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Biodegradation Capacity of Bacillus Sp. VC2 for Polythene Degradation

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***Abstract*- Plastics are vital hydrocarbons occurring both in natural as well as in synthetic forms. The present study describes the isolation of bacteria from soil and sludge with the ability to degrade biodegradable plastics. Biodegradable plastic was buried in the glass bottle with three layers of different soil like Rhizospheric soil, Sludge and Garden soil for 4 months. The bacteria was then isolated and identified on the basis of biochemical studies as *Bacillus* sp. VC2. The bacteria *Bacillus* sp. VC2 breaks the polymers and used as sole carbon source for their metabolic activities was estimated by FTIR analysis.**

***Keywords* :- Hydrocarbons, Biodegradable plastic, FTIR analysis, Bushnell's broth.**

I. INTRODUCTION

Biodegradable plastics are fully degraded by microorganisms, without leaving visible toxic remainders. The term "biodegradable" refers to materials that can disintegrate or break down naturally into biogases and biomass (mostly carbon dioxide and water) as a result of being exposed to a microbial environment and humidity (Jain et al., 2010).

Many animals are dying because of waste plastics either by being caught in the waste plastic traps or by swallowing the waste plastic debris to exert ruinous effects on the ecosystem (Usha et al., 2011). Some of the plastic products cause human health problems because they mimic human hormone. Vinyl chloride is classified as carcinogenic to humans by the International Agency for the Research on Cancer (IARC) (Rudel Ruthann et al., 2007). It has also shown to be a mammary carcinogen in animals.

Microorganisms can also play a vital role in this process, as over 90 genera of bacteria, fungi and actinomycetes have the ability to degrade plastic (Mahdiyah et al., 2013).Generally, the biodegradation of plastic by microorganisms is a very slow process, and some microorganisms cannot degrade certain plastics (Singh et al., 2014). Biodegradable plastics are materials designed to degrade under environmental conditions or in municipal and industrial biological waste treatment facilities, and thus open the way for new waste management strategies (Gouda et al., 2012). The present study aimed to isolate polythene degrading bacteria from the soil after soil burial of biodegradable plastic and analysis of its degradation through FTIR analysis.

II. MATERIALS AND METHOD

1. Materials:

Polyolefin's - collective term for the kinds of plastics that include polyethylene, namely low-density polyethylene (LDPE), linear low-density polyethylene (LLDPE), high-density polyethylene (HDPE) and polypropylene (PP) free plastic was procured from commodity selling bags of JABONG having 0.8 g/ml density was used in the present study. Nutrient agar and Nutrient broth were also used during this study. Bushnell's broth (g/l: $MgSO_4$.$7H_2O$ 0.2, $CaCl_2$ 0.02, KH_2PO_4 1, K_2HPO_4 1, $NH_4NO_3$1, $FeCl_3$ 0.05 pH adjusted to 7.0) devoid of any carbon sources, was used for the degradation experiments.

2. Isolation of biodegradable polythene degrading bacteria:

Biodegradable plastic was buried in the glass bottle with three layers of different soil like Rhizospheric

soil, Sludge and Garden soil for 4 months at room temperature amended with Bushnell's broth (g/l: $MgSO_4$.$7H_2O$ 0.2, $CaCl_2$ 0.02, KH_2PO_4 1, K_2HPO_4 1, NH_4NO_3 1, $FeCl_3$ 0.05 pH adjusted to 7.0) to maintain the availability of trace elements and moisture. Soil suspension was prepared from the three layers and diluted as per the requirement. The soil suspension was spreaded over the Nutrient agar plates by spreading method. The bacteria were incubated at room temperature; the isolated bacteria were then purified and proceed further for degradation study.

3. Identification of selected isolates:

The bacterial isolates were then identified macroscopically (colony morphology, surface pigment, shape, size, margin, surface), microscopically (Gram staining, shape, cell arrangement, granulation, presence of spore, motility) and biochemically on the basis of Bergey's Manual of Determinative Bacteriology.

4. FTIR analysis:

Test flask contained pieces of plastic as substrate and an inoculum in Bushnell's Broth. The test was performed at room temperature for 45 days with continuous stirring.

After the termination of biodegradable experiment plastic was recovered from the broth and washed with sterile water and surface sterilization with alcohol to remove all material attached on the surface of the sample. The plastic sample was air dried and proceeds for FTIR analysis. A Bruker FTIR spectrometer was used for FTIR analysis. The sampling station was equipped with an overhead ATR accessory. The ATR diamond crystal was carefully cleaned with pure isopropanol between measurements.

Plastic sample was carefully placed on the diamond crystal surface and each spectrum was recorded as absorbance under 100 N. The spectra were scanned between 4000 and 650 cm –1 (Ali et al., 2016).

III. RESULTS AND DISCUSSIONS

The present study deals with the isolation of Biodegradable Polythene degrading bacteria from the soil and sludge, analysis of biodegradation by FTIR. In our study out of 6 isolates, 4 were obtained through enrichment technique utilizing Biodegradable Polythene as sole carbon source.

1. Identification of selected isolates:

The bacterial isolate was identified as *Bacillus* sp. VC2 on the basis of colony and morphological characteristics are shown in (Table 1) and biochemical test are shown in (Table 2).

Table 1. Colony and cell morphology of biodegradable polythene degrading bacterial strain.

Characteristics	VC2
Shape	Round
Size	Big
Colour	Light Peach
Margin	Uneven
Surface	Flat
Straight rod	+
Cocci	-
Gram stain	+
Cell arrangement	Single/Chain

Note: -, negative; +, positive;

Table 2. Biochemical test of biodegradable polythene degrading bacterial strain.

Biochemical test	VC2
GLUCOSE	+
FRUCTOSE	-
MALTOSE	-
LACTOSE	-
SUCROSE	-
MANNOSE	+
MANNITOL	+
TREHALOSE	+
Indole	-
Citrate	+
MR Test	-
VP Test	+
TSI	-
Catalase	+

Note: -, negative; +, positive;

2. FTIR analysis:

In these present study, FTIR analysis of biodegradable plastic sample treated with *Bacillus* sp. showed removal of peaks at wavenumber 2964.83 cm-1 corresponding to alkane (C-H), 1515.55 cm-1

corresponding to nitro compound (N-O), 1374.44 cm-1 corresponding to sulfonate (S=O), 1030.30 cm-1 corresponding to anhydride (CO-O-CO) as compared to control plastic sample. Some peak show the stretching vibration from wavemumber 3910.83 cm-1 to 3667.71 cm-1 corresponding to O-H hydrogen bond stretching of functional group alcohol, 2311.71 cm-1 corresponding to O=C=O bond stretching of functional group carbon dioxide, 1715.75 cm-1 corresponding to C=O bond stretching of functional group α, β – unsaturated ester.

Some peaks show the bending vibration at wavenumber 1381.58 cm-1 corresponding to C–H bending of functional group alkane. Formation of new peaks shows the accumulation of products of biodegradation from wavenumber 673.87 cm-1 to 799.09 cm-1 corresponding to formation of C=C of functional group alkene, 2043.31 cm-1 corresponding to N=C=S of functional group isothiocyanate, 2209.77 cm-1 corresponding to C≡C of functional group alkyne, 3086.55 cm-1 and 3456.63 cm-1 corresponding to O-H of functional group alcohol. There is a shift on left and right side of the FTIR spectra of existing peak at wavenumber 3731.39 cm -1 and 2964.83 cm-1 , respectively.

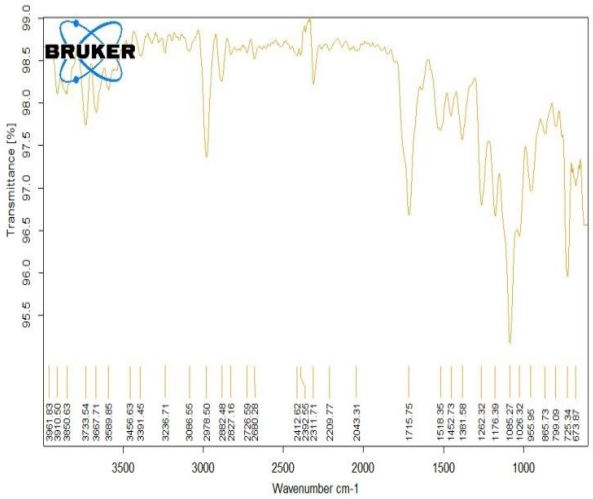

Fig 1. Amount of CO_2 evolution (g/l) as product of degradation.

Biodegradability of polyethylene by Bacillus cereus strain it was found that some new peaks arose after the period of biodegradation. They can be assigned to specific peaks, such as dehydrated dimer of carbonyl group (1720 cm-1), CH_3 deformation (1463 cm-1) and C=C conjugation bend (862 cm-1). The increase in carbonyl peak (1720 cm-1) as the increase in incubation period of pre-treated LDPE with *Bacillus cereus* (C1). The FTIR spectra of pre-treated BPE10 shows, the introduction of ketocarbonyl functional group (1718 cm-1) after 1 month of biodegradation and the intensity increases with irradiation period up to 3 months and at the same time a broadening of the bend which indicates the presence of more than one oxidation product (Sudesh et al., 2007).

Degradation of polyester polyurethane by *Bacillus subtilis* strain MZA-75 the peak at 1725 cm-1 representing carbonyl group of esters almost disappeared in the FTIR spectrum of test sample. The peaks at 1164.9 cm-1 and 1136.9 cm-1 represent C–O stretching of the ester functionality, are disappeared in test samples. Both these indicate that ester hydrolysis took place as a result of microbial treatment (Shah et al., 2013).

IV. CONCLUSIONS

Now-a-days, biodegradable plastics are used in packaging, paper coatings, bottles, bags, etc. Biodegradable plastic, soil and sludge sample were procured from Ahmedabad, Gujarat, India. The bacterial isolate VC2 *Bacillus* sp. was able to degrade the polymer sample by removal of functional group such as Thiol (S-H bond), Nitro compound (N-O bond), Alochol (O-H bond), Alkane (C-H bond). There was a stretching vibration in functional group such as Carbon dioxide (O=C=O bond), α, β -unsaturated ester (C=O bond), Amine salt (N-H bond), alkyl aryl ether (C-O bond) in laboratory studies. There was a change in transmittance (%) of polymer after biodegradation. It can be concluded that the soil and sludge contains potential bacteria for bioremediation of plastic waste and it will reduce polymer waste which causes environmental issues.

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