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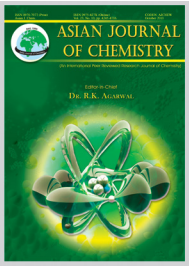

Zero-Valent Iron Supported Microbial Reductive Dechlorination of 2,4-Dichlorophenol

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The experiment was conducted to examine the potential of zero-valent iron (Fe^0) as the sole electron donor for reductive dechlorination of 2,4-dichlorophenol by mixed culture. The optimal conditions and the sustainability of 2,4-dichlorophenol dechlorination in Fe^0+microbe system were also investigated. The results showed that, Fe^0 was an effective electron donor to stimulate reductive dechlorination of 2,4-dichlorophenol and 2,4-dichlorophenol (30 mg L^{-1}) could be transformed completely within 68 h. The rate was similar to that with acetate or lactate as electron donor, while the rate was slow with glucose as electron donor. The quantity of Fe^0, inoculation added and pH had a significant effect on 2,4-dichlorophenol dechlorination. The optimal quantity of Fe^0, inoculation added and initial pH was 2.0 g L^{-1}, 646.4 mg volatile suspended solid L^{-1} and 8, respectively. Fe^0 sustained 2,4-dichlorophenol dechlorination over a long period. 4-Chlorophenol was the intermediate roduct from 2,4-dichlorophenol, but it was difficult to be further degraded.

Key Words: Chlorinated compounds, Anaerobic biotransformation, Reductive dechlorination, Electron donor, Zero-valent iron.

INTRODUCTION

2,4-Dichlorophenol (2,4-DCP) is a compound that is widely used in large quantities in industries for production of wood preservatives, pesticides, herbicides and plastic materials. Its discharge to the environment is of great concern because of its toxicity and potential carcinogenicity[1,2]. Dai *et al.*[3] suggested that chlorophenols are extremely resistant to oxidative degradation, whereas it become easier in anaerobic conditions. Said *et al.*[4] advised that anaerobic dechlorination could be considered as a promising means for bioremediation treatments of persistently polluted environments. Mineralization of chlorophenols often starts with reductive dechlorination to phenol and ends with formation of methane and carbon dioxide. Chlorinated compounds can be used as electron acceptors and energy for the growth of the dechlorinating microorganisms under anaerobic conditions[4,5]. Ballapragada *et al.*[6] and Smatlak *et al.*[7] studied the effect of electron donor on reductive dechlorination of chlorinated compounds and indicated that the type of electron donors might affect the ability to sustain dechlorination activity through providing the electrons required for chlorinated compounds reduction. Many different organic substrates such as lactate, acetate, glucose and methanol had successfully used to enhance the effect of reductive dechlorination of chlorinated compounds[8-10]. These electron donors were converted by fermenting organisms into acetate and hydrogen (H_2), either of which might be used by dechlorinating microorganisms[11]. However, Carr *et al.*[8] and Aulenta *et al.*[12] suggested that adding organic substrates as electron donors consumed much energy and the effect of reductive dechlorination was mostly inferior to hydrogen.

Hydrogen is considered to be an effective electron donor for biological reductive dechlorination[6,11,12]. Many dechlorinating isolates such as *Dehalospirillum multivorans*, strain CBDB1, *Desulfitobacter sp*. Strain PCE1showed high dechlorinating activity utilizing H_2 as electron donor[13-15]. Nevertheless, adding H_2 directly has some practical problems such as dangerous to handle. In addition, H_2 is also a kind of energy source and relatively expensive.

Another way to produce H_2 attracts our attention is the anaerobic corrosion of Fe^0. Fe^0 is relatively inexpensive and nontoxic. The corrosion of iron under anaerobic condition produces cathodic hydrogen as indicated below in eqn. 1[16]:

$$Fe^0 + 2H_2O = Fe^{2+} + 2OH^- + H_2 \quad (1)$$

Karri *et al.*[17] and Till *et al.*[18] advised that cathodic hydrogen could be used as an electron donor for methanogens, sulfate-reducing bacteria and nitrate-reducing bacteria. Rysavy *et al.*[19] demonstrated that Fe^0 could as a source of hydrogen for anaerobic polychlorinated biphenyl dechlorinators. The literature indicated that, Fe^0 could also serve as an electron donor to support the reductive dechlorination of chlorinated aliphatics[20-23].

Anaerobic culture was shown to improve the degradation of nitrobenzene and the explosive, hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the presence[24,25] of Fe^0. Whereas so far, Fe^0 supported microbial reductive dechlorination of chlorophenols has not been reported yet. In addition to serve as electron donor, Matheson *et al.*[26] and Kim *et al.*[27] advised that Fe^0 reacted directly with chlorinated compounds to achieve dechlorination, which decreased the toxicity of contaminants. This suggested that, the combined use of Fe^0 and anaerobic microorganism may be a cost-effective means for bioremediation treatments of chlorophenols polluted environments. The objective of this research was to determine if anaerobic microorganism could stimulate reductive dechlorination of 2,4-dichlorophenol using Fe^0 as electron donor. And if so, to investigate the optimal conditions for dechlorination of 2,4-dichlorophenol in presence of Fe^0. The sustainability for 2,4-dichlorophenol dechlorination in the integrated Fe^0 + microbe system was also evaluated.

EXPERIMENTAL

Microorganisms: A mixed anaerobic culture used for this research was developed from a full-scale internal circulation reactor treating paper mill wastewater. The anaerobic culture was first fed with 2 g L^{-1} glucose as the carbon source, which enhanced the biological activity and the chemical oxygen demand removal rate was over 85 % before the experiments. Batch experiments were inoculated with mixed culture without acclimation.

Geneal approach: Batch experiments were conducted using serum bottles (250 mL) at 37 ºC. The microorganism was transferred to serum bottle with 50 mL nutrient medium and 1 mL trace element solution. The nutrient medium in bottle contained (mg L^{-1}): KH_2PO_4 54.0, K_2HPO_4 70.0, NH_4Cl 106.0, $CaCl_2{\cdot}2H_2O$ 15.0, $MgCl_2{\cdot}6H_2O$ 20.0. The trace element solution contained (g L^{-1}): $CoCl_2{\cdot}6H_2O$ 0.5, $NiCl_2{\cdot}6H_2O$ 0.05, Na_2SeO_3 0.05, $CuCl_2{\cdot}2H_2O$ 0.03, $ZnCl_2$ 0.05, H_3BO_3 0.05, $MnCl_2{\cdot}4H_2O$ 0.5, $(NH_4)_6Mo_7O_{24}{\cdot}2H_2O$ 0.01. Zero-valent iron used in this study was iron fillings and iron fillings or organic substances were added directly to bottle. Each bottle was spiked with 2,4-dichlorophenol stock solution to give an initial concentration of 30 mg L^{-1}. $NaHCO_3$ (1.0 g L^{-1}) was also added to maintain a buffering capacity. Bottles were then filled with deionized water and pH was adjusted with HCl and NaOH solution. The liquid was purged with nitrogen for 10 min to remove any residual dissolved oxygen completely, then bottles were sealed with rubber stoppers and placed on a platform shaker and shaken continuously at 120 rpm over the course of experiments.

The experiments were first conducted to investigate the effect of Fe^0 and other three organic substrates (glucose, acetate and lactate) as electron donor on the reductive dechlorination of 2,4-dichlorophenol by a mixed culture. Biotic, abiotic and medium control treatments were also conducted with contaminant to evaluate the effect of individual microorganism without electron donor, the adsorption of microorganism and the volatile losses of contaminant, respectively. Moreover, to study the optimal conditions for 2,4-dichlorophenol transformation in the presence of Fe^0, three factors (the quantity of Fe^0 added, initial pH and the quantity of inoculation) that were investigated. Furthermore, the sustainability for 2,4-dichlorophenol transformation in the integrated Fe^0 + microbe system was evaluated with a semi-continuous experiment.

Effect of different electron donors on 2,4-dichlorophenol transformation: An experiment to investigate the effect of Fe^0 as an electron donor on the reductive dechlorination of 2,4-dichlorophenol was conducted in a 250 mL serum bottle and bottle contained 2 g L^{-1} iron fillings. To compare the effect of dechlorination in presence of different electron donors, glucose, sodium acetate and sodium lactate were introduced to experiment separately and the initial concentration was 790 mg L^{-1}. Meanwhile, three bottles were prepared for biotic, abiotic and medium control treatments. Bottle prepared for biotic control treatments contained live microorganism and no electron donor and abiotic control treatments contained autoclaved microorganism and no electron donor also. Medium control treatments contained no microorganism or electron donor. The initial pH was adjusted to 8 in all experiments. The biomass concentration in each bottle was approximately 323.2 mg L^{-1} based on the volatile suspended solids (VSS) contents of anaerobic sludge. Bottles were spiked with 2,4-dichlorophenol stock solution and sampled periodically for 2,4-dichlorophenol and 4-chlorophenol.

2,4-Dichlorophenol transformation at different conditions: The experiment was to investigate the optimal conditions for dechlorination of 2,4-dichlorophenol in presence of Fe^0. The effect of Fe^0 doses/initial pH/quantity of inoculation on 2,4-dichlorophenol transformation were performed as follows: (1) To study how the different Fe^0 doses affected 2,4-dichlorophenol transformation. The concentration of Fe^0 added was 0.1, 1.0, 2.0, 5.0 and 10.0 g L^{-1}, respectively. The initial pH in each bottle was 8.0 and the biomass concentration was 323.2 mg volatile suspended solid L^{-1}. (2) Six bottles were used to investigate the effect of pH on 2,4-dichlorophenol transformation. The initial pH was set up at 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5, respectively and each bottle contained 2.0 g L^{-1} iron and 323.2 mg volatile suspended solid L^{-1} biomass. (3) Different quantity of inoculation was studied in five bottles and the biomass concentration was 80.8, 161.6, 323.2, 484.8 and 646.4 mg volatile suspended solid L^{-1}, respectively. The initial pH in each bottle was 8 and the addition of iron was 2 g L^{-1}. Bottles were spiked with 2,4-dichlorophenol stock solution and sampled periodically for 2,4-dichlorophenol.

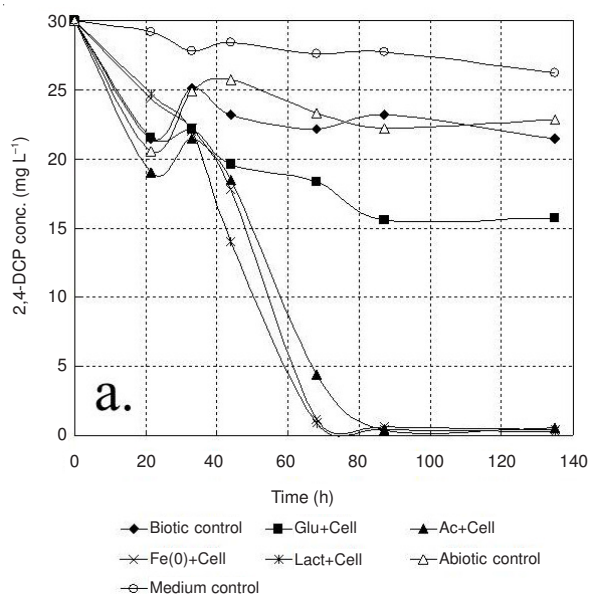
2,4-Dichlorophenol transformation in semi-continuous experiments: The sustainability for dechlorination of 2,4-dichlorophenol in the integrated Fe^0 + microbe system was conducted with a semi-continuous experiment. Repeated spiking of 2,4-dichlorophenol to the treatments containing Fe^0 (2.0 g L^{-1}) and microorganism (323.2 mg volatile suspended solid L^{-1}) when 2,4-dichlorophenol transformed completely. At this time, 15 mL nutrient medium and 1 mL trace element solution were injected also. In addition, acetate as an electron donor in the long-term dechlorination was conducted in comparison to Fe^0. Bottles were sampled periodically for 2,4-dichlorophenol and 4-chlorophenol.

Analytical methods: The sample was removed from bottle using a glass syringe. Analysis for 2,4-dichlorophenol and 4-chlorophenol were performed using a Hitachi HPLC (Tokyo, Japan) system, equipped with a Lichrospher C_{18} inverse phase column. An L-2400 UV detector was used for

the analysis and the detection wavelength was 280 nm. The HPLC mobile phase was the mixture of 2 % HAC (30 %) and methanol (70 %) at flow rate of 1.0 mL min^{-1}. The injection volume was 10 µL with an auto-sampler. Prior to HPLC analysis, sample solutions were filtered by 0.45 µm membrane. The biomass was measured as volatile suspended solids using standard methods.

RESULTS AND DISCUSSION

Effect of different electron donors on 2,4-dichlorophenol transformation: Fig. 1 showed the effect of different electron donors on the transformation of 2,4-dichlorophenol (a) and 4-chlorophenol (b). Fig. 1 showed that, 2,4-dichlorophenol remained at a high level (with about 50 % accumulated after 135 h) in the treatments containing glucose and cell and only a small quantity of 4-chlorophenol was formed. In contrast, 2,4-dichlorophenol was transformed most rapidly in the treatments containing Fe^0 and cell and 2,4-dichlorophenol was completely transformed within 68 h. At this time, the quantity of 4-chlorophenol was increased sharply, which did not degrade further. The behaviour of 2,4-dichlorophenol and 4-chlorophenol in the treatments containing acetate and cell or lactate and cell were similar to the behaviours in the treatments containing Fe^0 and cell. While the 2,4-dichlorophenol behaviour in the biotic or abiotic control treatments (without electron donor) remained at a high level (with above 70 % accumulated after 135 h) and 4-chlorophenol was detected barely. Similarly, the slow disappearance of 2,4-dichlorophenol in the medium control treatments and 4-chlorophenol was not detected. This indicated that the decrease of 2,4-dichlorophenol in the treatments added electron donor was not due to the adsorption or the losses of volatilization and 2,4-dichlorophenol transformation was enzymatic. In addition, in the first 20 h, a sharply decrease of 2,4-dichlorophenol concentration was observed and soon afterwards (33 h) the concentration of 2,4-dichlorophenol changed to increase. This is due to some adsorption of microorganism and that can be demonstrated by abiotic control treatments containing killed cell and no electron donors. Fig. 1 suggested that an external electron donor was required



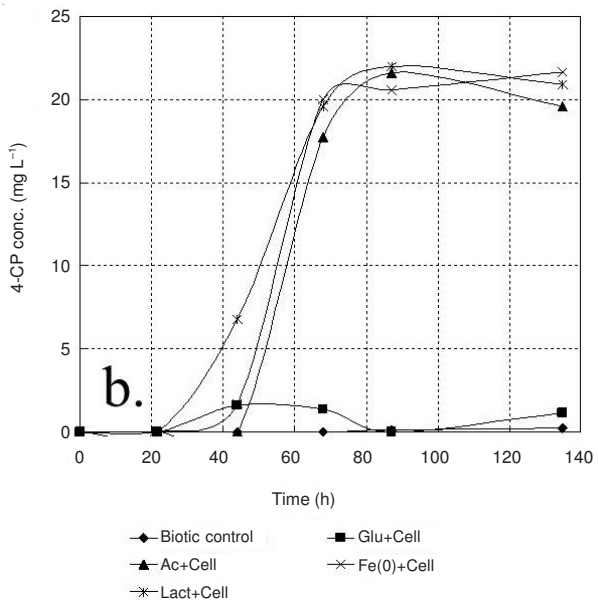


Fig. 1. Effect of different electron donors on 2,4-dichlorophenol(a) and 4-chlorophenol (b) transformation

for 2,4-dichlorophenol transformation and Fe^0, acetate and lactate were effective electron donors in comparison to glucose.

2,4-Dichlorophenol transformation at different conditions: Figs. 2-4 presented the effect of Fe^0 doses, initial pH and the quantity of inoculation on the transformation of 2,4-dichlorophenol in the treatments containing Fe^0 and microorganism, respectively. Emphasis was placed on evaluating the optimal conditions for 2,4-dichlorophenol transformation in the presence of Fe^0 and how these factors influenced the transformation of 2,4-dichlorophenol.

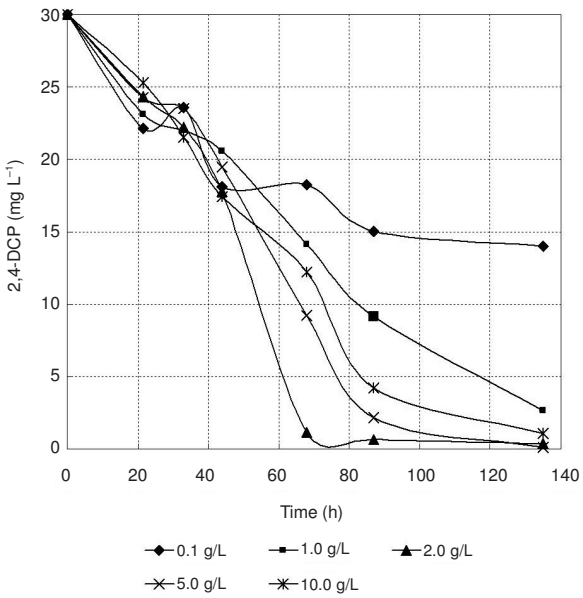


Fig. 2. Effect of iron doses on 2,4-dichlorophenol transformation

Fig. 2 showed that the Fe^0 doses had a distinguishable effect on 2,4-dichlorophenol transformation. Increasing the Fe^0 doses from 0.1 to 2 g L^{-1} enhanced the transformation of 2,4-dichlorophenol significantly. The rate of transformation for 2,4-dichlorophenol was very slow in the treatments

containing 0.1 g L^{-1} iron, only about 53 % of 2,4-dichlorophenol had been transformed after 135 h. While in the treatments containing 1 g L^{-1} iron, 2,4-dichlorophenol transformed about 90 % after 135 h. Moreover, 2,4-dichlorophenol was transformed completely within 68 h in the treatments containing 2.0 g L^{-1} iron. However, the rate of transformation for 2,4-dichlorophenol was decreased when adding higher doses and 2,4-dichlorophenol was transformed completely within 87 and 135 h in the treatments containing 5.0 and 10.0 g L^{-1} iron, respectively. This indicated that there existed an optimal Fe^0 doses in the integrated Fe^0 + microbe system and the optimal Fe^0 doses was 2.0 g L^{-1}. Small amount of Fe^0 perhaps could not produce enough hydrogen for the dechlorinators and increasing Fe^0 doses may increase the production of hydrogen, which served as an electron donor for reductive dechlorination and enhance the activity of dechlorinators. While higher Fe^0 doses than 2.0 g L^{-1} resulted in a high pH in the effluent (the end pH was 8.36 when added 10.0 g L^{-1} of Fe^0, data was not shown), which may be beyond the optimal range for dechlorinators and therefore inhibit the transformation of contaminant.

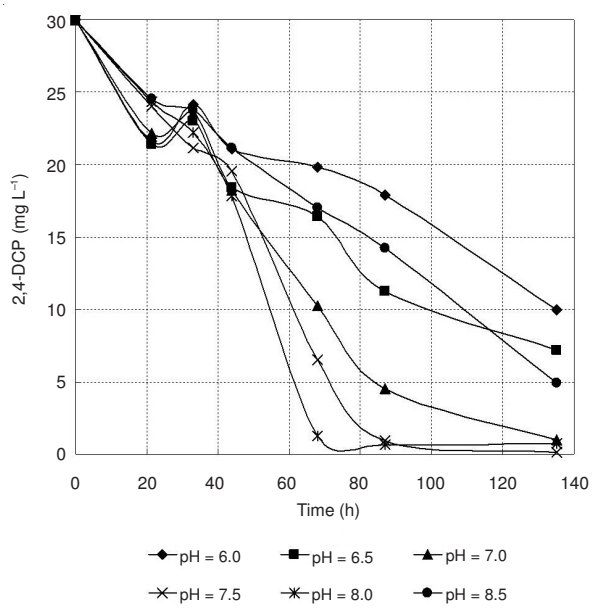


Fig. 3. Effect of initial pH on 2,4-dichlorophenol transformation

Fig. 3 showed the effect of initial pH on the transformation of 2,4-dichlorophenol in the presence of Fe^0. As shown in Fig. 3, pH had a significant impact on the transformation of 2,4-dichlorophenol and the optimal initial pH was 8. The rate of transformation for 2,4-dichlorophenol increased with pH when pH ranged from 6 to 8. In the treatments at pH of 6 and 6.5, 2,4-dichlorophenol transformed slowly, with 67 % and 76 % transformed after 135 h, respectively. In contrast, 2,4-dichlorophenol transformed rapidly in the treatments at initial pH of 7.0, 7.5 and 8.0 and complete transformation of 2,4-dichlorophenol within 135, 87 and 68 h, respectively. While in the treatments at initial pH of 8.5, the rate of transformation decreased dramatically, with only about 83 % of 2,4-dichlorophenol transformed after 135 h. This suggested that, the transformation of contaminant in neutral or slightly alkaline condition was superior to acid or alkaline condition in the integrated Fe^0 + microbe system. The anaerobic corrosion of Fe^0 produced hydrogen as shown in eqn. 1 and the concentration of hydrogen was an important factor to influence the activity of dechlorinators[28,29]. Yang *et al.*[29] and Fennell *et al.*[30] suggested that other H_2-consuming organisms might compete for H_2 with dechlorinators in the mixed culture and dechlorinators exhibited a lower threshold for H_2 [6,7,30]. Perhaps the slowly corrosion of Fe^0 produced a lower concentration of hydrogen in neutral or slightly alkaline condition in comparison to acid condition and that gave the dechlorinators a competitive advantage in mixed culture. While alkaline condition may be beyond the suitable condition for dechlorinators and influenced the transformation of contaminant.

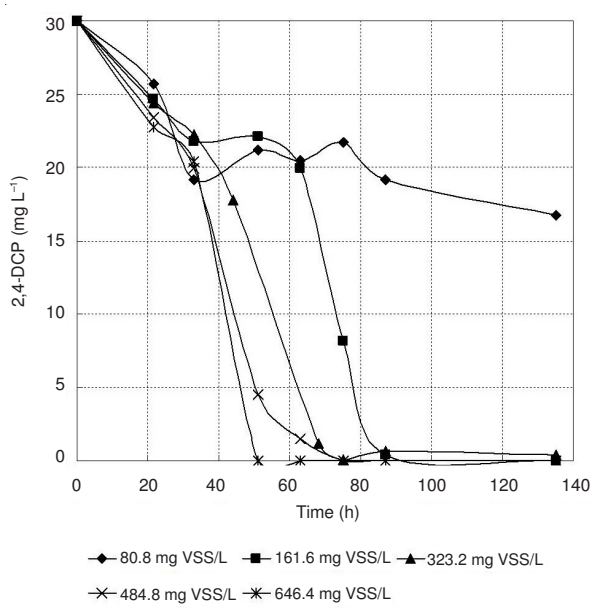


Fig. 4. Effect of the quantity of inoculation on 2,4-dichlorophenol transformation

The quantity of inoculation had also a significant impact on the transformation of 2,4-dichlorophenol (Fig. 4). Fig. 4 showed that, the rate of transformation for 2,4-dichlorophenol increased with the quantity of inoculation. In the treatments containing 80.8 mg volatile suspended solids L^{-1} biomass, 2,4-dichlorophenol transformed slowly, only with 44 % transformed after 135 h. However, after increasing the quantity of inoculation, the rate of transformation for 2,4-dichlorophenol enhanced dramatically. When the concentration of biomass was 161.6, 323.2 and 484.8 mg volatile suspended solids L^{-1}, the transformation of 2,4-dichlorophenol completed within 87, 68 and 63 h, respectively. With the most rapid 2,4-dichlorophenol transformation occurring in the treatments containing 646.4 mg volatile suspended solids L^{-1} biomass and 2,4-dichlorophenol was transformed completely within 51 h. These indicated that, the more quantity of inoculation, the faster transformation of contaminant. The transformation of 2,4-dichlorophenol was enzymatic as described before. Perhaps it generated more enzyme or enzymatic series when increasing the quantity of inoculation and catalyzed the degradation of contaminant. This trends were similar to previous study with 2,4-dichlorophenol in the treatments containing Fe^0 and mixed acclimated microorganism[31].

2,4-Dichlorophenol transformation in semi-continuous experiments: The sustainability for 2,4-dichlorophenol

dechlorination in the treatments containing Fe^0 and cell or acetate and cell was evaluated through repeating spiking of 2,4-dichlorophenol. Fig. 5 showed the transformation profiles of 2,4-dichlorophenol (a) and 4-chlorophenol (b) in semi-continuous experiments.

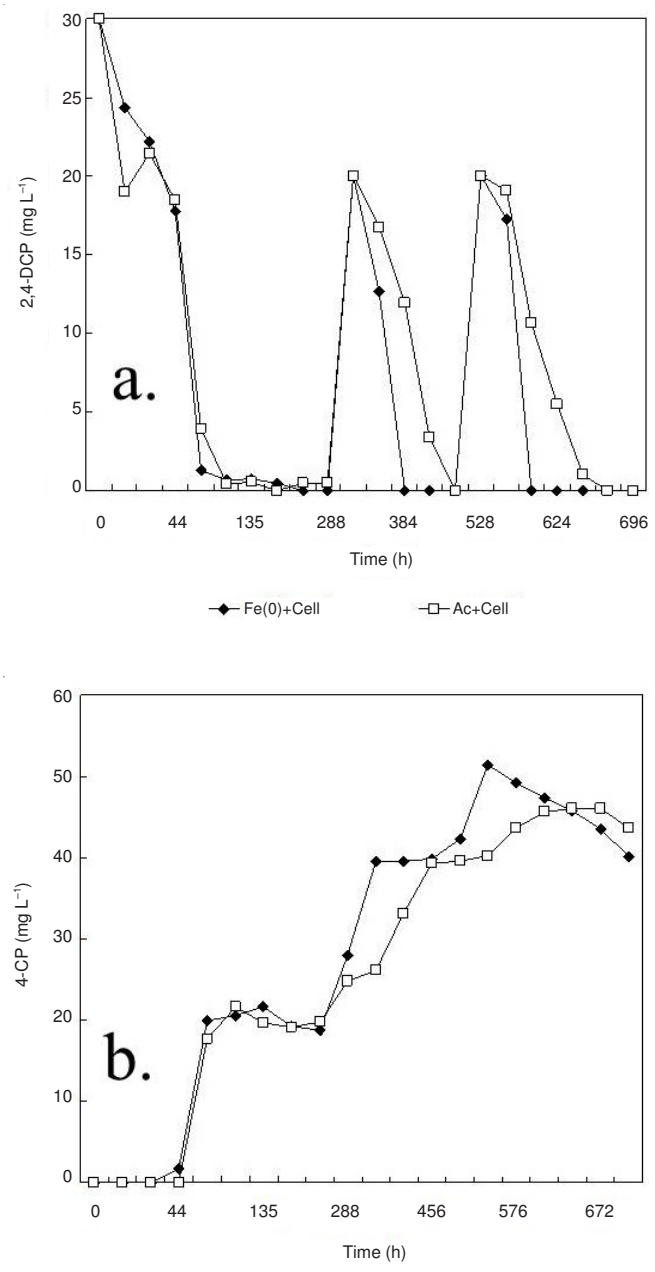


Fig. 5. 2,4-Dichlorophenol (a) and 4-chlorophenol (b) transformation in semi-continuous experiments

As shown in Fig. 5, compared to acetate as an electron donor, Fe^0 sustained the complete transformation of 2,4-dichlorophenol over a long period and kept a high speed. Perhaps the rate of consumption for acetate was rapid in anaerobic condition and could not support enough energy for dechlorinators. In addition, the quantity of 4-chlorophenol increased sharply with the complete transformation of 2,4-dichlorophenol, but it was difficult to be further degraded. Through the mass balance, 4-chlorophenol was the main intermediate product from 2,4-dichlorophenol.

Conclusion

Fe^0 was an effective electron donor to stimulate reductive dechlorination of 2,4-dichlorophenol by a mixed culture and complete transformation 30 mg L^{-1} of 2,4-dichlorophenol within 68 h. The rate of transformation was similar to that observed with acetate or lactate as electron donor. However, the transformation rate for 2,4-dichlorophenol was slow when glucose as electron donor.

The quantity of Fe^0, inoculation added and initial pH had distinguishable effect on reductive dechlorination of 2,4-dichlorophenol in the integrated Fe^0 + microbe system. Increasing Fe^0 doses at a suitable range enhanced the transformation of 2,4-dichlorophenol dramatically and the optimal Fe^0 doses was 2 g L^{-1}, higher Fe^0 doses had an inhibitory effect on 2,4-dichlorophenol transformation. Similarly, the rate of transformation for 2,4-dichlorophenol increased with pH when initial pH from 6 to 8 and the optimal initial pH was 8, but higher pH had a disadvantage to 2,4-dichlorophenol transformation. However, in the treatments containing different concentration of biomass, the more quantity of inoculation, the faster transformation of contaminant and the optimal quantity of inoculation was 646.4 mg volatile suspended solid L^{-1}.

Repeated spiking of 2,4-dichlorophenol to the treatments containing Fe^0 and microorganism showed that, Fe^0 could support the electron for reductive dechlorination of 2,4-dichlorophenol continuously and the rate of transformation remained at a high rate. While acetate as electron donor, the effect of dechlorination was inferior to Fe^0 with the consumption of acetate. Moreover, the production of 4-chlorophenol increased rapidly with the complete transformation of 2,4-dichlorophenol and 4-chlorophenol was the main intermediate product from 2,4-dichlorophenol, but it was difficult to be further degraded.

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REFERENCES

1. M.L. Krumme and S.A. Boyd, *Water Res.*, **22**, 171 (1988).
2. Z. Ning, K.J. Kennedy and L. Fernandes, *Water Sci. Technol.*, **35**, 67 (1997).
3. Y.Z. Dai, H.C. Shi and J.P. Ji, *Environ. Sci.*, **21**, 40 (2000). (in Chinese).
4. E.F. Said, N. Henry and N.A. Spiros, *Biotechnol. Prog.*, **14**, 167 (1998).
5. W.W. Mohn and J.M. Tiedje, *Microbiol. Res.*, **56**, 482 (1992).
6. B.S. Ballapragada, H.D. Stensel and J.A. Puhakka, *Environ. Sci. Technol.*, **31**, 1728 (1997).
7. C.R. Smatlak, J.M. Gossett and S.H. Zinder, *Environ. Sci. Technol.*, **30**, 2850 (1996).
8. C.S. Carr and J.B. Hughes, *Environ. Sci. Technol.*, **32**, 1817 (1998).
9. L. Xiao-Xia, L. Guang-He and S. Tao, *J. Environ. Sci. Heal.*, **37**, 439 (2002).
10. R. Doong and S. Chang, *Chemosphere*, **40**, 1427 (2000).
11. J. He, Y. Sung and M.E. Dollhopf, *Environ. Sci. Technol.*, **36**, 3945 (2002).
12. F. Aulenta, J.M. Gossett and P.M. Petrangeli, *Biotechnol. Bioeng.*, **91**, 743 (2005).
13. H. Scholz-Muramatsu, A. Neumann and M. Messmer, *Arch. Microbiol.*, **163**, 48 (1995).
14. L. Adrian, U. Szewzyk and J. Wecke, *Nature*, **408**, 580 (2000).
15. J. Gerritse, V. Renard and T.M. Pedro Gomes, *Arch. Microbiol.*, **165**, 132 (1996).
16. L. Liang, N. Korte and B. Gu, *Adv. Environ. Res.*, **4**, 273 (2000).
17. S. Karri, R. Sierra-Alvarez and J.A. Field, *Biotechnol. Bioeng.*, **92**, 810 (2005).

18. B. Till, L.J. Weathers and P.J.J. Alvarez, *Environ. Sci. Technol.*, **32**, 634 (1998).
19. J.P. Rysavy, T. Yan and P.J. Novak, *Water Res.*, **39**, 569 (2005).
20. L.J. Weathers, G.F. Parkin and P.J.J. Alvarez, *Environ. Sci. Technol.*, **31**, 880 (1997).
21. K.J. Lampron, P.C. Chiu and D.K. Cha, *Water Res.*, **35**, 3077 (2001).
22. T. Lee, T. Tokunaga, A. Suyama and K. Furukawa, *J. Biosci. Bioeng.*, **453**, 92 (2001).
23. H. Rosenthal, L. Adrian and M. Steiof, *Chemosphere*, **55**, 661 (2004).
24. W. Zhang, L. Chen, H. Chen and S.-Q. Xia, *J. Hazard. Mater.*, **143**, 57 (2007).
25. J.M. Fernandez-Sanche, E.J. Sawvel and P.J.J. Alvarez, *Chemosphere*, **54**, 823 (2004).
26. L.J. Matheson and P.G. Tratyek, *Environ. Sci. Technol.*, **28**, 2045 (1994).
27. O. Kim and E.R. Carraway, *Environ. Sci. Technol.*, **34**, 2014 (2000).
28. G. Kassenga, J.H. Pardue and W.M. Moe, *Environ. Sci. Technol.*, **38**, 1024 (2004).
29. Y. Yang and P.L. Mccrty, *Environ. Sci. Technol.*, **32**, 3591 (1998).
30. D.E. Fennell and J.M. Gossett, *Environ. Sci. Technol.*, **32**, 2450 (1998).
31. T. Cheng, Y.Z. Dai and Z.Y. Liu, *Microbiology*, **35**, 332 (2008). (in Chinese)