Study of Synthetic Plastic Degradation Capacity of VC6

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Abstract: *Synthetic polymers are typically prepared from monomers derived from oil or gas by polymerization and by addition of various chemical additives; plastics are usually made from these. There are currently some 20 different groups of plastics, each with numerous grades and varieties. Biodegradation capacity of VC6 was studied using various parameters such as Strum test, % weight loss determination, statistical analysis and tensile strength before and after incubation with VC6.*

Keywords: *Biodegradation, Plastic, Synthetic, Analysis*

1. INTRODUCTION

The Greek word plastios, which means plastic - 'able to be molded into varied shapes' (Joel, 1995). It is defined as the polymer which become mobile on heating and thus can be cast into varieties of moulds. The plastic is made up of silicon, oxygen, carbon, hydrogen, chloride and nitrogen. For extraction of the basic materials of plastics; coal, oil and natural gas are used (Seymour, 1989). Synthetic polymers are typically prepared from monomers derived from oil or gas by polymerization and by addition of various chemical additives; plastics are usually made from these. There are currently some 20 different groups of plastics, each with numerous grades and varieties (APME 2006). Plastics are incredibly versatile materials. They are inexpensive, lightweight, strong, durable, corrosion resistant, with high thermal and electrical insulation properties. The diversity of polymers and the versatility of their properties facilitate the production of a vast array of plastic products that bring technological advances, energy savings and numerous other societal benefits (Andrady & Neal, 2009).

From last three decades uncontrolled use of the plastics for packaging (e.g. fast food), transportation, industry and agriculture in rural as well as urban areas, has elevated serious issue of plastic waste disposal and its pollution. Light-weight, inertness, durability, toughness and low cost are the main advantages of plastic while it has disadvantages such as, it is recalcitrant to biodegradation and difficult to degrade naturally. The global use of plastic is growing at a rate of 12% per year and around 0.15 billion tones of synthetic polymers are produced worldwide every year (Premraj and Doble 2005; Leja and Lewandowicz 2010; Das and Kumar 2014). Under the natural condition degradable or non-degradable organic materials are considered as the major environmental problem, e.g. plastics. The accumulation of these plastic wastes created serious threat to environment and wild life (Chua et al., 2003). The environmental concerns include air, water and soil pollution. The dispersal of urban and industrial wastes contaminates the soil. The soil contaminations are mainly made by human activities (Ghosh et al., 2005). Environmental pollution is caused by synthetic polymers, such as wastes of plastic and water-soluble synthetic polymers in wastewater (Premraj & Mukesh, 2005).

Synthetic plastics are extensively used in packaging of products like food, pharmaceuticals, cosmetics, detergents and chemicals. Approximately 30% of the plastics are used worldwide for packaging applications. This utilization is still expanding at a high rate of 12% per annum (Sabir, 2004). They have replaced paper and other cellulose-based products for packaging because of their better physical and chemical properties, such as their strength, lightness, resistance to water and most water-borne microorganisms. The most widely used plastics used in packaging are polyethylene (LDPE, MDPE, HDPE and LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), poly (ethylene terephthalate) (PET), poly (butylene terephthalate) (PBT), nylons. The widespread applications of plastics are not only due to their favorable mechanical and thermal properties but also mainly due to the stability and durability (Rivard et al., 1995). Because of their durability and visibility in litter, plastics (polymers) have attracted more public and media attention than any other component of the solid waste stream. In 1993, the total world demand for plastics was over 107 million tones and it was estimated about 146 million tones in 2000 (Sabir, 2004). The plastic wastes are disposed off through landfilling, incineration and recycling. Due to their persistence in our environment, several communities are now more sensitive to the impact of discarded plastic on the environment, including deleterious effects on wildlife and on the aesthetic qualities of cities and forests. Improperly disposed plastic materials are a significant source of environmental pollution, potentially harming life (Jayasekara et al., 2005). Some of synthetic plastics like polyester, polyurethane, polyethylene with starch blend are biodegradable, although most commodity plastics used now are either non-biodegradable or even take decades to degrade (Kawai F, 1995).

2. MATERIAL AND METHODS

Sample collection: **(a)** Soil samples from different places of Ahmedabad and Dahod were collected and brought to the lab. **(b)** Plastic sample of three types were collected from different places such as Polyethene bag from vegetable vendor (PS-1), Zip lock bag from market (PS-2) and Black plastic bag from nursery (PS-3).

Preparation of Submerged Activity for Biodegradation: The plastic samples were washed to remove the oils and spread out to air dry. After drying, it was cut into a piece of 10 cm x 2 cm for the laboratory study. Glass Bottle was used for submerged activity. A piece of plastics were added in the soil solution in different soil solution flask, Minimal salt Medium [$(NH_2)_2SO_4$, 5 g; KH_2PO_4, 2 g; K_2HPO_4, 2 g; $MgSO_4.7H_2O$, 250 mg; $FeSO_4.7H_2O$, 10 mg; $MnSO_4.7H_2O$, 10 mg; $CaCl_2.7H_2O$, 10 mg; $ZnSO_4.7H_2O$, 30 mg; H_3BO_3, 100 mg; $CoCl_2.7H_2O$, 117 mg; $CuCl_2.2H_2O$, 30 mg; $NiCl_2{\cdot}2H_2O$, 10 mg; $NaMO_4.7H_2O$, 100 mg; Deionized water, 1L] was added to the system for enrichment, kept at room temperature for 3 month under shaking condition.

Screening of Bacteria: Soil suspension was prepared from incubated bottles as per requirement. The soil suspension was spreaded on the Nutrient agar plates by spreading method. The bacteria were incubated at room temperature. 40 isolates of bacteria were isolated. The isolated bacteria were then inoculated on Mineral salt Medium (MSM) and Guaiacol media. Out of 40 isolates 16 isolates were able to grow on MSM + Guaiacol plates, from which VC6 was selected for further study.

Identification of selected isolate: The bacterial isolate was 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.

Bacterial degradation of plastic samples: The isolated bacteria were then purified and inoculated in Minimal salt Medium [$(NH_2)_2SO_4$, 5 g; KH_2PO_4, 2 g; K_2HPO_4, 2 g; $MgSO_4.7H_2O$, 250 mg; $FeSO_4.7H_2O$, 10 mg; $MnSO_4.7H_2O$, 10 mg; $CaCl_2.7H_2O$, 10 mg; $ZnSO_4.7H_2O$, 30 mg; H_3BO_3, 100 mg; $CoCl_2.7H_2O$, 117 mg; $CuCl_2.2H_2O$, 30 mg; $NiCl_2{\cdot}2H_2O$, 10 mg; $NaMO_4.7H_2O$, 100 mg; Deionized water, 1L; devoid of carbon source; pH adjusted to 7.0) with a piece of plastic samples. The bacteria were then allowed to grow at room temperature for 3 and 6 months in anaerobic condition. Then biodegradation was evaluated using from methods:

1) **STRUM test:** Three test flask were prepared which contained pieces of plastic as substrate and an inoculum in Minimal salt Medium. The test was performed at room temperature for 3 and 6 months 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 bacterium (Aamer et al., 2008).
2) **Determination of %Weight loss of plastic samples:** To examine biodegradation of Plastic samples by bacterial isolated, plastic samples were recovered after exposure to bacterial isolates and washed with 2% Sodium Dodecyl Sulphate solution followed by distilled water. The weight loss of each sample was determined by comparing the weight before and after bacterial inoculation. The weight difference of plastic samples between initial and final weight illustrates the amount of plastic utilization by bacterial isolates. The average percentage weight loss of sample films were determined as follows:

$$\%\ \text{weight loss} = (w_i - w_f / w_i) \times 100$$

Here, w_i – initial weight of the sample
w_f – final weight of the sample

3) **Statistical analysis:** Statistical analysis was performed using IBM SPSS (Statistic Software) version 20 for determination of change in keto and ester carbonyl index. All experiments were performed in triplicate and numerical data for experiments were expressed as Mean ± Standard deviation. The data obtained were analyzed by using Analysis of Variance (ANOVA) and Interaction mean. The experiments were run at 95% significance level indicating that there might be a chance of 5% error.
4) **% Elongation at break of plastic sample:** To measure changes in mechanical properties, after biodegradation by bacterial isolate, elongation tests were performed for all the samples on INSTRON at a crosshead speed of 50mm/min and a gauge length of 10 cm according to ASTM D 638. At least, three samples of each film were tested and average value was calculated.

3. RESULTS AND DISCUSSION

The present study deals with the isolation of synthetic plastic degrading bacteria from the soil, analysis of biodegradation by STRUM test, Determination of %Weight loss of plastic samples, Statistical analysis and % Elongation at break of plastic samples.

Identification of selected isolate: The bacterial isolate was identified as Bacillus sp. VC6 on the basis of colony and morphological characteristics are shown in (Table 1) and biochemical test are shown in (Table 2). Bacterial isolates was identified using 16S-rRNA gene sequencing MEGA 10.0 software BLAST tool. The nucleotide sequence has been deposited in NCBI gene bank with Accession No. **OQ538154** (as Bacillus cereus VC6).

Table 1: Colony and Morphological Characteristics of VC6

Characteristics	**VC6**
Shape	Round
Size	Big
Colour	Off-white
Margin	Even
Surface	Convex
Opacity	Opaque
Straight rod	+
Cocci	-
Gram stain	+
Cell arrangement	Single/ Chain

Note: +, positive; -, negative;

Table 2: Biochemical Test of VC6

Biochemical test	**VC6**
Motility	+
Urea Utilization	+
Nitrate reduction	+
Lipid	**v**
Starch	+
Indole	**-**
Citrate	+
MR Test	**-**
VP Test	**-**
TSI	+
Catalase	+

Note: +, positive; -, negative;

STRUM test: In these present study, amount of CO_2 evolved as of product of plastic biodegradation by bacterial isolate for PS-1, PS-2 and PS-3 is 1.008 g/L, 0.884 g/L and 1.625 g/L; respectively (Chart 1).

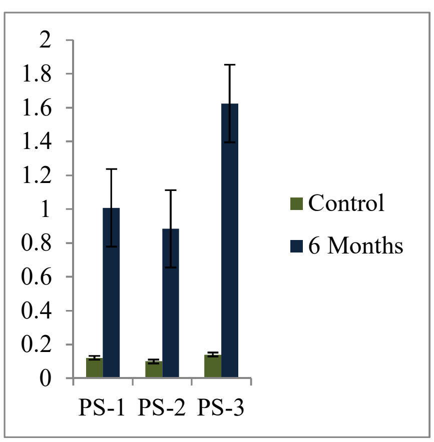

Chart 1: Amount of CO_2 Evolved as a Product of Biodegradation

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).

Determination of % Weight loss of plastic samples: After being treated with isolated bacterial strain B. cereus VC6 for 6 months, plastics sample showed significant % weight loss. It indicates that this strain is quite effective in degrading plastic films. It showed % weight loss (Chart 2) as for PS-1 (1.234%), PS-2 (0.856%) and PS-3 (1.821%). In previous study, bacterial isolates (isolated from sea water) led to biodegradation of low density polyethylene and weight loss after 30 days of incubation with M16, M27, and H1584 bacteria was 1%, 1.5%, and 1.75%, respectively (Harshvardhan and Jha, 2013).

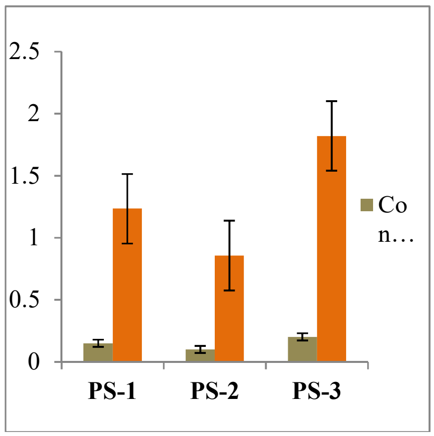

Chart 2: % Weight Loss of Plastic Samples

Statistical analysis: Result of ANOVA after 6 months of plastic samples biodegradation revealed that Keto-Carbonyl Index (K-C Index) in PS-1, PS-2 and PS-3 was recorded as 0.545, 0.654 and 0.484; respectively. The Ester-Carbonyl Index (E-C Index) was recorded as 0.655, 0.798 and 0.593 for PS-1, PS-2 and PS-3; respectively (Chart 3).

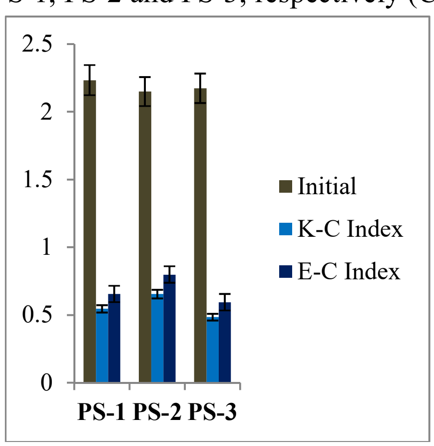

Chart 3: Statistical Analysis of K-C and E-C Index

% Elongation at break of plastics sample: Tensile strength was decreased in PS-1, PS-2 and PS-3 after 6 months of plastic samples biodegradation with bacterial isolate, according to ANOVA result the tensile strength was recorded as 28.35, 35.65 and 25.23; respectively (Chart 4).

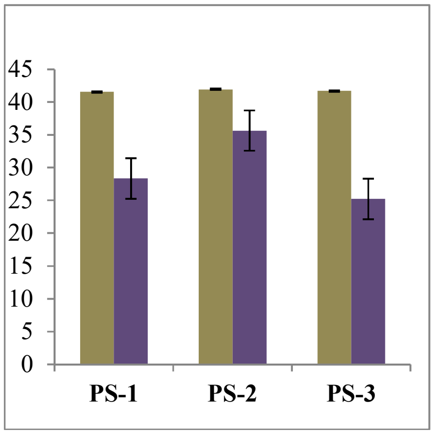

Chart 4: Decrease in Tensile Strength After Bacterial Incubation

Previously studied, LLDPE/starch blends composite showed decrease in tensile strength and elongation at break as starch content in polymer composite were increased (Chandra and Rustgi, 1997). It had been shown in a study that, Incubation of polyethylene with starch (6% starch) composite treated with Streptomyces and other isolated fungi recorded only small changes in tensile strength and also decrease in percent elongation values (El-Shafei et al., 1998).

4. CONCLUSION

Synthetic plastics are extensively used in packaging of products like food, pharmaceuticals, cosmetics, detergents and chemicals. Based on the result of present study it can be conclude the plastic biodegradation is effective process as it degrade plastics naturally in the environment. The complete mineralization of plastic to carbon dioxide requires more time and needs to be fully understood.

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