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Effect of metals on biodegradation kinetics for methyl *tert*-butyl ether

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Abstract

Many ground waters are polluted with a complex mixture of organic and metal contaminants. Especially, the presence of heavy metals may be of particular concern, since they may be toxic for microorganisms and may interfere with the biodegradation process. A study of methyl *tert*-butyl ether (MTBE) biodegradation in the presence of heavy metals with the purpose of determining the effects of type and concentration of metals on reaction kinetics was undertaken. The experiments were performed as batch biodegradation experiments, and the kinetic parameters were determined by a nonlinear regression technique. Experimental results showed that the presence of Mn^{2+} offers a slight stimulating effect on MTBE degradation; however, Cr^{3+} and Zn^{2+} resulted in less biodegradation at metal concentrations of 1 mg l^{-1}. The inhibitory effect of adding metals was obvious, with constantly decreasing value of MTBE degradation rate in the following order $Cu^{2+} > Cr^{3+} > Zn^{2+} > Mn^{2+}$ at metal concentrations 10 mg l^{-1} and 50 mg l^{-1}. The kinetic parameters of μ_m were found to be virtually unaffected until a threshold concentration (10 mg l^{-1}) of metal was reached. However, a significant inhibitory effect on bacterial growth, as manifested by a decrease in both K_i values for Cu^{2+} (at 1 and 10 mg l^{-1}), and both Cr^{3+} and Zn^{2+} (at 10 mg l^{-1}).

Keywords: Inhibition; Kinetic parameters; Metal ions; MTBE

1. Introduction

Methyl *tertiary* butyl ether (MTBE) is one of several fuel oxygenates added to gasoline to replace the tetraethyl lead and reduce harmful tailpipe emissions, such as CO, unburned hydrocarbons, etc. Between 1979 and the turn of the century, the production of MTBE grew rapidly in the United States. The massive production of MTBE, combined with its mobility, persistence, and toxicity, poses adverse effects on the human health, quality of groundwater aquifers and drinking water reservoirs. The present guideline limit established by the US Environmental Protection Agency (EPA) is 20–40 $\mu g\, l^{-1}$ for MTBE in drinking water [1], and MTBE is classified by EPA as a possible human carcinogen [2].

Numerous studies have demonstrated that MTBE can be biodegraded under aerobic or anaerobic conditions [3–7]. During recent years, the inhibitory effects on MTBE biodegradation have been investigated by additional researchers. These studies have focused mainly on substrate inhibition [8], by-product inhibition [9,10], or competitive inhibition [11,12]. However, little has been published on the effect of heavy metals on MTBE biodegradation. It is well known that heavy metals such as cadmium (Cd), nickel (Ni) and zinc (Zn) have been disposed of in dumps and landfills for years. The mobility of these heavy metals and high solubility of certain organic compounds, such as MTBE, pose a potential risk to groundwater and surface water quality. Delzer and Ivahnenko [13] also reported that bodies of groundwater have been contaminated by a complex mixture of MTBE and inorganic contaminants.

Many reports indicate that metal ions contamination may inhibit microorganisms by decreasing bacterial growth and biodegradation rates. The levels required to inhibit bacterial growth and their effect on biodegradation rates are dependent on the concentration and type of heavy metals [14,15]. Furthermore, the introduction of heavy metals can generally play an inhibitory role on microorganisms by displacing essential metal ions, or blocking vital functional group [16]. Therefore, it is assumed that certain co-contaminants such as heavy metals might preclude MTBE bioremediation.

The substitution of methyl groups around the central carbon and the ether linkage make MTBE relatively resistant to microbial attack. Most microorganisms cannot utilize MTBE as a sole growth substrate and at one time it was thought to be non-degradable [17,18]. However, strains of organisms have

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since been identified that rapidly degrade MTBE aerobically under suitable conditions of oxygen. The biodegradation rate for MTBE may be further diminished in the presence of heavy metals, but little has been reported on their effect on biodegradation or on modeling such effects.

The toxicity of metals on microorganisms has been studied by inhibition of growth, while the kinetics of the toxic effects of heavy metals has been studied to a lesser extent. To fully exploit MTBE bioremediation technology for field application, the kinetics of biodegradation in the presence of heavy metals must be better understood. Therefore, the objective of this work was to investigate the inhibitory effect for selected metal ions (Mn^{2+}, Cr^{3+}, Cu^{2+}, Zn^{2+}) at various concentrations (1, 10, 50 mg l^{-1}) on the rate of MTBE degradation by an MTBE-degrading pure culture under aerobic conditions. Based upon the batch experiments, the kinetic parameters associated with MTBE degradation were obtained using a nonlinear regression technique and a trial-and-error procedure. The kinetic parameters obtained were based on Haldane's model, and used as a tool for simulating the change of substrate and biomass concentrations as a function of time for field application.

2. Materials and methods

2.1. Bacterial strains and batch microcosm experiments

The bacterial cultures used in this study were obtained from a petrochemical wastewater treatment plant. They were initially grown in a liquid medium and were adapted to the targeted compounds and working conditions before the biodegradation experiments. After a series of screening and isolating procedures, a pure culture was identified as *Pseudomonas aeruginosa*. The microorganism was grown at pH 6.8 ± 1 in a mineral salt medium as described elsewhere [19]. Media and materials were sterilized by autoclaving at 121 °C for 20 min. The media were then inoculated under sterile conditions. The inoculated media were incubated at 30 ± 1 °C in a thermostat rotary-shaker for future use.

During the experiments, screw cap amber glass bottles of 250 ml volume, each containing 100 ml of an autoclaved phosphate-buffered mineral salts solution, sealed with Teflon Mininert valves were kept in the dark under constant shaking at 150 rpm and a temperature of 30 ± 1 °C. A stainless steel needle fitted to a gas-tight syringe was used to inject MTBE, and the bottles were then re-sealed and shaken for 12 h. This procedure ensured complete dissolution of the MTBE and equilibrated the headspace with the solution. Subsequently, the inoculum was then introduced using the same final biomass concentration in each bottle. Concentrated stock solutions of the heavy metals (1000 mg l^{-1}; Merck) were prepared with distilled water. No pH adjustments of the metal solutions were made since this could result in precipitation of some of the metal ions. Test solutions were prepared by making serial dilutions of the stock solutions to yield the desired metal concentrations. Metals (Mn^{2+}, Cr^{3+}, Cu^{2+}, Zn^{2+}) were added to provide a concentration of 1, 10 and 50 mg l^{-1} in the bottles. Test cultures, control cultures with added metals, and uninoculated control cultures were prepared in triplicate to examine if any abiotic removal of MTBE occurred and to confirm for metal precipitation in the absence of biomass.

2.2. Analytic methods

Key physical and chemical properties of MTBE including Henry's law coefficient (0.018 at 20 °C), vapor pressure (245–251 mmHg at 25 °C), and water solubility (43,000–54,300 at 20 °C) show MTBE is a volatile organic compound (VOC), mass transfer rates between the gas and aqueous phases of MTBE are rapid, and gas concentrations approach equilibrium with the liquid phase. Changes in liquid phase concentrations are slow compared to the rate of mass transfer between gas and liquid phase than discussing intrinsic biological reaction rates. Therefore, use of gas-phase measurements for biodegradation experiments satisfactorily captures the substrate concentrations in the liquid. In all biodegradation experiments, gas samples were periodically collected from the headspace of each bottle to monitor the degradation of MTBE using 250 μl gastight syringes equipped with Teflon Mininert valve fittings. Samples were then injected onto a gas chromatograph equipped with a RTX-1 capillary column (30 m × 0.53 mm) and a flame ionization detector (GC-14B, Shimadzu, Japan). Helium (99.98% purity) was used as the carrier gas and nitrogen was used as a makeup gas. Oven temperature was controlled at a constant temperature of 105 °C, injector temperature at 200 °C, and detector temperature at 250 °C. Concentrations of MTBE were quantified against primary standard curves. Biomass was measured either gravimetrically or spectrophotometrically. Gravimetric analysis consisted of measurement of total suspended solids, using a 47 mm diameter filter. Growth was monitored with optical density measurement at 600 nm using a UV spectrophotometer (U-2000, Hitachi, Japan). Biomass concentration was estimated from a correlation of optical density to the dry weight. Metal ion concentrations were determined using an inductively coupled plasma atomic emission spectrophotometer (ICP-AES, Optima 2000DV, Perkin-Elmer, Wellesley, MA).

2.3. Kinetic study

Batch experiments were conducted to estimate the kinetic parameters associated with MTBE degradation by *P. aeruginosa* in the presence of heavy metals. The most commonly used kinetic expression is that of Monod, which relates the rates of bacterial growth and substrate removal to the concentration of the substrate being removed [20]. The Haldane equation was used to model the degradation of MTBE accounting for the presence of metal ions by adding a substrate inhibition term into the Monod equation. The Haldane equation for inhibition is written as

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{-\mu_\mathrm{m} S X}{(K_\mathrm{s} + S + (S^2/K_\mathrm{i}))Y} \qquad (1)$$

where K_i is the inhibition coefficient (mg l^{-1}); μ_m the maximum specific growth rate (h^{-1}); K_s the half-saturation constant (mg l^{-1}); Y the yield coefficient (mg biomass/mg substrate); S the concentration of substrate at t (mg l^{-1}); X the concentration