



Graduated characterization method using a multi-well microplate for reducing reactivity of nanoscale zero valent iron materials



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ABSTRACT

Even though nanoscale zero valent iron (nZVI) has been intensively studied for the treatment of a plethora of pollutants through reductive reaction, quantification of nZVI reactivity has not yet been standardized. Here, we adapted colorimetric assays for determining reductive activity of nZVI and its composites with other metals. The assay quantifies reduction products to avoid interfering reactions, such as sorption and volatilization. Three different reaction products, ammonium, phenol, and aniline, generated as the result of reduction of nitrate, *p*-halophenols, and nitrobenzene, respectively, could be quantified using the same reagent for all reactions. The colorimetric assays were further adapted to the 96-well microplate format, thus minimizing sample and reagent use, as well as lowering color development time to 2 h. The substrates showed graduated reactivity, and thus, reduction potency and kinetics of different materials and reaction mechanism was distinguished. The applicability was successfully proven by determining the reactivity of a commercial nZVI sample, and investigating the effect of nickel content on dehalogenation. Therefore, the suggested reactivity test with different compounds, combined with the use of a multi-well microplate based color assay, promises to be a useful and simple tool in various nZVI related research topics.

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1. Introduction

Nanoscale zero valent iron (nZVI) has been intensively studied for environmental remediation, due to its strong reductive power and reactivity, which is derived from its small size and high specific surface area [1]. Its standard redox potential ($E^0 = -0.44$ V) makes nZVI an effective material when reacting with reducible contaminants. The removal mechanism by nZVI involves the direct transfer of electrons to the contaminants, transforming them into non-toxic, less toxic, or more biodegradable species [2,3]. A wide variety of pollutants, including halogenated organics, nitro aromatic compounds, heavy metals, and oxy-anions, have been successfully remediated, by utilizing the reduction reaction mechanism [4].

Although a number of different nZVI materials have been prepared and compared, across many intensive studies, different properties of prepared nZVIs make it hard to compare and standardize. Depending on the aim of research and laboratory

environment, the synthesis conditions of nZVI can be significantly varied; and, with it brings about considerably different nZVI properties. The effect of synthesis conditions, such as reduction time and precursor concentration, on nZVI characteristics and reactivity has been previously investigated [5]. It has also been determined that nZVI properties can be greatly influenced by solvent conditions during synthesis [6,7]. Moreover, surface modification of nZVI has been intentionally performed, in order to enhance nZVI properties desirable for environmental remediation. In order to prevent aggregation for in situ subsurface remediation, particle stabilization or nZVI immobilization on various support materials has been achieved in many studies [8,9]. Recently, remarkable enhancement of stability and reactivity was reported by applying an organo-functionalized clay, i.e. aminoclay [10,11]. Additionally, bimetallic particles with iron as the primary metal and the deposition of a thin layer of noble or transition metals, such as palladium (Pd), copper (Cu), nickel (Ni) or platinum (Pt) on the iron surface have been frequently applied to enhance the reactivity of nZVI, especially for reductive dehalogenation [12–14].

However, the evaluation of nZVI reactivity towards dehalogenation has not yet been standardized. The use of advanced

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chromatographic techniques has been the predominant method to determine the treatment target and subsequent degradation products [15,16]. These techniques are powerful tools to analyze the compounds, both qualitatively and quantitatively. At the same time, it generally requires lengthy analysis procedures and complicated set-ups. In this light, a simple color assay for use in laboratories, requiring only a basic spectrophotometer for determining color intensity would be a very useful tool.

In our previous study, the modified indophenol method for detecting phenol, which is the result from dehalogenation of 4-chlorophenol, was suggested to determine nZVI reactivity [17]. The aforementioned method was successfully applied to determine dehalogenation reactivity of lab synthesized monometallic and bimetallic nZVI. Throughout the previous study, analysis was focused on determination of the reduction product, rather than the treatment target. This gave a more accurate observation of the reducing activity of nZVI, due to the fact that other reactions, e.g., sorption, oxidation, or co-precipitation cannot interfere. In this study, an extended range of substrates, i.e. nitrate, nitrobenzene, and other halophenols (4-bromophenol and 4-iodophenol), was applied to give better understanding of nZVI reactivity. It was hypothesized that the reduction kinetics and capacity of these substrates would be different, depending on the involved redox reaction and chemical bond. Therefore, the graduated reducing reactivity towards different reducible compounds can be used to determine reduction kinetics and capacity.

Herein, the colorimetric assay to determine nZVI reactivity was further developed and optimized through a multi-well microplate based analysis. Firstly, the previously developed phenol detection method was further miniaturized and optimized for quantification in a 96-well microplate. The expanded range of target compounds, e.g., nitrate, nitrobenzene, and para-positioned halogenated phenols, which can be analyzed by the same reagent to create color, was applied, and subsequently optimized in the same manner. The range of target compounds provided a graduated characterization of reducing activity, with respect to our previously published method using 4-chlorophenol. Lastly, the applicability of the suggested method for investigation on the effect of different factors during the reaction and production of nZVI was demonstrated. Based on the overall results, a simple and effective assay to determine nZVI reactivity is suggested.

2. Methods

2.1. Color assay for analyzing reaction products

In this study, the same regime of reagents was applied to ammonium, phenol, and aniline to create a colored product. The use of similar reagents gives simplicity and versatility on analyzing several reaction products at the same time. The following sections describe in-detail procedures of the color assays.

2.1.1. Ammonium analysis as a product of nitrate reduction

The indophenol reaction is the name given to the reaction of monochloramine and a phenol, which results in the formation of a blue indophenol dye, which can then be determined spectrophotometrically [18,19]. This reaction is highly specific for ammonium, and in the presence of a suitable catalyst, extremely high sensitivity is attainable. Ammonium analysis was conducted according to standard methods, with a slight modification (4500-NH₃-D) [20]. Basically, this consisted of ammonium measurement, using ammonium as a limiting reagent, with excess amounts of phenol and hypochlorite being applied to ensure all ammonium forms indophenol, when catalyzed by sodium nitroprusside. 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 deionized water. Reagent B was prepared as 26 g/L of phenol and 0.3 g/L of sodium nitroprusside. Samples of 2.3 mL are transferred to a cuvette and 0.1 mL of each reagent is added to the sample. The mixture is then allowed to react in the dark for 2 h and subsequently quantified with a UV-vis spectrophotometer (Cary 50, Agilent Technologies Inc., USA) at 630 nm.

2.1.2. Phenol analysis as a product of halogenated phenol reduction

In our previous study, phenol detection by the indophenol reaction was successfully developed by simple substitution of phenol as the limiting substrate in the entire reaction [17]. In this color assay, phenol acted as the limiting reagent, and excess amounts of ammonium and hypochlorite were applied, in order to have the color development dependent on the concentration of phenol in samples. For the color assay, reagent A was modified to have a lower concentration of hypochlorite, which was optimized as described in Section 2.2.2. Reagent A was prepared by diluting 0.2 mL of 10% hypochlorite solution and 66 mL of 0.5 M NaOH solution to 100 mL with deionized water. Reagent C was prepared as 100 mg-NH₄⁺-N/L of ammonium chloride and 0.3 g/L of sodium nitroprusside in deionized water. A drop of hydrochloric acid was added into Reagent C to minimize ammonia stripping into the headspace. Equal volumes of reagents A and C were pre-mixed to make the reagent AC. Then, 2.3 mL of sample was transferred into a cuvette, and 0.2 mL of reagent AC was added to the sample. The mixture was allowed to react in the dark for 2 h and subsequently quantified with a spectrometer at 680 nm.

2.1.3. Aniline analysis as a product of nitrobenzene reduction

Reagents A and C were used for aniline analysis, as described in Section 2.1.2. In short, 0.1 mL of reagent A was added to 2.3 mL of the sample, and subsequently 0.1 mL of reagent C was added after 15 min of reaction. The mixture allowed to react in the dark for 2 h and then quantified with a spectrometer at 680 nm.

2.2. Miniaturization and optimization for quantification in 96-well microplate

2.2.1. Use of multiwell plate and plate reader for simple analysis

The multi-well microplate was applied in order to simplify the analysis procedure. Using a multi-well microplate offers the advantage of reading multiple samples with an automated plate reader and having a small sample volume for quantification. In this study, the samples were prepared in a 96-well plate (Nunc polypropylene flat bottom microwell plate, Thermo Fisher Scientific Inc., USA) and were measured over the range of 400–800 nm, using a Synergy MX microplate reader (BioTek Instruments Inc., USA). The 96-well plates can hold a maximum volume of approximately 400 μ L. In our assays, the volume of the sample was 230 μ L and 10 μ L for each of the reagents, which is an order of magnitude less volume than when using a normal cuvette. The remaining space in the well was assessed as sufficient for mixing of the mixture.

2.2.2. Optimization of reaction conditions

In order to evaluate the optimal conditions for the reagents, we tested the color assay at different concentration levels of active chlorine for reagent A, 0.01–0.4% active chlorine, through adding 0.1–4 mL of 10% hypochlorite solution in 100 mL reagent A and ammonium for reagent C (50–5000 mg-NH₄⁺-N/L). The reaction time was also further optimized by obtaining the calibration curve at different time intervals, up to 24 h. Since ammonium analysis has already been standardized and optimized as a standard method, further optimization was not considered.

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