Tert-butyl alcohol (TBA), a metabolite of the gasoline additive methyl tert-butyl ether (MTBE), is a common groundwater contaminant encountered at petroleum remediation sites. This work explored the capability of YZ2, a novel pure culture, to completely degrade TBA aerobically in both batch studies and in continuous-flow columns containing biologically activated granular carbon (bio-GAC), simulating an ex-situ remedial system. YZ2 mineralized TBA from 0.5mM to 50mM, which is significantly higher than previously reported cultures. The level at which TBA limited the cellular activity was 40mM; however, mineralization still proceeded up to 50mM. Mineralization experiments showed almost 80% recovery of uniformly radiolabeled TBA as carbon dioxide. YZ2 growth and corresponding TBA oxidation rates within saturated activated carbon were compared with rates quantified in pure aqueous media; results demonstrate that GAC decreases oxidation kinetics for TBA. Batch studies quantified the kinetics of abiotic TBA sorption to activated carbon versus biological TBA oxidation using bio-GAC to compare the rates and efficiencies of physical removal processes to biological strategies. Data demonstrate that adsorption may be more efficient at high TBA concentration. In addition, pH levels increased to as high as 10.2 once activated carbon was added to solutions containing TBA, which inhibited microbial growth.

The continuous-flow bio-GAC columns mimicked a field bio-GAC unit and focused on different potential inoculation strategies and the long-term capabilities of YZ2 to degrade TBA as a continuous culture. Current data indicate lower effluent TBA concentrations within the bio-GAC column for 70 days, compared to a sterile GAC control column. However, the overall stoichiometry of the mineralization pathway indicates that dissolved oxygen concentrations in influent water may limit TBA degradation in the bio-GAC column, preventing complete degradation. We are using these data to develop a strategy for biological regeneration of GAC, which may be the most effective use of inoculated, TBA degrading cultures.
To my parents with love.
ACKNOWLEDGEMENTS

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I am especially grateful to the following people who offered support throughout this project: My advisor, Dr. Finneran, for his guidance and positive support, members of the Finneran lab for countless ideas and suggestions, and my fiancé Sean for his patience and friendship.
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INTRODUCTION

The Clean Air Act Amendments of 1990 mandated the use of reformulated and oxygenated gasoline in certain urban regions to reduce air pollution from motor vehicles (47). Initially introduced in the 1970s as a low-cost octane replacement for tetraethyl lead, MTBE—methyl tert-butyl ether—was employed as the primary oxygenate to lower carbon monoxide emissions in accordance with this legislation (6). Subsequently, MTBE was used in 30% of all gasoline and constituted 11% by volume of oxygenated fuels in the United States in 2000 (43, 11). While it did indeed improve air quality, MTBE has since been cited as the current second most abundant groundwater contaminant in shallow drinking water wells (2). Highly water soluble and resistant to many technologies currently utilized for gasoline remediation, MTBE often forms very large and deep groundwater plumes (22). Groundwater aquifers beneath areas of high MTBE use have between five and eight times greater probability of MTBE contamination than areas of low MTBE use (47), and regions that have been using it continually since the 1980s and early 1990s have detected MTBE and its primary metabolite, tert-butyl alcohol, in multiple subsurface aquifers (28).

Tert-butyl alcohol (TBA, C₄H₉OH), a clear liquid with a strong odor, is a prevalent and recalcitrant groundwater contaminant of environmental concern (7). It has also been proven to cause a carcinogenic response in rats (9), while MTBE is only a suspected carcinogen (23), suggesting that TBA may be of even more concern due to its higher toxicity (38). Shown in Figure 1, it is primarily formed as an intermediate in the degradation of MTBE but also has been used as a fuel oxygenate itself and has been added as an impurity in commercial-grade MTBE, composing a volume to up to 11% of the total volume (5, 38). TBA has been cited as a hydrolysis product of alternate fuel additive ethyl tert-butyl ether (17) and a byproduct of additive tert-amyl methyl ether (24). It has also been in the manufacturing of a variety of cosmetic products, in perfumes as an alcohol denaturant, as a dehydration agent, and as an industrial solvent (9). Alternate sources of TBA contamination may originate from atmospheric deposition, storm water runoff, and releases directly to surface water systems by industrial activities, but the primary source is the leaking of MTBE from underground storage tanks (5). In a survey of 500 sites sampled, TBA had the greatest geometric mean site maximum groundwater concentration of all common gasoline contaminants excluding MTBE, indicating that is very prevalent (40). While the EPA has established a health advisory taste and odor threshold of 20-40 μg/L for MTBE, TBA is currently under no federal regulation (48). Sixteen states have either established or
proposed regulatory limits; the most stringent is California’s provisional action goal of 12 parts per billion, or ppb (12).

![Chemical Structure of TBA, CAS Number 75-65-0](image)

Figure 1:  Chemical Structure of TBA, CAS Number 75-65-0

Rapid migration of TBA and parent compound MTBE in the subsurface is due in part to its high aqueous solubility, low octanol-water partition coefficient, and its chemical structure, which makes it resistant to microbial attack (38). Chemical properties of TBA are listed in Table 1.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight (g/mol)</td>
<td>74.12</td>
</tr>
<tr>
<td>Boiling Temperature</td>
<td>83 degrees C</td>
</tr>
<tr>
<td>Specific Gravity (Dimensionless)</td>
<td>0.79</td>
</tr>
<tr>
<td>Vapor Pressure (mm Hg)</td>
<td>41 at 25 deg C</td>
</tr>
<tr>
<td>Log $K_{OW}$</td>
<td>0.35</td>
</tr>
<tr>
<td>Log $K_{OC}$</td>
<td>1.57, 0.41</td>
</tr>
<tr>
<td>Henry’s Constant (dimensionless)</td>
<td>$4.9 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Table 1:  Chemical and Physical Properties of tert-butyl alcohol. Values obtained from the EPA’s Technologies for using MtBE and Other Fuel Oxygenates (48), except as indicated.

1 Calculated through extrapolation of a single parameter linear free energy relationship derived for other compound classes (38).

2 Calculated through a multi-parameter approach (33)

The high solubility of TBA results in lower efficiencies and higher remedial costs in comparison to the removal of other gasoline hydrocarbons from water (10). Consequently, conventional gasoline in-situ treatment methods such as air sparging are ineffective on TBA; such strategies are better suited towards highly volatile gasoline constituents such as BTEX (benzene, toluene, ethylbenzene, and xylene). Biological recalcitrance to TBA prohibits the effective development of subsurface TBA degrading biomasses at many sites (11). When required, remediation actions are implemented at costs usually 10-30% higher than those at sites without MTBE/TBA contamination (15). While TBA has been reported to degrade faster than MTBE aerobically (15), MTBE contamination often exists in anaerobic environments, where TBA degradation is limited (4, 38). Furthermore, the retarded degradation of TBA may lead to the further spreading of contamination (38).
The emphasis of most oxygenate studies has been on MTBE, with only a few studies specifically focused on TBA. As TBA is even more soluble and harmful than MTBE, effective remedial strategies should reduce the accumulation of this unwanted intermediate. From a regulatory standpoint, pump and treat options may be favorable for TBA remediation because they lack the variability that subsurface treatment methods often encounter. A study done in Maine concluded that an estimated 16% of randomly selected household potable wells had MTBE/TBA contamination (22), indicating a need for smaller, decentralized treatment systems. Carbon adsorption using granular activated carbon (GAC) is the most widely employed method of treatment to remove MTBE/TBA from small, private wells and is also utilized at many sites with highly TBA-contaminated waters (48). This method, however, is a physiochemical treatment method that accumulates the alcohol on the surface of the carbon, requiring the carbon to either be regenerated or disposed of as solid hazardous waste, thus resulting in high carbon replacement and disposal costs to the operator. Biofilms have been detected on these systems (35); however, these biofilms often do not develop in a timely manner.

The purpose of this research is to develop a process that can effectively treat waters containing tert-butyl alcohol in a continuous, sustainable, and timely fashion utilizing pure culture YZ2 (55), previously isolated from an activated carbon treatment system (35, 36). This study aims to develop an inoculation strategy for this culture into existing activated carbon systems used for on-site TBA remediation. Successful inoculation would result in the development of a biofilm over the carbon granules that would continuously transform TBA to inert products instead of TBA simply adsorbing to the carbon. This would allow the carbon to be continually utilized, prolonging the life of the carbon and eliminating the need for carbon replacement. It would bioaugment systems where native TBA degraders are not present and would shorten the acclimation period for systems where native communities are slow to grow. This investigation plans to characterize TBA biodegradation (kinetics, pathways, affecting factors and limitations) under oxic conditions and stimulate aerobic biodegradation within columns mimicking an ex-situ remediation system. These studies are targeted towards developing a clearer understanding of activated carbon inoculation methods, TBA transformation on activated carbon, and TBA sorptive properties that may ultimately aid in the design of more effective remediation strategies for TBA and other oxygenates.
LITERATURE REVIEW

Although the recalcitrance of tert-butyl alcohol was first documented as early as 1960 (17), most studies focusing on its degradation and removal have been conducted within the last fifteen years. Experiments have studied both abiotic and biotic TBA removal mechanisms; however, the focus has been primarily on the latter.

Non-Biological Treatment Methods

Currently, the most utilized treatment system for gasoline pollution is air sparging, or injecting air containing oxygen into the ground. Studies conducted by the EPA estimate that the air flow rates for these systems must be 5 to 10 times greater for MTBE than benzene (a common gasoline pollutant) to compensate for MTBE’s lower Henry’s constant, but some of these systems have been successful (48). For TBA, however, flow rates of 100 to 1000 times greater than those required for benzene are needed because of the alcohol’s miscibility in water. High flow rates such as these have led to fractures in the soil, air breakthrough, plume migration, and extremely large energy outputs— but no real removal of TBA (48). It can thus be concluded that air sparging is not an effective treatment method for TBA. Other conventional treatment systems such as soil vapor extraction and air stripping are limited in the same way by its high solubility (13).

Ex-situ treatment of extracted groundwater (i.e. pump and treat) can be used to treat oxygenates such as TBA by removing water from the subsurface before treatment. Different effluent treatments can then be applied with various efficiencies (13) but require accounting for a low affinity for organic carbon (K_{oc}) and a high affinity for water. Presently, activated carbon adsorption is the most widely employed method for treatment of these waters. Dispersed and disputed data with respect to the adsorptive properties of TBA to activated carbon were found in the literature. As cited in Table 1, two papers were found that calculated a K_{oc} value for TBA using linear free energy relationships; one used a multi-parameter approach (33), while the second calculated the constant through extrapolation of a single parameter linear free energy relationship derived for other compound classes (38). This latter value has been widely cited and contested in the literature, and other scientists estimate this value to be approximately zero (29, 38). Another study calculated the K_{oc} value of TBA using a linear transformation of data. They obtained a log K_{oc} value of 0.762 +/- 0.088, which they stated is likely more accurate since it is closer to the reported K_{ow} and nearer to zero than the frequently cited single parameter.
extrapolation (18). A study using bituminous coal observed no TBA adsorption at all (53), but this may be due to the selection of bituminous coal as the carbon media.

Coconut-shell granular activated carbon (GAC) has been determined to work better for MTBE (8, 41) than other activated carbon types, and while no studies analyzing TBA sorption to coconut shell carbon have been found for TBA, TBA may in fact bind to the carbon. Shih et al. evaluated GAC systems for the removal of MTBE from drinking water using rapid small-scale column tests containing coconut shell GAC and using TBA as a co-contaminant. Their results, however, focused upon the competitive properties of natural organic matter and more sorptive co-contaminants such as benzene, which outcompete both MTBE and TBA for adsorptive sites on granular activated carbon (41). An extension of this study concluded that a resin specifically designed to adsorb MTBE showed increased MTBE sorption as compared to coconut shell GAC when used with water samples containing both MTBE and TBA. This is because the resin showed no competition between MTBE and TBA, and the coconut shell carbon did, indicating that TBA may have been sorbing to the GAC (47).

As pores on activated carbon granules become saturated, the efficiency of the carbon is reduced, and the carbon needs to be removed and replaced. No specific papers on the removal of TBA from carbon granules could be found, but typically spent carbon granules need to be either regenerated, or exhausted carbon needs to be disposed of as hazardous waste. Carbon is usually regenerated through either chemical or thermal processes. The operation and maintenance involved in both carbon regeneration processes is very costly; furthermore, it is estimated that up to 30% of activated carbon may be lost in the thermal regeneration process (3).

Additional abiotic treatment methods include chemical oxidation, but these methods are not as frequently utilized at field sites as those listed above (48). Information on chemical treatment methods is sparse and mainly focused on MTBE. A study reported that effective removal of MTBE and its degrading intermediates—including TBA—was achieved via heat-assisted persulfate oxidation in aquifers (21). Ozone and hydrogen peroxide have been used in a laboratory setting to transform MTBE into TBA and then into further byproducts (1). Zeolites have also been proven to work for MTBE, but TBA is not mentioned anywhere in the study (27). These treatment methods are beyond the scope of this study.
Biological Treatment Methods

Although one study suggested the possibility of remediating TBA using phytoremediation and weeping willows (54), biological research in general is focused on a consortia of mixed and pure cultures isolated from petroleum remediation sites (23), streambeds (4, 6) wastewater treatment systems (25, 32), and biofilters (19) that are capable of biologically transforming TBA into less harmful products. The complete mineralization of TBA can be written as a half reaction (written below as a reduction) showing the oxidation of TBA to bicarbonate (14).

\[
4\text{HCO}_3^- + 4e^- + 28\text{H}^+ \rightarrow \text{C}_4\text{H}_{10}\text{O} + 11\text{H}_2\text{O}
\] (1)

Among the environmental factors that can affect biodegradation kinetics and metabolic pathways of recalcitrant compounds are dissolved oxygen and redox potentials (2); however, under all environmentally relevant standard reduction-oxidation conditions, mineralization of TBA is thermodynamically favorable (38). Therefore, there is no thermodynamic rationale for accumulation of TBA at sites. Proposed TBA intermediates include 2-methyl-2-hydroxyl-1-propanol, 2-hydroxyisobutyric acid (HIBA), 2-propanol, acetone, hydroxyacetone, and eventually carbon dioxide (11, 38). The primary proposed metabolite is HIBA (20, 25), and TBA oxidation has been linked to a soluble P-450 enzyme (44).

Both aerobic and anaerobic degradation have been reported; anaerobic degradation is generally slower and more site-specific than aerobic TBA metabolism (14), and therefore more studies have focused on aerobes. Several researchers have investigated the possibility of aerobically biodegrading TBA using both pure and mixed cultures. To date, there are six classified aerobes capable of oxidizing tert-butyl alcohol. Current pure cultures that degrade TBA are: *Hydrogenophaga flava*. ENV735 (20), *Mycobacterium austroafricanum. IFP2012* (16), *Aquincola tertiaricarbonis* L10^T and L108 (17, 25), *Burkholderia cepacia* CIP I-2052 (32), *Methylibium petroleiphilum* PM1 (31), and isolate YZ2, whose proposed name is *Hydrogenophaga carboriundus* (55).

Fixed biofilms constitute an effective alternative to accelerate the biodegradation rate of xenobiotic compounds such as TBA (2). Aerobic mixed cultures have been used to degrade both MTBE and TBA in a variety of reactor configurations such as an upflow fixed-bed biofilm reactor (2, 24), a fluidized bed bioreactor (49), and a continuously stirred tank reactor with biomass retention (52). In reactors containing both TBA and MTBE, TBA degradation varies with MTBE concentration. In some examples, TBA was shown to accumulate until MTBE was completely degraded (37, 44); one study showed the conversion of MTBE to TBA at nearly stoichiometric ratio before any TBA was utilized for growth (18).
In other studies, MTBE was incompletely degraded to TBA as a dead-end product (11). Other mixed cultures have degraded MTBE and TBA concurrently (37); in some situations, TBA has retarded the degradation of MTBE (51). Mineralization of TBA to below detection limits was obtained using a sequencing batch reactor only treating TBA containing well-settling aerobic granules that were able to oxidize TBA concentrations up to 600mg/L (46, 56).

The ability of TBA to biologically degrade under oxic conditions has been well documented; however, limitations on oxygen transport in surface and groundwater systems undermine the importance of this process as a natural attenuation mechanism (6, 7). Anoxic biodegradation overall is incredibly slow (42). Complete biomineralization of [U-\textsuperscript{14}C] TBA to \textsuperscript{14}CO\textsubscript{2} within anoxic riverbed sediment amended with oxygen have been documented within 81 days; comparatively, only 5% recovery of CO\textsubscript{2} was obtained with the same sediment over 198 days using sulfate-reducing conditions (7). Yet other studies indicate that adding oxygen to anoxic sediments does not always result in TBA degradation (23).

TBA is strongly recalcitrant under methanogenic conditions, meaning that MTBE transformation to TBA under these conditions is likely to result in the accumulation of TBA and little expectation of efficient TBA biodegradation (4, 38). Overall, the failure to stimulate MTBE or TBA degradation in the majority of studies indicate that, though anaerobic microbial communities capable of degradation of TBA/MTBE can be stimulated in some instances, there are many sites where anaerobic transformation may not happen in a timely manner (38, 49, 50). Consequently, the ability of aerobic bacteria to rapidly degrade TBA to carbon dioxide make them the best candidate for the biological portion of the proposed treatment system.

**Pure Culture YZ2**

A British Petroleum remediation site located in Fountain Valley, California used a bio-GAC reactor to treat an initial TBA concentration of 350ppb to below detection limits (35, 36, 55). From the biofilm on the reactor, two mixed cultures—YZ1 (55) and KR1 (35, 36)—were sequestered. YZ2 (previously denoted as KRH_YZ) is a pure culture isolate from mixed culture KR1. This culture was believed to be the first that has been derived from a GAC system and may be best suited for bioaugmentation into other GAC systems (36).

YZ2 is a tert-butyl alcohol-oxidizing microorganism, and TBA is used as the sole carbon and energy source. Based on its 97% similarity to the most closely related *Hydrogenophaga*, this isolate has been proposed as a new species, *Hydrogenophaga*.
*carboriundus*, within the genus *Hydrogenophaga* (55). Strain YZ2 is the type strain and has been submitted to both the American Type Culture Collection (ATCC) and the Deutsche Sammlung von Mikroorganismen (DSMZ) (55).

Previous studies determined that the optimal growth temperature of YZ2 is 30°C, but the culture grows well and can degrade TBA at temperatures between 4-30°C, making it psychrotolerant. This makes YZ2 the first reported psychrotolerant *Hydrogenophaga* or TBA degrader (55). This is very important to ex-situ remedial strategies, since it means that groundwater will not have to be heated in colder climates. Limited oxygen concentration inhibited YZ2 growth and TBA mineralization, but as a *Hydrogenophaga*, it was also able to utilize hydrogen as an electron donor. The optimal pH range was 6.5, but the cells grew well in a pH range from 6-9.5 (55). Due to its robustness, versatility, and growth within a wide range of temperatures and pH values, all biological studies presented in this paper were conducted using pure culture YZ2.
MATERIALS AND METHODS

Materials

TBA (ACS reagent > 99.3%) was supplied by Sigma-Aldrich Inc. All chemicals used for nutrients (ACS Grade) and solvents (HPLC grade) were obtained from Sigma-Aldrich Inc. Uniformly radiolabeled $^{14}$C-TBA (250µCi, 10.7mCi/mmol) was purchased from Moravek Chemicals. A NANO pure water purification system was used to produce de-ionized (DI) water. Sterile syringes and needles were purchased from Fisher Scientific. All samples were pre-filtered prior to analysis with sterile 0.2µm PTFE filters obtained through VWR.

British Petroleum (BP) provided all granular activated carbon (GAC) used for experiments. All activated carbon experiments were conducted using AquaCarb 1230C high activity 12x30 mesh Virgin Coconut Shell Granular Activated Carbon produced by US-Filter (currently Siemens). This brand of activated carbon is commercially used for drinking water treatment and groundwater remediation.

Borosilicate glass columns with an internal diameter of 25mm and a length of 150 mm (73.63mL total volume) purchased from Chromtech were used in the continuous flow biological experiments. All tubing was Masterflex silicone and Tygon tubing, size 13. Chromtech also supplied the glide fit end caps and 25-micron PTFE fittings at the end of the columns.

Analytical Methods

Gas chromatography equipped with mass spectrometry (GC/MS; Varian 4000, Varian Inc.) and a combiPAL autosampler was used to analyze TBA. 0.2mL aliquots of sample were added to the autosampler vials. Vials were individually heated at 90 °C for 10 min and shaken at 500 rpm. A 1mL headspace sample was taken from the heated vial and injected into a capillary column (Varian, fused silica, VF-5MS 0.25x 0.25µM x 30 m) via gas-tight syringe. The oven was held at 30 °C for 3 minutes and increased by 25 °C/min to 220 °C for 10.6 minutes. Ultra high purity helium, maintained at 3.0psi, was used as the carrier gas.

Gas chromatography connected to a flame ionization detector (GC-FID) was used for TBA analysis via heated headspace when GC/MS equipment was unavailable. In this method, 0.2mL of experimental samples containing TBA was added to a small, septa-sealed crimped vial containing 0.2mL saturated calcium chloride. The bottle was incubated at 90°C for 12 minutes before 0.2mL of headspace was removed using a gas lock syringe and
analyzed using the GC/FID equipped with an HP-1 capillary column (Hewlett Packard). The oven was held at 40°C for 4 minutes and temperature was increased by 40°C/min to a final setting of 160°C.

A spectrophotometer was used to quantify cell growth. The instrument was set to measure optical density at 600 nm; these values were used as a relative measurement of bacterial growth. DI water was used as a blank. A scintillation counter was used to measure dpm/mL in all radiolabeled samples; to prepare, 0.5mL of sample was added to 10mL of scintillation fluid and analyzed on the machine. Aqueous phase pH was measured by a Semi Micro pH probe (Thermo Scientific, Inc.)

Continuous flow tracer studies using bromide were quantified using an ion chromatograph (ICS 1000, Dionex) equipped with an Ion Pac AS14A 4 x 250mm column (Dionex) using an isocratic 8mM Na₂CO₃/1mM NaHCO₃ eluent at a flow rate of 1mL/min.

**Microbial Culture**

Pure culture YZ2 was used for all biological experiments conducted. As stated earlier, YZ2 was isolated from a mixed culture found growing on activated carbon granules in use at a tert-butyl alcohol remediation site in California. Since isolation, YZ2 has been continuously maintained on a fresh water media broth containing the following components (g/L unless specified otherwise): NaHCO₃, 0.21; NH₄Cl, 0.25; NaH₂PO₄•H₂O, 0.6; KCl, 0.1; modified Wolfe’s vitamin and mineral mixtures, and 1mL of 1mM Na₂SeO₄. The final vitamin amounts within the freshwater media are as follows: 20µg biotin, 20µg folic acid, 100µg pyridoxine HCl, 50µg riboflavin, 50µg thiamine, 50µg nicotinic acid, 50µg pantothenic, 1µg B-12, 50µg p-aminobenzoic acid, and 50µg thioctic acid; the final mineral concentrations are: 15mg NTA, 30mg MgSO₄, 5mg MnSO₄•H₂O, 10mg NaCl, 1mg FeSO₄•7H₂O, 1mg CaCl₂•2H₂O, 1mg CoCl₂•6H₂O, 1.3mg ZnCl₂, 100µg CuSO₄•5H₂O, 100µg AlK(SO₄)₂•12H₂O, 100µg H₃BO₃, 250µg Na₂MoO₄, 240µg NiCl•6H₂O, and 250µg Na₂WO₄•2H₂O. Freshwater media broth was autoclaved for 1 hour at 121°C to ensure sterility. Immediately before inoculation, aqueous TBA was added to room-temperature media broth. For all transfers, TBA served as the sole carbon source and electron donor within the broth. All transfers were conducted aseptically.

Cultivation of YZ2 was conducted in 125mL conical flasks (50mL culture solution) with Teflon-lined mini-nert screw tops (Supelco) in the dark on a rotary shaker. Cells were incubated at 30°C. Prior to the work presented here, YZ2 was maintained on freshwater
media broth containing 2mM TBA through 2% vol/vol transfers (55). In this work, YZ2 was maintained on freshwater media broth though 5% transfers and was continuously cultivated on both 5mM and 50mM TBA. Cells were transferred to fresh, sterile flasks containing media during late growth phase when turbidity suggested cell growth, which occurred at approximately 60 hours (media amended with 5mM TBA) and at 10-12 days (media amended with 50mM TBA).

**Microbial Batch Experiments**

All microbial batch studies were conducted aerobically. In addition to experimental flasks, all studies also contained triplicate sterile controls. These controls were autoclaved for 1 hour at 121°C prior to use to ensure sterility. All samples were incubated at 30°C and continuously shaken on a rotary shaker at 150 rpm in the dark to ensure oxygen circulation within the flasks and tubes. The rotary shaker also prevented the formation and settling of cell flocks within the vessels.

**5mM YZ2 Growth Curve**

Microbial batch experiments were conducted in conical flasks (250mL) sealed with screw-top lids containing Teflon liners. This was done to maintain the purity of YZ2 and prevent potential contamination, but flasks were sealed loosely to allow for oxygen transfer. TBA samples and cell density measurements were taken simultaneously every 12-24 hours to generate a growth curve.

**TBA Inhibitory Concentrations**

This study was conducted to determine inhibitory effects that incremental amounts of tert-butyl alcohol had on the growth of YZ2. Throughout the analysis, all components were kept constant except the TBA concentration. During this experiment, cultures were maintained in 26mL test tubes sealed with thick blue stoppers. YZ2 was continuously cultivated in 5mM TBA as described in the Microbial Culture section of Methods and Materials. Following eight successive transfers, YZ2 was then transferred to tubes containing 10mL of freshwater media broth and TBA concentrations ranging from 0-50mM, in increments of 5mM. All tubes were replicated in triplicate. Cell densities in each of the tubes, measured as OD 600, were measured daily. 0.5mL of pure oxygen was added to each tube daily to ensure that oxygen limitations were not preventing TBA mineralization. Measurements continued until all tubes showed at least three successive decreases in optical density measurements, indicating that cells had reached decay stage. The highest optical
density value obtained for each TBA concentration was plotted. This experimental 
procedure was then repeated for YZ2 continuously cultivated in 50mM TBA.

*Co-Contaminants*

The ability of YZ2 to mineralize TBA in the presence of other organic contaminants 
was determined in this study. Benzene and MTBE were selected because they are typically 
present in conjunction with TBA at remediation sites. Cells were grown in 1L of freshwater 
media broth containing 5mM TBA. During late logarithmic growth phase, the broth was 
distributed into four vessels, and cells were harvested by centrifuging each vessel at 5000G 
for 15 minutes at 4°C. Resultant pellets were washed, resuspended in 30mL of 250uM 
bicarbonate buffer, and centrifuged again. Final pellets were resuspended in 1mL of the 
bicarbonate buffer, making a total of 4mL of dense cellular solution. 0.3mL of these cells 
were added to each tube. Samples were analyzed for benzene, MTBE and TBA every two 
hours using the GC/MS method described above for TBA analysis. Peak times for benzene, 
TBA, and MTBE were 1.4, 2.1, and 2.5 minutes, respectively.

*TBA Mineralization Curves*

10mL of freshwater media amended with [U-\(^{14}\)C]-TBA (10.7mCi/mmol) to a final 
concentration 0.10mM TBA of was added to test tubes to precisely quantify recovery as CO\(_2\). 
Cells were grown in 1L of freshwater media broth containing 5mM TBA. During late 
logarithmic growth phase, the broth was distributed into four vessels, and cells were 
harvested by centrifuging each vessel at 5000G for 15 minutes at 4°C. Resultant pellets 
were washed, resuspended in 30mL of 250uM bicarbonate buffer, and centrifuged again. 
Final pellets were resuspended in 1mL of the bicarbonate buffer, making a total of 4mL of 
dense cellular solution. 0.3mL of these cells were added to each tube. The tubes were 
sealed with thick butyl rubber stoppers and crimped. Tubes were placed on a shaker 
table and continuously mixed at 30°C.

Samples were collected using sterile, aerobic syringes and needles. 1mL gaseous 
headspace samples were taken periodically, and \(^{14}\)CO\(_2\) was monitored using a gas 
chromatograph (Hewlett Packard Series 6890A) equipped with a gas proportional 
radiochromatography detector (IN/US Systems, Inc., GC-Ram) as previously described 
(50). Partitioning of carbon dioxide between the headspace and media was measured to 
determine the total \(^{14}\)CO\(_2\) production; 0.3 uCi H\(^{14}\)CO\(_3\)^- was added to an autoclaved tube of 
media and was maintained and stored under the same conditions as the experimental tubes. 
Headspace samples was taken and \(^{14}\)CO\(_2\) was measured after at least 24 hours to let
partitioning reach equilibrium. A partitioning coefficient for the distribution of \( ^{14}\text{CO}_2 \) was calculated to determine total \( ^{14}\text{CO}_2 \) production.

**Activated Carbon Studies**

All batch studies containing activated carbon were conducted in serum bottles sealed with thick, blue butyl stoppers and were maintained at room temperature, approximately 25°C. This was done to minimize TBA losses due to volatility. Activated carbon granules were first washed with nanopure water to remove any fines. The carbon was then baked at 90°C for 24 hours to remove the bulk of the water. All prepared carbon was stored in a sealed container at room temperature prior to analysis. Activated carbon mass is listed as dry weight.

**Activated Carbon pH Studies**

Freshwater media was prepared as described above without sodium bicarbonate. Sodium bicarbonate was added after all other components had mixed; final bicarbonate concentrations used were 0.5, 1.0, 2.0, 30, and 45mM. 100mL of freshwater media was distributed into 160mL serum bottles, sealed, autoclaved, and allowed to cool to room temperature. 20mM TBA was added to serum bottles containing 30 and 45mM bicarbonate; 0 to 55 grams (in 5g increments) activated carbon was then distributed into each serum bottle\(^1\) to determine how GAC loadings and buffer concentrations affected solution pH. TBA and pH samples were taken after 20 minutes of equilibration. 40mM TBA was added to serum bottles containing 0.5, 1.0, and 2.0mM bicarbonate to measure whether TBA alone affected the pH of solutions containing low buffer concentrations. 10g of GAC were then added to three of the flasks containing 1mM bicarbonate buffer; pH and TBA samples were taken periodically over 400 minutes to monitor how parameters changed over time. Samples for TBA analysis were obtained by syringe withdrawal. All experiments were conducted in triplicate with the average of the three results used for analysis.

**Batch Adsorption Isotherms**

Precisely weighed quantities of activated carbon were added to 35mL serum bottles. 30mL of nanopure water amended with \([U-^{14}\text{C}]-\text{TBA} \) (10.7mCi/mmol) to a final concentration 0.0768mM TBA of was added to the serum bottles to precisely quantify sorption kinetics. The bottles were sealed with thick butyl rubber stoppers and crimped.

\(^1\) Experiments testing GAC masses that exceeded 30g utilized 250mL conical flasks because volume exceeded capacity in the 160mL serum bottles.
Three control flasks containing saturated TBA solution without carbon, and three control flasks containing carbon but no TBA were run simultaneously. Vessels were placed on a shaker table and continuously mixed at ambient room temperature.

Samples were collected using sterile, aerobic syringes and needles. 0.5mL aliquots of aqueous solution were removed from each flask at each of the time points. 0.5mL of gaseous oxygen were added to the each flask prior to withdrawing samples to maintain ambient pressure conditions. Samples were taken at various time intervals to develop a complete sorption curve until three successive sampling points indicated asymptotic values. The total volume extracted from each vial for analysis did not exceed 4mL, or 13.3% of the total aqueous volume.

**Continuous Flow Studies**

The continuous flow studies described below are designed to assess how a bioaugmented system may enhance tert-butyl alcohol removal using GAC. The objective was to develop a system where pure culture YZ2 could be added and, after a short acclimation period, could continuously remove TBA.

**Experimental Setup**

The continuous flow studies were conducted in two sealed borosilicate glass columns. Glass columns were identical and are referred to in this text as Column 1 and Column 2. Both columns were filled with saturated activated carbon and were operated at room temperature. Columns were sealed with glide fit end caps and contained ports located at the inlet and the outlet of the reactor for sampling. Feed was continuously passed through the columns in an upflow direction using a Masterflex L/S peristaltic pump drive. Feed flow for all experiments was maintained at 1mL/min. Feed was stored in 5L flasks and was continuously supplied; as feed solution was depleted, it was replaced with a fresh flask. No influent/effluent samples were taken within 12 hours of the replacement of a feed flask.

The continuous flow setup was separate but identical for both columns as seen in Figure 2. A tube originating from the feed flask was passed through the Masterflex pump and a pipeline composed of 1/8” Tygon and 1/8” stainless steel tubing before entering the column. Immediately before the column inlet, a small 10mL conical flask sealed with a thick, blue butyl stopper was connected in series to prevent air bubbles from potentially entering the column. Each feed flask was sealed with a vacuumed sealed screwtop fitting.
containing two holes; this was done to minimize flow interruptions. One hole allowed the feed tube to enter; the second was sealed with a .2um PTFE filter.

Figure 2: Schematic of the continuous flow column setup.

Feed was designed to mimic natural groundwater. This “artificial groundwater” was composed of (g/L): MgSO₄·7H₂O, 0.06; NaBr, 0.0013; KCl, 0.0019; NaHCO₃, 0.212; CaCl₂ 0.012. Bromide was used as the conservable tracer at a concentration of 0.1029 g/L, or 5mM. “Aquifers”- feed flasks- were autoclaved prior to use to prevent growth of bacteria outside of the reactor. TBA served as the sole carbon source and electron donor within the artificial groundwater and was added after autoclaving as .0472mL neat liquid, making feed concentration 0.5mM TBA except when specified otherwise.

Influent and effluent samples were taken approximately every 72 hours for TBA analysis and every 144-160 hours for bromide analysis. For this, 1mL of solution was extracted and filtered. 0.2mL of the filtrate was transferred to a gas chromatograph vial and analyzed to detect the breakthrough of tert-butyl alcohol; the remaining filtrate was analyzed for bromide concentrations on the ion chromatograph when analyzed. Both gas chromatograph and ion chromatograph vials were stored at 4 °C prior to analysis.

Inoculation Studies

Column 1 was used as the experimental “Bio-GAC” column throughout the column studies; Column 2 was treated as a sterile control. Columns were treated identically with
regard to temperature and feed constituents as described above. Prior to use in the columns, activated carbon granules were first washed with nanopure water to remove any fines. The carbon was then baked at 90°C for 24 hours to remove the bulk of the water. 15g of baked activated carbon was distributed into each conical flask (250mL) sealed with screw-top lids containing Teflon liners and autoclaved for 1 hour at 121°C to ensure sterile conditions. Two separate inoculation strategies were assessed. The more successful strategy was determined as the strategy that had the greatest amount of TBA degraded over 20 days.

Inoculation Strategy 1: Four vessels containing 100mL of nanopure water were autoclaved, cooled, and amended with TBA to a final concentration of 20mM. Each vessel was added to a conical flask containing autoclaved GAC. Flasks were incubated at 30°C in the dark on a rotary shaker for 24 hours. Excess fluid was then decanted, and GAC was added aseptically via spatula to columns that had been sterilized immediately prior using ultraviolet light. GAC-filled columns were capped, connected to the continuous flow setup, and feed flow was initiated. Samples were taken daily at both the influent and effluent ports until TBA concentrations reached steady-state. Once equilibrium was established, flow was stopped. 1mL of YZ2 grown to late growth phase in freshwater media broth amended with 5mM TBA was injected into the base of Column 1 via syringe. Flow was then resumed, and experimental samples were taken from both columns.

Inoculation Strategy 2: Five vessels containing 100mL of freshwater media broth were autoclaved, cooled, and amended with TBA to a final concentration of 50mM. Each vessel was added to a conical flask containing 15g of autoclaved GAC. 5mL of YZ2 grown to late growth phase in freshwater media broth amended with 5mM TBA was added to one of the flasks. Flasks were incubated at 30°C on a rotary shaker for 10 days. Columns were sterilized using ultraviolet light, and sterile GAC saturated with media was added aseptically to Column 2. The GAC from the flask containing YZ2 was transferred to the base of Column 1. GAC with YZ2 composed approximately 5g, or approximately one-sixth, of the total mass of GAC placed in Column 1. The rest of the column was filled with sterile GAC saturated in media. GAC-filled columns were capped, connected to the continuous flow setup, and feed flow was initiated immediately afterwards. Columns were each fed 5L of freshwater media amended with 5mM TBA before feed was switched to the artificial groundwater described previously. Before experimental data was collected, Column 2 was sampled daily to ensure steady-state conditions. Once equilibrium was obtained, experimental samples were taken from both columns.
Continuous Column Studies

This study assessed the long-term capability of YZ2 to degrade TBA as a continuous culture. Using Inoculation Strategy 2, Columns 1 and 2 were monitored for 207 days for TBA removal. Feed consisted solely of artificial groundwater amended with 0.5mM TBA, except for the 95 days described below. Bromide data was analyzed periodically for indications of flow abnormalities.

Effects of TBA Concentration

This study determined whether reducing the influent TBA concentration could increase the fraction of contaminant removed and whether TBA removal is limited by dissolved oxygen concentrations. In this experiment, the TBA concentration was reduced to 40uM after 70 days of being continuously fed 500uM TBA. 40uM is the theoretical maximum amount of TBA that could be removed assuming influent dissolved oxygen concentration was held constant at 8mg/L.

After the TBA concentration was reduced, the influent and effluent concentrations were monitored every 12 hours until the new steady-state was reached in each reactor; following re-equilibration, the influent and effluent concentrations were monitored every 72 hours for a period of 95 days. Following this experimental period, the amount of TBA was returned to its original concentration of 500uM and allowed to re-equilibrate. The influent and effluent concentrations were again monitored every 12 hours; when steady state was achieved, 72-hour sampling resumed.

Tracer Studies

Tracer studies were conducted to determine the hydraulic residence time within each of the columns. In the continuous column studies summarized above, flow abnormalities were indicated by fluctuations in bromide, a conservative tracer. To calculate residence times, columns were initially flushed with artificial groundwater containing no bromide for a period of 10 days. Effluent samples were collected to confirm the absence of bromide within the columns. The pump was stopped, and artificial groundwater containing bromide was connected to the influent line. Flow was resumed, and samples were taken every 3 minutes for 60 minutes. Samples were analyzed using the IC for bromide, and breakthrough curves were generated. This data was used to estimate the pore volume within each of the columns.
RESULTS

Microbial Batch Experiments

YZ2 degraded 5mM TBA when incubated at standard conditions (30°C, pH 7, and ambient air) after 60 hours (Figure 3). No cellular growth was observed in media that did not obtain TBA, and no TBA decrease was observed in media without cells. As previously investigated, no TBA degradation products were observed on the GC/MS (55). Cells entered decay stage after TBA concentrations decreased to below detection limits. Based on this growth curve, cells used for subsequent experiments were harvested between t = 50 and t = 58 hours.

![Standard Growth Curve using 5mM TBA](image)

Figure 3: Standard Growth Curve using 5mM TBA. Experiment was conducted in triplicate; values shown are averaged values.

Concentration of TBA influenced cellular density. Figure 4 depicts the highest optical density obtained for each concentration of TBA. This figure shows how increased TBA concentrations can yield higher cellular densities. TBA concentrations ranging from 0 to 50mM were investigated twice, once with cells maintained on 5mM TBA and once with cells maintained on 50mM TBA. Data was collected over a 20-day period for both experiments. Higher densities of YZ2 were obtained using cells that were maintained on 50mM TBA than with cells that were maintained on 5mM TBA concentrations. Cells
maintained on 5mM TBA obtained a maximum optical density in media containing 35mM TBA; cells maintained on 50mM TBA obtained a maximum optical density in media containing 50mM TBA. While little difference in optical density was observed in cells maintained on 50mM TBA inoculated into tubes containing 40, 45, or 50mM TBA, these concentrations show decreasing optical density values for cells maintained on 5mM TBA.

![Graphs showing cell density maximums for 5mM and 50mM TBA transfers.](image)

**Figure 4: Cell Density Maximums, 5mM and 50mM Transfers.** Optical density shown in the figure is the highest optical density obtained during a 20-day experiment. Tubes were measured daily for optical density.

High optical density values observed in TBA concentrations greater than 25mM were obtained in less time for cells maintained in 50mM TBA than for 5mM TBA (Table 2).
<table>
<thead>
<tr>
<th>Conc. TBA (mM)</th>
<th>Highest 5mM CD (time elapsed)</th>
<th>Highest 50mM CD (time elapsed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0mM</td>
<td>0 (0 day)</td>
<td>0.021 (0 days)</td>
</tr>
<tr>
<td>5mM</td>
<td>0.674 (3 days)</td>
<td>0.640 (3 days)</td>
</tr>
<tr>
<td>10mM</td>
<td>1.206 (5 days)</td>
<td>1.306 (5 days)</td>
</tr>
<tr>
<td>15mM</td>
<td>1.228 (8 days)</td>
<td>1.582 (7 days)</td>
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<tr>
<td>20mM</td>
<td>1.847 (8 days)</td>
<td>1.907 (7 days)</td>
</tr>
<tr>
<td>25mM</td>
<td>2.008 (11 days)</td>
<td>2.183 (9 days)</td>
</tr>
<tr>
<td>30mM</td>
<td>2.025 (13 days)</td>
<td>2.420 (10 days)</td>
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<tr>
<td>35mM</td>
<td>2.249 (14 days)</td>
<td>2.626 (10 days)</td>
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<tr>
<td>40mM</td>
<td>2.120 (16 days)</td>
<td>2.832 (11 days)</td>
</tr>
<tr>
<td>45mM</td>
<td>2.237 (14 days)</td>
<td>2.872 (12 days)</td>
</tr>
<tr>
<td>50mM</td>
<td>1.809 (16 days)</td>
<td>2.906 (12 days)</td>
</tr>
</tbody>
</table>

Table 2: Cell Density (CD, measured as OD 600) Maximums, 5mM and 50mM Transfers. Experiment was conducted in triplicate; values shown are averaged values. Numbers in parentheses represent time in days it took to reach that optical density value.

The effects of MTBE and benzene as co-substrates on TBA degradation and culture growth are not shown due to inconsistencies in benzene and MTBE in the controls that continuously occurred after multiple replications. However, under resting conditions, YZ2 mineralized TBA in sixteen hours, regardless of the presence of MTBE or benzene. This data indicates that the presence of other contaminants does not significantly affect the ability of resting cells to mineralize TBA. MTBE and benzene data is not shown; benzene was not detected in any samples or controls after 10 hours, and MTBE was not detected in any samples after 25 hours. No discrepancy was observed between the decrease of benzene and MTBE in the controls and the experimental flasks, indicating that YZ2 did not affect their concentrations. Negligible growth was observed in the tubes containing only YZ2 and MTBE and tubes containing YZ2 and benzene, respectively, during the experimental period of two weeks. However, growth in one of the benzene-containing tubes was noted after a period of 200 days. The culture was transferred to a fresh tube containing benzene but did not grow a second time.

An average 78.3% recovery as CO$_2$ was obtained through mineralization experiments as shown in Figure 5, indicating that YZ2 is capable of completely mineralizing TBA to carbon dioxide with no accumulation of undesirable and toxic daughter products. It is believed that the remaining 21.7% of radioactivity was taken up by the cells and used for metabolism.
Activated Carbon Studies

The pH of aqueous solutions containing TBA increased after the addition of activated carbon. The data presented in Figure 6 shows the pH increases in solutions only 20 minutes after GAC is added. Mass of GAC ranges from 0g per 10mL buffered water to 5.5g per 10mL buffered water (upper range, barely enough water to saturate activated carbon). pH increases vary directly with the amount of GAC added; the more GAC that is added, the more drastic the pH shift. The presence of bicarbonate does buffer against the pH shift; pH change in the 45mM bicarbonate solution is less pronounced than in the 30mM solution. Quantity of TBA adsorbed varies with the mass of GAC added; almost all the TBA is removed with 5.5g GAC per 10mL buffered water. More TBA is removed in the 30mM bicarbonate buffered water than in the 45mM bicarbonate buffered water.
Figure 6: TBA sorption in saturated GAC after 20 minutes. Open squares and diamonds represent TBA concentrations; closed diamonds and circles represent pH values. Experiment was conducted in triplicate; values shown are averaged values.

<table>
<thead>
<tr>
<th>Grams GAC per 10mL Media</th>
<th>0.5mM Buffer</th>
<th>1.0mM Buffer</th>
<th>2.0mM Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>No TBA</td>
<td>6.98</td>
<td>7.08</td>
<td>6.92</td>
</tr>
<tr>
<td>TBA</td>
<td>6.94</td>
<td>7.00</td>
<td>6.91</td>
</tr>
<tr>
<td>No TBA</td>
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<td>7.25</td>
<td>6.90</td>
</tr>
<tr>
<td>TBA</td>
<td>6.92</td>
<td>7.04</td>
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<tr>
<td>No TBA</td>
<td>6.93</td>
<td>7.05</td>
<td>6.85</td>
</tr>
<tr>
<td>TBA</td>
<td>6.84</td>
<td>7.05</td>
<td>6.93</td>
</tr>
</tbody>
</table>

Table 3: TBA effects on pH. "No TBA" columns represent pH values post-autoclaving; "TBA" columns represent pH after 40mM TBA added. pH of all bottles was 7.00 prior to autoclave.

The effect of autoclaving and adding TBA to nanopure water containing three different concentrations (0.5, 1.0, and 2.0mM) of bicarbonate buffer is recorded in Table 3 (above). Experiment was conducted in 250mL flasks sealed with vented, screw top lids. All flasks of media was titrated to a pH of 7.00 prior to autoclaving, and the column labeled “No TBA” represents pH levels after autoclaving and cooling. An increase in pH was observed after autoclaving and cooling. The addition of 40mM TBA to solutions containing bicarbonate buffer resulted in the overall slight lowering of pH levels.

The effects of Figure 7 (below) shows how the pH of a solution containing 1g GAC per 10mL media buffered with 1mM bicarbonate buffer increases over time. Sorption and
pH kinetics correspond; pH levels reach asymptotic values when all of the TBA is adsorbed. No pH change is observed in the control.

Granular activated carbon sorption studies were conducted to evaluate the ability of activated carbon to remove TBA from aqueous solution. Kinetic results, showing the decrease in TBA concentration with time, are presented in Figure 8 (next page).

Figure 7: TBA sorption in 100mL media containing 10g GAC and 1mM bicarbonate buffer over time. Open circles and crosses represent TBA concentrations; closed diamonds and triangles represent pH values. Experiment was conducted in triplicate; values shown are averaged values.
Figure 8: TBA sorption curves plotted for incremental amounts of GAC.

Continuous Flow System

Columns were inoculated with the two different strategies as described in the Methods section. Strategy 1 (injection of 1mL of YZ2 into Column 1 via syringe) was tested first. Prior to injection, both columns were filled with GAC saturated in aqueous solution containing 20mM TBA, and feed flow was initiated. Samples were taken daily until the effluent concentration decreased to or below a concentration of 0.5mM in both columns. This took 7 days; in this time, effluent concentrations of TBA in the columns ranged from 14.3 to 0.49mM while the influent concentration remained within 0.42 to 0.56mM, close to the targeted 0.5mM. Once an effluent concentration of 0.49mM was obtained, flow was stopped, and 1mL of YZ2 in late growth phase was injected via glass syringe with a 2-inch needle through the base of Column 1. Although YZ2 was injected quite slowly, much of the culture was seen trickling out of the needle piercing in the column fret as the column was being reconnected to the column setup. Flow was resumed, and influent and effluent concentrations were taken from each column every 1-2 days (Figure 9). After 19 days of continuous flow and 14 time points, no discernable difference was noted between Column 1 (inoculated) and Column 2 (sterile control.) Flow was then stopped, all GAC was removed and discarded, and the system was sterilized.
Figure 9: Fraction of TBA Remaining verses Time, Inoculation Strategy 1. Experiment was conducted for 19 days before determined to be unsuccessful.

Figure 10: Fraction of TBA Remaining verses Time, Inoculation Strategy 2. Experiment was conducted for 22 days before determined to be successful. All subsequent continuous flow system figures use the inoculation strategy depicted here.
Strategy 2 (GAC incubated in media containing YZ2 was placed in the bottom of Column 1) was tested next. As described in the Methods section, both columns were filled with GAC saturated in 50mM media and 5g of media containing YZ2 was placed in the bottom of Column 1. Columns were both fed 5L of freshwater media broth containing 5mM TBA before switching to artificial groundwater amended with 0.5mM TBA. Samples from Column 2 (control column) were taken daily until the effluent concentration decreased to or below the concentration of the influent. This took 11 days; in this time, effluent concentrations of TBA in Column 2 ranged from 33.8 to 0.55mM while the influent concentration remained within 0.43 to 0.61mM after the initial bottle of 5mM TBA media had been depleted.

The influent and effluent concentrations in Column 1 and Column 2 for the first 22 days after steady-state conditions were obtained are plotted in Figure 10 (previous page). Column 1 showed a marked decrease in TBA concentrations as compared to Column 2. Although visible evidence of biofilm was never observed in Column 1, this reduction in TBA concentration was used as indication of the successful transfer of the biofilm. Inoculation Strategy 2 was determined to be the more successful of the two strategies tested, and Figures 11-14 all depict continuing data from this inoculation.

Figure 11 (next page) shows data obtained from 207 days of continuous operation of the two columns. Two periods of equilibration (immediately after the feed concentration was lowered to 0.040mM at day 69 and immediately after the feed concentration was returned to 0.5mM at day 164) occurred; the times required for the effluent TBA concentration in Column 2 to return to the influent concentration and were 2 and 3 days, respectively. Only two periods of TBA removal were observed in Column 1; the first was observed in the first 22 days of column setup, and the second was seen during the 90 days that the columns were operated at the lowered TBA concentration. Table 4 outlines a summary of the trends observed in Figure 11.
Figure 11: Fraction of TBA Remaining verses Time, 207 days of continuous operation. Values indicated are percent degradation in the continuous flow reactors. No recirculation of effluent. Flow rate = 1mL/min.
### Periods

<table>
<thead>
<tr>
<th>Period</th>
<th>Time Period</th>
<th>TBA Conc</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>11 Day Equilibration Period</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Days 0-22</td>
<td>0.5mM</td>
<td>Enlarged in Figure vv. Clear discrepancy between Columns 1 and 2; 20-100% TBA mineralized</td>
</tr>
<tr>
<td>2</td>
<td>Days 22-69</td>
<td>0.5mM</td>
<td>No discrepancy between Columns 1 and 2; minimal mineralization, if any.</td>
</tr>
<tr>
<td><strong>Days 69-74 Re-equilibration to new TBA concentration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Days 74-164</td>
<td>0.04mM</td>
<td>Enlarged in Figure hh. Clear discrepancy between Columns 1 and 2. Some mineralization observed in Column 2; complete mineralization observed in Column 1 for 21 days (Days 130-151) and again at Days 158-164.</td>
</tr>
<tr>
<td><strong>Days 164-169 Re-equilibration to new TBA concentration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Days 169-207</td>
<td>0.5mM</td>
<td>Some mineralization occurred in both columns, but values fluctuate over a huge range and success cannot be determined.</td>
</tr>
</tbody>
</table>

*Table 4: Trends displayed in Figure 13 over 207 days of continuous operation.*

![Figure 12: TBA fraction removed over time, TBA Concentration = 40uM. I need a better description of this data, but hey- it’s at least formatted correctly. Replace plot when fixed.](image)

Figure 12 shows the fraction of TBA removed in the columns when the TBA concentration in the aquifers was reduced to 0.040mM, the maximum amount of TBA that
can be mineralized based on the stoichiometric relationship between TBA and oxygen, as stated in the Methods section. Sampling began after a 5-day equilibration period. During the first fifty days of operation at a TBA concentration of 40μM, sharp variations in TBA degradation were observed, but TBA effluent averaged approximately 50% of the influent concentration in Column 1. This period represents an acclimation period for the culture. After Day 134, effluent TBA concentrations in Column 1 dropped to below 10% of influent concentrations. For the 35 days\(^2\) following, YZ2 completely mineralized 100% of the TBA. This trend discontinued when the TBA concentration was returned to 0.5mM TBA at \(t = 164\) days.

The toxic/inhibitory effect of 500μM TBA was again observed immediately after the re-equilibration period. While some TBA degradation was observed, it was not continuous, 100% degradation as observed with a TBA concentration of 0.040mM.

Tracer studies were performed on both columns to determine the void volume or hydraulic retention time. Due to equipment malfunction, only the tracer data for Column 2 was analyzed at the time this text was written. An interstitial volume of 55.5mL was determined for Column 2; as the empty bed volume for the glass columns was 73.63mL, this represents a pore volume of 75.3%. Effluent tracer concentrations were recorded with respect to time and presented at \(C(t)/C_0\) verses time as depicted on Figure 13. The residence time distribution curve \(E(t)\), obtained by differentiating the \(C(t)/C_0\) are presented in Figure 14. The maximum or peak of each residence time distribution (RTD) curves (\(E\) curves) represent the average residence time within the system between the aquifer and the influent and effluent valves. The interstitial or pore volume can be calculated by multiplying the flow rate in mL/min by the average residence time in minutes.

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\(^2\) One data point during this period indicated otherwise. The point taken at \(t = 154.8\) days indicated 31.0% removal instead of 100% removal, but consecutive data points before it and after it indicate 100% removal. The reason for this spike is not known.
Figure 13: Column 2 experimental breakthrough data. Continuous input of 5.0mL TBA starting from $t = 0$ min. $Q = 1.0$ mL/min.

Figure 14: Column 2 residence time distribution. $Q = 1.0$ mL/minute, $t_{avg}$ from the aquifer to the influent valve = 12 minutes, $t_{avg}$ from the aquifer to the effluent valve = 67.5 minutes.
DISCUSSION

The focus of the studies conducted was to investigate the application of isolated culture YZ2 in bio-GAC treatment systems used to remediate tert-butyl alcohol (TBA). Such a system would prolong the lifespan of the activated carbon, produce less waste, and would transform TBA into inert carbon dioxide instead of simply transforming it to new media. Results presented demonstrate that culture YZ2 is capable of degrading TBA in a variety of conditions, including a continuous flow setup mimicking an ex-situ remediation system.

Preliminary batch studies conducted early in this investigation indicated that YZ2 was not able to grow in solutions containing high quantities of GAC and tert butyl alcohol. While later data suggests that growth likely was inhibited by a GAC-induced pH change, it was initially believed that high concentrations of tert-butyl alcohol were toxic to the pure culture. This was further explored, and the data presented in Figure 3 is the product. The cell density measurements taken in triplicate at 11 different TBA concentrations suggest that as TBA concentration increases, so does aqueous cell density. The linear and identical OD 600 values in 0-10mM tubes for cells grown at 5mM TBA and cells grown at 50mM TBA indicate that the consecutive transfers in high concentrations of substrate did not affect growth at lower TBA levels; likely, cells in these tubes grew until TBA was depleted and then stopped growing. Data from the cells maintained in 5mM TBA and transferred to tubes containing 15-50mM TBA suggests that higher concentrations of TBA may have affected cellular growth. Unlike tubes containing lower TBA concentrations, data in this range is not linear, and direct correlation between TBA concentration and maximum cell density cannot be established. Furthermore, optical density values in tubes containing 25-40mM TBA appear asymptotic, and optical density values in tubes containing 40-50mM decrease as TBA increases, indicating that these TBA concentrations may be toxic to the culture.

In contrast, cells maintained on 50mM TBA did not display evidence of TBA toxicity at any of the concentrations tested. Optical density values in TBA concentrations from 0-10mM and TBA concentrations from 10-40mM show a linear correlation. Optical density values in TBA concentrations between 40-50mM appear asymptotic but, unlike cells maintained on 5mM TBA, optical density values did not decrease at any point over the 0-50mM range. While it does contain the nutrients essential for bacterial growth, freshwater media broth is not considered a nutrient-rich media, and an optical density at 600nm of 3.100
is quite high for bacterial growth. Therefore, the asymptotic values observed between 40-50mM might be due to nutrient limitations in the media, not substrate toxicity. Cells in tubes containing 15-50mM previously maintained in 50mM reached their highest optical density values in a shorter period of time than the cells maintained in 5mM, indicating that cells grown at lower TBA concentrations may experience an adjustment lag time when introduced to the higher TBA concentrations. TBA concentrations of 50mM are quite high; no other literature sources citing culture growth at such levels could be found. Overall, cellular growth was more successful over the TBA range in cells maintained in the higher TBA concentration. This indicates that YZ2 is robust and adaptive to varying levels of toxicity, and that the culture may be applied to a wide range of TBA-contaminated sites. Figure 5, showing almost 80% recovery as CO₂, affirms that the observed TBA degradation is indeed mineralization to carbon dioxide, indicating that YZ2 does indeed transform high levels of TBA to innocuous products.

The objective of studies exploring TBA degradation by resting cells in the presence of benzene and MTBE was to determine whether the presence of co-contaminants adversely affected YZ2’s ability to mineralize TBA. Earlier studies conducted in this investigation (data not shown) and by previous investigations (55) determined that YZ2 is not able to utilize either MTBE or benzene as a substrate. TBA, MTBE, and benzene were all monitored over the experimental period. Experiments were conducted in triplicate in tubes sealed with thick, blue butyl stoppers to prevent losses due to volatility; however, the decrease of benzene and MTBE concentrations to below detection limits in both the experimental and control tubes indicates contaminant losses through means other than biological degradation. Both benzene and MTBE are considerably more volatile than TBA; although the bottles were sealed, it is believed that they volatilized and were absorbed by the butyl stoppers.

Cellular growth in one of the benzene-containing tubes that did not contain TBA was noted after a period of 200 days. The culture was transferred to a fresh tube containing benzene but did not grow a second time. Experimental data indicated that benzene in this tube depleted after 10 hours, and growth was only observed in one of the three tubes; therefore, it is likely that this growth was an abnormality and is not attributed to YZ2 using benzene as a substrate.

Despite the loss of the co-contaminants, very little discrepancy is observed between the control TBA decay curve and the TBA curves in the presence of benzene and MTBE. As both co-contaminants were present in the tubes for at least the first 10 hours of the experimental period, their initial presence did not affect culture growth and TBA
mineralization. This indicates that YZ2 may be used to biologically degrade TBA in waters containing other contaminants frequently encountered at petroleum remediation sites, and that co-contaminants should not affect mineralization rates.

GAC-induced pH shifts were investigated after numerous experiments yielded no cellular growth in the presence of TBA and GAC, and the final pH of such cellular solutions were highly alkaline, ranging from pH 10 to 11.5 (data not shown). In both previous investigations and in these studies, growth of YZ2 has been documented in highly alkaline solutions; however, minimal growth has been observed in pH values from 9.5 to 10.2, and no growth has been observed in solutions with a pH greater than 10.2 (55). The addition of TBA does not significantly affect the pH of a solution; in Table 3, experimental data shows that the addition of TBA slightly lowers the pH of aqueous solutions. Therefore, TBA is not believed to contribute directly to the pH increases observed. Incremental amounts of bicarbonate buffer increase the pH with autoclaving because bicarbonate produces carbon dioxide gas and hydroxide ions with heating in accordance with the following equation,

\[
HCO_3^- \rightarrow CO_2 + OH^- 
\]

(2)

and carbon dioxide gas then escapes unsealed bottles, leaving the hydroxide ion that subsequently increases the pH. All experiments were conducted with bicarbonate buffers since this buffer is most reflective of natural buffer within groundwater, and all experiments requiring concentrations of bicarbonate buffers greater than 2mM were autoclaved in sealed containers to prevent the escape of carbon dioxide gas. The pH levels then returned to around 7 as bottles cooled and carbon dioxide reformed into bicarbonate.

The addition of activated carbon to solutions containing TBA produced the highly alkaline solutions observed in experiments where no cellular growth was observed in the presence of TBA and high concentrations of GAC. This can be minimized using high concentrations of bicarbonate buffer as shown in Figure 6, but these levels of buffer are not reflective of natural groundwater. Groundwater usually contains naturally present bicarbonate concentrations below 1mM; the buffer concentrations used in Figure 6 are 30 and 45mM, respectively.

pH shifts yielding highly basic solutions are a function of TBA depletion, as shown in Figure 7. As TBA is rapidly removed from solution by sorption to carbon, pH exponentially increases from 7.36 to 9.78. The bicarbonate buffer for this reaction is the same as the buffer used for the continuous flow system. Although the TBA concentration (40mM) in this figure is considerably higher than the TBA concentration (0.5mM) maintained in continuous flow system, it does indicate that the pH inside of the glass columns will likely
increase considerably if flow is stopped and the columns are left to operate like a batch system. The system should therefore be continuously operated, with minimal flow interruptions.

It is not known why the interactions between activated carbon and aqueous TBA solutions produce such a drastic pH increase. This may be related to the method used to activate the carbon. The manufacturer of the carbon has been contacted for more information, which will be explored further in future studies.

The abiotic sorption reaction between TBA and activated carbon granules was studied to compare the abiotic reaction typically utilized in remedial pump and treat systems against the proposed biological treatment system studied in this text. In the proposed treatment process, TBA is removed by biological treatment processes, not though physiochemical reactions. Therefore, the GAC adsorption capacity is studied to determine the optimal use of carbon for the process.

Adsorption to GAC is currently the default treatment method for TBA in the remediation industry, but as stated previously the ability of TBA to adsorb to GAC is greatly disputed in the literature. Data shown in Figure 8 demonstrates TBA removal from aqueous solution via sorption and shows that mass of TBA removed is a function of mass of GAC added to solution. Sorptive properties often vary with the type of activated carbon used; parameters such as surface charge (related to how the carbon was activated) and overall surface area per mass of carbon can greatly affect a contaminant’s sorptive properties. The only carbon used in the experiments presented in this text was virgin coconut shell carbon; other experiments that have concluded TBA is not sorptive have used bituminous carbon. Physical properties of these two carbon types differ considerably. Bituminous carbon is produced by coal and is frequently utilized in bulk contaminant removal; coconut shell carbon is typically utilized in drinking water and remediation purposes and is better at removing micropollutants. This finding corresponds with suggestions from the literature that coconut shell carbon works well for MTBE (8, 41).

Like the pH studies, more properties specific to Siemen’s 1230C 12x30 mesh Virgin Coconut Shell Granular Activated Carbon must be understood before concluding why sorption using this carbon was observed in this study but was not observed in previous studies using other carbon types. Future studies will also replicate the sorption experiments conducted here with other carbon types to see how carbon type affects TBA removal from solution for comparative purposes. However, two general observations can be made about the sorptive data presented in this text that may be related to why other studies did not
observe similar characteristics. Firstly, the reaction in Figure 8 occurred over the course of 5.2 days. Other compounds that are sorptive will adsorb to carbon much faster, usually in a matter of hours or even minutes. Secondly, the highest mass of carbon used- 0.210g- in Figure 8 corresponds to a concentration of 6g/L, which is significantly higher than typical concentrations used in drinking water or remediation purposes and is considerably higher than the mass of TBA actually removed.

Other experiments (data not shown) were conducted to test TBA sorption to 1230C. Higher masses of GAC (corresponding to the quantities used in Figure 7) led to sorption that occurred so rapidly it could not be sampled fast enough to generate sorption curves. Lower masses of GAC (below the .012g GAC or 342mg/L mass presented in the figure) were also studied, but significant fluctuations in results and subsequent large error bars prevented the use of this data.

The sorption data presented in Figure 8 demonstrates that TBA can be removed from solution physiochemically via virgin coconut shell activated carbon. When using a small volume of aqueous solution containing TBA and a large amount of GAC, the sorption reaction occurs rapidly; biological mineralization of TBA occurs over a much longer period of time and therefore cannot compete with this reaction. For example, 40mM of TBA can be mineralized by YZ2 in 13 days; Figure 7 shows how the same quantity of TBA can be removed using 100g/L GAC in 1.6 hours. However, Figure 8 reinforces that TBA sorption does not occur rapidly or completely in a batch system unless an abnormally large amount of carbon is used. Using less GAC (a more field-appropriate ratios of GAC mass to volume of water treated) will mean an even longer retention time than the 5.2 days presented in the figure, and for batch studies make YZ2 a competitive—and perhaps even preferable—alternative. However, batch studies, though useful, are not an adequate prediction of carbon utilization in continuous flow systems; rapid small-scale column tests (RSSCT) are more reliable and therefore will be conducted in future studies.

Breakthrough curves for Column 2 of the continuous flow system were calculated but are unavailable for Column 1 at this time. Discrepancies in breakthrough curves between the two columns may indicate different retention times, and any difference in retention time is significant when comparing degradation rates the two columns. Breakthrough curves will be contrasted when the curve for Column 1 is developed. Mineralization of TBA within the activated carbon columns may be increased with increased retention time.

Prior to inoculation and the addition of GAC, artificial groundwater containing 0.5mM TBA was passed through both of the columns in the continuous flow system. The
effluent concentration of TBA in both columns was equivalent to the influent feed concentrations. It was concluded that the glide end caps, end filters, borosilicate glass, and silicone tubing do not absorb a detectable amount of TBA. Therefore, all disappearance of TBA was attributed solely to either sorption or microbial degradation.

Eventual field applications were considered when the two inoculation strategies tested in the continuous flow system were selected. Both the techniques tried were very simple and would be easy to replicate using an actual remedial system. The objective of this study was to bioaugment YZ2 in a remedial system to increase TBA degradation and to reduce the lag time typically associated with the natural development of biofilm. A period of 20 days was used as a trial period, beginning as soon as the control column stabilized. In the first strategy tried, the injection of the microorganism in the sealed column increased the internal pressure of the column; only 1mL of injection liquid caused pressure buildup and ejection of the fluid via gravity through the tiny pierced hole before the column could be recapped. It is unknown how much of the 1mL actually made it into the column verses what escaped. Regardless, the data in Figure 8 suggests that the overall inoculation attempt was unsuccessful; TBA concentrations in inoculated Column 1 ranged from 0.5 to 1.75 times the inoculated concentration with no visible trend identified. Column 9 also never stabilized and TBA values fluctuated in the same range over the 20-day period.

After the first 22 days of operation, the results from the second inoculation (Figure 10) strategy were more promising. Unlike the first strategy, TBA data from the control column stabilized, and the data indicated that almost the same mass of TBA was exiting the columns as was entering. Data in Column 1 showed a marked decline in TBA concentrations, and TBA removal ranged from 20-100%. The data in Figure 10, however, does not continue that trend. Four distinct trends over the lifespan of the experiment; these are summarized in Table 4.

Minimal TBA degradation was observed during days 22-69 of operation, yet TBA degradation was observed for the first 22 days. No operating parameters were changed during this time period, and the effluent pH did not change during this time period. It is suspected that the decrease in TBA degradation is due to oxygen depletion in the column. YZ2 is an aerobic culture, and 0.5mM TBA exceeds the estimated stoichiometric value of TBA needed. Likely the column was filled with excess oxygen as GAC was added to the system; during packing, saturated GAC was compacted as much as possible to minimize the presence of air bubbles, but small bubbles were observed. The increased amount of oxygen may have increased the amount of TBA that was degraded during the 11-day equilibration
(sampling not conducted because control was equilibrating) and the first 22 days of operation. If this did occur, the column became anoxic once excess oxygen was depleted, and dissolved oxygen within the column was limited to dissolved oxygen within the feed. Previous investigations using YZ2 documented that less mineralization occurred as oxygen decreased (55). Consequently, little TBA degradation was observed during days 22-69.

The influent concentration of TBA was lowered to test this theory. After an acclimation period of 50 days, complete mineralization of TBA was observed in Column 1. When the concentration was reamended to 500uM, 100% removal was no longer observed. TBA at 40uM appears to be a threshold value for the culture. This correlation between influent concentration and fraction mineralized further suggests that oxygen depletion may inhibit degradation. Future studies will be conducted to explore this more; dissolved oxygen measurements will be taken with influent and effluent samples to calculate the quantity of oxygen consumed by the column as TBA is mineralized. If oxygen depletion is confirmed, these later studies will also include exploration of supplemental oxygen sources, because this laboratory observation also represents a significant field concern. Pump and treat waters are often anaerobic; assuming that waters entering a biological reactor contain saturated levels of dissolved oxygen will not always be a valid assumption. Oxygen will need to be supplied to anoxic waters to successfully apply this treatment method.

Unlike the first 69 days of operation where effluent concentrations resembled influent samples, Column 2 also displayed evidence of TBA reduction when the TBA concentration was lowered to 0.040mM. This corresponded with the visual observation of a biofilm and fluctuations in tracer data in for Column 2. The culture appeared as a green growth seen within all influent piping within five days of lowering the TBA concentration. Since TBA was the sole carbon and energy source provided in the feed, this mysterious culture was assumed to degrade TBA. Immediately before increasing the TBA concentration, 3mL of solution was extracted from one of the regions containing biofilm. All piping was replaced, and the 10mL conical degassing flask was rinsed with ethanol. The ethanol was allowed to vaporize, and the flask was UV-sterilized before being returned to the system. No further growth was observed in the system once the TBA concentration returned to 0.5mM, and no fluctuations in tracer data have since been observed.

The 3mL of extracted biomass was distributed equally into three flasks containing freshwater media broth amended with 0.040, 0.50, and 5.0mM TBA. Flasks were incubated

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3 Tracer data for both columns between days 79 and 122 is unavailable due to laboratory error.
at 30 °C in the dark. Cultivation was unsuccessful; no biomass growth was observed in any of the flasks. This may indicate that the biomass is only able to continuous flow systems containing low concentrations of TBA. The biomass may have also used light as an energy source; if so, growth was inhibited due to incubation in the dark.

0.50mM TBA is a very high concentration and is equivalent to a field concentration of 33,000 parts per billion (ppb); at high TBA concentrations, YZ2 will likely outcompete most native communities. However, the rapid development of the biofilm in Column 2 immediately after lowering the TBA concentration to 0.40mM (3,000ppb) reinforces that native microbial species will play an integral part in any bioremediation system and will increasingly affect the system as contaminant concentrations decrease. The green biofilm appeared in a control column despite the fact that all components including feed flow were kept sterile. Since sterile conditions could not be maintained in an idealized, controlled laboratory setting, contamination is inevitable in the field, and expecting a monoculture biofilm to remain pure is not practical from an application perspective. Native microbial communities are encouraged in treatment systems; they make systems more versatile and promote longevity. If implemented in a field study, it is reasonable to assume that YZ2 biomass would remain pure only for a short period of time, and that biomass properties and reactor characteristics would evolve over time as the microbial community dynamics in the biomass change.

Pure culture YZ2 was selected for this investigation because it was believed that its robustness coupled with its ability to rapidly degrade TBA made it a viable candidate for bioaugmentation into GAC remedial systems with minimal lag time. Its ability to tolerate high concentrations of TBA made it well suited for a continuous flow system operating at 0.50mM TBA. No initial adaptation time using the second inoculation strategy was noted in the experimental period; any lag occurred during the starting 11-day equilibration period. Batch studies show that YZ2 is capable of mineralizing TBA concentrations up to 50mM, yet continuous degradation in the continuous flow systems has only been achieved at a TBA concentration of 0.040mM This indicates YZ2 was successful in reducing the lag time associated with biofilm development in remedial systems, but as a biotechnology, it is not being utilized to its greatest extent. Future studies hope to explore desorption kinetics of TBA from activated carbon granules to identify whether biological regeneration is a viable option.

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4 For reference, the maximum contaminant level allowed in the state of California is 12ppb.
CONCLUSIONS

TBA, an organic contaminant typically associated with leaking underground storage tanks, was treated biologically and physiochemically in a laboratory scale investigation over a 20-month period. Two parallel columns were operated using TBA as the sole organic contaminant and oxygen as the electron acceptor. Results show that this continuous flow process combining activated carbon and oxic biological degradation, is effective only when treating low levels of TBA contaminated waters, but that continuous flow, aerobic biodegradation can effectively reduce inlet concentrations by up to 100% in a single pass using the stoichiometric threshold of oxygen concentration. The rate is achieved with oxygen as the terminal electron acceptor at a reactor temperature at room temperature, or approximately 24 °C. In these column studies, an increased TBA concentration to 500uM produces extreme inhibition of the culture YZ2’s ability to degrade TBA. This concentration is not lethal as full recovery of the culture is attainable when the TBA concentration is again reduced. The overall stoichiometry of the mineralization pathway indicates that dissolved oxygen concentrations in influent water may limit TBA degradation in the bio-GAC column; oxygen delivery strategies will be explored in future studies.

Batch studies show that carbon adsorption using virgin coconut-shell GAC can effectively remove TBA from water, though this reaction may not occur in a timely fashion. Studies also show that the sorptive reaction produces alkaline conditions in solution when GAC granules are introduced to waters containing TBA. Pure culture YZ2 mineralized TBA in solutions containing 0.5mM to 50mM TBA, which is significantly higher than previously reported cultures. Data also suggest that the culture is able to adapt to high TBA concentrations if maintained on them as the sole carbon and energy source. This tolerance, coupled with TBA’s affinity for water, indicates that this culture may be a good candidate to biologically regenerate activated carbon saturated in TBA.
REFERENCES

1. Acero J; Haerlein S; Schmidt T; Suter M; Von Gunten, U. (2001) MTBE Oxidation by Conventional Ozonation and the Combination Ozone/Hydrogen Peroxide: Efficiency of the Processes and Bromate Formation. *Environmental Science and Technology* 35: 4252-4259 (61)


9. Cirvello, JD; Radovsky, A; Heath, JE; Farnell, DR; Lindamood, C. (1995) Toxicity and Carcinogenicity of T-Butyl Alcohol in Rats and Mice Following Chronic Exposure in Drinking-Water. *Toxicology and Industrial Health* 11:151-165. (9)


15. Fortin, NY; Morales, M; Nakagawa, Y; Focht, DD; Deshusses, MA. (2001) Methyl Tert-Butyl Ether (Mtbe) Degradation by a Microbial Consortium. *Environmental Microbiology* **3**: 407-416. (18)


19. Hanson, JR; Ackerman, CE; Scow, KM. (1999) Biodegradation of methyl tert-butyl ether by a bacterial pure culture. *Applied and Environmental Microbiology* **65**: 4788-4792 (20)

20. Hatzinger, PB; McClay, K; Vainberg, S; Tugusheva, M; Condee, CW; Steffan, RJ. (2001) Biodegradation of Methyl Tert-Butyl Ether by a Pure Bacteria Culture. *Applied and Environmental Microbiology* **67**: 5601-5607 (21)


22. Johnson, RJ; Pankow, D; Bender, C; Zogorski, J. (2000) MTBE - To what extent will past releases contaminate community water supply wells? *Environmental Science and Technology* **34**: 210-217 (22)


50. Wei, N.  (2008) *Anaerobic Methyl tert-Butyl Ether (MTBE) and Tert Butyl Alcohol (TBA) Biodegradation under Shifting Biogeochemical Conditions.* Master’s Thesis, University of Illinois at Urbana-Champaign. (48)


52. Wilson, GJ; Richter, AP; Suidan, MT; Venosa, AD.  (2001) Aerobic biodegradation of gasoline oxygenates MTBE and TBA. *Water Science and Technology* 43(2): 277-284 (42)


55. Zhang Y; Reinauer, KM; Finneran, KT.  (in press) *Hydrogenophaga carboriundus*, sp. Nov. a tertiary butyl alcohol-oxidizing, psychrotolerant aerobe derived from
granular activated carbon. *International Journal of Systematic and Evolutionary Microbiology* (59, 60)