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Short Communication

An approach to biological degradation of polystyrene

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Abstract

Protein production increases from five types of bacteria namely Pseudomonas fluorescens, Bacillus firmus, Brevundimonasdiminuta, Bacillus subtilis, Pseudomonasputida at the time of Biodegradation of plastic cup were studied in sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) containing a Plastic cup substrate. SDS PAGE studies were done at 76 days of biodegradation. Bacteria with plastic cup substrate have number of protein bands of BF>BD>PF>PP>BS. The Molecular weights of the each protein bands were estimated. The optimum temperature and pH for the biodegradation of plastic were between $30^0C – 32^0C$ and pH 7.5.

Keywords: Biodegradation, Substrate, Plastic cup.

Introduction

Plastics are artificial long chain polymeric molecules[1]. More than five decades ago artificial polymer started to additional usual resources in nearly each zone and nowadays plastics have become an indispensable part of our life. The plastics we utilize nowadays are prepared from inorganic and organic raw ingredients. The ingredients used for making plastics are obtained from oil, coal and natural gas[2]. Plastics are resist to microbial attack, there is handful information of information of new enzyme which acts on degradation of polymer till today[3]. Currently, a number of synthetic polymers from petroleum are formed worldwide to the range of nearly one hundred and forty million tons per year and notable amounts of these plastics are familiarized in the environment as industrial unused products[4].

Polystyrene (PS) is an artificial plastic used in the production of coffee and tea cups, packing materials, in laboratory ware, in some electronic uses. Polystyrene type of plastic is used for its lightweight, stiffness and outstanding thermal insulation. When it is degraded by thermal or chemical means it produces styrene, benzene, toluene and acrolein.

There are rare research work on the biodegradation of polystyrene but the microbial decomposition have been stated by few researchers[5,6].

Enzymes produced by bacteria are responsible for oxidized plastic cup degradation in pure culture. n the present study the synthetic plastic polystyrene i.e. plastic cup were used to study their biodegradation by the microorganisms *Pseudomonas fluorescens, Bacillus firmus, Brevundimonas diminuta, Bacillus subtilis, Pseudomonas putida.* At the time of biodegradation study of protein bands and their molecular weight are involved.

Material and methods

Microorganisms: The microorganisms we plan to use are the bacteria *Pseudomonas fluorescens, Bacillus firmus, Brevundimonas diminuta, Bacillus subtilis, Pseudomonas putida*. These five kinds of bacteria have been selected because previous studies have demonstrated that they possess greater potential to degrade plastics[7].

Selection of plastic: Polystyrene i.e. plastic cup are selected for the experimental work[8]. Plastic cup each having approximately 70 to 75 microns thickness was used for experimental work.

Pre-treatment of plastic: The plastic cups were cut in small strips of 3 x 3 cm, after weighing they were aseptically added to ethanol solution of 70% v/v for 30 min. lastly, the strips were relocated to a petri dish and incubated at 45 to 50^oC overnight. Ethanol was used to disinfect the plastic and remove any organic matter adhering to its surface.

Treatment of plastic tea cup: The five types of bacteria *Pseudomonas fluorescens, Bacillus firmus, Brevundimonas diminuta, Bacillus subtilis, Pseudomonas putida* were inoculated in 30 ml of nutrient broth in bottle with 60 mg of the Plastic tea cup pieces added to all flask except the control. For every type of bacteria six replicates setup were done. The entire bottle was incubated on shaker at 37^0C for 76 days[9].

SDS PAGE for molecular weight determination: For determination of molecular weight the samples and molecular weight markers were separated on 12.0% separating acrylamide gel (pH 8.8) and a 5% stacking gel (pH 6.8) containing 0.1% SDS. Electrophoresis was worked out by using trisglycine buffer (pH 8.3) polyacrylamide gel according to the procedure

of Laemmli. The Baglore Genei medium range molecular weight markers for staining with Coomassie Brilliant Blue R-250 were used containing proteins, Myosin, Rabbit Muscle 205,000 Phosphorylase b 97,400 Bovine Serum Albumin 66,000, Ovalbumin 43,000 Carbonic Anhydrase 29,000, Soyabean Trypsin Inhibitor 20,100, Lysozyme 14,300, Aprotinin 6,500 and Insulin 3,000. Silver staining was used to visualize the bands. The molecular weight of the sample proteins was estimated by comparing its Relative Mobility (RF) value with the molecular weight markers with the help of Fire reader software (Uvitec, UK).

Results and discussion

The result of SDS PAGE analysis of protein from *Pseudomonas fluroscence* suggested that the treated sample had five protein bands having different molecular weight whereas in control three protein bands were observed. The molecular weights of the five band of protein were estimated to be about 192, 176, 146, 107, 63kDa by SDS PAGE (Table-1).

The result of *Bacillus firmus 2637* suggested that were nine proteins band were observed having different molecular weight whereas in control three protein bands were observed. The molecular weight of the nine bands of proteins was estimated to be about 192, 179, 166, 146, 130, 102, 71, 62, and 46 kDa.

The *Brevundimonas dimunita* shows presence of eight proteins bands whereas in untreated five proteins bands were analysed. The molecular weights of eight proteins band were 197, 181, 171, 156, 115, 62, 55, and 47 kDa.

When *Bacillus subtilis* 2063 was used for treatment, it showed the presence of three protein bands by SDS –PAGE analysis in treated as well as control samples.

The analysis of *Pseudomonas putida* showed the presence of five bands in treated sample whereas as in control four bands were observed. The molecular weight of five proteins band were197, 187, 174, 153, and 66kDa.

Thus from the above studies, *Bacillus firmus 2637*and *Brevundimonas dimunita* shows nine protein bands and protein bands respectively, as compare to other bacteria that were used. Hence, they showed best result for degradation of plastic cup. *Pseudomonas fluroscence* and *Pseudomonas putida* both showed five bands of protein. And *Bacillus subtillus*2063 showed three protein bands in both treated as well as control samples.

From this we can estimate that, there is increase in protein bands in treated experiment because the bacteria uses plastic as a carbon source.

Bacillus firmus 2637, *Brevundimonasdimunita, Pseudomonas fluroscence* and *Pseudomonas putida* has the ability to utilize plastic as a carbon source and energy material to yield proteins. The production level of proteins of bacteria depended on the substrate used by the cells[10, 11].

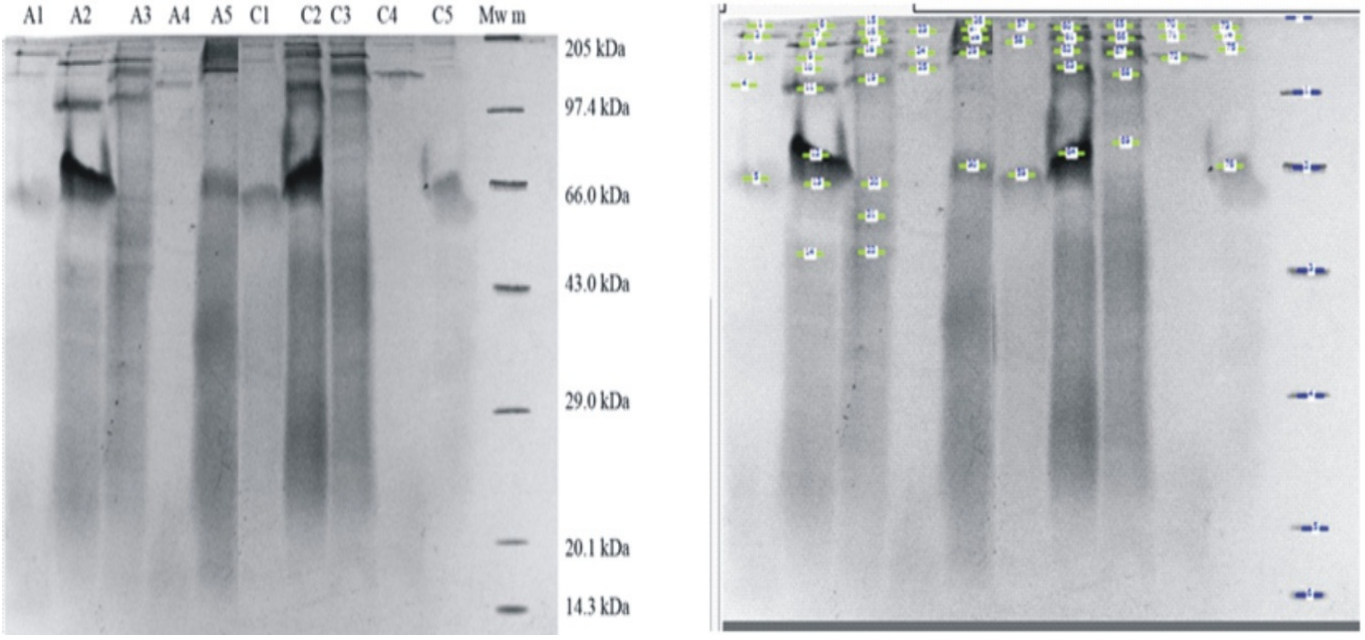

Figure-1: SDS-PAGE examination of the protein from the *Pseudomonas fluorescens, Bacillus firmus* 2637*, Brevundimonas diminuta, Bacillus subtilis* 2063*, Pseudomonas putida.* Lane A1, A2, A3, A4, A5: Treated. Lane C1, C2, C3, C4, and C5: control lane SDS PAGE, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Table-1: Shows SDS PAGE Results of five types of bacteria produces number of protein bands with the presence of Plastic cup

Name of Species	Symbol	Substrate conc. (Plastic cup)	Lane	Band	Position	Molecular Weight (KD)	Symbol of Control	Lane	Band	Position	Molecular Weight
Pseudomonas fluroscence	A1	60 mg	1	1	10	192	C1	11	1	10	192
				2	22	176			2	28	169
				3	46	146			3	176	64
				4	76	107			-	-	-
				5	180	63			-	-	-
Bacillus firmus 2637	A2	60 mg	2	1	10	192	C2	12	1	12	189
				2	20	179			2	24	174
				3	30	166			3	38	156
				4	46	146			4	56	133
				5	58	130			5	152	71
				6	80	102			-	-	-
				7	154	71			-	-	-
				8	186	62			-	-	-
				9	264	46			-	-	-
Brevundimonas dimunita	A3	60 mg	3	1	6	197	C3	13	1	10	192
				2	18	181			2	24	174
				3	26	171			3	40	153
				4	38	156			4	64	123
				5	70	115			5	140	76
				6	186	62			-	-	-
				7	222	55			-	-	-
				8	262	47			-	-	-
Bacillus subtillus 2063	A4	60 mg	4	1	16	184	C4	14	1	10	192
				2	40	153			2	22	176
				3	58	130			3	46	146
Pseudomonas putida	A5	60 mg	5	1	6	197	C5	15	1	12	189
				2	14	187			2	22	176
				3	24	174			3	36	158
				4	40	153			4	166	66
				5	166	66			-	-	-

Conclusion

The present work on biodegradation of polystyrene, analysed the ability of *Bacillus firmus 2637*, *Brevundimonas dimunita, Pseudomonas fluroscence* and *Pseudomonas putida* to utilizing carbon source and energy material to produce proteins, hence the increase in production of protein in samples is supportive for degradation of polystyrene by the microorganisms.

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