Microbial Biodegradation of Used Motor Oil on Concrete Surfaces

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Abstract

In the present study, the ability of a *Pseudomonas aeruginosa* to biodegrade used motor oil was tested on dried used motor oil on concrete surfaces. This was done to determine whether the use of the *Pseudomonas aeruginosa* is a viable method of biochemical cleanup of used motor oil on roadways and automobile parking, preventing environmental used motor oil pollution. Used motor oil and other used automobile fluids were collected from the Conejo 76 auto repair shop. The contents of the oil collected were on known as they came from multiple sources. Infrared spectroscopy was conducted on the collected used motor oil to determine its contents. The bacteria was cultured in tryptic soy broth and applied to sixty-four sample cases of used motor oil on concrete pavers and compared to sixty-four sample controls which were not inoculated. Results show the potential of *Pseudomonas aeruginosa* to aid in environmental protection. Further work may include developing practical means of application so that the bacterium can be used commercially to prevent motor oil pollution.

Introduction

It is commonly accepted that used automobile motor oil is a hazardous pollutant that can result in environmental and health risks. Automobiles expel used motor oil, as well as other fluids, as they are operated, leaving visible puddles and stains on parking lots and roadways. Rainfall lifts the oil and grease off these stains, and water runoff carries used motor oil into the watershed. If left unaddressed, this occurrence has potential to pollute freshwater supplies, fertile soil, and ocean environments. Used oil contamination to aquatic environments has potential to disrupt primary producers, resulting in adverse effects to the food chain (Ramadass, Megharaj, Venkateswalu, & Naidu, 2013). Such damage poses health risks to wildlife, livestock, and public health (Abdulsalam, Bugaje, Adefila, & Ibrahim, 2011). However, these risks maybe mitigated by microorganisms.

The characteristics some microorganisms have shown potential to remediate such risks. Previous research has shown that certain spore and non-spore forming bacteria, such as *Bacillus* and *Paenibacillus* exist in crude oil (Gong et al., 2012). Crude oil is a natural resource used in the production of manufactured goods such as plastic, kerosene, and motor oil. Previous work by Gong et al. (2012) identified multiple bacterial strains found to inhabit crude oil reserves in China. These strains were found to survive in natural and autoclaved crude oil. These findings showcase certain bacteria's abilities to resist high heat and pressure. The metabolic activity of some strains was also shown to aid in hydrocarbon-degradation, a process used in microbial enhanced oil recovery (MEOR) (Mokhatab & Giangiacomo, 2016). The metabolic activity and by-products of some microorganisms, when inoculated in oil reservoirs, allows them to clog pores, redirect flow, or mobilize oil via reduced interfacial tension. Such microbial behaviors

have shown MEOR to be an effective method of tertiary oil recovery (Armstrong & Wildenschild, 2012). While MEOR may be an effective method of resolving crude oil pollution, it is not as effective in treating unrecyclable pollutants which cannot be recycled, such as crude oil products.

The metabolic properties of MEOR capable microorganisms show the potential to resolve some environmental threats posed by crude oil products. As crude oil is the original unrefined source material for processed motor oil and resultant used automobile motor oil, it is possible that identified microorganisms able to metabolize used automobile motor oil may have the potential to remove used automobile motor oil from improper deposits and its danger as a pollutant. Work by Obayori, Salam, and Ogunwumi (2014) showcased such potential in *Pseudomonas aeruginosa*.

The microorganism *Pseudomonas aeruginosa* is a hydrocarbon degrader which has been found to inhabit petroleum contaminated soil. When inoculated in liquid cultures of both fresh and used motor oil, *Pseudomonas aeruginosa* was shown to degrade over ninety-percent of all types of oil tested. The current effort evaluated the ability of *Pseudomonas aeruginosa* to degrade dried used motor oil from concrete surfaces. Applications of this research include the prevention of used motor oil pollution from parking lots.

The purpose of the literature review was to gather background information in order to address the problem effectively. The literature review was conducted using the Broome library database at Cal State Channel Islands. This is not an exhaustive literature review.

Purpose

The purpose of the present work was to determine whether the exposure of *Pseudomonas aeruginosa* to used motor oil on concrete surfaces affected the oil and grease concentration in water runoff collected from such stains. As automobiles expel used fluids while operational, it is common to see used motor oil and grease stains on parking lots and roadways. During times of inclement weather, used oil and grease is lifted off these surfaces and will flow with rain runoff into the watershed, thus contaminating the watershed. Some of this rain runoff will find its way to the ocean or lakes, thus contaminating such bodies of water with used motor oil and grease. While soaps and detergents can be used to clean the concrete surfaces, it may not be entirely effective in removing the oil. This process is also labor intensive and can add additional chemicals to the watershed and water runoff. This method is also impractical for use in a large metropolitan setting. The problem is the used motor and grease continue to contaminate the watershed because there is no natural, biological, and easy method for removing these contaminants from concrete surfaces.

Hypothesis

Research question to be addressed by my work is as follows:

Does Pseudomonas aeruginosa exposure to used motor oil on concrete surfaces affect the oil and grease concentration in water runoff?

Null Hypothesis:

The Pseudomonas aeruginosa *exposure to used motor oil on concrete surfaces will not affect the*

oil and grease concentration in water runoff.

Alternative Hypothesis:

The Pseudomonas aeruginosa exposure to used motor oil on concrete surfaces will affect the oil and grease concentration in water runoff.

Dependent variable:

Oil and grease content (ppm) in simulated rainwater run-off.

Independent variable/Groups:

Cases will be concrete blocks stained with used motor oil and treated with Pseudomonas aeruginosa.

Controls will be concrete blocks stained with used motor oil NOT treated with Pseudomonas aeruginosa.

Statistical test: Independent Samples *t*-Test

Alpha: 0.05

Power (1-Beta): 0.80

Effect Size (d): 0.50-medium

Tail(s): Two

Sample size (minimum): N = 128 (Cases = 64, Controls = 64)

Power analysis and Sample size calculations conducted using G*Power 3.0.10. Statistical analysis was conducted using SPSS v.23

Safety Concerns

Pseudomonas aeruginosa is considered Biosafety Level 2 (BSL2), so proper personal protective equipment; these include gloves, lab coat, closed toed shoes, and eye protection; were worn at all times when working with this species. If the bacterium contacts eyes, mouth, or nose, rinsing for five minutes at an eyewash station is recommended. In the event of any other bodily contact, washing for five minutes with soap and water is recommended. While no such incidents occurred in the present work, these precautions are important to note for future work. The MSDS for *Pseudomonas aeruginosa* also recommends frequent hand washing and to avoid rubbing eyes as a precautionary measure against eye infections.

According to the ExxonMobil MSDS for motor oil, motor oil is not considered hazardous under normal conditions. Inhalation and ingestion are not expected to be a problem, but if complications follow physician contact is necessary. If oil gets into eyes, flush with running water. If oil gets onto the skin, it can be removed with soap and water. Affected clothing should be removed and washed.

Materials

Automobile Fluids

The used motor oil for the current effort was collected from the central waste collection barrel of Conejo 76 Union Station. The fluid collected from the central waste collection was obtained from multiple clients of the auto repair shop, so it was considered an unknown. The characterization of this fluid as an unknown prompted its analysis via Infrared Spectroscopy. Unused Prestone concentrate antifreeze/coolant was used as the antifreeze standard for the infrared analysis. Unused Mobil ATF D/M was used as the transmission fluid standard for the

infrared analysis. Unused Pennzoil 10W-40 was used as the unused motor oil standard for the infrared analysis.

Bacterial Culture

The stock culture of *Pseudomonas aeruginosa* was obtained from the lab stock of Cal State Channel Islands. The culture medium used was ATCC Medium 18: Trypticase Soy Broth. The cultures were shaken using a C25KC Incubator Shaker by New Bruinswick Scientific.

Modified EPA Analysis

For the purposes of this work, the distillation process originally included in EPA Method 1664, Revision A: N-Hexane Extractable Material (HEM Oil and Grease) and Silica Gel Treated N-Hexane Extractable Material (SGTHEM; Non-polar Material) was removed and replaced. The procedure used in place of the original distillation was an evaporation process done by using a BÜCHI Rotavapor R-114 with an attached BÜCHI Waterbath B-480.

Methods

Infrared Spectroscopy

The used motor oil used in the current effort was obtained from a local auto repair shop (Conejo 76 Union Station) which retrieved it from numerous unknown clients. As the exact contents of the collected fluid was unknown, infrared spectroscopy (IR) was conducted on the used motor oil to qualitatively determine its composition. This was conducted using a Thermo Scientific™ Nicolet iS™10 FT-IR Spectrometer. The used motor oil, as well as unused

automobile fluids (new motor oil, new antifreeze, and new transmission oil) were analyzed by IR. The inverted molecular peaks of all four samples were qualitatively compared to determine what was in the used motor oil sample.

Culturing of *Pseudomonas aeruginosa*

The *Pseudomonas aeruginosa* used was cultured using the following methods in a CSUCI laboratory located in Aliso Hall. The atmosphere was aerobic. The temperature of the culture environment was 37°C. Using the open flame of a bunsen burner, inoculating loop was burned until red hot and allowed to cool for 30 seconds. Stock culture of *Pseudomonas aeruginosa* opened and mouth of container immediately flamed to prevent fungal growth. Media in stock container scraped with previously mentioned inoculation loop. Mouth of stock container flamed again and closed. A one-liter sized cell media container was filled with ATCC Medium 18: Trypticase Soy Broth. Upon opening, the mouth of media container flamed with open flame. The loaded inoculation loop was then inserted into broth and swirled. Mouth of bottle containing inoculated broth flamed again and immediately closed. Inoculation loop reflamed in preparation for next use. This process was completed four times, so as to yield four liters of *Pseudomonas aeruginosa* culture.

Pilot Study

The preliminary study was conducted in order to evaluate feasibility, time, cost, and adverse events of the experimental analysis. It was necessary to determine appropriate exposure time of *Pseudomonas aeruginosa* to the dried used motor oil before beginning experimental

analysis. In order to do so, 5 ml of the used motor oil collected from Conejo 76 Union Station was applied centrally to six concrete pavers using a BD 2 oz. (60 ml) syringe with catheter tip. The concrete pavers were purchased from Home Depot, and were 6x6 inches in dimension. After the application of the used oil, the pavers were allowed to dry horizontally for a period of 6 hrs. To follow, 10 cc of *P. aeruginosa* culture was then applied on top of the dried oil stain on the five control blocks. The single control block was not inoculated. Each case sample was allotted a different exposure time (block 1 at 6 hrs., block 2 at 12 hrs., block 3 at 18 hrs., block 4 at 12 hrs., block 5 at 30 hrs.). After allotted exposure time, each block was held at approximately a 45-degree angle and was rinsed with 200 ml clean water to simulate rainfall. Runoff was collected for analysis of oil and grease content. Control block was rinsed after 30 hrs and runoff collected for analysis of oil and grease content.

Experimental Phase

The Experimental phase of this research was conducted following the pilot study. A total sample size of 128 concrete pavers were used ($N = 128$ is the minimum sample size required for a 2-tailed study of this nature). The pavers used for this phase are of the same source and are of the same dimensions as those described in the pilot study. One half of sample pavers (Cases, n= 64) were labeled to be case, while the other half (Control, n= 64) were labeled to be control. As 5 cc of used motor oil was shown in the pilot study to be too large of a volume for the pavers to handle, 3 cc of used motor oil was applied centrally on all samples using a BD 2 oz. (60 ml) syringe with catheter tip. After used oil application, pavers were allowed to dry horizontally for

6-7 hrs. To follow, 10 ml of *Pseudomonas aeruginosa* culture was applied to dried used motor oil on all case blocks (block no. 7-70). Control blocks (no. 71-134) were not inoculated. After *Pseudomonas aeruginosa* was exposed to case blocks for 48 hrs., all samples were held at approximately a 45 degree angle and rinsed with 200 ml clean water in rainfall simulation. Runoff was collected for EPA analysis of oil and grease content.

Analysis of water runoff

All runoff samples were analyzed for oil and grease content using a modified version of EPA Method 1664, Revision A: N-Hexane Extractable Material (HEM Oil and Grease) and Silica Gel Treated N-Hexane Extractable Material (SGTHEM; Non-polar Material) by Extraction and Gravimetry. The original protocol contained a distillation process that was too time consuming and labor intensive given the large sample size used in the present work. In order to save time and increase efficiency, the original distillation process was removed and replaced with an evaporation method conducted using BÜCHI Rotavapor R-114 with attached BÜCHI Waterbath B-480. This modification was equally (if not more) effective as the original procedure. The change was also significantly more efficient and reduced the amount of lab work required for the study. The modified procedure is as follows:

1. 250 mL round bottom flask baked for 15 minutes in 70℃ oven
2. Sample weighed with calibrated analytical balance
3. Sample transferred to 1 L glass bottle and volume of sample raised to 1 L with water
4. Sample acidified to pH>2 with HCL 6 molar solution
5. To verify pH:

   a. Glass stirring rod dipped into well mixed sample
   b. Allow drop of sample to fall off of stirring rod onto pH paper
   c. Stirring rod rinsed with n-hexane back into sample container so as to ensure no extractable material was lost on stir rod

6. Round bottom flask removed from oven and allowed to cool to room temperature (RT). Flask then weighed with calibrated analytical balance.
7. 30 mL of n-hexane (n-Hexane - 95% from Fisher Scientific) added to sample and shaken well with original cap closed
8. Sample poured into separatory funnel
   a. Aqueous layer drained back into original 1 L sample container
   b. Upper layer filtered with filter paper and sodium sulfate into previously mentioned round bottom flask
9. Steps 7-8b done in triplicate to ensure all extractable material was extracted
10. To replace original EPA distillation process, samples evaporated using BÜCHI Rotavapor R-114 (flask appeared dry when rotavapor process complete)
11. Flask baked again for 15 minute in 70℃ oven
12. Flask allowed to cool to room temperature and weighed again using calibrated analytical balance
13. Oil and grease content of original sample calculated in PPM

Results

Results of the Infrared Spectroscopy of the unknown used automobile fluid provided by Conejo 76 Station gasoline station show overlap of the inverted peaks of the known automobile fluids to the unknown automobile fluid. These findings qualitatively indicate the composition of the unknown used luid. Each sample tested showed distinct peaks in accordance to its molecular structure. As frequency is directly proportional to wavenumber, the peaks can be identified both qualitatively, by the frequency of light absorbed, and by the percent of transmittance of energy at any given wavenumber.

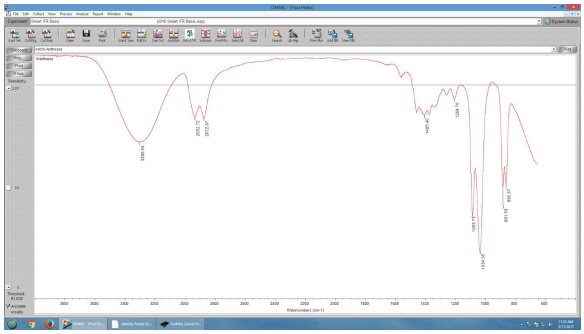

Figure 1: Spectrogram of Prestone concentrate antifreeze/coolant

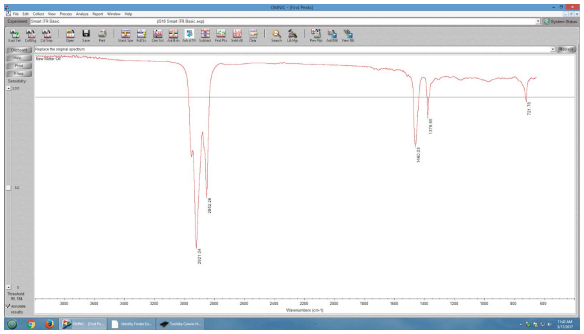

Figure 2: Spectrogram of Pennzoil 10W-40

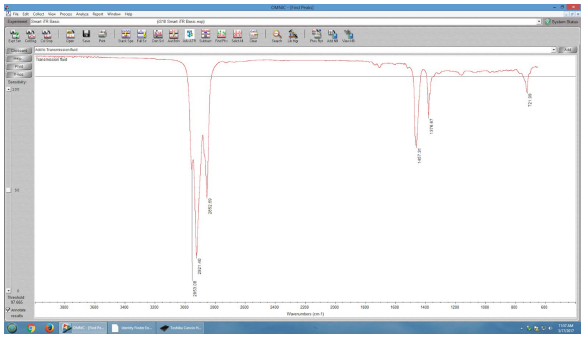

Figure 3: Spectrogram of Mobil ATF D/M

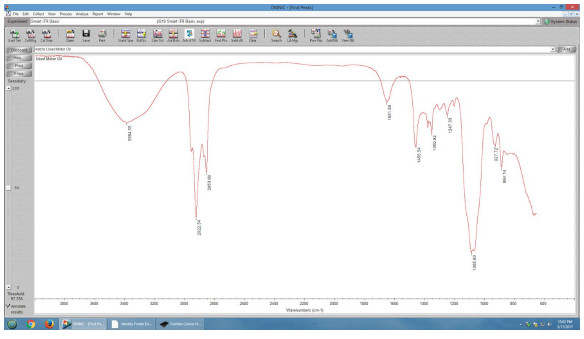

Figure 4: Spectrogram of used motor oil (unknown used automobile fluid

The spectrogram for antifreeze contains a distinct alcohol peak at wavenumber 3350 (approx.). This peak appeared for antifreeze because antifreeze contains alcohol. Unused motor oil and transmission fluid do not contain alcohol, so this peak does not appear on their respective spectrograms. However, this peak can be seen on the spectrogram for the used motor oil collected for the present work. From this, it was determined that the sample collected contained antifreeze. Transmission fluid has a pair of distinct peaks at wavenumbers 1450 (approx.) and 1400 (approx.). The same distinct peaks appear on the spectrogram for used motor oil. Therefore, the collected sample contained transmission fluid as well. Similar to transmission fluid, plain motor oil also has a pair of distinct peaks. These are located at wavenumbers 2925 (approx.) and 2875 (approx.). This pair of peaks can also be seen in the spectrogram for the collected sample. Based on all these observations, it was concluded that the used automobile fluid collected for the current effort contained motor oil, transmission fluid, and antifreeze.

Results of the Pilot Study indicated the feasibility, time, cost, and no adverse events of the analysis exposure of *Pseudomonas aeruginosa* to dried used motor oil on concrete surfaces. Additionally, the exposure of *Pseudomonas aeruginosa* to used motor oil on concrete surfaces affected the oil and grease concentration (ppm) in water runoff. As the modified EPA analysis for oil and grease content was conducted on an evening of inclement conditions, humidity error affected two pilot samples (labeled no. 4 and no.5). The error resulted in a negative result; impossible when correctly conducting such gravimetric methods as those described in the present work. Thus, samples no. 4 and no. 5 were rendered obsolete, and therefore the results collected

from these samples were not considered in the current conclusion. In addition, the *Pseudomonas aeruginosa* exposure time was determined to be no less than 30 hrs. for Experimental Analysis. Each experimental case sample was exposed to the bacteria for a different six-hour interval (6 hrs, 12 hrs, 18 hrs, 24 hrs, 30 hrs). After the 30 hr experimental case sample and the control (left for 30 hrs after initial 6 hr drying time without being inoculated) were rinsed with simulated rainfall for analysis of oil and grease content, all six control bricks were ignored for a number of days. After this time, the visual stains on the case bricks had lightened, whereas the control brick's stain had not diminished. From this observation, it was determined that the optimal exposure tie had to be greater than the longest interval tested, that being 30 hours.

Results of the Experimental Analysis (Independent samples t-test) transforming negative calculated EPA final ppm to zero [Final EPA Values less than zero = 0.0000] star how that the mean PPM of oil and grease content in water for the Control [No Exposure to *Pseudomonas aeruginosa*] samples [M = 157.23, SD = 171.42, n = 64] to be higher than that for the Cases [48 hrs. Exposure to *Pseudomonas aeruginosa*] samples [M = 136.77, SD = 162.48, n = 62] (note: two case samples lost to lab accident). However, these findings were not statistically significant at the .05 level of significance [$t(124) = .687$, $df = 124$, $p > .05$]. On average, the PPM of oil and grease content in water between the No Exposure samples and Exposure samples was [MD = 20.46, SED = 29.77, 95% C.I. (-38.47, 79.39)]. The null hypothesis which suggested that there was no significant difference in the mean PPM of oil and grease content in water between the No Exposure samples and Exposure samples cannot be rejected. This analysis was repeated after removing negative outliers [Final EPA Values less than zero].

Results of the Experimental Analysis (Independent samples t-test) after removing negative outliers [Final EPA Values less than zero] show that the mean PPM of oil and grease content in water for the Control [No Exposure to *Pseudomonas aeruginosa*] samples [*M* = 205.36, SD = 168.75, n = 49] to be higher than that for the Cases [48 hrs. Exposure to *Pseudomonas aeruginosa*] samples [M = 201.90, SD = 160.64, n = 42]. However, these findings were not statistically significant at the .05 level of significance [t(89) = .100, df = 89, p > .05]. On average, the PPM of oil and grease content in water between the No Exposure samples and Exposure samples was [MD = 3.46, SED = 34.70, 95% C.I. (-65.50, 72.42)]. These findings were validated by bootstrap. Bootstrap (Number of samples = 1000) results were [MD = 3.46, SED = 33.91, 95% C.I. (-68.33, 71.13). The null hypothesis which suggested that there was no significant difference in the mean PPM of oil and grease content in water between the No Exposure samples and Exposure samples cannot be rejected.

Discussion

As the composition of the used motor oil collected was unknown, Infrared Spectroscopy was necessary in determining what was actually being tested in the current effort. The fluid was collected from the central waste collection barrel of an auto repair shop that services many different clients. What is collected by this waste barrel is what is expelled by automobiles when they are operational. Because of this, the used fluid used in this study is an accurate representation of what would typically be found on roadways, parking lots, and driveways. It was important to note the composition of this fluid so as to know what automobiles expel into the environment, and therefore what causes pollution. The observation that used motor oil is not

the only substance released by automobiles is important to note for further work, as the present work was based on the ability of *Pseudomonas aeruginosa* to digest only used motor oil.

The estimate of optimal exposure time of *Pseudomonas aeruginosa* to the dried used motor oil was deduced from the Pilot Study. While oil and grease content was not analyzed after 30 hrs of exposure during the Pilot Study, the visual stains left by the used motor oil on the case samples was seen to be reduced a number of days after rainfall simulation of case block no.5 and the control block. The oil stain left on the control block was not visually diminished in comparison. Due to this observation, it was determined that the optimal exposure time had to be some point greater than the greatest interval tested (30 hrs). Because of time constraints, the exposure time used for the experimental analysis was 48 hrs. Given more time, a longer exposure time may have been optimal.

While the results of the independent samples t-test were not shown to be statistically significant; the results are notable nonetheless. The PPM for the control samples was higher than the *Pseudomonas aeruginosa* exposed samples for both corrected outliers, removed outliers, and removed outliers with bootstrap calculations. The non-statistically significant findings may have been a result of higher than normal humidity levels during the analysis. Of the original [N = 128] samples; n = 37 samples were removed from the calculations due to a negative final weight difference (removed outliers). This reduced the final sample size to n = 91 [Control = 49 and Exposed = 42]. However, the results are environmentally significant.

While the difference between case and control PPM of oil is small and seemingly insignificant in this sample size, it may make an influential impact when amplified in a metropolitan setting. Urban and suburban communities are heavily reliant on automobile

transportation, resulting in high amounts of used motor oil and other used automobile fluids expelled into the environment. When applied to such a large scale, the effect of *Pseudomonas aeruginosa* on dried used motor oil can provide significant aid in environmental protection.

The ability of *Pseudomonas aeruginosa* to prevent used motor oil on concrete surfaces from contaminating the watershed brings forth a new method of environmental protection and repair. Successful biodegradation of used motor oil waste from roadways and parking areas will limit the quantity of used motor oil found in rainwater runoff, thereby preventing or decreasing risk of water and environmental pollution. As used motor oil is known to be a hazardous waste, this environmentally and economically friendly method of environmental mediation will greatly benefit public health. Positive findings will further warrant additional research. Further research may include commercial development and application of *Pseudomonas aeruginosa* to increase and improve used motor oil cleanup. As the fluid expelled by automobiles was shown to be more than just used motor oil, it would be beneficial to investigate other MEOR capable organisms which can remediate the other components of the waste fluid. These microorganism could be used in conjunction with *Pseudomonas aeruginosa* to create a "microbial cocktail" to remove the entire stain and therefore prevent other contaminants from polluting the watershed.

Conclusion

The results of this study show *Pseudomonas aeruginosa* to have positive environmental implications for removal of unwanted automobile fluids. The small difference detected in the oil and grease in the water runoff in this study will be amplified in a major metropolitan setting. Additionally, the visual oil stain and residue was lessened in the days following *Pseudomonas*

aeruginosa exposure. These findings may be of significant interest to environmental scientists and the general public. Microbial biodegradation methodologies environmental protection and pollution control are beneficial due to their economic efficiency and environmental safety. Additionally, the simplicity of concept cannot be overlooked. Thus, the quantitative and qualitative findings of this study warrant further investigation. The next steps in this investigation is to explore which molecular components of the used oil and grease are digested by *Pseudomonas aeruginosa*. If the molecular components digested can be quantitatively identified, there is promise for investigation of the molecular digestion of components of used oil and grease by additional MEOR capable organisms.

This process can be repeated with other MEOR capable organisms. Once determined, combinations of MEOR organisms can be evaluated for effectiveness of the molecular digestion of components of used oil and grease. These combinations of MEOR organisms may then be applied as a cocktail to lessen the burden of used oil and grease in metropolitan and rural environments. The results of this study add to the conversation of knowledge of automobile population. Further, these results show promise to remove the threat of motor oil pollution from roadways and parking areas, increasing environmental safety and public health.

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