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Identification of Bacterial Species Capable of Degrading LDPE

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Abstract: ***The Plastic is polymeric material having capability of being molded or shaped by the application of heat and pressure. The low-density polyethylene(LDPE) is the type of plastic which is used in various purpose of our daily life. It is one of the major environmental problems because of its slow degradation rate .To control the pollution caused by low-density polyethylene, microbes are isolated from the dumping site of the Durg region of Chhattisgarh. These microbes are screened by the zone of clearance method for degradation of low-density polyethylene using PEG as substrate. The screened microbes were identified by morphological and biochemical characteristics.Nine bacterial isolates was selected for biodegradation studies.Confirmation of bacterial biodegradation of low-density polyethylene was performed by using weight loss measurement and pH change due to production of acidic product.One potential isolate were further identified through 16S rRNA sequences Alcaligenes faecalis was shown the high degradation rate of low-density polyethylene.***

Keywords: Plastic, Low-density polyethylene, zone of clearance, Biochemical characteristics, weight loss measurements, Alcaligenes faecalis .

I. INTRODUCTION

Plastic is a very big problem for environment because of its slow degradation rate. Plastics are the polymer which can be changed into variety of shapes by heating .It is used in various purposes such as packaging of food materials, cosmetics, pharmaceutical products.Plastics includes polyethylene {Low-density polyethylene(LDPE),High density polyethylene(HDPE)}, polypropylene, polystyrene, polyvinyl chloride, polyethylene terephthalate, nylon. Plastic degradation refers to the physical or chemical change in the polymer due to environmental factors like light, heat, moisture, chemical condition and biological activity [1].

Low-density polyethylene is the most important environmental problem because its chemical structure containing C-C and C-H bonds. Low-density polyethylene degradation is very difficult due to its recalcitrant nature. Recalcitrant nature is due to the hydrophobic property ,high molecular weight and three-dimensional structure [2].

Environmental factors like heat, moisture, light, chemical condition and biological activity results in the physical or chemical change of the polymer .Biodegradation is the process of degradation of substance by using microbes.Microorganism release some enzyme which change the polymer into oligomers, dimers and monomers. In natural condition the degradation rate of polyethylene is quite slow if it is in natural condition.Microorganisms such as Bacillus strain ,*Pseudomonas* species etc. have been reported to degrade low-density polyethylene.[2],[3],and [10].

The aim of the present study is to isolate and screen the indigenous microbial species capable of degrading low-density polyethylene.

II. MATERIALS AND METHODS

A. Collection of soil sample

The soil samples were collected from several sites of Durg district of Chhattisgarh such as waste dumping sites of potiya , chandkhuri , kolihapuri.

B. Collection of polyethylene films

The low-density polyethylene films (0.910-0.940g/cm^3) were collected from the local market of Durg [4] and [5].

C. Isolation of LDPE Degrading Bacteria

The low-density polyethylene degrading bacteria was isolated by using the synthetic medium containing NH_4NO_3 (1.0g), $MgSO_4$.7H2O (0.2g), K_2HPO_4 (1.0g), $CaCl_2.2H_2O$ (0.1 g), KCl (0.15g), Yeast extract (0.1g), $FeSO_4.6H_20$(1.0mg),$ZnSO_4.7H_2O$(1.0 mg),$MnSO_4$(1.0 mg),Tween 60,or 80 [0.01-0.50%(v/v)], and Agar by serial dilution and spread plate method and incubated at 37^0 C for 24 hrs.

(a) (b) (c)

Fig.1 (a) and (b) waste dumping site (c) Sub culturing plates of isolated bacteria

D. Screening of polyethylene degrading bacteria

The screening of low-density polyethylene degrading bacteria was done by zone of clearance methods where 0.5 concentration of PEG were used in minimal media containing salts of ammonium and potassium .The zone of clearance around the colonies were observed by staining with Coomassie blue [6].

E. Biochemical characterization

The bacterial isolates were initially identified by morphological and biochemical characteristics. Morphological characterization of the isolates was done by the Gram staining Biochemical characterization was done through IMViC, Carbohydrate fermentation, Gelatine hydrolysis, Starch hydrolysis, Catalase, Urease production tests [6] and [7].

F. Biodegradation studies

1) Weight loss measurement

The weight loss was measured by inoculating the isolated bacteria into the minimal medium.The thin film of polyethylene plastic (2 × 2 cm) was aseptically inserted and incubated in rotatory flask shaker at 120 rpm for 30 Days.After incubation polyethylene films were removed from the medium and washed with 2 %(v/v) sodium dodecyl sulfate solution and washed with distilled water.The LDPE is then dried overnight at 50 ^{0}c and weight loss was calculated by using the formula [8].

$$\textbf{\textit{Weight loss}}\ \% = \frac{\textbf{initial weight} - \textbf{final weight}}{\textbf{initial weight}} \times \mathbf{100}$$

2) pH change

pH changes in a medium due to metabolic activity of the microbial strain shows the evidence of polyethylene degradation.

G. Molecular identification

The potentially best bacterial strain in weight loss and pH change(BS2) measurements was sequenced through 16S ribosomal RNA gene for molecular characterization as it shows similarity of 95.69 % with ***Alcaligenes faecalis*** .

G. Fourier transform infra-red (FTIR) spectroscopy

FTIR is the preferred method of infrared spectroscopy.It was used for the identification of functional groups present in the degraded low-density polyethylene. In this analysis infrared radiation is passed through the sample and analyze the percentage of degradation using FTIR[9].

III. RESULT

A. Morphological and biochemical characteristics

Three different samples were identified by microscopically and identified by various biochemical tests (Table 1).The identified strains were *Klebsiella* sp., *Alcaligenes sp.*and *Pseudomonas sp*. respectively.

TABLE 1

Morphological and Biochemical characteristics

Bacterial isolates	Gram staining	Indol	MR	VP	Citrate	Starch Hydrolysis	Catalase	Urease	Gelatine	Fermentation of carbohydrates	Probably identified organism
BS1	Gram –ve, Rod shaped	-ve	+ ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	*Klebsiella sp.*
BS2	Gram –ve Rod shaped	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	*Alcaligenes sp.*
BS4	Gram –ve bacilli	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	*Pseudomonas sp.*

B. Weight loss measurements

Weight loss measurement of polyethylene film was done by using mineral salt medium at the interval of "10 days" .Sample BS2, (Table 2) showed the high degradation of polyethylene in "30 days" i.e.50% .

TABLE 2
Weight loss measurements

Sample code	Strain	Weight loss in 30 Days
BS1	*Klebsiella sp.*	20 %
BS2	*Alcaligenes sp.*	50 %
BS4	*Pseudomonas sp.*	12.5 %

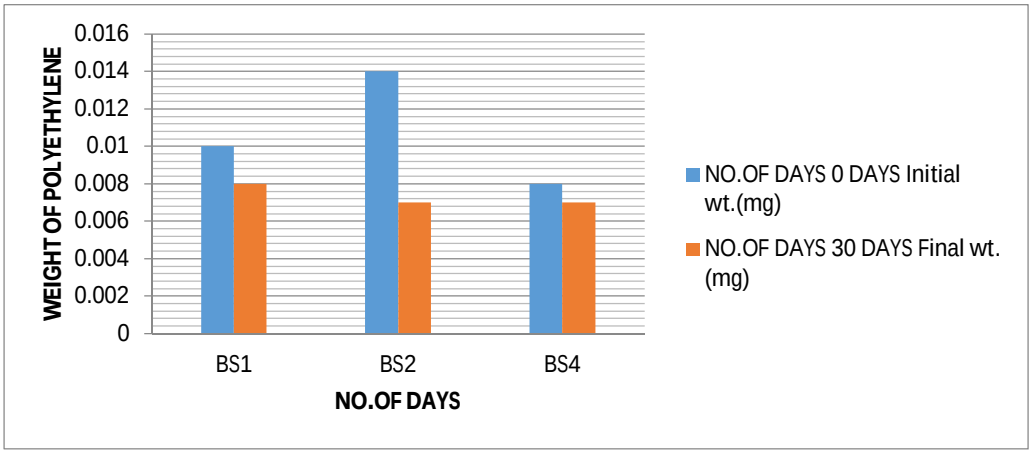


Fig.2.Graph represents the weight loss of low-density polyethylene at the interval of 30 days.

C. pH change

pH change was measured over "10-days".The change in pH (Table 3) confirmed the polyethylene degradation by the acid production.

TABLE 3
MEASUREMENT OF PH CHANGE

Sample code	Strain	Initial pH	pH Change in 10 days	pH Change in 20 days	pH Change in 30 Days
BS1	*Klebsiella sp.*	7.0	6.76	6.33	6.00
BS2	*Alcaligenes sp.*	7.0	6.73	6.34	5.90
BS4	*Pseudomonas sp.*	7.0	6.74	6.37	6.32

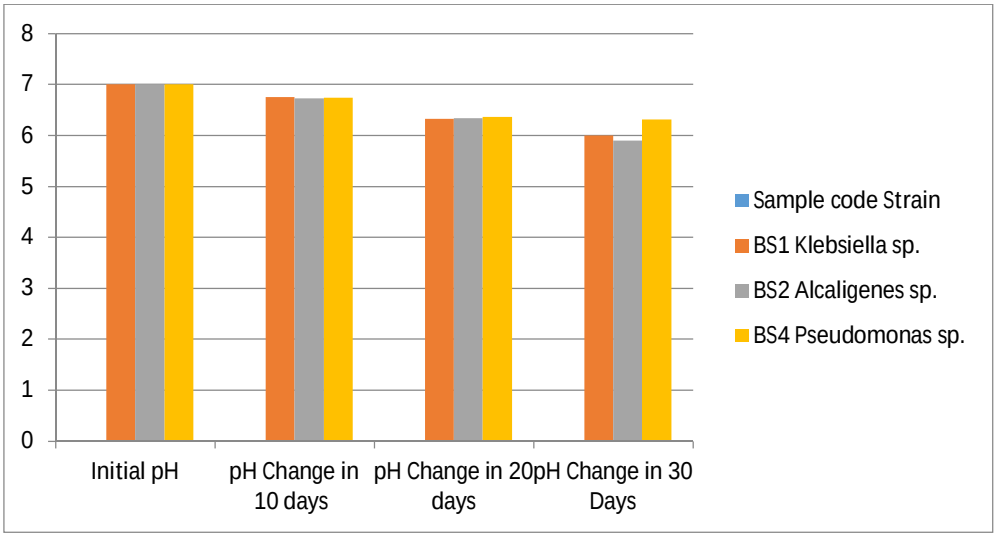


Fig.3.Graph shows the pH Change at the interval of 10 days

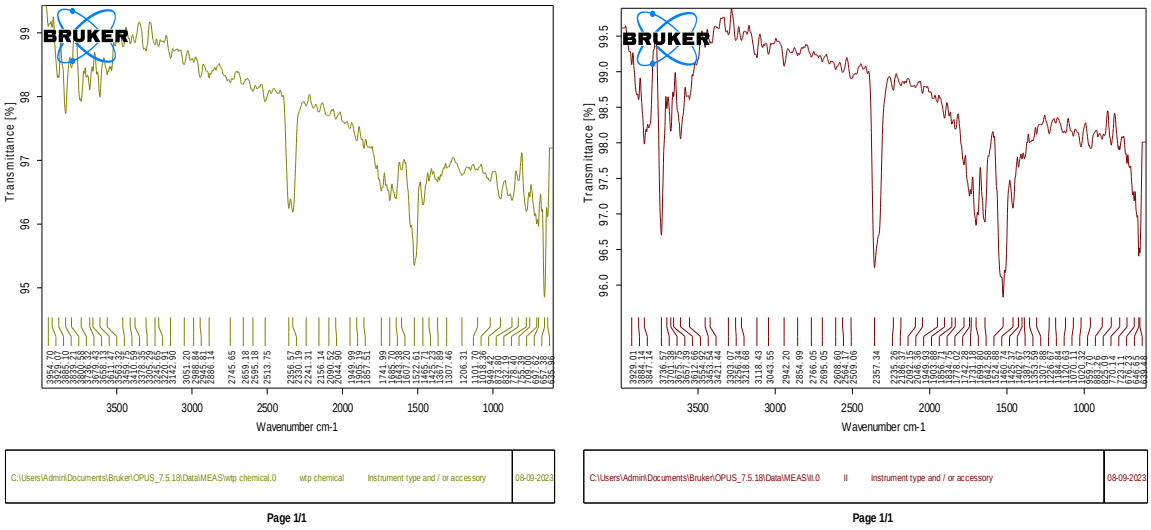
D. Molecular identification

The bacterial strain BS2, showed the high degradation rate which identified by molecular characterization and identified as ***Alcaligenes faecalis*** strain 1SJ128.The phylogenetic analysis is shown in fig.4.

Fig.4 Phylogenetic analysis of Alcaligenes faecalis strain 1SJ128 16S ribosomal RNA gene.

E. FTIR

Before degradation, the chemical structure of polyethylene film consists of repeated monomers made from carbon and hydrogen atoms containing C-C and C-H bonds but after degradation polyethylene sample(Fig.4) contain C-N,-C=O,-CH_3,-OH,-NH bonds show the polyethylene degradation.



(a) (b)

Fig.5. FTIR spectrum of biodegradation of low-density polyethylene film after 30 days of incubation (a) control (b)FTIR spectrum of Alcaligenes faecalis.

IV. DISCUSSION AND CONCLUSION

Microbial-based degradation of environmental pollutants is well known accepted phenomenon as the decomposer nature of naturally occurring microorganisms.In the present study the indigenous bacterial strains which examined for potential biodegradation of low-density polyethylene materials, causing primary pollution in common environments.The study reveals that the study site was having a potential consortium of bacteria capable to degrade low-density polyethylene.Weight loss and pH change studies at time intervals confirmed the biodegradation process. FTIR studies further demonstrated the chemical changes in the pollutant leading formation of simple compounds which can be further used or removed by other invaders from environment.The molecular characterization support for authentic nomenclature of potential isolate as ***Alcaligenes faecalis***.

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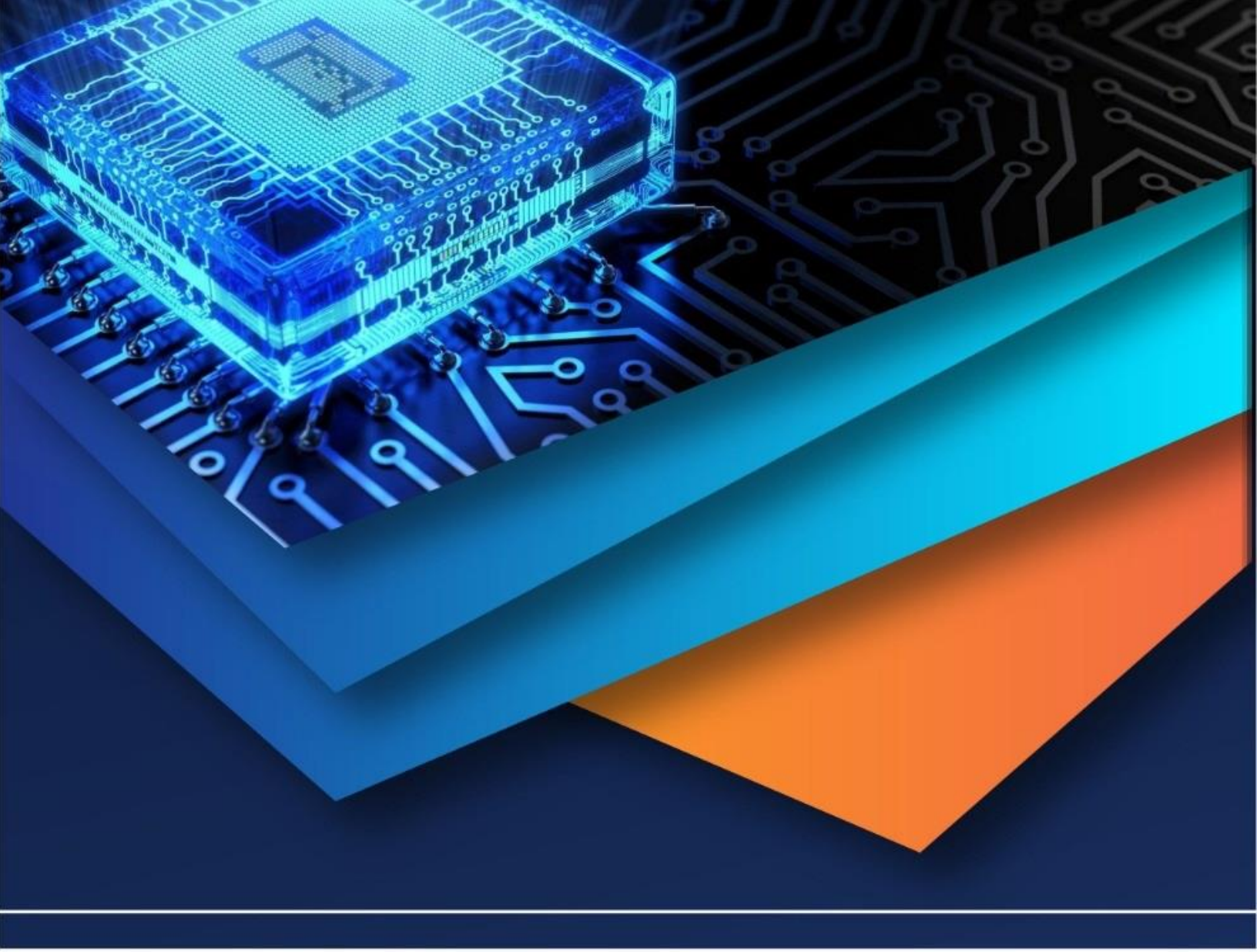

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