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Short communication

Microbial biosafety of pilot-scale bioreactor treating MTBE and TBA-contaminated drinking water supply

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ABSTRACT

A pilot-scale sand-based fluidized bed bioreactor (FBBR) was utilized to treat both methyl *tert*-butyl ether (MTBE) and *tert*-butyl alcohol (TBA) from a contaminated aquifer. To evaluate the potential for re-use of the treated water, we tested for a panel of water quality indicator microorganisms and potential waterborne pathogens including total coliforms, *Escherichia coli*, *Salmonella* and *Shigella* spp., *Campylobacter jejuni*, *Aeromonas hydrophila*, *Legionella pneumophila*, *Vibrio cholerae*, *Yersinia enterocolytica* and *Mycobacterium avium* in both influent and treated waters from the bioreactor. Total bacteria decreased during FBBR treatment. *E. coli*, *Salmonella* and *Shigella* spp., *C. jejuni*, *V. cholerae*, *Y. enterocolytica* and *M. avium* were not detected in aquifer water or bioreactor treated water samples. For those pathogens detected, including total coliforms, *L. pneumophila* and *A. hydrophila*, numbers were usually lower in treated water than influent samples, suggesting removal during treatment. The detection of particular bacterial species reflected their presence or absence in the influent waters.

1. Introduction

Biological treatment of contaminated groundwater is an emerging technology in the United States. Due to uncertainty about the safety of final water produced by biological systems, bioreactor effluent is usually discharged as wastewater. However, in cases where specific contaminants, such as methyl *tert*-butyl ether (MTBE) or perchlorate, are responsible for contamination, effective removal should generate high quality drinking water.

MTBE is very water soluble, and its plumes often extend far beyond those of other components of leaking underground storage tanks such as benzene, toluene, ethyl-benzene and xylene (BTEX) [1]. At concentrations greater than 1000 $\mu g\,L^{-1}$, bioreactor treatment of MTBE is competitive with other available treatment alternatives (i.e., carbon, air stripping with vapor-phase treatment, bioGAC, and chemical oxidation) [1].

Building on existing sand filtration and wastewater treatment technology, fluidized bed bioreactors were developed for nitrate removal from drinking water in Europe in the mid 1980s [2]. The technology has been shown to be superior to other suspended and attached growth biological systems, in part due to high biomass retention [3,4]. The potential for using FBBR technology for treatment of contaminated groundwater has been demonstrated for denitrification, as well as MTBE, trichloroethene and perchlorate biodegradation [5–8]. While the efficacy of fluidized bed systems for specific contaminant removal has been established, little attention has been paid to other water quality parameters in the treated water. For example, virtually nothing is known about the biological safety of the treated water, i.e. with respect to pathogens, information that is critical if the water is going to be used for irrigation or human consumption. Enteropathogenic *E. coli*, *A. hydrophila*, *L. pneumophila*, *V. cholerae*, *Y. enterocolytica* and the *M. avium* complex (MAC) have been identified as pathogens of chief concern for the groundwater environment [9–11]. The goal of this project was to determine selected groundwater pathogen load in a FBBR treating MTBE-contaminated groundwater aquifer in a small community in Glennville, CA. The community of Glennville was entirely supplied by private well water prior to aquifer contamination and has been without a local water supply since 1998. This was one of the first attempts to empirically determine the biological safety

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of final waters produced by a sand-based FBBR and to provide much needed data to help inform policies for re-use of treated groundwater.

2. Experimental

2.1. Glennville MTBE plume site

Glenville, California is located in northern Kern County in the foothills of the Sierra Nevada mountains, in a transition zone to higher elevation bedrock. An underground storage tank (UST) at 10,675 Highway 155 contaminated to a fractured bedrock aquifer in Glennville with MTBE in 1997. The fueling system, consisting of a 6000 gallon UST, fuel dispensers and related piping, was removed from the site in August 2002. Groundwater monitoring program consisting of quarterly sampling of up to 44 monitoring wells has been in effect at Glennville since July 1997. In addition to MTBE, benzene, toluene, ethylbenzene and xylenes (BTEX), and total petroleum hydrocarbons (TPH) have typically been detected in certain study area wells.

2.2. Bioreactors

Bioreactors studied were models ERI-500 (Bioreactor #1) and ERI-2000 (Bioreactor #2, #3) (Environmental Resolutions Inc. (ERI), Lake Forest, CA). Bioreactor parameters are summarized in Table 1. A 500 L capacity pilot-scale FBBR (Bioreactor #1) was established in a shed behind the former gas station at Glennville in December 2008 (Fig. 1). The protocol for Bioreactor #1 establishment involved bioreactor set up on location, filling with clean sand, filling with source water, and initial period of recirculation with added MTBE to establish the bioremediation community. If clear evidence of MTBE degradation could not be shown, inoculation from an established bioreactor would go ahead. Bioreactor influent was water from the well closest to the UST site, well W7. Following the establishment of MTBE degrading culture in the bioreactor, the bioreactor switched to treatment mode in March 2009. Bioreactor was decommissioned at the end of the pilot phase in September 2009. Samples from two established full-scale bioreactors (#2, #3) were used for comparison purposes.

2.3. Physical parameters

Physical conditions in the bioreactor were assessed on a weekly basis by certified technical staff. Throughout the Glennville bioreactor operation, pH, dissolved oxygen (DO) and temperature stayed close to desired values: pH = 7.4 ± 0.5; DO = 6 ± 1 mg L^{-1}; Temp. = 22 ± 4 °C. Total dissolved solids (TDS) in the reactor inflow rose rapidly from installation date, reaching over 2000 mg L^{-1} by the middle of January, and stayed very high while the reactor was in recirculation mode. The TDS dropped rapidly to below 1000 mg L^{-1} once the reactor was switched to flow-through mode on day 96. Average TDS during flow-through mode was 248 ± 118 mg L^{-1}.

2.4. Pathogen analysis

Waterborne pathogen analysis samples were collected in 100 mL sterile sample bottles. Samples were analyzed by Aemtek Inc., Fremont, CA. All samples were processed using USEPA standard methods. Enteric bacteria *Escherichia coli* (EPA 9223), *Salmonella* and *Shigella* (EPA 9260), *Yersinia enterocolytica* (EPA 9260K), and *Vibrio cholerae* (EPA 9260H) as well as opportunistic pathogens *Legionella pneumophila* (EPA 9260J), *Aeromonas hydrophila* (EPA 9260L), *Pseudomonas aeruginosa* (EPA 9260E) and *Mycobacterium avium* (EPA 9260M) were used as indicator organisms to assess potential pathogen growth within the bioreactor. Heterotrophic plate counts (HPCs) (EPA 9215B) were used to monitor microbial numbers in the influent and treated water from the bioreactor.

2.5. Nutrient analysis

Water samples for nitrate, phosphate and potassium analysis (EPA 300.0, SM4500P E and EPA 6010, respectively) were collected in 250 mL sterile sample bottles. Samples were analyzed by Kiff Analytical LLC, Davis, CA.

3. Results and discussion

3.1. Bioreactor establishment and MTBE removal

Bioreactor #1 was installed at Glennville on December 11, 2008 (Day 0). Although conventional and molecular methods (HPC and qPCR, respectively; data not shown) indicated the reactor was populated by bacteria very soon after installation, unchanging DO readings across the bioreactor indicated no MTBE degradation took place for 1 month. The bioreactor was inoculated with sand from an established bioreactor treating MTBE in Healdsburg, CA, on day 34.

Throughout the Glennville bioreactor operation, pH, DO and temperature stayed close to desired values: pH = 7.4 ± 0.5; DO = 6 ± 1 mg L^{-1}; Temp. = 22 ± 4 °C. Total dissolved solids (TDS) in the reactor inflow rose rapidly from day 0, reaching over 2000 mg L^{-1} by the middle of January (day 40), and stayed very high while the reactor was in recirculation mode. Due to regulatory concerns and freezing weather that prevented above ground water discharge, the reactor ran in recirculation mode from day 0 until day 96. The TDS dropped rapidly to below 1000 mg L^{-1} once the reactor was switched to flow-through mode on day 96. Average TDS during flow-through mode was 248 ± 118 mg L^{-1}. During recirculation mode, the microbial community was fed a mixture of MTBE and nutrients (N, P, K). We observed MTBE degradation in the bioreactor by day 55. During flow through mode, influent MTBE fluctuated between 1.3 and 7.2 mg L^{-1}. Treated water MTBE concentrations were always below detection limit. Although no nutrients were added to the bioreactor in run mode, low NO_3^- concentration persisted in the effluent for at least 48 days, before they decreased below detection limit by day 172. Aerobic bioreactors are not usually tested for effluent NO_3^- concentrations during the bioreactor establishment phase, and therefore comparison with prior studies was not possible. No clear explanation for the NO_3^- persistence was established.

3.2. Bioreactor pathogen analysis

Results of waterborne pathogen analysis of influent and treated water in Bioreactor #1 indicated that coliform numbers in the influent well water varied significantly over the testing period while the numbers in the treated water remained low or below detection limit (Table 2). We tested for *E. coli* whenever we tested for total coliforms. No *E. coli* were detected in any of our samples from the bioreactor. The HPC numbers varied in both the influent and treated water samples over the test period (Table 2) with a trend of lower counts in the treated water.

A full panel of 10 potential waterborne pathogens was analyzed in Bioreactor #1 during the initial recirculation period (day 61) and after the bioreactor was well established (day 167). *A. hydrophila* was the most numerous bacterium detected; its numbers were much lower in the treated water than influent aquifer water (Table 2). Low numbers of *P. aeruginosa* were also detected. No *L. pneumophila* was detected in the influent aquifer water or in the treated water.

Table 1
Bioreactor parameters.

Parameters	Units	Bioreactor #1	Bioreactor#2,3
Flow rates and mass loading			
Max VOC mass loading without oxygen booster	g/min	76	416
Max VOC mass loading with oxygen booster	g/min	151	946
Fixed recycle flow rate	L/min	38	189
Max hydraulic loading	L/min	26	132
Hydraulic residence time in FBBR tank	min	15	15
Dimensions		Pilot scale	Full scale
FBBR tank diameter	m	0.6	1.5
FBBR tank height (with fittings)	m	3.5	3.5
Capacity of FBBR tank	L	946	5677
Media			
bed height at rest	cm	105	~105
bed height expanded	cm	150	~150
sand type		Fine river sand	Fine river sand
Nutrient supply during bioreactor startup			
MTBE	L/week	0.5	NA[a]
fertilizer solution	L/week	95	NA

[a] Not applicable.

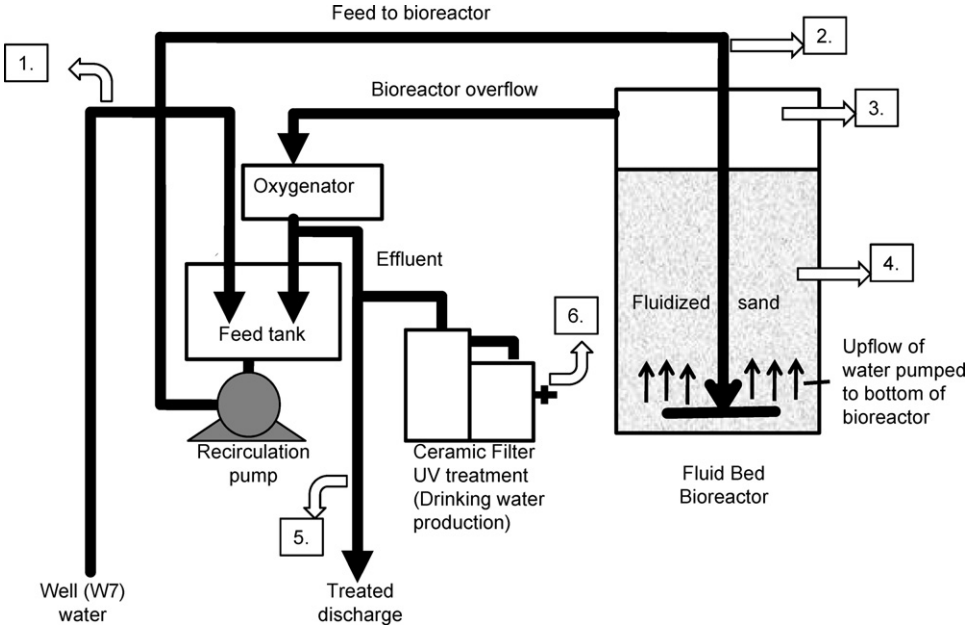

Fig. 1. Schematic diagram of Bioreactor #1. The fluidized-bed medium in this reactor was sand. Sampling points are indicated with arrows: (1) influent; (2) bioreactor influent; (3) effluent; (4) sand; (5) treated discharge; and (6) UV-treated effluent.

Table 2
Comparison of total coliforms, heterotrophic plate counts (HPC) and potential waterborne pathogens in bioreactor influent and treated discharge. Detection limit is 1 cfu 100 mL^{-1}. No representatives for *E. coli*, *Salmonella*, *Shigella*, *Camplyobacter jejuni*, *Yersinia enterocolitica*, *Vibro cholerae*, or *Mycobacterium avium* complex (MAC) were detected in any of the three bioreactors or the influent waters.

Microbiology test	USEPA limit[a] (cfu 100 mL^{-1})	Bioreactor #1				Bioreactor #2				Bioreactor #3			
		Initial (day 61)		Established (day 167)		(Oct 2008)		(Feb 2010)		(Oct 2008)		(Feb 2010)	
		inf.[c]	TD[c]	inf.	TD	inf.	TD	inf.	TD	inf.	TD	inf.	TD
Total coliforms	0 (MCLG[b])	178	25	2282	BDL[d]	1120	4	488	58	1	2	1	3
Heterotrophic plate count	50,000 (TT[e])	3010	6030	118	355	1470	209	271	38	2000	455	470	375
Legionella pneumophila	0 (MCLG)	BDL	BDL	BDL	BDL	4	96	5	BDL	BDL	2	BDL	BDL
Aeromonas hydrophila	No limit	153	2	832	2	470	24	1.5×10^5	BDL	2520	55	BDL	BDL
Pseudomonas aeruginosa	No limit	BDL	1	28	BDL	BDL	338	NT[f]	NT	BDL	BDL	NT	NT

[a] Colony forming unit.
[b] Maximum contaminant level goal.
[c] inf., influent; TD, treated discharge.
[d] Below detection limit.
[e] Treatment technology.
[f] Not tested.

To gain a broader understanding of the potential pathogen loads in these systems we also sampled two full-scale bioreactors (#2, #3) that were actively degrading MTBE plumes in two different locations (Healdsburg and Laguna Hills) for the presence of potential waterborne pathogens (Table 2). Both reactors showed similar trends in HPC counts, with significantly higher numbers in influent than treated water samples. Total coliforms were detected in both bioreactors but were either significantly lower in treated water than influent samples, or remained the same. Similarly, *A. hydrophila* was detected in both bioreactors; its numbers were significantly lower in the treated water (Table 2). In contrast, however, *L. pneumophila* numbers were higher in the treated water than influent of the Laguna Hills bioreactor. Very low numbers of this organism were also detected in the Healdsburg bioreactor.

To our knowledge, no comprehensive study of the health risks of waterborne pathogens in fluidized-bed bioreactor systems has been published to date. Recently, a static bed bioreactor for the treatment of perchlorate contaminated groundwater were certified for the production of drinking water [12]. However, this study only monitored for coliform bacteria and HPC's and no other potential pathogens were addressed [12]. Of the ten potential pathogens tested in our study, only *A. hydrophila* was present in all bioreactors, with significantly lower numbers in treated water than in influent aquifer water (Table 2). *P. aeruginosa*, a common environmental isolate, was sporadically detected in our samples. Health risks associated with exposure of the general population to *P. aeruginosa* in drinking water are thought to be insignificant [10,13]. No *L. pneumophila* were detected in the Glennville bioreactor.

The infrequent detection of *L. pneumophila* in the influent and treated water of Bioreactors #2 and #3 suggest that these bioreactors do not provide a conducive environment for *L. pneumophila* replication (Table 2). As *L. pneumophila* is present in some groundwaters [14] (Table 2), its detection in a bioreactor likely reflects source water contamination. In a comprehensive analysis of microbial communities in sand bioreactors, *Legionella* species were detected in a gravity fed slow sand filter used for treating horticultural irrigation water [15,16]. The top layer of this sand filter showed increased *Legionella* numbers, probably due to high temperature and long residence time, yet qPCR analysis also showed the number of *Legionella* bacteria decreased across the sand bioreactor [15]. The authors concluded that *Legionella* are a potential hazard in these types of gravity slow sand filters [15]. In contrast, the short residence time and upflow design of the FBBRs in our study are less likely to provide a suitable environment for *Legionella* replication.

A major limitation in routine analysis of the biological safety of biologically based treatment systems is the cost of monitoring for potential pathogens. Methods under development include multiplex PCR and qPCR, microarrays, and platforms that combine solid phase PCR with microarrays [17–19]. A recent study comparing the efficacy of traditional and qPCR methods for the detection of potential biological terror agents in large volume water samples found a high positive correlation between conventional and the less expensive qPCR-based results [20]. When EPA approved broad spectrum water borne-pathogen monitoring becomes available, it will be much more feasible to quickly assess the biological "safety" with respect to pathogen load. This will allow more accurate determination of suitability for potential downstream uses such as reinjection into groundwater, or drinking water use.

In our study, the detection of particular bacterial species appeared to reflect their presence in the influent waters and in most cases we observed decreases in both specific and total bacteria numbers tested within the bioreactor. These results could have significant implications for downstream uses of treated water, especially for re-injection into the contaminated aquifer. If approved, the aerated and degradative bacteria-enriched treated water could provide an important tool in a mixed ex situ–in situ treatment.

4. Conclusions

We found low counts of several potential waterborne pathogens in groundwaters contaminated with MTBE. Overall, our results show that the FBBR bioreactor successfully removed MTBE while not increasing the numbers of total bacteria or potential pathogens in treated water, therefore the quality of the treated water was significantly improved. Though pathogens were only occasionally detected in treated water, the fact that they are sometimes present indicates the importance of monitoring for potential pathogens in any treated water proposed to be reinjected into the aquifer. Currently accepted monitoring methods are too expensive and too slow to provide effective aquifer recharge management. The advent of molecular methods-based pathogen detection systems could provide acceptable risk management and allow safe implementation of ex situ–in situ mixed aquifer treatment strategies.

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