

Article

Enriched Co-Treatment of Pharmaceutical and Acidic Metal-Containing Wastewater with Nano Zero-Valent Iron

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Abstract: Among traditional hazardous waste sources, pharmaceutical-containing wastewater and acidic mine drainage need treatment to preserve the expected water supply quality. A nano zero-valent iron (nZVI)-enriched treatment of these two streams is evaluated for simultaneous removal of various heavy metal ions, organic pollutants, sulfates, the efficiency of the treatment system, and separation of reaction products in the fluidized-bed reactor. The reactor packed with silica sand was inoculated with sludge from an anaerobic digester, then 1–3 g/L of nZVI slurry added to cotreat a hospital feed and acid mine wastewater at 5:2 *v/v*. The biotreatment process is monitored through an oxidation–reduction potential (Eh) for 90 days. The removal pathway for the nZVI used co-precipitation, sorption, and reduction. The removal load for Zn and Mn was approximately 198 mg Zn/g Fe and 207 mg Mn/g Fe, correspondingly; achieving sulfate (removal efficiency of 94% and organic matter i.e., chemical oxygen demand (COD), biological oxygen demand (BOD), dissolved organic carbon (DOC), total dissolved nitrogen (TDN) reduced significantly, but ibuprofen and naproxen achieved 31% and 27% removal, respectively. This enriched cotreatment system exhibited a high reducing condition in the reactor, as confirmed by Eh; hence, the nZVI was dosed only a few times in biotreatment duration, demonstrating a cost-effective system.

Keywords: zero-valent iron; cotreatment; acidic mine drainage; fluidized-bed reactor



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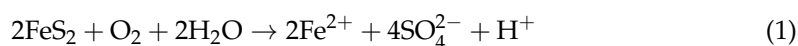
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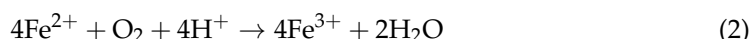
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1. Introduction

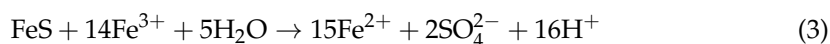
Acidic mine water resulting from the open mine tailings often contains sulfates and a complex mixture of heavy metal ions. These mine tailings are characterized to have a high concentration of sulfide containing minerals, such as pyrite (FeS₂). As a result, pyrite's bio-hydro-geochemical weathering with other sulfide containing minerals in oxidizing conditions produces acid mine drainage [1]. According to Masindi and Muedi [2], the formation of acid mine drainage (AMD) may be represented as:



Through sulfide oxidation to sulfate solubilizes the Fe²⁺ Equation (1), then oxidized to Fe³⁺ Equation (2):



Microorganisms, such as iron-oxidizing bacteria, can be catalyzed by microorganisms that draw energy from oxidation reaction. Then Fe³⁺ produced may also oxidize FeS₂ and also be reduced into Fe²⁺ Equation (3):



Generally, acid mine drainage contains metal concentrations that pose a risk to the environment and is strongly acidic. In South Africa, the mining industry is one of its economy's driving industries [3]; on the other hand, this industry's impact on the environment through the generation of acid mine drainage (AMD) is a call for concern. More so, a crisis in abandoned mining sites is the less attention given to remediate AMD, resulting in the significant environmental problem in South Africa (SA) and globally. AMD remediation for heavy metal removal includes adsorption, biosorption, ion exchange, filtration, precipitation, and stabilization [2].

AMD is not the only challenging water pollutant in SA; pharmaceutical compounds originating from hospital wastewater (HWW) are frequently found in natural water bodies and increasingly become a persistent pollutant requiring attention. Hospital wastewater is among the commonly found effluent in urban water streams, mainly pharmaceutical, toxic chemicals, and pathogens as dominant pollutants [4,5]. Regrettably, all these pollutants are typically pumped to the municipal wastewater treatment plants (WWTTPs), regardless of the call to hold the wastewater on-site for treatment before pumping to WWTTPs [6]. The on-site wastewater retention and prior treatment of HWW may help treat most hazardous pollutants before discharge to the natural environment. The pharmaceutical compounds' chemical and physical properties resulting in their polarity in nature hence resistant to degradation processes in WWTTPs. Hence an urge to find new treatment strategies that are more environmentally friendly and minimize acute health threats from HWW. Noticeably, the regulation on pharmaceuticals disposal or their treatment is not as stringent in emerging economies, including SA, in comparison to developed countries. Hence the current design of wastewater treatment facilities does not bear the capacity to remove pollutants like pharmaceuticals. To date, more methodologies have been investigated to support the integration of growing pharmaceutical pollutants into South Africa's water quality legislation.

A plan to employ a cotreatment strategy for simultaneous remediation of AMD and HWW is proposed after briefly providing the background of these two hazardous pollutants. This proposal is an extension of published studies that cotreated AMD and municipal wastewater, achieving success in reducing metal concentration and sulfates [7–9]. In these studies, municipal wastewater MWW offered carbon substrate bacterial oxidation to reduce the metals, while pathogens are removed from it as well [10]. The hospital wastewater is much richer in nutrients and organic carbon, making it slightly superior to municipal wastewater, hence promises higher alkalinity production. In the studies by Strosnider et al. [9,11,12], where MWW was cotreated with AMD, the organic matter from MWW was used to strip-off bacterial oxygen thus reduction in metal concentration and sulfate. As an alternative, an aerobic process is preferred to remove high strength organic matter. This cotreatment strategy's efficacy is better described in Makhathini et al. [13]; however, the heavy metals such as Pb, As, and Mn were not documented as they experienced a low removal rate in this system. As such, the cotreatment needs to be enhanced to assess its efficacy under the presence of nZVI, specifically to assess the heavy metals.

According to Dong et al. [14], the nZVI promises a beneficial influence on sulfate-reducing bacteria's development and activity. As such, it is expected that the wastewater under hypoxic groundwater environment, the Fe^0 will be oxidized to ferrous iron, thus increasing the pH value of the treatment system through the discharge of OH^- ion [15]. Kumar et al. [16] assert that the alkaline conditions emanating from Fe^0 oxidation are favorable for developing sulfate-reducing bacteria, and hydrogen and Fe^{2+} may act as energy sources SO_4^{2-} -reduction by sulfate-reducing bacteria. Moreover, $\text{Fe}(\text{II})$ has a beneficial effect in hydrogenase synthesis, which is intricate for the sulfate-reducing bacteria (SRB) metabolism process of electron transfer and mass transport [17]. Generally, the SRB systems are known to take a long time for treatment because of lacking the energy source; however, to increase the remediation efficacy, Fe^0 introduction could assist in speeding up the SRB process [17]. Therefore, the SRBs' possibility to gain from interacting with nZVI

for heavy metals' removal and stabilization of precipitates is to be tested in this treatment system.

Notably, the main reason for using fluidized bed reactors for this system rather than packed-bed or anaerobic fixed-bed bioreactors is their superiority in retaining the biomass and achieving large mass transfer with less pressure drop. While anaerobic fixed-bed and packed-bed bioreactors have demonstrated good remediation performances for wastewater [18], yet they are likely susceptible to blockages and channeling, which lessen their efficacy [19]. Furthermore, Sahinkaya et al. [20] and Kaksonen et al. [21] agree that the fluidized bed reactor (FBR) recycling stream decreases concentrations in the feed, thus rendering these bioreactors most fitting for acid mine drainage. However, just like most treatment systems, the cotreatment of two wastewater streams has shortcomings for one effluent's transportation cost to the shared facility [22]. Generally, AMD is formed some distance away from the urban area where hospitals might be situated; perhaps pumping cost may equip them with the cotreatment system's savings. However, some authors have constructed a wetland to treat hospital wastewater [23], as such, with an abundance of literature on wetlands from the treatment of AMD [7,24–26], this gives hope that such cotreatment strategy may be possible to set up.

This work reports on the application of nZVI as an enhancing reagent in the cotreatment of AMD and HWW using fluidized-bed reactors for the removal of heavy metals and toxic pollutants. A 2000 mL FBR was fed with nZVI slurry and operated continuously at a flow rate of 1.5–3.0 m³/h. The nZVI slurry was fed from the top of the column while the effluent's feed was from the bottom, hence the slurry's suspension. The main objective was to assess the treatment process's efficiency while treating the real stream of AMD with a high content of heavy metals and sulfates, together with a real stream of HWW with a high content of organic matter, including the pharmaceuticals. Furthermore, the study seeks to contribute to AMD remediation literature using cotreatment strategies, thus guiding future design.

2. Materials and Methods

2.1. Materials and Initial Experiments

nZVI supplied by Romachem (SA) with a specific surface area recorded as approximately 20 m²/g. Initially, to prepare the nZVI slurry, 250 g of nZVI particles were mixed to 1000 mL of deionized water to check the slurry's consistency. After this, the preparation was done to fill a 5000 mL container at a final *w/w* Fe to water ratio of 0.25 [27]. The Fe⁰ content in the slurry was measured to be 93%, based on the amount of hydrogen gas emitted when iron nanoparticles react at a volumetric volume of 60% with the addition of sulfuric acid [28]. There was no activation procedure investigated to enhance the reactivity of surface nZVI particles before application. The slurry was then continuously stirred at ±20 °C while monitoring electrode potential (E_h) and pH using oxidation–reduction potential and pH electrodes. Later after the E_h of the slurry was observed to have stabilized using the oxidation–reduction. Sampling was done at 60 min intervals, where at least 20 mL samples were withdrawn then filtered using 0.2-micrometer membrane filters.

The AMD samples were collected from an abandoned coal mine in Mpumalanga, South Africa. To investigate the nZVI concentration effect, batch experiments were set up in five 650 mL glass bottles sealed with plastic caps with a 5:2 *v/v* of HWW to AMD mixture (pollutant characteristics listed in Table 1) and an nZVI slurry prepared as the different mass dosage of nZVI between 0.5 and 12 g/L. Each mixture in a batch reactor of AMD and HWW was dosed with a different slurry concentration. An additional two bottles without nZVI were also set up in parallel to the five bottles as control experiments. The batch reactors were agitated at room temperature on a shaker at 280–310 rpm. A selected number of heavy metals like Pb, Zn, and Mn, which were not previously removed, were of interest here; hence they were measured using Inductively Coupled Plasma—Optical Emission Spectrometry ICP-OES (Varian 720-ES). After optimizing the slurry concentration, the slurry concentration was kept between 0.5 and 2.5 g/L. The fresh slurry was only made

twice in the biotreatment duration since there was less demand for it (more details are given in the discussion session).

Table 1. Characterization of the acidic mine water samples.

Parameters	Acidic Mine Water	Hospital Wastewater
pH	±2.1–3.8	±6.2–7.9
COD (mg/L)	±98	±156–1234
PO ₄ ³⁻	±11–31	±9.8–14
NH ₄ ⁺	±34–87	±34–87
Total nitrogen (mg/L)	±21–123	±45–223
Aluminum (mg/L)	±275	<2
Potassium	±34	<1
Iron (mg/L)	±5329	±12
Sodium (mg/L)	<0.05	-
Calcium (mg/L)	±210	<6
Copper (mg/L)	±112	±56
Zinc (mg/L)	±145	±23
Manganese (mg/L)	±113	-
Magnesium (mg/L)	±243	±29
Sulfate (mg/L)	±4900	±134
Turbidity (NTU)	±46–55	±11–52
Conductivity (µS/cm)	>82	>413
Naproxen (µg/L)	-	2.39 ± 0.75
Ibuprofen (µg/L)	-	8.72 ± 0.98

(-) undetectable.

2.2. Fluidized-Bed Reactors

An existing laboratory-scale fluidized bioreactor was converted to an nZVI reactor (Figure 1). The reactor used an effective bed volume of 1400 mL, which was packed with silica sand as a biomass carrier; further details on the reactor specification are discussed on Makhathini [13]. As part of the initialization stage, the bioreactor was on recycle operation for 24 h, and the bed fluidization was kept approximately 15–25% after the biofilm was developed.

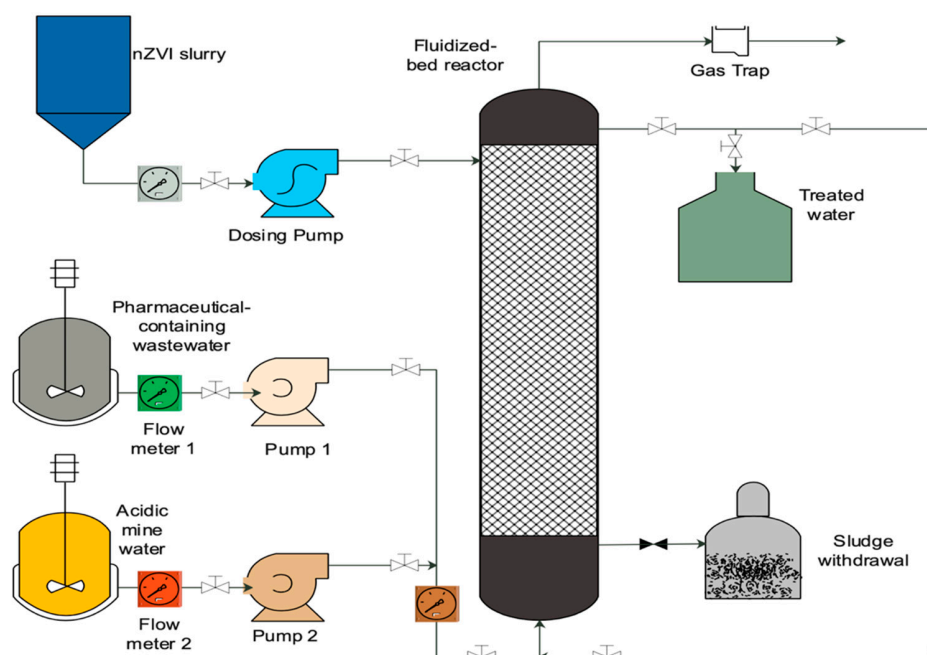


Figure 1. The experimental setup for the fluidized bed reactor.

The hydraulic retention time (HRT) was not rigorously varied since the previous study had given some insight on its effect [13]. This study gradually reduced the HRT from 24 to 16 then later to 8 h while maintaining the feed rate relatively constant. The mixed stream of pump 1 and pump 2 delivery lines were only sampled four times with only a few contaminants measured, for example, COD, pH, Zn, Pb, Mn, sulfates (Table 2). The pharmaceuticals were not measured in the reaction chamber, and the assumption was that there is no change in the initial values from HWW.

Table 2. Measurements of acid mine drainage (AMD) and hospital wastewater (HWW) at the mixing point of 2:5 *v/v*.

Parameter	HWW + AMD
Zinc (mg/L)	138 ± 11.2
Manganese (mg/L)	102 ± 7.8
Lead (mg/L)	114 ± 10.1
SO ₄ ²⁻ (mg/L)	5210 ± 12.3
COD (mg/L)	1523 ± 20.9
pH	±6.8–8.1

AMD and pharmaceutical-containing wastewater composite sample at a ratio of 2:5 was pumped to the reactor; simultaneously, the slurry of nZVI was pumped at 350 mL/min. The strength of reduction circumstance generated by Fe⁰ oxidation may support SRB development, which may already exist by adding highly organic pharmaceutical-containing wastewater.

2.3. Analytical Procedures

All samples collected from the field were filtered with 0.4 µm microfilters before analyses. Using the calibrated PHS-3BW pH meter maintained as recommended by the supplier, the temperature, conductivity, and pH were measured. To specify the alkalinity and acidity of the samples, the auto-titrator Thermo Science Orion Star T900 (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used. The unfiltered samples were analyzed for COD using the APHA [29] standard methods applicable to the Hach DR3900 spectrophotometer (Hach Company, Loveland, CO, USA). For NH₄-N, sulfide, sulfate, and metals, filtered samples were analyzed by APHA [29] standard methods.

A Hach DR3900 spectrophotometer was used to quantify the COD, sulfate, and sulfide. For sulfide measurement, each sample cell was filled with 10 mL of deionized water. Then 10 mL of sample was added to a second sample cell, mixed carefully to prevent sulfide loss. A 0.5 mL sulfide reagent one was pipetted to each sample cell, then swirled carefully. Then 0.5 mL of sulfide reagent two was added to each cell; again, the sample cells were then inverted to mix and then left to react for five minutes. After the time-lapse, the blank cell was used to reset the instrument (DR3900), then the cell with the sample was read.

For COD concentration measurement, the DRB200 (Hach Company, Loveland, CO, USA) was prepared by preheating the temperature to 150 °C. While waiting for the instrument to get ready, a 100 mL sample was homogeneously mixed in a blender for 30–60 s. Using the TNT plus vials, 0.3 mL of sample was pipetted inside then the vial was inverted several times for proper mixing. The vials were then left in a closed preheated DRB200 reactor for 2 h. After the lapse, the vials were taken out of the reactor, cooled to room temperature then inserted in the cell holder to produce a reading. The dissolved organic carbon (DOC) was measured using Hach standards methods.

Each sample cell was filled with 10 mL of sample for sulfate measurements; then, a SulfaVer 4 powder pillow was added to dissolve the powder completely. After this, the cell was left to complete the reaction for five minutes. After the time-lapse, the blank sample cell was inserted in the cell holder of the DR3900 to reset the instrument, and then the prepared sample was inserted in the cell holder to read the results.

The BOD measuring system required 300 mL incubation bottles and a nitrifying inhibitor (N-Allylthiourea). About 40–100 mL volume of sample was added into the incubation bottles, then few drops of ATH are added to the sample, after which the BOD system was placed for five days in an incubator at 20 °C. After five days, the dissolved residual oxygen was measured for all analyzed samples to estimate the BOD.

The total dissolved nitrogen (TDN) measurements were initiated by preheating the DRB200 reactor to 103 °C. A total nitrogen persulfate reagent powder pillow was added to two high range total nitrogen hydroxide digestion reagent vials. After this, 0.5 mL of sample was added to one of the vials, and the other vial, which was 0.5 mL of deionized water, was added. The prepared vials were put in the reactor for 30 min; after time elapsed, vials were cooled down to room temperature before second total nitrogen (TN) reagent A was added. A 3-min reaction took place, then TN reagent B was added, allowing a further 2-min for the reaction. On both the prepared sample and on the blank, a 2 mL TN reagent C was added. A 5-min reaction time was allowed then samples were read on the spectrophotometer.

For all dissolved samples, the pH was kept at <2 using concentrated HNO₃, then stored at 4 °C until use. Syringe filters with a 0.45 µm membrane were used to prepare the samples for metal concentration analysis of Al, Fe, Cu, Mg, Pb, Mn, and Zn before injecting into ICP-OES (Varian 720-ES, Varian Inc., Palo Alto, CA, USA). The analyses were performed in duplicate, as recommended by the USEPA protocol.

2.4. Pharmaceutical Analysis Procedure

Ibuprofen (98%) and naproxen (98%) were purchased from Sigma-Aldrich. In contrast, HPLC-grade ethyl acetate (>99.6%) and HPLC-methanol (>99%) was supplied by Macron Fine Chemicals used in the quantification of pharmaceutical compounds in the wastewater samples. Hydrochloric acid (37%) and hexane (approx. 60%) were purchased from Merck (SA). A flow rate of 1 mL/min was set for the mobile phase at acetonitrile to formic acid ratio of 3:2, v:v.

A solution was made in acetonitrile containing 100 mg/L concentration for the targeted compounds, using the high-performance liquid chromatography (HPLC) (Perkin Elmer, Wellesley, MA, USA) instrument. The detection limit, quantification limit, and linearity limit were determined during this test. The method validation was tested using a deionized water sample mixed with naproxen and ibuprofen at an instant addition of 50 to 5 µg/L concentration, in that order. The concentrations were determined from known values from SA WWTPs. Before each sample quantification, the solid-phase extraction (SPE) method was used to preconcentrate the sample. The method is described, and its results are presented in Section 3.6.

2.5. Sludge Characterization

The sludge samples were prepared for analysis using developed methods [30,31]. The sample was withdrawn at the bottom of the reactor at the end of the treatment period. The sludge was stopped from further reacting by adding 2% glutaraldehyde solution for approximately 15 min. After this, the loose sludge was filtered using a vacuum filter with 0.5 µm pores. The filtered residue was maintained under vacuum at −70 °C for at least three days. Before measuring SEM, the samples were allowed to warm to ambient conditions. Each sample was prepared in triplicate to ensure consistency and to evaluate the reliability of the results. Characterization of the sludge used for immobilizing the biological treatment biomass was carried out [32] a field emission gun (FEG) of the Thermo Fisher Nova NanoSEM (Thermo Fisher Scientific, Waltham, MA, USA). The EDS analysis was performed using the Oxford X-ma detector with Oxford INCA software (version 4.0, Oxford Instruments, Abingdon, UK).

3. Results and Discussion

There are influential factors that may drive metals concentrations and the growth of sulfate-reducing bacteria in the sulfidogenic environment for this cotreatment system with nZVI. As such, the results are presented to assess the optimum reaction conditions and influential factors thereof. These factors, include alkalinity, sulfide, organic ions, are given below.

3.1. Batch Experiments

Since this study continues from the published work of Makhathini et al. [13], there was no need to assess treatment process feasibility. However, nZVI in the biotreatment was to be explored for its possibility in this treatment system. The batch experiments were then conducted to evaluate the nZVI removal viability in the sulfidogenic bioreactor treating AMD and pharmaceutical-containing wastewater. The nZVI was tested for the removal of Pb(II), Mn(II), and Zn (II) ions simultaneously with sulfates as well and was found to be successful in this regard. The removal efficiencies recorded well over 90% of Mn, 92% of Zn, 94% of Pb, and 80% of sulfates in 60 min (Figure 2).

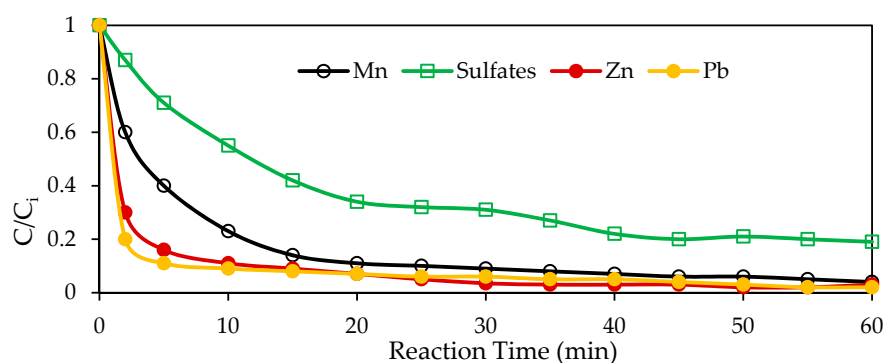


Figure 2. Removal of heavy metals from a mixture of acid mine drainage and pharmaceutical-containing wastewater at an initial pH of 5.6, using nano zero-valent iron (nZVI).

Guo et al., [17] warn that the presence of high nZVI concentration may potentially trigger oxidative stress on bacterial development. In agreement, the study of Kumar et al. [16] stated that when the nZVI dosage exceeded 1.0 g in a liter, the SO_4^{2-} the decline was significantly inhibited. While Velimirovic et al. [33] suggest that the microbial community activity can be hindered if the addition of nZVI concentration exceeds 0.05 g/L. In contrast, Kirschling et al. [34] demonstrate that a sufficient concentration of nZVI could stimulate SRB development, and it is helpful to eliminate pollutants. Since this current study sought to reduce the heavy metals and the sulfates, it is for this reason that the optimization of the nZVI dosage was initiated (Figure 3).

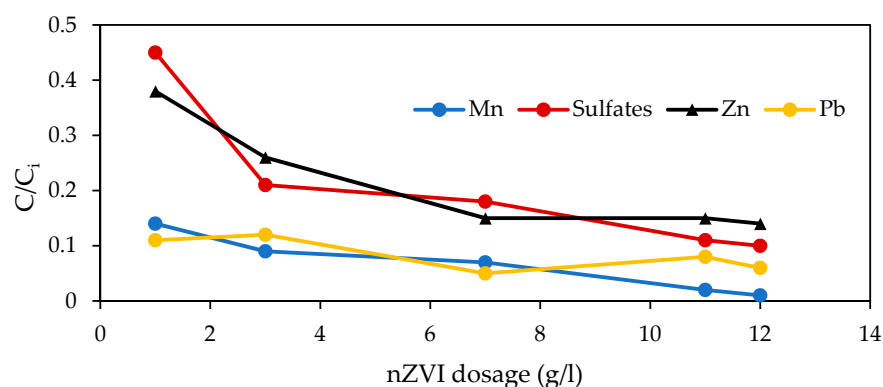


Figure 3. The dosage of nZVI with time testes over 24 h.

3.2. Reaction Mechanism

Organic carbon, mainly from the HWW, serves as the energy source for the sulfate reduction and removing metals from AMD by SRB in the bioremediation process. However, in the nZVI with HWW + AMD treatment configuration, hydrogen and Fe(II) ions originating from Fe⁰ oxidation may also act as an energy source for the sulfate reduction [33] in a shorter time. In a case where heavy metals are involved, as is in this study, several pathways of their removal are suggested by Dong et al. [14] as biosorption, co-precipitation, reductive, and sulfide precipitation (Figure 4).

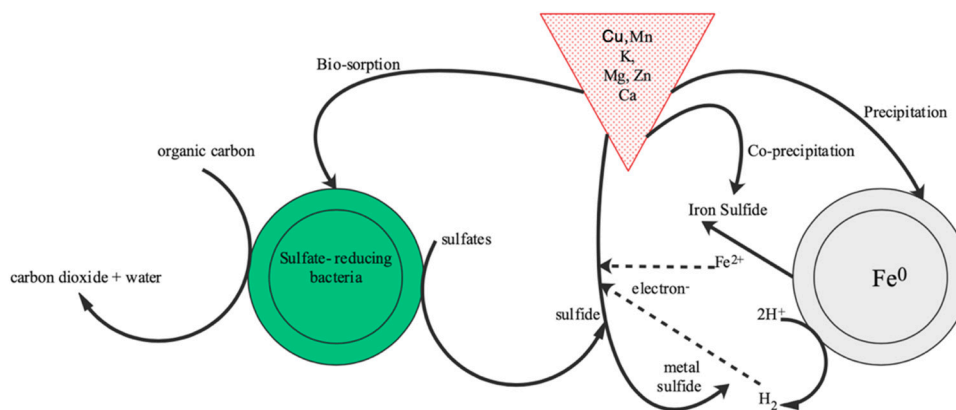
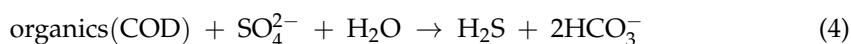


Figure 4. The elimination pathways of heavy metals in the (HWW+AMD)-nZVI system.

The SRB cell membrane and extracellular synthetic polymers will directly touch the heavy metals [35] and adsorb them by physical or chemical adsorption to the nanoparticle membrane [36]. The ferrous ions released from Fe⁰ corrosion may react to produce iron sulfides, which are otherwise relatively effective adsorbents for heavy metals [37]. For the reductive precipitation, as a reducing agent, nZVI reduces certain heavy metals like Pb²⁺, Zn²⁺, and Mn²⁺, then develops insoluble metal precipitates, disintegrate heavy metals, and mitigate environmental hazards [38]. The SO₄²⁻ can also be decreased by SRB metabolism to the sulfide, which may oxidize to the sulfide precipitates with metal ions [39]. Notably, the above removal pathways occur concurrently. However, the nZVI remedial strategy's primary removal pathway is reductive precipitation and adsorption, a critical elimination pathway in the SRB treatment process [40].

3.3. Influence of pH and the Source of Carbon

As mentioned earlier, this study used hospital wastewater as the organic substrate for SO₄²⁻ and microbial growth, basically using the following reaction Equation (4):



COD mass for desulfurization was based on the stoichiometry of 1 g of SO₄²⁻ be reduced, 0.67 g of COD is desired [41]; thus, the alkalinity of approximately 1.042 g is formed. The SRB development is optimal at a pH of 6.5 but still favorable from 5–9 [14]. In this study, the pH was at an average of 6.4, at least for phase II and III. In phase I, the pH was averaging at 6.9; thus, the metal removal was rapid. The addition of Fe⁰ raises the alkalinity value within a turn, improves the activity development of SRB. The response in alkalinity and pH is presented in Figure 5. Moreover, after phase I, which was mainly bacterial acclimation and reaching sorption capacity, from phase II, reducing conditions caused by high SRB start to increase the alkalinity, proving metal sulfide precipitation [42,43].

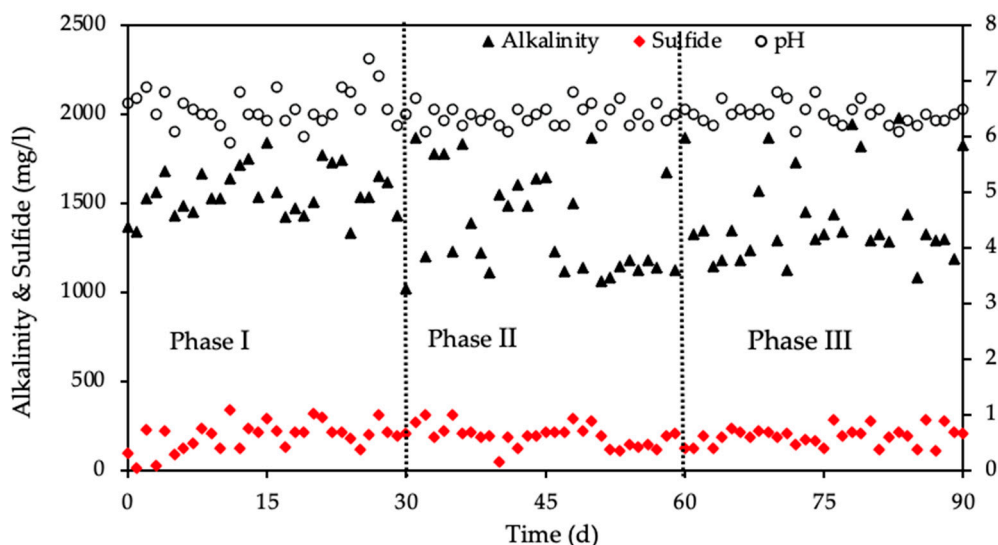
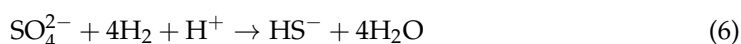


Figure 5. Alkalinity, pH, and sulfide response with time in the fluidized bed reactors.

When Kumar et al. [16] examined the impact of oxidation–reduction potential and the pH on the strength of Zn(II) precipitates in the ZVI and sulfate-reducing bacteria with ZVI systems. They found that column experiments signified Zn precipitates' strength as less sensitive to ORP change but significant sensitivity to pH change. Sulfide concentration was recorded as low as <0.05 mg/L for most of phases II and III. Simultaneously, the alkaline production increased during phases II and III, which could be attributed to a reduction in sulfate concentration.

The initial pH on SO_4^{2-} reduction and final pH indicates that Fe^0 positively affects pH and may increase it to support the SRB activity. In Fe^0 enhanced processes, like this current study, alkalinity may be generated by anoxic corrosion of Fe^0 Equation (5) and SO_4^{2-} Equation (6) since both equations use hydrogen ions.



Nitrogen and organic carbon concentrations were almost certainly influenced by precipitation in phase I of the treatment system. An overall observation of BOD, DOC, and TDN proved a decrease over the first 30 days, phase I (Figure 6). It is most likely that the bacterial SO_4^{2-} reduction facilitated processing of the BOD and DOC [12]. In the FBR, the inorganic nitrogen was introduced from the HWW sample in ammonia form; at the end of phase II, 9–28% NH_4^+ was measured. The DOC concentrations were evidently following the metal concentration decline trend. Nitrate and nitrite were recorded to be reasonably low at ≤ 0.1 mg/L and ≤ 5 g/L, in this order.

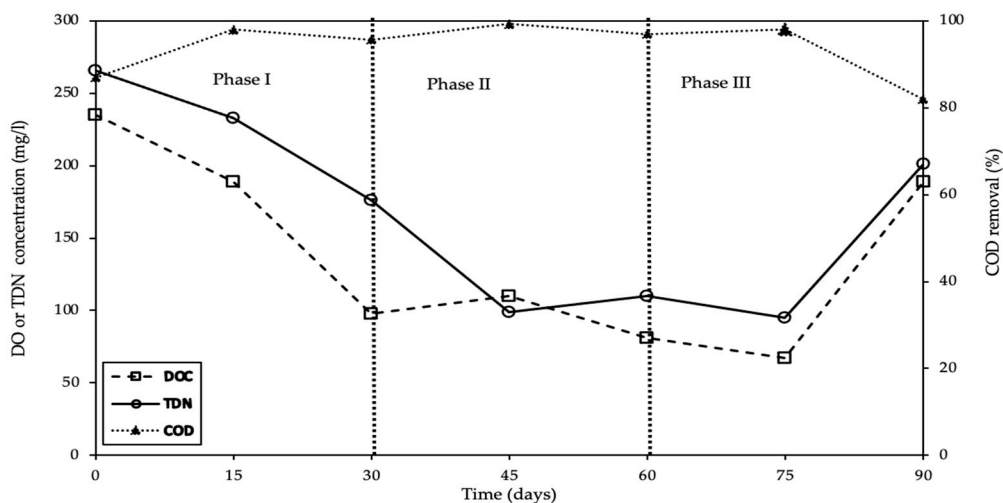


Figure 6. Total dissolved nitrogen (TDN), dissolved organic carbon (DOC) and organic matter (COD) concentrations at different phases of treatment.

3.4. Effects of COD and Sulfates

Throughout the first phase of treatment, there was a substantial reduction of sulfates and COD concentrations (Figure 7). Although the removal of metals and phosphates were high, 88% PO_4^{3-} , sulfate, and other organic matter were moderately reduced. Smyntek et al. [10] highlighted that SO_4^{2-} reduction rates are likely to be affected by establishing the microbial ecosystem or lack thereof. Evidently, during phase I, the FBR was not afforded enough time to develop a microbial ecosystem, which may have resulted in complete SO_4^{2-} reduction [44]. In addition, there is well-documented literature supporting the fact that sulfate reduction is dependent on COD, and the ratio of SO_4^{2-} /COD changes with the type of organics available [45,46].

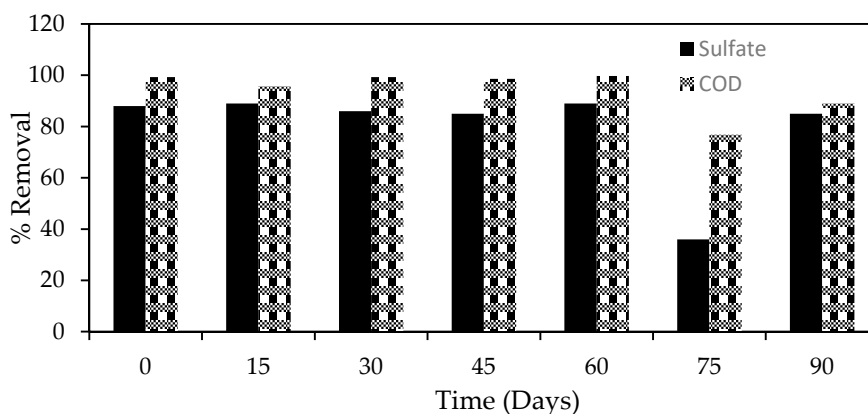


Figure 7. The removal rate for sulfate in comparison to COD.

The second phase of treatment confirmed the bioreactor’s reducing setting was given by redox potential measurements of -101 to -190 mV depending on the COD level on the FBR. Since the hospital wastewater samples had higher total suspended solids (TSS) than mine water samples, 478 and 2 mg/L, respectively, TSS significantly increased in the FBR during phase I. However, in phase II there was a reduction in TSS to the range of 5–24 gm/L. On the contrary, the total dissolved solids (TDS) decreased s phase I due to subsequent phase I formation of precipitates in the FBR. After this, there was no significant reduction of TDS in phase II observed.

3.5. Reactor Monitoring Using E_h

The nZVI was rapidly mixed using the laboratory mixer before its use to reduce conditions by even dispersing the resin in water. As such, the E_h in the FRB reduces below -310 mV instantly after the introduction of nZVI. There was no agitation in the FBR; influent was fed from the bottom, stabilizing fluidization and even dispersion on nZVI in the reactor. The control of the bed volume was carefully controlled by adjusting the influent while optimizing the dosage on nZVI (Figure 2). The E_h of the reactor contents was also used to monitor the FBR treatment performance, making necessary adjustments to nZVI dosage and the influent feed. It was evident that an automatic control strategy to regulate the nZVI and feed pump responding to E_h value could have made the treatment system more stable. This system's manual operation may compromise the reactor's performance, as there are times when the time elapsed without any response from the pumps, whereas the E_h was way below -200 mV. Li et al. [47] advised monitoring redox-potential in the nZVI enhanced reactor promises a quick, consistent, and efficient approach to managing the treatment performance. Consequently, by managing the treatment system, there may be less need for nZVI and, therefore, lower the cost of treatment [48].

3.6. Metal Concentration Removal

Two key factors may decide the toxic effects on metals for a biological treatment process that depends on SRB: the type of metals and their associated concentration. As such, the treatment process's performance is assessed by Fe^{2+} , Zn^{2+} , and Mn^{2+} removal, as highlighted in Table 3. The average concentration of Zn(II) was lowered from 144 to 0.55 mg/L and Mn(II) from 113 to 0.21 mg/L in the final reactor discharge, achieving an average of more than 99.8% removal efficiency. The results suggest that during phase I, the metal removal may be attributed to sorption on organic waste and precipitation of hydroxides and carbonates [49].

Table 3. Metal concentrations removal and standard deviation (SD).

Period	Days		Pb	Cu	Zn	Fe	Ca	Mn
Total metals in influent (mg/L)								
I	0–30	Concentration	33.12	112	421	3112	212	355
		SD	0.11	1.1	3.4	17	1.3	0.1
II	31–60	Concentration	18.1	59	81	589	119	117
		SD	9	0.2	2.1	7	1.2	0.2
III	61–90	Concentration	12	21	44	187	211	45
		SD	4	1.1	1.1	12	1	2.1
Total metals in effluent (mg/L)								
I	0–30	Concentration	12.1	1.02	0.2	11.9	2.1	<0.02
		SD	1.9	0.1	0.01	1.3	0.09	<0.02
II	31–60	Concentration	9.9	1.12	0.7	17	1.8	<0.05
		SD	2.3	0.4	0.3	1.4	0.01	<0.05
III	61–90	Concentration	-	<0.05	<0.05	19	<0.05	<0.05
		SD	-	-	-	1.1	-	-

For the whole of 90 days experimental run, the influent concentration of Mn, Fe, and Zn was spiked up and at times exceeded 350, 5000, and 420 mg/L, respectively. The spike was done deliberately to shock the system for simulation of the field experience. Regardless of these simulated unsteady state conditions, the effluent was consistently achieving less than 2 mg/L concentration for all metals, like K, Na, Ca, and Mg. These results demonstrate the reliability of the treatment process. Table 3 demonstrates that Pb, Mn, and Zn were removed 96%, >80%, and 92%, respectively, in the first treatment phase. This performance may be due to mine water-binding with organic molecules [26,50]. On the other hand, the sulfates were reduced by 70% in phase I, which is lower than the previous studies published for similar FBR application in the absence of nZVI [13]. At the

end of phase III, the increase in Fe could well be due to microbial Fe decrease of FePO_4 and $\text{Fe}(\text{OH})_3$ [12,51]. FeS precipitated due to the sulfate-reducing environment that is dominant in this period [52]. Lastly, metal biosorption to the organic molecules related to the metabolism mechanism could be the reason for metal removal [44,53,54].

Having incorporated nZVI as an enhancement tool for removing a wide variety of contaminants from chemical solutions; however, the current study focused on evaluating material efficiency for the bioremediation process of two real streams. The performance of nZVI for pollution prevention for groundwater depends on bioremediation as a parallel method to hinder metalloids and metal discharge [47]. Nonetheless, reactions that are microbially facilitated with Fe may assist and prevent removal reactions. Like, ferric ion decreasing microorganisms, it may lessen most high-valence metal pollutants, i.e., Cr, over intended enzymatic decrease and through unintended reduction catalyzed by biogenic ferrous ion [55].

3.7. Pharmaceutical Compounds Assessment

The chromatograms generated for the separation of naproxen and ibuprofen compounds are shown in Figure 8. These pharmaceutical compounds were separated using the HPLC column (C18) with the resolution values above 1.4. As described by Madikizela and Chimuka [56], the sensitivity of the method was measured by the limit of quantification (LOQ) and limit of detection (LOD).

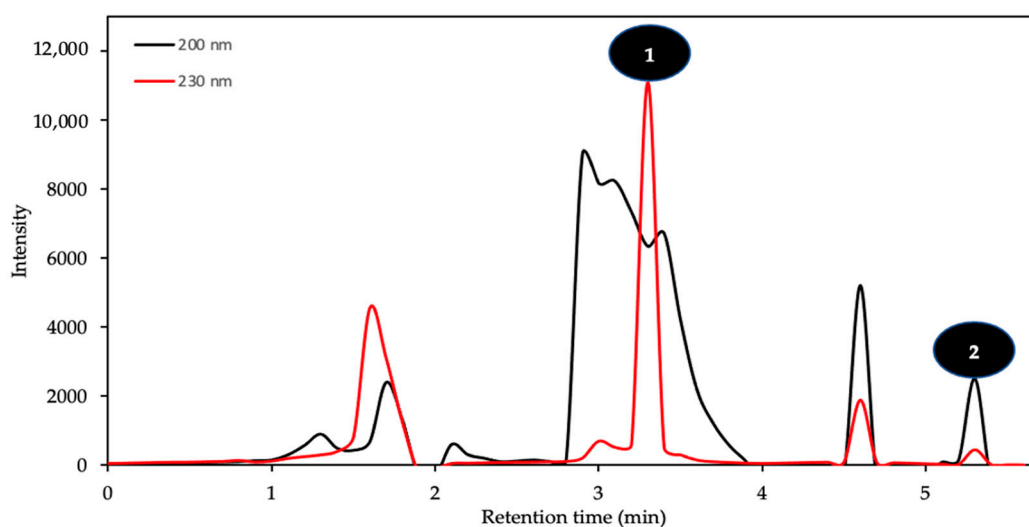


Figure 8. Naproxen (1) and ibuprofen (2) chromatograms $1 \times 10^3 \mu\text{g/L}$ at 200 and 230 nm.

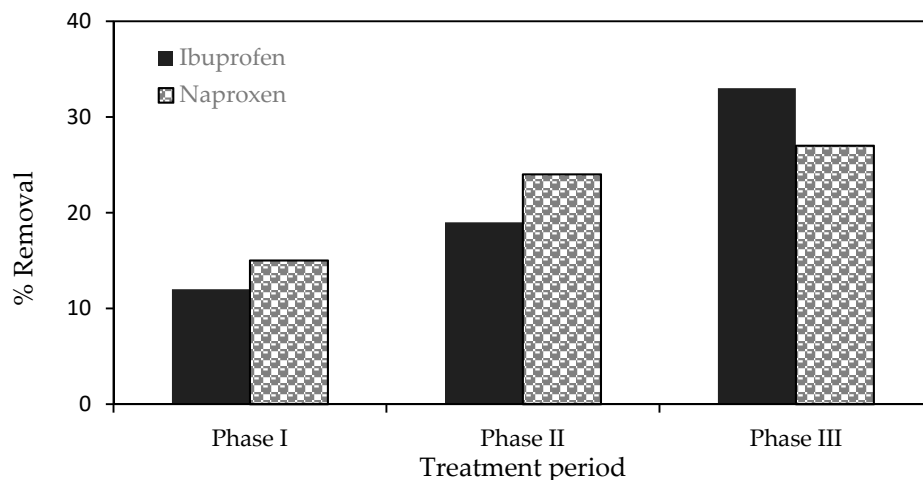
The results shown in Table 4 demonstrate that this method may be applied to target low levels of compounds, i.e., $\mu\text{g L}^{-1}$. Published studies quantified the same compounds using HPLC and diode array detection [56–58]. Solid-phase extraction (SPE) efficiency validated the analytical method, which was higher than 80 percent for naproxen and ibuprofen compounds. The linearity was found to be $r^2 \geq 0.97$, which demonstrates the acceptable method accuracy. The values of the SPE efficiency are given in standard deviation to ensure method precision further. When looking at the SPE production and linearity, the bioreactor effluent is identical to deionized water. Table 4 sets out the results of the validation.

Table 4. Analytical method validation.

Pharmaceutical Matrix	Effluent		Deionized	
	Ibuprofen	Naproxen	Ibuprofen	Naproxen
SPE efficiency (%)	82 ± 3.4	81 ± 8.7	83	84
Detection limit	1.2–1.9	0.2–1.1	0.3	0.2
Quantification limit	0.5–1.4	2.3–3.7	0.6	1.2
r ²	0.98	0.99	0.99	0.99

The pharmaceutical compounds like ibuprofen and naproxen are in the class of non-steroidal anti-inflammatory drugs [5]. These organic compounds are acidic and polar; naproxen and ibuprofen have a pKa value of 4.2 and 4.9, in that order [59]. These compounds' polarity nature makes it easier for them to escape the wastewater process and contaminate the receiving waters.

Pharmaceutical removal was not as successful as was initially anticipated; however, Figure 9 demonstrates a progressive increase in the removal as time lapsed. These results are worse than the previous study on cotreatment of AMD and HWW, but with no nZVI as an enhancing reagent. In this previous study, ibuprofen and naproxen achieved 51% and 34% removal, respectively, at the end of 90 days [13]. In this current work, the maximum removal achieved for ibuprofen is 33%. Again, the main factor for this low removal is not clearly understood yet, and it could be that biomass in the FBR consists of microorganisms that are utilizing pharmaceuticals as organic substrates.

**Figure 9.** The final removal for naproxen and ibuprofen at the end of each treatment period.

3.8. Sludge Management with nZVI

First, degradation efficiency for nZVI from the FBR is assessed by the ratio of heavy metals to the amount of nZVI dosed in the system. The pharmaceuticals removal was not reported here in the sludge as they showed less to no removal within this system. The removal load for Zn and Mn was approximately 144 mg Pb/g Fe, 198 mg Zn/g Fe and 207 mg Mn/g Fe, correspondingly. By the end of the 90 days treatment period, the combined load of metal ions removed for nZVI was estimated >600 mg metals/g Fe, which is higher than other reports on resins usually used for heavy metal separation.

As seen in Figure 10e, an elevated concentration of toxic elements was detected, which shows that nZVI is a flexible material for metal extraction [47]. However, the image of the morphology in the sludge was different than in other studies that used SRB and nZVI [60,61] in that it was not rod-shaped. It was expected that there would be small

particles on the surface of the cells, which are called extracellular biofilms. However, not identifying the extracellular polymeric substance responding to toxic compounds may be due to less availability of microorganisms [62] at the end of the treatment. As such, bacterial biofilms act as nucleation sites for sulfide mineral precipitation [63]; however, in this study, the SEM was only done at the end of phase III (at day 90), which may mean there were no longer available sites for precipitation. In addition, the inadequate removal of naproxen and ibuprofen could be attributed to no available nucleation sites.

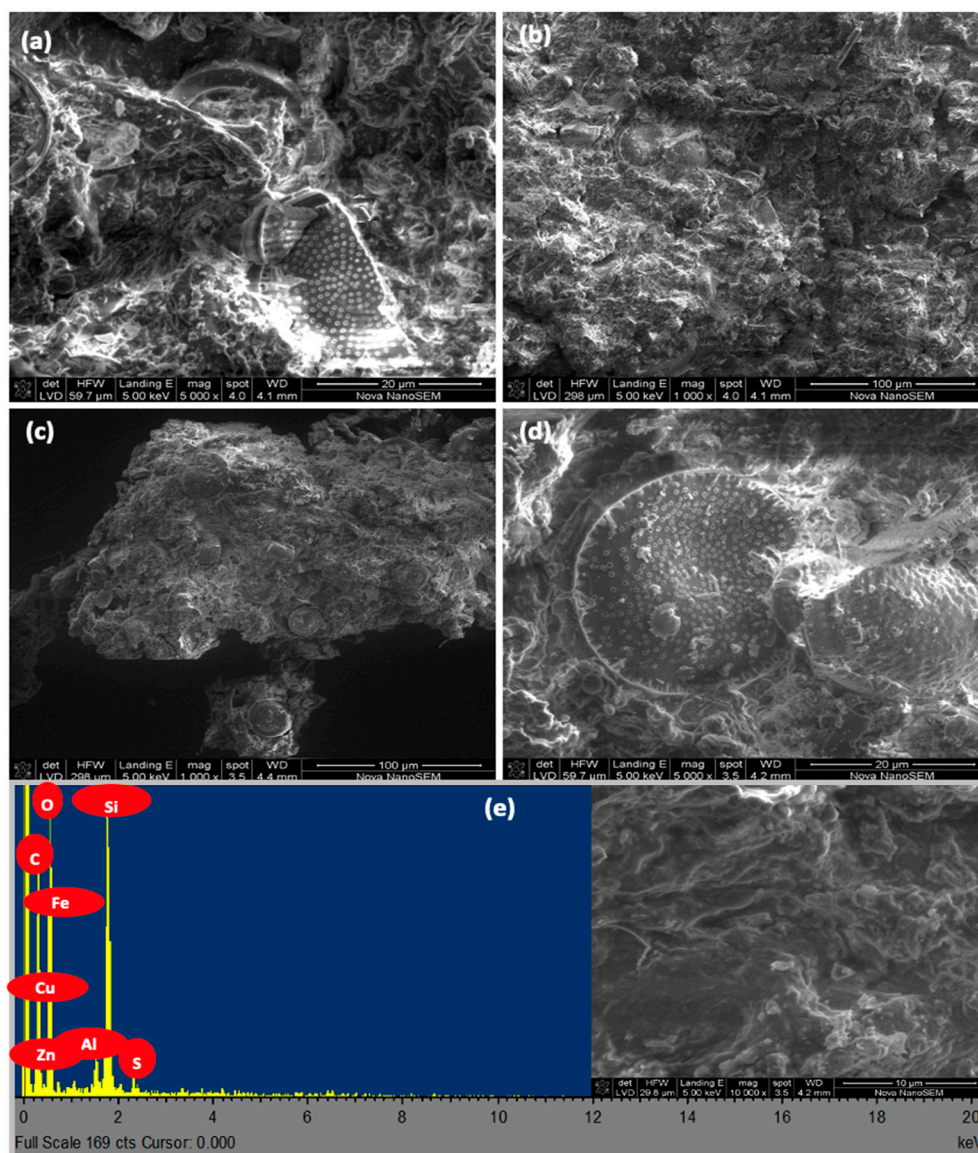


Figure 10. SEM photomicrograph of the sludge sample from the fluidized-bed reactor (FBR) (a,b) at 45 days; (c,d) at the end of 90 days and chemical element spectrum of the sludge (e) with an EDS scan inserted.

Since the study could not assess the solids recirculation of the spent nZVI because of its attachment to the silica sand, the hydraulic retention time was kept between 6 and 8 h. All the initially detected elements in the mine water samples were also found in the sludge (Figure 10). Both SEM and EDS analysis was performed to further characterize the sludge; when performed alone, the EDS has received an unwelcome reputation as a “semiquantitative” technique [31]. Furthermore, Figure 10a,d illustrates the SEM photomicrograph image of the sludge harvested in the FBR at the end of phase III. The EDS qualitative

analysis highlighted signals C, O, Al, Fe, Mn, and Si on the solid phase, in addition to small quantities of Zn in a range of 0.1–1%. These results are slightly different from Kumar et al. [64], who used zero-valent iron to enhance sulfate reduction, but permeable reactive barriers. Silicon originates from the silica sand used as biomass at the initial stage of the treatment.

3.9. General Overview of the nZVI Cost

One of the critical factors when choosing a treatment option after efficacy is the cost. The nZVI specifically is easily re-usable in the treatment process, then the dosage is reduced, which minimizes the consumption of fresh material. The reaction times are shortened, where nZVI is used to enhance the treatment process. Moreover, the process is simple in terms of monitoring since there are a few parameters to control. However, for a developing country like South Africa, this technology is yet to gain traction. It is an active treatment, energy-intensive since the mixing of nZVI powder makes slurry before use. South Africa is still struggling with energy reserves; this method may still be challenging to employ commercially. Even though some researchers assure that the effluent quality may offset the treatment cost [47].

3.10. The Overall Pollution Reduction in AMD

Regarding the treatment system performance, the sulfidogenic fluidized bed reactors removed over 85% of each metal measured in the initial samples before treatment (Figure 11). The iron removal rate was consistent if not superior with other SRB reactors [20,30,65,66], and achieved an average of 97%. At the observed removal rates, the Fe concentrations meet the water quality objectives. The removal of Mn was observed to be 90%, and this result was satisfactory since the manganese retention was evident in the FBR during phase I and phase II of treatment. As observed by Clyde et al. [51], the retention could have resulted from Mn sorption in the FBR. The removal mechanism may also be attributed to MnCO_3 and said not completely to remove manganese from the solution [67]. For copper, the removal rate was 88% in the FBR and may be considered a low removal compared to the other pollutants. However, the results are similar to the bench-scale study using SRB done by Clyde et al. [51] since copper was retained in the FBR throughout the treatment process. In terms of zinc removal, the average was found to be 92%. The result was satisfactory and better than a pilot-scale study done by Jarvis et al. [68]. The Zn result is somewhat pH-dependent; it has been reported that zinc removal increased with elevated pH > 4 [69].

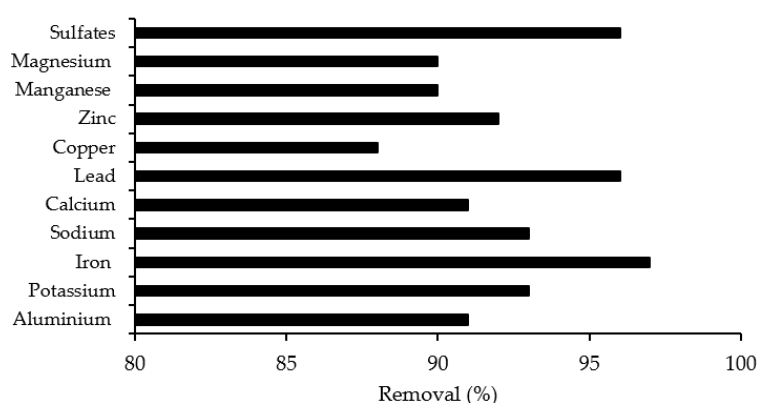


Figure 11. The average percent removal for pollutants, specifically in the FBR, over the treatment period.

Overall, the treatment process observed that the SO_4^{2-} concentration was gradually reducing every day, which was expected as per the study hypothesis. The effluent results showed an average of 96% decrease throughout the treatment process, with an average

area of removal rate of 7 g/m²/d. This system proved to be highly effective with sulfate removal, one of the persistent pollutants in mine water.

4. Conclusions

The degradation of pharmaceuticals in wastewater has recently received considerable attention as a potential solution to the growing demand for strict effluent standards. This work demonstrated nanoscale zero-valent iron's efficacy as an enhancing element for remediating real mine water and pharmaceutical-containing wastewater from a hospital. The laboratory-scale fluidized-bed reactor proved to simultaneously remove metal ions, like Mn and Zn, from wastewater while achieving a significant reduction of SO₄²⁻ and organic matter (COD, BOD, DOC), TDN. In terms of the treatment performance, effluent pH values were generally higher than the FBR influent. The generation of alkalinity corresponding to sulfate reduction in the sulfidogenic reactor demonstrates that SRB could maintain the biological activities. The cotreatment in FBR could be used to treat pharmaceutical wastewater containing nonsteroid inflammatory compounds and COD reduction of over 90%, suggesting biomass had acclimated to the compounds. The overall treatment effectiveness for AMD was found to be at an average of 92.5%. Since the COD degradation is directly affected by the complexity and variability of the hospital wastewater, long HRT in the FBR may lessen the effects. The reactor contents' redox potential was used to monitor the FBR treatment performance, making necessary adjustments to nZVI dosage and the influent feed. For the future, it is recommended that an automatic control strategy to regulate the nZVI and feed pump responding to E_h value be used to make the treatment system more stable. An investigation of the nZVI activation procedure may be necessary to enhance the reactivity of its surface-passivated particles in order to explore pharmaceutical removal in that case further. Finally, building from the work of Laber et al. [23], where they constructed a wetland to treat hospital wastewater, with such rich literature on wetlands from the point of treating AMD [7,24–26], this work contributes to a possibility of amalgamating the two-waste stream in one wetland.

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