SURFACE MODIFICATION OF ZERO-VALENT IRON NANOPARTICLES BY BIOLOGICAL ACTIVE SUBSTANCE - COMPLEX TESTS

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Abstract

Chlorinated solvents such as trichloroethene (TCE) are widespread groundwater contaminants. These contaminants can be transformed by combination of abiotic and biotic methods under anaerobic conditions. Nowadays nanoscale zero-valent iron (nZVI) is used for the treatment of chlorinated compounds via its strong reducing property. Biological reductive dechlorination of chlorinated ethenes (CEs) is contributed by dehalorespiration. The influence of nZVI in combination with carboxymethyl cellulose (CMC) and molasses on the specific dehalorespiring microflora and besides the influence of biological surface modification on reactive properties of iron was tested within this study.

Groundwater contaminated with CEs was collected from the chemical factory Spolchemie a.s. Batch tests (reactors) with iron and various concentrations of CMC and molasses were performed. The samples for gas chromatography (GC) analyses were taken regularly after defined time period for determination of CEs concentration and physical-chemical parameters were monitored simultaneously. DNA was extracted after filtration of the tested water and used as a template for a real-time PCR amplification. 16S rDNA gene was used as a total bacterial community marker. Specific genes were used for detection of ongoing reductive dehalogenation (vcrA, bvcA, Dre, DHC-RT and Dsb) and to monitor denitrifying and sulphate reducing bacteria (nirK and apsA).

Keywords: Iron particles, chlorinated solvents, biological surface modification, biological reductive dechlorination, dehalorespiration, molecular genetic analysis

INTRODUCTION

The different surface modification or stabilization techniques were used for decrease of ZVI (zero valent iron) particles aggregation and for increase its mobility in the subsurface. The typical stabilization is coating of particles by polymer substances. These surface macromolecules serve as a barrier which protects the particles against agglomeration using electrostatic or spatial repulsion [4]. Due to the project is the research focused on group of substances, which can be biological decomposed and can serve as a source of nutrients for dehalorespiring microorganisms [5,6].

The aim of this study was to develop a biological active substance supporting natural dehalogenation processes. A suitable biological substance has to be non-toxic, with minimum passivation effect on ZVI surface and with minimum negative effect on its reactivity properties. It should also support the migration capability of ZVI particles [9], [10].

To develop this material the laboratory tests with biological active substances were performed. The influence of tested materials on the reactivity properties of ZVI and on the quantity of soil bacteria was monitored. To test the influence of different substances on the specific soil bacteria the molecular genetic analyses were used. These analyses were focused mainly on the organohalide-respiring microflora.

1. LABORATORY TEST METHODOLOGIES

Methodology of reactor tests for testing of reactivity properties

The reactor tests were designed as a closed mixing system, with contaminated water and a remedial reagent. The tests were carried out in 2.5 l glass bottles fitted with a joint [2]. The reactors were mixed with mechanical stirrers, since magnetic stirrers cannot be used for testing magnetic particles. In addition, the reactors were equipped with sampling ports, electrodes and other electrodes for continuous measurement of physical and chemical parameters. The special stirrer (according to utility model 25752 of the Technical University of Liberec) [3] was placed on the central joint. The system was sealed as tightly as possible, but a certain degree of leakage was expected during the experiment and was monitored using a control reactor without the reagent (or electric field).

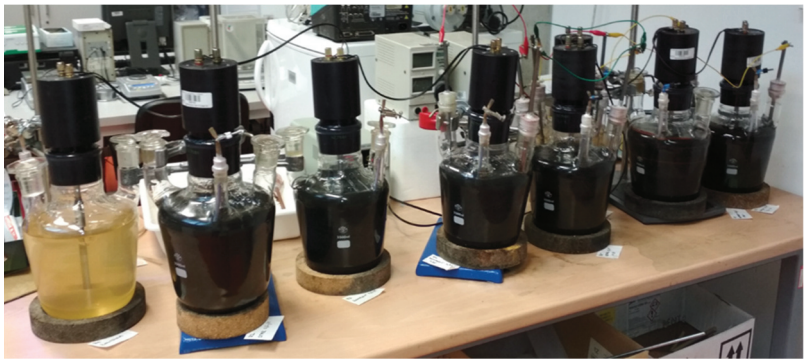

Figure 1 Photo of reactor tests

Reactor sampling took place in two modes. For control sampling, a Teflon tube was introduced into the sampling port and water samples were drawn through a glass syringe. In the first phase of the control sampling a water sample was taken for chlorinated hydrocarbons (CHC) analysis by GC/MS. Physical and chemical parameters (pH, redox potential and conductivity) were then measured. Samples were also regularly collected for the analysis of dissolved ions and metals. In the case of the enclosed electric field, the value of the electrical voltage and current in the system was deducted for each sample. The sampling frequency depended on the reagent used and the duration of the test. Sampling was usually more frequent at the beginning of the tests, and intervals were prolonged. These reactors were placed in laboratory temperature for periods ranging from 6 to 26 days. Direct current (DC) [1] was also applied in some reactors. **Figure 2** illustrates the possible reactor configurations for these types of test.

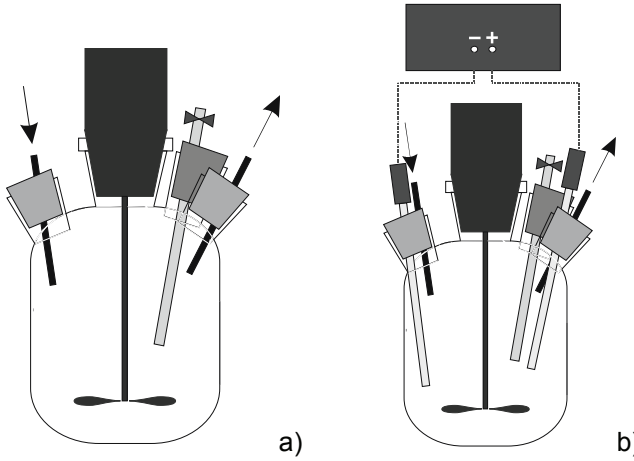

Figure 2 Possible reactor test configurations with a gas chromatogram connection: a) a simple reactor; (b) reactor with electrodes in a combined configuration

Methodology of molecular genetic analysis

0.5 L of water from each reactor was filtered through 0.22 µm pore membrane when reactor test was completed. DNA was isolated from filters with collected microorganisms by FastDNA SPIN Kit for Soil according to manufacturer's protocol.

Extracted DNA was used as a template for real-time PCR amplification. Real-time PCR assays were performed and analysed using LightCycler 480 SYBR Green system.

	Genes	Primers	Primer sequences (5'-3')
16S	16S rDNA broad-range	U16SRT-F	ACTCCTACGGGAGGCAGCAGT
		U16SRT-R	TATTACCGCGGCTGCTGGC
bvcA	vinyl chloride reductase	bvcA227F	TGGGGACCTGTACCTGAAAA
		bvcA523R	CAAGACGCATTGTGGACATC
vcrA	vinyl chloride reductase	vcrA880F	CCCTCCAGATGCTCCCTTTA
		vcrA1018R	ATCCCCTCTCCCGTGTAACC
Dre	Dehalobacter sp. 16S rDNA	Dre441F	GTTAGGGAAGAACGGCATCTGT
		Dre645R	CCTCTCCTGTCCTCAAGCCATA
DHC-RT	Dehalococcoides sp. 16S rDNA	DHC793F	GGGAGTATCGACCCTCTCTG
		DHC946R	CGTTYCCCTTTCRGTTCACT
Dsb	Desulfitobacterium spp. 16S rDNA	Dsb406F	GTACGACGAAGGCCTTCGGGT
		Dsb619R	CCCAGGGTTGAGCCCTAGGT
nirK	nitrite reductase	nirK876F	ATYGGCGGVCAYGGCGA
		nirK1040R	GCCTCGATCAGRTTRTGGTT
apsA	adenosine 5'-phosphosulfate reductase	RH1-aps-F	CGCGAAGACCTKATCTTCGAC
		RH2-aps-R	ATCATGATCTGCCAGCGGCCGGA

Figure 3 qPCR markers used in this study

16S rDNA gene was used as a total bacterial community marker. Specific genes were used for detection of ongoing reductive dehalogenation (vcrA, bvcA, Dre DHC-RT and Dsb) and to monitor denitrifying and sulphate reducing bacteria (nirK and apsA). All used markers are listed in **Figure 3**.

Cq value of each sample was compared with Cq value of feed water and relative quantity was given in the tables according to rules listed below:

- red colour - high increase of quantity (more than 80x),
- orange colour - lower increase of quantity,
- yellow colour - no changes,
- light blue colour - degrease of quantity,
- dark blue colour- no detection.

2. RESULTS

a) Carboxymethyl cellulose (CMC)

Iron particles (Fe) with diverse CMC concentration in combination with electric current (DC) were applied in this test.

Total bacterial abundance (16S rDNA) increased in the control reactor (contaminated water without treatment), in the reactor with CMC and in the reactor with Fe in combination with higher CMC concentrations (0.5 and 1 g/L). In the reactor with Fe in combination with the highest CMC concentration (1 g/l), the biggest increase of bacterial quantity was detected. In the reactors with Fe, the most of tested markers increased when higher CMC concentrations were used. These results indicated that CMC has probably a bacteria's protecting effect against Fe toxicity. (**Figure 4**).

The best growth conditions for dehalorespiring microflora were present in the control reactor and in the reactor with Fe particles in combination with high CMC concentration (1 g/l). According to these results, big decrease of DCE was also observed in this reactor. It appears that Fe could increase the amount of H_2 due to its strong reducing properties and H_2 serves as electron donor for dehalorespiring microflora. CMC seems to have a protecting effect on these bacteria [7], [8].

This test also demonstrated the devastating impact of electric current on all bacterial groups. It is visible on both total bacterial abundance (16S rDNA) and all specific markers. Although, this impact could be attenuated on contaminated sites due to bacterial recolonization of that site by groundwater flow.

			Fe + CMC			Fe + CMC + DC			
	Control	1 g/L CMC	0,25 g/L CMC	0,5 g/L CMC	1 g/L CMC	0,25 g/L CMC	0,5 g/L CMC	1 g/L CMC	Cq values
16S	6	1	0,04	10	83	0	0,0001	0,0005	12,25
bvcA	0,2	0	0	0,005	0,01	0	0	0	26,04
vcrA	0,6	0,01	0,001	0,01	0,1	0	0	0	19,4
Dre	3	1,4	0	0,1	0	0	0	0,8	33,38
DHC-RT	0,1	0	0	0,002	0,01	0	0	0	23,14
Dsb	0,5	0	0,0001	0,3	5	0,0004	0	0	25,36
nirK	70	0,18	0,01	1	44	0	0,01	0	25,03
apsA	0,3	0	0,2	10	6	0	0,001	0,01	19,95
	R1	R2	R3	R4	R5	R6	R7	R8	Feed water

Figure 4 Relative quantity of bacterial markers in reactors with CMC, ZVI composite and DC (5 V); Fe = 3 g/L; 21 period days

The following diagrams document the process of laboratory reactor tests. The **Figure 5** compares the normalized concentrations of DCE and TCE in time. The diagrams able easily to compare the influence of each system on dechlorination process.

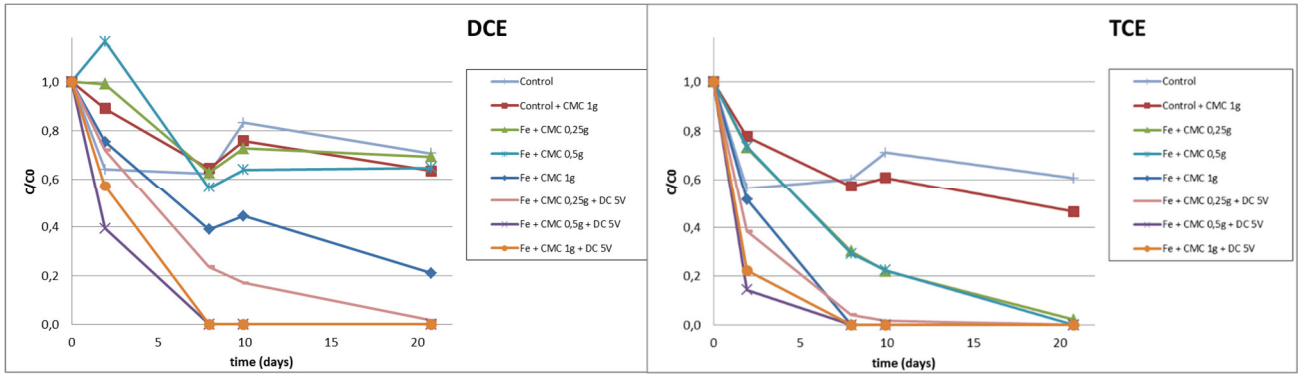

Figure 5 Degradation of TCE, DCE in reactors with CMC, ZVI composite and DC (5 V)

CMC had definitely positive effect on given microflora [7]. The surface modification by CMC passivated the surface of ZVI particles and so degreased its toxicity. However the surface passivation led to the rapid decrease of ZVI reactivity.

The systems with DC support achieved strongly better results from the point of view of dechlorination quickness and of total balance. The best results in CEs reduction were achieved in systems with high concentration of CMC and in combination with DC support.

b) Molasses

In this type of test the Molasses in combination with higher concentration of ZVI particles (10 g/l) were applied. The next configuration was the comparison of just ZVI influence and its combination with alternating current (AC) lower intensity. The sampling was performed twice after 11 and 19 days.

Total bacterial abundance (16S rDNA) increased in the control reactor (contaminated water without treatment), in the reactor with molasses and in the reactor with iron particles in combination with molasses. The highest increase of total bacterial quantity was observed in the reactor with molasses. In this reactor, the quantity of all tested markers increased, mostly denitrifying bacteria marker (nirK). Markers of dehalorespiring bacteria also increased in this reactor. It indicated that molasses served as the fermentable substrate and products of fermentation were utilised by dehalorespiring bacteria. The quantity of the most tested markers was lower in the second sampling due to ongoing substrate exhaustion.

In the reactor with the combination of Fe and molasses, the total bacterial abundance increased. The marker of denitrifying bacteria (nirK) was the only one of the specific markers whose amount increased in this reactor. This could be caused by toxicity of high Fe concentration used in this test (10 g/L).

	Control		Molasses		Molasses + Fe		Fe		Fe + AC	Cq values
16S	18,62	2,357	1298	512,7	824,2	88,03	3E-04	0	0,003	12,04
bvcA	0,039	0,064	3,029	0	0	0,216	0	0	0,012	27,11
vcrA	0,101	0,122	8,343	0,064	0,085	0,67	0,002	0	0,005	22,67
Dre	0,029	0,095	1,129	3,358	0	0,208	0	0	0,028	32,57
DHC-RT	0,006	0,03	1,788	0,005	0,002	0,177	0	0	0,001	23,90
Dsb	0,049	0,069	3,156	0,039	0,019	0,17	2E-04	0	0,000	24,82
nirK	20,63	5,994	114,3	1,44	1,334	5,871	0	0	0,005	28,29
apsA	0,161	0,179	2,034	0,39	0,164	0,201	0,003	0	0,006	22,71
	11 days	19 days	11 days	19 days	11 days	19 days	11 days	19 days	19 days	Feed water

Figure 6 Relative quantity of bacterial markers in reactors with molasses and ZVI composite; Fe = 10 g/L; Melasa = 10 g/L; two periods 11 and 19 days

This test also shoved lower impact of AC on bacteria in comparison with DC used in the previous test (above-mentioned). But the amount of all bacterial markers also significantly decreased when AC was used.

The following diagrams **Figure 7** compare the normalized concentrations of DCE and TCE in time. The diagrams able easily to compare the influence of each system on dechlorination process.

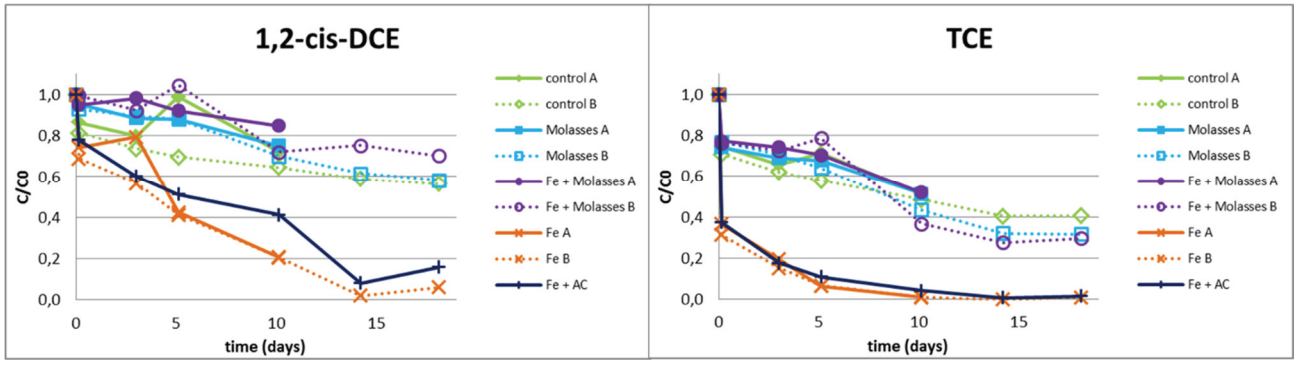

Figure 7 Degradation of TCE, DCE in reactors with molasses and ZVI composite

The development of monitored CEs concentration showed very well the influence of Fe surface modification by Molasses (Fe + Mel. A; Fe + Mel. B). Despite the using of high Fe concentration the combination with Molasses was not able to decompose the contamination. Applied Fe material served just for the equalizing of water pH, which had positive effect on biological processes. From the economical point of view this configuration does not make.

Separately applied Fe (Fe A; Fe B) achieved very good results because the CEs were during the experiment completely reduced. The reason was probably the high concentration of Fe - 10 g/L.

The results of the first testing of Fe application combinated with AC (Fe + AC) and its influence on dechlorination process were very interesting. Using AC showed lower impact on bacteria in comparison with DC but showed lower efficiency from the point of view of dechlorination rate of CEs.

3. CONCLUSIONS

Organic compounds used as ZVI modifiers could have large impact on the soil microflora of remediated sites.

Fe particles in combination with CMC increased the quantity of examined bacterial markers more than CMC by itself. This increasing was higher with higher CMC concentration. It confirmed positive influence of Fe particles on dehalorespiring bacteria when they are protected from its toxicity by CMC. The disadvantage of CMC usage could be its cost. Higher amount of ZVI would be necessary to apply because of the passivation effect of CMC on ZVI surface.

Molasses as carbon and electron source had positive effect on all studied groups of bacteria. Molasses is suggested to serve as the substrate for the fermentation which produces electrons utilised by dehalorespiration. Molasses supported the bacterial growth but had strong negative effect on ZVI reactivity due to its surface passivation.

Electricity (AC, DC) had devastating impact on tested bacteria in laboratory conditions. Remediated sites could be usually recolonised via ground water flow.

According to these results the use of organic compounds especially CMC as a surface modification of ZVI particles for the field applications does not represent a favourable product as was expected due to the literature sources. The CMC represents an expensive source of carbon and it passivates the surface of ZVI and decrease the reactivity of ZVI. The electricity prolongs the ZVI longevity but has a negative impact on given microflora. However at remediated sites is usually easy recolonised via ground water flow.

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