirochaetes*, declined to less than 1% post-treatment. Primary genera in pre-test MWTS included *Gallionella* (5.8%), *Rhodoferax* (3.8%), and others, with most decreasing in relative abundance after treatment, except *Rhodoferax* and *Devosia*. New genera such as *Escherichia* and *Prevotella* were detected in post-test MWTS, and *Methanobacterium* emerged post-treatment. The microbial communities in AMWTS and NMWTS were distinct, with specific genera appearing exclusively in one or the other. Several *Pseudomonas* species are known for their ability to degrade BTEX [69,70].

3.4.2. Changes in Microbial Community Composition of WTS

The only archaeal phylum in pre-test WTS was *Euryarchaeota* (0.19%), which was not found in AWTS or NWTS post-test (Figure 5b). The most abundant bacterial phyla in pre-test WTS included *Proteobacteria* (36.76%), *Verrucomicrobia* (17.19%), and *Acidobacteria* (13.35%). Post-test, *Verrucomicrobia* (40.32%) dominated in AWTS, while *Proteobacteria* (30.13%) remained predominant in NWTS. AWTS saw a significant decrease in *Acidobacteria*

and *Proteobacteria*, with increases in *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*. NWTS exhibited slight decreases in *Acidobacteria* and *Verrucomicrobia* but increases in *Actinobacteria*, *Bacteroidetes*, and *Planctomycetes*, along with significant increases in *Firmicutes* and *Chloroflexi*.
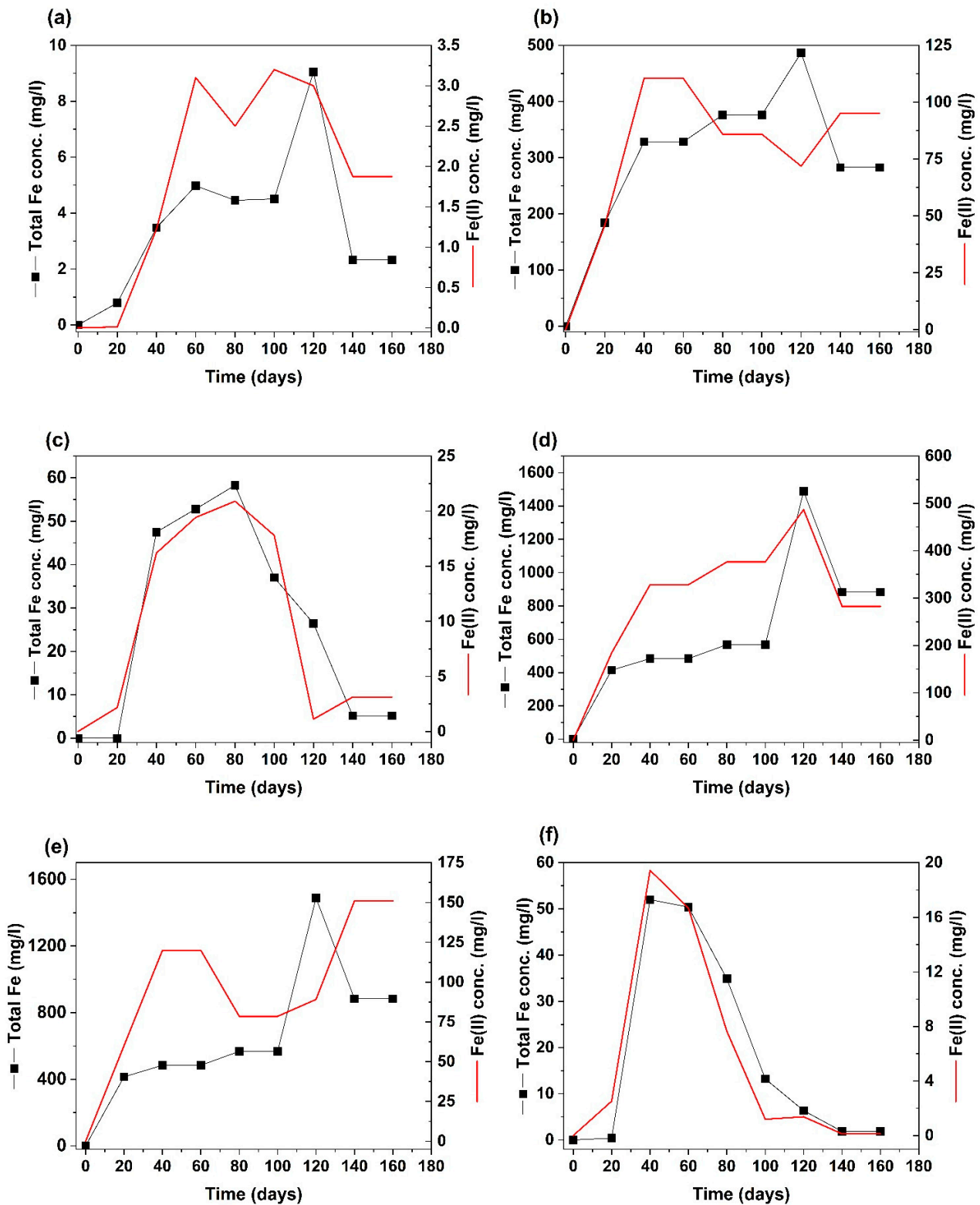

Figure 4. Changes in the total iron and ferrous iron [Fe(II)] concentrations during degradation of BTEX when treated with (**a**) AMWTS, (**b**) AWTS, (**c**) AMX, (**d**) NMWTS, (**e**) NWTS, and (**f**) NMX.
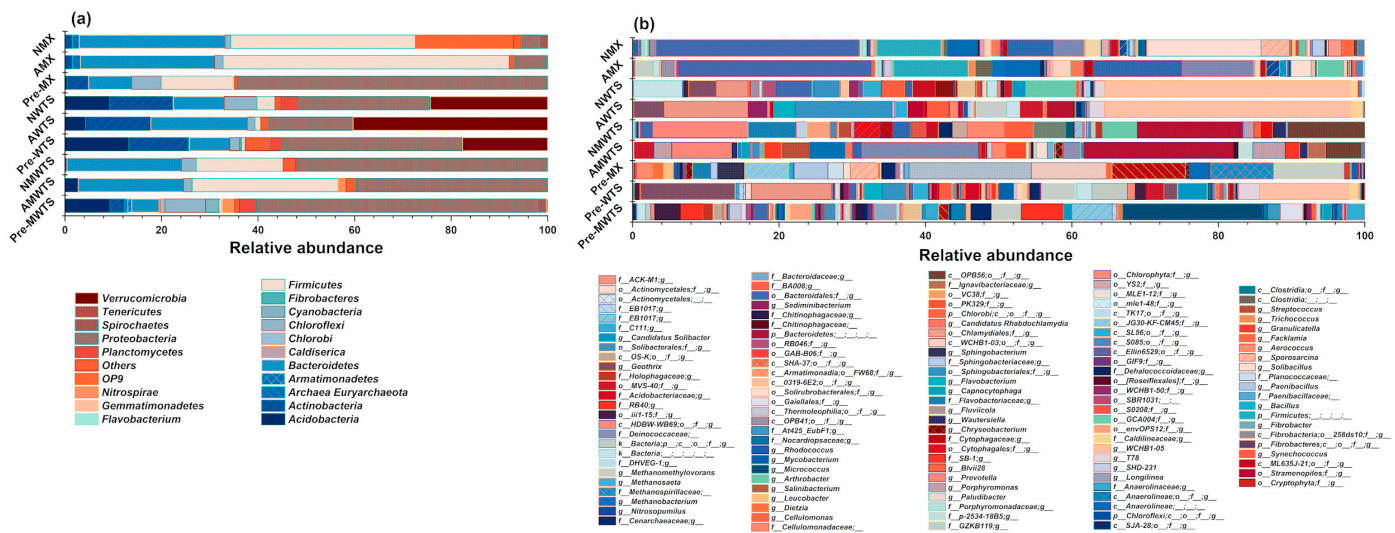
Figure 5. Changes in the diversity of the microbial community at (**a**) phylum level and (**b**) genus level in the media used to treat aerobically and anaerobically prepared BTEX wastewater. (AMWTS/AWTS/AMX—MWTS, WTS, and MX exposed to aerobically prepared BTEX wastewater, respectively; NMWTS/NWTS/NMX—MWTS, WTS, and MX exposed to aerobically prepared BTEX wastewater, respectively).

In pre-test WTS, the main genera included *Geothrix* (12.9%) and unclassified genera from family R4-41B (12.35%). Treatment with BTEX wastewater harmed *Gallionella* and *Rhodoferax*, while *Polynucleobacter* was negatively impacted by both treatments. BTEX wastewater significantly reduced *Geothrix* abundance but had a lesser effect on unclassified bacteria from family auto67_4W. Both AWTS and NWTS showed an increase in bacteria from family R4-41B, rising from 12.35% to ~33.70%. Certain genera responded differently to treatment; for example, *Flavobacterium* decreased in NWTS but increased in AWTS, while ACK-M1 increased in AWTS and decreased in NWTS. AWTS also showed the presence of *Escherichia* (3.68%) and *Veillonella* (1.17%), while NWTS enriched *Geobacter* (6.8%), *Desulfosporosinus* (2.43%), and WCHB1-05 (1.11%).

3.4.3. Changes in Microbial Community Composition of MX

The predominant phylum in pre-test MX was *Proteobacteria* (64.23%), which significantly decreased in AMX (6.62%) and NMX (1.69%), along with *Actinobacteria* and *Chloroflexi*. *Firmicutes* rose from 14.79% in pre-test MX to become the main phylum in AMX (59.28%) and NMX (38.05%). *Bacteroidetes* also increased, reaching 27.81% in AMX and 30.07% in NMX, compared to 8.91% in pre-test MX. NMX and AMX displayed distinct community structures; *Atribacteria*, absent in pre-test MX and AMX, appeared at 20.18% in NMX, while Spirochaetes rose from ~0.1% in pre-test MX and AMX to 1.69% in NMX. *Euryarchaeota*, not identified in pre-test MX, had abundances of 1.71% in AMX and 1.45% in NMX.

Key genera from *Proteobacteria* in pre-test MX included *Brevundimonas* (16.69%) and *Mycoplana* (10.25%). *Firmicutes* were represented by *Paenibacillus* (4.62%) and others, none of which were found in AMX or NMX. Post-exposure to BTEX, the community structure changed drastically at the genus level. The predominant genera in AMX and NMX were unclassified *Bacteroidales*, present at 26.43% and 27.57%, respectively, having proliferated from 0.43% in pre-test MX. Other notable genera included *Clostridium*, *Treponema*, and *Proteiniclasticum*. The phylum OP9 (*Atribacteria*) was identified only in NMX, featuring unclassified TIBD11 (15.58%) and SHA-1 (3.86%). Additionally, AMX contained the archaeal genus *Methanomethylovorans* at 2.35%. Notably, both AMX and NMX exhibited core species such as *Sedimentibacter* and *Methanobacterium*, which are involved in BTEX degradation [55], aligning with observed degradation trends.

3.4.4. Comparative Analysis of Differences in the Microbial Community Structure of HFO Sludges Exposed to BTEX Wastewater

Amongst the various phyla, *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* flourished to become the predominant phyla in all three sludges used in this study under both aerobic and anoxic conditions. Several studies have shown these phyla to be dominant during the biodegradation of BTEX [19,71–73]. *Proteobacteria* have been shown to degrade monoaromatic hydrocarbons such as benzene to CO_2 and H_2O [74], while *Firmicutes* and *Bacteroidetes* have been shown to degrade aromatic hydrocarbons [75,76]. *Chloroflexi* decreased in abundance in all the sludges except for NWTS, where it was enriched. This phylum has been shown to degrade aromatic hydrocarbons such as toluene and has been known to occur in numerous natural environments [70].

The wastes used in this study were rich in iron oxides/hydroxides, and, therefore, it is not surprising that several iron metabolising bacteria were identified in pre-MWTS, pre-WTS, and pre-MX. The iron-reducing genera *Geobacter* was only detected in pre-MX (6.8%), which, post-treatment, was halved in AMX and >1% in NMX. *Geobacter* was enriched only in NWTS and NMWTS. This genus is known to oxidise monoaromatic hydrocarbons while reducing Fe(III), which acts as an electron acceptor [24,77,78]. Similarly, *Geothrix*, an iron-reducing bacteria, was identified in all three pre-test sludges but was only present at >1% in AWTS (4.08%) and NWTS (3.72%) compared to 12.9% in pre-WTS. Although there is a dearth of reports on BTEX degradation by *Geothrix*, they have been isolated from hydrocarbon-contaminated aquifers [24,79]. *Gallionella*, an iron-oxidising bacteria, was detected in pre-MWTS (5.82%), pre-WTS (9.33%), and pre-MX (<1%) but was absent in post-test sludges, suggesting that the BTEX environment is detrimental to this genus, which agrees with the literature, as it has not been found so far to degrade aromatic compounds but is commonly found in ground- and wastewater [80]. *Clostridium* and associated genera like unclassified *Clostridiales*, which were not identified in any of the pre-test sludges, proliferated in all the sludges post-test. They have been known to reduce iron oxides while fermenting organic carbon [81] and have been isolated from mining lake ochres where iron-reducing conditions prevail [82,83]. In natural environments like aquifers, *Clostridium* along with other bacterial species such as *Geobacter* forming a microbial consortium degrade BTEX [84]. *Escherichia*, not identified in any of the pre-test sludges, was present at a significant abundance in AWTS (20.49%), NMWTS (14.06), and AWTS (3.68%). This genus was found in mine water treatment sludge as an indigenous genus [83] and is a genus effective in BTEX degradation [85,86]. Bacteria from the order *Sphingobacteriales* identified in AWTS (13.26%) and NWTS (3.50%) were found in the microbial community and were present in activated sludge that was capable of degrading BTEX [76]. *Prevotella* was enriched in AMWTS (8.14%) and NMWTS (13.08%) and bacteria from the order *Bacteroidales* were enriched in NWTS (4.92%), AMX (26.43%), and NMX (27.57%). These *Bacteroidetes* are commonly found in aquatic sediment systems [87] and have been shown to degrade aromatic hydrocarbons to the simplest compounds [88,89], even under iron-reducing conditions [90,91]. Similarly, the unclassified bacteria from the phylum *Firmicutes* and enriched in AMX (10.14%) and NMX (8.69%) were identified as the primary benzene degraders, with Fe(III) as the terminal electron acceptor [19]. *Methylibium*, an aerobic bacteria isolated from sites primarily contaminated with methyl tert-butyl ether (MTBE) or other aromatic hydrocarbons, are known degraders of BTEX [92,93]. *Desulfosporosinus* is a known toluene degrader and was only identified in NWTS (2.43%). All these organisms in concert under iron-reducing conditions bring about the degradation of BTEX. Both aerobic and anaerobic degradation of BTEX by microbes have been reported [59,77]. The distinct microbial community compositions of the HFO sludge used to treat aerobically prepared and anaerobically prepared BTEX wastewater are indicative of the distinct results obtained for BTEX degradation.

4. Importance/Implications/Significance of This Study

This study's use of iron oxide-bearing wastes has significant global implications for promoting sustainable waste management and environmental remediation, aligning with the principles of the circular economy by repurposing abundant waste materials. Its findings could reduce the economic costs associated with traditional remediation techniques and offer a cost-effective solution for treating contaminated groundwater and industrial wastewater, especially in regions with limited access to advanced treatment technologies.

5. Conclusions

The work presented in this paper demonstrates that the microbial community present within common iron oxide-rich sludges from the treatment of mine water (with or without amendment with wastewater treatment sludge) and drinking water treatment sludge (drinking water treatment residual) was effective in the biodegradation of BTEX. The native microbial community structure upon exposure to BTEX was completely modified, with microbes capable of (i) degrading hydrocarbons while reducing Fe(III) (*Clostridium*, *Geobacter*, unclassified bacteria from *Firmicutes*) and/or (ii) completely degrading hydrocarbons without reducing Fe(III) (*Escherichia*, *Methylibium*), being enriched within the iron oxide-rich waste. Given that these are abundant waste sludges with currently limited reuse potential, this work demonstrates that they may be useful media for the anaerobic biodegradation of BTEX-bearing wastewaters or groundwater and, given their abundance, may find useful application in permeable reactive barriers or as amendments to nature-based treatment systems.

Abbreviations

HFO—hydrous ferric oxide; MWTS—mine water treatment sludge; WTS—potable water treatment sludge; WWS—wastewater treatment sludge; MX—MWTS mixed with autoclaved MX at a mass ratio of 9:1; BTEX—benzene, toluene, ethylbenzene, xylene; AMWTS—aerobic MWTS; AWTS—aerobic WTS; AMX—aerobic MX; NMWTS—anoxic MWTS; NWTS—anoxic WTS; NMX—anoxic MX.

References

1. Bailey, M.T.; Moorhouse, A.M.L.; Byrom, A.J.; Kershaw, S. Applications for Hydrous Ferric Oxide Mine Water Treatment Sludge—A Review. In *Reliable Mine Water Technology, Proceedings of the International Mine Water Association Annual Conference 2013, Vols I & Ii, Golden, CO, USA, 6–9 August 2013*; International Mine Water Association: Lakewood, CO, USA, 2013; Volume 63, pp. 519–524.
2. Sapsford, D.; Santonastaso, M.; Thorn, P.; Kershaw, S. Conversion of coal mine drainage ochre to water treatment reagent: Production, characterisation and application for P and Zn removal. *J. Environ. Manag.* **2015**, *160*, 7–15. [CrossRef] [PubMed]
3. Ahmad, T.; Ahmad, K.; Alam, M. Sustainable management of water treatment sludge through 3'R' concept. *J. Clean. Prod.* **2016**, *124*, 1–13. [CrossRef]

4. Nguyen, M.D.; Thomas, M.; Surapaneni, A.; Moon, E.M.; Milne, N.A. Beneficial reuse of water treatment sludge in the context of circular economy. *Environ. Technol. Innov.* **2022**, *28*, 102651. [CrossRef]

5. Hodson, M.E.; Islam, M.; Metcalf, M.; Wright, A.C. Amendments of waste ochre from former coal mines can potentially be used to increase soil carbon persistence. *Appl. Geochem.* **2023**, *151*, 105618. [CrossRef]

6. Dias, R.; Daam, M.A.; Diniz, M.; Maurício, R. Drinking water treatment residuals, a low-cost and environmentally friendly adsorbent for the removal of hormones—A review. *J. Water Process. Eng.* **2023**, *56*, 104322. [CrossRef]

7. Emmanuel, S.S.; Olawoyin, C.O.; Ayodele, I.D.; Oluwole, O.J. Emerging nanosemiconductors for photocatalytic degradation of mono-aromatic volatile organic compounds (BTEX): A pragmatic review. *J. Organomet. Chem.* **2023**, *996*, 122767. [CrossRef]

8. Duan, X.; Li, Y. Sources and Fates of BTEX in the General Environment and Its Distribution in Coastal Cities of China. *J. Environ. Sci. Public Health* **2017**, *1*, 86–106. [CrossRef]

9. Yu, B.; Yuan, Z.; Yu, Z.; Xue-Song, F. BTEX in the environment: An update on sources, fate, distribution, pretreatment, analysis, and removal techniques. *Chem. Eng. J.* **2022**, *435*, 134825. [CrossRef]

10. Bolden, A.L.; Kwiatkowski, C.F.; Colborn, T. New Look at BTEX: Are Ambient Levels a Problem? *Environ. Sci. Technol.* **2015**, *49*, 5261–5276. [CrossRef]

11. Mitra, S.; Roy, P. BTEX: A Serious Ground-water Contaminant. *Res. J. Environ. Sci.* **2011**, *5*, 394–398. [CrossRef]

12. Ambaye, T.G.; Chebbi, A.; Formicola, F.; Prasad, S.; Gomez, F.H.; Franzetti, A.; Vaccari, M. Remediation of soil polluted with petroleum hydrocarbons and its reuse for agriculture: Recent progress, challenges, and perspectives. *Chemosphere* **2022**, *293*, 133572. [CrossRef] [PubMed]

13. Elgazali, A.; Althalb, H.; Elmusrati, I.; Ahmed, H.M.; Banat, I.M. Remediation Approaches to Reduce Hydrocarbon Contamination in Petroleum-Polluted Soil. *Microorganisms* **2023**, *11*, 2577. [CrossRef] [PubMed]

14. Firmino, P.I.M.; Farias, R.S.; Buarque, P.M.; Costa, M.C.; Rodríguez, E.; Lopes, A.C.; dos Santos, A.B. Engineering and microbiological aspects of BTEX removal in bioreactors under sulfate-reducing conditions. *Chem. Eng. J.* **2015**, *260*, 503–512. [CrossRef]

15. Mazzeo, D.E.C.; Levy, C.E.; Angelis, D.d.F.d.; Marin-Morales, M.A. BTEX biodegradation by bacteria from effluents of petroleum refinery. *Sci. Total Environ.* **2010**, *408*, 4334–4340. [CrossRef] [PubMed]

16. Farhadian, M.; Vachelard, C.; Duchez, D.; Larroche, C. In situ bioremediation of monoaromatic pollutants in groundwater: A review. *Bioresour. Technol.* **2007**, *99*, 5296–5308. [CrossRef]

17. Kelly, W.R.; Hornberger, G.M.; Herman, J.S.; Mills, A.L. Kinetics of BTX biodegradation and mineralization in batch and column systems. *J. Contam. Hydrol.* **1996**, *23*, 113–132. [CrossRef]

18. Weelink, S.A.B.; van Eekert, M.H.A.; Stams, A.J.M. Degradation of BTEX by anaerobic bacteria: Physiology and application. *Rev. Environ. Sci. Bio/Technol.* **2010**, *9*, 359–385. [CrossRef]

19. Aburto-Medina, A.; Ball, A.S. Microorganisms involved in anaerobic benzene degradation. *Ann. Microbiol.* **2014**, *65*, 1201–1213. [CrossRef]

20. Botton, S.; Parson, J.R. Degradation of BTEX compounds under iron-reducing conditions in contaminated aquifer microcosms. *Environ. Toxicol. Chem.* **2006**, *25*, 2630–2638. [CrossRef]

21. Lovley, D.R.; Woodward, J.C.; Chapelle, F.H. Stimulated anoxic biodegradation of aromatic hydrocarbons using Fe(III) ligands. *Nature* **1994**, *370*, 128–131. [CrossRef]

22. Lovley, D.R.; Coates, J.D.; Blunt-Harris, E.L.; Phillips, E.J.P.; Woodward, J.C. Humic substances as electron acceptors for microbial respiration. *Nature* **1996**, *382*, 445–448. [CrossRef]

23. Anderson, R.T.; Rooney-Varga, J.N.; Gaw, C.V.; Lovley, D.R. Anaerobic Benzene Oxidation in the Fe(III) Reduction Zone of Petroleum-Contaminated Aquifers. *Environ. Sci. Technol.* **1998**, *32*, 1222–1229. [CrossRef]

24. Rooney-Varga, J.N.; Anderson, R.T.; Fraga, J.L.; Ringelberg, D.; Lovley, D.R. Microbial Communities Associated with Anaerobic Benzene Degradation in a Petroleum-Contaminated Aquifer. *Appl. Environ. Microbiol.* **1999**, *65*, 3056–3063. [CrossRef] [PubMed]

25. Kunapuli, U.; Griebler, C.; Beller, H.R.; Meckenstock, R.U. Identification of intermediates formed during anaerobic benzene degradation by an iron-reducing enrichment culture. *Environ. Microbiol.* **2008**, *10*, 1703–1712. [CrossRef] [PubMed]

26. A Edwards, E.; E Wills, L.; Reinhard, M.; Grbić-Galić, D. Anaerobic degradation of toluene and xylene by aquifer microorganisms under sulfate-reducing conditions. *Appl. Environ. Microbiol.* **1992**, *58*, 794–800. [CrossRef]

27. Morasch, B.; Schink, B.; Tebbe, C.C.; Meckenstock, R.U. Degradation of o -xylene and m -xylene by a novel sulfate-reducer belonging to the genus Desulfotomaculum. *Arch. Microbiol.* **2004**, *181*, 407–417. [CrossRef]

28. Meckenstock, R.U.; Warthmann, R.J.; Schä¤Fer, W. Inhibition of anaerobic microbial o-xylene degradation by toluene in sulfidogenic sediment columns and pure cultures. *FEMS Microbiol. Ecol.* **2004**, *47*, 381–386. [CrossRef]

29. Rabus, R.; Widdel, F. *Anaerobic Degradation of Ethylbenzene and Other Aromatic Hydrocarbons by New Denitrifying Bacteria*; Springer: Berlin/Heidelberg, Germany, 1995.

30. Fries, M.R.; Zhou, J.; Chee-Sanford, J.; Tiedje, J.M. Isolation, characterization, and distribution of denitrifying toluene degraders from a variety of habitats. *Appl. Environ. Microbiol.* **1994**, *60*, 2802–2810. [CrossRef]

31. Häner, A.; Höhener, P.; Zeyer, J. Degradation of p-xylene by a denitrifying enrichment culture. *Appl. Environ. Microbiol.* **1995**, *61*, 3185–3188. [CrossRef]

32. Jahn, M.K.; Haderlein, S.B.; Meckenstock, R.U. Anaerobic Degradation of Benzene, Toluene, Ethylbenzene, and *o*-Xylene in Sediment-Free Iron-Reducing Enrichment Cultures. *Appl. Environ. Microbiol.* **2005**, *71*, 3355–3358. [CrossRef]

33. Botton, S.; Parsons, J.R. Degradation of BTX by dissimilatory iron-reducing cultures. *Biodegradation* **2006**, *18*, 371–381. [CrossRef] [PubMed]

34. Lovley, D.R. Potential for anaerobic bioremediation of BTEX in petroleum-contaminated aquifers. *J. Ind. Microbiol. Biotechnol.* **1997**, *18*, 75–81. [CrossRef]

35. Foght, J. Anaerobic Biodegradation of Aromatic Hydrocarbons: Pathways and Prospects. *Microb. Physiol.* **2008**, *15*, 93–120. [CrossRef] [PubMed]

36. Mello, J.M.M.; Brandão, H.L.; Valério, A.; de Souza, A.A.U.; de Oliveira, D.; da Silva, A.; de Souza, S.M.A.G.U. Biodegradation of BTEX compounds from petrochemical wastewater: Kinetic and toxicity. *J. Water Process. Eng.* **2019**, *32*, 100914. [CrossRef]

37. Meyer, C.L.; Sun, P.T. Iron-assisted activated sludge for removal of btex and other volatile organics from recovered groundwater. *Proc. Water Environ. Fed.* **2003**, *2003*, 471–490. [CrossRef]

38. Castro, A.R.; Martins, G.; Salvador, A.F.; Cavaleiro, A.J. Iron Compounds in Anaerobic Degradation of Petroleum Hydrocarbons: A Review. *Microorganisms* **2022**, *10*, 2142. [CrossRef]

39. Srivastava, P.; Al-Obaidi, S.A.; Webster, G.; Weightman, A.J.; Sapsford, D.J. Towards passive bioremediation of dye-bearing effluents using hydrous ferric oxide wastes: Mechanisms, products and microbiology. *J. Environ. Manag.* **2022**, *317*, 115332. [CrossRef]

40. Shariati, S.R.P.; Bonakdarpour, B.; Zare, N.; Ashtiani, F.Z. The effect of hydraulic retention time on the performance and fouling characteristics of membrane sequencing batch reactors used for the treatment of synthetic petroleum refinery wastewater. *Bioresour. Technol.* **2011**, *102*, 7692–7699. [CrossRef]

41. Schwertmann, U.; Cornell, R.M. The Iron Oxides. In *Iron Oxides in the Laboratory*; VcH: Weinheim, Germany, 2000; pp. 5–18.

42. Webster, G.; Newberry, C.J.; Fry, J.C.; Weightman, A.J. Assessment of bacterial community structure in the deep sub-seafloor biosphere by 16S rDNA-based techniques: A cautionary tale. *J. Microbiol. Methods* **2003**, *55*, 155–164. [CrossRef]

43. Kozich, J.J.; Westcott, S.L.; Baxter, N.T.; Highlander, S.K.; Schloss, P.D. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Appl. Environ. Microbiol.* **2013**, *79*, 5112–5120. [CrossRef]

44. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Gonzalez Peña, A.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [CrossRef] [PubMed]

45. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410. [CrossRef] [PubMed]

46. Cornell, R.M.; Schwertmann, U. (Eds.) Characterization. In *The Iron Oxides*; VcH: Weinheim, Germany, 2003; pp. 139–183.

47. Barnes, A. *The Rates and Mechanisms of Fe(ll) Oxidation in a Passive Vertical Flow Reactor for the Treatment of Ferruginous Mine Water*; Cardiff University: Cardiff, UK, 2008.

48. Singh, B.; Gräfe, M.; Kaur, N.; Liese, A. Applications of Synchrotron-Based X-Ray Diffraction and X-Ray Absorption Spectroscopy to the Understanding of Poorly Crystalline and Metal-Substituted Iron Oxides. In *Developments in Soil Science*; Elsevier: Amsterdam, The Netherlands, 2010.

49. Da, A. Adsorption from theory to practice. *Adv. Colloid Interface Sci.* **2001**, *93*, 135–224.

50. Gupta, V.K. Suhas Application of low-cost adsorbents for dye removal—A review. *J. Environ. Manag.* **2009**, *90*, 2313–2342. [CrossRef] [PubMed]

51. Foo, K.; Hameed, B. Insights into the modeling of adsorption isotherm systems. *Chem. Eng. J.* **2010**, *156*, 2–10. [CrossRef]

52. Kotoky, R.; Ogawa, N.; Pandey, P. The structure-function relationship of bacterial transcriptional regulators as a target for enhanced biodegradation of aromatic hydrocarbons. *Microbiol. Res.* **2022**, *262*, 127087. [CrossRef]

53. Gibson, D.T. Microbial Transformations of Aromatic Pollutants. In *Aquatic Pollutants*; Elsevier: Amsterdam, The Netherlands, 1978. [CrossRef]

54. Hidalgo, K.J.; Sierra-Garcia, I.N.; Dellagnezze, B.M.; de Oliveira, V.M. Metagenomic Insights Into the Mechanisms for Biodegradation of Polycyclic Aromatic Hydrocarbons in the Oil Supply Chain. *Front. Microbiol.* **2020**, *11*, 561506. [CrossRef]

55. Wu, Z.; Liu, G.; Ji, Y.; Li, P.; Yu, X.; Qiao, W.; Wang, B.; Shi, K.; Liu, W.; Liang, B.; et al. Electron acceptors determine the BTEX degradation capacity of anaerobic microbiota via regulating the microbial community. *Environ. Res.* **2022**, *215*, 114420. [CrossRef]

56. Baedecker, M.J.; Cozzarelli, I.M.; Eganhouse, R.P.; Siegel, D.I.; Bennett, P.C. Crude oil in a shallow sand and gravel aquifer—III. Biogeochemical reactions and mass balance modeling in anoxic groundwater. *Appl. Geochem.* **1993**, *8*, 569–586. [CrossRef]

57. Krumholz, L.R.; Caldwell, M.E.; Suflita, J.M. Biodegradation of 'BTEX' hydrocarbons under anaerobic conditions. In *Bioremediation*; Cambridge University Press: Cambridge, UK, 2009; pp. 61–99. [CrossRef]

58. Carmona, M.; Zamarro, M.T.; Blázquez, B.; Durante-Rodríguez, G.; Juárez, J.F.; Valderrama, J.A.; Barragán, M.J.L.; García, J.L.; Díaz, E. Anaerobic Catabolism of Aromatic Compounds: A Genetic and Genomic View. *Microbiol. Mol. Biol. Rev.* **2009**, *73*, 71–133. [CrossRef]

59. El-Naas, M.H.; Acio, J.A.; El Telib, A.E. Aerobic biodegradation of BTEX: Progresses and Prospects. *J. Environ. Chem. Eng.* **2014**, *2*, 1104–1122. [CrossRef]

60. De Nardi, I.R.; Zaiat, M.; Foresti, E. Kinetics of BTEX degradation in a packed-bed anaerobic reactor. *Biodegradation* **2006**, *18*, 83–90. [CrossRef] [PubMed]

61. Khodaei, K.; Nassery, H.R.; Asadi, M.M.; Mohammadzadeh, H.; Mahmoodlu, M.G. BTEX biodegradation in contaminated groundwater using a novel strain (*Pseudomonas* sp. BTEX-30). *Int. Biodeterior. Biodegrad.* **2017**, *116*, 234–242. [CrossRef]
62. Yadav, J.S.; A Reddy, C. Degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) by the lignin-degrading basidiomycete Phanerochaete chrysosporium. *Appl. Environ. Microbiol.* **1993**, *59*, 756–762. [CrossRef] [PubMed]
63. Kennes, C.; Cox, H.H.J.; Doddema, H.J.; Harder, W. Design and performance of biofilters for the removal of alkylbenzene vapors. *J. Chem. Technol. Biotechnol.* **1996**, *66*, 300–304. [CrossRef]
64. Aizpuru, A.; Malhautier, L.; Roux, J.-C.; Fanlo, J.-L. Biofiltration of a Mixture of Volatile Organic Emissions. *J. Air Waste Manag. Assoc.* **2001**, *51*, 1662–1670. [CrossRef]
65. Gorby, Y.A.; Yanina, S.; McLean, J.S.; Rosso, K.M.; Moyles, D.; Dohnalkova, A.; Beveridge, T.J.; Chang, I.S.; Kim, B.H.; Kim, K.S.; et al. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 11358–11363. [CrossRef]
66. Regberg, A.; Singha, K.; Tien, M.; Picardal, F.; Zheng, Q.; Schieber, J.; Roden, E.; Brantley, S.L. Electrical conductivity as an indicator of iron reduction rates in abiotic and biotic systems. *Water Resour. Res.* **2011**, *47*. [CrossRef]
67. Gusmão, V.R.; Martins, T.H.; Chinalia, F.A.; Sakamoto, I.K.; HenriqueThiemann, O.; Varesche, M.B.A. BTEX and ethanol removal in horizontal-flow anaerobic immobilized biomass reactor, under denitrifying condition. *Process. Biochem.* **2006**, *41*, 1391–1400. [CrossRef]
68. You, Y.; Shim, J.; Cho, C.; Ryu, M.; Shea, P.J.; Kamala-Kannan, S.; Chae, J.; Oh, B. Biodegradation of BTEX mixture by *Pseudomonas putida* YNS1 isolated from oil-contaminated soil. *J. Basic Microbiol.* **2012**, *53*, 469–475. [CrossRef]
69. Hu, Z.-F.; Dou, J.-F.; Liu, X.; Zheng, X.-L.; Deng, D. Anaerobic biodegradation of benzene series compounds by mixed cultures based on optional electronic acceptors. *J. Environ. Sci.* **2007**, *19*, 1049–1054. [CrossRef] [PubMed]
70. Rosenkranz, F.; Cabrol, L.; Carballa, M.; Donoso-Bravo, A.; Cruz, L.; Ruiz-Filippi, G.; Chamy, R.; Lema, J. Relationship between phenol degradation efficiency and microbial community structure in an anaerobic SBR. *Water Res.* **2013**, *47*, 6739–6749. [CrossRef] [PubMed]
71. Alfreider, A.; Vogt, C. Bacterial Diversity and Aerobic Biodegradation Potential in a BTEX-Contaminated Aquifer. *Water, Air, Soil Pollut.* **2007**, *183*, 415–426. [CrossRef]
72. Siddique, T.; Penner, T.; Klassen, J.; Nesbø, C.; Foght, J.M. Microbial Communities Involved in Methane Production from Hydrocarbons in Oil Sands Tailings. *Environ. Sci. Technol.* **2012**, *46*, 9802–9810. [CrossRef]
73. Huang, H.; Jiang, Y.; Zhao, J.; Li, S.; Schulz, S.; Deng, L. BTEX biodegradation is linked to bacterial community assembly patterns in contaminated groundwater ecosystem. *J. Hazard. Mater.* **2021**, *419*, 126205. [CrossRef]
74. van der Zaan, B.M.; Saia, F.T.; Stams, A.J.M.; Plugge, C.M.; de Vos, W.M.; Smidt, H.; Langenhoff, A.A.M.; Gerritse, J. Anaerobic benzene degradation under denitrifying conditions: *Peptococcaceae* as dominant benzene degraders and evidence for a syntrophic process. *Environ. Microbiol.* **2012**, *14*, 1171–1181. [CrossRef]
75. Takahata, Y.; Kasai, Y.; Hoaki, T.; Watanabe, K. Rapid intrinsic biodegradation of benzene, toluene, and xylenes at the boundary of a gasoline-contaminated plume under natural attenuation. *Appl. Microbiol. Biotechnol.* **2006**, *73*, 713–722. [CrossRef]
76. Huang, Y.; Li, L. Biodegradation Characteristics of Naphthalene and Benzene, Toluene, Ethyl Benzene, and Xylene (BTEX) by Bacteria Enriched from Activated Sludge. *Water Environ. Res.* **2014**, *86*, 277–284. [CrossRef]
77. Lueders, T. The ecology of anaerobic degraders of BTEX hydrocarbons in aquifers. *FEMS Microbiol. Ecol.* **2016**, *93*, fiw220. [CrossRef]
78. Prakash, O.; Gihring, T.M.; Dalton, D.D.; Chin, K.-J.; Green, S.J.; Akob, D.M.; Wanger, G.; Kostka, J.E. Geobacter daltonii sp. nov., an Fe(III)- and uranium(VI)-reducing bacterium isolated from a shallow subsurface exposed to mixed heavy metal and hydrocarbon contamination. *Int. J. Syst. Evol. Microbiol.* **2010**, *60*, 546–553. [CrossRef]
79. Coates, J.D.; Ellis, D.J.; Gaw, C.V.; Lovley, D.R. Geothrix ferrnentans gen. nov., sp. nov., a novel Fe(l1l)-reducing bacterium from a hydrocarbon-contaminated aquifer. *Int. J. Syst. Evol. Microbiol.* **1999**, *49*, 1615–1622. [CrossRef] [PubMed]
80. Sperfeld, M.; Rauschenbach, C.; Diekert, G.; Studenik, S. Microbial community of a gasworks aquifer and identification of nitrate-reducing Azoarcus and Georgfuchsia as key players in BTEX degradation. *Water Res.* **2018**, *132*, 146–157. [CrossRef] [PubMed]
81. Park, H.S.; Kim, B.H.; Kim, H.S.; Kim, H.J.; Kim, G.T.; Kim, M.; Chang, I.S.; Park, Y.K.; Chang, H.I. A Novel Electrochemically Active and Fe(III)-reducing Bacterium Phylogenetically Related to Clostridium butyricum Isolated from a Microbial Fuel Cell. *Anaerobe* **2001**, *7*, 297–306. [CrossRef]
82. Castro, L.; García-Balboa, C.; González, F.; Ballester, A.; Blázquez, M.L.; Muñoz, J.A. Effectiveness of anaerobic iron bio-reduction of jarosite and the influence of humic substances. *Hydrometallurgy* **2013**, *131–132*, 29–33. [CrossRef]
83. Wei, M.; Yu, Z.; Zhang, H. Molecular characterization of microbial communities in bioaerosols of a coal mine by 454 pyrosequencing and real-time PCR. *J. Environ. Sci.* **2015**, *30*, 241–251. [CrossRef]
84. Da Silva, M.L.B.; Alvarez, P.J.J. Effects of Ethanol versus MTBE on Benzene, Toluene, Ethylbenzene, and Xylene Natural Attenuation in Aquifer Columns. *J. Environ. Eng.* **2002**, *128*, 862–867. [CrossRef]
85. Morlett-Chávez, J.A.; Ascacio-Martínez, J.; Rivas-Estilla, A.M.; Velázquez-Vadillo, J.F.; Haskins, W.E.; Barrera-Saldaña, H.A.; Acuña-Askar, K. Kinetics of BTEX biodegradation by a microbial consortium acclimatized to unleaded gasoline and bacterial strains isolated from it. *Int. Biodeterior. Biodegrad.* **2010**, *64*, 581–587. [CrossRef]

86. Van der Zee, F.P.; Cervantes, F.J. Impact and application of electron shuttles on the redox (bio)transformation of contaminants: A review. *Biotechnol. Adv.* **2009**, *27*, 256–277. [CrossRef]

87. Ivanov, V. Microbiology of Environmental Engineering Systems. In *Environmental Biotechnology*; Wang, L.K., Ivanov, V., Tay, J.-H., Eds.; Humana Press: Totowa, NJ, USA, 2010; pp. 19–79. [CrossRef]

88. Plumb, J.J.; Bell, J.; Stuckey, D.C. Microbial Populations Associated with Treatment of an Industrial Dye Effluent in an Anaerobic Baffled Reactor. *Appl. Environ. Microbiol.* **2001**, *67*, 3226–3235. [CrossRef]

89. Zhu, Y.; Cao, X.; Cheng, Y.; Zhu, T. Performances and structures of functional microbial communities in the mono azo dye decolorization and mineralization stages. *Chemosphere* **2018**, *210*, 1051–1060. [CrossRef]

90. Smalley, J.W.; Silver, J.; Birss, A.J.; Withnall, R.; Titler, P.J. The haem pigment of the oral anaerobes Prevotella nigrescens and Prevotella intermedia is composed of iron(III) protoporphyrin IX in the monomeric form. *Microbiology* **2003**, *149*, 1711–1718. [CrossRef] [PubMed]

91. Grabowski, A.; Tindall, B.J.; Bardin, V.; Blanchet, D.; Jeanthon, C. Petrimonas sulfuriphila gen. nov., sp. nov., a mesophilic fermentative bacterium isolated from a biodegraded oil reservoir. *Int. J. Syst. Evol. Microbiol.* **2005**, *55*, 1113–1121. [CrossRef] [PubMed]

92. Joshi, G.; Schmidt, R.; Scow, K.M.; Denison, M.S.; Hristova, K.R. Effect of benzene and ethylbenzene on the transcription of methyl-tert-butyl ether degradation genes of Methylibium petroleiphilum PM1. *Microbiology* **2016**, *162*, 1563–1571. [CrossRef] [PubMed]

93. Hristova, K.R.; Schmidt, R.; Chakicherla, A.Y.; Legler, T.C.; Wu, J.; Chain, P.S.; Scow, K.M.; Kane, S.R. Comparative Transcriptome Analysis of *Methylibium petroleiphilum* PM1 Exposed to the Fuel Oxygenates Methyl *tert* -Butyl Ether and Ethanol. *Appl. Environ. Microbiol.* **2007**, *73*, 7347–7357. [CrossRef]