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# Biodegradation of polyethene by bacteria isolated from mangroves

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Original Article

## Abstract

The current study focused on the biodegradation of polyethene by three bacterial strains namely, *Acinetobacter pittii*, *Acinetobacter nosocomialis* and *Acinetobacter radioresistens* isolated from the mangrove ecosystem of Ayiramthengu, Kerala coast. The bacterial samples individually as well as in combination were studied for polyethene degradation for a period of 45 days. Percentage weight loss, FTIR analysis and carbonyl index studies suggested that *A. pittii* and *A. radioresistens* in combination showed the highest polyethene biodegradation.

***Keywords***: *Acinetobacter, FTIR analysis, carbonyl index, Ayiramthengu*

## Introduction

The versatility of plastics has led to the replacement of natural products and has become an essential part of human society (Danso *et al.*, 2019). The worldwide production of plastic was almost 6.3 billion tons in 2015 and it is increasing each year exponentially due to its competent multipurpose usage (Yuan *et al.*, 2020). It is used for packing food, pharmaceuticals, cosmetics, detergents, chemicals and so on (Sangale *et al.*, 2012). Even though plastic eases human life, the disposal of plastics has become a challenging threat to the ecosystem. About 92% of synthetic plastics are contributed by polyethene and polypropylene (Byuntae *et al.*, 1991). Degradation of polyethene is a major challenge as it is widely used in day-to-day life. In India, the Central Pollution Control Board (CPCB) under the Ministry of Environment and Forest banned plastic bags with less than 50-micron thickness. Also, many states in India made a complete ban on these plastic bags. Despite this ban, many small vendors and groceries used plastic bags illegally (Sangale *et al.*, 2019). Biodegradation of polyethene by microorganisms is a natural process (Rutkowska *et al.*, 2002). The mechanism of polyethene degradation starts with the microbial assemblage on the surface followed by the extracellular enzyme secretion by microbes. This leads to the process of biodegradation by the cleavage of polymeric chains (Mohanan *et al.*, 2020). The biodegradation process is characterized by weight loss of the sample, change in physical and chemical properties, alteration in tensile strength (TS), carbon dioxide production, difference in molecular weight distribution and bacterial counts, variations in infrared (IR) spectrum of the sample after biotic exposure (Kathiresan, 2003; Roy *et al.*, 2008). The present study focuses on the biodegradation of polyethene by three bacterial strains individually as well as in combination.

## Material and methods

### *Collection and Surface Sterilization of Polyethene*

Polyethene bags of 51 micron thickness were cut into small pieces (5x3cm) and washed with tap water followed by surface sterilization using ethanol (70%). These were then washed with distilled water followed by 0.1% mercuric chloride and distilled water (Begum *et al.*, 2015). Air-dried and shade-dried samples were weighed.

### *Isolation and Identification of Bacteria*

Three selected bacterial strains (B1, B2, B3) isolated from soil samples of the Ayiramthengu mangrove, Kerala coast were identified by morphological, biochemical and molecular characterization using pure culture. The phylogenetic tree construction was done using the Maximum Likelihood method and Kimura 2 parameter method (Kimura, 1980).

Evolutionary analyses were conducted in MEGA version software (Kumar *et al.*, 2018).

## *Degradation of Polyethene*

The sterile pre-weighed samples were inserted aseptically into seven 250 ml conical flask containing 200 ml nutrient broth, which was autoclaved, cooled and labelled. A loopful of each bacterial strain was introduced to three conical flasks individually. Also, two bacteria were combined and introduced to the remaining nine conical flasks separately. One control without bacteria was maintained along with the experiment (Kathiresan; 2003, Begum *et al.*, 2015). These were incubated at 37°C for 45 days. After the duration, the polyethene strips were collected and washed thoroughly using distilled water, shade-dried and weighed for final weight.

## *Determination of Polyethene Degradation*

The percentage of degradation was calculated using the formula below (Usha *et al.*, 2011).

$$\text{Percentage of weight loss} = \frac{\text{Initial Weight - Final Weight}}{\text{Initial Weight}} \text{ X } 100$$

After incubation, the control and bacteria-treated polyethene samples were analysed using FTIR to calculate the carbonyl index, presence or absence of functional groups and analyse stretches. The carbonyl index is a measure of the concentration of carbonyl groups like acids, aldehydes and ketones (Albertson *et al.*, 1987).

$$\text{Carbonyl Index} = \frac{\text{Absorbance at 1715 cm}^{-1}\text{ (peak wavelength)}}{\text{Absorbance at 1465 cm}^{-1}\text{ (peak wavelength)}}$$

# Results and discussion

The three bacterial strains identified were *Acinetobacter pittii* (B1), *Acinetobacter nosocomialis* (B2) and *Acinetobacter radioresistens* (B3). *Acinetobacter* is commonly observed in various hydrocarbon-contaminated sites, comprising soils, mangrove sediments, Antarctic marine sediments, and pristine environments, exhibiting the widespread capacity for alkane biodegradation (Kuhn *et al.*, 2009; Kang *et al.*, 2011; Rocha *et al.*, 2013). It is also involved in various metabolic abilities like the degradation of numerous long-chain dicarboxylic acid pathways aside from aromatic and hydroxylated aromatic compounds that relate to the plant degradation products (Yoshida *et al.*, 1975).

The phylogenetic tree was constructed from these isolated bacteria using 16SrDNA and compared with those in the nucleotide database (Fig. 1). *Bacillus subtilis* was used as an outgroup. The phylogenetic tree revealed that there is a homology between different strains of *Acinetobacter* sp. Out of the three selected bacteria, *A. radioresistens* was more closely related to *A. nosocomialis* than *A. pittii*.

The weight loss of polyethene samples after 45 days of incubation is represented in Table 1. The result indicated that the highest degradation was found in isolate B4 with a combination of *A. pittii* and *A. radioresistens* (16.8%) followed by isolate B3 with *A. radioresistens* (15%), isolate B5 with a combination of *A. pittii* and *A. nosocomialis* (14.4%), isolate B6 with a blend of *A. radioresistens* and *A. nosocomialis* (7.6%), isolate B1 having *A. pittii* (2.8%) and isolate B2 with *A. nosocomialis* (1.6%) (Fig. 2). Kathiresan and Bingham (2001) stated that polyethene biodegradation of mangrove sediment bacteria was 2.19% to 20.54% by *Pseudomonas* sp., *Staphylococcus* sp., Moraxella sp., *Micrococcus* sp. and *Streptococcus* sp.

During the process of biodegradation of polyethene, extracellular enzymes secreted by microbes help to perform various chemical reactions that may lead to several changes like oxidation, reduction,

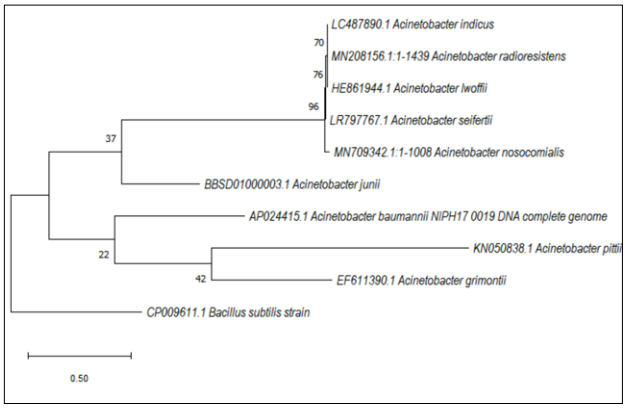


Fig. 1. A phylogenetic tree of *Acinetobacter* showing relationships between 16SrDNA sequences

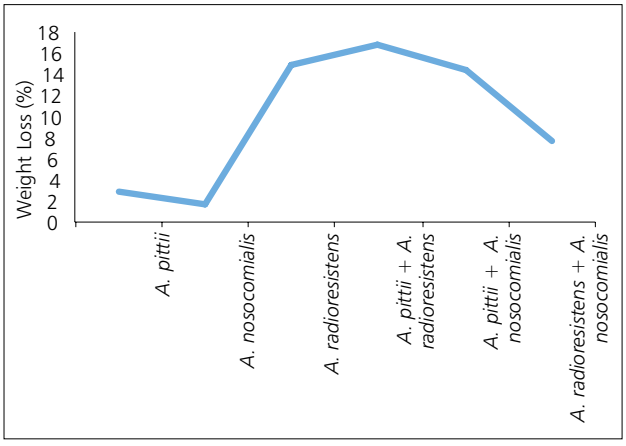


Fig. 2. Weight loss (%) of polyethene by different bacterial strains

esterification, hydrolysis and inner molecular conversion (Gupta and Devi, 2019). These alterations can be observed using FTIR analysis. The FTIR spectral figures of the present study indicated the formation of new functional groups because of new peaks on the polyethene sheets and the control (incubated without inoculum) which are represented in Fig. 3 to 9. FTIR spectra showed changes in chemical structure, and side chain modifications in almost all the tested samples confirming the biodegradation of polyethylene film due to bacterial activities. Also, band intensities of different regions of spectra varied in test samples which may be attributed to bacterial action.

Analysing the FTIR spectra at 3000-2500 $cm^{-1}$ representing single bond region showed variation in test samples from that of control. A very long peak was observed for treatment with *A. pittii* and *A. radioresistens* at 2500-2000 $cm^{-1}$ region, and *A. nosocomialis* alone with a long peak when compared with the control (small peak) whereas other test samples showed a short peak for other bacterial combinations. 2500-2000 $cm^{-1}$ region

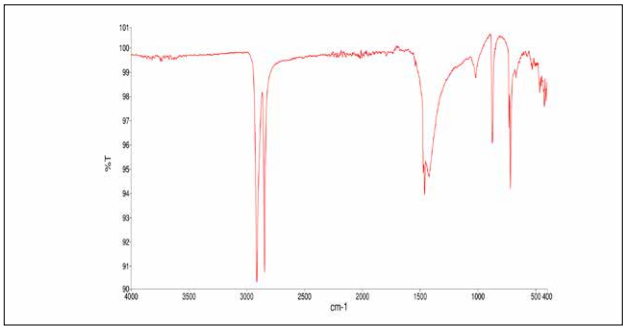


Fig. 3. FTIR spectra of polyethene as control

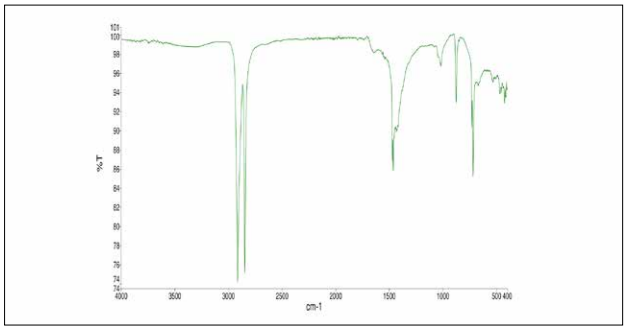


Fig. 4. FTIR spectra of polyethene treated with *Acinetobacter nosocomialis*

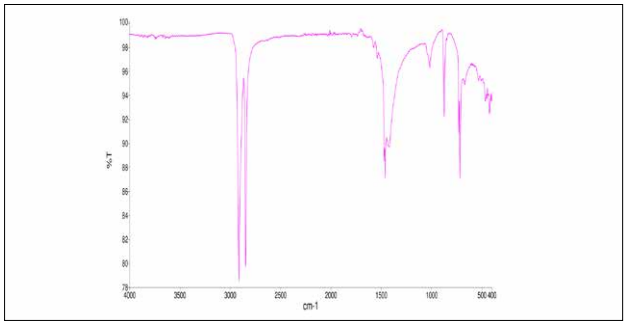


Fig. 5. FTIR spectra of polyethene treated with *Acinetobacter radioresistens*

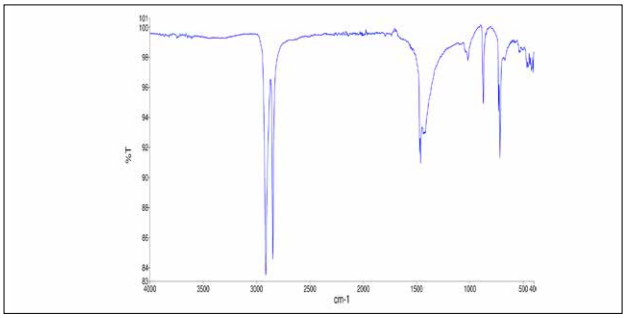


Fig. 6. FTIR spectra of polyethene treated with *Acinetobacter pittii*

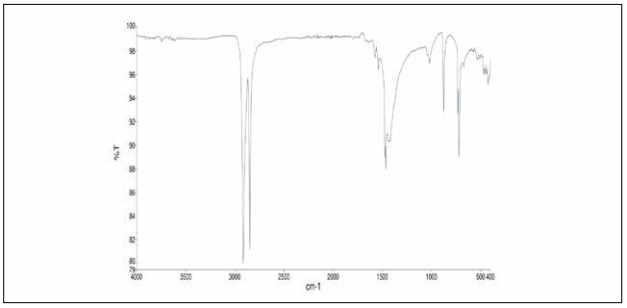


Fig. 7. FTIR spectra of polyethene treated with *A. pittii* and *A. nosocomialis*

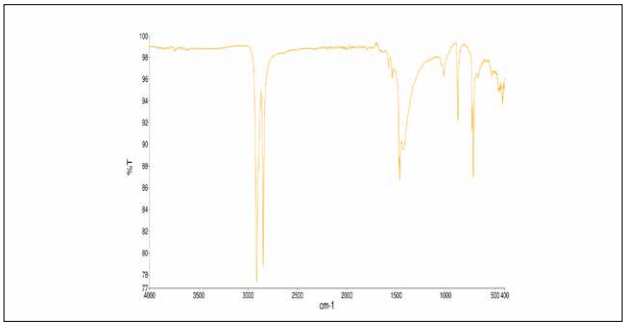


Fig. 8. FTIR spectra of polyethene treated with *A. pittii* and *A. radioresistens*

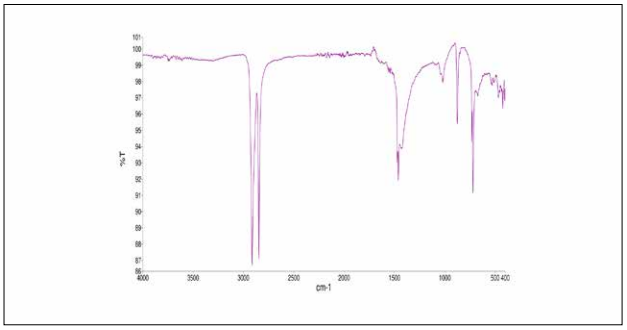


Fig. 9. FTIR spectra of polyethene treated with *A. radioresistens* and *A. nosocomialis*

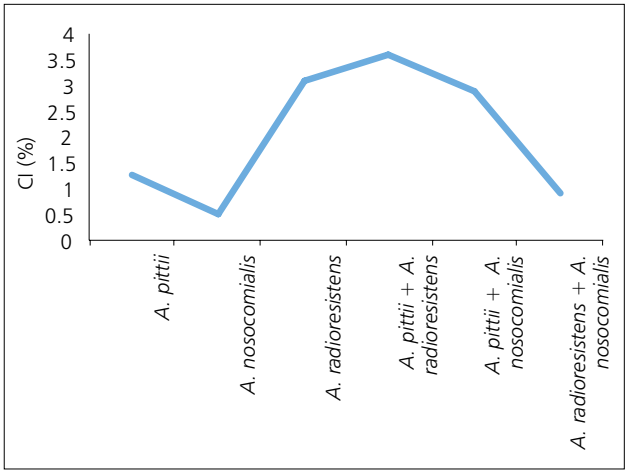


Fig. 10. Carbonyl Index (% increase) of polyethene by different bacterial strains

represents a triple bond and the spectra depicted a change in all the test samples by the bacterial action. At 2000-1500 $cm^{-1}$ region, *A. pittii* and *A. radioresistens* treatment on polyethene showed long and a greater number of peaks compared to the control sample. This region represents a double bond and all the test samples showed a greater number of peaks compared to the control sample.

The carbonyl index analysis indicated that all the tested samples showed an increase in carbonyl index which signifies the biodegradation process. A maximum percentage increase in carbonyl index was observed for treatment with *A. pittii* and *A. radioresistens* (3.58%), followed by *A. radioresistens* (3.1%), combination of *A. pittii* and *A. nosocomialis* (2.87%), *A. pittii* (1.28%), combination of *A. radioresistens* and *A. nosocomialis* (0.90%) and *A. nosocomialis* (0.50%) (Fig. 10). Similarly, Harshvardhan and Jha (2013) noted PE biodegradation with a rise in the carbonyl index calculated using FTIR spectra. These functional groups at the surface of polyethene are critical as the oxidized groups produce a rise in the hydrophilicity which in turn helps the microbe for effective attachment on the surface of polyethene and hence promotes biodegradation (Albertsson *et al.*, 1995; Tribedi and Sil, 2013).

Thus, the present study indicated that *A. pittii* and *A. radioresistens* showed the highest weight loss and carbonyl index. The reason for high polyethene degradation may be due to the cooperation between the two bacterial strains which activated certain biochemical events for the process of polyethene degradation. In the case of polyethene treated with *A. radioresistens* 3.1% carbonyl index increase was seen whereas in combination with *A. nosocomialis* showed 0.90% only. This decrease may be due to the inhibition between these two bacterial strains that happened between these two bacterial strains. Hibbing *et al.* (2010) stated that bacterial competition happens when they are in combination either to impair or kill other bacteria for survival or flourishing.

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