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Adaptation of *Pseudomonas aeruginosa* in Kansas oil fields for use in tertiary oil recovery

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ABSTRACT

Over half of the oil reservoirs in the United States are considered “empty” although they still contain approximately 50% of their potential yield (Exxon Corporation, 1983). *Pseudomonas aeruginosa* produces a rhamnolipid biosurfactant extracellularly that can drive this trapped oil from crevices into openings that can be easily pumped. This tertiary method of oil recovery is currently being used in Venezuela, but has not been proven to work in Kansas. This bacterial strain has adapted to survive in Venezuelan oil reservoirs. This research will prove that this strain will adapt to conditions (pH and temperature) that are prevalent in Kansas oil reservoirs. The environment for the research was a petri dish and a test tube with a nutrient agar amended to a specific pH and then placed at various temperatures. Typical deviations from these conditions and growth were analyzed. The results will show that the bacteria continued to grow and thus adapt to the new conditions. This proves that this bacterial strain would survive and be able to produce its biosurfactant in Kansas oil fields.

Keywords: *biosurfactant, microbial enhanced oil recovery, Pseudomonas aeruginosa, rhamnolipid, tertiary oil recovery.*

INTRODUCTION

Surfactants are widely used for various purposes in industry, but for many years were mainly chemically synthesized. It has only been in the past few decades that biological surface-active compounds (biosurfactants) have been described. Biosurfactants are gaining distinction and have already taken over for a number of important industrial uses, due to their advantages of biodegradability, production on renewable resources, and functionality under extreme conditions (Van Dyke, Couture, & Brauer, 1993).

Currently, biosurfactants are used mainly in the oil and petroleum industries, where they are used primarily for their emulsification capacity in both tertiary recovery and polluted sites remediation. Caution is frequently exercised with respect to their use because of possible subsequent microbial contamination of either underground oil reservoirs or products.

Biosurfactants are a heterogeneous group of surface-active molecules produced by microorganisms. These molecules reduce surface tension, critical micelle concentration (CMC), and interfacial tension in both aqueous solutions and hydrocarbon mixtures (Banat, 1995). “World-wide interest in biosurfactants has increased immensely due to their ability to meet most synthetic surfactants’ requirements” (Morkes, 1993). Chemically synthesized surfactants have been used by the oil industry to aid in the clean up of oil spills, as well as to enhance oil recovery. These compounds are not biodegradable and can be toxic to the environment. Biosurfactants, however, have been shown in many cases to have equivalent emulsification properties and are biodegradable. Thus, there is an increasing interest in the possible uses of biosurfactants in the oil industry (Venkata & Karanth, 1989).

A strain of *Pseudomonas aeruginosa* isolated from crude oil-associated injection water in Venezuelan oil fields was found to be adapted to the conditions prevalent in this oil reservoir (Rocha et al., 1992). Further, the biosurfactants (rhamnolipids) produced by this strain were not inactivated by pH, temperature, salinity, calcium, or magnesium at concentrations in excess of those found in many oil reservoirs in Venezuela (Rocha et al., 1992). Although this strain of bacteria has been tested extensively in Venezuela, it has not been used in Kansas oil fields. *Pseudomonas aeruginosa* is known for its adaptation qualities and thus the strain should adapt to the conditions prevalent in Kansas oil fields.

MATERIALS AND METHODS

The first step in this experiment is to obtain the microorganism. The microorganism used in this research was *Pseudomonas aeruginosa* 9027. This particular strain produces the rhamnolipid that can drive the residual oil out of the oil reservoir. The microorganism can be easily obtained through the American Type Culture Collection (ATCC). The microorganism was ordered through the use of an online catalog. The *P. aeruginosa* strain was grown in a nutrient agar (Difco Laboratories). Initial cultures were grown at 37° C at an optimum pH of 6.8 under static conditions. Data regarding the oil fields in Kansas was then obtained through the help of Texaco Incorporated in ElDorado, Kansas, and the National Cooperative Refinery Association in McPherson, Kansas. The data received include the pH of the wells,

the temperature of the wells, the salinity of the oil and water mixture, the density of the oil, and the projected percentage of oil still present in the wells. The data also included the date the sample was taken and the geographical location of the sample.

A simulated environment was then created for the bacterial strain to attempt to survive. This environment was a petri dish and a test tube with *Pseudomonas aeruginosa* Agar F (Difco Laboratories) amended by laboratory-grade glacial acetic acid to a pH level of 4.3. The petri dish was used to be able to test and isolate colonies, whereas the test tube was used to show only growth in the simulated environment. Preparation of the agar was accomplished by using 100 mL of distilled water and titration with glacial acetic acid until the pH of the water was approximately 4.0 (Collins & Lyne, 1984). Then 3.8 grams of the dehydrated *Pseudomonas aeruginosa* Agar F (Difco Laboratories) was placed into the flask with the water and 10 grams of glycerol was added to this solution. The solution was then mixed and the final pH adjustment was made. After the pH was adjusted to 4.3, the solution was heated to boiling for the powder to dissolve completely. The solution was then placed in the autoclave for 15 minutes at 250° F in order to neutralize all bacteria in the nutrient agar. After cooling to 45° C, the agar was then poured into the petri dishes and the test tubes and allowed to cool to room temperature. After cooling, each petri dish and test tube received a part of the original colony growth and was then placed into a different temperature.

The temperature of the ovens in which the cultures were grown was set at 30° C, 37° C, 45° C, and 85° C. One of the cultures was taken and placed in a petri dish with only the nutrient agar at 37° C and a pH of 6.8 to act as a control for the experiment. After 96 hours, the cultures were taken out of their respective environments and analyzed. The analysis consisted of, first, recognition of the bacteria, then the comparison of size and density of the bacterial colonies. The nutrient agar was then analyzed to ensure that it retained its original pH level.

RESULTS

After plating the bacteria and allowing it to grow for 96 hours, the petri dishes and test tubes were removed and analyzed. The bacteria were recognized in the *Pseudomonas aeruginosa* Agar F by the color of the colony. In this particular agar, the bacteria produce a fluorescent green color in its colony. The color of the colonies in each of the samples was a yellowish-green, which is a positive recognition of the bacteria. This proves that there was a growth of the bacteria in each of the different environments. The size of the bacterial colonies was very similar in all of the samples including the control.

The density of the colonies did not vary in any of the samples. Each sample seemed to have a similar colony density and distribution. The pH of the agar was tested after the experiment and the pH remained at 4.3. Also found in the samples was a yellowish-white clear liquid, which was determined to be the biosurfactant that *Pseudomonas aeruginosa* produces (Arino, Marchal, & Vandercasteele, 1996). This liquid is concluded to be the biosurfactant due to a physical description of the biosurfactant. This clear liquid was present in all of the experimental samples, but in a larger quantity in the control.

DISCUSSION

The analysis showed that the bacteria had grown in each of the four different environments. Because the growth of the bacteria was present, the bacteria must have adapted to its environment. This is consistent with the findings of Rocha, et al., in the Venezuelan oil reservoirs. The production of the biosurfactant by the bacteria shows that the bacteria begin to produce shortly after placement into the new environment. As shown in previous microbial enhanced oil recovery experiments, this biosurfactant would not possess a large enough concentration to drive the oil out of the rock until the bacteria had grown in the environment for at least four to six months.

By showing the bacterial growth, it was proven that *Pseudomonas aeruginosa* adapted to the different pH and temperature environments prevalent in Kansas oil fields. This would show that microbial enhanced oil recovery is possible in Kansas with the use of *Pseudomonas aeruginosa*. By using this method of tertiary oil recovery, “empty” oil wells can be reopened after a certain growing period of time for the bacteria. The process of injecting an oil well with a microbial solution and allowing the bacteria to not only grow, but also produce the biosurfactant is expensive, but given a period of time, the oil well could begin to produce at levels similar to previous lifts. The size and the density of the bacterial colonies were going to show a dependence of the bacteria on temperature in its ease of adaptation, but because of the similarity in size and density, the bacteria must not rely strongly on temperature alone. All cultures grew approximately the same regardless of temperature differences. Further study of the pH and temperature dependence of *Pseudomonas aeruginosa* could determine an optimum temperature, pH, and time period for the production of the biosurfactant. *Pseudomonas aeruginosa* adapted to an environment similar to the environment of a Kansas oil reservoir.

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