



## Research article

# Characteristics investigation on biofilm formation and biodegradation activities of *Pseudomonas aeruginosa* strain ISJ14 colonizing low density polyethylene (LDPE) surface



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## ABSTRACT

The accelerated population and industrial development have caused an extensive increase in the use of plastic products. Since polyethylene degrades slowly generating poisonous compounds, therefore, elimination of plastic from the environment is the prerequisite requirement today. Biodegradation of plastics seems to be a convenient and effective method to curb this problem. In view of this, the present study focuses on LDPE degradation capability of bacterial strain *Pseudomonas aeruginosa* ISJ14 (Accession No. MG554742) isolated from waste dump sites. Further, the stability of 16S rDNA of the isolate was determined by applying bioinformatics tools. For biodegradation studies, the polyethylene films were incubated with the culture of *P. aeruginosa* ISJ14 in two different growth medium namely Bushnell Hass broth (BHM) and Minimal Salt medium (MSM) for 60 days at 37 °C on 180 rpm. In addition, hydrophobicity and viability of bacterial isolate along with quantification of total protein content was also done. The microbial degradation was confirmed by surface modification and formation of fissures on polyethylene surface along with the variation in the intensity of functional groups as well as an increase in the carbonyl index using field emission scanning electron microscopy (Fe-SEM) and Fourier transform infrared spectrophotometry (FTIR). These results indicate that *P. aeruginosa* strain ISJ14 can prove to be a suitable candidate for LDPE waste treatment without causing any harm to our health or environment.

## 1. Introduction

The demand for plastic is increasing continuously from the last several decades due to its low production cost. The world plastic production was found to be increased from 204 million tonnes to 348 million tonnes in 2017, justifying an extensive usage of plastic products (Plastic Europe, 2018). The non-degradable nature of plastic waste leads to its continuous accumulation in the environment, therefore, poses serious threat to almost all life forms. The serious environmental issues resulting from these waste include all kinds of terrestrial ecosystems such as desert forest grassland and Polar Regions (Gregory, 2009; Zylstra, 2013). Plastics pollution has shown a deleterious effect on the aquatic environment also as evident by a reduction in marine fauna population (Browne et al., 2011).

The degradation of LDPE in natural conditions is a time taking process, subjected to various factors for example, environmental (i.e. temperature, air humidity, moisture content, pH, and solar radiation) polymer properties and physiological as well as biochemical nature of

microorganisms. Therefore, the elimination of these hazardous substances from the environment is the demand of the hour. Different methods currently in use for waste treatments such as incineration, landfilling, and recycling have certain limitations for instance, toxic gases are evolved during the thermal decomposition of plastic waste, which may adversely affect the humans by causing human allergies and other severe health problems. Landfilling may be considered as an effective approach, but it has certain limitations such as longer duration of degradation and release of toxic pollutants known to be associated with several diseases in humans such as cancer (Yang et al., 2011). However, recycling of plastic waste is very common now a days, but this is not a conventional approach due to difficulties with the collection and storage of plastic waste (Hopewell et al., 2009; North and Halden, 2013).

Biodegradation of these man made compounds by naturally occurring microorganisms seems to be a powerful method to curb the problem of plastic waste due to its substantial advantage over the chemical and physical processes (Ojha et al., 2017). The exploration of microorganisms towards polyethylene degradation is well studied by several researchers.

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A number of bacterial genera were reported for the degradation of LDPE including *Bacillus* spp. (Priyanka and Archana, 2011; Kumar Gupta and Devi, 2019), *Pseudomonas* spp. (Kyaw et al., 2012; Skariyachan et al., 2015), *Streptomyces* spp. (Usha et al., 2011), *Rhodococcus* sp. (Orr et al., 2004), *Acinetobacter* sp., *Brevibacillus* sp., *Flavobacterium* spp., *Ralstonia* spp., *Staphylococcus* spp., *Stenotrophomonas* spp., *Micrococcus* spp., *Microbacterium* sp. and *Nocardia* sp. (Kathiresan, 2003). However, the quest for novel microorganisms with enhanced biodegradation ability still continues. It has already been reported that the coupling of bacteria on the surface of hydrocarbons is a prerequisite step for the growth (Karthick et al., 2016). Biofilm formation by microorganisms on the polyethylene surface might be a relevant approach for finding the degradation capability of microorganisms. This work is in continuation of our earlier work (Kumar et al., 2016), where we isolated *Pseudomonas* sp. strain ISJ14 from the partially degrading plastic adhered soil. In this study, we have evaluated our strain for biofilm forming ability on the surface of polyethylene considering various parameters i.e., cell surface hydrophobicity, viability, and total protein content, with a prime objective to determine LDPE biodegradation.

## 2. Materials and method

### 2.1. Procurement of bacterial culture and growth conditions

The polyethylene-degrading bacterium *P. aeruginosa* strain ISJ14, previously isolated in our laboratory (Kumar et al., 2016) was maintained on nutrient agar at 4 °C and used for performing biodegradation experiments in the present work.

### 2.2. Identification of bacteria

#### 2.2.1. Isolation of genomic DNA

The isolated bacterial strain ISJ14 was identified by using 16S rRNA sequencing. PCR of the isolated genomic DNA was done by using forward and reverse 16S rRNA primers with DNTP, Buffer and Taq polymerase. The 16SrRNA genes were amplified by using universal primer sequence AGAGTTTGATCMTGGCTCAG and CGGTTACCTGTGTTAGACTT. A mixture of PCR amplified product contains 100ng of template DNA, 0.3µM forward primer, 0.3µM reverse primer by availing the volume up to 50µl by using an appropriate amount of DNTP, Taq polymerase, and PCR grade water. Thirty-five cycles of PCR were performed and the finally obtained product was stored at 4 °C. After this, the amplified product resolved on 1% agarose gel at 80V for 60 min and the gel was visualized under U.V light.

#### 2.2.2. 16S rRNA sequencing and phylogenetic assessment

All the related sequences were collected from the nucleotide database. The obtained DNA sequences were compared with the reference species of bacteria, contained in the genomic database to match the similarity between strains using the NCBI BLAST tool (<http://www.ncbi.nlm.nih.gov/BLAST>). A phylogenetic tree was constructed from the aligned DNA sequences based on the bootstrap test of phylogeny with neighbour-joining method using MEGA X software. The 16S rRNA sequence was submitted to the GenBank, NCBI, USA and accession number was obtained (Kumar et al., 2016).

### 2.3. RNA secondary structure prediction and determination of restriction sites

To analyze the structural stability in terms of Gibbs free energy, the secondary structure of the 16S rDNA was predicted by Mfold software (<http://unafold.rna.albany.edu/>) (Zuker, 2003). The sequences of *P. aeruginosa* ISJ14 were submitted to the web server (Mfold) and various parameters including exterior loop type, bulge loop size, base numbering frequency, structure draw mode, structure annotation and structure rotation angle were fixed. The folding process was predicted at 37 °C in

ionic conditions of 1M NaCl deprived of divalent ions. The restriction sites were identified by using NEB cutter online tool version 2.0 ([nc2.neb.com/nebcutter2/](http://nc2.neb.com/nebcutter2/)) (Ganesan et al., 2019).

### 2.4. Assessment of cell surface hydrophobicity of *Pseudomonas aeruginosa* ISJ14

Bacterial adhesion to hydrocarbon (BATH) test (Rosenberg et al., 1980) was used for the evaluation of hydrophobicity of bacterial cells toward the hydrophobic surface of LDPE. In this experiment, the log phase fresh culture of *P. aeruginosa* ISJ14 was centrifuged at 5000 rpm and washed (twice) with phosphate urea magnesium (PUM) buffer containing (per liter): 17 g K<sub>2</sub>HPO<sub>4</sub>, 7.26 g KH<sub>2</sub>PO<sub>4</sub>, 1.8 g urea and 0.2 g MgSO<sub>4</sub>. The bacterial cells were then suspended in PUM buffer to an optical density value of 1.0–1.2 at 600 nm (OD<sub>600</sub>). An aliquot of this suspension (1.2 ml of each) was transferred to a set of test tubes, to which an increasing volume of hexadecane (range 0–0.2 ml) was added. The test tubes were then shaken for 10 min and allowed to stand for 2 min to facilitate phase separation. The OD<sub>600</sub> of the aqueous suspension was then measured spectrophotometrically. A cell-free buffer maintained as the reference blank (Harshvardhan and Jha, 2013).

### 2.5. Investigation of polyethylene biodegradation

#### 2.5.1. Pretreatment of low density polyethylene film

Polyethylene (PE) films (3 × 3 cm) were soaked in the solution containing 7 ml of Tween 80, 10 ml of bleach and 983 ml of sterile water for 30–60 min with continuous stirring followed by washing with distilled water at room temperature. After this, PE films were surface sterilized with 70% ethanol for 30 min and allowed to dry overnight at 45 °C (Awasthi et al., 2017). PE films were weighed by using a weighing balance (Setra BL- 410S, India).

#### 2.5.2. Monitoring of planktonic cell growth

The growth of the bacterial culture was examined in the media every 20 days by serial dilution technique followed by plating on nutrient agar for the cell count of planktonic cells and the results were interpreted in the form of Colony Forming Units/ml of media (CFU/ml) (Arkatkar et al., 2010).

#### 2.5.3. Viability of surface attached bacteria on polyethylene surface

The viability of bacterial cells in biofilm over polyethylene surface was monitored at a regular interval of every 20 days through serial dilution technique in which the polyethylene films were removed from the liquid medium and washed with distilled water in a precise manner so as to liberate loosely attached bacteria. The polyethylene films were then subjected to water bath sonication in 1 ml of saline solution (0.85%) to remove the bacterial biofilm (Andes et al., 2004). An aliquot of this solution was serially diluted and plated on Nutrient agar plate and incubated at 35 °C for 48 h. Results were obtained in the form of colony forming units.

#### 2.5.4. Estimation of the biomass of bacterial biofilm on LDPE surface

The biomass of bacterial biofilm was estimated in the form of total protein content. After an interval of every 20 days the PE films were deliberately removed from the culture medium and gently washed with water for the removal of any medium debris or loosely adhered cells. The bacterial biomass obtained from the PE film was subjected to mild water bath sonication in 1 ml of 0.85% of saline for 4 min (Arkatkar et al., 2009). The saline solution thus obtained, was used to determine protein concentration spectrophotometrically at 595 nm by using Bradford assay (Bradford, 1976).

#### 2.5.5. Biodegradation assay

PE films (0.1g) were aseptically added to 100ml of medium (BHM and MSM) and inoculated with 5 ml active culture of *P. aeruginosa* ISJ14. The

assay was performed using two independent experimental sets: Set 1: MSM + *P. aeruginosa* ISJ14 + LDPE as treatment taking LDPE + MSM as control, Set 2: BHM + *Pseudomonas aeruginosa* ISJ14 + LDPE as treatment taking LDPE + BHM as control. The flasks were then kept on continuous shaking at 180 rpm at 35 °C. The polyethylene films were withdrawn after 20, 40 and 60 days of incubation respectively and checked for weight loss and surface analysis. The whole experiment was performed in triplicates. Fresh medium was added after every 20 days to provide the necessary nutrients to support microbial growth.

## 2.6. Determination of dry weight and half-life of residual LDPE

The degrade PE films were removed from the degradation medium and washed with the solution of sodium dodecyl sulfate (SDS) (2% v/v). The PE films were then rinsed with distilled water to remove any impurity on the polyethylene surface and dried overnight at 45 °C followed by weighing. The weight loss percentage of PE films was determined using the formula

$$\%Biodegradation = \frac{Initial\ weight - Final\ weight}{Initial\ weight} \times 100 \quad (1)$$

The obtained results were further processed to calculate the half-life of residual LDPE and reduction rate constant of LDPE using the first-order kinetic model as follows (Auta et al., 2017):

$$K = -\frac{1}{t} \left( \ln \frac{W}{W_0} \right) \quad (2)$$

where K is the rate constant for polymer uptake per day, t denotes time in days, W is the weight of residual polymer (g), and  $W_0$  is the initial weight of polymer (g). Following the generation of the LDPE removal rate constant, the half-life ( $t_{1/2}$ ) was calculated (Auta et al., 2018) according to Eq. (3):

$$\left( \frac{1}{t_{1/2}} \right) = \ln(2) / K \quad (3)$$

## 2.7. Surface analysis of PE films

### 2.7.1. Scanning electron microscopy

LDPE films treated with culture of *P. aeruginosa* ISJ14 for 60 days were removed from the medium and subjected to Field-emission Scanning Electron Microscopy (FE-SEM) for the observation of biofilm formation and surface erosion. Bacterial morphology of biofilm on PE surface was observed after washing PE films in 0.01 M phosphate buffer for 2 min in order to remove the excess medium adhered with bacterial colonies. To observe surface alteration, PE films were washed with 2% SDS along with warm distilled water for 10–20 min so as to remove bacterial biomass. After this, polyethylene films were fixed in 4 % of glutaraldehyde at 4 °C for 2 h and dehydrated with 50% ethanol for 30 min. The PE films were incubated overnight in 70% ethanol at room temperature, dried, mounted and sputter coated with gold for 40 s and scanned through FE-SEM.

### 2.7.2. Fourier Transform Infrared spectroscopy

Alteration in the structure and functional groups of polyethylene films were analyzed by Fourier Transform Infrared (FTIR) (Perkin Elmer, Spectrum EX) spectra in the frequency range of 4000–400  $\text{cm}^{-1}$  were used at a resolution of 1  $\text{cm}^{-1}$ . The relative absorbance intensities of the ester carbonyl bond, keto carbonyl bond, terminal double bond (vinyl) and the internal double bond to that of the methylene bond were

evaluated using the following formula: Keto Carbonyl Bond Index (KCBI) =  $I_{1715}/I_{1465}$ ; Ester Carbonyl Bond Index (ECBI) =  $I_{1740}/I_{1465}$ ; Vinyl Bond Index (VBI) =  $I_{1650}/I_{1465}$  and Internal Double Bond Index (IDBI) =  $I_{908}/I_{1465}$ . Carbonyl index was used to measure the degree of biodegradation has its value depends on the degradation. The crystallinity percentage of the PE film was measured based on the method suggested by (Zerbi et al., 1989) and calculated using the following formula:

$$\%Crystallinity = 100 - \left[ \frac{\left\{ \frac{1 - \frac{I_a}{1.233I_b}}{1 + \left( \frac{I_a}{I_b} \right)} \right\} \times 100 \right] \quad (4)$$

where  $I_a$  is the absorbance at 1473 and  $I_b$  is the absorbance at 1463.

## 3. Result and discussion

### 3.1. Molecular characterization and phylogenetic analysis of bacterial strain ISJ14

The bacterial strain ISJ14 was previously isolated from soil adhered to polyethylene waste collected from dumpsites. As this isolate was found positive for biodegradation activity in a clear zone assay (Kumar et al., 2016) the isolate was selected to perform further studies on biodegradation. The nucleotide sequence of *P. aeruginosa* was deposited to NCBI database and accession no MG554742.1 was obtained. The sequences with highest partial nucleotide sequence similarities were compare through CLUSTAL W. The phylogenetic tree and evolutionary analysis were conducted by MEGA-X using neighbor-joining algorithm. The alignment of the sequence with other sequences found in the database showed 100% similarity of this isolate with the sequence of *Pseudomonas aeruginosa* (JCM 5952). Based on these results, the isolated bacterium was identified as *Pseudomonas aeruginosa* ISJ14 (Figure 1).

### 3.2. 16S rDNA secondary structure prediction and restriction sites analysis

The 16S rDNA folding was predicted to comprehend the thermodynamic stability of the gene sequence (Figure 2A). The free Gibb's energy of 16S rDNA in its folded form for ISJ14 was observed to be  $-398.50$  kcal/mol. This study suggested the minimal energy level of 16S rDNA sequences, specifying high folding stability of nucleotides in the organisms. Similar Gibb's free energy values exhibiting in predicted structures of 16S rDNA from *Pseudomonas* sp., *Stenotrophomonas maltophilia* and *Bacillus vallismortis* were reported by Skariyachan et al. (2018). The concept of studying free energy associated with the folding of 16S rDNA gene sequence of bacteria might provide preliminary information to make a concurrent prediction on stabilities of the genes. However, whole genome analysis of the organism should be performed to predict the probable genes responsible for LDPE degradation. The restriction analysis of 16S rDNA sequence of ISJ14 indicated the presence of GCAT content to 59% & 41% respectively (Figure 2B).

### 3.3. Bacterial cell surface hydrophobicity

The interaction between the microorganism and PE film was evaluated through BATH test and the results are shown in Figure 3. The present work displayed high hydrophobicity of logarithmic cells than that of the stationary cells of ISJ14 towards PE Films. The utilization of any substrate depends on the adhesion ability of the particular microorganism which is governed by a number physical factors, i.e, the forces which help the bacterium to adhere with solid substratum, properties of

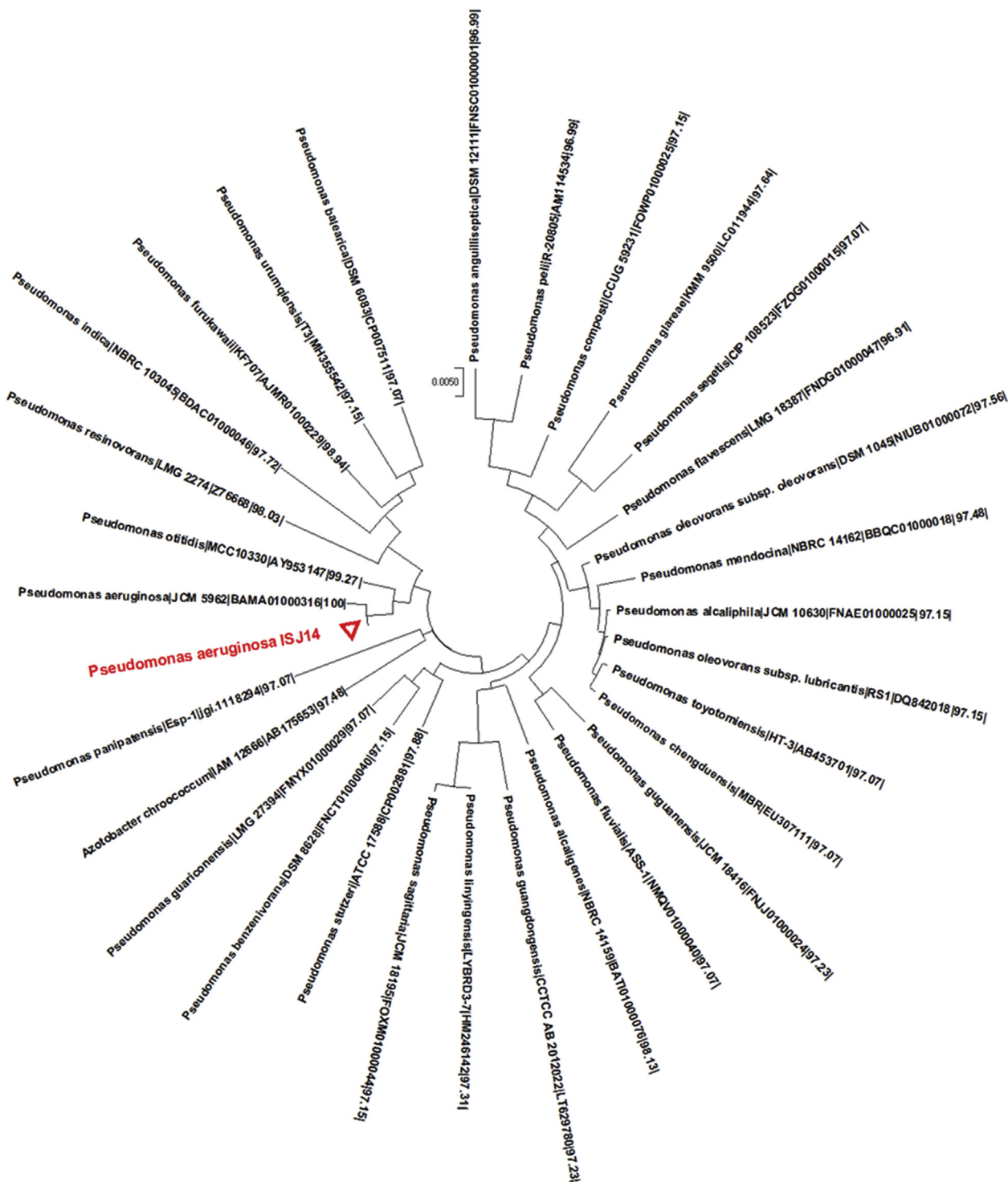


Figure. 1. Representation of phylogenetic tree showing the position of *Pseudomonas aeruginosa* ISJ14.

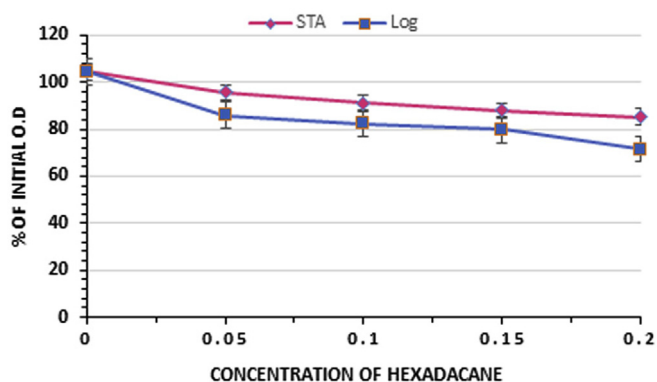
the substrate, and nature of microorganism. Generally, hydrophobic surface is preferred by a hydrophobic bacterium for the attachment however, a bacterium with hydrophilic properties like to attach on hydrophilic surfaces (Harshvardhan and Jha, 2013). In the present study, we observed a significant increase (31.5%) in the hydrophobicity of logarithmic cells of *P. aeruginosa* (Figure 3) as compared to the stationary phase (14.9%) at 0.2 µl concentration of hexadecane. These results are in agreement with Harshvardhan and Jha (2013), who reported that bacterial cells in their logarithmic phase are more hydrophobic than the stationary phase. Previous literature also documented similar findings,

where, maximum increase in hydrophobicity i.e, 20% and 10% was observed for *Rhodococcus ruber* and *Brevibacillus borstelensis* respectively at 0.2 µl concentration of hexadecane (Orr et al., 2004; Hadad et al., 2005).

3.4. Growth pattern of *Pseudomonas aeruginosa* ISJ14 planktonic cells and surface adhered cells on polyethylene films

The growth kinetics of surface-attached bacteria on the polyethylene film was monitored in planktonic cells and on the surface of the film by





**Figure 3.** The hydrophobicity of bacterial isolate ISJ14 was determined by the bacterial adhesion to hydrocarbon test. Aliquots of logarithmic (Log) and stationary cell (STA) suspensions were supplemented with increasing concentrations of hexadecane. The transfer of hydrophobic cells from the aqueous phase to hexadecane is reflected as a decrease in the turbidity (optical density at 600 nm) of the bacterial suspension. The data represent the means of three replicates, and the error bars indicate the standard error mean.

obtained from liquid cultures of planktonic cells. The data (CFU/ml) obtained in our study was analyzed using one-way ANOVA and found to be statistically significant ( $p < 0.05$ ).

The data suggest that incubation of *P. aeruginosa* in BHM and MSM containing polyethylene films as the sole source of organic carbon resulted in the adhesion of cells to the polyethylene surface and subsequent formation of biofilm indicating continuous proliferation and growth. It was also evident from these results that ISJ14 cells show better colonization, biofilm formation and, presumably partial biodegradation of polyethylene in BHM as compared to MSM. These findings not only indicate high affinity of ISJ14 cells for the polyethylene but also raise the possibility that the low carbon availability in ISJ14 cultures may enhance hydrophobic interactions leading to biofilm development. In view of consensus model, the formation of microbial biofilm is initiated when growth of planktonic culture achieves high cell density that help attachment of cells to the surface via quorum sensing signals, thereby resulting in the development of micro colonies that will ultimately form the structure of the mature biofilm (Costerton et al., 1995). However, this scenario is not necessarily common to all biofilm-producing bacteria. In our study polyethylene films served as a substrate for the attachment and formation of biofilms as well as a carbon source for ISJ14 cells. Therefore, employing carbon starvation by utilizing the medium where PE films are the only source of organic carbon. In contrast, prolonged incubation of ISJ14 with PE films resulted in a thick biofilm on the LDPE surface which may have led to partial degradation of the polymer. Similarly, the capacity of ISJ14, to form and maintain an active biofilm on polyethylene

surface, for 60 days during incubation, may be due to the consumption of low molecular substances in the polymer. These results suggested that the formation of biofilms on hydrophobic polymers, such as LDPE may be promoted by carbon starvation. Similarly, Sanin et al. (2003) stated alterations in cell surface hydrophobicity of bacteria in response to carbon starvation. Earlier studies showed that the biofilm of *Rhodococcus ruber* formed on polyethylene displayed great viability even after 60 days of incubation and remained adhered to the substratum without any external supplementation of carbon (Orr et al., 2004; Sivan et al., 2006; Gilan and Sivan, 2013).

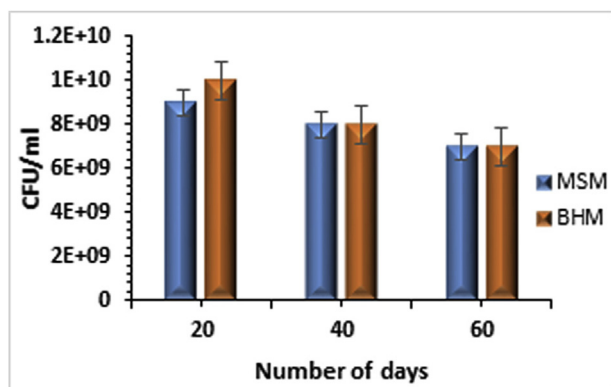
### 3.5. Quantitative estimation of surface adhered biomass

Quantification of surface adhered biomass in terms of proteins considered as an effective approach for determining the state of polyethylene colonization and biofilm formation (Orr et al., 2004). In this study, growth kinetics and biofilm development of ISJ14 cells on polyethylene surface was evaluated by quantifying the total protein content of bacterial biomass adhered to polyethylene film.

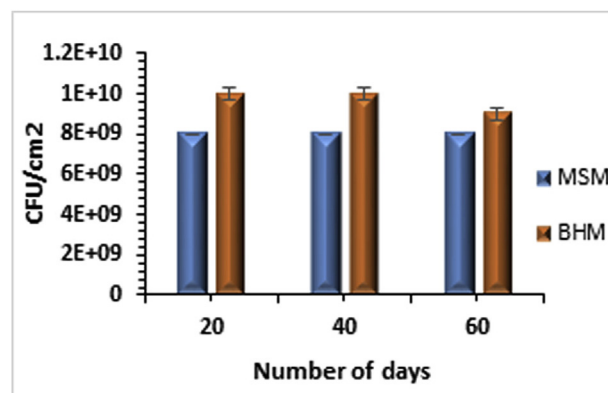
The data depicted in Figure 6 displays quantification of total biomass adhered to polyethylene surface in the form of total protein content ( $\mu\text{g}/\text{ml}$ ). On the 20<sup>th</sup> day of incubation, there was slight increase in protein content, reflecting the development of bacterial biomass on polyethylene surface for both the medium. On the 40<sup>th</sup> day of incubation period, there was a steep increase in protein content in both the medium indicating the strong affinity of bacterial isolates to the LDPE. This is due to the rate of biofilm formation over the surface of the LDPE film. But on the 60<sup>th</sup> day, a slow decrease in total protein content was observed, which may be due to the detachment of bacterial cells from the polyethylene surface resulting from prolonged incubation under shaking conditions. The extractable protein content was found to be higher in BHM than that of MSM. The continuous and slow increase in extractable protein suggests a regular growth of bacterial isolates over the LDPE surface. These results agree with the growth pattern of biofilm on polyethylene surface as described above.

### 3.6. Biodegradation assay by monitoring weight loss of PE films

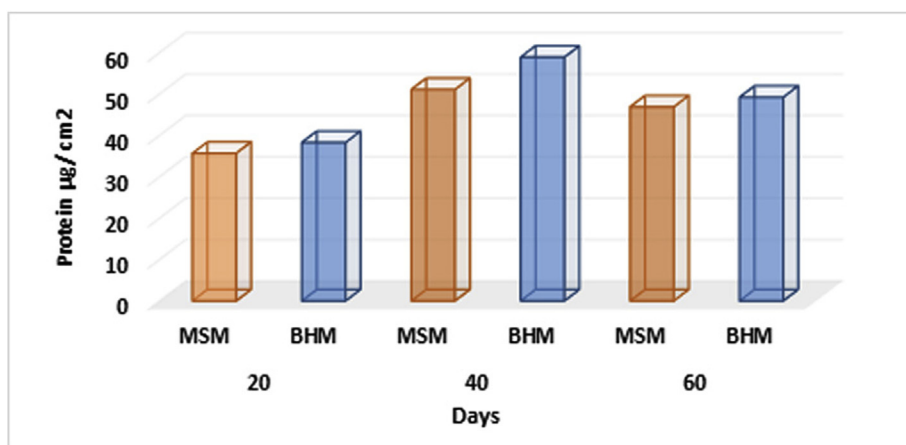
The biodegradation assay was carried out for 60 days and weight loss was recorded at regular intervals of 20 days. The final weight loss for ISJ14 in respective culture media (i.e. BHM and MSM) has been provided in Figure 7. These results suggested that ISJ14 has shown its better degradation capability in BHM (8.70%) as compared to MSM (6.5%) after 60 days of incubation. *P. aeruginosa* strain ISJ14 had a removal rate of  $0.0015 \text{ day}^{-1}$  with a longer half-life of 462 days in BHM. These results suggested that ISJ14 consumes 0.0015 g of LDPE per day and needed approximately 462 days to reduced PE films to its half (i.e., .1g-.05g). On the other hand, a removal rate of  $0.0012 \text{ day}^{-1}$  with a longer half-life of



**Figure 4.** Kinetics of *P. aeruginosa* ISJ14 planktonic cells. Data are means of three replicates  $\pm$ S.D.



**Figure 5.** Kinetics of *P. aeruginosa* ISJ14 surface adhered cell. Data are means of three replicates  $\pm$ S.D.



**Figure 6.** Biofilm protein contents ( $\mu\text{g}$ ) of ISJ14 cultured in MSM and BHM respectively, supplemented with polyethylene as a sole carbon source, values are the means of three replicates.

577.5 days was observed in MSM. Our results are in line with [Auta et al. \(2018\)](#), who reported  $0.0019 \text{ day}^{-1}$  reduction rate for PS microplastic with a half-life of 363 days. The growth kinetics of the ISJ14 in both media demonstrated its colonization on LDPE surface consequently, reduction in weight was observed as a result of utilization of PE film as carbon source. Similar findings have been documented by other researchers ([Kapri et al., 2010](#); [Negi et al., 2009](#); [Sah et al., 2010](#); [Satlewal et al., 2008](#); [Shah et al., 2015](#)). [Harshvardhan and Jha \(2013\)](#) also reported the weight loss of  $1 \pm 0.033\%$ ,  $1.5 \pm 0.038\%$  and  $1.75 \pm .06\%$  for *K. palustris* M16, *B. pumilus* M27 and *B. subtilis* H1584, respectively after 30 days of incubation. Whereas, [Kyaw et al. \(2012\)](#) reported 20% reduction in LDPE film treated with *Pseudomonas aeruginosa* after 120 days.

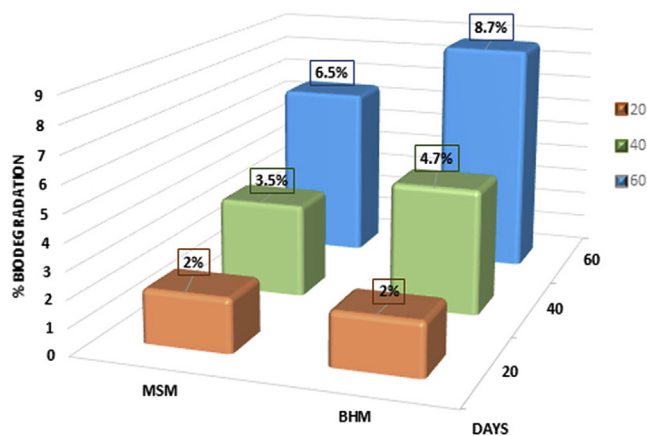
### 3.7. Surface morphology

Presence of *P. aeruginosa* ISJ14 on LDPE along with surface erosion, cracks and folding's were observed through SEM analysis, confirming the extensive colonization after 60 days of biological treatment ([Figure 8 \(A-G\)](#)). The PE film samples (Both MSM and BHM) exhibited alteration in surface morphology of LDPE resulting from the action of bacteria. The erosion of surface is considered as the primary cause of mass reduction from the surface, which is due to the secretion of enzymes and

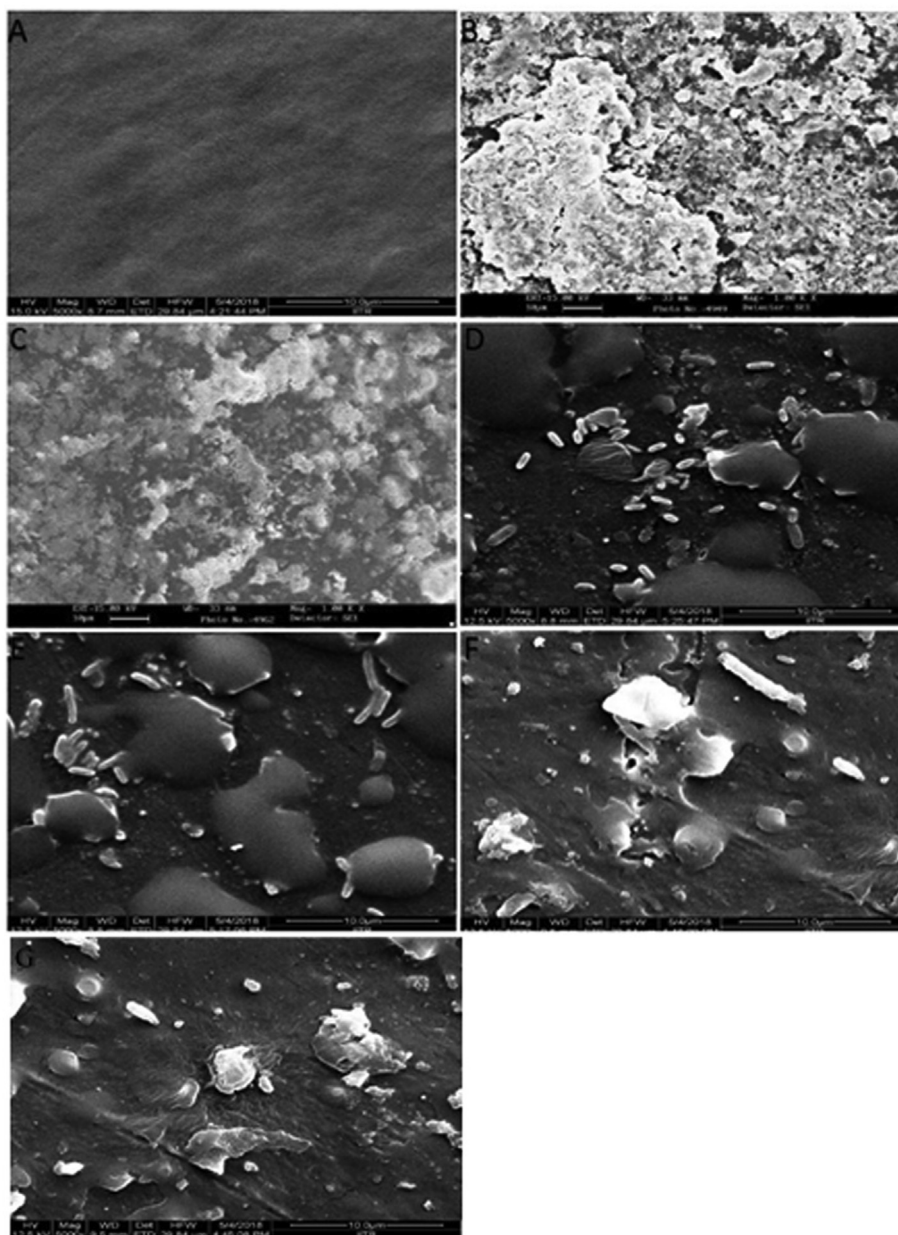
extracellular metabolites by the bacteria in response to carbon starved conditions. However, the control SEM micrographs did not show any changes on surface morphology and have an exceptionally smooth surface upon comparison with treated samples of PE films. On the other hand, in previous studies several observed similar morphological changes on LDPE surface treated with *Pseudomonas* sp. ([Kyaw et al., 2012](#); [Skariyachan et al., 2015](#)). Moreover, the presence of dense biofilm on the surface of PE films for both medium further validate the fact that the bacteria was involved in the mechanism of biodegradation. As evident in the SEM micrographs ([Figure 8 \(D, E\)](#)) we observed some localized degradation of the polyethylene around the bacterial cells. The bacterial biofilm showed rod shaped morphology of *P. aeruginosa* strain ISJ14 on the PE films after 60 days. After the removal of bacteria biofilm, treated LDPE films showed distorted images along with a number of cracks and grooves after 60 days of incubation ([Figure 8 \(F, G\)](#)). On the other hand, the control film retained a smooth surface under similar incubation conditions without bacterial culture. This indicates that bacterium *P. aeruginosa* ISJ14 secretes enzymes capable of degrading polyethylene resulting in grooves formation. Clear marks of degradation can be seen at places where initially microbes were attached along with the pockets and pits around.

### 3.8. FTIR

FTIR analysis of PE film treated with *P. aeruginosa* ISJ14 indicates changes in functional groups as compared to control ([Figure 9 A, B and C](#)). In contrast, the PE films treated with ISJ14 displayed variation in the intensity of bands in different regions for both mediums ([Figure 9 B, C](#)). For control spectrum, the characteristic absorption bands were assigned at  $729.01 \text{ cm}^{-1}$  (C-H bend),  $1,465.59 \text{ cm}^{-1}$  (C=C stretch),  $1713.55 \text{ cm}^{-1}$  (CHO stretch), and  $2916, 2855 \text{ cm}^{-1}$  (both due to C-H stretch). A decrease indicated the cleavage of C-H bonds peak was observed in spectra of LDPE sample treated with *P. aeruginosa* ISJ14 at  $2913.50, 2853.50 \text{ cm}^{-1}$  for MSM and  $2912.00, 2854.00 \text{ cm}^{-1}$  for BHM. An increase in already existing peaks along with the formation of some new peaks at  $1200-1400 \text{ cm}^{-1}$  region of FTIR spectra, indicates the formation of new intermediate products ([Figure 9 B and C](#)). A similar observation of biodegradation was reported by [Howard and Hilliard \(1999\)](#); [Nakajima-Kambe et al. \(1997\)](#). The appearance of new functional groups at the region of  $3445.58 \text{ cm}^{-1}, 2727.05 \text{ cm}^{-1}$  was observed in both culture mediums. FTIR analysis provides a close view of N-H stretching of aldehyde group at  $3445.61$  and  $3300.90 \text{ cm}^{-1}$ . The carboxyl absorption peaks were shifted as evident at  $1721.26 \text{ cm}^{-1}$  for MSM and  $1721.88 \text{ cm}^{-1}$  for BHM, because of the formation of ketone or aldehyde. Our results were supported by several previous reports [Das and Kumar, 2015](#)



**Figure 7.** The plot represents the gradual increase in percent weight loss of LDPE sheets after 60 days of incubation with *P. aeruginosa* ISJ14 in minimal salt medium (MSM) and bushnell hass broth (BHM) respectively. The results represent the average of three independent experiment.



**Figure 8.** Field emission scanning electron microscopic view of the LDPE degraded sheets. (A) LDPE films as seen on 0<sup>th</sup> day (control) (B) Biofilm of *P. aeruginosa* ISJ14 in BHM after 60<sup>th</sup> day (C) Biofilm of *P. aeruginosa* ISJ14 in MSM after 60<sup>th</sup> day (D) Morphology of ISJ14 in BHM after 60 days of incubation (E) Morphology of ISJ14 in MSM after 60 days of incubation (F) Formation of cracks on LDPE surface in BHM (G) Formation of cracks on LDPE surface in MSM.

noticed the formation and disappearance of functional groups in their LDPE degradation studies treated with *Bacillus amyloliquefaciens* strain. The alteration in the peak values of almost all functional groups evident the conformational change in LDPE film samples treated with *P. aeruginosa* in both the medium. On the other hand, an increase in the amount of carbonyl groups (KCBI, ECBI, VBI and IDBI) has been noticed after 60 days of incubation with *P. aeruginosa* ISJ14 further elucidating the role of biotic environment in the degradation of polyethylene (see Figure 10). These results are in agreement with Sudhakar et al. (2008) who, reported that carbonyl groups are the major products formed in the presence of enzymes involved in biodegradation such as oxidoreductases. During biodegradation process, enzymes catalyze various types of chemical reactions, for instance, oxidation, reduction, hydrolysis,

esterification, and molecular inner conversion. However, Albertsson et al. (1987) reported the change of keto carbonyl index, ester carbonyl index and terminal double bonds resulting from the action of microorganism on polyethylene. Other scientists have also observed visible changes in biodegradation of synthetic polymers before and after exposure to microbes using FTIR analysis (Gajendiran et al., 2016).

The same was observed for terminal double bonds after incubation with *P. aeruginosa* ISJ14. These results suggest the polymer degradation potential of our isolate *P. aeruginosa* ISJ14. Harshvardhan and Jha (2013) reported an increase in the keto carbonyl bond index, the ester carbonyl bond index and the vinyl bond index of FTIR spectra of polyethylene film treated with three bacterial isolates namely *Kocuria palustris* M16, *Bacillus pumilus* M27 and *Bacillus subtilis* H1584. We observed 23%, 22% and 18%



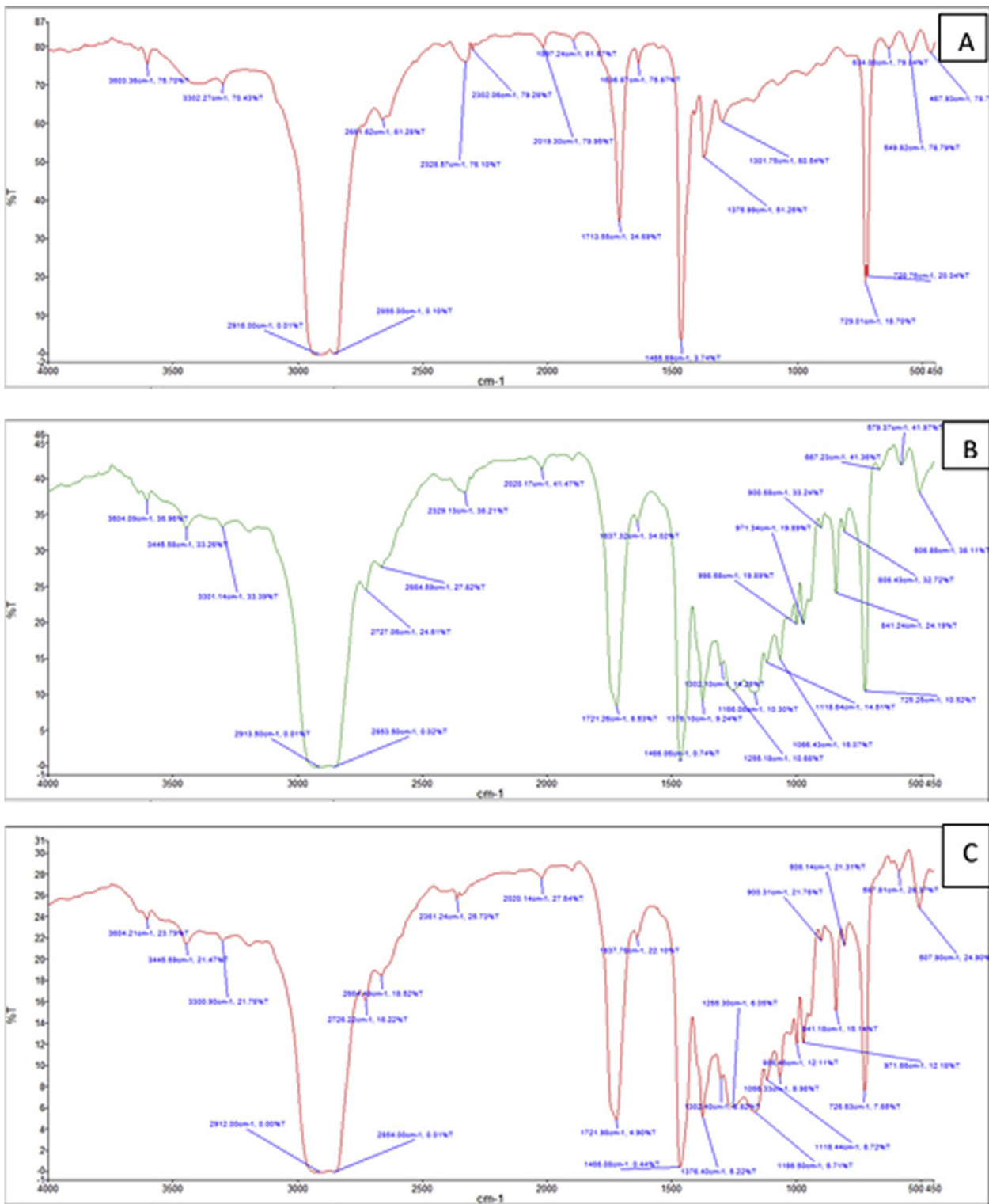
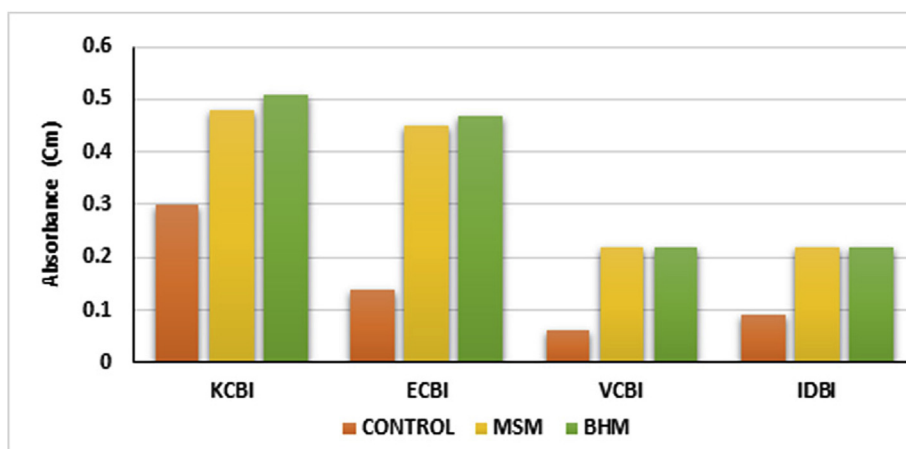


Figure 9. (A-C). Represents the peaks after the FTIR analysis of LDPE sheets post 60 days of incubation for *P. aeruginosa* ISJ14 (A) control polyethylene film (B) Represents the peaks after the FTIR analysis of LDPE sheets after 60 days incubated in MSM (C) Represents the peaks after the FTIR analysis of LDPE sheets after 60 days incubated in BHM.



**Figure 10.** Carbonyl indexes of polyethylene incubated with *P. aeruginosa* ISJ14 after 60 Days of incubation period (KCBI – Keto Carbonyl Bond Index; ECBI – Ester Carbonyl Bond Index; VBI – Vinyl Bond Index; IDBI – Internal Double Bond Index).

decrease in crystallinity for polyethylene sample in case of BHM, MSM and control respectively. There are previous reports of polyethylene bio-degradation utilizing UV irradiated polyethylene films (Hadad et al., 2005; Orr et al., 2004). Earlier studies suggested that exposure of polyethylene to UV radiation causes increased carbonyl and terminal double bond indices and confer functional groups to microbes for attachment on the polyethylene surface. This is in contrast to the previously published reports showing very slow degradation of LDPE and an enhancement of this degradation only after LDPE was subjected to prior oxidation (Albertsson, 1980; Chatterjee et al., 2002). Thus, compared to previous reports, the rate of LDPE degradation by *Pseudomonas* sp. is very high, even without the use of prior oxidation, these findings further validate the fact that our isolate has the ability to degrade LDPE films efficiently.

#### 4. Conclusion

The *in vitro* biodegradation of LDPE by *P. aeruginosa* ISJ14 is reported in the Minimal salt Medium (MSM) and Bushnell Hass Broth (BHM). We observed a tremendous efficiency of our isolate *P. aeruginosa* ISJ14 to degrade LDPE in BHM. The isolate was not only capable of forming biofilm on LDPE surface but also utilized it as a sole carbon source for growth, as reflected by weight loss of PE films in comparison to control. Although biodegradation is a slow process, however, high CFU counts and protein concentration values of the isolate reflects its high metabolic activity even after 60 Days of incubation. *P. aeruginosa* strain ISJ14 also demonstrated a decrease in the hydrophobicity and crystallinity percentage of PE films at the end of incubation period. Based on the results of FE-SEM as well as FTIR analysis, it is interpreted that *P. aeruginosa* ISJ14 is a suitable candidate for the degradation of polyethylene without any requirement of pretreatments (i.e. U.V, physical and chemical treatment). The study also indicates that partially degraded polyethylene from the waste disposal sites may act as a significant source for finding more microorganisms efficient in PE degradation. This limited study on LDPE degradation demonstrates that strain ISJ14 can be employed for eco-friendly management of plastic waste, however, further studies on enzyme based metabolic pathways of *P. aeruginosa* ISJ14 are also suggested to understand its potential role in the degradation process.

#### Declarations

##### Author contribution statement

Kartikey Kumar Gupta: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Deepa Devi: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

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##### Additional information

No additional information is available for this paper.

#### References

- Albertsson, A.C., 1980. The shape of the biodegradation curve for low and high density polyethylenes in prolonged series of experiments. *Eur. Polym. J.* 16, 623–630.
- Albertsson, A.C., Andersson, S.O., Karlsson, S., 1987. The mechanism of biodegradation of polyethylene. *Polym. Degrad. Stab.* 18, 73–87.
- Andes, D., Nett, J., Oschel, P., Albrecht, R., Marchillo, K., Pitula, A., 2004. Development and characterization of an *in vivo* central venous catheter *Candida albicans* biofilm model. *Infect. Immun.* 72, 6023–6031.
- Arkatkar, A., Arutchelvi, J., Bhaduri, S., Uppara, P.V., Doble, M., 2009. Degradation of untreated and thermally pretreated polypropylene by soil consortia. *Int. Biodeterior. Biodegrad.* 63, 106–111.
- Arkatkar, A., Juwarkar, A.A., Bhaduri, S., Uppara, P.V., Doble, M., 2010. Growth of *Pseudomonas* and *Bacillus* biofilms on pretreated polypropylene surface. *Int. Biodeterior. Biodegrad.* 64, 530–536.
- Auta, H.S., Emenike, C.U., Fauziah, S.H., 2017. Screening of *Bacillus* strains isolated from mangrove ecosystems in Peninsular Malaysia for microplastic degradation. *Environ. Pollut.* 231, 1552–1559.
- Auta, H.S., Emenike, C.U., Jayanthi, B., Fauziah, S.H., 2018. Growth kinetics and biodeterioration of polypropylene microplastics by *Bacillus* sp. and *Rhodococcus* sp. isolated from mangrove sediment. *Mar. Pollut. Bull.* 127, 15–21.
- Awasthi, S., Srivastava, N., Singh, T., Tiwary, D., Mishra, P.K., 2017. Biodegradation of Thermally Treated Low Density Polyethylene by Fungus *Rhizopus Oryzae* NS 5. *3 Biotech* 7.
- Bradford, M.M.A., 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72 (1-2), 248–254.
- Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines worldwide: sources and sinks. *Environ. Sci. Technol.* 45, 9175–9179.
- Chatterjee, J., Haik, Y., Chen, C.J., 2002. Synthesis of polyethylene magnetic nanoparticles. *Eur. Cell. Mater.* 3, 98–101.
- Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., Lappin, H.M., 1995. Microbial biofilms. *Annu. Rev. Microbiol.* 49, 711–745.
- Das, M.P., Kumar, S., 2015. An approach to low-density polyethylene biodegradation by *Bacillus amyloliquefaciens*. *3 Biotech* 5, 81–86.
- Gajendiran, A., Krishnamoorthy, S., Abraham, J., 2016. Microbial degradation of low-density polyethylene (LDPE) by *Aspergillus clavatus* strain JASK1 isolated from landfill soil. *3 Biotech* 6, 1–6.
- Ganesan, P., Reegan, A.D., Antony, R.H., Gandhi, M.R., Paulraj, M.G., Al, N.A., Ignacimuthu, S., Gandhi, M.R., Paulraj, M.G., Al-dhabi, N.A., Ganesan, P., Daniel, A.,

- Host, R., David, A., 2019. Antimicrobial activity of some actinomycetes from western ghats of Tamil nadu , India. *Alexandria J. Med.* 53, 101–110.
- Gilan, I., Sivan, A., 2013. Effect of proteases on biofilm formation of the plastic-degrading actinomycete *Rhodococcus ruber* C208. *FEMS Microbiol. Lett.* 342, 18–23.
- Gregory, M.R., 2009. Environmental implications of plastic debris in marine settings-entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 2013–2025.
- Hadad, D., Geresh, S., Sivan, A., 2005. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *J. Appl. Microbiol.* 98, 1093–1100.
- Harshvardhan, K., Jha, B., 2013. Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India. *Mar. Pollut. Bull.* 77, 100–106.
- Hopewell, J., Dvorak, R., Kosior, E., 2009. Plastics recycling: challenges and opportunities. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 2115–2126.
- Howard, G.T., Hilliard, N.P., 1999. Use of Coomassie blue-polyurethane interaction in screening of polyurethanase proteins and polyurethanolytic bacteria. *Int. Biodeterior. Biodegrad.* 43, 23–30.
- Kapri, A., Zaidi, M.G.H., Satlewal, A., Goel, R., 2010. SPION-accelerated biodegradation of low-density polyethylene by indigenous microbial consortium. *Int. Biodeterior. Biodegrad.* 64, 238–244.
- Karthick, T., Sumithra, B., Bhuvaneshwari, K.P., Gurujeya, B., Jaya Barath, J., 2016. Biodegradation of plastic by *Bacillus subtilis* isolated from polythene polluted soil. *World J. Pharmaceut. Res.* 5, 1148–1153.
- Kathiresan, K., 2003. Polythene and Plastics-degrading microbes from the mangrove soil. *Rev. Biol. Trop.* 51, 629–634.
- Kumar Gupta, K., Devi, D., 2019. Biodegradation of low density polyethylene by selected *Bacillus* sp. *Gazi Univ. J. Sci.* 32, 802–813.
- Kumar Gupta, K., Devi, D., Rana, D., 2016. Isolation and screening of Low Density Polyethylene (Ldpe) degrading bacterial strains from waste disposal sites. *World J. Pharmaceut. Res.* 5, 1633–1643.
- Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874.
- Kyaw, B.M., Champakalakshmi, R., Sakharkar, M.K., Lim, C.S., Sakharkar, K.R., 2012. Biodegradation of low density polythene (LDPE) by *Pseudomonas* species. *Indian J. Microbiol.* 52, 411–419.
- Nakajima-Kambe, T., Onuma, F., Akutsu, Y., Nakahara, T., 1997. Determination of the polyester polyurethane breakdown products and distribution of the polyurethane degrading enzyme of *Comamonas acidovorans* strain TB-35. *J. Ferment. Bioeng.* 83, 456–460.
- Negi, H., Kapri, A., Zaidi, M.G.H., Satlewal, A., Goel, R., 2009. Comparative in-vitro biodegradation studies of epoxy and its silicone blend by selected microbial consortia. *Int. Biodeterior. Biodegrad.* 63, 553–558.
- North, E.J., Halden, R.U., 2013. Plastics and environmental health: the road ahead. *Rev. Environ. Health* 28, 1–8.
- Ojha, N., Pradhan, N., Singh, S., Barla, A., Shrivastava, A., Khatua, P., Rai, V., Bose, S., 2017. Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimization. *Sci. Rep.* 7, 1–13.
- Orr, I.G., Hadar, Y., Sivan, A., 2004. Colonization, biofilm formation and biodegradation of polyethylene by a strain of *Rhodococcus ruber*. *Appl. Microbiol. Biotechnol.* 65, 97–104.
- Plastic Europe, 2018. Plastic- the Facts 2018. Retrieved from. [https://www.plasticseurope.org/application/files/6315/4510/9658/Plastics\\_the\\_facts\\_2018\\_AF\\_web.pdf](https://www.plasticseurope.org/application/files/6315/4510/9658/Plastics_the_facts_2018_AF_web.pdf).
- Priyanka, N., Archana, T., 2011. Biodegradability of polythene and plastic by the help of Microorganism: a way for brighter future. *J. Environ. Anal. Toxicol.* 1, 1–4.
- Rosenberg, M., Gutnick, D., Rosenberg, E., 1980. Adherence of bacteria to hydrocarbons: a simple method for measuring cell-surface hydrophobicity. *FEMS Microbiol. Lett.* 9 (1), 29–33.
- Sah, A., Kapri, A., Zaidi, M.G.H., Negi, H., Goel, R., 2010. Implications of fullerene-60 upon in-vitro LDPE biodegradation. *J. Microbiol. Biotechnol.* 20, 908–916.
- Sanin, S.L., Sanin, F.D., Bryers, J.D., 2003. Effect of starvation on the adhesive properties of xenobiotic degrading bacteria. *Process Biochem.* 38, 909–914.
- Satlewal, A., Soni, R., Zaidi, M., Shouche, Y., Goel, R., 2008. Comparative biodegradation of HDPE and LDPE using an indigenously developed microbial consortium. *J. Microbiol. Biotechnol.* 18, 477–482.
- Shah, A.A., Nawaz, A., Kanwal, L., Hasan, F., Khan, S., 2015. Degradation of poly ( $\epsilon$ -caprolactone) by a thermophilic bacterium *Ralstonia* sp. strain MRL-TL isolated from hot spring. *Int. Biodeterior. Biodegrad.* 98, 35–42.
- Sivan, A., Szanto, M., Pavlov, V., 2006. Biofilm development of the polyethylene-degrading bacterium *Rhodococcus ruber*. *Appl. Microbiol. Biotechnol.* 72, 346–352.
- Skariyachan, S., Megha, M., Kini, M.N., Mukund, K.M., Rizvi, A., Vasist, K., 2015. Selection and screening of microbial consortia for efficient and ecofriendly degradation of plastic garbage collected from urban and rural areas of Bangalore, India. *Environ. Monit. Assess.* 187.
- Skariyachan, S., Patil, A.A., Shankar, A., Manjunath, M., Bachappanavar, N., Kiran, S., 2018. Enhanced polymer degradation of polyethylene and polypropylene by novel thermophilic consortia of *Brevibacillus* sps. and *Aneurinibacillus* sp. screened from waste management landfills and sewage treatment plants. *Polym. Degrad. Stabil.* 149, 52–68.
- Sudhakar, M., Doble, M., Murthy, P.S., Venkatesan, R., 2008. Marine microbe-mediated biodegradation of low- and high-density polyethylenes. *Int. Biodeterior. Biodegrad.* 61, 203–213.
- Usha, R., Sangeetha, T., Palaniswamy, M., 2011. Screening of polyethylene degrading microorganisms from garbage soil. *Libyan Agric. Res. Cent. J. Int.* 2, 200–204.
- Yang, C.Z., Yaniger, S.I., Jordan, V.C., Klein, D.J., Bittner, G.D., 2011. Most plastic products release estrogenic chemicals: a potential health problem that can be solved. *Environ. Health Perspect.* 119, 989–996.
- Zerbi, G., Gallino, G., Del Fanti, N., Baini, L., 1989. Structural depth profiling in polyethylene films by multiple internal reflection infra-red spectroscopy. *Polymer* 30, 2324–2327.
- Zuker, M., 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* 31, 3406–3415.
- Zylstra, E.R., 2013. Accumulation of wind-dispersed trash in desert environments. *J. Arid Environ.* 89, 13–15.