Shyamala Gowri R et al. / Vol 6 / Issue 2 / 2016 / 64-68.

International Journal of

Innovative Drug Discovery

e ISSN 2249 - 7609
Print ISSN 2249 - 7617

www.ijidd.com

ASSESSMENT AND MICROBIAL DEGRADATION OF POLYETHYLENE GLYCOL

R. Shyamala Gowri[*], T. Kuberan[#] and V. Prabhakaran[$]

*Department of Microbiology and Biochemistry, Nadar Saraswathi College of Arts and Science, Theni 625 523, Tamil Nadu, India.
[#]UPASI Tea Research Foundation, Regional centre-Coonoor, The Nilgiris 643 101, Tamil Nadu, India
[$] Department of Zoology, Government Arts College, Melur 625 203, Madurai, Tamil Nadu, India

ABSTRACT

The polyethylene glycol 6000 degrading organism was isolated from the sewage sludge and it was determined as *Pseudomonas fluorescence* by morphological and biochemical characterization. The specific, relative and intrinsic viscosity of the polymer was determined by Ostwald Viscometric Method as 0.086, 1.086 and 0.43 respectively. Biodegradation of polyethylene glycol 6000 was carried out by *Pseudomonas fluorescence*, *Pseudomonas aeruginosa 2962* and *Pseudomonas stutzeri 2643* at various concentrations (100mg, 300mg and 500mg). Growth activity of organism in synthetic medium and soil medium was determined by turbid metric method and most probable number method. Among three types of microorganism, the *Pseudomonas fluorescence* showed better growth and activity in all concentration of polyethylene glycol. Among three different microorganism test, *Pseudomonas fluorescence* was found to be efficient in biodegradation compared to the *Pseudomonas aeruginosa 2962* and *Pseudomonas stutzeri 2643*. The residues of polyethylene glycol was observed in different samples was performed by High performance Liquid Chromatography (HPLC).The changes in the functional group was observed by Fourier Transform Infrared Spectrophotometer.

KEY WORDS: Polyethylene glycol, HPLC, FTIR, Biodegradation, *Pseudomonas.*

INTRODUCTION

Polyethylene Glycols (PEGs), $HO(CH_2\ CH_2O)_n\ H$ are an important group of nonionic synthetic water – soluble polymers of ethylene oxide. These compounds are widely used in the production of pharmaceuticals, cosmetics, lubricants and antifreeze for automobiles radiators, in the conservation treatment of ancient waterlogged wood, and in the manufacture of non-ionic surfactants. Every year, millions of tons of polyethylene glycol are manufactured worldwide and most of them reach conventional sewage disposal systems after industrial utilization. From the last three decades, concern has been expressed about the fate of these polymers in the environment [6]. Polyethylene glycol was once thought to be a recalcitrant xenobiotic with nondegradable ether bonds, but both aerobic and anaerobic biodegradation have been reported [2]. Under aerobic conditions, polyethylene glycol is degraded by dehydrogenation to either carboxylated intermediates or glycoldehyde by acetaldehyde production and by extra cellular hydrolytic cleavage with production of ethylene glycol and diethylene glycol [4]. Polyethylene Glycol also known as polyethylene oxide (PEO) or Polyoxyethylene (POE) is the most commercially important type of polyether. Polyethylene glycol, polyoxyethylene or polyethylene oxide refers to an oligomer or polymer of ethylene oxide. Polyethylene glycols are prepared by polymerization of ethylene oxide and are commercially available over a wide range of molecular weights from 300mg/mol to 10,000,000g/mol, polyethylene glycol is soluble in water, methanol, benzene, dichloromethane and is insoluble in diethyl ether and hexane.

Corresponding Author:- **R. Shyamala Gowri** Email:-siya_gowri@yahoo.in

Polyethylene glycol is produced by the interaction of ethylene glycol is produced by the interaction of ethylene oxide with water, ethylene glycol or ethylene glycol oligomers [7]

Most commercially produced nonionic detergents contain polyethylene glycols as hydrophilic moieties [1]. Although vast amounts of this material are produced by the chemical industry, information on its biodegradability is less.

Aerobic microorganisms use both ethylene glycol and polyethylene glycols as sources of carbon and energy [5]. The bacterial metabolism of polyethylene glycol was clarified by [8], who found that the first step of polyethylene glycol metabolism was catalyzed by polyethylene glycol dehydrogenase.

The present study was done on the biodegradation of recalcitrant compound polyethylene glycol 6000 by versatile organisms such as *Pseudomonas fluorescence, Pseudomonas stutzeri 2643, Pseudomonas aeruginosa 2962.*

MATERIALS AND METHODS

Chemicals and Materials

Polyethylene glycol 6000 and other chemicals used in this study was the product of Hi Media Lab Pvt Ltd., Mumbai and glass wares were the product of Borosil Laboratory, Mumbai.

Isolation and Characterization of bacteria

Polyethylene glycol 6000 degrading bacteria was obtained from municipal sewage sludge at Srivilliputtur. This isolated bacterial strain was maintained in a slant containing glycerol, 0.5%, polypeptone, 0.1%, K_2HPO_4, 0.2%, $NaHPO_4$, 0.1% $MgSO_4$.7H2O, 0.02%; Yeast extract, 0.02%; Polyethylene glycol 6000, 0.5% and agar, 2.0%. The pH of medium was adjusted to 7.0-7.2. The bacterial isolate undergo for Biochemical and Cultural characterization to identify the organism.

Determination of specific, relative and intrinsic viscosity of polymer (Polyethylene glycol 6000)

The specific, relative and intrinsic viscosity of polymer was determined by Ostwald viscometric method.

Stock preparation: 2gm of polyethylene glycol 6000 was dissolved in dimethyl sulfoxide and used as a stock solution.

From the above stock solution 0.2% polyethylene glycol 6000 solution was prepared.

Flow time of solvent and flow time of polymer solution was observed in Ostwald viscometer.

$\eta = nsp/c.$

η sp = specific viscosity.

C = concentration of polymer solution,

η = intrinsic viscosity

η sp = nr-1.

η r = relative viscosity

η r = Flow time of polymer solution(t)/ Flow time of solvent (to).

Biodegradation of polyethylene glycol 6000

Different concentration of polyethylene glycol 6000 was taken in both soil and synthetic medium (NH_4SO_4 - 0.5gm, $MgSO_4.7H_2O$ - 0.02gm, K_2HPO_4 - 0.2gm, yeast extract 0.05gm and NaH_2PO_4 -0.01gm in 100ml distilled water) and inoculated with MTCC (*Pseudomonas aeruginosa 2962* and *Pseudomonas stutzeri 2643*) and bacterial isolate.

Determination of bacterial growth and activity in soil medium

The growth and activity of organism in soil medium was determined by Most Probable Number Method. The MPN method involved decimal dilution of 1 ml sample in tubes containing the basal medium (in g/ l) of distilled water: bacteriological Peptone, 5,0; Yeast extract, 3.0; D-Glucose,1.0 (pH 6-6.5). Inoculated tubes were incubated for ten days at 25.c. A pH drop and pink colour of methyl red indicator were taken as evidence of bacterial activity in soil.

Determination of bacterial growth and activity of organism in synthetic medium

In synthetic medium the growth and activity of organism was determined by turbidimetric method. In this method, the turbidity of the inoculated medium was determined by turbid meter. In this method, distilled water or blank solution was taken into test tube and place in the test tube holder. The required range for measurement was selected and the display was adjusted to 000 by adjusting "set zero "knob, The test tube containing distilled water was removed and test tube containing standard solution was inserted into the test tube holder. Measurement of the solution suspension was taken and "calibrate" knob as adjusted top the select solution value 100 NTU. After adjusting the standard solution value, test samples were taken in the test tube and readings were taken.

Determination of Biodegradation of polyethylene glycol 6000 by High Performance Liquid Chromatography

Polyethylene glycol 6000 was extracted from soil medium and synthetic medium using methanol as a solvent. The extracted polyethylene glycol 6000 was analyzed by HPLC [C18 Silica Capillary column 10AT up Shimadzu Company].

Determination of biodegradation of polyethylene glycol 6000 by Fourier transform infrared spectrophotometer (FTIR) analysis

The inoculated samples were given for FTIR analysis (FTIR 8400S Shimadzu) to identify the biodegradation of polyethylene glycol 6000 by determining the chemical changes in the functional group.

RESULTS

Isolation and characterization

In the present study, different bacterial strains were isolated from municipal sewage at Srivilliputur by enrichment technique, nine colonies were observed in l00 mg concentration of polyethylene glycol 6000, five colonies were observed in 300 mg concentration of polyethylene glycol 6000 and one colony was observed in 500 mg concentration. The one colony obtained in 500 mg concentration was used or the further studies and it is identified as *Pseudomonas fluorescence* by morphological and biochemical characterization. The isolated organism was a gram negative rod and motile. It showed a positive result for citrate utilization, oxidase, catalase, nitrate reduction and gelatin liquefaction test. The isolated organism also showed a positive result to the carbohydrate such as Xylose, galactose, mannose, ribose and glycerol.

Determination of bacterial growth and activity in synthetic medium

The bacterial activity in synthetic medium was determined by turbidimetric method. Among the three strains *Pseudomonas aeruginosa 2962, Pseudomonas stutzeri 2643* and *Pseudomonas fluorescence,* inoculated sample (Table 1).

Determination of bacterial growth and activity in soil medium

The bacterial growth and activity in soil medium was determined by most probable number method. The formation of pink colour by the addition of methyl red indicator to the basal medium, indicates the bacterial growth and activity in soil (Table 2)

Analysis of biodegradation of polyethylene glycol 6000 by HPLC

Different concentration of polyethylene glycol 6000 in both soil and synthetic medium inoculated with *Pseudomonas fluorescence*, *Pseudomonas aeruginosa 2962* and *Pseudomonas stutzeri 2643* showed a different peak at retention time of 5 minutes. The HPLC result were tabulated in table 3

Analysis of biodegradation of polyethylene glycol 6000 by FTIR

The different intermediate compounds such as carboxylic acid, aldehydes and ketones are formed due to biodegradation of polyethylene glycol 6000 by inoculated organisms.

Table 1. Turbidimetric Method To Determine The Bacterial Growth In Synthetic Medium

S.NO	Concentration of polyethylene glycol (mg)	*Pseudomonas aeruginosa 2962*	*Pseudomonas stutzeri 2643*	Bacterial Isolate(*Pseudomonas fluorescence*)
1	100 mg	078	064	084
2	300 mg	067	057	072
3	500 mg	055	045	059

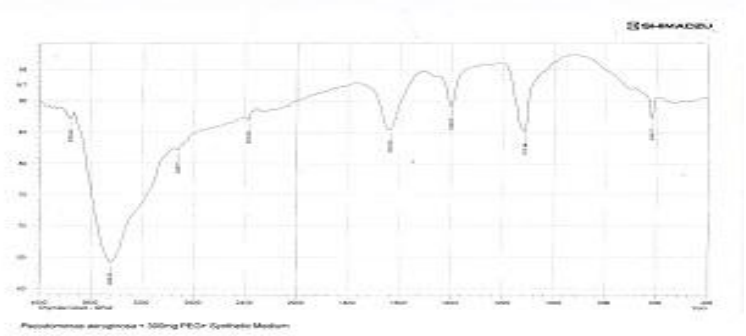
Table 2. Determination of bacterial Growth and activity in Soil Medium.

S.NO	Concentration of polyethylene glycol (mg)	*Pseudomonas aeruginosa 2962*	*Pseudomonas stutzeri 2643*	Bacterial Isolate (*Pseudomonas fluorescence*)
1	100 mg	Positive	Positive	Positive
2	300 mg	Positive	Positive	Positive
3	500 mg	Positive	Positive	Positive

Table 3. Analysis of biodegradation of polyethylene glycol 6000 by HPLC:

Organism	Peak formed for control sample	Peak formed for test sample Synthetic medium	Soil medium
100 Mg Concentration			
Pseudomonas aeruginosa 2962	5.180	5.140	5.067
Pseudomonas stutzeri2643	5.180	5.110	5.113
Pseudomonas fluorescence	5.180	5.030	5.013
300 Mg Concentration			
Pseudomonas aeruginosa 2962	5.243	5.230	5.230
Pseudomonas stutzeri2643	5.243	5.210	5.215
Pseudomonas fluorescence	5.243	5.207	5.200
500 Mg Concentration			
Pseudomonas aeruginosa 2962	5.287	5.280	5.280
Pseudomonas stutzeri2643	5.287	5.270	5.263
Pseudomonas fluorescence	5.287	5.250	5.243

Figure 1. Analysis of biodegradation of polyethylene glycol 6000 by FTIR



DISCUSSION

Bacteria can degrade and detoxify wide range of compounds. The bacteria can degrade the compounds such as polyethylene glycol, hydrocarbon and aromatic compounds. Some of the microbial catabolic pathways are responsible for degradation which have been extensively characterized and are generally located on large catabolic plasmid usually found in *Pseudomonas sp* [11].

Generally the natural degradation is shows due to various reasons; they may be bioavailability, physical, chemical factors unsatisfactory. pH, oxygen requirement, moisture, redox potential, concentration of pollutants, recalcitrance(basic chemistry of pollution responsible) , and xenobiotic nature of pollutant. Biodegradation ameliorates these factors through optimization of environmental conditions [9].

In this context, the efficacy of degradative by the bacteria, *Pseudomonas fluorescence, Pseudomonas aeruginosa 2962, Pseudomonas stutzeri 2643* were assessed in order to advocate these parameters for the restoration of environment. Eventhough selective studies were made with polyethylene glycol but the role of microorganism like *Pseudomonas sp* in environment degradation of polyethylene is not well documented.

Role of microbes on biodegradation has been clearly investigated in concept of enrichment culture. It is a powerful and simple technique. During enrichment, in 100mg, 300mg, 500mg concentration of polyethylene glycol, One colony was observed in the 500mg concentration polyethylene glycol. This strain was used for the biodegradation study. The strain was confirmed as *Pseudomonas fluorescence* by biochemical and cultural characterization.

In viscometric method the viscosity of the polymer was determined by Ostwald viscometric method. The relative viscosity of polymer was 1.0860, specific viscosity polymer was 0.0860 and intrinsic viscosity of polymer was 0.43. By turbidimetric method the growth rate of organism was analyzed. When the concentration of polyethylene glycol is increased, the growth of organism was decreased. It may be due to the inhibition of activity of microorganism in the medium at high concentration of polyethylene glycol

By most probable number method the activity of organism in soil was determined. The pink colour was observed when methyl red indicator was added to the medium. Due to degradation of polyethylene glycol and formation of acid end products leads to the decrease in pH and the medium colour was changed from yellow to pink.

Biodegradation of polyethylene glycol was analysed by High Performance Liquid Chromatography method. By determining the peak formation at retention time 5 for control and experimental sample, the effect of degradation was analyzed. Among three different strains such as *Pseudomonas fluorescence* and MTTC obtained culture (*Pseudomonas aeruginosa 2962* and *Pseudomonas stutzeri 2643*) biodegradation of polyethylene glycol at various concentrations was done effectively by *Pseudomonas fluorescence*. The biodegradation rate was high at 100mg concentration than 300mg and 500mg concentration of polyethylene glycol.

The reason probably may be due to microbial transformation. Microbial transformation occurs due to mineralization. Mineralization of compounds is most desirable because it generates carbon and energy for growth of organism and leads to disappearance of xenobiotic compound. Transformation in the environmental does not necessarily results in the complete oxidation of xenobiotic compound. But may lead to accumulation of transformation products with increased or decreased toxicity as compared to the original compound. By comparing the peak formation of functional group between the control and experimental sample, the biodegradation of polyethylene glycol was determined. In *Pseudomonas fluorescence, Pseudomonas aeruginosa 2962* and *Pseudomonas stutzeri 2643* treated sample additional peaks for O-H stretch carboxylic acid, S=O sulfones, C-H stretch epoxide, O-H stretch alcohols was formed where as these peaks are absent in the control sample. It is presumed that additional peaks obtained is due to break down of polyethylene glycol by microorganism.

Impact of degradation of polyethylene glycol by *Pseudomonas fluorescence ,Pseudomonas aeruginosa 2962* and *Pseudomonas stutzeri 2643* with the regard to the application of the sample to FTIR. Taking a clue from the

literature report by [10] and also in present study which indicate the effects of bacteria in the process of biodegradation. FTIR is used to determine the presence of groups in the polymer. The degradation products and chemical moieties are incorporated in to polymer molecules. Our results confirmed indirectly, a mechanism of polyethylene glycol degradation by *Pseudomonas sp* which is also checked out with similar work carried out by [3], where in the hydroxyl group of polyethylene glycol is shifted to sub terminal carbon atom by polyethylene glycol degrading enzyme by this reaction the cleavage of polyethylene glycol is preferred because hemiacetal which is formed unstable and it easily releases acetaldehyde. After degradation of polyethylene glycol chain the remaining residues of polyethylene glycol was cleaved by diol dehydratase, an enzyme responsible for cleaving polyethylene glycol into acetaldehyde and water.

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

REFERENCES

1. Bock KJ and H Stache. Surfactants. The hand book of environment chemistry, 3B, 1982, 163-199.
2. Cox DP. The biodegradation of polyethylene glycols. *Adv Appl Microboil*, 23, 1978, 173-194.
3. Frings J Schramm E and B Schink. Enzymes involved in anaerobic polyethylene glycol degradation by *Pelobacter venetianus* and *Bacteriodes*. *Appl. Environ Microbiol*, 58, 1992, 2164 – 2167.
4. Haines JR and M Alexander. Microbial degradation of polyethylene glycols. *Appl Microbiol*, 9, 1975, 217-229
5. Hosaya H, Miyazaki N, Sugisaki Y, Takanash E, T surufuji M, Yamasaki M and G Tamura. Bacterial degradation of synthetic polymers and oligomers with the special reference to the case of polyethylene glycol. *Agric Biol Chem*, 42, 1978, 1545-1552.
6. Huang YL, Li QB, Deng X, Lu YH, Liao XK, Hong MY and Y Wang. Aerobic and anaerobic biodegradation of polyethylene glycols using sludge microbes. *Process Biochemistry*, 40, 1980, 207-211.
7. Kahovec J, Fox RS and K Hatada. Nomenclature of regular single – strand organic polymers. *Pure and applied chemistry*, 74, 1921, 1956.
8. Kawai F, Kimura T, Tani Y, Yamada H and M Kurachi. Purification and characterization of polyethylene glycol dehydrogenase involved in the bacterial metabolism of polyethylene glycol. *Appl Environ. Microbiol.* 40, 1978, 701-705.
9. Romas JL, Duque E, Rodiguez JJ, Godiy P and A Fernandez Barrerro. Mechanisms for solvent tolerance in bacteria. *J.Biol. Chem*, 272, 1995, 3887-3890.
10. Sudhakar M, Trishu A, Syed Jahan S, Umadevi VR, Venkatesan R and P Sriyutha murthu. Biofouling and biodegradation of polyolefin's in ocean waters. *Science Direct*, 92, 2007, 1743- 1752.
11. Whyte LG, Bourbonniere L and CW Greer. Biodegradation of petroleum hydrocarbons by Pseudomonas strains possessing both alkane and naphthalene catabolic pathways. *Applied and Environmental Microbiology*, 63(9), 1997, 3719- 3723.