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Biodegradation of petroleum hydrocarbon by *Micrococcus* sp. in form of wax ball

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Hydrocarbon containing compounds like diesel, crude oil and petrol cause environmental pollution. Hydrocarbon contaminated sites contain large amount of microbial community adapted for utilizing these compound as a source of carbon and other metabolic activities. A study was conducted in order to isolate the micro-organism from hydrocarbon contaminated sites for petroleum degradation. The soil sample was collected from the bus terminal of Punjab University, Lahore. The isolate (*Micrococcus sp.*) was characterized microbiologically by using microbiological techniques such as staining and biochemical testing based on Bergey's manual. Isolated organism has an ability to degrade the petrol as well as paraffin wax as a sole carbon source. This ability shows clear evidence that genome of this isolate harbors gene for degradation. By combined both properties, we can reduce petroleum product from hydrocarbon impacted environment.

Key words: Petrol degradation, paraffin wax, biochemical tests, wax ball.

INTRODUCTION

In the modern world, hydrocarbon products are widely used as a source of fuel (Panda et al., 2013). One of the major environmental problems is contamination through hydrocarbon compounds resulting from the activities of the petrochemical industry. Oil spills are of increasing environmental alarm that has a direct impact on biotic, abiotic factors and wildlife. They affect a wide range of organisms that are connected in a food chain that includes human food resources (Pennings et al., 2014; Silliman et al., 2012).

The accidental release of such petroleum products is of particular concern on the abiotic and biotic factors of that environment, if not treated properly in time. Currently accepted disposal ways of incineration and landfills can become exorbitantly expensive when amounts of hydrocarbon waste are large. Chemical and mechanical methods generally used have limited efficiency and can be expensive.

Biodegradation is the auspicious technology for the treatment of these contaminated sites and it is cost-effective and will lead to complete mineralization. Microbes are the most important organisms which are cosmopolitan in nature so, exploitation of these microbes for human benefit is easy. Remediation of contaminated soil is critical to diminish the effect of accidental hydrocarbon release and biodegradation is a preferential

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and more economical method to remediate the polluted soil (Khan and Rizvi, 2011).

In this study, we aimed to use the various properties of isolated bacteria combined to diminish the amount of petroleum hydrocarbon from contaminated soil. The isolated strain has the ability to interact with hydrocarbon thus, removing them from soil. Both the properties (degradation of wax and petrol) (Abostate et al., 2011) of hydrocarbon degrading bacteria will help to reduce the amount of petroleum product from polluted soil by enhancing the bacterial amount in form of wax ball.

MATERIALS AND METHODS

Sterilization

Glassware and media were sterilized in an autoclave at 121°C for 20 min at 15 lbs pressure.

Soil sample collection

Soil sample was collected from a contaminated site at the bus terminal of Punjab University, Lahore. Soil was collected randomly 5 to 10 cm beneath the surface using the sterile spatula and packed into the sterile polybags and stored at 4°C until the end of work.

Culture media

For purification and enrichment of isolate, nutrient agar was used. Biodegradation experiment was done in mineral salt media (MSM) (pH 7.2) (Al Disi et al., 2016). Media were sterilized at 121°C for 20 min.

Isolation of bacterial strain from soil sample

Bacterial species was isolated from the collected soil sample by serial dilution and agar plating method. The collected sample was diluted from 10^{-1} to 10^{-5} dilutions, and the diluted sample was spread on sterile nutrient agar (NA) plates composed of peptone (5 g/L), meat extract (1 g/L), sodium chloride (8 g/L), yeast extract (2 g/L), and distilled water (1 L). The inoculated plates were incubated for 24 h at 37°C (Khan and Rizvi, 2011). The isolate obtained (*Micrococcus sp.*) was purified by quadrant streaking on sterile NA plates (Figure 1).

Staining and biochemical testing of purified culture

In order to identify the purified cultures on the basis of Bergey's manual (Aneja, 2003), various staining and biochemical test were performed; mainly gram staining, spore staining, oxidase test, mannitol fermentation and glucose fermentation (Table 1).

Enumeration of hydrocarbon (wax and petrol) degrading bacteria

The degrading activity of isolate was obtained by using mineral salt media (MSM) (Khan and Rizvi, 2011) in which 1% hydrocarbon (petrol and paraffin wax liquid) was added. Two plates of MSM agar were prepared and labeled as 1 and 2; 1% petrol and paraffin wax was added as a sole carbon sources, respectively. Media was inoculated with 24 h of respective pure culture. Then agar plates were incubated at 37ºC for 15 days. After 15 days, there was noticeable growth on both plates which indicated that isolated bacteria are able to degrade the petrol as well as paraffin wax (Figure 2) (Zaky et al., 2011).

Figure 1. Pure culture of *Micrococcus* sp.

Table 1. Staining and biochemical activity of Micrococcus strain.

S/N	Staining and biochemical test	Result
1	Pigmentation	Yellow
2	Simple staining	Cocci
3	Gram staining	Positive
4	Spore staining	Negative
6	Mannitol fermentation	Negative
7	Glucose fermentation	Negative
8	Catalase	Positive

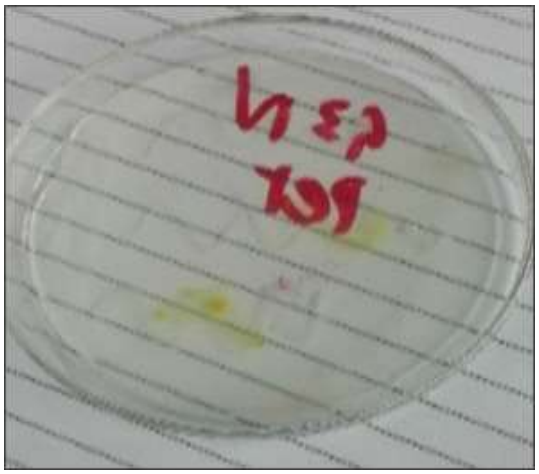

Figure 2. Degradation of petrol using *Micrococcus* sp.

Table 2. Petrol degradation (O.D at 600 nm).

S/N	Samples	O.D at 0 day (nm)	O.D at 5 day (nm)	O.D 10 day (nm)
1	Wax	0.004	0.004	0.004
2	Wax ball	0.01	0.049	0.09
3	Organism	0.05	0.106	0.5
4	Petrol	0.01	0.01	0.01

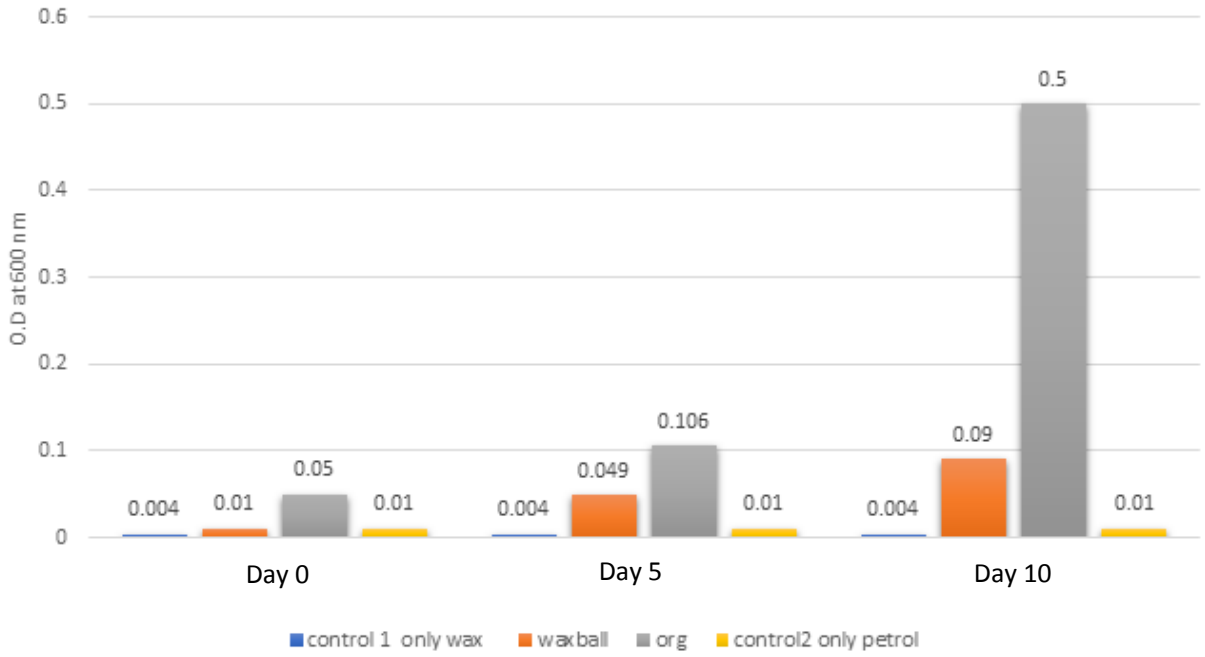

Figure 3. Effect of *Micrococcus* sp. on biodegradation of petrol at different time intervals (optical density at 600 nm).

Degradation of petrol with wax ball in vitro method

Four culture tubes were prepared in which 25 ml MSM media was added and labeled as 1, 2, 3 and 4. 20 ul of petrol was added in all four tubes. In the first tube, paraffin wax was added in tube two wax ball (containing isolated strain and 1 cm paraffin wax) while in tube three bacterial strain was added and tube four acted as the negative control. Tube one and four acted as control. The tubes were incubated at 37ºC for 10 days. Growth of organism was noticed by taking optical density (OD). The optical density (OD 600 nm) was read by using the spectrophotometer (Table 2, Figure 3).

RESULTS AND DISCUSSION

The isolated bacterial strain was biochemically characterized and a Gram positive coccus with yellow pigments in colonies was selected as the micrococcus (Table 1 and Figure 1). Petroleum degradation was checked using wax balls and without wax balls. It was found that if the organism was separately used for degradation, it had the same effect as that of when wax ball was used but as the time increased, the OD decreased for the wax ball contacted microbes compared to only microbes (Table 2, Figures 2 and 3).

Soil sample was collected from contaminated site from Punjab University, Lahore. Isolated sample was able to degrade petrol as well as petroleum product such as paraffin wax (Haritash and Kaushik, 2016) so, by making a wax ball having both *Micrococcus sp.* and wax, petroleum hydrocarbon can be reduced from contaminated soil by adding these wax balls and augmenting the amount of petroleum degrading bacteria. So, for this purpose, MSM is used in laboratory to check the ability of isolated strain to degrade wax and petrol. MSM is supplemented with 1% petrol and wax. After incubation at 15 days, growth on plates was observed. Following this technique, *in vitro* method is used to check the ability of wax ball to degrade the petroleum product (Marchand et al., 2017) by using the MS media. So, it has been proposed that *Micrococcus sp.* is able to degrade the petroleum product in the form of wax ball. Thus, by the amount of oil, petrol and diesel in contaminated soil by the use of the wax ball can be diminished.

Conclusion

Biodegradation of hydrocarbon compound holds a promising prospect to remove or alleviate the environmental impact of petroleum. Future research on the subject of biodegradation application at petroleum fields are required to fully utilize the opportunity in hand. Based on the above study, it is established, that *Micrococcus sp.* in form of wax ball can be an advantageous source for remediation of contaminated soil. Further research in this zone can make an obvious improvement.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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