Effect of pH on Fe⁰ and Anaerobic Microorganism Combined Degradation 2,4-Dichlorophenol

Ting Cheng, Chen Chen, Xiao Zhang, Wei-Fang Xie and Hai-Xia Liu

ABSTRACT.

Batch laboratory tests were conducted through inoculating the anaerobic mixed microorganism, to examine the effect of different initial pH on the process of anaerobic biodegradation of 2,4-dichlorophenol (2,4-DCP) in the presence of zero-valent iron (Fe⁰). Furthermore, the degradation mechanism of the combined system was investigated. The results showed that, The combined use of Fe⁰ and anaerobic microorganism could accelerate the degradation of 2,4-DCP. Different pH value had a significant impact on the 2,4-DCP degradation of the combined system. The degradation effect was better in neutral or slightly alkaline condition. The optimum initial pH for the degradation of target contaminant was 9 in the combined system. The OH⁻ from Fe⁰ anaerobic corrosion balanced the organic acid produced by fermenting of glucose effectively, that made the concentration of volatile fatty acid (VFA) remain at a lower level. The corrosion of iron was strong in acidic condition. Ferrous ion was the main corrosive product and the content of ferric ion was lower. Furthermore, the removal rate of COD in combined system at different pH had a relation to the biodegradation of contaminant in some extent.

INTRODUCTION

2,4-Dichlorophenol is an important chemical raw materials. Meanwhile, it is a kind of difficult degradation of environmental toxicity, persistent organic pollutants [1,2]. 2,4-DCP is included in the United States Environmental Protection Agency (USEPA) and the State Environmental Protection Administration of priority control of pollutants. Because of their aromatic ring structure and the presence of chlorine atoms, chlorophenols are highly toxic and anti-oxidative degradation [3,4]. In nature, chlorophenols are easily accumulated in the soil and organic matter in the sediment.
In the anaerobic environment, reductive dechlorination is the most potential technique for remediation of contaminated soil point and groundwater\cite{5-7}.

In recent years, another kind of reductive dechlorination technique, zero valent iron reduction of chlorinated organic compounds, got more attentions. Zero valent iron was readily available, inexpensive and non-toxic. The technology was used to repair the pollution point of chlorinated organic compounds \cite{8,9}. In the presence of a proton donor, reductive dechlorination of chlorinated organic compounds occurred on the surface of the metal iron. Equation was shown below, \( \text{Fe}^0 + \text{R-X} + \text{H}^+ = \text{Fe}^{2+} + \text{R-H} + \text{X}^- \). However, the corrosion of iron produced cathodic hydrogen under anaerobic condition as indicated below in equation, \( \text{Fe}^0 + 2\text{H}_2\text{O} = \text{Fe}^{2+} + 2\text{OH}^- + \text{H}_2 \). Cathodic hydrogen could be used as an electron donor for hydrogen consuming microorganism, such as methanogens, sulfate reducing bacteria and nitrate-reducing bacteria \cite{10,11}. On the other hand, the reaction of \( \text{OH}^- \) can change the pH value in the combined system of \( \text{Fe}^0 \) and microorganism, and then affecting the degradation of pollutants. Researchers pointed out, using anaerobic microorganisms to degrade nitrobenzene, chlorinated aliphatic hydrocarbons and polychlorinated biphenyls (PCBs), while adding zero valent iron, can achieve satisfactory treatment effect \cite{11}. Under anaerobic conditions, the combined effect of microorganism and zero valent iron could effectively degrade chlorophenols had also been confirmed\cite{12,13}. However, Researches related to the effect of pH on the degradation of chlorophenols in the combined system of \( \text{Fe}^0 \) and anaerobic microorganisms were rare. The degradation mechanism was also to be revealed. In this study, we focused on the degradation of 2,4-dichlorophenol with \( \text{Fe}^0 \) and anaerobic microorganism. On the basis of this study, the effect of pH on the degradation of 2,4-DCP in the combined system of anaerobic microorganism and \( \text{Fe}^0 \) was investigated. To reveal the mechanism of combined system, change of pH and the corrosion of \( \text{Fe}^0 \) in the process of reaction were studied.

**EXPERIMENTAL**

**Microorganisms**

A mixed anaerobic sludge used for this research was developed from a full-scale internal circulation reactor treating dye wastewater. The anaerobic sludge was first fed with 6,000 mg of glucose per liter as the carbon source for two weeks, which enhanced the biological activity of anaerobic microorganism. This period lasted 60 days and the COD removal rate was over 80% before the batch experiments. Batch experiments were inoculated with mixed culture without acclimation.

**Experimental Setup**

*Batch experiment set-up*

Batch experiments were conducted using serum bottles (250ml) at 37°C. The microorganism was transferred to serum bottle with 50ml nutrient medium and 1ml trace element solution. The nutrient medium in bottle contained (mg l\(^{-1}\)): \( \text{KH}_2\text{PO}_4 \) 54, \( \text{K}_2\text{HPO}_4 \) 70, \( \text{NH}_4\text{Cl} \) 106, \( \text{CaCl}_2\cdot2\text{H}_2\text{O} \) 15, \( \text{MgCl}_2\cdot6\text{H}_2\text{O} \) 20; The trace element solution contained (mg l\(^{-1}\)): \( \text{CoCl}_2\cdot6\text{H}_2\text{O} \) 500, \( \text{NiCl}_2\cdot6\text{H}_2\text{O} \) 50, \( \text{Na}_2\text{SeO}_3 \) 50,
The anaerobic degradation of 2,4-DCP in “Fe⁰+cell”, “biotic cell” and individual Fe⁰ systems

An experiment was first conducted to investigate the effect of Fe⁰ on the anaerobic degradation of 2,4-DCP by a mixed culture. The batch experiment was carried out through shake serum bottle study, and bottle contained 2.0 g l⁻¹ iron fillings and a mixed culture. To compare the effect of individual culture on the anaerobic degradation of 2,4-DCP, biotic cell was introduced to the experiment separately, and the initial concentration of 2,4-DCP was 30 mg l⁻¹. Meanwhile, one bottle was prepared for individual Fe⁰. Bottle prepared for biotic culture treatments contained live microorganism and no Fe⁰, and Fe⁰ chemical treatments contained iron fillings and no live microorganism. The Initial pH was adjusted to 7.0 in all experiments. The biomass concentration in each bottle was approximately 335 mg l⁻¹ based on the volatile suspended solids (VSS) contents of anaerobic sludge. Bottles were sampled periodically for 2,4-DCP.

Effect of pH on 2,4-DCP degradation in “Fe⁰+cell” systems

Experiments were carried out to investigate the effect of pH on 2,4-DCP anaerobic degradation in the presence of Fe⁰. The initial pH was set up at 6.0, 7.0, 8.0, 9.0 and 10.0, respectively. Each bottle contained 2.0 g l⁻¹ iron fillings and 335 mg VSS l⁻¹ biomass. Bottles were spiked with 2,4-DCP stock solution and sampled periodically for 2,4-DCP. In addition, to study the complicated degradation mechanism of Fe⁰ and anaerobic microorganism at different pH, the corrosion of Fe⁰ under anaerobic conditions was investigated. Change of pH during the reactive process and the production of iron ion in “Fe⁰+cell” systems were evaluated by monitoring pH, total iron ion, ferrous iron (Fe²⁺), ferric iron (Fe³⁺) and the concentration of the chemical oxygen demand (COD). Meanwhile, experiments were carried out to evaluate the fluctuation of volatile fatty acid (VFA) during the reactive process in “Fe⁰+cell” system and individual biotic cell system.

Analytical Methods

The sample was taken from bottle using a glass syringe. Analysis for 2,4-DCP was performed using a Agilent LC-1260 HPLC system, equipped with a Lichrospher C18 inverse phase column. An L-2400 UV detector was used for the analysis and the detection wavelength was 290 nm. The HPLC mobile phase was the mixture of purified water (15%) and methanol (85%) at flow rate of 1.0 ml min⁻¹. The injection volume was 10μL with an auto-sampler. Prior to HPLC analysis, sample solutions were filtered by 0.45μm membrane. The pH was determined by
RESULTS AND DISCUSSION

Effect of Fe0 on the Anaerobic Degradation of 2,4-DCP

Fig. 1 showed the degradation of 2,4-DCP in “Fe0+cell”, “Biotic cell” and individual Fe0 systems. Fig. 1 showed individual Fe0 had some chemical reduction to 2,4-DCP. In the reaction time of 70 hours, the degradation rate of 2,4-DCP was 12.8%. When the reaction time was extended to 260 hours, the degradation rate of 2,4-DCP was slightly increased, the 2,4-DCP degradation rate was 22.3%. Individual biotic cell had the potential of degrading 2,4-DCP, and the biotic cell began to degrade 2,4-DCP almost without lag phase.

In the degradation period of 70 hours, the degradation rate of 2,4-DCP in biotic cell system was 26.4%. The 2,4-DCP degradation rate was improved obviously with the increasing of reaction time. Fig. 1 also showed that the effect of Fe0 stimulated the anaerobic degradation of 2,4-DCP. The 2,4-DCP degraded effectively in “Fe0+cell” system compared to the individual biotic cell or individual Fe0 use. In the degradation period of 70 hours, the degradation rate of 2,4-DCP in “Fe0+cell” system was 46%. The degradation rate of 2,4-DCP increased rapidly with the extension of reaction time. When the degradation period was extended to 240 hours, the 2,4-DCP was almost complete degradation.
Effect of pH on the Degradation of 2,4-DCP in “Fe0+cell” Systems

Effect of pH on the degradation of 2,4-DCP in the combined Fe0 and cell system was shown in Fig. 2. The degradation effect of 2,4-DCP in combined system was lower when the initial pH was 6. When the initial pH value was 6 to 9, with the increasing of initial pH value, the 2,4-DCP degradation efficiency improved gradually in the combined Fe0 and cell system. The degradation effect of 2,4-DCP in combined system was the best while the initial pH was 9. The target pollutant was almost completely degraded in 120 hours. When the initial pH was 7 and 8, the time required for the complete degradation of the target pollutant was 238h and 166h, respectively. However, when the initial pH value increased to 10, the degradation effect of the combined system on the target was reduced.

Change of pH and VFA(C) of the Reaction Process in “Fe0+cell” Systems

The corrosion of Fe0 in anaerobic environment produced OH-, which made the change of pH value in the system. Fig. 3 showed the change of pH during the reactive process in the combined Fe0 and microbial systems. When the initial pH value was 6, the pH of combined system increased gradually during the reaction process, and the final pH value stabilized at about 6.5. When the initial pH value was 7, the pH value of combined system was relatively stable and remained at about 7. When the initial pH value is 8 and 9, the pH value of the combined system decreased firstly, and then tended to be stable, and the endpoint pH value stabilized at 7.2 to 7.4. When the initial pH value is 10, the pH value of the combined system was higher and stabilized at about 8.3. In addition, the pH value of individual microbial system stabilized at about 6.5 during the reaction process when the initial pH value was 7. Compared to the individual microbial system, the pH value of the combined system was always higher during the reaction process.

Figure 2. Effect of pH on the degradation of 2,4-DCP in “Fe0+cell” systems.

Figure 3. Change of pH during the reactive process in “Fe0+cell” system.
Fig. 4 showed the variation of VFA concentration during the reactive process in the combined and individual microbial system. The concentration of VFA in the combined system during the reaction process was always higher than that of the individual microbial system. In individual microbial system, a large amount of organic acids were generated through the glucose fermentation in a relatively short time, and the VFA concentration maintained at a high level. When the reaction time was 95 hours, the VFA concentration was up to 1189 mg/L. The corrosion of Fe\(^0\) occurred in a relatively short time under anaerobic environment in the combined system of Fe\(^0\) and microorganism. The product of OH\(^-\) could effectively balance the organic acids produced by fermentation of glucose. That made the VFA concentration of the combined system maintained at a lower level. Furthermore, the product of OH\(^-\) made the pH value of the combined system to rise and tended to be stable (Fig. 3). When the initial pH value of the combined system was 9, the concentration of VFA was always lower than the system of initial pH value of 7. That indicated the product of OH\(^-\) from Fe\(^0\) corrosion could balance the organic acids effectively in the combined system of initial pH value of 9. And the pH value was stable, thus contributed to the degradation of the 2,4-DCP (Fig. 2). In addition, the experimental results of the combination of Figure 1 and Figure 2 showed that, the 2,4-DCP degradation effect in partial alkaline or neutral environment of combined system was better than that of the acidic environment. However, high pH value was not conducive to the degradation of the target pollutant. That suggested the degradation of 2,4-DCP had its proper pH value, and the optimum pH value was between 7.2 to 7.4.

![Figure 4](image)

**Figure 4.** Variation of VFA concentration during the reactive process in “Fe\(^0\)+cell” and individual microbial systems.

**The Concentration of Iron Ions at the End of Reaction in “Fe\(^0\)+cell” Systems**

In addition to generate OH\(^-\), the corrosion of Fe\(^0\) in anaerobic environment also produced ferrous iron (Fe\(^{2+}\)) and ferric iron (Fe\(^{3+}\)). Fig. 5 showed the concentration of total iron, ferrous iron and ferric iron at the end of the reaction in the combined of Fe\(^0\) and microbial systems. The corresponding corrosion product of Fe\(^0\) in anaerobic conditions was mainly ferrous iron, and the concentration of ferric iron was less. When the initial pH was between 6 and 9, the concentration of Fe\(^{2+}\) was greater than 50 mg/L, and the Fe\(^{3+}\) concentration was less than 15 mg/L. The corrosion of Fe\(^0\) was closely related to the pH value.
Fig. 5 indicated with the increasing of initial pH value, the corrosion amount of Fe$^0$ decreased gradually. When the initial pH value was 6 and 7, the combined systems produced a large amount of Fe$^{2+}$, and the Fe$^{2+}$ concentration at the end of reaction was greater than 80 mg/L. The concentration of Fe$^{2+}$ was between 50 and 60 mg/L when the initial pH value of the combined systems was 8 and 9. However, the corrosion of Fe$^0$ was less when the initial pH value was 10, and the Fe$^{2+}$ concentration was about 40 mg/L. According to the change of pH value during the process of the combined systems in Figure 3, the pH value maintained at 6.5 to 7 when the initial pH value was 6 and 7, which was beneficial to the corrosion of Fe$^0$. When the initial pH was 8 and 9, the pH value of combined systems maintained at partial alkaline environment, and the corrosion of Fe$^0$ was relatively slow.

**Anaerobic Biodegradability of Organic Compounds in “Fe$^0$+cell” Systems**

By measuring the change of COD value of the reaction end point, the anaerobic biodegradability of organic compounds in the combined system of Fe$^0$ and microbial systems was determined in an indirect way. Fig. 6 showed the COD removal efficiency in the combined system of Fe$^0$ and microbial systems. Fig. 6 indicated the removal rate of COD had a certain correlation with the degradation effect of 2,4-DCP at different initial pH value (Fig. 2). The COD removal efficiency was higher in the combined systems when the initial pH value was between 7 and 9, and that was greater than 60%. The COD removal rate was up to 82% when the initial pH value was 9. However, when the initial pH was 6 and 10, the COD removal efficiency was lower, which was 45% and 39% respectively. The pH value of combined system maintained at acidic environment during the reaction process when the initial pH was 6 (Fig. 3). Combined with the variation of VFA concentrations of Figure 4, it suggested the accumulation of organic acids in acidic environment leads to lower COD removal rate. When the initial pH value was 10, the higher pH value of the combined system was not conducive to the degradation of the target pollutant.

The partial alkaline environment was conducive to the metabolism of microorganism. The low molecular organic acids produced by metabolism were easy to be used by other microorganism, and achieved higher COD removal rate. In addition, the COD removal rate of individual microbial system was determined, which was 43% (not shown in Fig. 6) and lower than the combined system when the initial pH value was 7. Rosenthal et al. indicated the transformation efficiency of PCE and its intermediate products in the combined system of Fe$^0$ and anaerobic.
microorganism was higher than that of the single microorganism system. We speculated that the presence of iron not only speeded up the degradation rate of 2,4-DCP, and its degradation products had also been further degraded.

![Graph](image.png)

**Figure 6.** The removal rate of COD in “Fe$^0$ + cell” system.

**SUMMARY**

Compared to the individual biotic cell or Fe$^0$ use, the combined system of Fe$^0$ and anaerobic microorganism could significantly improve the degradation efficiency of 2,4-DCP. The initial pH value had a significant impact on the degradation of the target pollutant in the combined system. The degradation effect of the combined system was better in the partial alkaline environment. Higher or lower pH value was not conducive to the degradation of the target pollutant. The 2,4-DCP degradation effect of the combined system was the best when the initial pH value was 9. The corrosion of Fe$^0$ occurred in anaerobic condition, and the product of OH$^-$ could effectively balance the organic acids produced by fermentation of glucose. That made the VFC concentration maintained at a lower level. The pH value of the combined system increased slightly and tended to be stable, which was beneficial to the degradation of 2,4-DCP. The corrosion of Fe$^0$ was closely related to the pH value. The corrosion product of Fe0 was mainly Fe$^{2+}$, and the content of Fe$^{3+}$ was less. Compared to the neutral or alkaline environment, acidic environment was conducive to the corrosion of Fe$^0$. The COD removal rate of the combined system had a certain correlation with the degradation effect of 2,4-DCP at different initial pH value. The COD removal rate was the best when the initial pH value of the combined system was 9, which was 82%.

This work was financially supported by the Natural Science Foundation of China (NSFC) (Grant No. 21407068), Science Foundation of Jiangsu Colleges and Universities (Project No. 14KJD610002), Jiangsu Science and Technology Program - a prospective joint research project (Project No. BY2016068-01), 2016 University of Jiangsu Province Blue Project of young academic leader training objects, Jiangsu College Students’ Innovation and Entrepreneurship Training Program (Project No. 201614000005Y).

**REFERENCES**
