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Aerobic and anaerobic microbial degradation of crude (4-methylcyclohexyl)methanol in river sediments

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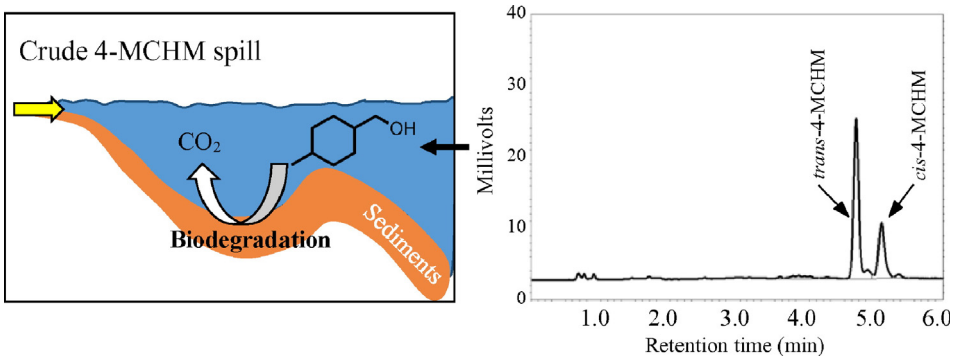
HIGHLIGHTS

- 4-MCHM isomers were readily biodegradable in aerobic river sediments.
- 4-MCHM isomers were reluctant to biodegradation in anaerobic river sediments.
- 4-MCHM isomers exhibited isomer-specific biodegradation.
- Nitrate addition markedly enhanced 4-MCHM mineralization into inorganic carbon.
- One 4-MCHM-degrading strain was identified as *Bacillus pumilus* at the species level.

GRAPHICAL ABSTRACT



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ABSTRACT

Cyclohexane and some of its derivatives have been a major concern because of their significant adverse human health effects and widespread occurrence in the environment. The 2014 West Virginia chemical spill has raised public attention to (4-methylcyclohexyl)methanol (4-MCHM), one cyclohexane derivative, which is widely used in coal processing but largely ignored. In particular, the environmental fate of its primary components, *cis*- and *trans*-4-MCHM, remains largely unexplored. This study aimed to investigate the degradation kinetics and mineralization of *cis*- and *trans*-4-MCHM by sediment microorganisms under aerobic and anaerobic conditions. We found the removal of *cis*- and *trans*-4-MCHM was mainly attributed to biodegradation with little contribution from sorption. A nearly complete aerobic degradation of 4-MCHM occurred within 14 days, whereas the anaerobic degradation was reluctant with residual percentages of 62.6% of *cis*-4-MCHM and 85.0% of *trans*-4-MCHM after 16-day incubation. The *cis*-4-MCHM was degraded faster than the *trans* under both aerobic and anaerobic conditions, indicating an isomer-specific degradation could occur during the 4-MCHM degradation. Nitrate addition enhanced 4-MCHM mineralization by about 50% under both aerobic and anaerobic conditions. Both *cis*- and *trans*-4-MCHM fit well with the first-order kinetic model with respective degradation rates of 0.46–0.52 and 0.19–0.31 day^{-1} under aerobic condition. Respective degradation rates of 0.041–0.095 and 0.013–0.052 day^{-1} occurred under anaerobic condition. One bacterial strain capable of effectively degrading 4-MCHM isomers was isolated from river sediments and identified as *Bacillus pumilus* at the species level based

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on 16S rRNA gene sequence and 97% identity. Our findings will provide critical information for improving the prediction of the environmental fate of 4-MCHM and other cyclohexane derivatives with similar structure as well as enhancing the development of feasible treatment technologies to mitigate these compounds.

1. Introduction

Chemical spills and accidents have been a well-known environmental problem for decades because of their widespread occurrence and multiple threats to human beings and the environment (Capel et al., 1988; Bryant and Abkowitz, 2007; Jiang et al., 2012; Lim and Huang, 2007). Thus, when a contamination event occurs, it is of significant importance to comprehensively assess the fate and transport of the spilled chemical in order to provide critical information for accurately evaluating its environmental risks and developing feasible cleanup strategies. Cyclohexane and its derivatives have been widely used in various manufacturing processes (US EPA, 1994) and some of them are found to exhibit significant adverse human health effects with large amounts of environmental releases; e.g. the release of cyclohexane in US totaled about 3.7 million pounds in 2014 according to the Toxics Release Inventory (US EPA, 2014). However, to date, limited data are available on the environmental fate of cyclohexane and its derivatives, making it difficult to predict environmental risks brought by these compounds.

(4-Methylcyclohexyl)methanol (MCHM, $CH_3C_6H_{10}CH_2OH$), one cyclohexane derivative, has been widely used in coal processing but largely ignored before the West Virginia chemical spill (Savoia et al., 2015). On January 9, 2014, an estimated 10,000 gal of crude 4-MCHM (a mixture that contains 68–89% *cis*- and *trans*-4-MCHM, 4–10% water, and other cyclohexane compounds) (Eastman Chemical, 2015) leaked from a storage tank, down the river embankment, and into a small river, the Elk River in West Virginia. Only 1.6 km downstream of the leak was the intake to the drinking water treatment plant. Thus, 4-MCHM contaminated drinking water for 300,000 people and cost at least $61 million losses for local businesses and residents (Manuel, 2014). A "do not use" order was issued to advise affected residents not to use tap water for any purpose, other than flushing toilets, to reduce the health impacts of 4-MCHM (Rosen et al., 2014). Residents were immediately and readily aware of the 4-MCHM contamination as its air odor threshold is in the range of 0.06 ppb-v (Gallagher et al., 2015). Due to the initial lack of gas chromatographic methods for monitoring, 4-MCHM (measured as the combined *cis* and *trans* isomers) concentration was first measured in water about two days after the initial spill; soon after monitoring began, concentrations as high as 3.8 mg L^{-1} were detected (WVDHSEM, 2014; Whelton et al., 2015). 4-MCHM concentrations were not measured in the soil or river sediment at the site of the spill. Monitoring in drinking water continued through April 2014; concentrations of several μg L^{-1} were detected (Foreman et al., 2015; Whelton et al., 2015; WVDHSEM, 2014).

More than 1900 patients reported symptoms like rashes and nausea after exposure to 4-MCHM-contaminated tap water (Toxicology Data Network, 2014). Since very limited data were available about crude 4-MCHM, various studies regarding the physical, chemical, biological, and toxicological properties of crude 4-MCHM were conducted soon after the spill. A recent study revealed that 4-MCHM at 10 mg L^{-1} can lead to DNA damage in human cells, indicating 4-MCHM can be related to genotoxicity and may cause chronic carcinogenesis (Lan et al., 2015). The National Toxicology Program (2015) reported that 4-MCHM was a developmental toxicant that decreased fetal weight and induced fetal malformations at an oral dose of 400 mg kg^{-1} day^{-1} using the rat model. Foreman et al. (2015) observed the presence of 4-MCHM in the Ohio River (Louisville, Kentucky) which is ~630 km downriver from the spill location, indicating that 4-MCHM could linger long and travel far to impact a large geographic area and population.

Possible fates for 4-MCHM released into the environment include hydrolysis, photolysis, biodegradation, volatilization, and sorption on sediments (Toxicology Data Network, 2014). Among them, biodegradation is a desirable pathway because it can lead to complete mineralization instead of transferring pollutants from one phase to another (Pandey et al., 2007). One of the few previous studies on 4-MCHM biodegradation was conducted in activated sludge. During a 28-day period, about 50% of the crude 4-MCHM [dissolved organic carbon (DOC) concentration of 20 mg L^{-1}] was degrade after a 9-day lag phase; degradation was based on carbon dioxide (CO_2) evolution (Beglinger, 1997). So far as we know, no effort has been made to understand the biodegradation of 4-MCHM by microorganisms in river sediments.

Given that river sediments exhibit both aerobic (surface layer) and anaerobic (significant quantities) conditions (Rhee et al., 1989), biodegradation of organic contaminants can be aerobic and/or anaerobic depending on the chemical structure and microbial community. Anaerobic biodegradation is generally much slower than aerobic biodegradation due to lack of sufficient amounts of electron acceptors (Shim et al., 2002; Zhang et al., 2010). Addition of oxygen (Zhi et al., 2015), nitrate (Burland and Edwards, 1999), sulfate (Kohring et al., 1989), or Fe(III) oxides (Villatoro-Monzón et al., 2008) to sites where anaerobic biodegradation predominates has proved to be feasible for enhanced biodegradation of many compounds. In particular, nitrate has an oxidative potential which is close to that of oxygen (Chen and Strous, 2013; Zhang et al., 2010). Few studies have addressed the biodegradation of 4-MCHM in river sediments under aerobic and anaerobic conditions, or investigated the effect of nitrate. Moreover, biodegradation of individual geometric isomers, *cis*- and *trans*-4-MCHM, is also little reported in the literature partly because of the previous lack of available analytical methods that were capable of detecting them individually. Once analytical methods for *cis*- and *trans*-4MCHM were established, investigations into their individual physicochemical properties demonstrated clear differences. The *cis*-isomer was more water soluble and less sorptive to organic matter than the *trans*-isomer. Specifically, for the *cis*-isomer, its room temperature properties were $K_{OW} = 225$, aqueous solubility = 1300 mg L^{-1}, and activated carbon capacity = 12.8 mg g^{-1}; the *trans*-isomer properties were $K_{OW} = 291$, aqueous solubility = 1010 mg L^{-1}, and activated carbon capacity = 26.5 mg g^{-1} (Dietrich et al., 2015). The *cis*-isomer was also less volatile than the *trans*-isomer; the room temperature unitless Henry's Law Constant for *cis*-4-MCHM was 1.42×10^{-4} while for *trans*-4-MCHM was 3.08×10^{-4} (Sain et al., 2015).

As reported by Dietrich et al. (2015), the difference in the physicochemical properties of *cis*- and *trans*-4-MCHM is expected to impact their biodegradation behaviors. Thus, the individual biodegradation of these two geometric isomers needs to be studied for more accurately assessing the environmental fate of 4-MCHM. The aim of this study was to investigate the biodegradation kinetics of *cis*- and *trans*-4-MCHM in aerobic and anaerobic river sediments as well as determine the effect of nitrate on the biodegradation behaviors of *cis*- and *trans*-4-MCHM. To reveal whether sediment microorganisms were able to completely mineralize 4-MCHM into inorganic carbon, production of CO_2 and methane (CH_4) were also measured at the end of the biodegradation period. Isolation and identification of 4-MCHM-degrading strain (s) were conducted to investigate which bacterial isolates contributed to the loss of 4-MCHM in river sediments. To the best of our knowledge, this is the first report on the degradation of *cis*- and *trans*-4-MCHM in natural aquatic systems under aerobic and anaerobic conditions and isolation of 4-MCHM-degrading bacteria.