BIODEGRADATION OF DIESEL ENGINE OIL BY MICROORGANISMS AND COMPARISON OF THEIR BIOREMEDIATION POTENTIAL: THE GRAVIMETRIC METHOD

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Abstract
As the use of hydrocarbon fuels increases, the pollution of engine oil becomes one of the environmental problems. The rate of biodegradation of diesel fuel by microorganisms isolated from sewage (oil-contaminated soil) was studied. A reformed diesel fuel system was used and two major microorganisms isolated from the car parking lot - Micrococcus sp. and Pseudomonas sp. They were found to be hydrocarbon decomposers and two bacteria were selected for degradation tests. Diesel fuel degradation is estimated over a period of five to twenty-five days, using a gravimetric method. After 25 days of the fall season, Pseudomonas sp. reduce 67.57% of oil in Micrococcus sp. 52.95% today. But the mixture of Micrococcus sp. and Pseudomonas sp. was found to have high capacity to degrade diesel oil i.e. 89.98% after 25 days degradation rate of diesel oil by Micrococcus sp. The study found that it is 7.48 x 10-4gm / hr and that of Pseudomonas sp. was 9.55 x 10-4gm/hr while the combination of the two bacterial isolates showed the highest rate of diesel fuel degradation i.e. 1.27 x 10-3gm/h.
Keywords: biodegradation, microorganisms, bioremediation, diesel engine oil, hydrocarbon

Introduction

As we enter the modern era of technological innovation, many aspects of people's lives are changing. People benefit greatly from the development of life and many live in prosperity, but prosperity has a price. This price is our country that suffers every day from various pollution and destruction. People are now looking for ways to heal this destruction. Oil pollution is one of the most dangerous causes of

pollution known today. It can pose a danger to the environment. Environmentalists are very afraid that it is difficult to control if it disappears.

As the use of hydrocarbon fuels increases, the pollution of engine oil becomes one of the environmental problems. Mineralization is the complete conversion of organic compounds into a more mobile energy state or mineral form (Baker and Herson, 1994). Diesel fuel, which is one of the main products of crude oil, is a major pollutant in our environment. With the increasing reliance on diesel fuel by some automakers and manufacturers, an increasing number of vehicles are being transported over long distances. Therefore, diesel oil can enter the environment through the destruction of oil tankers carrying diesel oil, washing of diesel tanks by consumers, military vehicles carrying diesel oil and cars (Hill and Moxey, 2000). Diesel fuel spilled on agricultural land often reduces plant growth. Suggested reasons for plant growth reduction in diesel fuel pollution are from direct toxic effects on plants (Baker, 2012) and reduced germination and poor soil conditions due to soil inefficiency due to air movement from the interstellar space. Advantages of diesel engine oil (Zahir, Malik and Arshad, 2011).

Oil-damaged soil can be remedied by removing motor oil residues produced by biodegradation and biotransformation of organic matter in motor oil. Microbial degradation of complex compounds does not always lead to mineralization. Incomplete decomposition, called structural changes, can occur due to microbial

activity (Ideriah., Tubonimi, Igwe., Chika., Stanley and Herbert, 2008). Most of the methods used to reduce oil pollution are costly, time consuming and rely on excavation of these soils, treatment in separate areas or better treatment facilities. These treatments include cremation and/or burial in a protected landfill. These are effective treatments but after fire, the soil loses most of its nutritional value and structure. These methods do not remove the contamination and actually create the problem (Lageman, Clarke and Pool, 2005). Bioremediation has been shown to be an effective method of increasing biodegradation of contaminated soils (Swannell, Richard, Lee, and McDonagh, 2016) and can restore contaminated soils through extensive biodegradation of unwanted organic compounds by microorganisms (Andreoni and Gianfreda, 2007).).

Among the different treatment methods available to remove petroleum hydrocarbons from soil and groundwater, bioremediation methods gain ground due to their simplicity, high efficiency, and cost effectiveness when used compared to other technologies (Adriano Pinto Mariano, Ana Paula de Arruda, Geraldes Kataoka, Dejanira de Franceschi). de Angelis Daniel Marcos Bonotto, 2017). Therefore, this study was carried out to evaluate the biodegradation rate of diesel fuel (hydrocarbon) by microorganisms from the parking lot (oil house), using the gravimetric method.

Material and Methods

Preparation of modified diesel engine: Modified diesel fuel machine contains 0.7 gm K2HPO4, 0.1 gm (NH4) 2SO4, 0.3 gm KH2PO4, 0.3 gm MgSO4 7H2O, 2.2 gm agar - agar5. The mineral complex is dissolved in half to 100 ml of hot water and mixed with 2 ml of Gulf diesel oil. The flask was incubated at 121 oC for 15 min.

The development of microorganisms: Microorganisms can spoil the diesel fuel by adding that the diesel fuel does not change by inoculating the soil and half of the 250 ml conical flask. 0.5 g of soil in storage put in 100 ml of a dish of diesel fuel is not well mixed and left to cool at 37oC for a week.

The determination of the number of microbial colony for the tumor was negative: 5 ml of the rare Nutrient broth was thoroughly infused in sub-region 1 (C1) in the first test tube and Colony 2 (C2) in the second test tube and the two tubes. It was mixed at 37oC for 24 hours. Afterwards, the volume of the contents in one ml of fermented broth was determined from the media plate. The amount of minerals in the two tubes is sorted in such a way that the two aggregates have about the same amount of microorganism in one milliliter of sample using the nutrient broth as a fragrance (Dong-ju Kim, Seung-gun Chung, Sang-hyup Lee and Jae-woo Choi, 2012).

Soil Collection and Preparation: The surface sample was taken from Gulshan, Dhaka; in a plastic jar. The autoclave was incubated at 121°C for 15 min, after which it was allowed to cool to room temperature to continue processing.

Definition and Organization of Experiment: i. Sample 12 x 15 g injection soil mixed with 1 ml (0.848 g) sterile Gulf diesel engine oil + 0.2 ml custom C1, ii. 12 x 15g samples of milled mix mixed with 1ml (0.848g) Sterile Gulf diesel engine oil + 0.2ml culture C2, iii. A 12 x 15 g sample of milled oil mixed with 1 mL (0.848 g) pure Gulf diesel oil + 0.1 mL culture C1 + 0.1 mL culture C2.

Control: 12 samples of 15 g of sterile soil mixed with 1 ml (0.848 g) of Sterile Gulf diesel fuel + 0.2 ml of sterile water.

Diesel damage study: The potency of C1, C2 and the combination of various bacterial reduction derivatives were examined on the first day (empty day) of the study and then between 5 days for 25 days and activated carbon tetrachloride. as puree. Every day, two samples for one treatment are evaluated for residual diesel fuel levels (Dong-ju Kim, Seung-gun Chung, Sang-hyup Lee, and Jae-woo Choi, 2012). Each 15 g sample of home treatment was mixed with 40 ml of carbon tetrachloride, placed in a separate conical tube, shaken for 3 minutes and allowed to stand for 5 minutes above power consumption (diesel fuel - carbon tetrachloride) to pass gradually through a funnel fitted with filter paper (Whatman No 1). Anhydrous sodium sulfate is dispersed on filter paper to remove water from

the mixture. Fluid levels were obtained in pre-doses of 50 mL. The beaker containing the puree is placed in the oven and allowed to rise to 50 ° C. The beaker containing the remaining diesel fuel is allowed to cool in the chamber and is measured to determine the amount of diesel fuel remaining at each exchange (Udeme in Antai, 2008).

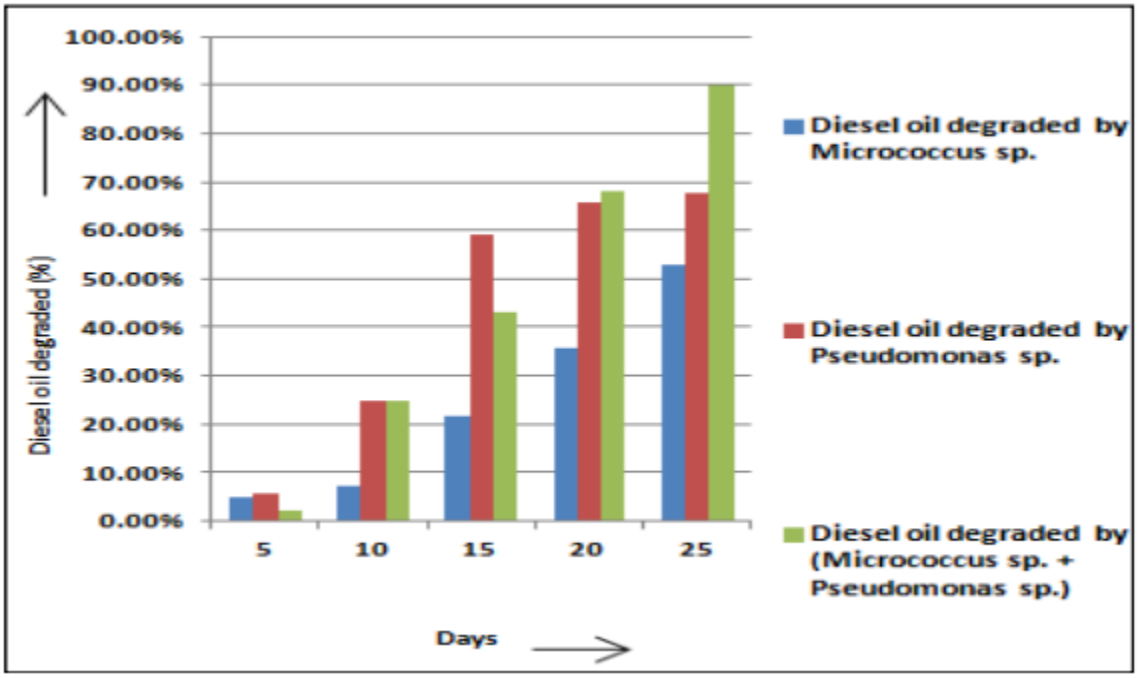
Results and Discussion

Table 1: Weight of diesel engine oil extracted (on various days) from 15 gm soil samples polluted with 1 ml (0.848 gm) of Sterilized diesel oil and 0.2 ml of culture

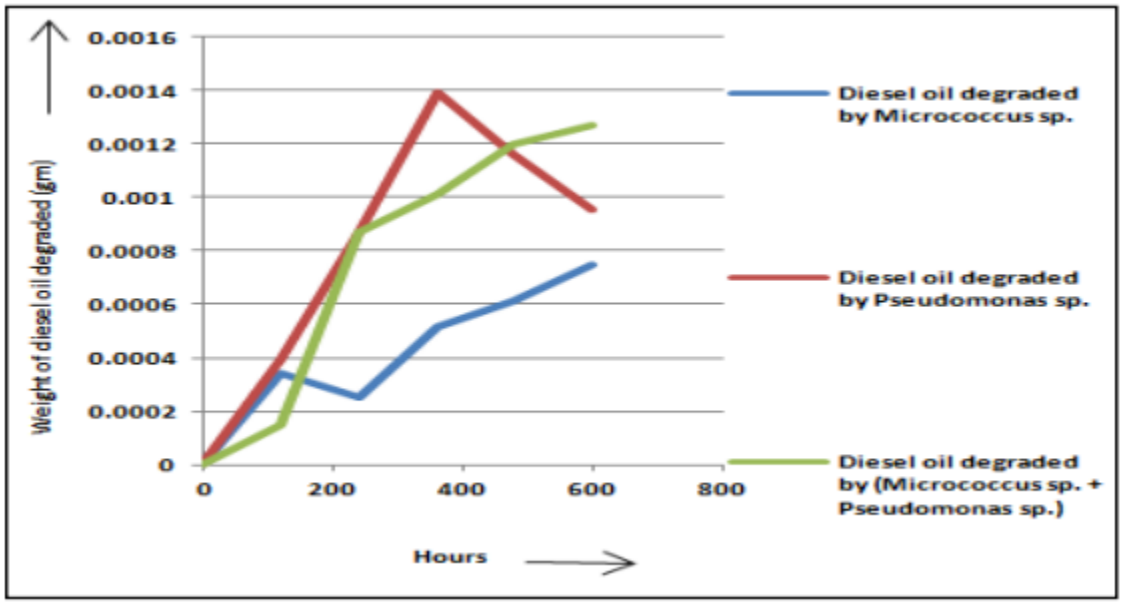
Day	Sample	Weight of diesel oil extracted (gm)	Weight of diesel oil degraded (gm)	Rate of degradation (gm/hr)
0	I	0.848 gm	0.000	0.00
	II	0.848 gm	0.000	0.00
	III	0.848 gm	0.000	0.00
	IV	0.848 gm	0.000	0.00
5	I	0.807 gm	0.041	3.42×10^{-4}
	II	0.801 gm	0.047	3.92×10^{-4}
	III	0.830 gm	0.018	1.50×10^{-4}
	IV	0.848 gm	0.000	0.00
10	I	0.787 gm	0.061	2.54×10^{-4}
	II	0.639 gm	0.209	8.71×10^{-4}
	III	0.639 gm	0.209	8.71×10^{-4}
	IV	0.848 gm	0.000	0.00
15	I	0.663 gm	0.185	5.14×10^{-4}
	II	0.348 gm	0.500	1.39×10^{-3}
	III	0.483 gm	0.365	1.01×10^{-3}
	IV	0.848 gm	0.000	0.00
20	I	0.545 gm	0.303	6.31×10^{-4}
	II	0.290 gm	0.558	1.16×10^{-3}
	III	0.271 gm	0.577	1.20×10^{-3}
	IV	0.848 gm	0.000	0.00
25	I	0.399 gm	0.449	7.48×10^{-4}
	II	0.275 gm	0.573	9.55×10^{-4}
	III	0.085 gm	0.763	1.27×10^{-3}
	IV	0.848 gm	0.000	0.00

*values are means of twice determinations. Key: i. Sterilized soil + Sterilized diesel oil + Micrococcus sp. ii. Sterilized soil + Sterilized diesel oil + Pseudomonas sp. iii. Sterilized soil + Sterilized diesel oil + Micrococcus sp. + Pseudomonas sp. IV. Sterilized soil + Sterilized diesel oil

The biodegraders which were Micrococcus sp., Pseudomonas sp., and Mixture of both the culture showed different abilities in the breakdown and utilization of the diesel engine oil.



Figure–1 Comparison of % Diesel engine oil degradation



Figure–2 Comparison: Rate of Diesel engine oil degradation (gm/hr)

Diesel Oil Contamination Study by Micrococcus sp: We found that the rate of diesel fuel pollution by Micrococcus sp. it is faster compared to the amount of diesel damage from Pseudomonas sp. and a mixture of Micrococcus sp. and pseudomonas sp. But the diesel oil degradation potential of Micrococcus sp. was continuously increasing as the time of contact between oil and organism increased. Diesel oil damage analysis by Pseudomonas sp: The performance of Pseudomonas sp. to reduce diesel fuel faster than that of Micrococcus sp. As aging increases the rate of damage to diesel fuel increases. But it was found that by the fifteenth day, the number of damages was very fast. This may be due to the late end of cell growth but after that, the sales rate decreases slightly. It is possible because the cells of Pseudomonas sp. close to cell growth. Investigation of diesel fuel pollution from a mixture of Micrococcus sp. and Pseudomonas sp: How much diesel oil is extracted from the soil containing diesel oil as well as a mixture of the two bacteria Micrococcus sp. + Pseudomonas sp. showed persistent obesity lasting up to 25 days of the fall period. After the 5th day of the design period, it was found that there was a significant increase in the diesel fuel rate up to 25 days of the installation period which was higher than that of a traditional Micrococcus sp. as well as that of Pseudomonas sp. In this case, it is found that around 90% diesel fuel

is damaged after 25 days and the rate of increasing diesel fuel damage continues ie 1.50 x 10-4gm / hr after the 5th day to 1.27 x 10-3gm / hr after the 25th day.

Conclusion

When Micrococcus sp. is used in combination with Pseudomonas sp. showed great potential for diesel degradation. This was probably due to the different enzyme system of two different bacterial isolates acting on the hydrocarbon at the same time, which turned out to be an excellent option to degrade this hydrocarbon if both the bacterial enzyme system has considerable efficiency to act on him and to degrade him. This was followed by a single culture of Pseudomonas sp and then Micrococcus sp. Oil degradation by Pseudomonas sp. was not surprising not only because it was isolated from garage floors already contaminated with oil and grease, but also because it is known to possess a more competent and more efficient hydrocarbon degrading enzyme system. active than Micrococcus sp. It is known to grow rapidly and is able to degrade a wide variety of organic compounds11. In the case of Micrococcus sp. which is also known to possess considerable efficiency to be used as an oil degrader, but it requires more time compared to that of Pseudomonas sp.

The use of biological methods, as in the case of bioremediation, generally reduces its cost compared to chemical treatment for many polluted areas. It is also a little unhealthy for the environment. However, since this is a creative process, it takes

time. The above experience shows that bioremediation can be used effectively to treat oil-damaged soils. The astonishing amount of diesel oil damage caused by bacteria isolation demonstrated by this process allows the safe and efficient use of this microorganism in the oil pollution zone. In addition, its results were obtained by comparing the diesel fuel consumption capacity of Pseudomonas sp., Micrococcus sp. and the second combination helps to use them in bioremediation systems based on their effectiveness. The benefits of using a blended culture against pure culture and bioremediation have been demonstrated.

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