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Bioaugmentation of an anaerobic biotrickling filter for enhanced conversion of trichloroethene to ethene

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ABSTRACT

During biological reduction of trichloroethene (TCE), incomplete reduction to partially dechlorinated intermediates *cis*-1,2-dichloroethene (*cis*-DCE) and vinyl chloride (VC) can occur due to kinetic and inhibitory limitations. In this study, an anaerobic biotrickling filter was inoculated initially with a mixed culture containing *Dehalococcoides* spp. that contained the TceA and VcrA reductive dehalogenases. After significant accumulation of *cis*-DCE and VC was observed in the bioreactor effluent, it was hypothesized that bioaugmentation with *Dehalococcoides* strain BAV1, which contains the BvcA dehalogenase responsible for the metabolic dechlorination of *cis*-DCE and VC, would improve the conversion of TCE to ethene. It was found that at TCE loadings of 8–9 g m_{bed}^{-3} h^{-1}, bioaugmentation with strain BAV1 resulted in 45% conversion of TCE to ethene, as opposed to less than 10% prior to bioaugmentation. Strain BAV1 was found to grow to the same density (10^6–10^7 cells per g of packing material) as *Dehalococcoides* strains containing the TceA and VcrA dehalogenases. Strain BAV1 was also confirmed to be active, as determined by RT-qPCR of the BvcA mRNA. This study shows that it is possible to enhance the performance of continuously fed dechlorinating bioreactors by using a consortium that contains all three known reductive dehalogenases in the TCE dechlorination pathway. This is also the first study where a gas-phase biotrickling filter has been bioaugmented with a single strain to result in improved performance.

1. Introduction

Trichloroethene (TCE) is a toxic soil and groundwater pollutant which poses significant remediation challenges [1]. The recent discovery of microorganisms from the *Dehalococcoides* genus provides an excellent opportunity for using bioremediation to treat contaminated sites, as these microorganisms can reduce TCE completely to non-toxic ethene coupled with oxidation of hydrogen [2]. Recently, a novel technique for treatment of contaminated sites was proposed [3]. It combines physical removal of TCE from contaminated sites using soil vapor extraction or dual-phase extraction using nitrogen gas to provide an oxygen-free environment, followed by biological treatment of waste gas or/and liquid streams thus generated in anaerobic biotrickling filters inoculated with *Dehalococcoides* spp., and fed with a fermentable substrate via the recirculating liquid. The most important advantage of this technique, compared to in situ treatment, would be a better control of conditions for growth of *Dehalococcoides* spp., which are known to be very sensitive to environmental conditions [4].

In the proof of concept study [3], high volumetric rates of TCE removal were achieved (10 fold greater compared to typical in situ removal rates) using a lab-scale anaerobic biotrickling filter inoculated with a mixed culture containing *Dehalococcoides* spp., and fed with lactate as the fermentable substrate. However, significant accumulation of toxic intermediates in the reductive dechlorination pathway, *cis*-1,2-dichloroethene (*cis*-DCE) and vinyl chloride (VC), was observed in the effluent gas streams. A subsequent modeling study [5] showed that the specific rates of conversion of *cis*-DCE and VC were lower than that of TCE, and that TCE competitively inhibited the dechlorination of *cis*-DCE, and also had a detrimental effect on the dechlorination of VC.

The culture that was used in both studies contained *Dehalococcoides* strains that have two known reductive dehalogenases, TceA and VcrA. Strains that contain TceA as the only known reductive dehalogenase (for e.g. *Dehalococcoides ethenogenes* strain 195) are capable of metabolic dechlorination of only TCE and *cis*-DCE [6], while those than contain VcrA as the only known reductive dehalogenase (for e.g. *Dehalococcoides* strain VS and strain GT) can metabolically dechlorinate all TCE, *cis*-DCE and VC [7,8]. The

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modeling study mentioned above showed no inhibition of *cis*-DCE on VC or vice versa, suggesting that strains harboring different known reductive dehalogenase were responsible for the dechlorination of each. The competitive inhibition of TCE on *cis*-DCE points to the involvement of strains containing only TceA in the dechlorination of TCE and *cis*-DCE, while the minor inhibition of TCE on VC suggests that strains containing VcrA dechlorinate primarily VC, and only to some extent TCE. A similar specificity of strains containing different known reductive dehalogenases towards dechlorination pathways was also observed by Holmes et al. [9] and Lee et al. [10] in cultures enriched from contaminated sediments.

As a solution to the incomplete dechlorination of TCE that was observed in the biotrickling filters, it was hypothesized in this study that bioaugmentation of a biotrickling filter with a strain containing BvcA, another known reductive dehalogenase which is responsible primarily for metabolic dechlorination of *cis*-DCE and VC [11] would favorably shift the overall conversion of TCE to ethene. To test this hypothesis, a laboratory-scale anaerobic biotrickling filter inoculated with a mixed culture containing *Dehalococcoides* strains with the TceA and VcrA dehalogenases was operated at high TCE loading rates resulting in less than 10% conversion to ethene, and then bioaugmented with *Dehalococcoides* strain BAV1. The performance of the biotrickling filter was monitored before and after bioaugmentation, and *tceA*, *vcrA* and *bvcA* gene copies and transcripts per cell were quantified throughout the experiment.

2. Materials and methods

2.1. Culture selection and growth

SDC-9™ (Shaw Environmental Inc., Lawrenceville, NJ), a commercially available reductively dechlorinating mixed culture that contains *Dehalococcoides* spp. that have the TceA and VcrA dehalogenases was used in this study for initial inoculation of the anaerobic biotrickling filter. The original culture contained approximately 10^8 *Dehalococcoides* cells mL^{-1}, and was stored at 4 °C prior to use for inoculation. *Dehalococcoides* strain BAV1 was obtained from the laboratory of Dr. Frank Löffler (then at Georgia Institute of Technology, Atlanta, GA). The pure culture was grown on *cis*-DCE, as described elsewhere [12] to approximately 2×10^7 cells mL^{-1}, prior to use for bioaugmentation.

2.2. Biotrickling filter setup

The anaerobic biotrickling filter setup was the same as described by Popat and Deshusses [3]. Briefly, the biotrickling filter was constructed from a clear PVC pipe 60 cm in height and 10 cm in internal diameter (Harrington Plastics, Riverside, CA). It was packed with cattle bone Porcelite (CBP), a porous spherical packing material with slow-release nutrients incorporated (Aisin Takaoka Co., Ltd., Japan). The spherical beads had an average diameter of 3 mm. The active bed height was 26 cm, and thus a bed volume of 2 L. The initial bed porosity was 0.42. The biotrickling filter contained two biomass sampling ports, one 5 cm from the top, and the other 5 cm from the bottom of the bed. The bed was inoculated on day zero with 50 mL of SDC-9 culture, and with 200 mL of *Dehalococcoides* strain BAV1 on the 21st day of operation, by adding the cultures to the recirculating liquid, and operating the biotrickling filter in a closed loop mode with respect to the liquid for two days.

TCE vapors in humidified nitrogen gas were fed to the biotrickling filter from the top, resulting in down-flow co-current operation mode. The loading rate of TCE over the entire experimental duration was 8–9 $g_{TCE}\ m_{bed}^{-3}\ h^{-1}$. This was achieved using an inlet gas-phase concentration of TCE of 500–600 mg m^{-3}, and a gas empty bed residence time (EBRT) of 4 min. A nutrient solution was continuously recirculated from the sump at the bottom of the biotrickling filter (500 mL in this study) to the top of the bed. The recirculation rate was 50 mL min^{-1}. Fresh nutrient solution (modified RAM media) supplemented with 2.0–3.0 g L^{-1} sodium lactate (60% w/w sodium lactate solution, Fisher Chemical, Fairlawn, NJ) was continuously added to the sump at a flow rate of 0.55 mL min^{-1}, resulting in a liquid hydraulic retention time (HRT) of 15 h. This lactate feed rate was a result of adjustment to 10 times the stoichiometric requirement for producing enough hydrogen necessary for complete TCE dechlorination within the range of loadings imposed, and had been used in previous studies as well [3,5]. During closed loop operation after inoculation, sodium lactate was added to the liquid sump directly everyday (8 g day^{-1}). Because lactate was supplied in excess, its fate was not measured in this study.

2.3. Chemical analyses

Gas phase concentrations of TCE, *cis*-DCE, VC, ethene and methane were determined using an SRI gas chromatograph fitted with a 30 m GS-Q column (having internal diameter 0.32 mm) and a flame ionization detector. The detection limits for the compounds were: 0.002 g m^{-3} for TCE, 0.001 g m^{-3} for *cis*-DCE and VC, and 0.0005 g m^{-3} for ethene and methane. Dissolved oxygen (DO) concentration, redox potential (ORP), and pH were determined using Vernier (Beaverton, OR) probes, to ensure optimum conditions for bacterial growth and activity.

2.4. DNA and RNA isolation

4 g of packing samples were taken from the top and bottom sampling ports for each biomass sampling event, and were immediately suspended in 10 mL nuclease-free water. Biomass was extracted from the packing samples into the liquid by first sonicating the suspension for 3 min, followed by vortexing for 3 min. Following biomass extraction, cell pellets for DNA analysis were stored at −20 °C, and those for RNA analysis were stored at −80 °C, for no more than two weeks before further processing. DNA was isolated from cell pellets using MO BIO UltraClean™ microbial DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA), according to the manufacturer's instructions. The quality of extracted DNA was assessed by measuring A260/A280 ratios using a ND-1000 Spectrophotometer (NanoDrop Products, Wilmington, DE). DNA aliquots were stored at −20 °C for no more than one month before qPCR analysis.

RNA was isolated from cell pellets using MO BIO UltraClean™ microbial RNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA), according to the manufacturer's instructions. Contaminating DNA was removed using DNA-free™ kit (Applied Biosystems, Austin, TX) according to the manufacturer's instruction. Selected RNA aliquots after DNA removal were used for qPCR analysis as controls to ensure the absence of DNA in final RNA aliquots. The quality of purified RNA was assessed by measuring A260/A280 ratio using a ND-1000 Spectrophotometer (NanoDrop Products, Wilmington, DE). RNA aliquots were stored at −80 °C for no more than one week before further processing. cDNA was synthesized from RNA using SuperScript® III reverse transcriptase (Invitrogen Corporation, Carlsband, CA) by following the manufacturer's instructions. Aliquots of cDNA were stored at −20 °C for no more than one month before qPCR analysis.

2.5. qPCR and data analysis

Real-time qPCR amplification and quantification was performed on a Stratagene Mx3000P® QPCR system (Agilent Technologies, La Jolla, CA), using iQTM SYBR® Green Supermix (Bio-Rad

Table 1
List of primers used for qPCR [13].

Name	Target gene	Sequence (5′ → 3′)
tceA511F	tceA	GCCACGAATGGCTCACATA
tceA817R		TAATCGTATACCAAGGCCCG
vcrA880F	vcrA	CCCTCCAGATGCTCCCTTTA
vcrA1018R		ATCCCCTCTCCCGTGTAACC
bvcA277F	bvcA	TGGGGACCTGTACCTGAAAA
bvcA523R		CAAGACGCATTGTGGACATC
pceA877F	pceA	ACCGAAACCAGTTACGAACG
pceA976R		GACTATTGTTGCCGGCACTT

Laboratories, Inc., Hercules, CA) and gene specific primers for the different reductive dehalogenases (Table 1) [13]. Each reaction mixture contained 12.5 μL of the Supermix, 8.5 μL nuclease-free water, 1 μL of 5 μM forward primer, 1 μL of 5 μM reverse primer, and 2 μL of DNA or cDNA template. Real-time PCR conditions were: 3 min at 95 °C, followed by 40 cycles of 10 s at 95 °C and 45 s at 62 °C.

Standard curves were generated with purified PCR products of the individual genes targeted against the fluorescence corresponding to initial DNA concentration in PCR reactions, as determined using the LinRegPCR software developed by Ramakers et al. [14]. This assumption-free method allowed more accurate quantification of the gene copies, since it takes into consideration the reaction efficiency for each sample. The purified PCR products were obtained with ExoSAP-IT PCR Product Clean-up Kit (Affymetrix, Santa Clara, CA) that eliminates unincorporated primers and excess salts, that may interfere with the standard curve. In the case of RNA analyses, it was not possible to determine exactly the number of transcripts per cell for each gene or the fold change in transcripts of individual genes with time, because of the absence of an internal control, and thus uncertainty in extraction efficiency. However, it was possible to compare reasonably accurately the ratios of expressions of various genes within one particular sample, and thus these are reported in the results.

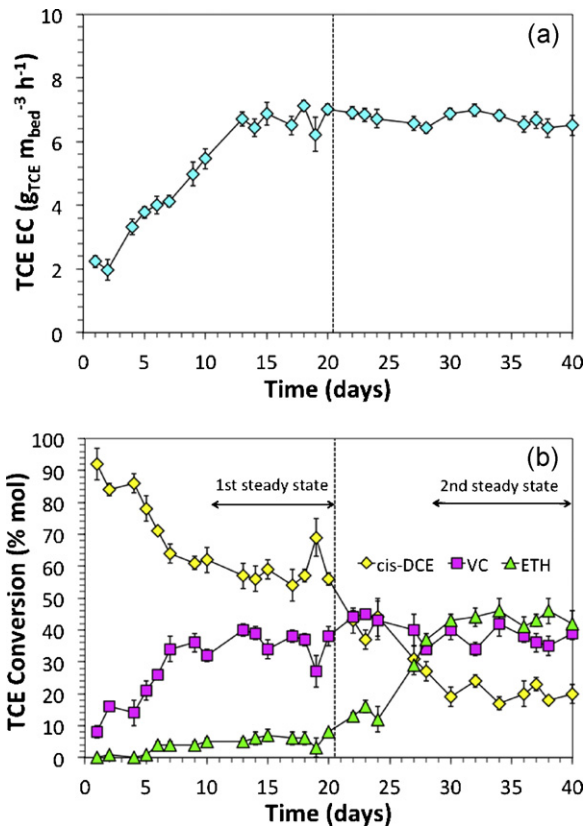

Fig. 1. (a) TCE elimination capacity (EC = gas flow × (concentration in–out)/bed volume) and (b) conversion of the TCE removed to intermediates vs. time in the biotrickling filter. The dotted line represents the time point of bioaugmentation with strain BAV1. Error bars represent standard deviations determined as errors propagated from standard deviations of three measurements of inlet and outlet gas composition (see Supporting information for details).

3. Results and discussion

The TCE elimination capacity (EC) of the anaerobic biotrickling filter during the entire duration of operation is shown in Fig. 1(a). As mentioned in the methods section, a higher loading rate of TCE was used here compared to previous studies [3,5] to better observe possible differences in the distribution of reductive dechlorination intermediates before and after bioaugmentation of the biotrickling filter with strain BAV1. Based on our previous studies, at the high TCE loading rate imposed, conversion of TCE to ethene would be minimum prior to bioaugmentation. As shown in Fig. 1(a), the TCE EC reached 6–7 g_{TCE} m_{bed}^{-3} h^{-1} before bioaugmentation, and remained at about that value after addition of strain BAV1. It is known that strain BAV1 cannot metabolize TCE, and even in the presence of lower chlorinated ethenes, the rates of co-metabolic dechlorination of TCE by strain BAV1 are orders of magnitude smaller than compared to strains containing the TceA and VcrA dehalogenases [12]. This explains the observation that at the high loading rate of TCE used, there was no effect of bioaugmentation with strain BAV1 on the TCE EC.

The distribution of the intermediates of reductive dechlorination of TCE in the anaerobic biotrickling filter before and after bioaugmentation with strain BAV1 is shown in Fig. 1(b). Prior to bioaugmentation, a pseudo-steady state with respect to the distribution of intermediates was reached. Conversion to *cis*-DCE was the highest at about 55% of the TCE removed, while 40% of the TCE removed was converted to VC, and less than 10% was recovered as ethene. Good closure of the TCE mass balance was achieved with a total of 95–105% of the reduced TCE recovered as *cis*-DCE, VC and ethene (on a molar basis). The low conversion to ethene, and significant accumulation as *cis*-DCE is consistent with earlier studies, where a loading of around 4–5 g_{TCE} m_{bed}^{-3} h^{-1} resulted in only about 10% conversion of the removed TCE to ethene [3]. The large conversion to *cis*-DCE and low conversion to ethene prior to bioaugmentation was expected in light of the inhibition of *cis*-DCE and, to some extent, VC dechlorination by TCE [5]. With TCE concentrations approaching the half-saturation constant of TCE reductive dehalogenation, this inhibition was even more pronounced.

Following bioaugmentation with strain BAV1, a distinct change in the distribution of the intermediates was observed (Fig. 1(b)). The conversion to *cis*-DCE started to decrease, while ethene production increased immediately. After a phase of about 10 days after bioaugmentation, during which conversion to ethene increased linearly, a new pseudo-steady state with respect to the conversion of removed TCE to intermediates was reached, with 45% conversion to ethene, 40% to VC and 15% to *cis*-DCE. Conversion of the removed TCE to VC increased immediately after bioaugmentation to 45% but then onwards remained around 40% until the end of the study. Again, good closure of the TCE mass balance was achieved (95–105% on molar basis of the reduced TCE was recovered as *cis*-DCE, VC and ethene). It is obvious that the distribution of the intermediates of reductive dechlorination of TCE changed favorably after bioaugmentation, even so, because of the high TCE loadings imposed, conversion of the removed TCE to ethene could not be increased further than 45%.

The concentrations of *tceA*, *vcrA* and *bvcA* gene copies in the top of the biotrickling filter bed at various biomass sampling time points during the experimental duration are shown in Fig. 2(a). Note that the TceA, VcrA and BvcA dehalogenases are present as a single

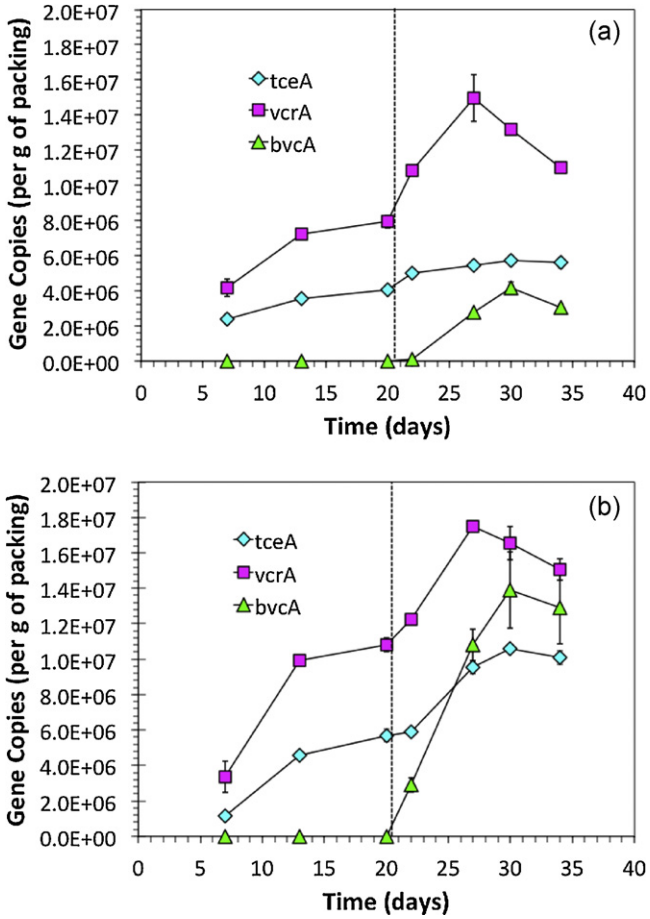

Fig. 2. Abundance of *tceA*, *vcrA* and *bvcA* gene copies with respect to time during operation of the biotrickling filter in (a) top and (b) bottom of the biotrickling filter bed. The dotted line represents the time point of bioaugmentation with strain BAV1. Error bars represent standard deviations of 2–4 measurements of DNA concentration per sample (two qPCRs each for two separate DNA extractions in most cases); they are not visible when they are smaller than the datapoint (see Supporting information for details). Recall that the biotrickling filter is operated with gas in down-flow mode, in co-current with the trickling liquid.

copy in the genomes of the various *Dehalococcoides* strains [10], and thus each gene copy corresponds to one cell. We also performed qPCR analysis for the *Dehalococcoides* spp. 16s rRNA gene (data not showed), and found that the sum of the *tceA* and *vcrA* gene copies consistently exceeded the total number of expected 16s rRNA genes by 1.2–1.4 times (which too exists as one copy per *Dehalococcoides* genome). This suggests that at least one strain in the culture that was used contains both the dehalogenases. In the past, it had been shown that the sum of all dehalogenases gene copies usually equals the total *Dehalococcoides* spp. 16s rRNA gene copies (i.e. one known reductive dehalogenase per genome) [10]. However there are more recent studies (e.g., [15]) in which qPCR data indicates that strains containing both the *tceA* and *bvcA* genes possibly exist.

Prior to bioaugmentation with strain BAV1, *tceA* and *vcrA* gene copies in the top of the bed reached up to 4×10^6 and 8×10^6 copies per g of packing, respectively (day 20), while no *bvcA* genes could be detected. This is about an order of magnitude higher than found by Behrens et al. [13] after bioaugmentation of a column packed with aquifer material, through which synthetic groundwater was passed. Possibly, the conditions in our biotrickling filter were more conducive to bacterial growth than in the aquifer material column. Following bioaugmentation, both *tceA* and *vcrA* gene copies increased, and reached up to 5.6×10^6 and 1.1×10^7 copies per g of packing respectively (day 34); however since strain BAV1 does not contain either TceA or VcrA dehalogenases and because a pseudo-steady state was observed with respect to treatment data, this was unexpected. These increases could possibly be explained by the fact that there were two instances of accidental increases in TCE loading, due to fluctuations in inlet gas flow rate, on days 18 and 24, in response to which there was likely increased growth of the *Dehalococcoides* spp. possessing these two dehalogenases. These reactor upsets were corrected as soon as detected (<12 h).

It is interesting to note that there was a significant increase in *vcrA* gene copies in the top of the bed, and that its abundance by the end of the study was higher (~2 times) than that of *tceA* gene. Recall that the biotrickling filter was operated cocurrently, i.e., that the inlet gas was fed at the top of the bed. The concentrations of the various compounds were measured along the length of the biotrickling filter bed, and TCE, *cis*-DCE, VC and ethene could all be detected even in the first quarter of the bed (data not shown). Part of this is due to the recirculating liquid which carries dissolved compounds from the bottom of the bed, back to the top of the bed. Thus, the large abundance of *vcrA* genes copies in the top of the bed does not necessarily mean that strains containing the VcrA dehalogenase are more active in TCE dechlorination than the strains containing the TceA dehalogenase. In fact, it has been shown that the pure extracts of the TceA dehalogenase dechlorinate TCE at a rate at least an order of magnitude higher than the VcrA dehalogenase [16,17]. The abundance of *bvcA* gene copies increased in the top of the bed following bioaugmentation with strain BAV1 to 2×10^6 copies per g of packing. Similar to the increase in *vcrA* gene copies, this increase can also be attributed to the presence of the lower chlorinated compounds at the top of the biotrickling filter, resulting from the recirculating liquid.

The concentrations of *tceA, vcrA* and *bvcA* gene copies in the bottom of the biotrickling filter bed at various sampling time points during the experimental duration are shown in Fig. 2(b). Prior to bioaugmentation, *tceA* and *vcrA* gene copies in the bottom of the bed reached up to 5.5×10^6 and 1.1×10^7 copies per g of packing respectively, while again no *bvcA* genes could be detected (day 20). Similar to the top of the bed, both *tceA* and *vcrA* gene copies increased in the bottom of the bed following shock-loading events on days 18 and 24. The final concentrations of *tceA* and *vcrA* genes were 1.1×10^7 and 1.5×10^7 copies per g of packing respectively (day 34).

Following bioaugmentation with strain BAV1, the abundance of *bvcA* gene copies in the bottom of the bed increased exponentially with time, confirming that the observed change in reactor performance was directly related to the growth of strain BAV1, and its retention on the packing material. DNA concentrations in the liquid were not determined because in previous studies [3,5], it was shown that no significant dechlorination occurs in the liquid at the HRTs (15 h) used in these reactors, and thus the majority of TCE reduction is achieved by attached biomass. The pseudo-steady state concentration of *bvcA* gene copies in the bottom of the bed reached 1.3×10^7 copies per g of packing (day 34). This is an order of magnitude higher than that in the top of the bed. This can be explained by the fact that TCE concentrations approached zero at the bottom of the bed, and the primary electron acceptors available were *cis*-DCE and VC, which are conditions reported to result in optimum growth of strain BAV1 [18,19].

To confirm that strain BAV1 was as active as the *Dehalococcoides* strains in the originally inoculated culture, RT-qPCR analyses were done for the *tceA*, *vcrA* and *bvcA* genes for each biomass sampling event. As mentioned before, because an internal control was not used, it is not possible to derive quantitative information regarding specific expression of each gene individually, as a function of duration of operation, as is normally reported in literature [9,10]. The raw data of cDNA concentrations from RT-qPCR analyses are shown in Supporting information in Fig. S1. These confirm that strain BAV1

Table 2
RNA analyses data represented as ratio of transcripts per cell of two genes. The biotrickling filter was bioaugmented with strain BAV1 on day 21, and thus RNA analyses for *bvcA* is not available (N/A) for earlier time points. The uncertainty in the calculated ratios reported here was between 5 and 10%, determined as errors propagated from standard deviations of 2–4 measurements of cDNA concentration per DNA concentration (see Supporting information for details).

Day	Ratio of *vcrA* transcripts per cell to *tceA* transcripts per cell		Ratio of *vcrA* transcripts per cell to *bvcA* transcripts per cell	
	Top	Bottom	Top	Bottom
7	44	254	N/A	N/A
13	18	64	N/A	N/A
20	35	47	N/A	N/A
22	14	32	0.26	0.45
27	19	95	2.5	0.05
30	6.3	165	2.7	0.28
34	14	36	9.7	0.45

was as active as the other strains, primarily in the bottom of the biotrickling filter. However the specific expression (i.e., cDNA concentrations normalized to the DNA concentrations) would appear low for all genes, and this is likely an artifact of low RNA extraction and processing efficiency. Even so, within a particular sample, the specific expression of different genes can be compared, since essentially, these were subjected to the same efficiencies during sample processing, and results from these are reported in Table 2 and discussed next.

The mRNA analyses data are thus presented in Table 2 as ratios of *vcrA* transcripts per cell to *tceA* transcripts per cell, and *vcrA* transcripts per cell to *bvcA* transcripts per cell. A ratio of 1 here suggests that the expressions of *vcrA* and the other gene being compared are equal, while a ratio >1 suggests higher expression of *vcrA*, and a ratio <1 a lower expression of *vcrA*. In both the top and bottom of the biotrickling filter bed, the ratio of *vcrA* transcripts per cell to *tceA* transcripts per cell was >10 under all conditions (except on day 30 in the top of the bed, when it equaled 6), highlighting that *vcrA* expression was at least an order of magnitude higher than that of *tceA*. As mentioned earlier, this expression, especially at the top of the bed, is not only a response to TCE, but also to the other chlorinated intermediates present because of the recyled trickling liquid. The ratio of *vcrA* transcripts per cell to *bvcA* transcripts per cell was >1 in the top of the bed after bioaugmentation under all conditions, and <1 in the bottom of the bed. More drastic gradients of reductive dehalogenases transcripts copy numbers were observed in the Behrens et al. study mentioned earlier [13], with up to several orders of magnitude differences depending on the location in their aquifer column. Differences in the mode of operation and in the nature of the systems that were investigated are probably responsible for the differences between the two studies. Here, the high density of *bvcA* transcripts confirms that strain BAV1 was not only growing in the biotrickling filter, but also actively participating in dechlorination. The high abundance of *bvcA* gene copies and its large expression observed here at the bottom of the biotrickling filter suggests that in a flow-through reactor that develops gradients of concentrations of TCE and of the various dechlorination intermediates, strain BAV1 can play an important role in enhancing the conversion of TCE by localizing in regions which receive primarily the lower chlorinated compounds.

This study shows that it is possible to improve the conversion of TCE to ethene in anaerobic biotrickling filters treating waste gases through reductive dechlorination by adding *Dehalococcoides* strain BAV1 to a mixed culture already containing the TceA and VcrA dehalogenases. Strain BAV1 was confirmed to grow in the biotrickling filter, and its reductive dehalogenase BvcA was shown to be highly active as demonstrated by detection of *bvcA* gene copies and transcripts respectively concomitant to improvement in TCE conversion to ethene. Approximately 45% of the inlet TCE was converted to ethene at loadings of around 8–9 $g_{TCE}\ m_{bed}{}^{-3}\ h^{-1}$. The high EC observed in this study with significant conversion to ethene suggests that it may be possible to reach higher TCE degradation rates with complete conversion to ethene by further optimization of the process culture.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cej.2011.12.026.

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