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GCMS Analysis of *Pseudomonas* sp., Mediated Degradation of Polystyrene

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ABSTRACT

Accumulation of polysytrene waste is of environmental concern. Although several strategies have been adopted to dispose off these waste, an eco-friendly and cost effective method has to be evolved. Microorganisms have been reported to degrade polystyrene waste. In this study, bacteria prevalent in polystyrene waste dumped soil have been screened for their potential to degrade polystyrene waste. The bacteria adapted to such environment could utilise polystyrene as a carbon source for their growth. Hence, we have traced the degradative metabolites of polystyrene on incubation of polystyrene in minimal salt media inoculated with Pseudomonas sp., isolated from polystyrene waste dumped soil. Polystyrene was degraded to p-xylene, Ethylbenzene, (3-chloro-1-propynyl)-cyclohexane, (3-chloropropyl)methylene-cyclopropane, 1-cyclopropyl-2-nitro-Benzene, Bis(2-methylpropyl)ester, 1,2-Benzenedicarboxylic acid, 2-(Heptyloxycarbonyl)benzoic acid, 2-(octyloxycarbonyl)benzoic acid, Dihexyl ester, 1,2-Benzenedicarboxylic acid and Butyl octyl ester, 1,2-Benzenedicarboxylic acid in MSM after a period of one month. Comparatively, inoculation of polystyrene with Pseudomonas sp., in MSM resulted in the formation of 2-(Nonyloxycarbonyl)benzoic acid, 1-chloro-2-methyl-cyclohexene, Dihexyl ester 1,2-Benzenedicarboxylic acid besides these compounds.

Keywords: Polystyrene, *Pseudomonas* sp., GCMS

INTRODUCTION

Rigid polystyrene and polystyrene related plastics, which are used as food packaging materials, have longer history of use than poly vinyl chloride [1]. Further, Polystyrene and expanded polystyrene (EPS) are commodities used in packaging, insulation materials in the form of a foam or bead in construction sectors [2,3]. Polyethylene, Polypropylene and Polystyrene are the major plastics in municipal waste, with chlorinated polymers such as poly

(vinyl chloride) (PVC) present in small amounts. The processing of this waste has become a technological issue that has attracted the attention of researchers. Since both landfill and incineration cause secondary pollution problems, novel disposal technologies are in high demand by the industry and regulators to provide for more energy efficient and environmentally and economically sound solution [4]. Microorganisms present in the environment also attack the polymer indirectly to utilise it as a carbon source [5]. Microorganisms could be a sustainable option in degrading these wastes. In our previous study, we have observed through FTIR that *Micrococcus* sp., and *Pseudomonas* sp., induce chemical changes in polystyrene in MSM after a period of one month [6]. This study aims to tap the potential of soil bacteria to degrade polystyrene. Inoculation of polystyrene with *Pseudomonas* sp., in MSM resulted in the formation of 2-(Nonyloxycarbonyl) benzoic acid, 1-chloro-2-methyl-cyclohexene, Dihexyl ester 1,2-Benzenedicarboxylic acid besides these compounds when compared to the control.

MATERIALS AND METHODS

Isolation of bacteria from polystyrene waste dumped soil

Soil samples were collected from polystyrene waste dumped sites. 1 g of soil was dissolved in 99 ml sterile distilled water and serially diluted. The diluted samples were inoculated on nutrient agar plates and the bacterial isolates were identified using Bergeys manual of Determinative Bacteriology [7].

Preparation of Polystyrene samples

The polystyrene foam were cut into beads of equal sizes and used for degradation studies.

Degradation of PS by bacteria in MSM

Pseudomonas sp., were used for PS degradation studies. 10 µl of the broth culture of Pseudomonas sp. were inoculated each in 100 ml sterile minimal salt medium (MSM) containing polystrene and kept in the shaker at 37°C and 120 rpm for a period of 1 month. The PS samples were subjected to GCMS studies.

Instrumental analysis

The residues present in sample were detected by GCMS (45 X GC-44, Brucker) equipped with auto injector (8410). The analyses separation was performed in a 30 m × 0.25 mm I.D × 0.25 µm film thickness BR 5 ms column (made in USA) and helium was used as a carrier gas at a flow rate of 1ml/min. the column temperature was programmed as 70°C to 150°C at 10°C/min, to 250°C at 5°C/min to 280°C at 2/min, finally to 320°C at 5°C/min and hold for 10 min. 1 µl of the extract was injected into the injection port (at 280°C) using autoinjector. The mass spectrometer was operated was in scam mode and the ion source temperature was kept at 250°C. The electron ionization (EI) unit was operated at 70eV and at an emission current of 60 µA. Full scan data was obtained in a mass range of m/z 50 - 950. Scanning interval and sample rate were 0.5 and 0.28, respectively.

RESULTS AND DISCUSSION

Polystyrene was degraded to p-xylene, Ethylbenzene, (3-chloro-1-propynyl)-cyclohexane, (3-chloropropyl)methylene-cyclopropane, 1-cyclopropyl-2-nitro-Benzene, Bis(2-methylpropyl)ester, 1,2-Benzenedicarboxylic acid, 2-(Heptyloxycarbonyl)benzoic acid, 2-(octyloxycarbonyl)benzoic acid, Dihexyl ester, 1,2-Benzenedicarboxylic acid and Butyl octyl ester, 1,2-Benzenedicarboxylic acid in MSM after a period of one month. Comparatively, inoculation of polystyrene with *Pseudomonas* sp., in MSM resulted in the formation of 2-(Nonyloxycarbonyl)benzoic acid, 1-chloro-2-methyl-cyclohexene, Dihexyl ester 1,2-Benzenedicarboxylic acid besides these compounds (Table 1).

Naima Atiq et al., [8] have isolated *Microbacterium* sp., *Paenebacillus urinalis*, Bacillus sp., and *Pseudomonas aeruginosa* from expanded polystyrene film buried in soil for a period of 8 months. Further, through HPLC technique `they have demonstrated that these bacterial isolates degraded polystyrene inoculated in mineral salt media incubated with polystyrene. 1-phenyl 1,2 ethandiol was degraded product detected in the extracellular media of strains *Paenebacillus urinalis* and (9.8 ppm), Bacillus (14.31 ppm), *Pseudomonas aeruginosa* (0.36 ppm) and 2-phenylethanol was detected in the samples of *Paenebacillus urinalis* (3.16 ppm) and *Pseudomonas aeruginosa* (0.85 ppm) after 4 weeks of incubation with polystyrene films. Przbulewska et al., [9] have reported that *Streptomyces halstedii*, *Bacillus megaterium*, *Sphingobacterium spiritivorum*, B cereus were capable of utilising styrene as a carbon source. A large number of microorganisms are capable of aerobic growth with styrene as a sole

source of carbon and energy [10-16]. Under aerobic conditions, styrene is generally metabolised via oxidation of its vinyl side chain [17,18]. Oxidation of aromatic ring was also reported [16].

Treatments	Retention Time (min)	Compound name	Moleculer weight	NIST Library Number
Polystyrene	3.090	P-xylene	106	161
	3.488	Ethylbenzene	106	55527
	15.063	(3-chloro-1-propynyl)-cyclohexane	156	91305
	16.288	(3-chloropropyl)methylene-Cyclopropane	130	45293
	17.552	1-cyclopropyl-2-nitro-Benzene	163	106947
	18.257	Bis(2-methylpropyl)ester, 1,2-Benzenedicarboxylic acid	278	121239
	18.481	2-(Heptyloxycarbonyl)benzoic acid	264	122941
	19.671	2-(octyloxycarbonyl)benzoic acid	278	122887
	19.898	Dihexyl ester, 1,2-Benzenedicarboxylic acid	334	121062
	21.250	Butyl octyl ester, 1,2-Benzenedicarboxylic acid	334	121044
Polystyrene + *Pseudomonas* sp.,	3.092	P-xylene	106	161
	3.486	Ethylbenzene	106	55527
	15.055	(3-chloropropyl)methylene-Cyclopropane	130	45293
	16.284	1-chloro-2-methyl-cyclohexene	130	61780
	17.348	1-cyclopropyl-2-nitro-Benzene	163	106947
	18.425	2-(Nonyloxycarbonyl)benzoic acid	292	122946
	18.659	butyl octyl ester 1,2-Benzenedicarboxylic acid	334	121044
	19.670	2-(octyloxycarbonyl)benzoic acid	278	122887
	21.251	Dihexyl ester 1,2-Benzenedicarboxylic acid	334	121062

Table 1: Metabolites of polystyrene degradation by *Pseudomonas*

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