

ENHANCEMENT OF TRICHLOROETHYLENE DEGRADATION VIA CARBON DONOR AND MICROBIAL AMENDMENT ADDITION

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ABSTRACT: Microcosm experiments were conducted to identify a carbon donor that can be used at OU5, Hill AFB to stimulate optimal TCE dechlorination while minimizing methanogenesis and arsenic release from the native aquifer material. Microcosms contained aquifer solids, site groundwater amended with approximately 7 mg/L TCE, nutrient solution, and various carbon donor amendments. Three soluble carbon donors, three low solubility vegetable oils, and an emulsified oil were added to biotic and abiotic reactors incubated under anaerobic conditions for up to 280 days. Carbon donor addition of 1,000 mg C/L was necessary to initiate and maintain reducing conditions conducive to TCE dechlorination. Even at these high donor levels, however, only minimal TCE dechlorination was evident, and high levels of iron and associated As were released from the aquifer material in some treatments. Microcosms incubated with carbon donor plus microbial inocula did, however, demonstrate complete TCE dechlorination in less than 100 days without the apparent interference from iron reduction and with low arsenic release rates. Successful TCE dechlorination at OU5 will only be possible through the addition of both high dose carbon donor plus microbial inocula.

INTRODUCTION

Slow release carbon donors in the form of vegetable oils have been promoted for a number of years as a means of controlling carbon delivery rates, subsequent hydrogen production, and relative electron flow to dechlorination reactions versus methane production in groundwater remediation systems (Dybas et al., 1997; Boulicault et al., 2000 among many others). These low solubility vegetable oils are seen as economical, long-lasting, slow-release carbon donor materials that can be applied at a site in a single, high-dose to eliminate the cost of multiple, low-dose applications required with soluble donor amendments.

In response to field evidence of the production of dechlorination products following placement of a guar-lined sparge trench to intercept a TCE plume at OU5 at Hill AFB, this study was initiated to investigate the feasibility of controlled stimulation of TCE dechlorination using a range of both soluble and slow release carbon donor materials. In addition, two known dechlorinating microbial consortia were evaluated for their effectiveness in accelerating TCE dechlorination rates above those of the native microbial populations that were stimulated using select carbon donor amendments alone.

MATERIALS AND METHODS

Microcosm Preparation. Microcosms used for the evaluation of TCE dechlorination and intermediate product formation were prepared using 20-mL crimp-top, glass headspace

vials with Teflon-lined rubber septa. Each vial contained 3 g dry weight of OU5 aquifer material and 9 mL of site groundwater with approximately 8 mL of reactor headspace. All of the reactors were amended with TCE to a nominal initial concentration of 7 mg/L. Three soluble carbon donors (whey, HRC[®] and lactic acid), three low solubility carbon donors (a low melting point vegetable oil, a high melting point vegetable oil, and coconut oil), and an emulsified oil were evaluated for their effectiveness in stimulating TCE dechlorination in OU5 aquifer material. The soluble donors and emulsified oil were spiked directly into the microcosms at a nominal dose of 1,000 mg C/L liquid volume. The low solubility vegetable oils were added by first coating a washed fine sand with the individual donors at a concentration of 1 mg donor/g inert media and then adding the coated sand to the microcosms to yield a nominal dose of 150 mg C/L liquid volume to minimize the formation of a separate oil phase in the reactors.

Microcosms used for the evaluation of water quality changes produced in response to the stimulation of CAH dechlorination were constructed from 125-mL screw-top sample vials with Teflon-lined septa, that contained aquifer solids (25 g dry weight), groundwater (75 mL), and carbon and CAH amendment in the same proportion used within the smaller microcosms. These larger reactors provided sufficient sample volume for the analysis of groundwater pH, ORP, EC, DO, Fe²⁺, As³⁺, nitrate, sulfate, and DOC.

Triplicate reactors amended only with carbon donor were prepared with nutrient addition (2 mg/L yeast extract per microcosm), while an identical set were prepared without nutrient amendment to evaluate the impact of nutrient addition on biologically mediated process taking place during reactor incubation. All reactors amended with both carbon donor and microbial inocula also contained the nutrient solution. Both biotic (TCE but no carbon donor addition) and abiotic (TCE and carbon donor addition to reactors containing autoclaved aquifer solids and filter sterilized OU5 groundwater) controls were carried through the microcosm study to allow delineation of contaminant removal associated with abiotic losses and microcosm storage and handling losses. All microcosms were constructed and stored at 15°C, inverted, in an anaerobic glove box that was constantly purged (1.5 volumes/day) with a pure nitrogen gas stream. Seven sampling events were used to evaluate TCE transformation rates and water quality changes in the reactors over time in response to carbon donor and microbial amendment addition.

Microbial Amendments. Two microbial cultures were supplied to USU from the RETEC Group, Inc., (Lansing, MI) containing different consortia of known dechlorinating organisms. The first was a proprietary anaerobic granular mixed culture developed by MBI International (Lansing, MI), and grown by RETEC in an upflow anaerobic sludge bed reactor grown at ambient temperature on sodium lactate as the sole carbon source with an inlet TCE concentration of 500 mg/L. The second was a suspended growth mixed culture first described by Löffler et al. (2000) and enriched from samples collected from a field site in Michigan referred to as the “Bachman Road” culture. This Bachman Road (BR) culture is also grown on sodium lactate at room temperature with feed TCE concentrations of 25 mg/L. Both cultures were transferred to the USU in sealed, glass containers and were stored within the anaerobic chamber prior to addition to the microcosms. The MBI granular structure was disrupted by high-speed blending for a 2-minute period in the anaerobic glove box, while the BR culture was

added directly to the microcosms without further preparation. Both cultures were added directly to the TCE amended groundwater in the microcosms at a 10 vol% concentration (0.9 mL to the headspace vials, 7.5 mL to the 125-mL microcosms).

Sampling and Analytical Methods. Samples collected for CHC and degradation product analysis were removed from the anaerobic glove box at the appropriate sampling intervals, were immediately placed in a Tekmar 7000 Headspace Autosampler and were processed for quantitation of TCE, cis-and trans-DCE, and VC concentrations using an HP 6890/5973 GC/MS according to SW-846 Methods 5021 and 8260 (U.S. EPA, 1996). Following headspace analysis, the sample vials were manually transferred to another Tekmar 7000 Headspace Autosampler for quantitation of carbon dioxide, methane, ethane, and ethene using a Shimadzu GC-14 with dual Flame Ionization and Thermal Conductivity detection using procedures adapted from the methods of Smatlak et al. (1996) and Bradley and Chapelle (1999). Water quality analysis was carried out by opening the larger reactors within the glove box, sampling them for Fe^{2+} (Lovely and Phillips, 1986) and As^{3+} (Wilkie and Hering, 1998) resealing them, removing them from the glove box, immediately analyzing DO (U.S. EPA, 1979) and ORP (APHA, 1998), then processing the samples for nutrient and DOC analysis (U.S. EPA, 1979), followed by measurements of the remaining sample volume for pH and EC (U.S. EPA, 1979).

RESULTS AND DISCUSSION

Carbon Donor Treatments. Figure 1 summarizes the changes in TCE concentration measured in the carbon donor only microcosm systems over a 280-day incubation period. ANOVA results for these microcosms indicated that the decline in TCE concentrations in all reactors was significant over time, that significant differences in biotic versus abiotic treatments only occurred in the soluble donor reactors, and nutrient addition inconsistently increased TCE loss rates only in the biotic Whey and Lactic Acid treatments. TCE loss from all of these reactors over time can be attributed to irreversible sorption and reactor losses, as well as to potentially more significant abiotic transformation by iron sulfides in the aquifer material (Lee and Batchelor, 2002).

First order TCE loss rates for the carbon donor only reactors were estimated by converting TCE mass concentration data to molar concentrations, normalizing these molar concentrations to C_0 values for each treatment, and determining the slope of the linear regression of the natural log transformed normalized data. TCE degradation rates were then calculated by subtracting the first order loss rate for the Control microcosms from each of the other treatments. The resulting first order, Control-corrected degradation rates for TCE in the carbon donor only reactors are summarized in Table 1, and confirm results in Figure 1, that only the soluble donor materials stimulated TCE degradation in the OU5 aquifer material, and then only to a marginal extent.

Only limited stimulation of TCE dechlorination occurred despite high levels of DOC within the reactors, and reducing conditions that resulted in the complete removal of both nitrate and sulfate in all of the treatments (Figure 2). What is also evident from Figure 2 is the significant release of dissolved iron that occurred in response to soluble carbon donor addition to the reactors. Dissolved iron concentrations as high as 97 mg/L Fe^{2+} were observed during incubation, and although they did not persist throughout the

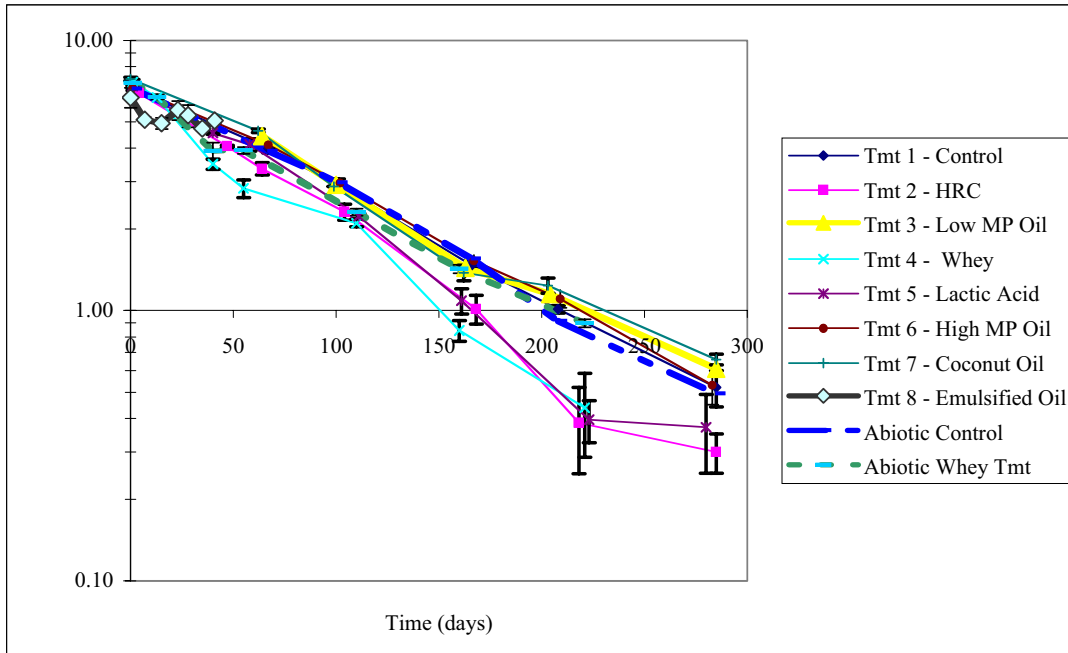


FIGURE 1. TCE concentration change with time in carbon donor only treatments. Error bars indicate 95% Confidence Interval of replicate measurements.

TABLE 1. Control-corrected TCE degradation rates in carbon donor only treatments.

Treatment	Control-Corrected TCE Degradation Rate, 1/d	r ² of Linear Regression
HRC [®]	-0.0024	0.9786
Whey	-0.0025	0.9745
Lactic Acid	-0.0023	0.9765
Control loss rate = -0.0092/d, r ² = 0.9979		

duration of the study, there was a concern that what appeared to be a large bioavailable iron pool was inhibiting TCE degradation in these OU5 sediment systems. In addition, OU5 soil is naturally high in arsenic, and arsenic release coupled to ferrous iron generation was of great concern, particularly at the levels observed in the carbon donor amended systems (Figure 2).

Maximum Potential Bioavailable Iron Determination. To assess the quantity of the maximum bioavailable iron pool in the OU5 soil, a series of short-term experiments were carried out to monitor dissolved iron release from the OU5 soil when incubated in the presence of excessive quantities of readily biodegradable carbon. Biotic microcosms containing OU5 groundwater were incubated in an anaerobic glove box at 15°C in the presence of 0.05, 0.5 and 5 wt% glucose and were sampled for Fe²⁺ and arsenic concentrations over a 21-day period. Results shown in Figure 3 indicate that the OU5 soil does indeed have a very large pool of bioavailable iron, generating over 1,000 mg/L

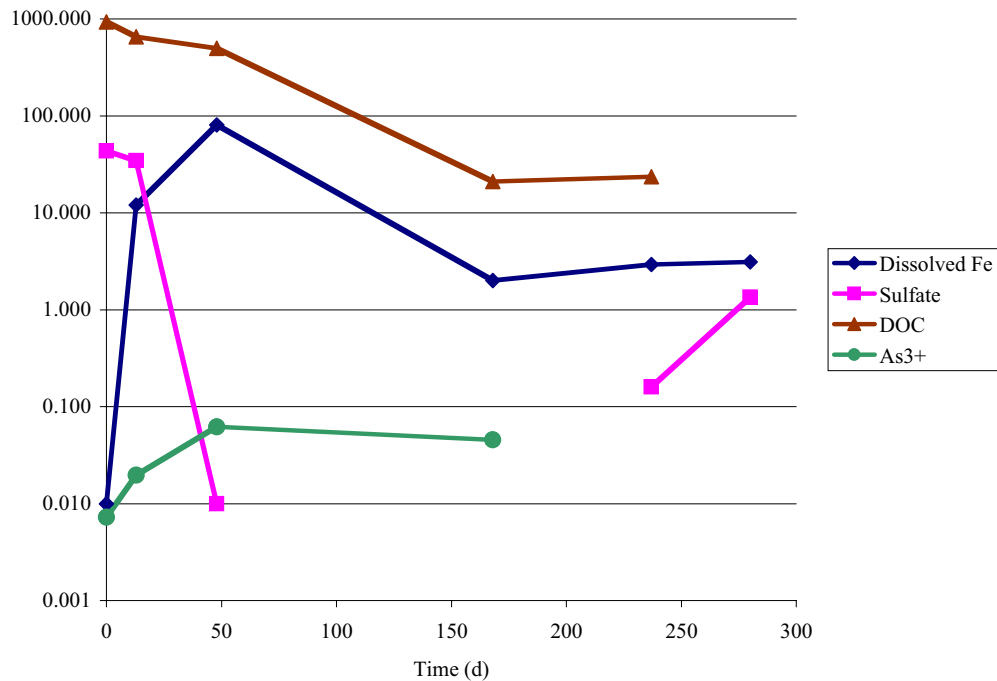


FIGURE 2. Typical change in water quality parameters over time as shown by response to whey addition to OU5 soil.

of dissolved Fe^{2+} with high carbon donor dosing rates. Very high levels of dissolved arsenic also result from high concentrations of carbon donor addition, reaching over 350 $\mu\text{g/L}$ in the 5% glucose amended reactors.

Carbon Donor Plus Microbial Inocula Treatments. Because of the large bioavailable iron pool and the potential for excessive release of arsenic from the aquifer solids during carbon amendment, the site was considered as a candidate for bioaugmentation. Microcosms were sampled over an 80-day period using the procedures described above to define enhancements to TCE and daughter product degradation, and reductions in undesirable intermediate product formation in response to microbial inocula addition. Figure 4 summarizes the impact microbial inocula addition had on the rate and extent of TCE transformation in OU5 soil, expressed as the mean chlorine number, the weighted average of the molar concentrations of TCE (Cl # = 3), cis-DCE (Cl # = 2), VC (Cl # = 1), and ethane (Cl# = 0) in the microcosms at each sampling interval. As dechlorination proceeds, the mean chlorine number of the mixture decreases from 3 (100% TCE) to 1 (100% VC), and is a simple indicator of the status of dechlorination at a given point in time during incubation. As shown in Figure 4, dechlorination rates were significantly accelerated in the OU5 soil with the addition of both a carbon source and the microbial inocula. Complete TCE transformation to ethane was observed within a 7-week period in the Bachman Road + Emulsified Oil treatment. Figure 5 shows the time course of TCE and its chlorinated degradation products within this inocula + Emulsified Oil treatments, once again highlighting the effectiveness of TCE transformation in these microbially amended reactors.

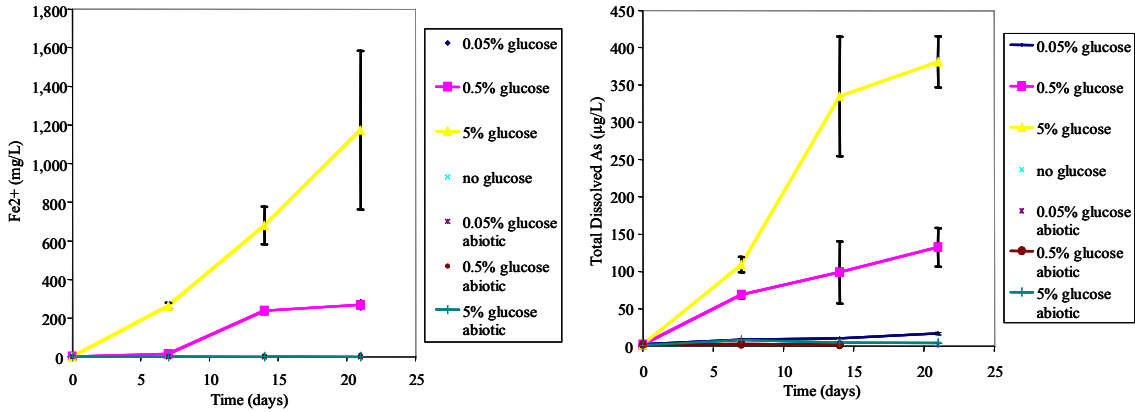


FIGURE 3. Determination of bioavailable iron and arsenic release over time as shown by response to glucose addition to OU5 soil. Error bars indicate 95% Confidence Interval of replicate measurements.

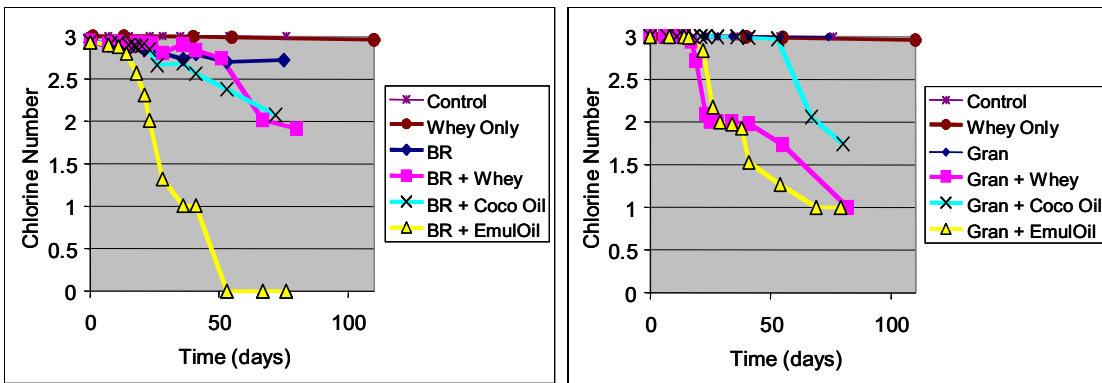


FIGURE 4. Changes in mean chlorine number in carbon donor + inocula amended microcosms during incubation.

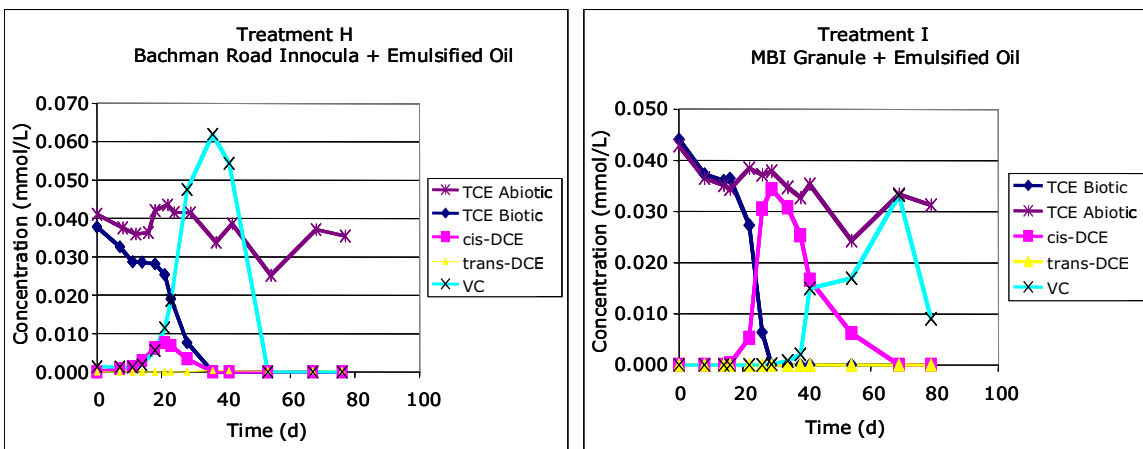


FIGURE 5. Time course of TCE and its chlorinated daughter products in the inocula + Emulsified Oil amended microcosms during incubation.

The microbial inocula amendments were also evaluated in terms of their ability to mitigate the release of iron and arsenic from the aquifer sediments while still providing accelerated TCE transformation. The results of these measurements are shown in Figure 6 and indicate that with microbial inocula, levels of both dissolved iron and arsenic were generally lower than with carbon donor alone. Whey produced the largest metal release of all donors, and despite the release of iron and arsenic being transitory as indicated in Figure 2, large metal release excursions would be problematic for full-scale implementation of carbon donor addition alone at this site.

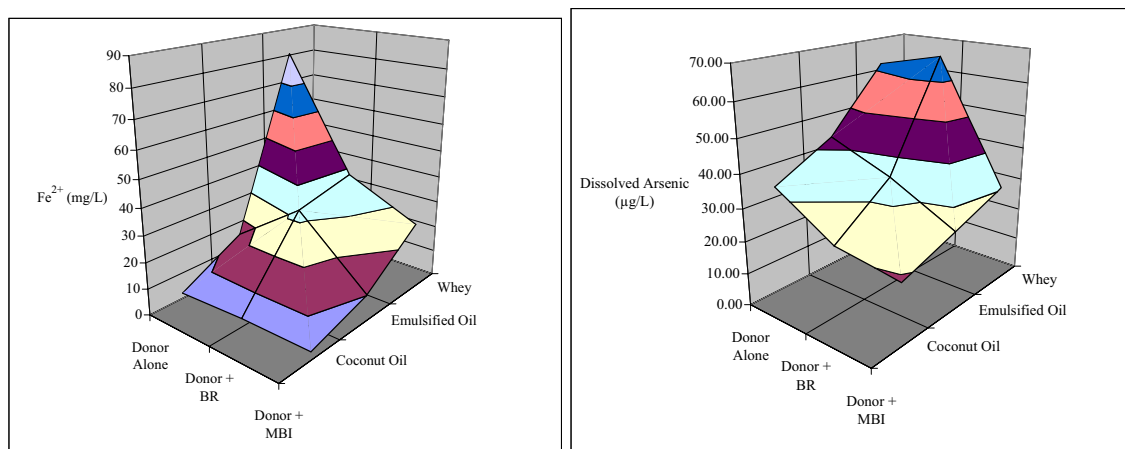


FIGURE 6. Maximum dissolved iron and arsenic in carbon donor only versus innocula + carbon donor amended microcosms during incubation.

CONCLUSIONS

TCE, and dissolved iron and arsenic concentration data observed in microcosms anaerobically incubated over a 280-day period have demonstrated that simply adding even high doses of easily degradable carbon does not effectively stimulate TCE dechlorination in OU5 sediment. Extremely high levels of bioavailable iron in the OU5 aquifer material, as determined from bioassay procedures, is likely the cause of this inhibition of TCE dechlorination. Effective enhancement of dechlorination capacity that persisted over an 80-day incubation period was observed, however, with the addition of both carbon donor and known dechlorinating microbial consortia. In the presence of the microbial inocula, coconut oil was the most effective slow-release carbon donor at stimulating TCE degradation, while the emulsified oil yielded the most rapid and complete dechlorination of all donors tested. Dissolved iron and arsenic production from aquifer solids during dechlorination was also shown to be mitigated with the addition of the microbial inocula. Successful TCE dechlorination at OU5 will only be possible, then, through bioaugmentation along with the addition of high doses of carbon donor.

ACKNOWLEDGMENTS

Funding for this project was provided by Hill AFB under Subcontract 0979-02-10-01 SA 001, with Sverdrup Technology, Inc., and from Tyndall AFB under Contract F08630-02-C-0002 through MBI, Inc. Additional assistance and technical support with field sampling of soil and groundwater was provided by URS and is greatly appreciated. Samples of emulsified oil were kindly provided by Bill Newman.

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