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Potential biodegradation of low density polyethylene (LDPE) by *Acinetobacter baumannii*

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Acinetobacter baumannii **was isolated from municipal landfill area, Pallikaranai, Chennai, Tamilnadu. The degradation ability of the bacteria was determined by performing Fourier Transform Infrared Spectroscopy (FTIR). The by-products of polyethylene degradation were monitored by gas chromatography-mass spectrometer (GC-MS) analysis. The toxicity of degradation by-products of low density polyethylene (LDPE) was tested on the plant *Vigna radiata* by determining the morphological parameters such as root length, shoot length and chlorophyll content. After 30 days of degradation process, the FTIR results revealed an increase in carbonyl index and formation of peaks and occurrence of stretches. Alkane compounds were analyzed in GC-MS analysis. Determination of toxicity level of intermediate degraded products showed no changes in morphological characters.**

Key words: Biodegradation, Low density polyethylene (LDPE), *Acinetobacter baumannii*, Fourier transform infrared spectroscopy (FTIR), gas chromatography-mass spectrometer (GC-MS), *Vigna radiata.*

INTRODUCTION

Polyethylene plays an important role in our everyday life. It is a synthetic polymer, made of long chain of monomers of ethylene. Its density ranges from 0.915-0.9359 gcm^3. Polyethylene is classified into different types such as low density polyethylene (LDPE), high density polyethylene (HDPE), linear low density polyethylene (LLDPE), etc. Among these, LDPE has been used for various purposes such as packaging, making carry bags, disposable cups etc. In contrast, when considering disadvantages of polyethylene it poses one of the worst environmental problems. Polyethylene products tend to accumulate in the land areas and remain inert for several decades. This reduces the fertility of the soil, water percolating capacity into the plants and it also threatens animal life. On burning, it produces toxic chemicals polluting the environment, leading to diseases affecting the lungs and skin.

Numerous activities are carried out to reduce the usage of polyethylene and plastic, however less attention is focused on the degradation of polyethylene. Recent research focuses on biodegradation of polyethylene. Biodegradation is the process by which organic substances are broken down by living organisms like bacteria and fungi. During biodegradation process of polymers, two categories of enzymes are actively involved; extracellular and intracellular depolymerases. During degradation, exoenzymes from microorganisms break down complex polymers into smaller molecules, for example oligomers, dimers, and monomers that are small enough to pass the semi-permeable outer bacterial

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membranes, and then utilized as carbon and energy sources and release end products such as CO_2 and H_2O.

Biodegradation of LDPE was studied earlier (Albertsson et al., 1987; Shah, 2007; Suresh et al., 2011; Negi et al., 2011) however the results of these reports were based on pre-treating the LDPE with UV irradiation, thermally oxidized fragments and pro-oxidant additives containing LDPE and starch blended polyethylene.

Gilan et al. (2004) and Hadad et al. (2005) have reported the degradation of LDPE by pretreatment with UV-irradiation and subsequent incubation with *Rhodococcus ruber* and thermophilic bacteria *Brevibacillus parabrevis.*

Sudhakar et al. (2008) and Harshavardhan and Jha (2013) have isolated marine bacteria and utilized them for degradation study of thermally pretreated and starch blended LDPE. Mahalashmi et al. (2012) and Kyaw et al. (2012) have reported the degradation of untreated LDPE by *Pseudomonas* species.

A bacterial culture was isolated from a municipal land fill area and identified as *Acinetobacter baumannii* during previous study (Pramila et al., 2012). The preliminary degradation ability of *A. baumannii* was studied by measuring CO_2 evolution, calculation of generation time, protein estimation, and Bacterial adhesion to hydro-carbon (BATH) test. The significance of chosen municipal dump soil for isolation of bacteria was associated with the fact, that the cultures already had stressful conditions and could develop tolerance towards such environmental conditions.

The current study was focused on determina-tion of physical changes by tensile strength and chemical changes in LDPE by FTIR analysis to measure carbonyl Index (CI). Measuring the carbonyl index (CI) is neces-sary to elucidate the mechanism of biodegradation process where the initial step involves oxidation of the polymer chain and leads to the formation of carbonyl groups, since these groups undergo β-oxidation and are totally degraded via citric acid cycle resulting in formation of CO_2 and H_2O (Albertsson et al., 1987). Additionally, the current study also aimed to study the formation of intermediate by-products by GC-MS analysis and to test the toxicity level of the degraded by-products on plants by *A. baumannii.*

MATERIALS AND METHODS

Preparation of LDPE powder

LDPE sheets were cut into bits and immersed in xylene. It was boiled for 15 min as xylene dissolves the LDPE film and the residue was crushed while it was warm by using band gloves. The LDPE powder so obtained was washed with ethanol to remove residual xylene and allowed to evaporate to remove ethanol. The powder was dried in hot air oven at 60°C over night.

Isolation of microorganism

Bacterial culture was isolated by spread plate method in sterilized synthetic medium (SM) at 37ºC for 24 h. SM contains the following constitutions in 1000 ml distilled water (K_2HPO_4, 1 g; KH_2PO4, 0.2 g; NaCl, 1 g; $CaCl_2.2H_2O$, 0.002 g; $(NH_4)_2SO$, 1 g; $MgSO_4.7H_2O$, 0.5 g; $CuSO_4.5H_2O$, 0.001 g; $ZnSO_4.7H_2O$,0.001 g; $MnSO_4.H2O$, 0.001 g and $FeSO_4.7H_2O$, 0.01 g, amended with 500 mg LDPE powder. Synthetic mineral medium had LDPE as the sole carbon source.

Degradation study

The degradation study was carried out in synthetic medium broth. LDPE films were cut into 2×2 cm. The films were disinfected with 95% ethanol and washed with sterile distilled water. One full inoculation loop of isolated culture were inoculated in 5 ml SM broth and incubated at 37°C for 24 h. After 24 h, the broth was compared with McFarland scale ($CFU\times10^9$/ml) and poured into 45 ml of SM broth in a 100 ml conical flask. Four pieces of equally weighing LDPE films were placed in SM broth. The flasks were incubated at 37°C for 30 days with shaking at 100 rpm. SM broth with LDPE films without culture was maintained as control.

Tensile strength

For tensile strength measurement, test strips were retrieved after 30 days of incubation, washed with 2% sodium dodecyl sulphate (SDS) followed by distilled water and dried in oven overnight at 50°C. The strips were subjected to tensile strength tests as per ASTM A.370 (2012).

FTIR study

After 30 days of incubation, the LDPE sheets were taken and washed with 2% SDS followed by sterile distilled water. The LDPE sheets were dried in oven overnight at 50°C. The films were subjected to FTIR analysis to calculate carbonyl index, presence or absence of functional groups, stretches. The carbonyl index is a measure of the concentration of carbonyl group (acids, aldehydes, ketones) (Albertsson et al., 1987).

$$\text{Carbonyl index (CI)} = \frac{\text{Absorbance at 1715 cm}^{-1}\text{(Peak wavelength)}}{\text{Absorbance at 1465 cm}^{-1}\text{(Peak wavelength)}}$$

GC-MS study

After 30 days of incubation, 10 ml broth was centrifuged at 1000 rpm for 10 min. Supernatant was extracted with 10 ml dichloromethane using a separating funnel. Simultaneously, LDPE films were extracted with 5 ml dichloromethane. Both the extracts were determined by GC-MS (JOEL GCMATE II GC-MASS SPECTROMETER IIT CHENNAI) using HP5 column, helium gas, temperature from 70 to 200°C, injection liquid 1 µl. By retention time the compounds were identified by NIST library.

Toxicity study

Culture broth was analyzed for its toxicity after 30 days, towards plant *V. radiata*. 10 g of garden soil was placed in a pot. Seeds were sown and the soil was wetted regularly with 5 ml of the culture broth. The pots were kept in room temperature with normal condition. After 7 days, the seedlings were harvested and morphological parameters such as root length, shoot length and chlorophyll content of the plant were estimated by Arnon (1949) method. SM with LDPE without culture and SM alone served as controls.

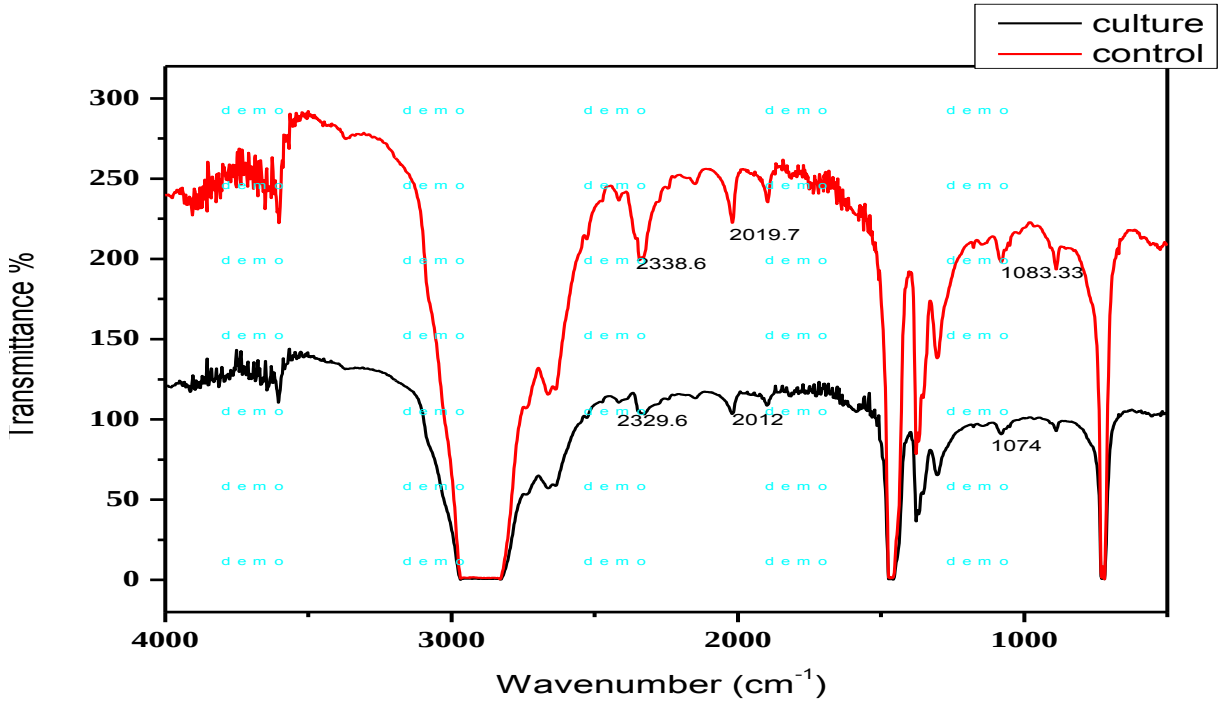


Figure 1. FTIR study of LDPE treated and untreated with *Acinetobacter baumannii* after 30 days of incubation.

RESULTS

FTIR study

Increase in carbonyl index (CI) of LDPE treated with *A. baumannii* after 30 days of incubation indicates the formation of carbonyl groups (Figure 1).

GC-MS study

Figures 2 and 3 indicate the formation of new peaks and compounds in 7.464- as 2-butene, 2-methyl, 8.250- Acetone and 17.288- ethene.

Toxicity test

Table 1 shows the toxicity results of LDPE biodegraded by-products after 30 days of incubation with *A. baumannii*. There are no changes in germination percentage as well as root length and shoot length when compared to control.

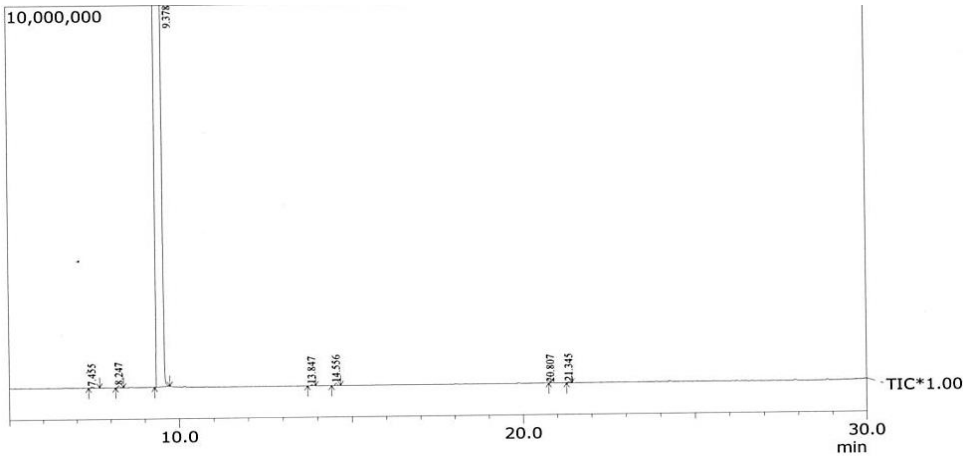
DISCUSSION

Biodegradation of polyethylene has been known for several years. In the previous study, the LDPE degradation ability of *A. baumannii* was reported (Pramila et al., 2012). The current study focused on monitoring the chemical changes of LDPE by FTIR analysis by measuring the carbonyl index (CI). The obtained results indicate the CI was increased by 0.1% after 30 days of incubation without pretreating the LDPE film.

Previous reports on polyethylene degradation utilized UV-irradated LDPE films and showed increase in CI after 30 days of incubation (Gilan et al., 2004; Hadad et al., 2005). Albertsson et al. (1987) has reported the 0.3% increase in CI after 10 years of incubation in soil burial method by pretreating with UV. Sudhakar et al. (2008) and Harshavardhan and Jha (2013) revealed the result of 0.15% increase in CI by incubating with marine bacteria for 30 days.

Suresh et al. (2011) and Negi et al. (2011) have reported the FTIR results by monitoring the changes in peaks such as formation or disappearance of peaks of LDPE film containing pro-oxidant additives by incubation with *Bacillus cereus* and soil burial method for 3 months. Mahalakshmi et al. (2012), studied the degradation of unblended or untreated LDPE using *Pseudomonas* spp. after two months of incubation and reported slight changes in peak wave numbers.

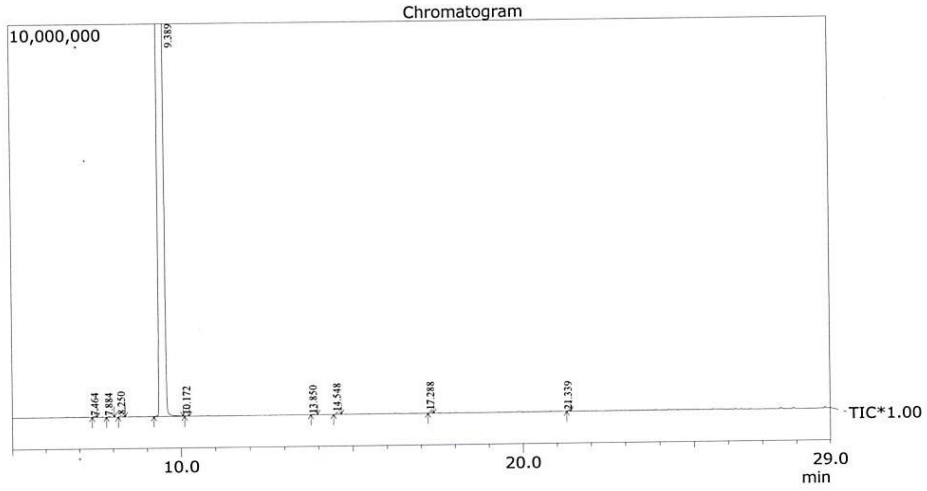
Kyaw et al. (2012) reported the result of 16-80% decrease in CI after 120 days of incubation in mineral based medium by *Pseudomonas* spp. The decrease was presumably due to the prolonged incubation time where the culture entered the Norrish II type mechanism (Albertsson et al., 1987). No changes were observed in tensile strength.



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	7.455	150367	0.02	CYCLOPROPANE, 1,1-DIMETHYL-
2	8.247	283870	0.04	2-PROPANONE
3	9.378	734843731	99.88	METHANE, DICHLORO-
4	13.847	117086	0.02	ACETIC ACID ETHYL ESTER
5	14.556	260309	0.04	METHANE, TRICHLORO-
6	20.807	5404	0.00	2-(3'-PHENYLSULFONYLBUT-3'-ENYL)-
7	21.345	97452	0.01	HEXANAL
		735758219	100.00	

Figure 2. Compounds detected after GC-MS study of untreated LDPE after 30 days.



Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	7.464	94385	0.01	2-BUTENE, 2-METHYL-
2	7.884	45743	0.01	ETHENE, 1,2-DICHLORO-, (Z)- $$ 1,2-DIC
3	8.250	207912	0.03	Acetone
4	9.389	646176618	99.84	METHANE, DICHLORO
5	10.172	100101	0.02	ETHENE, 1,1-DICHLORO-
6	13.850	77234	0.01	ACETIC ACID ETHYL ESTER
7	14.548	241284	0.04	METHANE, TRICHLORO-
8	17.288	200178	0.03	ETHENE, TRICHLORO-
9	21.339	91222	0.01	Hexanal
		647234677	100.00	

Figure 3. Compounds detected after GC-MS analysis of LDPE after 30 days of incubation with *Acinetobacter baumannii.* Figures 2 and 3. Indicates the formation of new peaks and compounds in 7.464- as 2-butene, 2-Methyl, 8.250- acetone, 17.288- ethene.

Table 1. Toxicity results of LDPE biodegraded by-products after 30 days of incubation with *Acinetobacter baumannii.*

Culture	Germination percentage %	Root length (cm)	Shoot length(cm)	Chlorophyll content (mg/g)
Control	80	2.05±0.59	10.07±1.54	0.152
Culture Broth	80	2.52±0.45	11.47±2.16	0.177

Mean ± S.D n=3. There are no changes in germination percentage as well as root length and shoot length when compared to control.

GC-MS results presented in the framework of this study reveals the presence of compounds such as 2-butene, 2-methyl-, acetone, ethene. Presence of acetone indicates the formation of carbonyl groups. Kyaw et al. (2012) has reported GC-MS result of formation of alkanes, aromatic compounds and fatty acid such as hexadecanoic acid and octanoic acid after 120 days incubation.
The byproducts did not reveal any toxicity towards the tested plant characteristics.

Conclusion

Accumulation of polyethylene is becoming a serious environmental issue. Biodegradation of polyethylene process can be viewed as one of the strategic studies to overcome this problem. The current study focused on degradation of LDPE by *A. baumannii*. This isolate grows by utilizing LDPE as a sole carbon source. The bacteria are able to degrade LDPE without any additives and pretreatment in short time duration. This is the first report on degradation of non-pretreated LDPE by *A. baumannii*.

Conflict of interests

The author(s) did not declare any conflict of interest.

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