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Isolation and Study of Biodegradation Capability of Hydrocarbonoclastic Bacteria from Industrial Waste Lubrication Oil Contaminated Sites

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A research was carried out on Isolation and study of biodegradation capability of hydrocarbonoclastic bacteria from industrial waste lubricant oil contaminated sites. 12 different bacterial cultures are isolated from the soil samples collected from liquid effluent dump site of black galaxy granite, chimakurthy, prakasam Dist, and auto motive lubrication oil replacement garages, Auto Nagar, Vijayawada. Among the 12isolates 10 isolates were found to have capability of degrading used industrial lubrication oil VG 320 collected from NSL textiles LTD, Guntur. Among the isolates tested the isolate no BG4 obtained from liquid effluent dump site gave the maximum biodegradation potential of 18.78mm^{2}followed by BG7 of 15.36, BG1 of 14.07and BG8 of 10.56 mm^2 and the isolate no AL1 from automotive lubrication oil replacement garage soil sample found to be 9.03mm^2 degradation potential. These isolates were identified based on physical and biochemical characters as *Pseudomonas*, *Bacillus*, *Proteus*, *Flavobacterium* and *Enterococcussps* respectively. For the future, our study will be focused on several data like the species of isolates, the optimal activity of isolates to degrade several Industrial lubricant oils.

Introduction

Some products are essential for humans to sustain living standards and comfort. Lubricant oil is one of those products. Their main function is to reduce friction and wear of metal surfaces, extend the service life of the equipment and save resources and energy. Scientific research has shown that the direct costs of friction and wear phenomena may be responsible for nearly 10% of the gross national product (GNP) in many industrialized countries (Stachowiak & Batchelor, 2014). In addition, it is estimated that 1% of GNP could be saved in terms of energy if current tribological knowledge (the science of friction) was applied to lubricated systems (Bronshteyn & Kreiner, 1999).

In 2017, the world lubricant market was estimated at around 35.7 Mt. Accounting for 43% of the global market, Asia-Pacific has the highest share and is the region with the fastest growth in the consumption of lubricants.

The Indian lubricants market was valued at over 2,610 kiloton in 2020, and the market is projected to register a CAGR of more than 1.5% during the forecast period (2021-2026). This, in turn, affected the demand for lubricants in 2020. According to the application, lubricants may be classified into automotive, industrial, process, and marine oils. Indeed, the automotive segment accounted for 57% in 2016 (Fuchs, 2017). Industrial lubricants are sub-divided into industrial oils and industrial specialties (metalworking fluids and greases). The term lubricant has been loosely applied to many other fluids that do not specifically perform the main function of reducing friction and wear. Some examples include power and heat transmission fluids, hydraulic fluids, dielectric fluids, specific process oils, and others. Process oils correspond to raw materials, for instance used as plasticizers agents in plastic and rubber industries. Process oils are only linked with lubricants because they are mineral oil products resulting from the refining of crude (Totten *et al.,* 2003). Nowadays, the sustainability principles are considered a driving force for the industry, and thus saving energy, resources and cutting emissions have become central issues. Also, lubricants production and its recovery are increasingly drawing public attention, since good practices may contribute to the sparing use of resources and sustainable development. With ever-growing global environmental awareness, many countries have developed regulations for guiding the management of hazardous waste. In the European Union (EU), demanding regulations led to the employment of the best available techniques (BAT) regarding waste oil management and to increasing collection and recycling rates over the years in several countries (GEIR, 2016). However, some Member States still need to improve the performance of the integrated waste oil management systems to reach the goals of the EU directives (Pinheiro, Ascensão, Cardoso, *et al.,* 2017). Other countries around the world have also been trying to implement reliable management practices. As an example, (El-Fadel and Khoury 2001) reported the situation in Lebanon, and in Indian automotive industries (Jhanani and Joseph 2011).

Used engine oil can be considered as one of the sources responsible for polluting the soil with hydrocarbons. Used engine oil consists of Petroleum ether or Benzine, Gasoline, Naphtha, Mineral spirits, Kerosene, Fuel oil, Lubricating oil, Paraffin wax, Asphalt or Tar. Used motor oil typically has much higher concentrations of PAHs (polycylic aromatic hydrocarbons) than new motor oil. Chronic effects of naphthalene, a constituent in used motor oil, include changes in the liver and harmful effects on the kidneys, heart, lungs, and nervous system. Due to their relative persistence and potential for various chronic effects (like carcinogenicity), PAHs (and particularly the alkyl PAHs) can contribute to long-term (chronic) hazards of jet fuels in contaminated soils, sediments, and groundwaters (Irwin *et al.,*1997). One of the most significant impact associated with workshop seepage of used engine oil includes loss of soil fertility, water holding capacity, permeability and binding capacity. (Moorthi *et al.,* 2008). They are really harmful to living organisms including human beings and also indirectly contribute to the economic losses in developing countries (Ismail *et al.,* 2013). It's a very costly approach to treat oil contaminated site by conventional methods such as use of chemicals or peat moss (a plant which absorbs hydrocarbons). These conventional methods can be replaced by modern methods such as micro-organism or engineered micro-organism which can detoxify the contaminants in to lesser toxic compounds. Bacteria have the incredible ability to degrade the natural or synthetic materials. This degradation process is known as biodegradation. Nowadays, bacteria or other microorganisms is utilized for clean up the polluted environment. This activity is known as bioremediation.

Waste lubricant oil is one of the environment pollutants(Bhattacharya *et al.,* 2015, Ibrahim HMM 2016 and Salam LB 2016). It could damage our health when it is oxidated, nitration, cracking of polymers and decomposition of organometallic compounds in diesel engine for long period and high temperature (Salam LB 2016). Lubricant oil is one of the petroleum derivatives. Waste lubricating oil also contain other dangerous compound like polyaromatic hydrocarbons (PAHs), toxic heavy metal dan chlorinated hydrocarbons (PCBs) (Ibrahim HMM 2016). These compounds are carcinogenic dan neurotoxic on human life system (Ray *et al.,* 2008 and Obayori *et al.,* 2008). Uncontrolled disposal of waste lubricant oil to environment cause human and other living organisms to be contaminated. Regarding of high risk of waste lubricant oil to living organisms especially human being, it is an urgent way to do bioremediation. Many studies found some potential genus of bacterial for waste lubricant oil biodegradation like *Acinetobacter, Achromobacter, Arthrobacter, Flavobacterium, Pseudomonas* and many others (Adelowo *et al.,*2006; Mandri T and Lin J 2007; Bagherzadeh- Namzi *et al.,* 2008; Basuki *et al.,* 2011; Obayori *et al.,* 2014; Salam LB *et al.,* 2015). Bioremediation method is considered to be more economical and safe method for the treatment of oil contaminated site. Enriched biodegradation can be speed up growth of microorganisms for easy isolation. Referring the previous studies on bioremediation (Moorthi *et al.,* 2008; Emtiazi *et al.,* 2005; Bragg, *et al.,* 1994; Singh and Lin 2008; Udeani *et al.,* 2009; Barathi and Vasudevan., 2001; Head and Swannell., 1999; Ortega *et al.,* 2003) of contaminated soils.

In this context the study is extended based on the biodegradation of used industrial lubricant oils in order to find the indigenous bacterial cultures in oil contaminated soils for their biodegradation abilities.

Materials and Methods

Sample collection

Soil samples were collected from lubricant oil contaminated sites of Liquid effluent dump sites of black galaxy granite, Chimakurty, Prakasam District and Auto motive lubricant oil replacement garages located in Autonagar, Vijayawada.

Used Industrial lubrication oil (VG 320) collected from NSL textiles LTD, Guntur

Oil condition when collected is blackish brown, oxidation status 2 AB/CM, pH 10, Water -0.019%, PQ index-7, Al-2, Cr-1,Cu-1, Fe-53,K-16, Na-5,Si-7,B-1,Ca-12, Mg-4,P-238,Zn-28 PPM

Isolation of bacteria

Bacterial species were isolated from the collected soil samples by serial dilution and agar plating method wherein the soil sample was diluted from 10^{-1} to 10^{-8} dilutions, and the diluted soil samples were spread on sterile Nutrient agar plates. The inoculated plates were incubated at 37^{o}C for 24 hours. Colonies formed were picked and purified by streaking on Nutrient agar slants. 12 isolates were collected and named as BG1, BG2. BG3, BG 4, BG 5, BG 6, BG 7, BG8, AL1, AL 2, AL 3, and AL4.Mineral salt medium (MSM) with the following composition (g/L): 1.2 g NH4Cl; 1.6 g K2HPO4; 0,4 g KH2PO4; 0.1 g NaCl; 1 g KNO3; 20 g MgSO4.7H2O; 10 g CaCl2.2H2O; 0.05 g FeCl3, 1 mL of trace element solution [20,21] per litre containing 50 mg MnCl2.H2O, 300 mg H_3BO3, 1.1 mg FeSO4.7H2O, 190 mg CoCl2.6H2O, 2 mg CuCl2.2H2O, 24 mg NiCl2.6H2O, 18 mg NaMoO4.2H2O, 42 mg ZnCl2.7H2O, 20 g Agar, 1 ml vitamin solution (M Rassi *et al.*, 2015) were used as selective medium for hydrocarbonoclastic bacteria isolation

Screening of Isolates for the capability of biodegradation of Industrial Lubrication oil

Oil degradation capability of purified cultures were performed by streaking each colony on Nutrient agar enriched with 10% waste lubricating oil, Tween 80 and 0.5 g neutral red (Yolantika H *et al.*, 2015). Isolates then incubated at 37^0C till found colonies with clear zones. The degradation - potential of bacteria in vitro is revealed through measuring the zone of hydrolysis around the isolates.

Results and Discussion

Among the 12 Bacterial isolates 10 are capable of degradation of industrial lubrication oil while the remaining two are not capable of degrading lubrication oil. Depending upon the zone of clearance isolates with high degradation activity were selected on the basis of relative growth and degradation of lubrication oil. The results are presented in Table 1, Table 2 and Fig 1. The 10 Bacterial isolates have the capability of degradation of lubrication oil at varying levels from 4.04 to18.78 $mm^{2.}$ Among the isolates tested the isolate no BG4 obtained from liquid effluent dump site of black galaxy granite, Chimakurthy, Prakasam DIST gave the maximum biodegradation potential of 18.78 mm^2, followed by BG7 of 15.36, BG1 of14.07and BG8 of 10.56 mm^2respectively,whereas isolates from auto motive lubricant oil replacement garages namely AL1. AL2 and AL4. AL 1 is potential in degradation of Industrial lubrication oil with 9.03 $mm^{2.}$ Potentiality. The Isolate Nos BG1,4,7,8 and AL1 were selected for Cultural identification. The cultures are identified based on the Characteristics summarized in Table 3. on the basis of Bergey's manual the isolates are identified as BG4 *Pesudomonas*, BG7 *Bacillus*, BG1 *Proteus*.BG8 Flavobacterium and AL1 as Enterococcus *sps*. Many studies have revealed that there is a large number of hydrocarbon-degrading bacteria in oil-rich environments, such as oil spill areas and oil reservoirs (Hazen *et al.*, 2010; Yang *et al.*, 2015), and that their abundance and quantity are closely related to the types of petroleum hydrocarbons and the surrounding environmental factors (Fuentes *et al.*, 2015; Varjani and Gnansounou, 2017). Recent studies have identified bacteria from more than 79 genera that are capable of degrading petroleum hydrocarbons (Tremblay *et al.*, 2017). Several of these bacteria such as *Achromobacter*, *Acinetobacter*, *Alkanindiges*, *Alteromonas*, *Arthrobacter*, *Burkholderia*, *Dietzia*, *Enterobacter*, *Kocuria*, *Marinobacter*, *Mycobacterium*, *Pandoraea*, *Pseudomonas*, *Staphylococcus*, *Streptobacillus*, *Streptococcus*, and *Rhodococcus* have been found to play vital roles in petroleum hydrocarbon degradation (*Margesin et al.*, 2003; Chaerun *et al.*, 2004; Jin *et al.*, 2012; Nie *et al.*, 2014; Varjani and Upasani, 2016; Sarkar *et al.*, 2017; Varjani, 2017; Xu *et al.*, 2017). *Bacillus subtilis* to effectively accelerate the degradation of crude oil. (Wang C. *et al.*, 2018).

Table.1 Bacterial isolates from different locations

S.no.	Source	Isolate no	Degradation potential
1	Liquid effluent of black granite dump site, chimakurthy	BG1	Yes
2	Liquid effluent of black granite dump site, chimakurthy	BG2	No
3	Liquid effluent of black granite dump site, chimakurthy	BG3	Yes
4	Liquid effluent of black granite dump site, chimakurthy	BG4	Yes
5	Liquid effluent of black granite dump site, chimakurthy	BG5	Yes
6	Liquid effluent of black granite dump site, chimakurthy	BG6	Yes
7	Liquid effluent of black granite dump site, chimakurthy	BG7	Yes
8	Liquid effluent of black granite dump site, chimakurthy	BG8	Yes
9	Auto motive lubricant oil replacement garage	Al1	Yes
10	Auto motive lubricant oil replacement garage	Al2	Yes
11	Auto motive lubricant oil replacement garage	AL3	No
12	Auto motive lubricant oil replacement garage	AL4	Yes

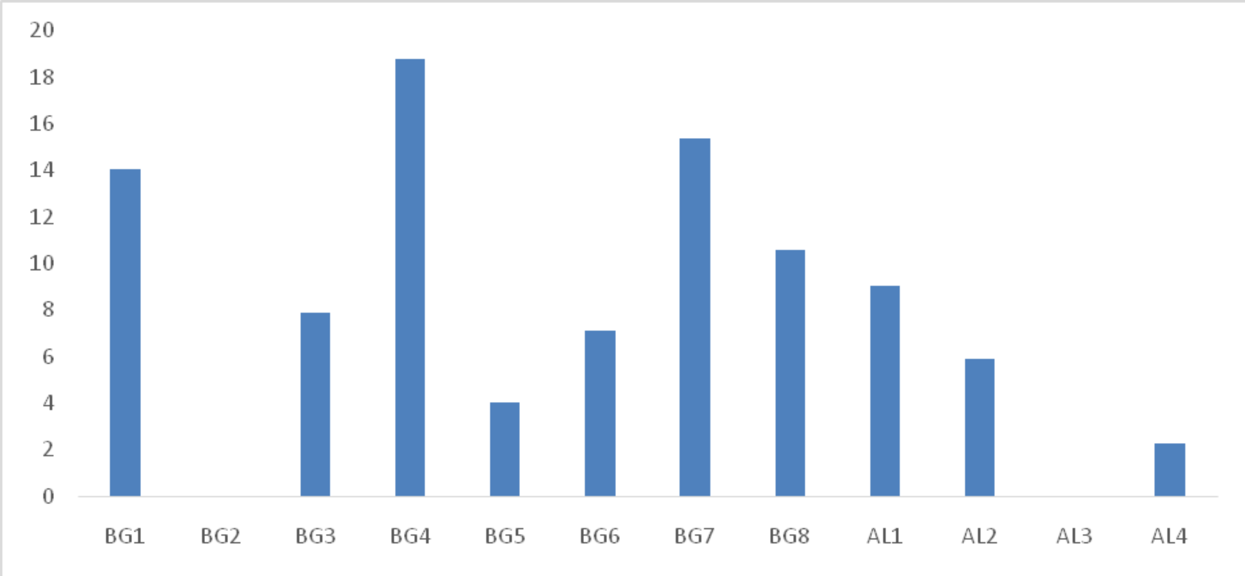
Table.2 Degradation potential of isolates

Isolate No	Colony Growth area (G) (mm^2)	Lysis Zone Area(C) (mm^2)	C/G
BG1	50.4	708.75	14.07
BG3	78.5	617.4	7.87
BG4	28.35	532.35	18.78
BG5	78.5	315.0	4.04
BG6	113.4	806.4	7.11
BG7	28.35	453.6	15.36
BG8	50.4	532.35	10.56
Al1	78.5	708.75	9.03
Al2	154.35	910.35	5.9
AL4	113.4	255.15	2.25

Table.3 Physical and Biochemical characterization of Isolates

Tests	BG4	BG2	BG1	BG8	AL1
Cell morphology					
Colony colour	Greenish blue	Creamy white	Cream	Yellow to orange	Light brown
configuration	Metallic sheen	Waxy	Mucoid	Mucoid	smooth
Elevation and margin	Large opaque, irregular	Convex Smooth round and opaque	Convex circular	Smooth, Raised and circular	Raised, circular
Grams reaction	Negative, slightlycurved rods	Positive Moderate rods arranged in chains	Negative, rod shaped, single or pairs	Negative, rods with parallel sides and rounded ends	Positive Cocci, pairs and chains
Spore	NO	Yes, oval and subterminal	No	No	NO
Motility	Motile	Motile	Motile	Non motile	Non motile
Growth at 15^0	_	+	_	_	+
Growth at 37^0	+	+	+	+	+
Growth at 45^0	_	+	_	_	+
Growth at pH 8	+	+	+	+	+
Growth at pH 9	+	+	+	_	+
Growth at pH 10	-	+	_	_	+
Growth on NaCl at 6%	NA	+	_	_	+
Oxidase test	+	+	_	+	_
Coagulase	+	+	_	_	_
Catalase test	+	+	+	+	+
Nitrate reduction test	_	+	+	_	_
Indole test	_	_	Varies	_	_
MR	_	_	+	_	_
VP	_	_	Varies	_	_
Citrate utilization test	_	+	Varies	_	_
H_2S production	_	_	+	_	_
Urea hydrolysis	_	_	+	_	_
KCN test	_	+	+	_	_
Gelatin liquification test	_	+	+	_	_

Fig.1 Degradation potential of Isolates



Pseudomonas aeruginosa strain RM1 and strain SK1 could digest zinc, iron and nickel (Salam LB 2016). Those three metals were found in large quantities in lubricant oil. In the present study the results are in accordance with the above stated literature.

This research was established 10 bacterial isolates that have ability to degrade Industrial waste lubrication oil. Highest activity is shown by isolate *Pseudomonas* followed by *Bacillus, Proteus, Flavobacterium* and *Enterococcus*. For the future, our study will be focussed on several data like the species of isolates, the optimal activity of isolates to degrade several Industrial lubricant oils.

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