JOURNAL OF
ENVIRONMENTAL
SCIENCES

ELSEVIER

Science Press

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Reductive transformation and detoxification mechanism of 2,4-dinitrochlorobenzene in combined zero valent iron and anaerobic-aerobic process

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Abstract

A combined zero valent iron (ZVI) and anaerobic-aerobic process was adopted for the treatment of 2,4-dinitrochlorobenzene (DNCB)-containing wastewater. The transformation pathway, reduction of acute toxicity and enhancement of biodegradability were investigated. After pretreatment by ZVI, DNCB in wastewater could be completely converted into 2,4-diaminochlorobenzene (DACB). The ratio of BOD_5/COD increased from 0.005 ± 0.001 to 0.168 ± 0.007, while $EC_{50, 48 hr}$ (V/V) increased from 0.65% to 5.20%, indicating the enhancement of biodegradability and reduction of acute toxicity with the pretreatment by ZVI. DACB was further dechlorinated to m-phenylenediamine during the anaerobic process using methanol as electron donor, with $EC_{50, 48 hr}$ increasing from 5.20% to 48.2%. After the subsequent anaerobic-aerobic process, m-phenylenediamine was degraded completely, with effluent COD of 67.5 ± 10.8 mg/L. This effluent of the subsequent anaerobic-aerobic process was not toxic to zebrafish. The combined ZVI and anaerobic-aerobic process offers bright prospects for the treatment of chlorinated nitroaromatic compound-containing wastewater.

Key words: 2,4-dinitrochlorobenzene; zero-valent iron; toxicity; biodegradability

DOI: 10.1016/S1001-0742(11)61054-1

Introduction

Chlorinated nitroaromatic compounds are widespread in the environment due to the manufacturing and processing of a variety of industrial products, such as pharmaceuticals, explosives, dyes and pesticides (Lin et al., 2011; Liu et al., 2011). They are of interest to environmental scientists because they are known or suspected to be human carcinogens, mutagens or toxins (Lin et al., 2011; Shen et al., 2008). In addition, they have been listed as a class of priority environmental pollutants. Therefore, there is interest in removing them from contaminated sites.

Many treatment technologies such as photocatalysis (Ye et al., 2010; Zhang et al., 2006), adsorption (Guo et al., 2009), ozonation (Shen et al., 2008), and biological treatment (Niu et al., 2009; Wu et al., 2009) have been developed to remove chlorinated nitroaromatic compounds from contaminated environments. Physico-chemical methods have proven to be costly and have inherent drawbacks due to the tendency for the formation of secondary toxic materials. Biological treatment, which is environmentally friendly and cost-effective, has turned

out to be a favorable alternative (Zhang et al., 2009; Shen et al., 2009). However, due to the pronounced electron-withdrawing character of the nitro group and chloro group, chlorinated nitroaromatic compounds, such as 2,4-dinitrochlorobenzene (DNCB), harbor a highly electron deficient π-electron system (Lin et al., 2011). Electrophilic attack, which is usually the first step in oxidation or aerobic biodegradation, becomes more difficult (Zhang et al., 2009). What's more, with increasing numbers of nitro and chloro groups, chlorinated nitroaromatic compounds become more resistant to mineralize through oxidative pathways and are subject to initial reductive transformation (Shen et al., 2009; Susarla et al., 1998).

In order to develop a cost-effective wastewater treatment system for chlorinated nitroaromatic compounds, studies have examined biological or chemical processes to reductively transform the nitro functional groups or dechlorinate the compounds to overcome the hindrance to oxidation (Barreto-Rodrigues et al., 2009). Among the reductive transformation processes, anaerobic reduction and zero-valent iron reduction have turned out to be promising.

Under anaerobic conditions, some anaerobic microorganisms are able to reduce chlorinated nitroaromatic compounds into their corresponding chlorinated aminoaro-

matic compounds. However, the process is usually very slow and requires an electron donor (organic cosubstrate) to create the necessary reductive conditions (Mu et al., 2009). The competition for electron donors by other microorganisms may upset the enrichment of the nitro-reducing population and the nitro reduction of chlorinated nitroaromatic compounds (Lin et al., 2011; Häggblom et al., 2000; Li et al., 2008). Therefore, in this process, an additional organic cosubstrate is often required as an electron donor, in amounts which may far exceed the stoichiometric requirements (Mu et al., 2009; Le et al., 2011).

Zero-valent iron (ZVI), as an inexpensive, reliable and moderately strong reducing agent, has been widely used to treat many kinds of environmental contaminants, such as halogenated organic compounds, azo dyes, and nitroaromatics (Lin et al., 2011; Fan et al., 2010; Arnold and Roberts, 2000; Shu et al., 2010; Ma and Zhang, 2008). An assessment of the literature on zero-valent iron reduction shows a predominance of articles with the focus of interest mainly on the preparation of nanoscale zero-valent iron (nZVI) and the reductive pathway. However, to date, the application of nZVI process on a large scale in wastewater treatment is rare, and articles with technological orienta-tion are in the minority (Ma and Zhang, 2008). There are still some properties of nZVI which reduce its effectiveness in reductive reactions. There are technical challenges asso-ciated with its use (Tseng et al., 2011; Hoch et al., 2008). For real industrial wastewater treatment, iron shavings may be a better choice, compared with nZVI. Ma and Zhang (2008) have undertaken a major research and development project to investigate the technical and economic feasibility of iron shavings for the enhanced treatment of industrial process wastes, with success achieved.

In recent years, attention has been focused on stud-ies using ZVI to enhance the reduction of chlorinated nitroaromatic compounds. Lin et al. (2011) reported the reductive transformation and dechlorination of chloroni-trobenzenes in an upflow anaerobic sludge blanket (UASB) reactor enhanced with the addition of zero-valent iron. In the coupled ZVI-sequencing batch reactor (SBR) system, 2-chloronitrobenzene was readily transformed into 2-chloroaniline, which was completely biodegrad-ed in the following SBR (Xu et al., 2011). Le et al. (2011) described the effective degradation of *para*-

chloronitrobenzene through a sequential treatment using zero-valent iron reduction and Fenton oxidation. The re-ductive product *para*-chloroaniline was more susceptible to oxidation by Fenton agent than *para*-chloronitrobenzene itself.

In the present study, iron shavings were used as ZVI for the pretreatment of DNCB-containing wastewater. The combined biotreatment processes of anaerobic baffled reactor (ABR) and aerobic moving bed biofilm reactor (MBBR) were also investigated for the post-treatment. The objectives of this work were: (1) to identify intermediate products of the coupled process and to ascertain the degradation pathway, (2) to evaluate the efficiency of ZVI for enhancing biological transformation and reducing the acute toxicity of 2,4-dinitrochlorobenzene, (3) to assess the feasibility of the combined ZVI and anaerobic-aerobic process for the treatment of chlorinated nitroaromatic compound waste.

1 Materials and methods

1.1 Synthetic wastewater

The synthetic wastewater contained DNCB as target compound. The composition was as follows: phosphate buffer (Na_2HPO_4 and KH_2PO_4, 14 mmol/L, pH 7.0), $MgSO_4 \cdot 7H_2O$ (0.2 g/L), $CaCl_2$ (0.05 g/L), SL-4 (10 mL/L), DNCB (200 mg/L). The composition of SL-4 was as described previously by Shen et al. (2009).

1.2 Apparatus and materials

The combined ZVI-ABR-MBBR process is illustrated in Fig. 1. The ZVI bed was a plug flow reactor with a total volume of 6.75 L. The ZVI bed was filled with 2 kg of iron shavings, reaching an empty bed volume of 3.5 and 1.75 L as headspace, with a bottom hole. To improve the performance of the iron shavings, 0.2 wt.% of copper was applied to the iron surface by reductive precipitation to form the so-called bimetallic ZVI structures (Ma and Zhang, 2008). A peristaltic pump drove the direct upflow of wastewater through the reactor, with influent entering at the base, and with the treated effluent leaving near the top. Air was introduced from the bottom of the ZVI bed to provide the oxygen for accelerated corrosion of the iron. In order to increase the flow velocity and improve
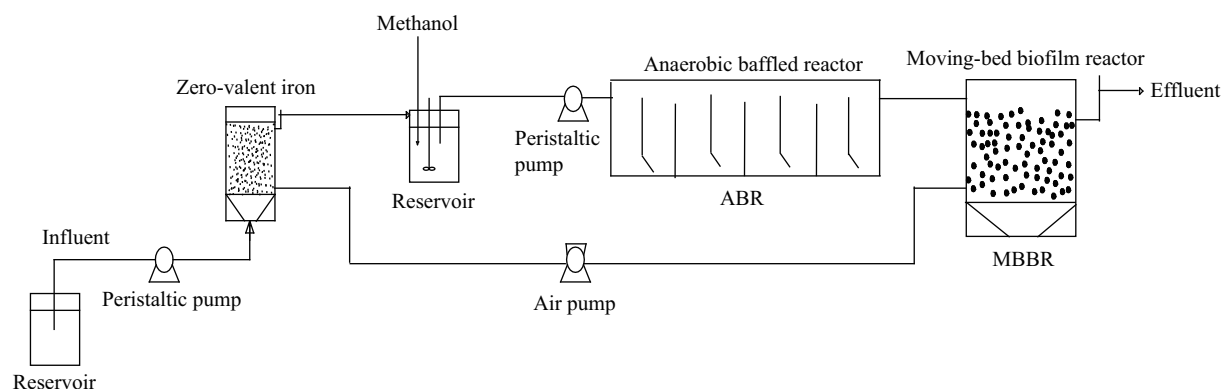

Fig. 1   Experiment installation.

the wastewater distribution within the reactor, effluent was recirculated through a peristaltic pump with effluent recirculation rates of 500%. For the purpose of ZVI bed washing, the patching was loosened by means of water and air in cross-current flow.

The effluent of the ZVI bed entered a reservoir by gravity. A certain amount of methanol was added into the reservoir as electron donor for dechlorination in the subsequent biotreatment process and as carbon source to facilitate biomass growth. Water in the reservoir entered the biotreatment process through a peristaltic pump.

The biotreatment process was designed in two parts: an anaerobic baffled reactor (ABR) and a moving-bed biofilm reactor (MBBR). The ABR was a rectangular box with active reactor volume of 40 L. It was divided into eight equal compartments by vertical baffles with 45 slanted edges. The effluent of the ABR entered the MBBR through a gravity drain. The MBBR had an effective water depth of 25 cm and working volume of 20 L. Polyhedral hollow ball fillers with 25 mm diameter, density of 144.5 kg/m^3, and specific biofilm surface area of 500 m^2/m^3 were added into the MBBR and occupied 50% of the reactor volume. Both the ABR and MBBR were installed in a temperature-controlled chamber maintained at 35°C. Both ABR and MBBR were initially inoculated with sludge taken from the secondary sedimentation tank of a municipal wastewater treatment plant.

1.3 Experimental procedure

Before the combined ZVI and biotreatment process in our study could be operated, it was necessary to investigate the effectiveness of ZVI in DNCB wastewater pretreatment. DNCB, TOC, COD, BOD$_5$ concentrations, and EC$_{50, 48 hr}$ (%, V/V) of the influent and effluent were monitored at empty bed contact time (EBCT) of 3 and 6 hr, respectively. The reduction intermediates in the effluent were also analyzed through HPLC and GC-MS.

The combined ZVI and biotreatment process was operated for 3 months. To facilitate the biomass growth and reduction of DNCB and its metabolites, methanol was added into the influent of the biotreatment process as the carbon and energy source, giving a final methanol concentration of 1000 mg/L in the ZVI effluent. The ZVI reactor was operated at an empty bed contact time (EBCT) of 6 hr under an influent flow rate of 14 L/day, with effluent recirculation rates of 500%. After the acclimation phase of the ABR-MBBR (25 days), steady-state was achieved. The ABR and MBBR were operated at HRT of 8 and 4 days, respectively. DNCB and its metabolites, TOC, COD, Cl$^-$, and EC$_{50, 48 hr}$ (%, V/V) of the influent and effluent were monitored during the operation period of 3 months. Only the results obtained under steady-state conditions were reported.

1.4 Analytical methods

Before analysis, water samples were passed through a 0.22 μm filter. COD was analyzed according to standard methods (GB 11914-89). BOD was determined according to the HACH BODTrak^{TM} II procedure. TOC was mea-

sured on a vario TOC analyzer (Elementar, Germany). The toxicity of wastewater to the Zebrafish was assessed with 48 hr lethality tests and performed using a static procedure, according to standard method (GB/T 13267-91). The toxicity was described as EC$_{50, 48 hr}$ (%, V/V). DNCB and its degradation products in the supernatant were identified and quantified by HPLC (Waters 2996, Waters Incorporation, USA) through authentic standard and UV-Visible light analysis. The HPLC analysis was conducted at room temperature using a Waters RP18 column (5 μm, 4.6×250 mm) and a UV-Vis detector. The mobile phase was a mixture of 45% methanol and 55% water pumped at a flow rate of 1.00 mL/min. The analysis was performed at 254 nm, with column temperature at 35°C. The analysis of chloride ion was performed on an ion chromatograph (ICS-2100, DIONEX) using an Ion Pac® As11-HC (4×250 mm) column and a suppressed conductivity detector. The eluent was 30 mmol/L NaOH (with anion self-regenerating suppressor) pumped at a flow rate of 1.5 mL/min. GC-MS analysis was conducted according to the procedures described by Xiong et al. (2011). Dissolved iron in the effluent was determined by ICP-OES Optima 7000DV.

2 Results and discussion

2.1 Transformation pathway of DNCB in coupled ZVI-ABR-MBBR process

Study of the intermediates and pathway for the transformation of DNCB is important for investigation of the effectiveness of the coupled ZVI-ABR-MBBR process. The influent and effluent samples of the ZVI process were analyzed using HPLC, as shown in Fig. 2. The main peaks in the effluent with retention time of 21.5 and 5.4 min match exactly the retention times of DNCB and DACB (Fig. 2), respectively. In addition, the mass balance calculated from the measured DNCB and DACB concentration in the effluent versus the inlet DNCB concentration was as high as 93.1% ± 3.1% (EBCT = 6 hr). These results suggested that DACB might be the dominant product of DNCB reduction by ZVI. The GC-MS spectra results further confirmed the dominance of DACB in the ZVI effluent (EBCT = 6 hr). The components with the retention time of 4.6 min could be attributed to DACB (data not shown). No other intermediates was observed in the ZVI effluent (EBCT = 6 hr) from GC-MS. It was very interesting to note that a peak with retention time of 2.7 min appeared at low EBCT (3 hr) which may be an intermediate of DNCB reduction, such as 2-amino-4-nitrochlorobenzene or 2-nitro-4-aminochlorobenzene (Fig. 2b). Some possible intermediates of DNCB, such as hydroxylamino compounds and nitroso compounds, have been reported to be unstable in water solution and were therefore not expected to be present in the solution. However, the hypothesized dechlorination of DACB did not occur at the presence of ZVI, even with the increase of EBCT, indicating that DACB could not be further transformed to m-phenylenediamine by ZVI. Hence, at
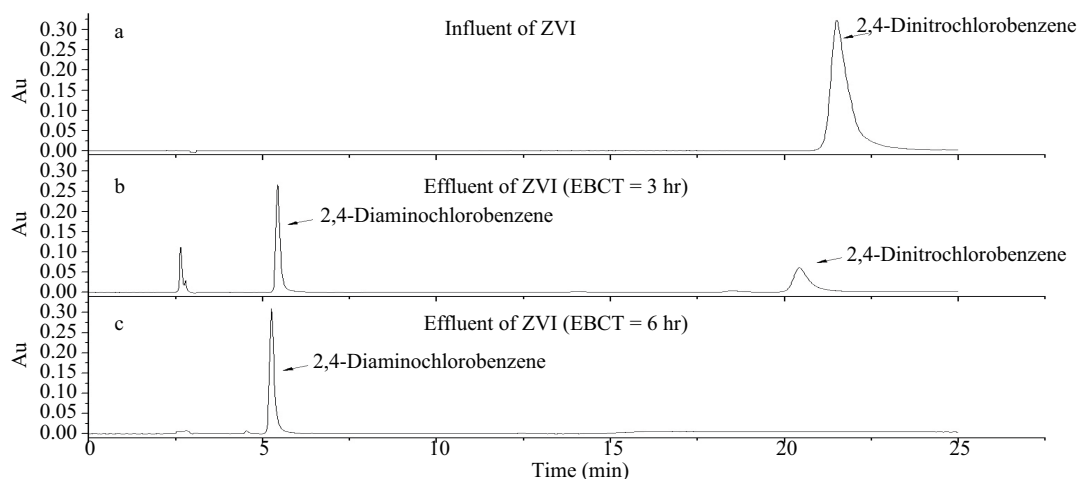
Fig. 2   Identification of intermediates in effluent of ZVI by HPLC.

EBCT of 6 hr, complete transformation of DNCB to DACB by Fe-Cu bimetallic systems was confirmed in this study (Fig. 2c). To ensure complete reduction of DNCB, EBCT of 6 hr was adopted in the coupled ZVI-ABR-MBBR process.

Several studies have reported that ZVI was very effective for transforming nitro aromatic compounds into the corresponding aromatic amines (Agrawal and Tratnyek, 1996; Mu et al., 2004). Catalytic dechlorination of *para*-nitrochlorobenzene (*p*-NCB) was achieved in Ni/Fe bimetallic nanoparticles, *p*-NCB was quickly reduced to *p*-chloroaniline (*p*-CAN) and aniline. Ni played the role of catalyst, decreasing the activation energy of the hydrogenolysis of C–Cl bonds (Xu et al., 2009). In a continuously stirred hydrogen-based hollow fiber membrane biofilm reactor, *p*-NCB was first reduced to *p*-CAN and then to aniline by hydrogenotrophic bacteria with H_2 as electron donor (Xia et al., 2011). Previous publication reported that 2-chloronitrobenzene could be transformed rapidly into chloroanillines (ClAns) by ZVI with minor amounts of intermediate products (Lin et al., 2011; Xu et al., 2011). However, dechlorination in the ZVI system was not observed in their study, which was consistent with our results, indicating that nitrochloroaromatic compounds were quite difficult to be dechlorinated by Fe^0 or Fe-Cu bimetallic system.

Since DACB as the final product of ZVI reduction was non-degradable in the ZVI system, the subsequent ABR-MBBR process was adopted for further treatment. The HPLC chromatogram of the samples during the bioprocess is presented in Fig. 3. The DACB concentration decreased along the ABR compartments, while the *m*-phenylenediamine concentration increased along the first 4 compartments of the ABR (Fig. 3a and b), indicating the reductive dechlorination of DACB into *m*-phenylenediamine in the ABR system. In addition, the removal of DACB was also accompanied with the release of chloride ion, and 78.3% ± 3.8% chloride recovery efficiency was achieved in the effluent of the ABR. The good chloride balance confirmed that the reductive dechlorination of DACB occurred. The *m*-phenylenediamine concentration decreased along the following 4 compart-

ments of the ABR (Fig. 3c). The HPLC analysis also revealed that, after the aerobic treatment, the chromatographic peak area decreased significantly and shifted to lower retention times (Fig. 3d). These findings indicated the formation of less aromatic and more polar compounds during the treatment process in the ABR-MBBR.

Based on these findings, a possible transformation pathway for DNCB in the coupled ZVI-ABR-MBBR system is suggested in Fig. 4. In the ZVI bed, Fe^0 served as an electron donor to produce electrons. As the electron acceptor, DNCB was first reduced to DACB through 2-amino-4-nitrochlorobenzene or 2-nitro-4-aminochlorobenzene in the ZVI bed. In the ABR, DACB was further transformed via the dechlorination pathway to yield the product *m*-phenylenediamine in the presence of anaerobic microorganisms, using methanol as electron donor. *m*-Phenylenediamine could be further degraded in the subsequent anaerobic and aerobic process.

2.2 Treatment performance of coupled ZVI-ABR-MBBR process

2.2.1 Characterization of effluent of ZVI process

As shown in Table 1, in the effluent of the ZVI bed (EBCT = 6 hr), DNCB was always below 10 mg/L for influent DNCB concentration of 200 mg/L. The DNCB removal efficiency was generally over 95% during the test period of 3 months. The DACB concentration increased to (126.3 ± 6.4) mg/L after treatment by ZVI. The mass balance calculated from the measured DNCB and DACB concentration in the effluent versus the inlet DNCB concentration was as high as 93.1% ± 3.1%. The high mass balance confirmed the dominance of DACB in the ZVI effluent during the 3-month operation, while constantly high removal efficiency of DNCB proved the process stability of the ZVI process.

Table 1 also reveals the variation of COD and BOD_5 before and after treatment by ZVI. As observed from Table 1, the initial BOD_5/COD ratio was only 0.005 ± 0.001, which indicated that the DNCB wastewater had high toxicity and this kind of effluent could not be treated effectively by traditional biological methods. COD removal and BOD_5 production were observed after treatment by ZVI, resulting in an increase in the BOD_5/COD ratio.
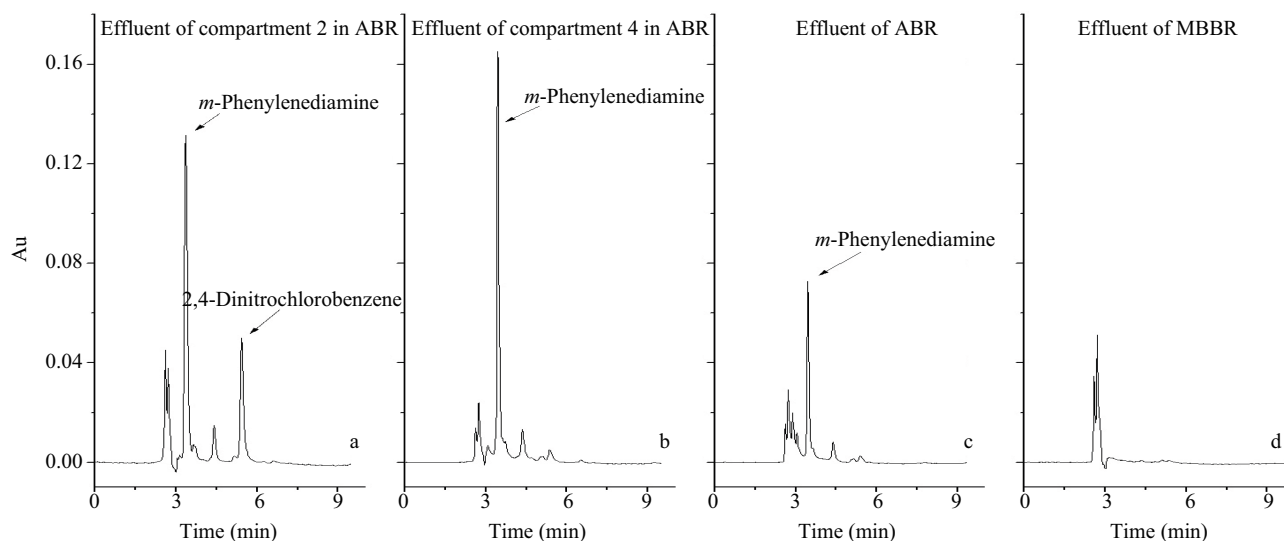
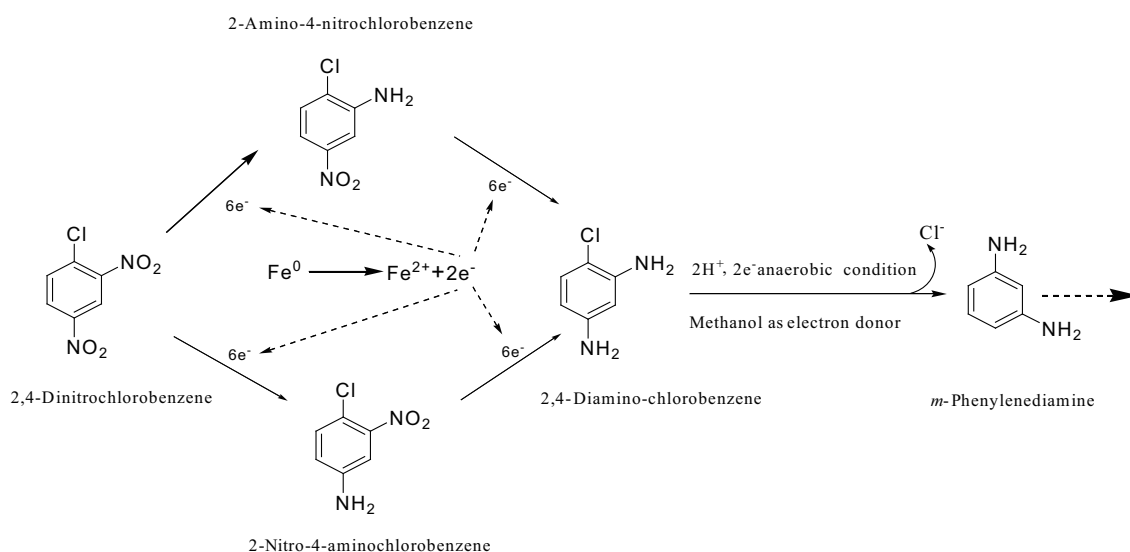**Fig. 3** HPLC of effluent from the bioprocess.

Fig. 4 Proposed transformation pathway of DNCB in the coupled ZVI-ABR-MBBR process.

Table 1 Characterization of the influent and effluent from the ZVI process (EBCT = 6 hr)

	COD (mg/L)	BOD$_5$ (mg/L)	BOD$_5$/COD	TOC (mg/L)	DNCB (mg/L)	DACB (mg/L)	EC$_{50, 48 \, hr}$ (V/V)
Influent of ZVI	215.7 ± 18.0	1.1 ± 0.2	0.005 ± 0.001	105.7 ± 2.8	199.8 ± 1.6	0	0.65%
Effluent of ZVI	189.8 ± 16.8	31.8 ± 1.5	0.168 ± 0.007	95.7 ± 3.3	6.8 ± 2.9	126.3 ± 6.4	5.20%
Removal efficiency	12.0% ± 1.1%	–	–	9.5% ± 0.8%	96.6% ± 1.4%	–	–

–: not evaluated.

The BOD$_5$/COD ratio increased from 0.005 ± 0.001 to 0.168 ± 0.007 after treatment by ZVI at EBCT of 6 hr. The increase of BOD$_5$/COD ratio indicated the increase of biodegradability. The BOD$_5$/COD ratio after pretreatment by ZVI was much higher than that before pretreatment by ZVI, suggesting the effectiveness of ZVI in improving the biodegradability of DNCB wastewater.

Although the BOD$_5$/COD value increased, BOD$_5$/COD of the ZVI effluent (0.168 ± 0.007) was still lower than 0.2. It is accepted that low BOD$_5$/COD ratio (less than 0.2) indicates toxic wastewater which needs further treatment before being subjected to biochemical treatment (Vlys-

sides et al., 2005). Toxicity reduction during the coupled process was also included in Table 1. Higher EC$_{50}$ values indicated lower toxicity to Zebrafish. The EC$_{50, 48 \, hr}$ (V/V) value of ZVI influent with 200 mg/L DNCB was only 0.65%, indicating that the DNCB wastewater was highly toxic and was very resistant to mineralization by biological processing. After pretreatment by ZVI (EBCT = 6 hr), the biological toxicity was greatly lowered as revealed by the EC$_{50}$ values increasing from 0.65% to 5.20%, probably due to the almost complete conversion of DNCB into DACB. Due to the reduction of the electron-withdrawing nitro group, the high electron deficiency of the π-electron

system on DNCB was reduced (Shen et al., 2009). In addition, it was found that with the presence of ZVI, TOC decreased slightly, which was consistent with the results of COD (Table 1), confirming that the reduction of toxicity was mainly due to the conversion of DNCB into DACB, not due to the removal of toxic compounds from the water phase through electrophoretic deposition, electrochemical flocculation or adsorption (Fan and Ma, 2009). The improvement of biodegradability reflected the reduction of biological toxicity (Xiong et al., 2011), so that the results of the reduced biological toxicity agreed with the finding of increased biodegradability, which demonstrated the effectiveness of ZVI in the detoxification of the tested DNCB wastewater.

2.2.2 Characterization of effluent from ABR-MBBR process

After the acclimation phase of the ABR-MBBR (about 25 days), steady state was achieved. As was described previously, in the ABR, DACB produced in the ZVI bed was further transformed via a dechlorination pathway to yield the product m-phenylenediamine using methanol as electron donor. As shown in Table 2, 126.3 ± 6.4 mg/L of DACB could be completely removed in the ABR, with DACB undetected in the effluent. However, along the ABR compartments, the m-phenylenediamine concentration increased first to 67.4 ± 8.4 mg/L in compartment 4 and then decreased, with the effluent m-phenylenediamine concentration as low as 21.7 ± 6.2 mg/L. The residual COD and TOC concentration in the ABR effluent was also not remarkable. Noticeably, after the anaerobic reduction process in the ABR, BOD_5/COD increased greatly from 0.168 ± 0.007 to 0.375 ± 0.012, demonstrating great enhancement of biodegradability by the anaerobic reduction process. It was found that most of the added methanol could be degraded rapidly in the first two compartments of the ABR, while complete methanol removal occurred in subsequent compartments. Accordingly, it could be inferred that the increase of the BOD_5/COD ratio was not due to residual methanol in the effluent of ABR, but probably due to the dechlorination reactions generating m-phenylenediamine, which could be easily degraded by aerobic microorganisms.

Under steady-state conditions, the residual m-phenylenediamine in the ABR effluent was completely removed by the subsequent MBBR process, as indicated in Table 2. The average COD concentration in the effluent of the MBBR was as low as 67.5 ± 10.8 mg/L, and the residual TOC concentration was as low as 21.3 ± 4.2 mg/L.

Based on the EC_{50} data presented in Table 2, the ABR-MBBR process used in this study was effective for reducing the toxicity of the ZVI effluent. After the ABR process, the $EC_{50, 48 \, hr}$ (V/V) values increased from 5.20% to 48.2%, whereas the samples taken at the end of the MBBR were not toxic, since all Zebrafish subjected to the undiluted effluents of the MBBR survived. Thus, an EC_{50} value for the MBBR effluent could not be ascertained. These results confirmed the complete elimination of acute toxicity in the coupled ABR and MBBR process.

2.3 Implications

Since conventional biological treatment was not effective in removing chlorinated nitroaromatic compounds, such as DNCB, the goal of this research was to look into the effectiveness of the ZVI-ABR-MBBR coupled process as a treatment method for DNCB-containing wastewater.

Overall, the results of the BOD_5/COD, toxicity tests indicated that DNCB exhibited obvious toxic or inhibitory effects on microorganisms. On the other hand, DACB, as the major ZVI-treated product of DNCB, had less inhibitory effect and showed enhanced biodegradability. Although chloro-substituents could not be changed or discharged through ZVI pretreatment, the conversion of highly electron-deficient DNCB to less electron-deficient DACB significantly reduced its toxicity (Xu et al., 2011). Accordingly, the first step in the coupled ZVI-ABR-MBBR process involving the reduction of DNCB to DACB using ZVI was necessary, and reduced the toxicity of DNCB, creating favorable conditions for degradation of the target pollutant in the subsequent biological process.

In the control ABR-MBBR system (without ZVI pretreatment), 2.35 ± 0.39 mg/L of chloride ions was detected in the effluent, corresponding to only 8% of the chloride in DNCB, indicating that the control ABR-MBBR system had poor removal capability for DNCB. In addition, HPLC of MBBR effluents showed that after the aerobic treatment, the chromatographic peak areas were still very large. The large chromatographic peak area might be attributed to serious intermediate metabolite accumulation. It could be concluded that pre-reduction of DNCB by ZVI was necessary, and degradation of DNCB was efficiently enhanced in the coupled ZVI-ABR-MBBR system.

For the reductive transformation of nitroaromatic compounds, the ZVI process offers bright prospects, due to a number of advantages, such as small footprint and excellent performance at much higher loading rates than conventional anaerobic reductive processes. Compared with an anaerobic reductive process, the ZVI reactor in this

Table 2 Characterization of influent and effluent from the ABR-MBBR process under steady-state conditions

	COD (mg/L)	BOD_5/COD	TOC (mg/L)	DNCB (mg/L)	DACB (mg/L)	m-Phenylenediamine (mg/L)	$EC_{50, 48 \, hr}$ (V/V)
Effluent of ZVI	189.8 ± 16.8	0.168 ± 0.007	95.7 ± 3.3	6.8 ± 2.9	126.3 ± 6.4	0	5.20%
Influent of ABR (with methanol added)	1697.6 ± 41.3	–	489.6 ± 22.9	6.8 ± 2.9	126.3 ± 6.4	0	–
Effluent of ABR	128.4 ± 13.1	0.375 ± 0.012	45.2 ± 5.9	Undetected	Undetected	21.7 ± 6.2	48.2%
Effluent of MBBR	67.5 ± 10.8	–	21.3 ± 4.2	Undetected	Undetected	Undetected	Not toxic

–: not evaluated.

study is much smaller, with EBCT of only 6 hr. In addition, the ZVI process is also very stable, with no specialized pH and/or temperature requirements and no need for any nutrient and alkalinity amendment (Ma and Zhang, 2008).

What's more, the transformation of contaminants by ZVI was accompanied by the release of ferrous iron and hydroxyl. In this study, the effluent iron concentration was generally below 0.65 mg/L. The low iron concentration in the effluent indicated the reduced consumption of iron, leading to easy maintenance and low operating cost. In addition, the low concentration of ferrous and ferric iron in the ZVI effluent was beneficial to the microorganisms in the bioprocess. The low iron concentrations in the column effluent might be due to the formation of iron hydroxides and some other iron oxides which remained and precipitated within the ZVI bed (Xu et al., 2011). To prevent agglomeration, passivation and clogging, period backwashing by means of water and air in cross-current was necessary.

As the post-treatment step, the ABR-MBBR coupled bioprocess exhibited excellent polishing performance. The dechlorination reaction occurred in the ABR using methanol as the cosubstrate, with BOD_5/COD ratio increased from 0.168 ± 0.007 to 0.375 ± 0.012, while the dechlorinated product *m*-phenylenediamine was further degraded in the subsequent bioprocess. In other words, the applied anaerobic process in the ABR improved the biodegradability of the ZVI effluent for further aerobic treatment by transforming DACB to its dechlorinated product, which could be mineralized aerobically. The positive effect of the anaerobic reductive process on the aerobic biodegradability of some recalcitrant organics, such as azo dyes, was also proved by other researchers (Koupaie et al., 2011; Sponza and Işik, 2002; An et al., 1996).

The good quality of the effluent from the coupled ZVI-ABR-MBBR process was indicated by low residual COD concentration, TOC concentration, acute toxicity, and complete removal of DCAB and its products. Thus, the coupled ZVI-ABR-MBBR process gave excellent degradation performance when treating wastewater containing DNCB. This technology could also be considered as a potential process for the treatment of wastewater containing other chlorinated nitroaromatic compounds.

2.4 Conclusions

A coupled ZVI-ABR-MBBR system was established for the treatment of synthetic wastewater containing DNCB. The following conclusions were derived: (1) The ZVI column used for pretreatment could rapidly transform DNCB to DACB, with biodegradability of effluent significantly enhanced and toxicity reduced. (2) In the subsequent ABR process, DACB generated in the ZVI process could be reduced to *m*-phenylenediamine through dechlorination with methanol as the electron donor. (3) As the post-treatment step, the ABR-MBBR coupled bioprocess exhibited excellent polishing performance, ensuring good quality of effluent. (4) The present findings indicate that the coupled ZVI-ABR-MBBR system offers bright prospects for the treatment of chlorinated nitroaromatic compound-

containing wastewater.

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