White Paper Research Report

Biodegradation of Hydrocarbons in
Petroleum Produced Water Using Microbes

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Research Project Overview

The primary purpose of this research project is to determine the efficacy of a specialized microbial inoculant to cleanse and remove high levels of hydrocarbon pollutants found in petroleum wastewaters. The US Department of Energy estimates that over 87% of petroleum extracted from oil production activities is what the industry calls **"produced waters"**[1]. This is water trapped in underground formations that is brought to the surface along with oil or gas. It is by far the largest volume byproduct associated with oil and gas production.

Increasingly stringent environmental regulations require extensive treatment of produced water from oil and gas productions before discharge. Hence, these treatment and disposal costs can be extremely high for the industry, which are simply passed onto the consumer. Consequently, if there is a cost-effective way to cleanse and remove the hydrocarbon contaminants contained in produced waters, then this would dramatically reduce the treatment expenses, allow for an effective means to recharge the aquifers, and turn a hazardous waste material into a value-added commodity that can provide green business opportunities for local economies.

Experimental Procedure

Prescribed treatments, run in triplicate, were initiated to assess the capability of the microbial inoculant known as **"Pristina BioCleanse"** to reduce hydrocarbon concentrations in petroleum produced water samples extracted from a holding pond located near Jal, New Mexico. Approximately 500 ml of produced water, stirred before each addition, was placed in 1000 ml glass containers, then inoculated with the prescribed treatments listed below in **Chart 1.**

Treatment	Mixture	Dosage
B1	Control	No inoculant
B2	Control + Accelerant	No inoculant
B3	Inoculant Only	0.1g/500mL
B4	Inoculant + Accelerant	0.1g/500mL

Chart 1: Classifications for treatment protocols.

Inoculant Treatment Mixtures

As recommended, an additional treatment included a biological accelerant blend containing a special mixture of vitamins, minerals, amino acids, enzymes, and other proprietary ingredients. These treatments were amended using 1 mL/500mL produced water of a 16:4 ratio of H_2O. The treatment reactors, containing the 500 mL of produced water and inoculant (or control) were allowed to sit covered with a fine mesh nylon cloth in normal atmospheric conditions subjected to ambient temperatures (56^{o}F to 101^{o}F) and indirect sunlight.

1. https://publications.anl.gov/anlpubs/2009/07/64622.pdf

Twelve hours previous to sampling, reactors were stirred and allowed to settle. As each sample was taken, a new liquid level was marked on the glass containers to indicate remaining produced water to allow replenishment of water using distilled water to offset losses from evaporation and to better reflect the original hydrocarbon content. Each successive sampling was subjected to the same sampling protocol.

Hydrocarbon content was quantified using Gas Chromatography-Mass Spectroscopy (GC-MS) analysis. A total of thirty mL of treated produced water was collected as three 10 mL samples from each of the triplicate within the treatments and placed into a 40 mL vial with a Teflon seal and cap. This 30 mL was then inoculated with 2 uL of an internal standard of 1-chloroocto-decanol and then mixed with 3 mL of hexane. The sample bottles were mixed thoroughly on a rotary shaker for one hour at 60 rpm. Initial assessment of the produced water revealed that several of the hydrocarbons detected by the GC/MS were similar to the chemical makeup of diesel; therefore, a diesel standard was used to quantitate the hydrocarbons detected in the produced water/microbial samples. Based on GC/MS peak areas, approximately 40% of the hydrocarbons in the produced water were similar to the hydrocarbon makeup of diesel.

Therefore, this assay was determined to reflect a large portion of the hydrocarbon content and a potentially reliable method to track microbial reduction of hydrocarbons in produced water. Hydrocarbon concentrations were unexpectedly low in the untreated produced water and initial concentrations demonstrated ~1.7 ppm (mg/L) initial hydrocarbon concentrations. Detection range of samples from after-treatment sample concentrations ranged down to 0 hydrocarbons detected with sensitivity in the 2 ppb (ug/L) range. Calibration curves for diesel standards exhibited linear correlations with r_2 of 0.98 to 0.99 providing support for accuracy of the quantitative estimates of hydrocarbon concentrations in the treatments.

The Carbon:Hydrogen (C:H) content in the diesel standard are listed in the **Chart 2** and the quantity by percent of abundance in the produced water samples are listed in **Figure 1** below:

Hydrocarbon Types	Chemical Structure
n-docosane	$C_{22}H_{46}$ (solid)
n-dodecane	$C_{12}H_{26}$ (355 isomers) (liquid)
n-eicosane	$C_{20}H_{42}$ (366,319 isomers) (solid)
n-hexacosane	$C_{26}H_{54}$ (solid)
n-hexadecane	$C_{16}H_{34}$ (cetane) (liquid)
n-octacosane	$C_{28}H_{58}$ (solid)
n-octadecane	$C_{18}H_{38}$ (solid)
n-tetracosane	$C_{24}H_{50}$ (14,490,245 isomers) (solid)

Chart 2: Diesel standard hydrocarbon components with corresponding Carbon:Hydrogen ratios.

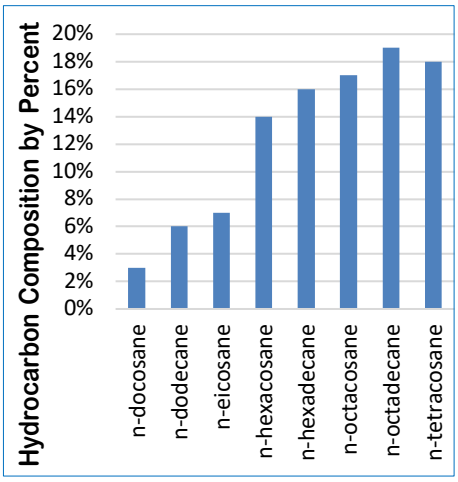

Figure 1: Relative % of diesel standard components in tested produced water.

Biodegradation Test Results

After the first week, the results from the GC/MS analysis of the microbial reduction of the diesel components of the produced water are shown in **Chart 3**. There were moderate hydro-carbon reductions during this timeframe averaging 25% using the prescribed inoculant alone, and an additional 3% reduction when adding the specialized nutrient accelerants. However, the

hydrocarbon reductions in the control treatments remained relatively low indicating that the microbial consortium in the produced water was lacking a sufficiently diverse microbial community structure to enable efficient hydrocarbon reductions.

After four weeks, tests showed that the inoculant combined with the accelerant nutrients had effectively removed **100%** of the hydrocarbon contaminants with the exception of the *n-octacosane* compound being reduced by **92%**. As shown in **Chart 4**, the grand total of hydrocarbon reductions was **over 99%**. The results of this experimental survey to determine the potential of beneficial microbes successfully biodegrading the hydrocarbons in produced water demonstrated conclusively that this specially formulated inoculant can effectively cleanse and remove the hydrocarbons present in produced waters.

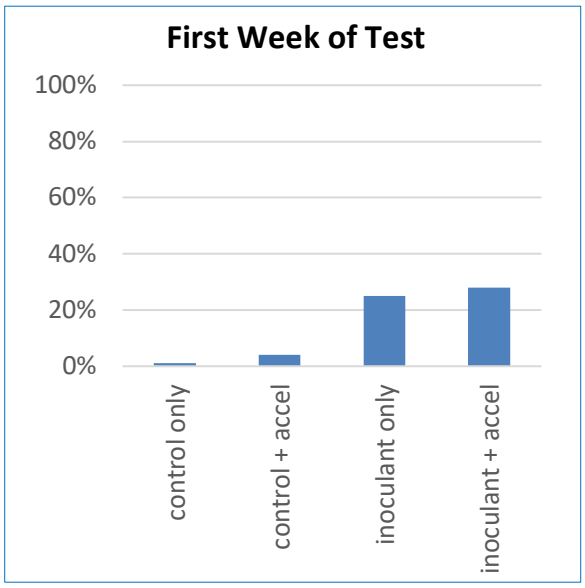

Chart 3: Percentage reduction of hydrocarbons after the first week.

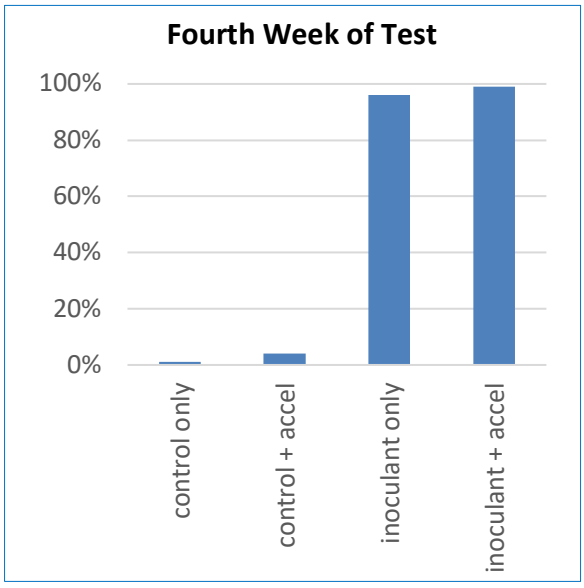

Chart 4: Percentage reduction of hydrocarbons after the fourth week.

Conclusions

It is recommended that further analytic tests be conducted to not only remove the hydrocarbon pollutants from produced waters, but to thoroughly test the microbiological applications for remediating the primary petroleum crude products in order to significantly reduce their harmful health and environmental impacts.This would essentially turn the noxious crude oils into nontoxic vegetable oils that would have a broad range of commercially viable and ecologically sustainable applications.