Simple colorimetric assay for dehalogenation reactivity of nanoscale zero-valent iron using 4-chlorophenol

Yuhoon Hwang [a], Paul D. Mines [a], Mogens H. Jakobsen [b], Henrik R. Andersen [a,*]

[a] Department of Environmental Engineering, Technical University of Denmark, Miljøvej, B113, DK-2800 Kgs. Lyngby, Denmark
[b] Department of Micro- and Nanotechnology, Technical University of Denmark, Ørsteds Plads, B 345E, DK-2800 Kgs. Lyngby, Denmark

ARTICLE INFO

ABSTRACT

Despite the wide application of nanoscale zero valent iron (nZVI) for the treatment of a plethora of pollutants through reductive reactions, reactivity evaluation of nZVI toward dehalogenation has not been standardized. In this light, it was desired to develop a simple colorimetric assay, for versatile laboratory application, using merely a spectrophotometer for color intensity determination. A modification of the indophenol reaction, which is well known for its use in ammonia detection, is suggested for this color assay. Being that 4-chlorophenol can be reduced to phenol by nZVI but cannot react according to the indophenol reaction, as its para-position is blocked; the simple colorimetric assay for reducing reactivity determination of nZVI to organically bound halogens was developed, using 4-chlorophenol as the model substrate that is selectively reduced to phenol by nZVI. The sensitivity and selectivity of the color assay for phenol detection was initially investigated. Since a shift of the peak absorbance was observed at higher concentrations of indophenol formed from phenol, the appropriate concentration range for analysis was determined as 0–50 μM, with a peak absorbance at 680 nm. The suggested color assay showed superior selectivity toward phenol in the presence of 4-chlorophenol. However, the high concentration of soluble iron ions produced throughout the reaction caused positive interference on absorbance. Addition of $NaCO_3$ for the pre-treatment of samples was suggested to eliminate the effect of residual iron ions and acidity from buffers used in nZVI reactivity tests. Several types of nZVI were investigated with the reaction, including monometallic nZVI and bimetallic nZVIs of Ni/Fe and Cu/Fe; and only Ni/Fe particles were able to produce phenol, indicating the reducing reactivity of Ni to produce atomic hydrogen along with an electron supplied by the core of nZVI. Throughout this study, the 4-chlorophenol based reactivity assay, using the modified indophenol reaction, successfully determined reducing reactivity of nZVI in varied experimental conditions.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Zero valent iron (ZVI), by utilizing reductive reaction mechanisms, has been applied in the removal of a wide variety of pollutants, especially halogenated organics, including chlorinated hydrocarbons, chlorinated phenols, polychlorinated biphenyls (PCBs), etc. [1]. In particular, it has been found that nanoscale ZVI (nZVI) exhibited much higher activity for dehalogenation than commercially available micro-iron powder, due to the difference in specific surface area [2]. Moreover, the further enhancement of nZVI reactivity has been intensively investigated. A wide range of stabilizers, e.g. surfactant [3], sodium carboxymethyl cellulose [4],

starch [5], and guar gum [6], have been applied to prevent aggregation of nZVI, although this can lead to a decrease in actual surface area available for reaction [7]. The application of a second metal is another approach for enhancement of nZVI reactivity. In bimetallic materials, the primary metal, having a lower standard redox potential, Fe, acts as an electron donor to reduce the contaminants; while the secondary metal (Pd, Pt, Ag, Ni, Cu, etc.), acting as a catalyst and having a high standard redox potential, promotes the reactivity via hydrogenation and accelerates corrosion [8–10].

However, the evaluation of nZVI reactivity toward dehalogenation has not yet been standardized in previous studies. The use of advanced chromatographic techniques has mostly been applied to determine the parent compound and subsequent degradation products [11,12]. These techniques are powerful tools to analyze the compounds both qualitatively and quantitatively. Nevertheless, it generally requires long analysis times and complicated set-ups. In

this light, a simple color assay for use in laboratories, requiring only a basic spectrophotometer, for determining color intensity should be developed.

Chlorinated phenols, which are broadly used as wood preservatives, anti-rust agents, and pesticides, have become an note-worthy contaminant in the environment [13], being that most of them are toxic, carcinogenic, and intractable [14]. Due to the aryl structure and presence of the chlorine atom, chlorinated phenols are exceptionally recalcitrant toward chemical reactions aimed at their reduction [15]. In the last decade, application of ZVI for reduction of chlorophenols has been extensively studied [8,15–18]. Chlorophenol can be reduced to phenol via dehalogenation, driven from electron transfer, and it is generally accepted that phenol is the only product during this process [16,17].

The indophenol reaction is the name given to the reaction of monochloramine and a phenol, which, under suitable conditions, results in the formation of a blue indophenol dye, which can be determined spectrophotometrically [19]. This reaction is highly specific for ammonia, and in the presence of a suitable catalyst, extremely high sensitivity is attainable. The reaction mechanism, as it's understood, is outlined below in Eqs. (1)–(3) [20]

$$NH_3 + NaOCl \rightarrow NH_2Cl + NaOH \qquad (1)$$

(2)

(3)
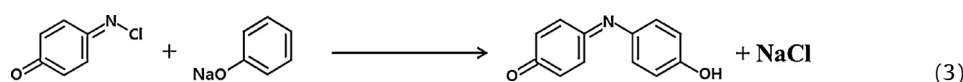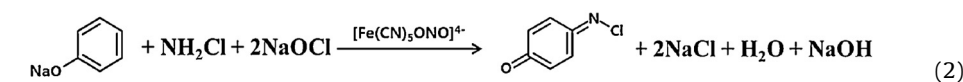
The produced monochloramine in Eq. (1) reacts with the para position of phenol, and N-chloro-p-hydroxybenzoquinone monoamine is produced. This intermediate reacts with phenolate to produce a blue colored indophenol. Since the para position of phenol is involved in this reaction, it is generally accepted that only phenols with un-substituted para-positions can make this reaction [19]. Therefore, it can be hypothesized that phenols with a substituted para position will not be detected by this reaction; whereas the reduction product of it, phenol, will produce color. Because it is known that para-chlorophenol (4-chlorophenol) is able to be reduced to phenol by the reaction with nZVI; in this study, 4-chlorophenol is chosen as a target compound for the development of a color assay.

Herein, the simple colorimetric assay for reducing reactivity determination of nZVI to organically bound halogens was developed, using 4-chlorophenol as the model substrate that is selectively reduced to phenol by nZVI. Firstly, the sensitivity of the color assay was optimized for phenol quantification. Secondly, the selectivity of the color assay toward phenol, in the presence of 4-chlorophenol, was investigated to test the feasibility of the assay. Several types of nZVI and bimetallic nZVI (Ni/Fe, Cu/Fe) were tested for degradation of 4-chlorophenol to phenol; in order to demonstrate the applicability of the method and the feasibility to distinguish reducing reactivity. The matrix effect, namely pH and residual iron ions concentration, was investigated and carbonate addition is applied to eliminate any interferences. Based on the overall results of bimetallic nZVI reactivity and the selectivity of the color assay, a simple and effective assay to determine reactivity of nZVI is suggested.

2. Methods

2.1. Chemicals

Ferrous sulfate heptahydrate (>99%, Fluka), sodium borohydride (>98%, Sigma–Aldrich), copper sulfate pentahydrate (>99%, Fluka)

and nickel sulfate hexahydrate (>99%, Sigma–Aldrich), ethanol (>99.9%, Sigma–Aldrich) were used for nZVI synthesis. Phenol (>99%, Fluka), 4-chlorophenol (>98%, Merck), sodium hypochlorite solution (10%, Fluka), ammonium chloride (>99.5%, Sigma–Aldrich), sodium nitroprussid (>99%, Merck) were used for the modified indophenol reaction. Hydrochloric acid (37%, Sigma–Aldrich), sodium hydroxide (>98%, Sigma–Aldrich), sodium citrate (>98%, Sigma–Aldrich), citric acid (>99.8%, Fluka), and acetate (>99.8%, Sigma–Aldrich) were used for pH adjustment. Ferric chloride hexahydrate (>98%, Sigma–Aldrich) was used for investigating effect of residual iron ions in the sample. Sodium carbonate (>99%, Sigma–Aldrich) was used for sample pretreatment. Unless otherwise stated, all of the chemicals were used as received from the supplier. All solutions were prepared using degassed deionized water (DDIW) by purging with nitrogen gas for 3 h.

2.2. Reagents and optimized assay procedure

Typical reaction conditions for ammonium measurement using the indophenol reaction had ammonium as the limiting reagent, and excess amounts of phenol and hypochlorite were applied to ensure all ammonium forms indophenol, catalyzed by sodium nitroprusside. In this study, phenol was chosen as the limiting reagent, in order to have the color development dependent on the concentration of phenol.

In this study, reagent A, hypochlorite-NaOH solution, and reagent B, ammonium-sodium nitroprusside solution, were prepared for the suggested color assay. Reagent A was prepared by diluting 1 mL of 10% hypochlorite solution and 66 mL of 0.5 M NaOH solution to 100 mL with DDIW. Reagent B was prepared as 1250 mg-N/L of ammonium chloride (4.78 g NH_4Cl/L) and 0.3 g/L of sodium nitroprusside in DDIW. A drop of hydrochloric acid was added to minimize ammonia stripping to the headspace in the reaction vessel. Reagent C was prepared by dissolving 106 g/L Na_2CO_3 to make a 1 M solution. Reagent C is used to neutralize any acidic buffer that might be used in the experiments with nZVI reactivity and to precipitate residual soluble iron ion (Fe^{2+} or Fe^{3+}) produced throughout reaction, in order to remove interferences with the color reading.

The following procedure was used to quantify phenol in the reaction mixture: 5 mL of homogenized samples were pre-treated by 250 μL of carbonate solution (reagent C) to neutralize the acidic buffer and to precipitate interfering iron ions. Produced iron carbonate was well precipitated in 30 min, and the supernatant was taken for phenol analysis. Equal volumes of reagents A and B were mixed to make the reagent AB, prior to sample addition. Then, 2.3 mL of diluted sample, having an expected phenol concentration range of 0–50 μM, was transferred into a tube, and 0.2 mL of reagent AB was added to the diluted sample. The mixture was gently shaken and allowed to react in the dark for 2 h and subsequently quantified with a photometer at 680 nm in 1.0 cm polycarbonate cuvette. Depending on the desired precision of quantification, a 5 point calibration curve (Blank, 5, 10, 25 and 50 μM of phenol) was obtained in parallel. If a high precision is not required (kinetic or competitive studies), quantification is performed using the determined calibration curve: [phenol, μM] = $(Abs - 1.43 \times 10^{-2} \, cm^{-1})/(7.62 \times 10^{-3} \, cm)$.

## 2.3. Sensitivity and selectivity of color assay for phenol detection

In order to investigate the sensitivity of the color assay suggested in this study, the adsorption spectra of phenol and 4-chlorophenol solution, in the concentration range of 0–2000 μM, was monitored. The color assay's selectivity toward phenol was investigated using a mixture of phenol and 4-chlorophenol, in the concentration range of 0–50 μM. The mixture composition was designed as a full factorial design. Adsorption spectra of reacted samples were monitored using a UV–vis spectrophotometer (Cary 50, Agilent Technologies Inc., USA), and the absorbance at 680 nm was chosen as the response variable. Design-Expert 7.0.0 (Stat-Ease, Inc., USA) was used for analysis of variance (ANOVA), mathematical regression models, and to plot response surfaces.

The effects of pH and residual iron concentration in the sample on the color assay were also investigated. The phenol concentration was fixed at 20 μM. Iron concentration was controlled by ferric chloride stock solution, and the sample pH was adjusted using 0.1 M hydrochloric acid and 0.1 M sodium hydroxide. Additionally, the total iron concentration was monitored by an atomic absorption spectrometer (AAnalyst 200, PerkinElmer, USA).

2.4. Reduction of 4-chlorophenol by monometallic and bimetallic nZVI

2.4.1. Preparation of monometallic and bimetallic nZVIs

In this study, the mild chemical reduction of metal salts in the solution phase was used to prepare nZVI, as stated in previous research [21]:

$$2Fe^{2+} + BH_4^- + 3H_2O \rightarrow 2Fe^0 + H_2BO_3^- + 4H^+ + 2H_2 \qquad (4)$$
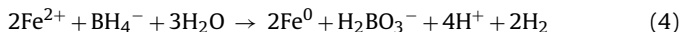
The concentrations of ferrous (Fe^{2+}) solution and sodium borohydride solution were 35.8 and 89.6 mM, respectively. Ferrous sulfate and sodium borohydride solutions were introduced via peristaltic pump with a constant delivery rate of 5 mL/min. Collection of nZVI was performed via centrifugation (4500 rpm, 5 min, Heraeus Multifuge X1, Thermo Fisher Scientific Inc., USA) of the solution, and the collected particles were then washed twice with DDIW. In order to achieve a dry nZVI powder, the solution was washed with ethanol was and subsequently dried in an anaerobic chamber (N$_2$:H$_2$ = 95:5, Coy laboratory products, USA).

Bimetallic particles were prepared via chemical reduction, similar as with nZVI preparation. The co-reduction of FeSO$_4$ and NiSO$_4$ was performed, as described by Zhang et al. [8]. The detailed preparation procedure was the same as with the monometallic nZVI preparation, except that a mixture of ferrous sulfate and nickel sulfate was reduced by sodium borohydride. Being that nickel has a higher standard redox potential, the nickel (Ni^{2+}) ion in solution will take the form of reduced metallic nickel, prior to iron reduction. Therefore, the nickel will be located in the core of the resultant bimetallic Ni/Fe particles. In order to ensure that the secondary metal ends up on the surface of the nZVI, a step-wise reduction of metal salts was conducted. The bimetallic nZVIs were prepared by mixing the secondary metal solution (CuSO$_4$, NiSO$_4$) with freshly prepared nZVI particles [22,23]. After 5 min of redox reaction between the second metal and nZVI, the resulting Ni/Fe and Cu/Fe bimetallic particles were washed twice with DDIW. In all cases, the amount of second metal was calculated to be 5 wt% of Fe weight.

2.4.2. 4-Chlorophenol dechlorination by different nZVI

Batch tests for 4-chlorophenol degradation by nZVI were conducted in a 50-mL glass serum bottle. The bottle was initially filled with 4-chlorophenol stock solutions, and prepared nZVI was added to the bottles to initiate the reaction. The nZVIs tested in this study were monometallic nZVI (in both dried and slurry form),
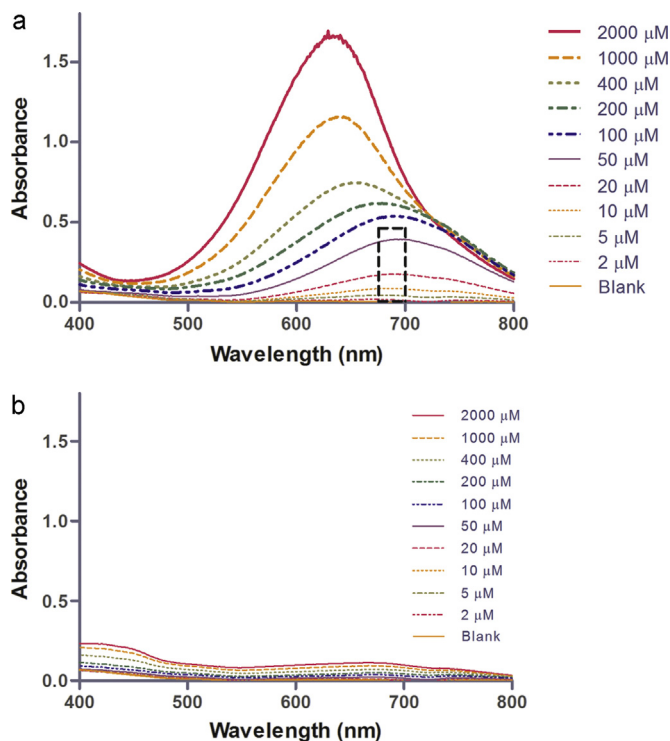

Fig. 1. Spectra evolution with different concentrations (μM) of (a) phenol and (b) 4-chlorophenol after reaction with NH$_2$Cl (reagent AB).

co-reduced Ni/Fe, stepwise-reduced Ni/Fe, and Cu/Fe. Solution pH was controlled by either adjustment of initial pH or buffering with citrate or acetate. The nominal concentrations of 4-chlorophenol, buffer, and nZVI were 500 μM, 10 mM, and 2 g/L, respectively. The headspace of the bottle was purged with nitrogen gas and securely capped with a PTFE/silicon septum and aluminum crimp seal. The bottle was mixed on a shaker at 125 rpm. Samples of 5 mL were taken periodically and filtered with 0.45 μm syringe filters. The phenol concentration in the sample was measured as described in Section 2.2.

3. Results and discussion

3.1. Sensitivity and selectivity of color assay for phenol detection

The suggested color assay, i.e. the modified indophenol reaction, was tested for detection of both phenol and 4-chlorophenol in the concentration range of 0–2000 μM. The adsorption spectra of the color product by phenol are detailed in Fig. 1(a). The shift of peak absorbance was observed at the concentration range of 100–400 μM. The peak absorbance in concentrations lower than 100 μM was consistent at 680 ± 7 nm, while the peak absorbance in concentrations higher than 400 μM was 640 ± 2 nm. In this sense, the appropriate concentration range for analysis was determined as 0–50 μM, since linearity was not maintained after 50 μM. The standard curve of absorbance at 680 nm versus phenol concentration is plotted in Fig. 2. The slope of standard curve was determined as 7.62×10^{-3}, with a R^2 value of 0.998.

The color development by 4-chlorophenol is much lower than phenol, as is detailed in Fig. 1(b), which elucidated the higher sensitivity of color assay toward phenol. The slope of standard curve was determined as 4.00×10^{-4}, with a R^2 value of 0.990. The slight color development from 4-chlorophenol might be attributed to
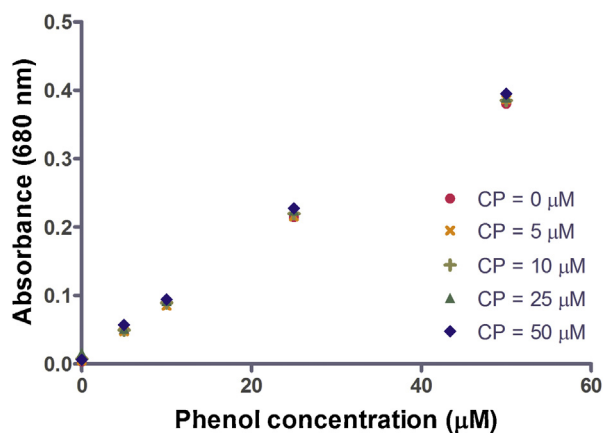
Fig. 2. Standard curve for phenol (0–50 μM) after reaction with NH$_2$Cl in the presence of 0, 5, 10, 25, and 50 μM 4-chlorophenol.

the non-para substituted phenol, due to the impurities in the 4-chlorophenol, which has a purity of >98%.

The selectivity of the color assay toward phenol detection in the presence of 4-chlorophenol was evaluated. The concentrations of phenol and 4-chlorophenol were adjusted to 0, 5, 10, 25, and 50 μM, the same as in the previous standard curve; and the full factorial design was applied to investigate the assay's selectivity. Standard curves, having different concentrations of 4-chlorophenol, are presented in Fig. 2. The slope of the standard curves were $7.61 \pm 0.08 \times 10^{-3}$, and the slope did not show a positive correlation with added 4-chlorophenol. In this sense, it was demonstrated that the selectivity of the color assay adequately distinguished phenol in the presence of 4-chlorophenol.

The response surface obtained from full factorial design is presented in Fig. S1. The derived equation is given in Eq. (5).

Absorbance at 680 nm

$$= 7.56 \times 10^{-3} \cdot [\text{Phenol}] + 1.19 \times 10^{-4} \cdot [\text{4-chlorophenol}]$$

$$+ 3.25 \times 10^{-6} \cdot [\text{Phenol}] \cdot [\text{4-chlorophenol}]$$

$$+ 1.21 \times 10^{-2} (R^2 = 0.9960) \tag{5}$$

Absorbance at 680 nm

$$= 7.62 \times 10^{-3} \cdot [\text{Phenol}] + 1.43 \times 10^{-2} (R^2 = 0.9954) \tag{6}$$

The *p*-value of the model is <0.0001, which indicates that the derived equation is significant. The *p*-value of model terms, [Phenol], [4-chlorophenol], and [Phenol]·[4-chlorophenol] are <0.0001, 0.1008 and 0.5830, respectively. Since model terms having *p*-value greater than 0.1000 indicates insignificant model terms, the term [Phenol] is the only significant model term in the equation. Therefore, the model can be rearranged as Eq. (6), and the slope is in the range of the values obtained using the individual standard curves with various 4-chlorophenol concentrations. Therefore, it can be concluded that the indophenol reaction used in this study exhibits high selectivity toward phenol, in the presence of 4-chlorophenol.

3.2. Reducing reactivity of nZVI for reduction of 4-chlorophenol to phenol

The reactivity of several nZVIs was evaluated through a 4-chlorophenol reduction test. Dried nZVI powder was applied under buffered and un-buffered conditions, with initial pH's of 3, 5, and 7.
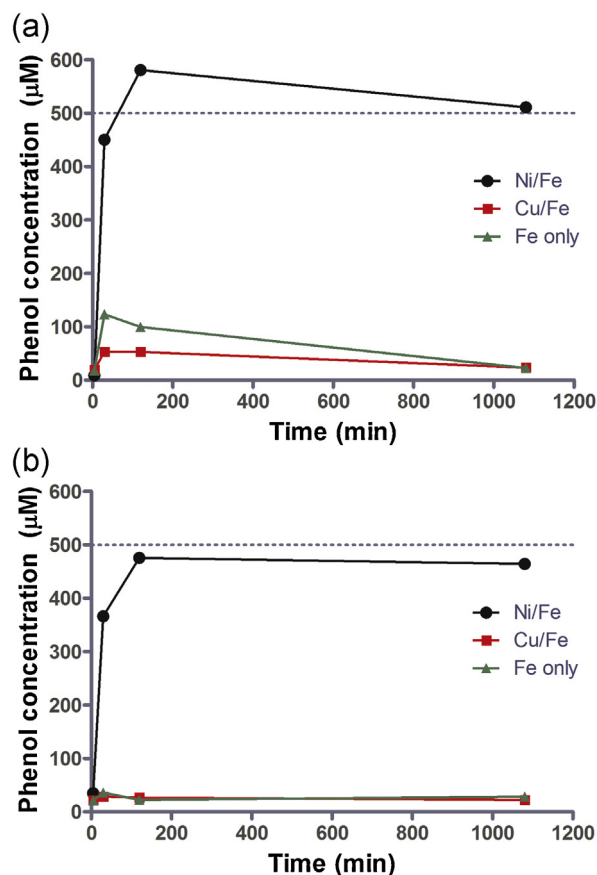

Fig. 3. Effect of the second metal on the 4-chlorophenol reduction by nZVI (a) without sample pretreatment, and (b) with sample pretreatment. Dotted line indicate theoretical yield.

Phenol production was not detected in any sample. The slurry form of nZVI, i.e. without drying, was also tested under the same conditions as with dried nZVI powder. However, no phenol production was detected in any sample.

Bimetallic nZVI particles have been reported to have superior reactivity for 4-chlorophenol reduction compared to monometallic nZVI [8]. In this study, co-reduction of both metal precursors and stepwise-reduction of metal precursors were tested. Fig. S2 presents the phenol concentration in the case of co-reduction. Since the initial concentration of 4-chlorophenol was 500 μM, the theoretical maximum of produced phenol would be 500 μM as explained by Li et al. [15]. The produced phenol after 2 h of reaction time was 30.5% of the theoretical maximum. Therefore, it is determined that certain types of nZVI, specifically Ni/Fe prepared in this study, have an affinity for reduction reactivity with 4-chlorophenol, which can be detected by the suggested method.

Being that the standard redox potential of the second metal, nickel in this case, is higher than iron; it could be assumed that nickel would mainly be located in the core of bimetallic particles as it reduces first. Given this fact, the stepwise-reduction of iron and nickel was performed to position nickel on the surface of the nZVI, which can subsequently promote the surface reaction of nickel. Fig. 3(a) presents the phenol concentration through 4-chlorophenol reduction by monometallic and stepwise-reduced bimetallic Ni/Fe and Cu/Fe. As is illustrated in Fig. 3(a), the Ni/Fe exhibits an ability to produce phenol, whereas Cu/Fe and monometallic Fe exhibit limited production of phenol.

In general, the enhancement of dehalogenation activity by a bimetallic system would be understood as a combination of galvanic effects and surface properties of the second metal. A higher

standard redox potential of the second metal would develop a galvanic cell where iron corrosion accelerates. In addition, some dopant metals, e.g., Pd, are also well-known hydrogenation catalysts [24]. In fact, the enhanced reactivity of Pd/Fe nanoparticles for dehalogenation hydrogenation of organic contaminants has been previously well reported [9,25]. Hydrogenation occurring on the surface of the second metal is outlined in reactions Eqs. (7)–(9) [26]. The second metal can enhance the formation of active hydrogen on the particle surface (Eq. (8)) and the reactive hydrogen generation depends on the absorption capacity of the second metal [10]. Generation of different degradation products in different bimetallic systems has been previously reported and was explained as the difference in the affinity of the contaminant on the metal [22,27].

$$Fe \rightarrow Fe^{2+} + 2e^- \tag{7}$$

$$2H^+ + 2e^- \rightarrow 2H^* \tag{8}$$

$$RX + 2H^* \rightarrow RH + HX \tag{9}$$

The results outlined that nickel has the ability to reduce 4-chlorophenol, as described in Eqs. (7)–(9). It was previously reported that Ni is a suitable second metal for 4-chlorophenol degradation, which can provide reducing reactivity for atomic hydrogen production [8,17]. The higher catalytic reactivity of Ni was also reported in several studies on 1,1,1-trichloroethane reduction [25] and hydrogen generation in the reaction with water [28]. Therefore, it was detailed that the modified indophenol reaction coupled with 4-chlorophenol, as suggested in this study, can selectively determine reactive nZVI that can reduce 4-chlorophenol.

However, the phenol concentrations in the Ni/Fe samples were higher than the theoretical maximum. Furthermore, some development of color was observed in the case of Cu/Fe and monometallic Fe, as well. It could be explained with adsorption spectra in the range of 400–800 nm, as is detailed in Fig. 4(a). Immediately after reagent addition, precipitates, as well as a strong yellow color in the solution, were noticed; and, consequently interfered with the absorbance measurement at 680 nm, which was used for the assay. As the sample taken after 5 min of reaction time did not show significant color development by interference, it was hypothesized that the changing of pH or residual soluble iron ions in the sample could bring interference on the suggested color assay.

3.3. Sample pretreatment using carbonate to minimize interference from residual iron ions

3.3.1. Effect of pH and residual iron ions on the phenol determination

In order to investigate the interference caused by the sample matrix, the effect of pH and residual iron ions on the color development was evaluated. Plotted in Fig. S3(a), the color development is stable in the pH range of 2.3 through 11, with 20 μM of phenol solution. The absorbance at 680 nm was measured to be 0.1736 $\pm$ 0.0008, and could be explained by the presence of a sufficient amount of alkali in the reagent. When the reagent is mixed with the sample, the total concentration of sodium hydroxide is 13.2 mM, enough to bring the solution to a basic condition. Searle [19] previously reported that the indophenol assay was stable in basic pH conditions, with an optimum pH range of 10.4–13.1. Therefore, it was clear that the effect of pH could be minimized by supplying a sufficient amount of alkali, depending on the buffer used in the reactivity test.

On the other hand, the clear positive interference from residual iron ions was observed, and is detailed in Fig. 4(b). At concentrations up to 100 mg/L of ferric ion (Fe^{3+}), the measured absorbance was stable; however, the interference drastically increased, which can be observed in Fig. S4. Due to the development of color and turbidity by soluble iron, the absorbance measurement at the peak
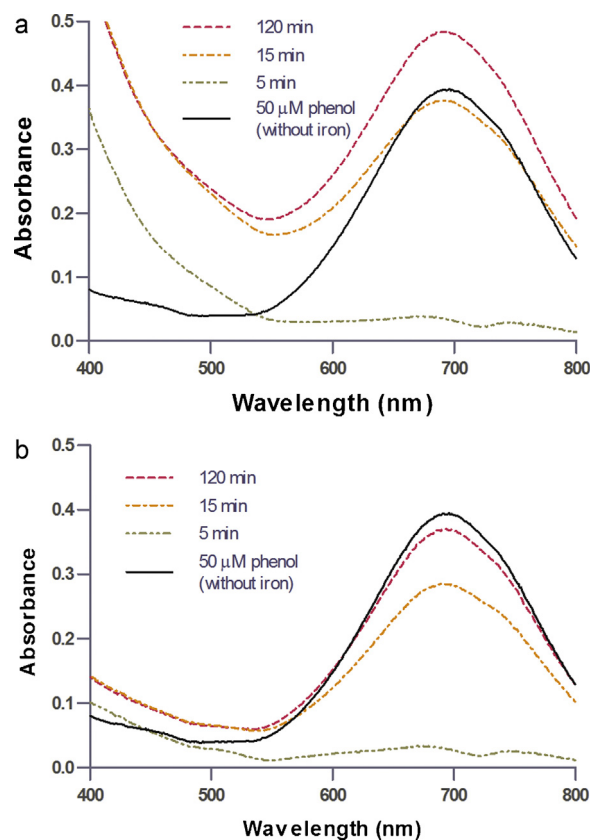

Fig. 4. Spectra change by sample pretreatment using Na_2CO_3 (reagent C) (a) without (b) with addition of Na_2CO_3.

representing phenol was interfered. The iron concentration having significant interference corresponded with the measured iron concentration in samples from the 4-chlorophenol reduction test (192.8 $\pm$ 9.7 mg/L). Some metals were reported to have interference on the indophenol reaction, if present in sufficient amounts [19]; in particular, iron was reported as having a positive interference on the indophenol reaction [27]. Therefore, it was concluded that sample pretreatment to minimize residual iron ions was required, in order to develop a suitable color assay for nZVI reactivity determination.

3.3.2. Performance of sample pre-treatment by carbonate for residual iron ions removal

Sample pretreatment with carbonate solution was used to remove residual iron ions in the sample, by adding 250 μL of 1 M Na_2CO_3 solution to 5 mL of sample. Sample color changed from a greenish yellow to a dark green, indicating the formation of iron carbonate. The produced iron carbonate precipitates in the bottom of the test tube; resultantly, a clear supernatant could be obtained after 30 min.

Fig. 4 illustrates the changes of spectra by this pre-treatment, and a positive bias with the absorbance at 680 nm could be observed in the untreated sample. On the other hand, a stable baseline could be achieved by the suggested pre-treatment. Referentially, the spectrum of the 50 μM phenol solution is also illustrated in Fig. 4(b). The similar trend of a baseline confirmed the effectiveness of pre-treatment for residual iron removal.

An additional experiment was performed in order to investigate the interference of buffers on the color assay. Commonly, both buffered and un-buffered conditions, have been applied in studies of nZVI reactivity. Buffered conditions have been used for ensuring a stable pH condition during the reaction [9], while un-buffered
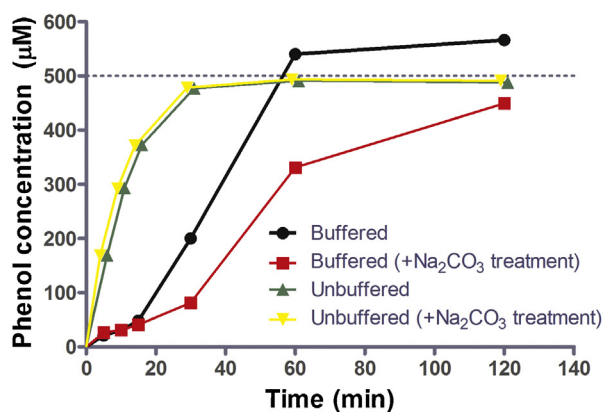
Fig. 5. Measured phenol production by Ni/Fe under different buffered condition. Dotted line indicate theoretical yield.

conditions have also been frequently applied, due to the ease of reaction control [19]. However, each condition brought a considerably different nature of resultant sample, in terms of buffering capacity, soluble iron ions concentration, form of iron, etc.

Fig. 5 plots the measured phenol production by stepwise-reduced Ni/Fe under different buffered conditions, with and without sample pretreatment. A stable baseline of the spectrum could be achieved by pretreatment, and reasonable phenol concentration was detected. The maximum concentration of detected phenol after 2 h of reaction was 490.9 mM. Therefore, 98.2% of phenol existed in aqueous phase. The only remained 1.8% could be either unreacted 4-chlorophenol or adsorbed phenol.

Depending on buffered condition significance of pretreatment was varied. In case of the condition buffered with 10 mM acetate, the same pattern was obtained as was observed in the long term test, plotted in Fig. 3. A significant difference of absorbance by pretreatment was observed after 30 min of reaction. It revealed the necessity of pre-treatment for residual iron ions removal, since the total iron concentration was measured as 218.5 ± 28.0 mg/L in the filtered samples. This high concentration soluble iron ions was attributed to the acidic pH (3.5–4.0) maintained during the reaction under buffered condition.

Contrastingly, the change of absorbance by pretreatment was negligible under un-buffered condition. High pH could be expected under un-buffered conditions, due to hydroxide produced in the reaction between nZVI and 4-chlorophenol, as described in Eq. (9). Consequently, an iron hydroxide coating could be generated in this high pH environment, which can prevent the loss of iron in the soluble ionic form [29]. The low concentration of soluble iron ions concentration in the filtered sample (7.9–19.2 mg/L) ensured the insignificant positive interference on the color development.

This suggested pretreatment scheme is strongly related with iron speciation. Since alkaline condition was provided by adding carbonate, iron would be insoluble. Moreover, the redox condition plays a major role on iron speciation. When sample was taken from sealed reactor, it turned oxic condition which make more insoluble Fe(III) precipitation. By adding excess carbonate it is ensured that the pH becomes high enough that both Fe(II) and Fe(III) will not stay in solution. Additionally the $FeCO_3$ quickly forms heavy crystal grains that adds weight to the fluffy Fe(III)hydroxides so they settles in a reasonable time. Since the interference caused by pH and residual iron can be eliminated by carbonate treatment of the sample, it is therefore suggested to use carbonate pretreatment as a standard procedure for ensuring a high pH and a low iron ions concentration, which is favorable for the indophenol reaction.

4. Conclusion

In this study, the modified indophenol reaction that is originally used for ammonia detection was suggested for phenol detection in the mixture of 4-chlorophenol and phenol. Since 4-chlorophenol can be reduced to phenol by nZVI and 4-chlorophenol cannot be detected by the indophenol reaction, the simple colorimetric assay for reducing reactivity determination of nZVI to organically bound halogens was developed, using 4-chlorophenol as the model substrate. The suggested color assay showed superior selectivity toward phenol in the presence of 4-chlorophenol, which showed the applicability of the assay based on the suggested purpose.

The sensitivity and selectivity of the color assay for phenol detection was investigated. Being that a shift in the peak absorbance was observed at the concentration range of 100–400 μM, the appropriate concentration range for analysis was determined to be 0–50 μM with peak absorbance of 680 nm. The suggested color assay displayed superior selectivity toward phenol in the presence of 4-chlorophenol, which demonstrated the feasibility of the assay, as suggested.

However, the high concentration of soluble iron ions produced throughout the reaction caused a positive interference on absorbance, due to baseline increase on the spectra. Addition of Na_2CO_3 as a sample pretreatment was suggested to eliminate the effect of residual iron in the color assay. The residual iron ions in the sample were successfully removed by precipitation after 30 min. Additionally, experiment results revealed that the addition of Na_2CO_3 served to eliminate interference from buffers used in reactivity experiments at different pHs.

Among several types of nZVI applied, the bimetallic Ni/Fe, produced either by co-reduction or stepwise-reduction, showed the production of phenol, indicating a promotion of reducing reactivity by nickel via production of atomic hydrogen with electrons supplied by the Fe core. Throughout this study, the 4-chlorophenol based reactivity assay using the modified indophenol reaction successfully determined reducing reactivity of nZVI in varied experimental conditions. Further optimization with microplate analysis will increase the simplicity and applicability of the suggested method for testing nZVI reactivity.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apcatb.2014.10.059.

References

[1] R.A. Crane, T.B. Scott, J. Hazard. Mater. 211–212 (2012) 112–125.
[2] C.B. Wang, W.X. Zhang, Environ. Sci. Technol. 31 (1997) 2154–2156.
[3] N. Saleh, K. Sirk, Y. Liu, T. Phenrat, B. Dufour, K. Matyjaszewski, R.D. Tilton, G.V. Lowry, Environ. Eng. Sci. 24 (2007) 45–57.
[4] H.-J. Kim, T. Phenrat, R.D. Tilton, G.V. Lowry, Environ. Sci. Technol. 43 (2009) 3824–3830.
[5] H. Dong, I.M. Lo, Water Res. 47 (2013) 419–427.

[6] A. Tiraferri, K.L. Chen, R. Sethi, M. Elimelech, J. Colloid Interface Sci. 324 (2008) 71–79.
[7] N. Sakulchaicharoen, D.M. O'Carroll, J.E. Herrera, J. Contam. Hydrol. 118 (2010) 117–127.
[8] W.-H. Zhang, X. Quan, Z.-Y. Zhang, J. Environ. Sci. 19 (2007) 362–366.
[9] Y. Liu, G.V. Lowry, Environ. Sci. Technol. 40 (2006) 6085–6090.
[10] R.J. Barnes, O. Riba, M.N. Gardner, T.B. Scott, S.A. Jackman, I.P. Thompson, Chemosphere 79 (2010) 448–454.
[11] Y. Liu, S.A. Majetich, R.D. Tilton, D.S. Sholl, G.V. Lowry, Environ. Sci. Technol. 39 (2005) 1338–1345.
[12] S. Bae, W. Lee, Environ. Sci. Technol. 48 (2014) 2368–2376.
[13] R. Cheng, J.-L. Wang, W.-X. Zhang, J. Hazard. Mater. 144 (2007) 334–339.
[14] P.J. Dorathi, P. Kandasamy, J. Environ. Sci. 24 (2012) 765–773.
[15] Y. Li, Y. Zhang, J. Li, G. Sheng, X. Zheng, Chemosphere 92 (2013) 368–374.
[16] P.L. Searle, Analyst 109 (1984) 549–568.
[17] A. Afkhami, R. Norooz-Asl, J. Braz. Chem. Soc. 19 (2008) 1546–1552.

[18] Y. Hwang, Y.-C. Lee, P.D. Mines, Y.S. Huh, H.R. Andersen, Appl. Catal. B: Environ. 147 (2014) 748–755.
[19] Y.H. Liou, S.-L. Lo, C.-J. Lin, W.H. Kuan, S.C. Weng, J. Hazard. Mater. 127 (2005) 102–110.
[20] F. Alonso, I.P. Beletskaya, M. Yus, Chem. Rev. 102 (2002) 4009–4091.
[21] A. Ghauch, A. Tuqan, Chemosphere 73 (2008) 751–759.
[22] Y.H. Hwang, D.G. Kim, H.S. Shin, J. Hazard. Mater. 185 (2011) 1513–1521.
[23] W.-J. Liu, T.-T. Qian, H. Jiang, Chem. Eng. J. 236 (2014) 448–463.
[24] B.P. Chaplin, M. Reinhard, W.F. Schneider, C. Schuth, J.R. Shapley, T.J. Strathmann, C.J. Werth, Environ. Sci. Technol. 46 (2012) 3655–3670.
[25] D.M. Cwiertny, S.J. Bransfield, K.J. Livi, D.H. Fairbrother, A.L. Robertst, Environ. Sci. Technol. 40 (2006) 6837–6843.
[26] K.-F. Chen, S. Li, W.-X. Zhang, Chem. Eng. J. 170 (2011) 562–567.
[27] J.A. Russell, J. Biol. Chem. 156 (1944) 457–462.
[28] C.J. Lin, S.L. Lo, Y.H. Liou, J. Hazard. Mater. 116 (2004) 219–228.
[29] G.C.C. Yang, H.-L. Lee, Water Res. 39 (2005) 884–894.