IRON OXY/HYDROXIDE STIMULATION OF ANAEROBIC BIOREMEDIATION OF GROUNDWATER CONTAMINATED WITH DIESEL/BIODIESEL BLEND (B20)

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Abstract
Field experiments were conducted to assess the biostimulation of anaerobic biodegradation of diesel B20 (20:80 v/v biodiesel and diesel) by indigenous iron oxy-hydroxide reducing bacteria. Iron reduction process and BTEX removal were observed during the 7.4 month study. Chemical and microbial analyses documented the simultaneous degradation and microbial community shifts.

Keywords
Anaerobic bioremediation; groundwater; biodiesel; diesel, iron oxy-hydroxide

INTRODUCTION
Environmental and economic concerns about the use of fossil fuels stimulated the development of biofuels (*e.g*., diesel/biodiesel blends) in the world energy matrix. The increasing use of diesel/biodiesel blends increases the probability of groundwater contamination by leaks in storage tanks and accidental releases during transportation. Thus, the study of the environmental impact of diesel/biodiesel blends on groundwater and appropriate treatment for contaminated aquifers could reduce future environmental risks.

Although biodiesel is commonly referred to as a harmless and readily biodegradable biofuel (Zhang et al., 1998), the use of new diesel formulations contain BTEX (benzene, toluene, ethyl benzene and xylenes), which creates toxic and carcinogenic potential risks (USEPA, 1998) and therefore would require remedial action. Previous field experiment that evaluated the monitored natural attenuation of diesel B20 in groundwater demonstrated that strongly anaerobic conditions were established in the subsurface environment and the preferential biodiesel biodegradation delayed the onset of BTEX degradation (Chiaranda, 2011). Therefore, the biostimulation of indigenous bacteria by the addition of electrons receptors (*e.g*., nitrate, Fe (III), and sulfate) to biodiesel contaminated groundwaters could reduce the chance of reduced BTEX degradation.

High Fe (II) concentrations present in groundwaters contaminated with organic matter illustrate the potential importance of iron reduction processes during natural attenuation (Chapelle, 2001). Moreover, a experiment under fermentative methanogenic conditions in groundwater containing diesel B20 observed an increase of *Geobacter* spp (Ramos et al, 2013), which is known to carry out iron reduction (Lin et al., 2009). Despite the potential efficiency of the iron reduction process to degrade biodiesel, the quantity and cost of the iron required could preclude the use of this process *in situ*. Thus, this study applied relatively inexpensive iron oxy-hydroxide recovered from the treating acid mine drainage for biostimulation process. A controlled field experiment has been conducted to assess the potential for iron reducing conditions (by iron oxy-hydroxide (Goethite) recovered from acid mine drainage) to enhance biodegradation BTEX in groundwater contaminated with diesel B20.

MATERIAL AND METHODS

Experimental setup and biostimulation system

This study was conducted at the Ressacada Experimental Farm in Florianópolis, SC, Brazil. The field experiment was performed in a monitored area of $180m^2$, containing 30 monitoring wells (Figure 1). Each well has 5 sampling depths (located 2, 3, 4, 5 and 6 m below ground surface). 100 L of diesel B20 (20 % palm biodiesel and 80 % diesel) was released into a source-zone area of 2x1.5x1.8 m deep down to the water table. After the biofuel release, biostimulation was performed by adding 100 kg of iron oxy-hydroxide recovered from acid mine drainage (Goethite). Furthermore, 2 kg of ammonium acetate was added to accelerate the initial microbial growth, since this compound is readily assimilated by microorganisms. Lastly, the experimental area was covered with gravel and tarp to minimize rainfall infiltration. Two monitoring samplings were conducted to date (3 and 7.4 months after the controlled release and biostimulation).

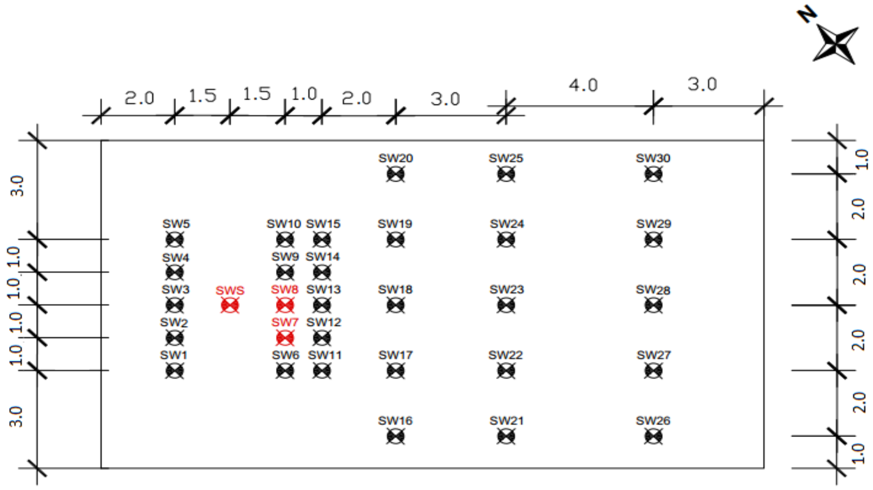

Figure 1. Representation of the experimental area. Legend: SW = Sampling well

Experiment Monitoring

Physical chemical analysis (Temperature, pH, redox potential, dissolved oxygen, total iron, iron II, BTEX) and microbial analysis (polymerase chain reaction (PCR) and ribosomal rRNA intergenic spacer analysis (RISA)) were used to assess the biogeochemical and microbial community structure changes.

Temperature, pH, redox potential and dissolved oxygen were measured on site using a Micropurge Flow Cell (MP20). BTEX was analyzed by gas chromatography. Total iron and iron II analysis were conducted according to the methods established by APHA, 1998. PCR analysis targeted to the 16S-23S rRNA Gene Intergenic Spacer Region was performed using the primers (RISA-fw: 5'-TGCGGCTGGATCCCCTCCTT-γ', RISA-rvμ 5'-CCGGGTTTCCCCATTCGG-γ').The amplified products were loaded and migrated in gel by using Agilent DNA 1000 Kit to analyze the bacterial community changes. Rstudio version 3.1.3 software was used to analyze RISA results through principal component analysis (PCA).

RESULTS AND DISCUSSION

The points that have the most representative results (for biodegradation) are located in source zone, as expected since diesel and biodiesel blends behave as a fixed source of contamination with limited capacity of migration in groundwater (Ramos et al., 2013). Therefore, this study contemplates the discussion of sampling well source (SWS) (at 2 and 3 m below ground surface), sampling well 7 (SW7) (at 2 m below ground surface) and sampling well 8 (SW8) (at 3 m below ground surface). The location of SWS, SW7 and SW8 is shown in Figure 1 (marked in red).

Temperature and pH ranges varied from 21.8°C to 24.6°C and 4.05 to 6.63, respectively. The high biochemical oxygen demand exerted by biodiesel (Chiaranda, 2011) resulted in rapid decrease in dissolved oxygen (dissolved oxygen next 0.5 $mg.L^{-1}$ in most of the sampling wells, apart from SWS, at 3m depth), which led to the development of anaerobic degradation processes such as iron reduction. This finding was also reflected by the decrease in oxidation–reduction potential (ORP) in most of the sampling wells.

The biostimulation with goethite resulted in the development of the iron reduction process, as intended. An increase in total iron concentration was observed initially (3 months after the release) due to the addition of iron oxy-hydroxide (goethite). The decrease in total iron concentration observed 7.4 months after the release, concomitantly with a decrease in BTEX concentration (Figure 2) are indicatives of the occurrence of iron reduction process, once B20 compounds oxidation can be occurring while the iron III, present in total iron, is being reduced. These results corroborate with the increase in iron II concentration (Figure 2), which provides evidence of iron reduction occurrence.

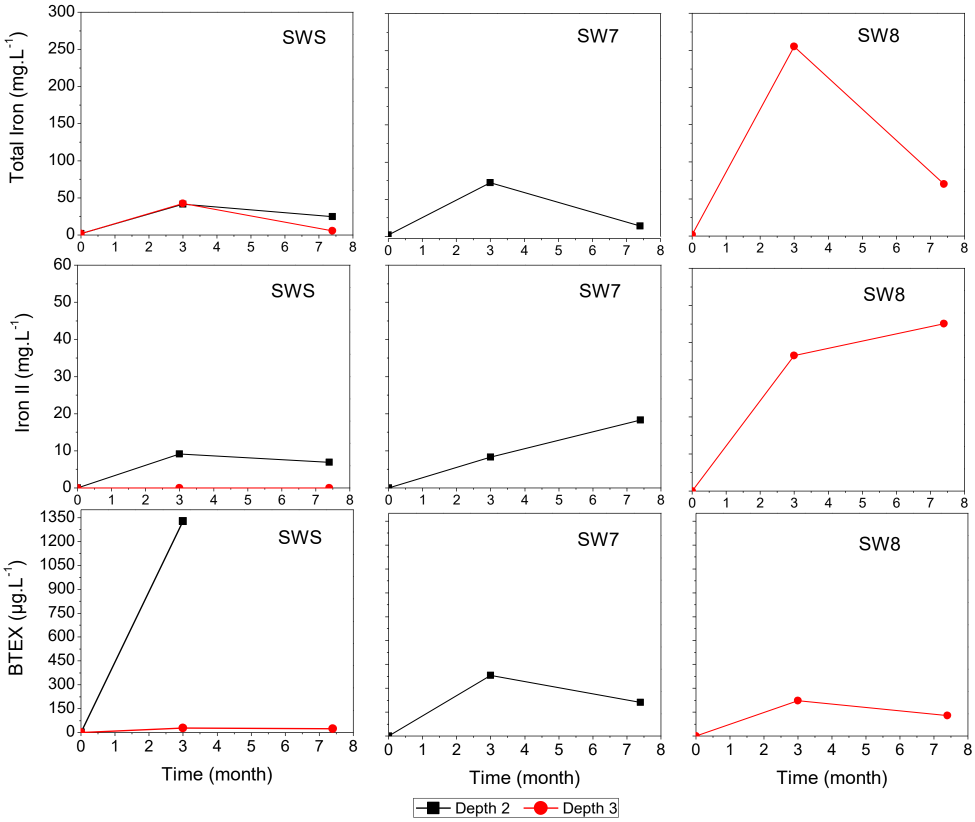

Figure 2. Variations in the total iron ($mg.L^{-1}$), iron II ($mg.L^{-1}$) and BTEX ($\mu g.L^{-1}$) over time.

A decrease in BTEX concentrations was noticeable 7.4 months after biostimulation while monitored natural attenuation of diesel B20 started BTEX degradation only in 34.8 months years after the release (Ramos et al, 2013). The biodegradation process is demonstrated by the changes observed over time in bacteria community structure (Figure 3).

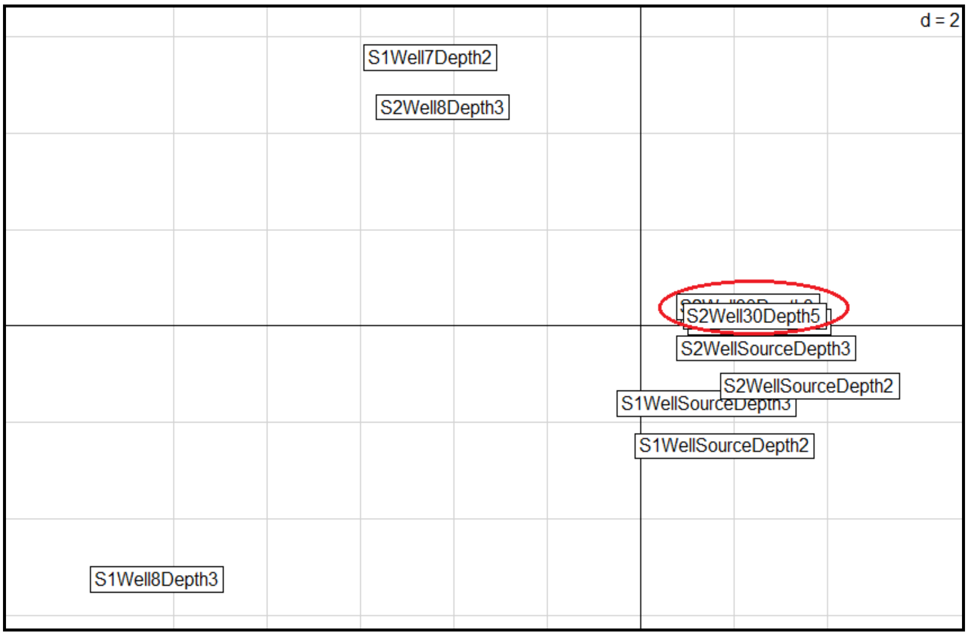

Figure 3. PCA analysis of community structure changes over. Legend: S1= Sampling 3 months after the controlled release and S2 = Sampling 7.4 months after the controlled release. Well 30 was used as control (marked in red).

CONCLUSIONS

The results indicate that the development of the iron reduction process achieved by biostimulation with goethite in the source zone of the diesel B20 accelerated the BTEX degradation when compared to monitored natural attenuation.

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