Nature Environment and Pollution Technology
An International Quarterly Scientific Journal
p-ISSN: 0972-6268
(Print copies up to 2016)
e-ISSN: 2395-3454
Vol. 20 No. 1 pp. 275-280 2021

Original Research Paper
https://doi.org/10.46488/NEPT.2021.v20i01.030
Open Access Journal

Plastic and Petroleum Hydrocarbon Degrading Potentials of Single and Mixed Bacterial Cultures Isolated from Garbage Areas of Darrang, Assam

R. S. Ahmed and M. D. Swargiary†
Department of Biosciences, Assam Don Bosco University, Tapesia Gardens, Sonapur-782 402, Assam, India
†Corresponding author: M.D. Swargiary; maitrayee.zoo@gmail.com

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 01-03-2020
Revised: 13-03-2020
Accepted: 27-05-2020

Key Words:
Bacterial isolates
Biodegradation
Plastics
Petroleum hydrocarbons

ABSTRACT

The ability of bacterial isolates viz., *Enterococcus cloacae* and mixed bacterial isolates, to degrade plastic was studied. The bacteria were isolated from waste effluent sites viz., industrial waste sites of Nilon's pickle factory, Dalgaon and market waste sites of Balugaon vegetable market, Kharupetia, Assam. Plastic degradation was carried out at different time intervals within 15 and 30 days. It was observed that degradation increased with an increase in the time interval and hence effective observation was recovered after 30 days interval. Polythene bags showed maximum degradation by *Enterococcus cloacae* (85.25%). Plastic degradation was observed by Scanning Electron Microscope (SEM). In biodegradation of petroleum hydrocarbon, *Enterococcus cloacae, Pseudomonas putidia and Ralstonia pickettii* were found to degrade oil as well as they were able to grow in presence of petroleum hydrocarbons.

INTRODUCTION

Nowadays plastic pollution has become a threat to global ecology and pollution arises from both terrestrial and marine sources. Plastic pollution occurs mainly for two reasons, firstly due to the illegal or inappropriate dumping of domestic and industrial refuse and secondly due to the poorly contained static and transported waste (Webb et al. 2013). The main disadvantage of plastics is that they are resistant to biodegradation. Plastics and thermocols cause environmental pollution and have ecological damaging effects by getting accumulated in the environment as they are stable (Sharma et al. 2014). Biodegradation is a change in a material caused by the biological activity of microorganisms like bacteria, yeast, fungi and actinomycetes (Bhardwaj 2012, Sharma 2014). It has been documented that microbial biodegradation increases the rate of degradation of plastics without causing any harm to the environment (Yoon 2012).

Petroleum hydrocarbon contamination occurs due to accidents or leaks and accidental spills during the exploration, production, refining, transport, and storage of petroleum and petroleum products which drastically disturbs the marine life (Atlas 1981). Petroleum hydrocarbons generally spread to vast area which may span across hundreds of squares of miles (Mandri & Lin 2007). The degradation of petroleum hydrocarbons by microorganisms is a complex process which depends on nature and the amount of the hydrocarbons present (Swift et al. 1997). One of the important factors which limit biodegradation of oil pollutants in the environment is their limited availability to microorganisms. Petroleum hydrocarbon compounds generally bind to the soil components which are difficult to be removed or degraded. Hydrocarbons are susceptible to microbial attack and individual microorganisms are capable of degrading only a limited number of crude oil and depend on the presence of metabolically diverse microbial communities. The bioremediation technologies applied to hydrocarbon-polluted environments highly depend on the biodegrading capabilities of native microbial populations or exogenous microorganisms used as inoculants. Oil biodegradation of subsurface does not require oxygen; it does require certain essential nutrients like nitrogen, phosphorus, potassium, etc. Microbes present in the soil and their catabolic activity play a vital role in degrading soil hydrocarbon contaminants like petroleum, crude oils, etc. Since the different microorganisms such as bacteria, fungi, protozoa, algae and actinomycetes present in the soil have varying capacity to degrade the petroleum hydrocarbons, the present study is aimed to study the degradation of plastics and petroleum hydrocarbon by Scanning Electron Microscope.

MATERIALS AND METHODS

Sample Collection

Plastic samples (polythene bags) were collected from the industrial garbage (waste disposable site dumped with a

polythene bag and plastic cup) from Nilon's pickle industrial area of Dalgaon, Darrang (Assam).

Surface Sterilization of Samples

The collected polythene bags were cut into 1×1 cm pieces and cleaned with tap water and surface sterilized with ethanol. It was then washed with distilled water, 0.1% mercuric chloride and again washed with distilled water (Begum et al. 2015).

Plastic Biodegradation Procedure

For biodegradation of plastic (polythene bags) minimal media (Alexander 1981, Atlas 1981, Ahmed et al. 2014, Akpe et al. 2015, Aneta et al. 2018), having a composition of 0.3g of NH_4NO_3, 0.5g of K_2HPO_4, 0.1g of NaCl, 0.02g of $MgSO_4 . 7H_2O$ and 2g of agar was prepared. Then from the plates of pure culture bacterial colonies were picked up with the help of inoculating loop and streaked on minimal salt agar. Polythene bags of 1×1 cm were cut and placed on the minimal salt agar plates. The sterile polythene bags pieces were weighed and recorded before inoculating into the culture medium. The control was maintained with plastic (polythene bags) in the microbe-free medium. After 1 month incubation, the growth of microorganisms was observed on the polythene bags strips.

Determination of Dry Weight of Residual Polythene

To measure the biodegradation residual plastic, polyethene bags were initially weighed. From the culture plate containing bacteria, after 1 month of incubation, and plastics were collected with a sterile loop, washed thoroughly with distilled water, shade dried and weighed again for final weight. It was calculated as follows (Usha et al. 2011):

$$\text{Weight loss \%} = \frac{(\text{Initial weight} - \text{Final Weight})}{\text{Initial weight}} \times 100 \quad \ldots(1)$$

Screening of Polythene Biodegradation by Scanning Electron Microscope (SEM)

Three samples consisting of a mixed culture of bacterial strains that degrade plastic, the best plastic degrading strain, *Enterococcus cloacae* considered as the isolates of interest and control with only media was taken for further analysis through Scanning Electron Microscope (SEM). SEM analysis was carried out for obtaining more structural information about polyethene strips. The samples were smeared on a small piece of adhesive carbon tape which was fixed on a brass stub and general hair dryer was used to dry the polythene samples. The samples were then subjected to gold coating using sputtering unit (Model: Q150RES) with $1\times e^{-1}$ mB pressure and 2nm of resolution. The gold coated samples were placed in SEM (Gemini) chamber and scattered electron images were recorded at different magnifications.

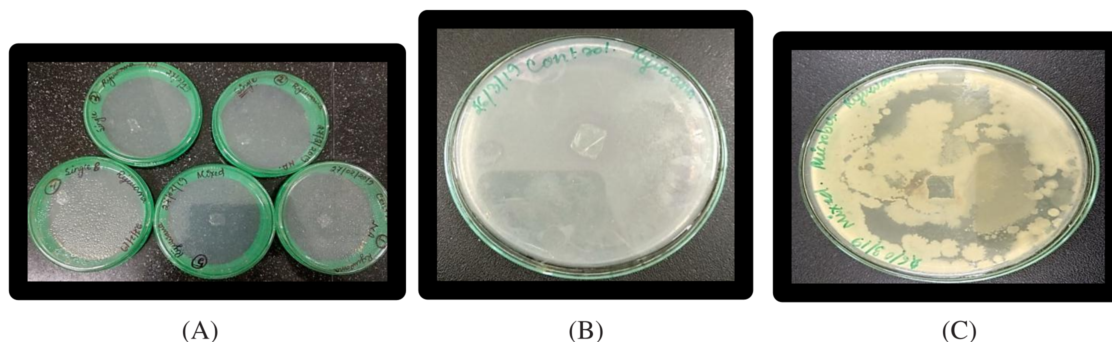
Biodegradation of Petroleum Hydrocarbons

Petroleum was collected from a petrol pump in Khanapara, (Meghalaya). 28g of nutrient agar (Hi media) was dissolved in 1000mL of distilled water and sterilized in an autoclave for 20 minutes at 121 psi. 100µL of overnight bacterial culture in nutrient broth (Hi media) was measured and poured in a sterile Petri plate, 100µL of petroleum hydrocarbon was then poured over the bacterial culture and in the same plate autoclaved and cooled nutrient agar was poured over both culture and oil. Immediately the plates were kept for incubation at 37°C for 3-4 days and bacterial growth was observed on the oil containing Petri plates.

RESULTS AND DISCUSSION

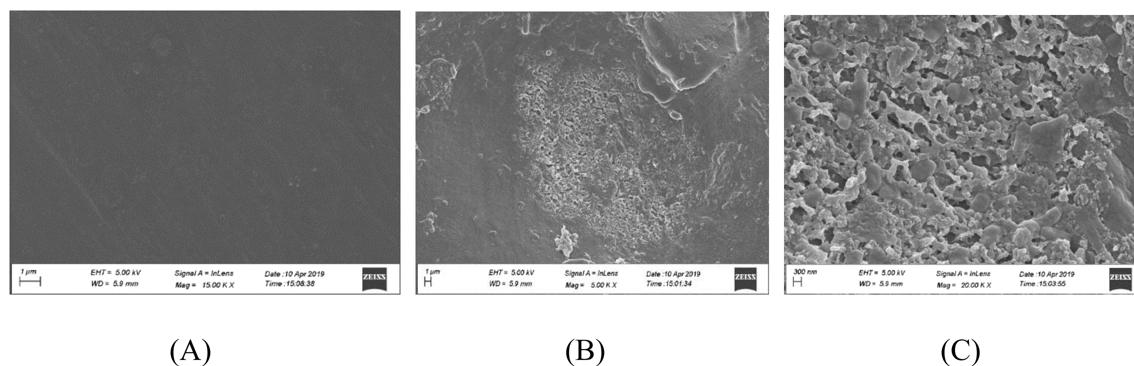
Biodegradation of polythene sheets was observed by using *Enterococcus cloacae* and a mixed population of bacterial isolates which were isolated from both the soil samples. After 30 days of incubation at 37°C, microbial growth (Fig. 1) was observed in polythene containing areas. Scanning Electron Microscope (SEM) analysis (Fig. 2 and Fig. 3) showed more degradation of polythene sheets, presence of roughness, degradation portions in *Enterococcus cloacae* cultured sheets when compared to the sheets which were cultured with mixed bacterial culture. eZAF Smart quadrant SEM results (Table 1 to Table 5) shows the amount of elements present on K shell and M shell as well as their weight percentage and atomic percentage. It was observed from the tables that the amount of elements in the control polythene sheet (Table 1) was less compared to the polythene sheets inoculated with *Enterococcus cloacae* (Table 2 and Table 3). The weight percentage and atomic percentage of elements present on the polythene sheet inoculated with mixed bacterial isolates (Table 4 and Table 5) were also found less as compared to the elements present on the polythene sheet taken as control (Table 1) and the polythene sheet inoculated with *Enterococcus cloacae* (Table 2 and Table 3).

In case of weight loss graph (Fig. 4) degradation was observed within 15 days in *Enterococcus cloacae* containing polythene sheet (59.02%) and in mixed culture containing sheet (51.22%). Thirty days time interval degradation studies (Fig. 4) showed that the plastic sheets were degraded by *Enterococcus cloacae* (85.25%) and mixed culture (73.18%) and there was weight loss whereas, in control sample without bacterial culture did not show any change in their weight and no roughness observed in SEM micrographs. However, in this study plastics took longer degradation by bacteria which needs further study because polythene sheet degradation



(A) (B) (C)

Fig. 1: (A) Day 1; Biodegradation of polythene sheets; (B) & (C) Day 30; Biodegradation of polythene sheets.



(A) (B) (C)

Fig. 2: SEM images of the polythene sheets after 30 days of the biodegradation at 28°C in the sterilized media inoculated with *Enterococcus cloacae.* (A) Control, (B) & (C) inoculated with *Enterococcus cloacae.*

Table 1: eZAF smart quant results selected area 1 (control polythene sheets).

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	84.79	95.80	3931.82	4.60	0.6179	1.0471	0.6960	1.0000
O K	2.59	2.20	41.47	38.82	0.0044	0.9973	0.1694	1.0000
MgK	1.18	0.66	83.38	8.10	0.0084	0.9156	0.7741	1.0003
SiK	1.34	0.65	105.48	6.76	0.0112	0.8990	0.9277	1.0014
AuM	10.09	0.70	247.78	8.44	0.0767	0.5657	1.3449	0.9982

Table 2: eZAF smart quant results selected area 1 from polythene sheet 2 (incubated with *Enterococcus cloacae*).

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	83.43	93.69	3683.25	4.53	0.6103	1.0430	0.7014	1.0000
O K	5.31	4.48	82.21	20.37	0.0091	0.9932	0.1733	1.0000
MgK	1.09	0.61	72.25	8.81	0.0077	0.9114	0.7706	1.0004
SiK	1.28	0.62	95.04	6.87	0.0107	0.8948	0.9276	1.0015
AuM	8.89	0.61	206.40	8.96	0.0673	0.5629	1.3483	0.9981

Table 3: eZAF smart quant results selected area 1 from polythene sheet 2 (incubated with *Enterococcus cloacae*).

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	81.68	92.81	2780.06	4.68	0.5924	1.0476	0.6923	1.0000
O K	6.42	5.47	78.84	18.46	0.0113	0.9978	0.1761	1.0000
MgK	0.66	0.37	34.01	13.23	0.0046	0.9159	0.7632	1.0004
SiK	1.36	0.66	78.45	6.65	0.0113	0.8993	0.9256	1.0014
AuM	9.88	0.68	178.75	8.38	0.0750	0.5659	1.3439	0.9982

Table 4: eZAF smart quant results selected area 1 from polythene sheet 2 (incubated with mixed bacterial isolates).

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	67.22	81.66	2460.77	6.04	0.4070	1.0660	0.5680	1.0000
O K	16.00	14.59	297.85	13.71	0.0331	1.0159	0.2037	1.0000
NaK	1.73	1.10	74.56	10.27	0.0093	0.9186	0.5852	1.0001
MgK	1.01	0.60	63.56	10.05	0.0067	0.9332	0.7190	1.0003
SiK	1.16	0.60	85.23	7.38	0.0096	0.9166	0.8957	1.0013
AuM	11.34	0.84	265.14	7.82	0.0865	0.5768	1.3181	1.0027
ClK	0.74	0.30	34.16	15.08	0.0059	0.8517	0.9389	0.9976

Table 5: eZAF smart quant results selected area 2 from polythene sheet 2 (incubated with mixed bacterial isolates).

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	80.88	93.20	3437.52	4.76	0.5829	1.0540	0.6838	1.0000
O K	5.49	4.75	85.81	24.67	0.0098	1.0042	0.1773	1.0000
NaK	0.42	0.25	18.39	28.36	0.0024	0.9079	0.6178	1.0001
MgK	0.89	0.51	57.13	10.22	0.0062	0.9223	0.7584	1.0003
SiK	1.00	0.49	72.75	8.62	0.0084	0.9057	0.9197	1.0013
AuM	11.32	0.80	257.41	8.07	0.0861	0.5700	1.3368	0.9983

studies with plastic and thermocol by using microbes revealed that thermocol degrades faster as compared to plastics. This study conforms with studies of various workers (Begum et al. 2015, Dey et al. 2016), where polythene degradation by *Desulfotomaculum nigrificans* and *Pseudomonas alcaligenes* increased with incubation period and there was a dramatic increase in weight loss of polythene bags (Usha et al. 2011). Their studies also established that polythene bags and plastic cups incubated for 2, 4 and 6 months with the microbial culture of *Pseudomonas* sp., *Bacillus* sp., *Staphylococcus* sp., *Aspergillus nidulans, Aspergillus flavus*, *Streptomyces* sp. and *Pseudomonas* sp. degraded 37.09% of polythene and 28.42% of plastics in 6 months period. So, the results established in this study bear similarity as the elements which were present in the polythene sheets as depicted in Fig. 2 and Fig. 3 were also found to change after 30 days of incubation.

In presence of petroleum hydrocarbon and minimal required medium as depicted in Fig. 5, the growth of *Enterococcus cloacae* (isolated from soil sample 2), *Ralstonia pickettii* (isolated from soil sample 1) and *Pseudomonas putidia* (isolated from soil sample 1) was observed which indicates that the bacterial strains can consume oil and tolerate oil and grow in petroleum hydrocarbon containing media. The results in this study are thus in conformity with other workers (Horowitz et al. 1975, Latha & Kalaivani 2012, Marjadi & Dharaiya 2012, Vinothini et al. 2015), which established that *Bacillus subtilis* and *Pseudomonas aeruginosa* were able to degrade the oil. Earlier studies (Ahmed et al. 2014) showed that the biodegradation of crude oil is possible in contaminated water by local isolates of *Enterobacter cloacae* and observed that *E. cloacae* E1 degraded 70.00 ± 0.40% of the crude oil as well as (Dey

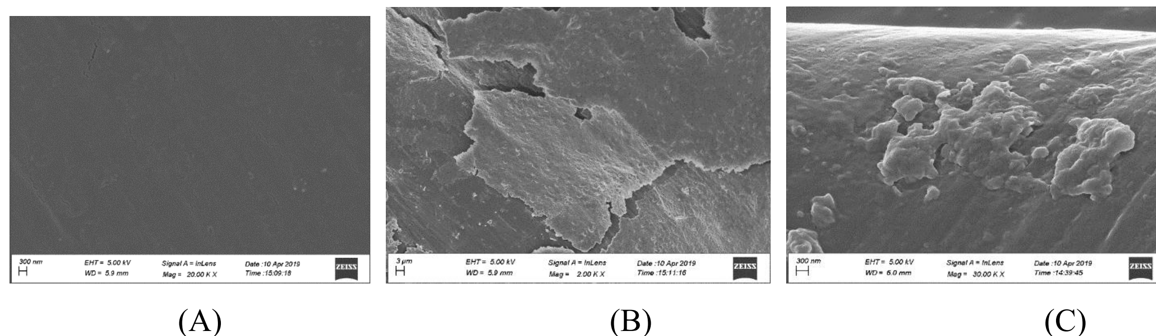


Fig. 3: SEM images of the polythene sheets after 30 days of the biodegradation at 28°C in the sterilized media inoculated with (B) & (C) mixed bacterial strains vs (A) control.

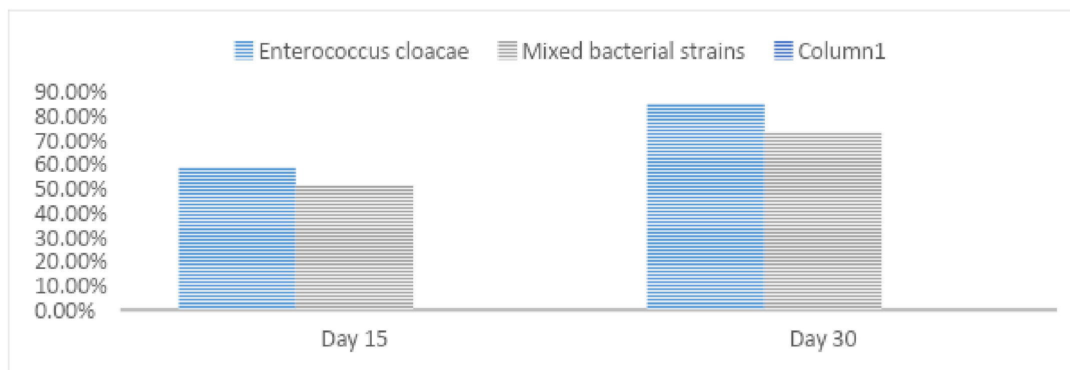


Fig. 4: Weight loss graph of bacterial degradation of polythene bag.

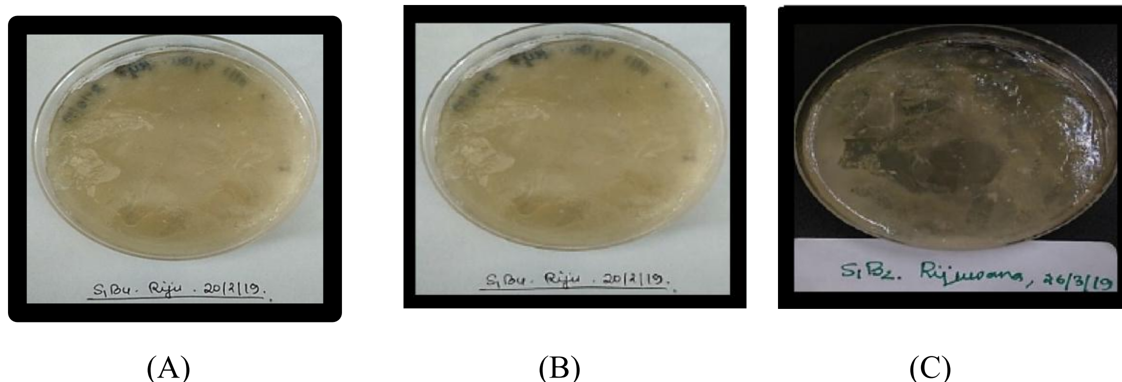


Fig. 5: Biodegradation of petroleum hydrocarbon**.** (A) The plate shows the growth of *Enterococcus cloacae,* (B) Plate shows the growth of bacterial strain *Ralstonia pickettii,* (C) Plate shows the growth of bacterial strain *Pseudomonas putidia* in presence of petroleum hydrocarbon and minimal required medium.

et al. 2016) biodegradation of petroleum and crude oil by *Pseudomonas putida* and *Bacillus cereus,* which supports this study for biodegradation of petroleum hydrocarbon.

CONCLUSION

Garbage areas are mostly involved in environmental pollution and due to the presence of wastes like industrial, biological and household may contain many hazardous substances which can cause dangerous health issues in human. It also contains some non-degradable waste like plastics, polythene bags, petroleum hydrocarbons, etc. which play a major role in soil infertility. In this study bacteria viz., *Enterococcus cloacae*, *Pseudomonas putidia* and *Ralstonia pickettii* were isolated from two garbage areas of Darrang, Assam during the period July 2018 to April 2019 and used for plastic and petroleum hydrocarbon degradation, and through Scanning Electron Microscope studies the polythene degradation was observed.

ACKNOWLEDGEMENT

Our sincere thanks to the Head, Department of Chemistry, Gauhati University for the Scanning Electron Microscope facility.

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