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Biodegradation of Polythene by Novel Bacteria Sphingomonas sp. VC1

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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 were then isolated and identified on the basis of biochemical studies as Sphingomonas sp. VC1. The novel bacteria Sphingomonas VC1 sp. break the polymers and used as sole carbon source for their metabolic activities and results in release of carbon dioxide as end product was estimated by STRUM test.*

Key Words*: *Hydrocarbons, Biodegradable plastic, STRUM test, Bushnell's broth.

1. 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 by the International Agency for the Research on Cancer (IARC) as carcinogenic to humans (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 STRUM test.

2. MATERIALS AND METHOD

2.1 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 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.

2.2 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 on 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.

2.3 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.

2.4 STRUM Test:

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 culturing, the amount of CO_2 produced was calculated in the test flask gravimetrically.

Evolution of CO_2 as a result of degradation of polymeric chain was trapped in the absorption flasks containing 1 M KOH. $BaCl_2$ solution (0.1 M) was added to the CO_2 containing KOH flasks and as a result precipitates of $BaCO_3$ (using CO_2 released from breakdown of polymer) were formed. CO_2 produced was calculated gravimetrically by measuring amount (g) of CO_2 precipitates evolved by addition of $BaCl_2$.

The precipitates of $BaCO_3$ were then washed and dried. The weight of precipitates ($BaCO_3$) was noted for each bacterium (Aamer et al., 2008).

3. RESULTS AND DISCUSSIONS

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

3.1 Identification of selected isolates:

The bacterial isolate was identified as *Sphingomonas* sp. VC1 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	VC1
Shape	Round
Size	Small
Colour	Cream white
Margin	Even
Surface	Convex
Straight rod	+
Cocci	-
Gram stain	-
Cell arrangement	Single/Chain

Note: -, negative; +, positive;

Table -2: Biochemical test of biodegradable polythene degrading bacterial strain

Biochemical test	VC1
GLUCOSE	-
FRUCTOSE	-
MALTOSE	-
LACTOSE	-
SUCROSE	-
MALTOSE	-
MANNITOL	-
GLUCOSE	-
FRUCTOSE	-
Indole	-
Citrate	-
MR Test	-
VP Test	-
TSI	+
Catalase	+

Note: -, negative; +, positive;

3.2 STRUM Test:

In these present study, amount of CO_2 evolved as a product of degradation by bacterial isolates is 1.324 g/l by *Sphingomonas* sp. these result were obtained after 45 days of incubation at room temperature (Chart 1).

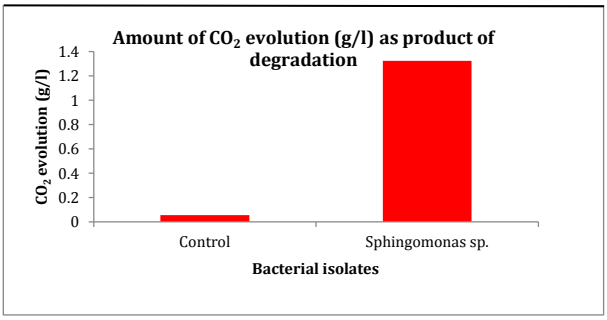

Chart -1: Amount of CO_2 evolution (g/l) as product of degradation

Biodegradation of Cellulose Blended Polyvinyl Chloride Films gravimetric analysis of CO_2 produced in test and control was 21.28 g/l and 11.07 g/l, respectively. There was also a significant change in the dry cell mass of *Phanerochaete chyrosporium* PV1 in test higher (0.136 g/l) than in control (0.056 g/l) (Muhammad et al., 2009).

Degradation of polyester polyurethane by *Pseudomonas* and *Bacillus* sp. A bacterial consortium was used in case of Sturm test and its degradation efficiency was studied in terms of difference in CO_2 evolved both in test and control vessels (biotic as well as abiotic). High amount of CO_2 (8.1675g/l) was recovered after quantification in test vessel as compared to both biotic and abiotic control (Shah et al., 2016).

According to Shah et al., 2010 at the end of the degradation experiment of Polyisoprene Rubber by *Bacillus* sp. AF–666, gravimetric analysis of CO_2, evolved in case of test was higher 4.43 g/1, than control 1.57 g/1. The CO_2 produced after mineralization of polymer for 30 days was found to be 10.21 g/l, which showed significant degradation of the polymer.

4. 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 production of CO_2 as a product of biodegradation analyzed through STRUM test showed 1.324 g/l by *Sphingomonas* sp. Genomic study can be done and strategies can be developed using bacteria to degraded plastics, the fate of these biodegradable polymers in the environment and the time required for their complete mineralization to carbon dioxide needs to be fully understood.

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