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Comparative Study of Nickel Removal from Synthetic Wastewater by a Sulfate-Reducing Bacteria Filter and a Zero Valent Iron—Sulfate-Reducing Bacteria Filter

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ABSTRACT

Nickel removal from wastewater by a zero valent iron sulfate-reducing bacteria (ZVI-SRB) filter and a sulfate-reducing bacteria (SRB) filter were investigated in this study on the basis of the assumption that integrating SRB with the ZVI system should enhance the removal efficiency of heavy metals from solution. The variation in oxidation–reduction potential (ORP) and pH in the effluent indicated that ZVI provided favorable anaerobic conditions for SRB growth. Measurement of sulfate concentrations confirmed that ZVI promoted sulfate-reducing activity of SRB. When the nickel concentration of influent was 150 mg/l with a HRT of 19h, the nickel-removal efficiency was $>$ 98% for the ZVI-SRB filter and 94.3–96.5% for the SRB filter; with a hydraulic retention time (HRT) of 12h, the nickel-removal efficiency was 95.3–97.0% for the ZVI-SRB filter and 84.3–88.2% for the SRB filter, with a HRT of 6h, the nickel-removal efficiency was 94.2–96.5% for the ZVI-filter and 78.2–81.4% for the SRB filter. This result demonstrated that the ZVI-SRB filter had apparent advantages over the SRB filter, especially when filters were run with a shorter HRT. The scanning electron microscope-energy dispersive X-ray analysis (SEM-EDXA) analysis showed that nickel in the ZVI-SRB filter was mainly removed as nickel sulfide and zero valent nickel. This study demonstrated that a ZVI-SRB filter could be a promising technology for treating wastewater containing heavy metals and bioremediation of contaminated groundwater.

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KEYWORDS
Biofilm; bioremediation; sulfate-reducing bacteria

Introduction

Nickel (II) ion is a toxic metal widely present in raw wastewater streams from industries such as non-ferrous metals, mineral processing, paint formulation, electroplating, porcelain enameling, copper sulfate manufacture and steam-electric power plants (Dermentzis 2010; Palmer et al. 2015). In this type of effluent, there is usually a high concentration of nickel ions (Ni^{2+}) (Peng et al. 2014). Exposure to nickel compounds of high concentration can produce a variety of adverse effects on human health including allergies in the form of contact dermatitis, cardiovascular and kidney diseases, and carcinogenic activity (Kasprzak et al. 2003).

Sulfate-reducing bacteria are obligate anaerobes that are notable for their end product, hydrogen sulfide, which is produced from dissimilatory sulfate reduction (Kieu et al. 2011). This biogenically produced sulfide can react with dissolved metals to form insoluble metal sulfides. Bacterial sulfate reduction has been increasingly applied to bioremediation technology for removing heavy metals from wastewaters and groundwater (Chang et al. 2000; Hockin and Gadd 2007; Martinsa et al. 2009; Wang et al. 2013; White et al. 1997; White et al. 1998; White and Gadd 1997; White and Gadd 1998).

A variety of SRB reactors, such as anaerobic filter, fluidized bed, batch reactors, up-flow anaerobic sludge bed, anaerobic baffled reactor, alga-SRB anaerobic pond, were employed to treat wastewaters containing heavy metals, particularly acid mining drainage (AMD). Most studies demonstrated that various reactors using SRB were effective in removing heavy metals (Goncalves et al. 2007; Lenz et al. 2008; Sahinkaya, 2009; Viggi et al. 2010; White et al. 1997; White et al. 1998; White and Gadd 1997; White and Gadd 1998).

Zero valent iron (Fe^0) is a strong reducing agent and is non-toxic and inexpensive. A few studies demonstrated that a ZVI-PRB (Zero valent Iron-Permeable Reactive Barrier) was effective at remediation of groundwater contaminated by a variety of contaminants such as halogen hydrocarbons, uranium and chromium (Bartzas et al. 2006; Moraci and Calabrò 2010). Some studies showed that microbiological processes may play an important role in the long-term performance of zero valent iron (Fe^0)-based permeable reactive barriers (Gu et al. 2002; Qiu et al. 2000).

Conversely, ZVI corrosion depleted dissolved oxygen and produced H_2 and thus provided a reducing environment favorable for many H_2-consuming anaerobic microorganisms, such as sulfate and metal-reducing bacteria, methanogens, and

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denitrifying bacteria, which may stimulate the biotransformation of many redox-sensitive contaminants. The interaction between ZVI and microbes indicates the possibility that combination of a specific type of microbe with ZVI should enhance the removal efficiency of pollutants.

For example, although a high concentration of sulfate in solution can not be directly reduced by ZVI, ZVI can react with water and produce hydrogen, which can be utilized by H_2-utilizing SRB. Consequently, much more H_2S is produced and formation of metal sulfide precipitation is promoted. Some studies have shown that a combination of Fe^0 with microbes significantly enhanced both the rate and extent of biotransformation of many pollutants such as chloroform, carbon tetrachloride and chromium (Bai et al. 2012; Fernandez-Sanchez et al. 2004; Kumar et al. 2015).

Few studies were conducted, however, on removing toxic metals from wastewater or groundwater by combining ZVI with microbes. This study was based on the assumption that ZVI might stimulate growth and sulfate-reducing activity of SRB and combination of ZVI with SRB should thus enhance removal efficiency of heavy metals from solution. The primary objective of this work was to comparatively investigate the removal efficiency of nickel by the ZVI-SRB filter and the SRB filter.

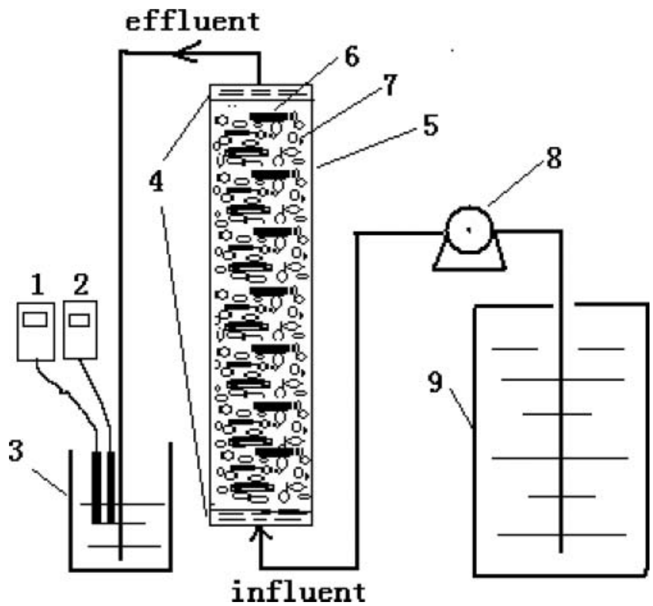

Figure 1. Diagram of the ZVI-SRB filter system (1, pH meter; 2, Redox potential meter; 3, Sampling container; 4, Glass wool; 5, PVC filter; 6, Iron scraps; 7, Sands; 8, Constant flow pump; 9, Nickel-bearing wastewater tank).

Materials and methods

SRB sludge preparation

The mixed SRB population was enriched from activated sludge collected from the anaerobic digester at BeiXiaoHe sewage treatment plant, Beijing. Five grams of anhydrous sodium sulfate was added into a sterile 1-L Schott bottle containing 1 L of anaerobic sludge. The bottle was incubated at 35°C for 7 days. Then 50 ml of sludge was transferred to another sterile 1-L Schott bottle containing 950 ml of autoclaved Postgate's B Medium (Postgate 1984). The SRBs were subcultured 8 times before they were enriched. Light microscopy and scanning electron microscopy (SEM) showed that motile vibrios dominated the population. The presence of SRB was further confirmed by direct observation of the precipitation of black iron sulfide.

Filters

Two PVC tubes were used as filters. The total filter volume was 912 ml (inside diameter 44 mm, height 600 mm) and the working volume was 760 ml. One filter was packed with coarse sand (diameter 2–5 mm) mixed with iron scraps. The ratio of sand to iron scraps was 9:1 by volume. The other filter was used as the control and packed only with sand. The void volume of filter was between 342–380 ml. Cast iron scraps were collected from the machine manufactory at the Institute of Nuclear and New Energy Technology, Tsinghua University, Beijing.

The iron scraps were about 1-mm thick and were further cut into 3×1-cm slices. Iron slices and sands were pretreated by soaking in 10% HNO_3 for 48 h to remove organic material, thoroughly rinsed with deionized water and dried at 60°C before use. The influent was fed in continuously at the bottom of the filter and the effluent port was at the top (Figure 1). The whole filters packed with sand and/or iron scraps were sterilized with 10% sodium hypochlorite solution for 48 h.

Biofilm development

Both sterilized filters were inoculated with 250 ml enriched SRB culture. Sterile Postgate's C Medium (Postgate 1984) was fed in the filters in batch mode. After a period of 4 weeks at 25°C, the biofilm was observed on the surface of the sand. The biofilm was allowed to develop for a further 4 weeks in batch mode.

Composition of synthetic wastewater

The chemical composition of synthetic wastewater is shown in Table 1, with lactate serving as the organic carbon source for growth. Lactate at a high concentration was used as the electron donor for sulfate reduction because lactate is an ideal nutrition for SRB and would keep the SRB biofilm in good condition (Kaksonen et al. 2006; Postgate 1984). All chemicals used were analytical grade. Synthetic wastewater was prepared with deionized water. The final Ni(II) concentration was 150 mg/L and the SO_4^{2-} concentration was 3065.6 mg/L. The pH value of synthetic wastewater was adjusted to 7.5 with 0.1M HCl and 0.1M NaOH.

Table 1. Chemical composition of synthetic wastewater.

Component	Concentration g/l
KH_2PO_4	0.05
NH_4Cl	1.0
Na_2SO_4	4.5
$CaCl_2$	0.06.
$MgSO_4 \cdot 7H_2O$	0.06
Lactate acid	6.0
$Na_3C_6H_5O_7 \cdot 2H_2O$	0.3
$NiCl_2 \cdot 6H_2O$	0.61

Operation of filters

Continuous flow was started 8 weeks after inoculation with the enriched SRB culture. The nickel-bearing synthetic wastewater was fed in at a constant flow rate. The filters were operated at 25°C for 90 days including three different hydraulic retention times (HRT) every 30 days, 19 h, 12 h and 6 h, in 3 different months. Then 5-ml water samples were periodically collected from the effluent. The concentrations of Ni(II), SO_4^{2-}, pH, ORP, and dissolved sulfide were measured. Chemical oxygen demand (COD) was not be a reliable parameter for assessing the performance of the filters in this study because COD measurement would inevitably be interfered with by the presence of dissolved sulfide.

Analytical methods

Sulfate in the effluent was measured using a DX-100 DIONEX ion chromatograph (IC). All samples were prefiltered using 0.22-μm-pore size syringe-tip filters. Ferrous ions and Ni(II) in the effluent were measured using atomic absorption spectrophotometry (Vario 6 AAS, Jena, German). ORP and pH were measured with an ORP/pH meter.

Scanning electron microscope-energy dispersive X-ray analysis (SEM-EDXA) study

At the end of the experiments, sand samples of both filters were collected under N_2 purging and immediately dried with acetone to avoid oxidation prior to sputter coating with carbon. A CSM-950 scanning electron microscope (Opton Corporation, German) and EDXA (Opton Corporation, German) were used to analyze element composition of the precipitation found on the surface of sand in the ZVI-SRB filter.

Results and discussion

ORP and pH of effluent

Figure 2 shows that both filters operated under strict anaerobic conditions during the 90 days' operating term. When the filters ran with a HRT of 19 h, the redox potential of the ZVI-SRB fitlter was below –200 mV and the ORP of the SRB filter was between −170 to −200 mV. The ORP of the SRB filter was slightly higher during the first few days and this can be attributed to a higher ORP of influent without purging of N_2. As the SRB filter ran, ORP decreased gradually and values remained between –180 to −200 mV. In comparison, the ORP of the ZVI-SRB filter rapidly dropped to −210 mV after 8 h and maintained this level during the first 30-day operation period indicating that ZVI facilitates the filter to reach ideal anaerobic conditions within a shorter time.

As the HRT decreased, the ORP of the ZVI-SRB filter increased to –196 to −204 mV at a HRT of 12 h and –192 mV to −204 mV at a HRT of 6 h. Similarly, the ORP of the SRB filter also increased to –169 to −171 mV at a HRT of 12 h and –159 to −166 mV, respectively. The large difference in ORP between the ZVI-SRB filter and the SRB filter indicated that ZVI created stricter anaerobic conditions that were more favorable SRB. Gandhi et al. (2002) reported similar results. The control column (packed with glass beads and without ZVI) exhibited an ORP of ±10 mV whereas the ZVI columns had a

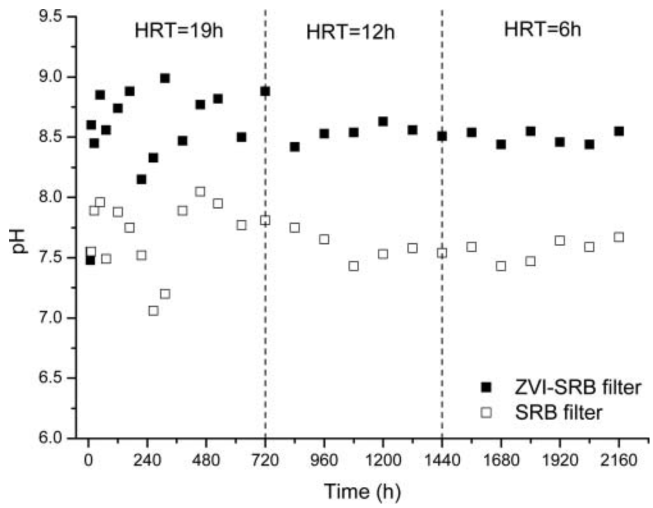

Figure 3. Variation in pH of effluent.

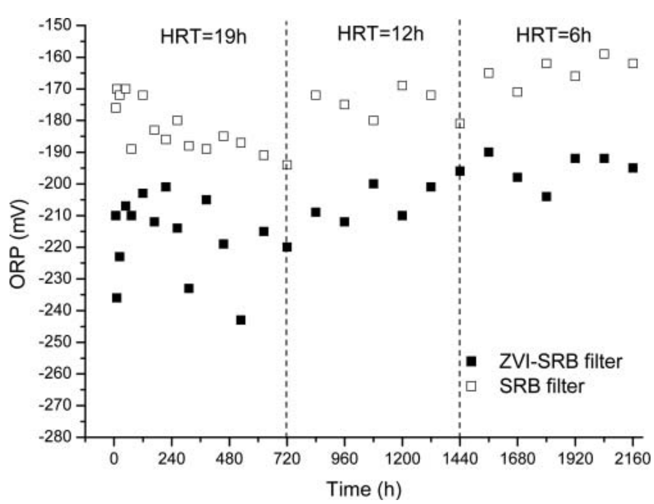

Figure 2. Variation in ORP of effluent.

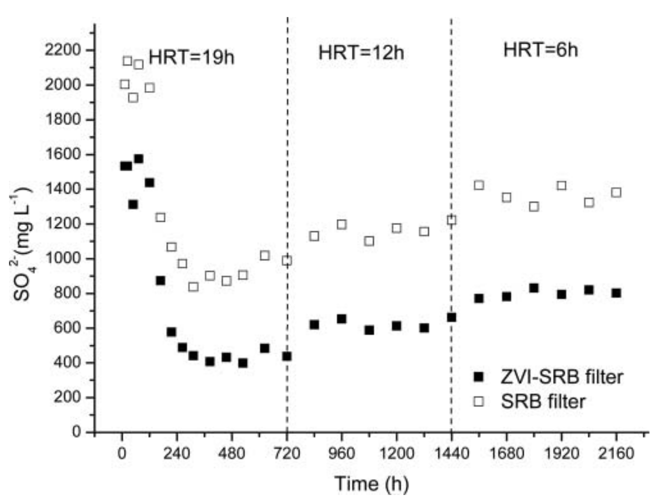

Figure 4. SO_4^{2-} concentration of effluent with time.

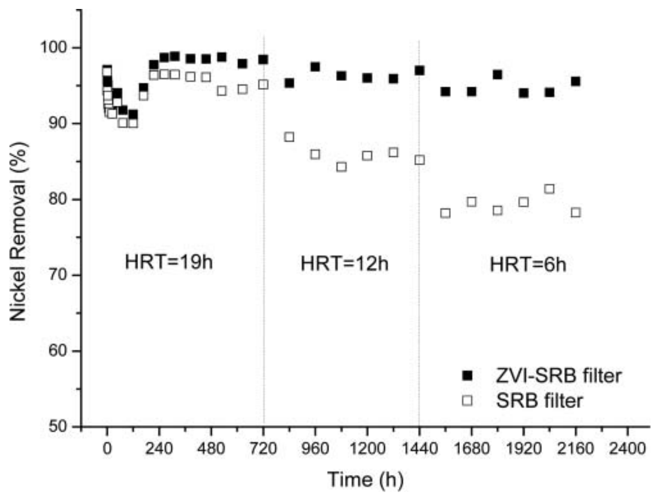

Figure 5. Nickel-removal efficiency.

low ORP below −200 mV. Gu et al. (2002) held that the strongly reducing environment was a result of rapid depletion of dissolved oxygen by ZVI corrosion.

The pH values of effluent of ZVI-SRB filter were higher than that of the SRB filter during the whole operation time. The pH values of the ZVI-SRB filter were about 8.5 at most times. In contrast, the pH values of the SRB filter were 7.5–8.0 (Figure 3). The higher pH values of the effluent from the ZVI-SRB filter could be explained by H_2O being reduced by ZVI as H_2, and OH^- was released and thus the pH increased (Equation 1):

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + 2OH^- + H_2. \qquad [1]$$

Sulfate reduction

The ZVI-SRB filter had much higher sulfate-reducing activity than the SRB filter regardless of HRT (Figure 4). At the beginning of operation (0–120 h) with a HRT of 19 h, sulfate was poorly reduced and the average sulfate-reduction efficiencies of ZVI-SRB filter and SRB filter were about 50% and 35%, respectively. At the late stage of operation (60 d–90 d), the sulfate concentrations in the effluent of the ZVI-SRB filter and the SRB filter decreased from 3065.6 mg/l of influent to 422.4–488.9 mg/l and 802.5–1018.1 mg/l, respectively. The average sulfate-reduction efficiencies of the ZVI-SRB filter and the SRB filter were 87.7% and 75.3%, respectively.

This could be explained by nickel having an inhibitory effect on sulfate reduction by the mixed SRB population when the nickel concentration was up to 150 mg/L. As the filters continued to run, the number of SRB that could not tolerate nickel the toxicity decreased gradually with tolerant cells coming predominant (White et al. 1997) and thus sulfate-reducing activity increased. The results also showed that sulfate-reducing activity increased as the HRT decreased. For the ZVI-SRB filter, sulfate-reducing efficiency decreased from 84.1–86.2% to 78.4–80.4% and to 72.9–74.8% as the HRT decreased from 19 h to 12 h and to 6 h. Similarly, the sulfate-reducing efficiency of the SRB filter also decreased from 66.8–73.8% to 60.1–64.1% and to 53.6–57.6% as the HRT decreased from 19 h to 12 h and to 6 h.

The average sulfate-reducing efficiency of the ZVI-SRB filter was 10.3–21.2% higher than that of the SRB filter during the whole operation time. Because sulfate reduction is considered primarily as a microbiologically-mediated reduction process (Kaksonen et al. 2006) and there is little or no direct evidence showing an abiotic reduction of sulfate by ZVI so far (Gu et al. 2002), the much higher sulfate-reducing activity in the ZVI-SRB filter could be attributed to the stimulation of growth and sulfate-reducing activity of SRB by ZVI. ZVI is a strong reducing agent ($E^0 = -0.44$ V) and can provide more favorable anaerobic condition (below −192 mV in this study).

In addition, production of cathodic H_2 by ZVI corrosions (Equation 1) is favorable to many H_2-consuming anaerobic microorganisms including sulfate-reducing bacteria (Gu et al. 2002). Many SRB have a great affinity for H_2 being an efficient energy source (Muyzer and Stams 2008). The microbial population was able to adapt to the strongly reducing ZVI environment that resulted in an increased sulfate reduction over time in a laboratory column flow-through experiment using simulated groundwater (Gu et al. 1999; Kumar et al. 2014). A few

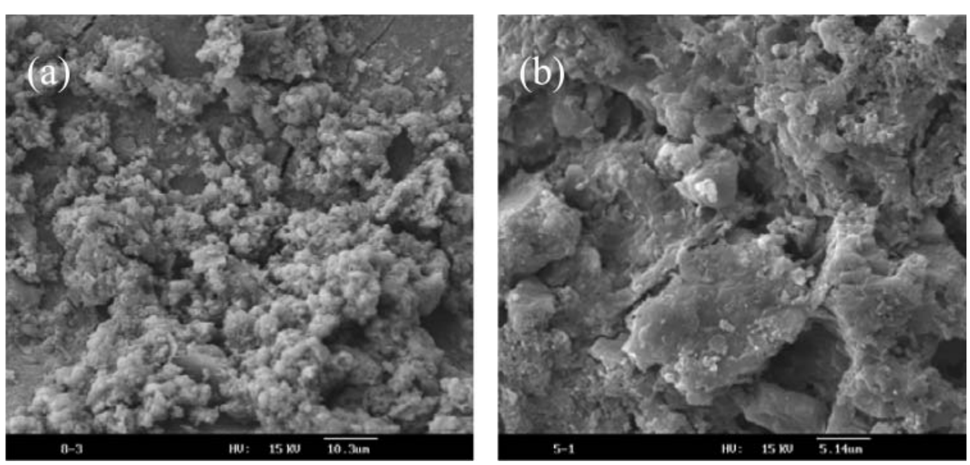

Figure 6. SEM micrographs of precipitation on the surface of sand particles in (a) the ZVI-SRB filter and (b) the SRB filter.

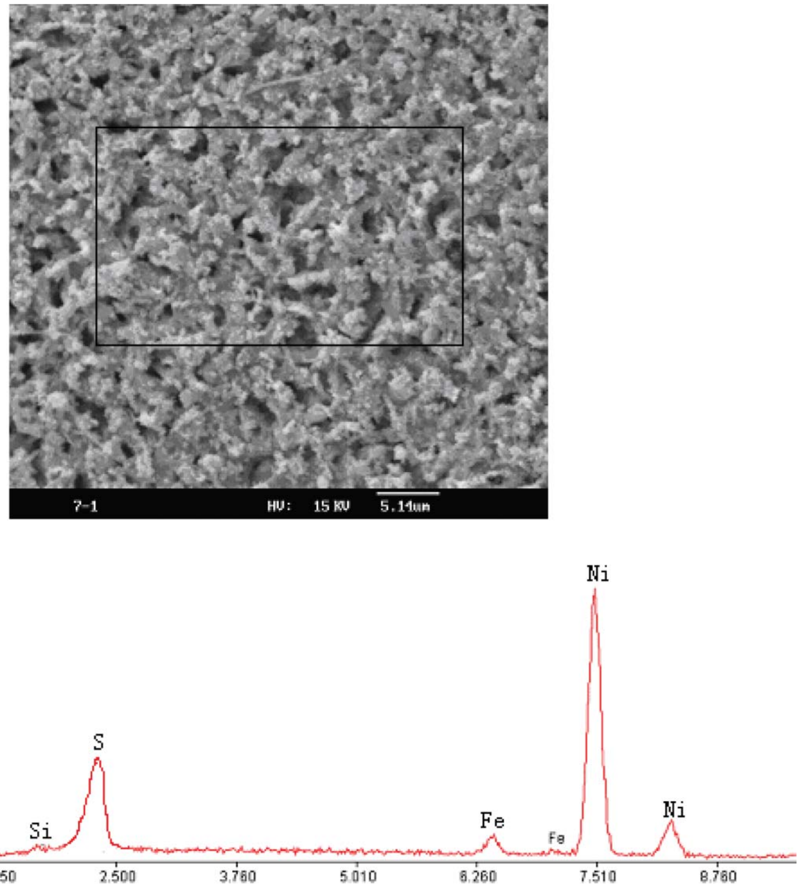

Figure 7. SEM-EDXA analysis of the off-white precipitation in the ZVI-SRB filter.

studies also showed significant decrease of sulfate concentrations in the reducing zone of a ZVI barrier and increased the level of sulfide and sulfide-mineral precipitates as a result of enhanced microbial reduction of sulfate to sulfide (Bai et al. 2012; Gu et al. 1999; Kumar et al. 2015; Phillips et al. 2000).

Nickel removal

When ran with a HRT of 19 h, both filters were effective in removing nickel from synthetic wastewater and their removed efficiencies were more than 90% (Figure 5). However the removal efficiency of the ZVI-SRB filter was on average 1–2% higher than that of the SRB filter. At the beginning of operation (0-8h), the removal efficiencies of the ZVI-SRB filter and SRB filter dropped rapidly from 97.1% and 96.9% to 93.3% and 92.0%, respectively. Nickel-removal efficiencies of the ZVI-SRB and SRB filter continued to decrease from 8–120 h and maintained at a level of 91.2–94.1% and 90.0–92.8%, respectively. In the subsequent 96 h, removal efficiencies of the ZVI-SRB filter and SRB filter recovered and increased from 91.2% and 90.0% at 120h to 97.7% and 96.4% at 216 h, respectively. After 11 days operation, both the ZVI-SRB filter and the SRB filter ran steadily and their nickel-removal efficiencies were more than 98% and 94.3–96.5%, respectively. The higher removal efficiencies at the beginning of operation (0–8 h) and subsequent decrease was attributed to sorption of Ni^{2+} by the SRB biofilm on the sand and ZVI. As filters ran, a sorption equilibrium was gradually attained.

After equilibrium was reached, nickel was removed mainly by forming sulfide precipitation, which depended on the sulfate-reducing activity of SRB. The lower removal efficiencies of both filters from 8–120 h showed that nickel at a concentration of 150 mg/L was clearly toxic to SRB, sulfate-reducing activity

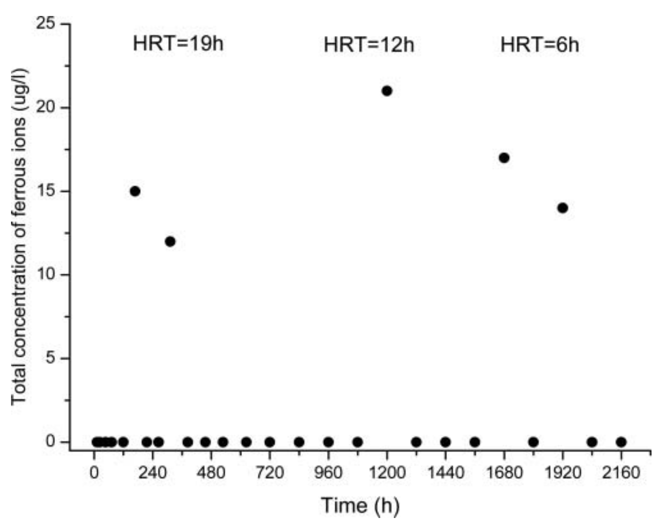

Figure 8. Ferrous ion concentration in effluents of the ZVI-SRB filter.

was greatly inhibited, and the average sulfate-reducing efficiencies of the ZVI-SRB filter and the SRB filter were nearly 50% and 35%, respectively. After about 120 h, sulfate-reducing efficiencies of the ZVI-SRB filter and SRB filter increased to 87.7% and 75.3%. The nickel-removal efficiencies of both filters correspondently increased.

As the HRT decreased, the ZVI-SRB filter demonstrated apparent advantages over the SRB filter in nickel removal. For the SRB filter, the nickel-removal efficiency decreased significantly as the HRT decreased. The nickel-removal efficiencies decreased from 94.3%–96.5% to 84.3%–88.2% and to 78.2%–81.4% as HRT decreased from 19 h to 12 h and 6 h. Comparatively, the nickel-removal efficiencies of the ZVI-SRB filter decreased slightly as the HRT decreased. The nickel-removal efficiency was still over 94% even when the HRT decreased to 6 h.

SEM-EDXA analysis

At the end of the operation, a substantive off-white precipitation was observed in the upper part of the ZVI-SRB filter and on the surface of the iron scraps but not found in the SRB filter. Figure 6 shows that much more precipitation was found in the ZVI-SRB filter than in the SRB filter. SEM-EDXA analysis (Figure 7) showed that the chemical composition of the off-white precipitation in the ZVI-SRB filter was mainly NiS, Ni^0 and iron sulfides. The zero valent nickel formed might be a reduction product of Ni^{2+} by ZVI. Iron sulfides found in the precipitation may be because a very low concentration of Fe^{2+} was occasionally detected in the effluent of the the ZVI-SRB filter (Figure 8). In the ZVI-SRB filter, nearly all of the Fe^{2+} produced by ZVI reacted with sulfide and formed ferrous sulfide. Similarly, sulfide mineral precipitation, such as FeS and mackinawite (Fe_9S_8), were also reported in laboratory ZVI columns after addition of a mixed microbial inoculum (Gu et al. 1999; Kaksonen et al. 2006).

Conclusions

The ZVI-SRB filter had apparent advantages over the SRB filter for nickel removal from synthetic wastewater. The presence of ZVI created a more favorable anaerobic environment for SRB growth and enhanced sulfate-reducing activity. The ZVI-SRB filter had about 10.3–21.2% higher sulfate-reducing activity than that of the SRB filter. When the nickel concentration of the influent was 150 mg/l with a HRT of 19 h, the nickel-removal efficiency was > 98% for the ZVI-SRB filter and 94.3–96.5% for the SRB filter; with a HRT of 12 h, the nickel-removal efficiency was 95.3–97.0% for the ZVI-SRB filter and 84.3–88.2% for the SRB filter; with a HRT of 6 h, the nickel-removal efficiency was 94.2–96.5% the ZVI-filter and 78.2–81.4% for the SRB filter. The SEM-EDXA analysis showed that nickel in the ZVI-SRB filter was mainly removed as nickel sulfide and zero valent nickel. This study demonstrated that a ZVI-SRB biofilm filter could be a promising technology for treating waters containing heavy metals.

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