BIODEGRADATION OF THE FUEL OXYGENATE METHYL TERT-BUTYL ETHER IN A FLUIDIZED BED BIOREACTOR

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Abstract
Industrialization, increasing motorization and rapid urbanization have led to extensive soil and groundwater contamination. The main pollutants are fuel hydrocarbons and various gasoline additives. Microbial bioremediation is a cost-effective and sustainable way to promote the remediation of affected sites.
Our experiments focused on testing key parameters of a universal and promising biological treatment technology, using a lab-scale fluidized bed bioreactor and a previously isolated bacterial consortium. The aim of our work was to test our setup and provide data for the technological design and optimization of field-scale bioreactors.

Introduction
Gasoline additives such as ether oxygenates and their tertiary alcohol metabolites are common groundwater contaminants, mainly due to leaks from storage tanks. Compared to other components of gasoline, ether oxygenates, like methyl *tert*-butyl ether (MTBE), are well-soluble in groundwater and able to form an extensive contaminant plume [1].
Bioremediation is an effective and affordable way of environmental remediation. Studies have focused on the investigation of aerobic and even anaerobic biodegradation of MTBE. Many individual isolates and bacterial consortia are able to grow using ether oxygenates as a sole carbon and energy source. In some cases, natural attenuation was observed, however, with stimulation and optimized conditions enhanced biodegradation is achievable[2].
Fluidized bed bioreactor is an *ex situ* bioremediation technology that can provide the necessary nutrients and optimal growth conditions (pH, temperature, dissolved oxygen) for microbes. As a consequence of fluidization, the specific surface area is increased, enhancing bioconversion efficiency by giving an opportunity for the formation of high biomass concentration and promoting retainment. This type of reactor design is capable of treating large volumetric flow rates, as well [3].

Experimental
The total volume of our lab-scale fluidized bed bioreactor is approximately 13 L. As an affordable biomass carrier, we used sterilized, fractionated medium sand with a diameter of 1-2 mm.
In advance of the initiation, the bioreactor was sterilized with sodium-hypochlorite solution and washed several times with softened water. The volatilization of MTBE has been monitored for several days with analytical measurements (HS-GC-MS).
The reactor was inoculated with a previously grown consortium culture maintained in minimal media containing MTBE as a sole carbon source.
Influent water was delivered to the bioreactor by a peristaltic pump. To ensure the needed recirculation flow for fluidization we used a membrane pump in order to reduce shear force and

avoid the heating effect of centrifugal pumps.
We previously tested the optimal temperature for the chosen consortium at 18 °C, 20 °C, 22 °C, 23 °C 24 °C, 26 °C, 28 °C, and 30 °C, respectively. We also investigated the optimal nutrient composition for the consortium, focusing on nitrogen and phosphate supplementation, respectively.
Firstly, we were testing the effect of increasing MTBE concentration in contaminated water, while influent flow was set to a middle-low level and was unchanged during the experiment.
Our next approach was using fixed MTBE concentration in contaminated water, which was typical of an averagely polluted field. Inflow of contaminated water with unchanged MTBE concentration was raised gradually in order to find out the maximum efficiency of our experimental setup. The upper limit for MTBE concentration in effluent-treated water was 0,4 mg/l.

Results and discussion

Based on our results, the optimal temperature range for growth and efficient MTBE biodegradation is 24-27 °C. The results of preliminary experiments indicate that ten times decreased nitrogen and phosphate supplementation compared to basic minimal media was perfect for efficient workflow.
Within days after the inoculation of the bioreactor, MTBE concentration decreased by 80% compared to the starting point, thus we were able to start the continuous phase (starting the intake of contaminated water and release of treated water). MTBE concentration was monitored daily in the influent and in effluent water, respectively.
During the continuous phase, we applied necessary modifications to eliminate and prevent technical issues, for instance redesigning the aeration in order to increase its efficiency and using different types of pumps to find out, which one is the best choice.
As a result of increasing the intake gradually, we observed stable MTBE removal efficiency of 98% at a loading rate of up to 175 L/day. This achievement was much better than we expected, additionally, based on our results, there are more opportunities to improve efficiency in the near future. We are also planning to develop our bioreactor setup, making it more robust and being able to work automatically.

Conclusion

Our prototype was developed to optimize the fundamental conditions for an MTBE-degrading bacterial consortium. Our results verified that the prototype is far suitable for maintaining the optimal conditions for several microorganisms, furthermore, gives the possibility to grow a large amount of biofilm on the surface area of fluidized carrier particles in order to enhance biodegradation.
Additionally, this setup is useful for testing newly isolated microbes and microbial communities even for other types of biodegradation methods or optimizing operational parameters of a fieldscale bioreactor. The main advantage of our lab-scale bioreactor is that it gives us the possibility to model and test all of the parameters which are needed for designing and developing full-scale bioreactors, which are universally usable, efficient, and economical solutions for bioremediation of even large volumes of contaminated groundwater.

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