Isolation and Identification of Petroleum Degrading Bacteria

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Abstract— Petroleum oils are being used drastically in advanced world and at the same time cause pollution by getting entry into soil and water through spillage and other ways. Natural auto bioremediation by native bacterial population is the most effective way to get rid from such pollution. From petroleum contaminated soil some bacteria were isolated with petroleum (diesel, petrol, kerosene) as sole carbon source to detect petroleum utilizing bacteria. After biochemical identification of these bacteria Bushnell Haas agar medium was incorporated with 1%, 3%, 5% and 7% petroleum oil was used to determine the tolerance of them. In the current study we isolated fourteen bacterial isolates from petroleum oil contaminated soil and they were found to be *Bacillus* spp., *Pseudomonas* spp., *Serratia* spp., *Acinetobacter* spp., *Enterobacter* spp., *Morganella* spp., *Burkholderia* spp., *Corynebacteria* spp., *Citrobacter* spp. and *Proteus* spp. after biochemical identification. *Burkholderia* spp., *Corynebacteria* spp. and *Enterobacter* spp. were most tolerant to all three types of oil between 1% to 7% concentrations. On an average all of these isolates were capable of using petroleum oil diesel, petrol and kerosene with different effectiveness. Biodegradation result was best for diesel with the opposite scenario for petrol. Only *Bacillus* spp. was found to degrade petrol.

Index Terms— Bioremediation, Carbon source, Degradation, Petroleum, Tolerance,Pollution, Contamination.

1 Introduction

Every year huge amount of petroleum products are produced which is readily accessible to come in contact with the soil and water environment by various ways (Erdogan, 2011). An average estimate of crude oils entering the environment has been found out about 6 million tons damaging both human life, wild life and environment itself (Kafilzadeh, 2011). There are several sources from where petroleum products can get entry into the environment. Some such sources include domestic wastes, underground storage tank seepage, vehicles on roads (low amount of petroleum), storage tank/tanker/pipeline spillage (high amount of petroleum product), slow seepage from natural soil reservoirs etc. (Hussein, 2012; Sarma et al., 2010; Pramanik et al., 2013). Petroleum and hydrocarbon components in the environment are cleaned up naturally by native microbial population followed by metabolic pathways resulting in the biodegradation of the complex molecules (Al-Hawash et al., 2018). Bacter ıd microalgae mainly do the job of biodegradation (Sanscartier et al., 2010). Rather than working a single species of bacteria or fungi in degradation of the complex molecules, combined activity of several bacterial of fungal population shows the best result due to availability of broader range o activity in soil, water or marine environment (Spini et al., 2018; Sarma et al., 2010; Al-Wasify, 2014; Ojo-Omoniyi, 2018; Das, 2011). These microorganisms utilize the carbon in hydrocarbons as their energy sources and are naturally distributed in the environment (Adeline et al., 2009). Degree of biodegradation depends on some factors like type of microorganisms, composition of hydrocarbon, envi conditions, bioavailability of the hydrocarbon etc. (Sanscartier et al., 2010).
Active degrading out to investigate their capability of degradation (Tazaki, 2018; Das *et al*. 2011; Ekanem, 2017). Biodegradation of petroleum products finally end up with the production of CO_2, H_2O and biomass with the complex and combined metabolic/enzymatic reaction of various microorganisms (Islam et al., 2013). This is a step by step process proceeding from minor organic molecule change with intact core structure, fragmentation while it is still readily identifiable as a petroleum product and finally complete mineralization into inorganic substances or end products (Islam et al., 2013). There are many harmful effects of petroleum in the environment. In a study carried out in the Niger Delta Region of Nigeria revealed that adverse effects on soil (degraded agricultural lands) and water resources (surface water pollution with difficulty in oxygen transportation in deep water), aquatic lives (reduced availability of fish), crops (destruction of crops), livestock (death of animals) and plants. As a result farm productivity and animal farm income has been reduced a lot (Ugwu, 2009; Eneh, 2011a, b; Tabieh and Al-Horani, 2010; Al-Turki, 2010). Human health can also be hampered. Development of kidney and liver diseases, cancer, bone marrow damage etc. can result from exposure to high oil concentrations (Islam et al., 2013).
In currents study was conducted to isolate and identify the bacteria from petroleum contaminated soil from Dhaka city, Bangladesh with the determination of the biodegrading capabilities of those isolates.

2 Materials and Methods

2.1 Study Area And Sample Collection

Soil samples in 5 cm depth of surface soil were collected in pre sterilized bottle from different petroleum oil contaminated areas of Dhaka city. Collected samples were taken back to the microbiology laboratory as soon as possible and stored at -4°C

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until further processing. Three petroleum oil samples were selected for this study including diesel, petrol and kerosene which were collected from local petroleum oil station.

2.2 Isolation of Bacterial Cultures

Ten gm of soil sample was homogenized with 90 ml distilled water and serially diluted up to 10^{-5} dilution. 0.1 ml suspension from each dilution was transferred into nutrient agar plate following spread plate technique and all the plates were incubated at 37°C for 24 hours. Distinct isolated colonies were selected for further study to determine their capability to degrade hydrocarbon compounds.

2.3 Isolation of Petroleum Degrading Bacteria

For the isolation of petroleum degrading bacteria Bushnell Haas (BH) agar medium was used. Because this medium contains all nutrients except the carbon source, petroleum oil must be supplemented in the media to serve as the only source of carbon and energy. Each pure culture isolated from soil earlier were streaked on BH agar plate. In addition, 100 µl of petroleum (diesel, kerosine, petrol) was spread onto the surface of agar plate separately as sole source of carbon. After that the plates were incubated at 37°c for 2-3 weeks. By this period of time only petroleum degrading bacteria will remain on the surface of the plates (Atlas and Richard, 1946).

2.4 Isolation of Bacterial Isolates

Bacteria which grew ion Bushnell Haas medium with different petroleum oils as sole carbon and energy sources were selected for microscopic and biochemical identification. Triple sugar iron agar test (TSI), catalase, oxidase, indole production test, nitrate reduction test, methyl red test (MR), voges proskauer test (VP) and citrate utilization test were performed and based on the test results the bacterial isolates were identified.

2.5 Tolerance at Different Petroleum Concentrations

Tolerance towards different petroleum (diesel, petrol, kerosine) concentrations were determined by the ability of organisms to grow on BH agar plate with different concentrations of petroleum (1%, 3%, 5% and 7% concentrations). Each bacterial culture was spread onto the plates with different concentrations of petrol, diesel and kerosine separately and incubated at 37°C for 7 days. On the basis of the growth of bacteria, tolerance to different petroleum concentrations was found out.

2.5 Screening for Petroleum Degradation

For this study Bushnell Haas (BH) broth medium was used. At first biochemically identified bacteria were enriched into peptone broth. 1 ml of enriched culture was added into the test tube containing 9 ml BH broth and mixed thoroughly. 50 µl of each petroleum (diesel, petrol and kerosine) was added as sole source of carbon and energy in all of the test tubes. All the test tubes were incubated at 37°C for 2-3 weeks. Gas bubbles will be produced on the petroleum-water interface in case of petroleum degradation.

3 RESULTS AND DISCUSION

At first some soil microorganisms were isolated and subjected to BH agar medium containing petrol, diesel and kerosine separately as sole source of carbon and observed for positive result for growth of the soil bacteria in the media during the 2-3 weeks of incubation time. About fourteen separate bacterial isolates from soil sample were used in this procedure.

TABLE 1
DETECTION OF BACTERI CAPABLE OF USING PETROLEUM AS SOLE CARBON SOURCE

ISOLATES FROM CONTAMINATED SOIL	GROWTH WITH PETROL	GROWTH WITH DIESEL	GROWTH WITH KEROSINE
01	+	++	+
02	+	++	++
03	+	++	++
04	+	++	+
05	+	++	++
06	-	++	+
07	+	++	-
08	-	++	-
09	+	+	+
10	+	+	+
11	-	+	-
12	+	++	++
13	+	++	++
14	-	++	++

TABLE 2
BIOCHEMICAL IDENTIFICATION OF THE ISOLATES AS SOLE CARBON SOURCE

ISOLATES	TSI				CITRATE	INDOLE	NITRATE	MR	VP	OXIDASE	CATALASE	BACTERIAL IDENTIFICATION
	SLANT	BUTT	GAS	H_2S								
01	R	Y	+	-	+	-	+	+	-	+	+	*Burkholderia* spp.
02	R	Y	+	+	+	-	+	+	-	-	+	*Corynebacteria* spp.
03	Y	Y	+	-	+	-	+	-	+	-	+	*Enterobacter* spp.
04	R	Y	-	-	+	+	-	+	-	-	+	*Morganella* spp.
05	R	Y	-	-	+	-	+	-	+	+	+	*Bacillus* spp.
06	R	Y	+	-	+	-	+	-	+	-	+	*Serratia* spp.
07	R	Y	-	-	+	-	-	-	-	-	+	*Acinetobacter* spp.
08	R	R	-	-	+	-	-	-	-	+	+	*Pseudomonas* spp.
09	R	Y	-	-	+	-	+	+	-	-	+	*Proteus* spp.
10	Y	Y	+	+	+	-	+	+	-	-	+	*Citrobacter* spp.
11	R	R	-	-	+	-	-	-	-	+	+	*Pseudomonas* spp.
12	Y	Y	+	-	+	-	+	-	+	-	+	*Enterobacter* spp.
13	R	Y	-	-	+	-	+	-	+	+	+	*Bacillus* spp.
14	R	Y	+	-	+	-	+	-	+	-	+	*Serratia* spp.

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Almost all of the isolates grew well with petrol, diesel and kerosene. Isolates 6, 8, 11 and 14 were unable to grow with petrol. Other isolates showed little growth. With diesel all bacterial isolates found to grow very well. In case of kerosene, isolates showed adequate growth except isolates 7, 8 and 11. So it can be said that the isolates which grew properly in presence of petroleum oils can utilize the oils as their source of carbon.

Then the bacteria which were found to be capable for growing with the petroleum as carbon source were identified by conventional biochemical methods. From the fourteen isolates we found ten different bacterial isolates.

The tolerance of each of the bacteria was determined with different concentrations (1%, 3%, 5% and 7%) of petrol, diesel and kerosine. Capability to grow up to 7% concentration of petroleum was observed in this study. The microorganisms which can tolerate the petroleum at various concentrations which present as only carbon source are able to utilize them which is eventually degrading the complex compound unlike other bacteria.

Biodegradation was detected by the production of gas bubble between BH broth and petroleum interface. For petrol, only *Bacillus* spp. showed positive result for degradation. All of the bacterial isolates were capable to degrade diesel except *Bacillus* spp. *Serratia* spp. and *Proteus* spp. showed no gas bubble formation for kerosene. Other eight bacteria were identified as potent degraders with the production of gas bubble. Biodegradation is the only way of getting away from the petroleum oil pollution both in soil and marine environment as it is natural way without investment. As a single species the bacteria might not be able to degrade properly but in combination the degradation result will be a lot better. The gene involved in the degradation of petroleum can be identified and the expression of the gene can be increased to get rapid result. The identified bacteria capable of degrading petroleum oil can also be augmented in the polluted areas to increase their number in the natural environment which simultaneously increase the rate of degradation.

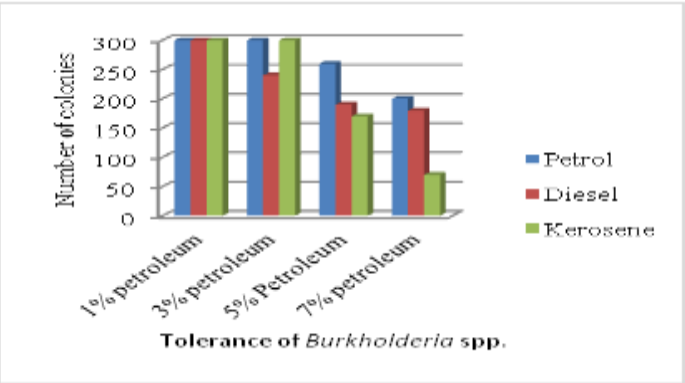

Figure 01: Tolerance of *Burkholderia* spp. with 1%, 3%, 5% and 7% petroleum (petrol, diesel and kerosine).

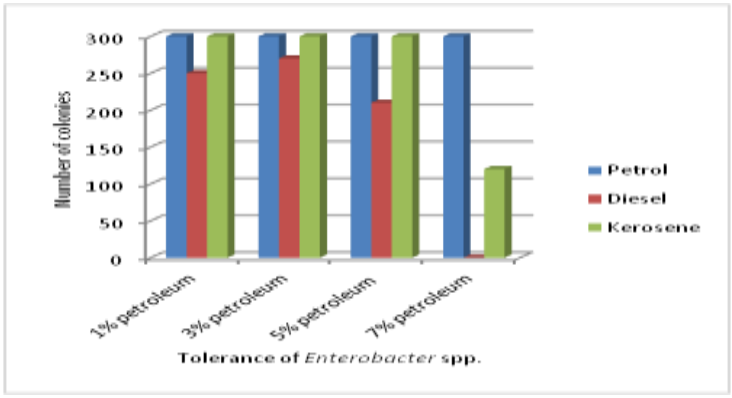

Figure 02: Tolerance of *Enterobacter* spp. with 1%, 3%, 5% and 7% petroleum (petrol, diesel and kerosine).

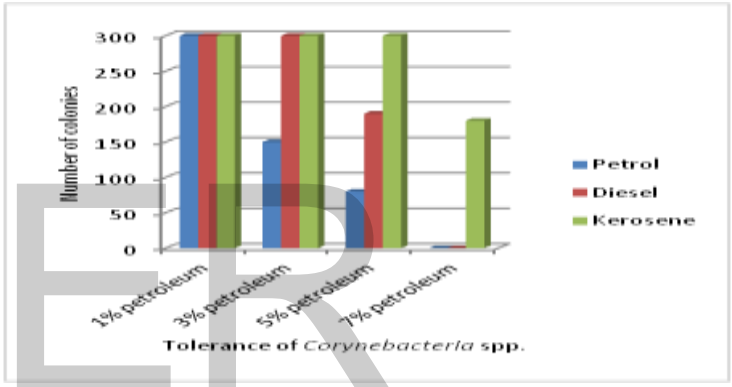

Figure 03 Tolerance of *Corynebacteria* spp. with 1%, 3%, 5% and 7% petroleum (petrol, diesel and kerosine).

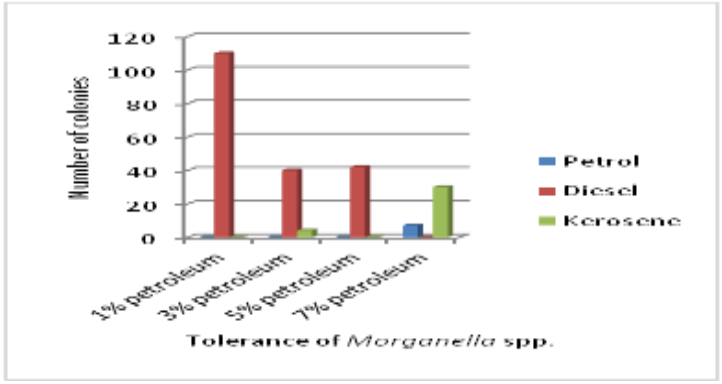

Figure 04 :Tolerance of *Morganella* spp. with 1%, 3%, 5% and 7% petroleum (petrol, diesel and kerosine).

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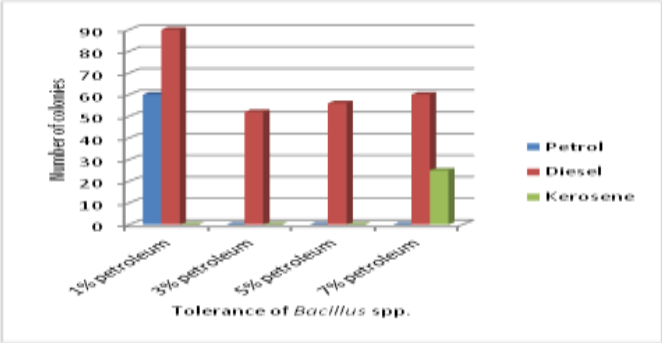

Figure 05:Tolerance of *Bacillus* spp. with 1%, 3%, 5% and 7% petroleum (petrol, diesel and kerosine).

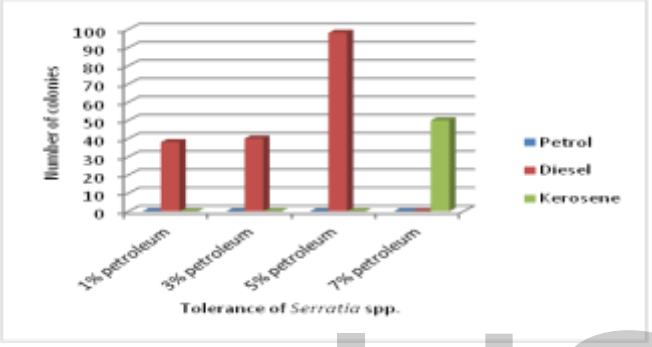

Figure 06:Tolerance of *Serratia* spp. with 1%, 3%, 5% and 7% petroleum (petrol, diesel and kerosine).

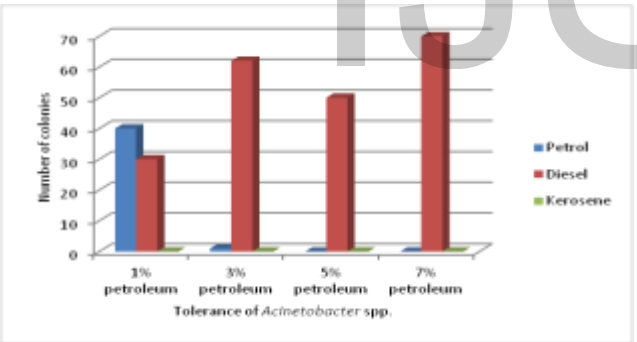

Figure 07:Tolerance of *Acinetobacter* spp. with 1%, 3%, 5% and 7% petroleum (petrol, diesel and kerosine).

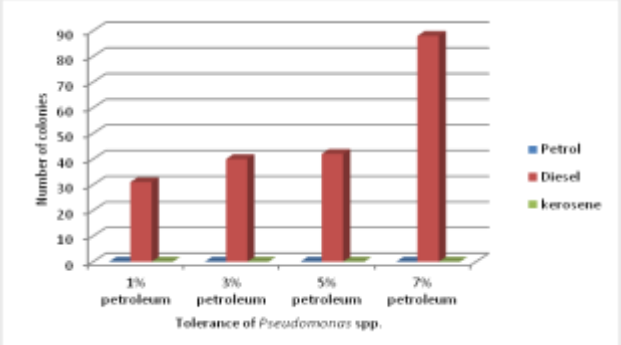

Figure 08:Tolerance of *Pseudomonas* spp. with 1%, 3%, 5% and 7% petroleum (petrol, diesel and kerosine).

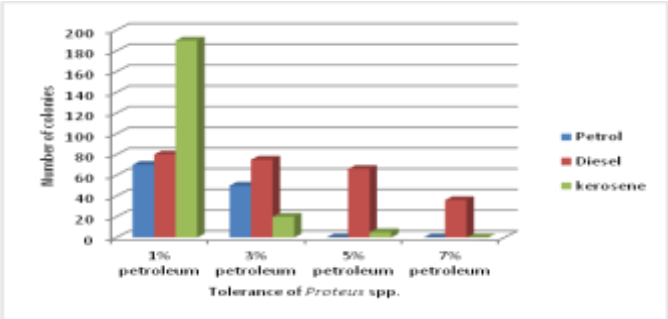

Figure 09:Tolerance of *Proteus* spp. with 1%, 3%, 5% and 7% petroleum (petrol, diesel and kerosine).

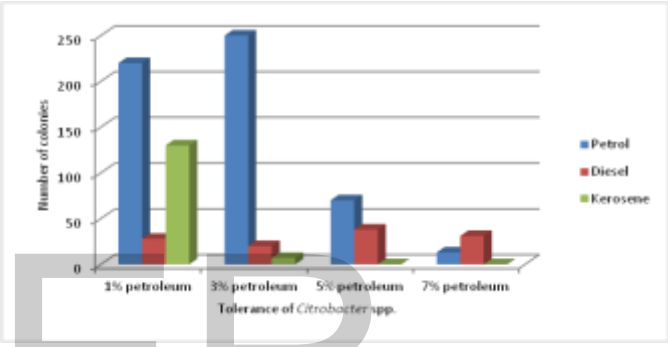

Figure 10:Tolerance of *Citrobacter* spp. with 1%, 3%, 5% and 7% petroleum (petrol, diesel and kerosine).

TABLE 3
BIODEGRADATION OF PETROLEUM

ISOLATES	PETROL BIODEG-RADATION	DIESEL BIODEGRADA-TION	KEROSINE BIODEG-RADA-TION
Burkholderia spp.	-	+	+
Corynebacteria spp.	-	+	+
Enterobacter spp.	-	+	+
Morganella spp.	-	+	+
Bacillus spp.	+	-	+
Serratia spp.	-	+	-
Acinetobacter spp.	-	+	+
Pseudomonas spp.	-	+	+
Proteus spp.	-	+	-
Citrobacter spp.	-	+	+

4 CONCLUSION

Bacillus spp., *Serratia* spp., *Acinetobacter* spp., *Pseudomonas* spp. showed the least capability to tolerate the petroleum at higher concentrations and *Burkholderia* spp., *Corynebacterium* spp., *Enterobacter* spp. showed the greater ability to withstand

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high petroleum concentration and ultimately degrade them. These potent bacterial isolates can be used in clearing the contamination from petroleum oil spillage in soil and water bodies.

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