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Bacteria Isolated from an MTBE Mineral Medium Culture

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ABSTRACT

Ground water pollutant from accidental gasoline spills have found to have traces of Metyl *tert*-butyl ether (MTBE). This study involved the isolation of a bacteria found in the soil around gasoline spills and its capabilities of utilizing MTBE for growth. The process was growing the bacteria in a liquid broth and then transferring to a selected mineral medium containing MTBE. Within five days the results showed a gram positive aerobic bacteria strain that was able to grow with methyl *tert*-butyl ether (MTBE) as its sole carbon source. The shape of the bacteria were cocci and arrangement was expressed in single and in clusters. This bacteria was similar to previous isolates. Alike the *Rubrivirex gelatinosus* PM1 and *Hydrogenophaga flava* ENV 735 its aerobic and able to grow with MTBE as its energy source. The difference was these two bacteria where gram negative. A third bacteria the *Mycobacterium austroafricanum* IFP 2012 is the only gram positive that has been identified.

Keywords: metyl *tert*-butyl ether, MTBE

INTRODUCTION

Methyl tertiary-butyl ether, also known as MTBE, is a compound that was developed in the late 1970's as an octane enhancer. It was to reduce emissions of toxic chemicals (carbon monoxide and hydrocarbon are two chemicals released by gas powered vehicles) that are discharged as pollutant gases into the atmosphere. MTBE as an oxygenate was also used to meet the vehicle emissions requirements of the 1990 Clean Air Act Amendment (Hatzinger P.B., et al, 2001). Within years MTBE became the world's most dominant fuel oxygenate in reformulated gasoline, and reached production of over twenty five million tons (Francois A., et al, 2002). Some reformulated gasoline contains approximately 11% (vol/vol) MTBE (Hatzinger P.B., et al, 2001).

Due to its wide usage and massive production, MTBE has been involved in accidental spills that have led to its discharge in residential soil that eventually progressed to groundwater near many service stations. Its concentrations were first detected in ground water in 1986. MTBE is now the second most commonly detected contaminant in urban ground water in the United States as stated by the US Geological Survey's National Water Quality Assessment program (Salanitro J.P., et al, 2001).

The reason for this is that MTBE acts differently in the given environment. MTBE, released in exhaust, reacts with precipitants that carry it to soil or surface water. In the soil and other organic matter MTBE has a low absorption. The MTBE that is not biodegraded in the soil or in the air eventually reaches the ground water where its molecular structure allows it to move rapidly with the constant flow of water. Its structure consists of an oxygen atom that makes it polar therefore making it hydrophilic (Figure 1).

Its structure contains an ether bond and a high steric hindrance, which makes it intractable to microbial degradation.

Through cultivation of soil samples in a selected Mineral Medium bacteria where isolated expressing

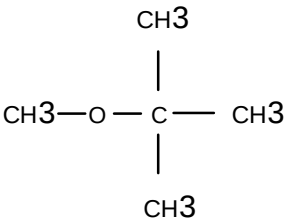

Figure 1. MTBE Molecular Structure

many of the characteristics found in previous studies. Two of three bacteria isolated in previous studies where gram negative as for the third one it was Gram positive. After certain cultivation techniques an aerobic gram positive bacteria was successfully isolated.

MATERIALS AND METHODS

The following materials were purchased from Aldrich: Methyl tertiary-butyl ether and Murashige and Skoog (MM). Test tubes, incubators, petri dishes, auto-claver, microscope, chemicals used for staining, 2000ml flask and 500ml flasks, refrigerator and inoculating loops where provided by the McPherson Science department.

Soil samples where abstracted from two McPherson gas stations. Prior to any procedures all tubes and flasks where autoclaved for 53 minutes at 121° C.

Preparing Culture

The application of 4.3 g of Murashige and Skoog was mixed with 1L of ionized water in a 2000ml flask. Once mixed it was covered with aluminum foil and autoclaved at 121° C for 50 minutes to purify the culture. Once out 500 ml was used for the liquid broth culture and 500 ml was used for the solid agar media. Once in separate flasks the 500 ml used for the liquid culture was injected with 15ml of MTBE.

A pinch of soil sample were placed into 24 test tubes. The liquid broth culture was poured into 24 test tubes. Three fourths of the tube was filled with Mineral Media, MTBE and soil samples. These tubes where then placed in an incubator at 30° C until growth is present.

The other 500 ml was used for the solid agar media. 7.53 g of agar was placed into the 500ml of mineral medium. This was then heated and stirred constantly. After mixture the flasks was placed in the auto clave for 15 minutes at 121° C. Once out it was placed into a 45° C incubator to cool down and for better handling. While in the incubator an hour later 15 ml of MTBE was injected and mixed with the solid culture.

The agar was then placed into 10 petri dishes and allowed to solidify at room temperature. The dishes where then refrigerated until used.

Growing of Bacteria

After a week in the incubator the test tubes containing bacteria in the broth where ready for growth onto to the agar media. Using a sterile inoculating loop samples of the liquid broth media was placed onto the agar dishes. After smearing 4 dishes the plates where sealed tightly and placed into a zip-lock bag into an incubator at 30° C until growth was expressed. The environment was aerobic. After 5 days growth o f bacteria was visible.

Isolation of Bacteria

A colony was then abstracted from the agar media using a sterile inoculating needle and smeared onto a separate petri dish. The quadrant method was used to efficiently separate bacteria.

After 5 days of growing bacteria were abstracted from the agar mineral medium and a single bacteria was placed on a slide (prepared 3 separate slides). Once the slide was fixed it was placed onto a stain rack over a sink or stain pan and gram staining procedures where applied.

Viewing Bacteria

Once stained bacteria where viewed under a microscope and viewed for characterization.

RESULTS and DISCUSSION

After viewing the bacteria under high magnification it was found to be gram positive. The size of the bacteria were .5µm or one division equivalent to 1µm at 100x oil immersion. The bacteria appeared in one shape and arrangement was displayed in several groupings. The shapes of the bacteria were cocci and most abundant arrangement was single. Also visible were diplococci, chains of cocci and clusters of cocci.

The characterization of this bacterium was similar to to previous isolates showed (*Rubrivirex gelatinosus* PM1 and *Hydrogenophaga flava* ENV 735). This isolate, like the others, was aerobic and able to grow as MTBE as its sole carbon source and energy source. Though these two known bacterium where found to be gram negative. A third bacterium *Mycobacterium austroafricanum* IFP 2012 is the only gram positive MTBE utilizing organism yet identified. The bacterium that has been isolated is gram positive and aerobic. The present results where consistent with the hypothesis that a MTBE utilizing bacteria can be isolated from soil samples from a public gas station.

The name of the bacteria is still on ongoing project and future studies would include the rate that this bacterium biodegrades MTBE.

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