

Physiological Characterization of *tertiary* Butyl Alcohol-Degrading Bacteria

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Tertiary butyl alcohol (TBA) is a metabolic byproduct of the degradation of the gasoline oxygenate methyl tertiary butyl ether (MTBE). Both MTBE and TBA are recognized as important ground water pollutants in the United States and around the world. MTBE, a branched alkyl ether, is included in gasoline products in order to enhance octane as well as to assist an oxygenating compound that decreases the amount of automobile exhaust emissions of carbon monoxide and photochemical smog. What was unknown about MTBE until recent years was that once allowed to enter the environment, it is broken down into TBA and other toxic chemicals that eventually make their way into the groundwater and are extremely dangerous in high concentrations. It is very difficult to detect levels of MTBE and TBA in the water because they both have low taste thresholds and are difficult to detect unless a chemical analysis is run on the water. This is dangerous for people who take their water from a well because there is no filter system in place and because the government does not regulate the levels of TBA in drinking water and therefore does not test for it in any primary water source. Recently, TBA-contaminated water has been treated using biological systems, such as bacteria, to break down the TBA in the water source. These systems, called Bio-GAC systems, use microorganisms in biofilms that are attached to granulated activated carbons to degrade the TBA into nontoxic chemicals. The Biofilms are placed in water treatment plants and contaminated water is allowed to run over them. The microorganisms on the Biofilm break down the TBA into smaller, nontoxic compounds. The microorganisms studied in this research project, S1B1 and G2B2, were isolated from a Bio-GAC system because not much is known about TBA oxidizing

organisms or the biochemistry involved in the oxidation process. The goal of the research project was to begin characterizing these bacteria to try to determine what other types of carbon-containing compounds they grow on and to determine if the two types of bacteria have similar ways of oxidizing TBA.

S1B1 forms small, clear, dot-like colonies on a 1% TBA R.K. plate, the medium on which it was isolated. G2B2 forms large, mucous, round, raised, yellow colonies on an LB medium plate. Since both strains of bacteria were previously isolated, colonies were used from the plates on which the bacteria were originally isolated. Not much more about these two bacteria was known before the beginning of this experiment besides the fact that they both oxidized TBA and were able to grow and multiply in its presence; therefore the physiological characterization of these two bacteria began with determining on which carbon structures are these two types of bacteria able to grow. In order to do so, a solution must be prepared in a test tube in which to add the bacteria and allowed to incubate for a set amount of time to allow for growth to occur. The solution is a mixture of 25 mL of mineral salt medium and 12.5 uL of whatever growth substance was being tested in the particular test tube. A colony (or part of a colony) of bacteria was then added to the test tube and the tube was then sealed. The tubes were then placed sideways between two test tube racks in a shaker set at a constant temperature of 30° Celsius and allowed to incubate for one week. After a week, the tubes were removed from the shaker and their contents were analyzed with the use of a spectrophotometer. The spectrophotometer compares the amount of light shining through a specific sample with a zeroed blank cartridge to determine if there was any growth in the sample. If growth occurred, the amount of light shining through the sample was diminished and the number read-out from the spectrophotometer would show exactly by how much the light was diminished. After all the

cultures had been analyzed, they were properly disposed of and new cultures were made. The same substrate was tested at least twice to maintain continuity and to be sure that no contamination occurred. If there was any doubt about a certain substrate, it was tested a third, or even a fourth time until it provided a constant reading on the spectrophotometer. A control group was also tested every time, containing the medium, TBA, and the bacteria, to make sure that the bacteria were viable. LB plates were also prepared every week from growth from randomly chosen substrates to make sure that no contamination occurred and that the bacterium being tested was still a pure culture.

Through this research we found that S1B1 is able to grow on numerous carbon-containing substrates such as primary alcohols and other branched alcohols besides TBA, but G2B2 is very finicky and is only able to grow on very specific substrates such as 2-methyl-1,2-propanediol, which is similar to TBA. Because these organisms grow on completely different substrates besides TBA, it is safe to say that they are most definitely not the same organism and that they may have different means of TBA oxidation. The projected TBA oxidation pathway is as follows: $\text{TBA} \rightarrow \text{2-methyl-1,2-propanediol} \rightarrow \text{2-hydroxyisobutyrate} \rightarrow \text{2-propanol} \rightarrow \text{acetone} \rightarrow \text{hydroxyacetone}$. S1B2 grew on most of these compounds, while G2B2 only grows on the first two listed, therefore they must have a separate way of breaking down these compounds. Future research into both of these bacteria will investigate the pathways they use to oxidize TBA as well as hopefully isolate the enzymes used within these pathways. This will hopefully lead to a better water filtration system in water treatment and purification plants.

Throughout this experience I have learned many valuable skills pertaining to working in a lab as well as earned the trust and respect of not only my advisor but of some of the graduate students in the lab as well. My knowledge of aseptic technique, bacterial cultures, basic

laboratory procedures, and some of the technological devices in the lab has grown immensely and I am very grateful for Dr. Hyman for allowing me to work in his laboratory under the guidance of one of his graduate students. I have learned a lot and hope to continue to expand my knowledge in the microbiology field.