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*Full Length Research Paper*

# Isolation and identification of polystyrene biodegrading bacteria from soil

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**With the increased production of municipal solid waste by the disposal of plastic materials, there is a need to develop new biodegradable materials and biodegrade existing plastic materials in daily use. Polystyrene and expanded polystyrene are commodity plastics that are extensively used in packaging and other applications. Six bacterial isolates were isolated from soil buried expanded polystyrene films showing adherence and growth with the polystyrene as a sole carbon source. Scanning electron microscopy (SEM) of the film surface used for isolation showed extensive microbial growth. The preliminary screening of biodegradation capability was done by Fourier transform infrared (FTIR) spectroscopy for surface chemical changes and high pressure liquid chromatography (HPLC) for analysis of biodegradation products. Bacterial isolates NA26, NB6, NB26 showed the production of biodegradation products in the extracellular media indicating biodegradation process.**

**Key words:** Polystyrene, bacteria, biodegradation, soil burial, FTIR.

## INTRODUCTION

With the development of new synthetic polymers, plastics have found applications in every field of life. A worldwide increase in the use of these materials has generated issue of solid waste disposal (Al-Salem et al., 2009). Millions of tons of solid waste is disposed off annually in the world and a large proportion consists of plastics (Encinar and González, 2008). Synthetic plastics do not biodegrade in natural environments due to the complexity of their structure, high molecular weight and hydrophobic nature (Schlemmer et al., 2009; Rahmat et al., 2009). Polystyrene is a rigid plastic which is a most commonly used packaging material (Khaksar and Khansari, 2009).

The expanded polystyrene is extensively used in fast food take-out restaurants for its excellent thermal insulation properties. The ultimate fate of such packaging material is municipal solid waste (Aarnio and Hamalainen, 2008). Microorganisms play key role in the biodegradation in the environment (Gu, 2003). The extra-cellular enzymes of microorganisms play a key role in biodegradation process of polymers. They convert long chains of polymers into smaller ones and then subsequently into small molecules that are easily absorbed and metabolised inside the microorganism by intracellular enzymes. Soil burial is employed as a field test for biodegradation studies because it is similar to natural environmental conditions (Eubeler et al., 2009). The aim of the present study was to isolate soil bacteria able to colonise and biodegrade polystyrene films.

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## MATERIALS AND METHODS

### Soil burial

Expanded polystyrene (EPS) solution (2%) in chloroform was casted on petri plates to get thin films (0.3 - 0.5 mm). Similar procedure was used to get films of pure polystyrene (Fluka, Germany, Mol. Wt. 100,000). Garden soil from Quaid-i-Azam University, Islamabad, Pakistan, was mixed with manure (1:0.25). The films (6 x 2.5) were placed in soil contained in an earthen flower pot at 6 inches depth. 2% glucose solution (400 ml) was added to the soil to enhance the microbial growth and population.

The films remained buried for eight months (May - November 2006)

**Figure 1.** The visible growth of microbial consortia growing on expanded polystyrene film used for isolation of bacteria.

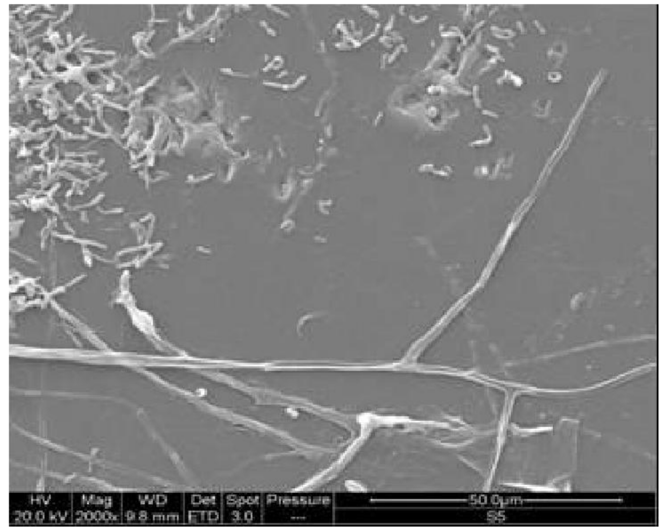


**Figure 2.** The scanning electron micrograph shows mixed microbial population on the surface of expanded polystyrene film after incubation (30˚C) for 3 months on mineral salts media agar plate without any other carbon source.

#### Isolation of bacteria

The buried films were recovered after 8 months to isolate the adhered bacteria. The films were cut into pieces, washed with sterilised water and placed on mineral salts media agar plates (Motta et al., 2009). The mineral salts media contained $K_2HPO_4$, 1 g; $KH_2PO_4$, 0.2 g; NaCl,1 g; $CaCl_2.2H_2O$, 0.002 g; Boric Acid, 0.005 g; $(NH_4)_2SO_4$, 1 g,; $MgSO_4.7H_2O$, 0.5 g; $CuSO_4.5H_2O$, 0.001 g; $ZnSO_4.7H_2O$, 0.001 g; $MnSO_4.H_2O$, 0.001 g and $FeSO_4.7H_2O$, 0.01 g per litre distilled water. The plates were incubated at 30˚C. Environmental scanning electron microscopy (FEI Quanta 200) was used to visualize the adhering microbes on expanded polystyrene film. For isolation of bacterial strains loop full of inoculum was taken from MSM plates and streaked on nutrient agar plates. Nutrient agar plates were incubated at 30˚C. To exclude the growth of Fungi antifungal agent Nystatin 0.5 ml (1% (w/v) stock sol.) was added to nutrient agar media. Sub-culturing many times was done to get pure cultures. Serial dilution and plating onto nutrient agar plates was also used to isolate bacteria.

#### Molecular identification

Bacterial DNA was extracted manually by boiling a loop full of culture in sterilized distilled water and centrifugation at 13, 000 rpm for 10 min. The supernatant containing the extracted DNA was used to amplify 16S ribosomal DNA segments by PCR (Bio-Rad i cycler) using 16S- 27F and 16S- 1492R. Bioline, Biotaq$^{TM}$ DNA Polymerase kit and dNTPs set was used. DNA was visualised at 80 V and 400 mA for 35 min on agarose gel (0.8% (w/v) in TAE buffer 1x, 0.1 µl Ethidium Bromide solution).

Concentration of DNA was determined by nanodrop spectrophotometer (Nanodrop™ 1000). The amplified PCR products were purified by QIAquick® PCR Purification Kit (Qiagen Ltd., Crawley, United Kingdom). The sequencing was done at the facility of University of Manchester. The obtained sequences were subjected to BLAST search in NCBI database for phylogenetic relationship.

#### Biodegradation studies

The bacterial isolates were subjected to shake flask incubation (30˚C, 120 rpm) with pure polystyrene in mineral salts media in 250 ml Erlenmeyer flasks. Inoculums were prepared in nutrient broth.

#### Analysis of biodegradation

FTIR spectroscopy (Bio- Rad Merlin Excalibur) was employed to study surface changes of polystyrene films. Biodegradation products were analyzed by High Pressure Liquid Chromatography (Shimadzu) . Standards used were 2-phenyl ethanol, 1-phenyl-1, 2-ethanediol, Phenylacetaldehyde, Styrene oxide and Styrene (Sigma Aldrich).

## RESULTS AND DISCUSSION

### Isolation and identification of microorganisms

The growth of microbial consortia was visible by naked eye on the expanded polystyrene film in the absence of any other carbon source as shown in Figure 1. Electron micrograph also showed extensive growth of mixed microbial population on polymer surface as presented in Figure 2. The bacterial isolates were identified on the basis of 16S ribosomal RNA conserved sequences. The bacterial isolated strains were identified as *Microbacterium* sp. NA23, *Paenibacillus urinalis* NA26, *Bacillus* sp. NB6, *Pseudomonas aeruginosa* NB26. The sequences were submitted to NCBI Gene bank and accession numbers were obtained (Table 1). Bacterial growth and adherence with the expanded polystyrene film for longer period of time without any other carbon source indicate that isolated soil bacteria are able to

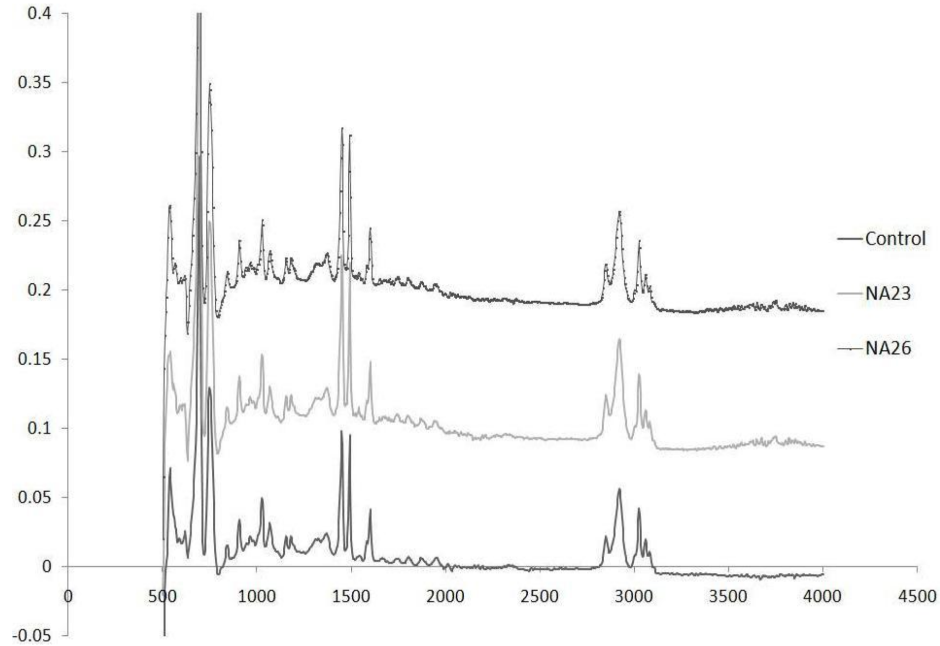


**Figure 3.** FTIR spectra of control and 8 weeks treated polystyrene films with bacterial isolates NA23 and NA26 at 30˚C, 120 rpm.

colonize and use expanded polystyrene as a sole carbon source. The microbial colonization of a polymer surface is the first requirement for its biodegradation (Yabannavar and Bartha, 1993). Soil burial is employed to study the biodegradability of polymers (Yabannavar and Bartha, 1993; Orhan and Büyükgüngör, 2000; Rizzarelli et al., 2004; Alvarez et al*.,* 2006; Schlemmer et al., 2009). Soil burial is much close to the natural conditions encountered by the waste polymer materials (Alvarez et al., 2006) and act as a field test for further application of biodegradation studies.

The microbial population is influenced by the materials in the surrounding environment. Those soil microbes that will be able to best utilise the carbon contained in the polymer will be abundant while others will not survive. There is a wide variety of degradation pathways employed by a large biodiversity of microorganisms to metabolize aromatic hydrocarbons. Such organisms are the main focus of research for clean- up of environmental pollution (Atlas and Cerniglia, 1995; Van Hamme et al*.,* 2003). Natural physical and chemical spoilage processes in various materials are characterized as biodegradation. The organisms involved are called biodeteriogens that possess the saprotrophic ability of using substrata to sustain their growth and reproduction (Pinzari et al*.,* 2006).

**Biodegradation studies**

FTIR spectroscopy was used for the analysis of films recovered from the shake flask experiments. There was no increase of area of absorption peaks in the treated and control films of polystyrene as illustrated in Figures 3 and 4 indicating that no significant surface changes had occurred during 4 weeks of incubation with bacterial isolates. FTIR spectroscopy is used as analytical technique in many biodegradation studies (Kiatkamjornwong et al., 1999; Klrbas et al., 1999; Arboleda et al., 2004; D ímal et al., 2007). Synthetic polymers especially polyolefins, made up of only carbon and hydrogen atoms, are generally less susceptible to microbial attack. Their inertness is probably due to a total lack of carbon-to-oxygen bonds (C = O, C–OR, C–OH), which are the sites of microbial enzymes attack (Motta et al*.,* 2007).

Polystyrene structurally consists of aliphatic chain with aromatic ring attached to every other carbon atom. Styrene is the monomer of polystyrene and its biodegradation by bacteria and fungi is well established in the literature (Mooney et al., 2006). Styrene biodegradation intermediates were used as standards for the HPLC analysis. The results of HPLC analysis are summarised in Figure 5. 1-phenyl1, 2 ethandiol was detected in the extracellular media of the strains NA26 (9.88 ppm), NB6 (14.31 ppm) and NB26 (0.36 ppm). 2-phenylethanol was detected in the samples of strains NA26 (3.16 ppm) and NB26 (0.85 ppm) after 4 weeks of incubation with polystyrene films. Extracellular media was used to study the biodegradation products as the polymer molecule can not be taken up by the microorganism as such inside the cell. The long chains of the polymer are

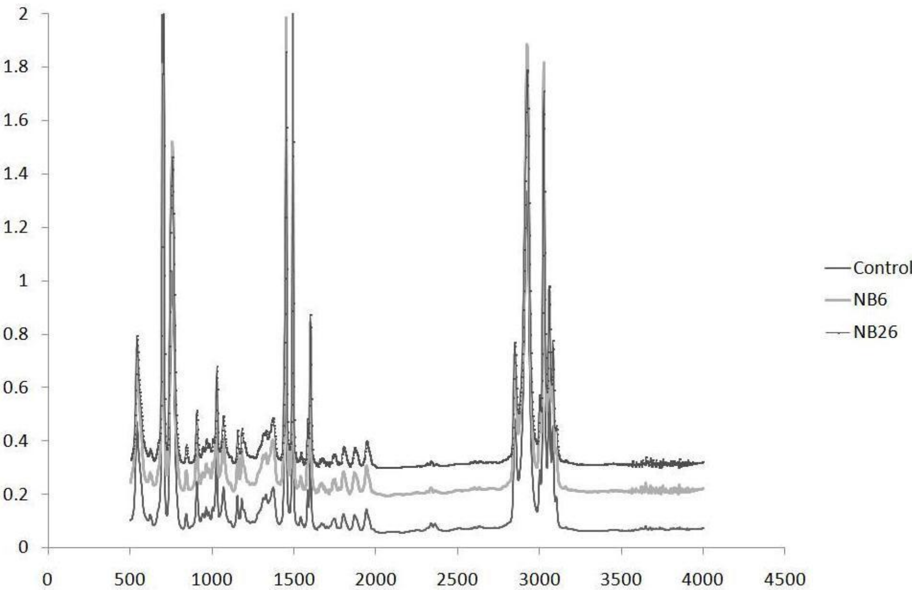


**Figure 4.** FTIR spectra of control and 8 weeks treated polystyrene films with bacterial isolates NB6, NB26 at 30˚C, 120 rpm.

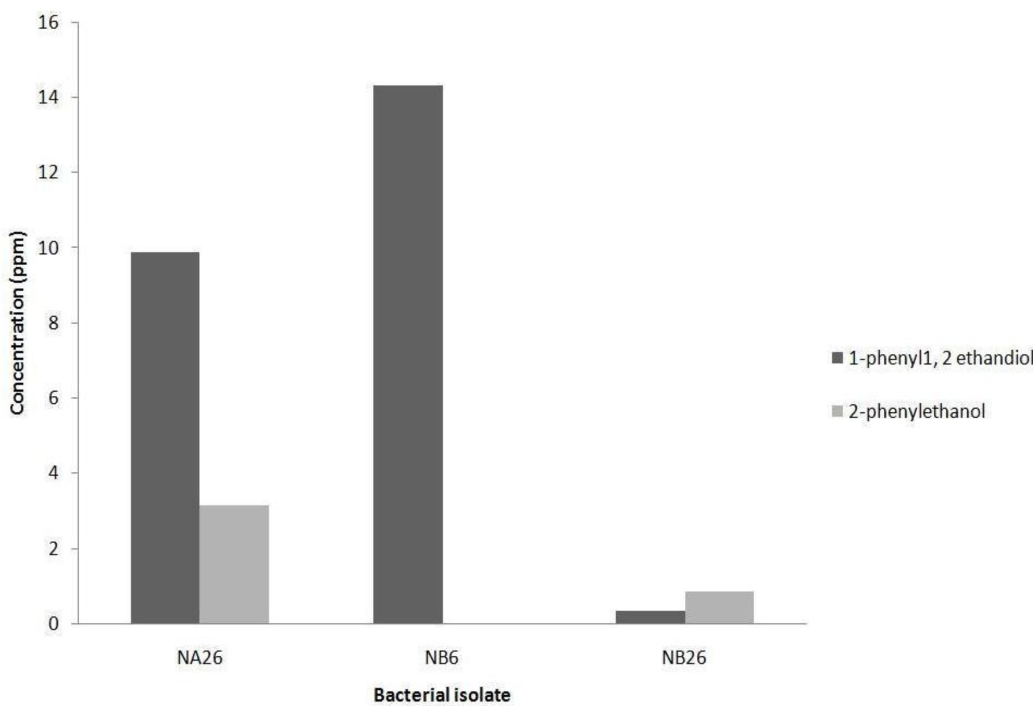


**Figure 5.** HPLC Analysis of biodegradation products of polystyrene by bacterial isolates after 8 weeks incubation at 30˚C, 120 rpm.

broken down into small molecules by extracellular enzymes that are absorbed for further metabolism inside the cell. The biodegradation of polymers is usually started by oxidation process. Oxidases and peroxidases oxidize appropriate substrates to carbonyls, alcohols or aldehyde groups. Peroxidases reduce dissolved oxygen to peroxide. Laccases reduce oxygen to water and oxidize phenolic and non-phenolic substrates with the formation of quinones or phenoxy radicals and cation radicals (Moen and Hammel, 1994; Rabinovich et al., 2004).

The detection of metabolites in the extracellular environment is the indication that the bacterial isolates NA26, NB6, NB26 were able to extract some carbon from the complex molecules of polystyrene but the process is very slow and causes no significant chemical changes on the surface. The strain development by molecular techniques can be employed to improve the biodegradation potential of the isolated strains.

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