*J. Int. Environmental Application & Science,* Vol. 10(4): 399-407 (2015)

# Optimum Conditions for LDPE Strips Biodegradation by Local Bacterial Isolates

Amal A. Hussein[1,*], Saad H. Khudhair[2], Ithar K. Al-Mayaly [3]

[1]*Applied of Science Department, University of Technology. Baghdad, IRAQ.* [2]*Ministry of Science and Technology. Baghdad, IRAQ.* [3]*Department of Biology. University of Baghdad. Baghdad, IRAQ*

*Received July 20, 2015; Accepted November 16, 2015*

**Abstract:** Optimum conditions (Temperature, pH and incubation time) were studied for selected bacterial isolates *Pseudomonas fluorescens, Pseudomonas aeruginosa* and *Acinetobacter ursingii* to biodegrade the low density polyethylene (LDPE) by using mineral salts medium (MSM) contains one pieces of LDPE strips (1cm ×5cm) using bacterial growth and amount of dissolved carbon dioxide as an indicators for ability of degradation. The results have been revealed that the above three bacterial isolates were shown high ability to degrade the LDPE strips after 15 days of incubation in liquid mineral salts medium pH 7 in 30˚C, the growth was 0.351, 0.272 and 0.241, respectively, while the amount of dissolved $CO_2$ was 1.18, 1.05 and 0.95 g/l, respectively. The results of FTIR spectrum of LDPE strips which treated with three bacterial isolates were shown different changes in transmission bands for active groups of LDPE strips. *Pseudomonas fluorescens* isolates was shown the highest % of C-H group biodegradation (40.47%) compared with the other two isolates 35.89% and 30.55%, respectively.

***Keywords****: LDPE biodegradation, optimum condition, $CO_2$, FTIR*

## Introduction

Low density polyethylene (LDPE) is an important commodity plastic and has a wide applicability in the current time. In virtue of their wide applicability, the generation of the huge plastic waste became a conundrum for environment and public health (Harshita Negi *et al*., 2011). Plastics waste lay enormous burden on the environment, because their recalcitrance to degradation accelerates the accumulation in nature. Plastics waste buried in soil cause the water clogging phenomena and devastates soil for agricultural cultivation. Many animals die of plastics waste either by being caught in the plastics waste trap or by swallowing the plastics waste debris to exert ruinous effects on the ecosystem (Usha *et al*., 2011).

There is lot of interest in the microbial degradation of polyethylene waste material (Kambe *et al*., 1999). Biodegradation resulting from the utilization of polyethylene as nutrient may be more efficient if the degrading microorganism forms a biofilm on the polyethylene surface (Shah *et al*., 2009). The microbial species are associated with the degrading materials were identified as bacteria (*Pseudomonas, Streptococcus, Staphylococcus, Micrococcus, Moraxella*), fungi (*Aspergillus niger, Aspergillus glaucus*), *Actinomycetes* sp. and *Saccharomonospora* genus (Swift, 1997; chee *et al*., 2010). Microbial degradation of plastic caused by oxidation or hydrolysis using microbial enzymes that lead to chain cleavage of the high molecular weight polymer into low molecular weight oligomer and monomer by aerobic or anaerobic metabolism (Kumar *et al*., 2013). In the present study optimized condition for polyethylene degradation using three selected isolates was studied and the FTIR analysis was used to confirm this ability.

## Materials and Methods

### Polyethylene (PE)

The low-density polyethylene (LDPE) strips used in this study were obtained from local plastic bags. LDPE was cut into small pieces of about (1cm×5cm).

### LDPE degrading bacterial isolates

Three bacterial isolates have been isolated from sites contaminated with plastic waste and selected according from previous study and this three isolates were identified as a *Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Acinetobacter ursingii* (Amal *et al*., 2015).

[*] *Corresponding: E-Mail: amelali71@yahoo.com; Tel: 00947902609453*

**Biodegradation assay of Polyethylene by bacterial isolates**

To determine the ability of the selected isolates to degrade Low Density Polyethylene, 25 milliliter of mineral salt medium (MSM) (Shah, 2007) was dispensed in Erlenmyer flasks (100ml) and supplemented with LDPE strips (1cm×5cm).as a substrate and sole source of carbon, flasks were autoclaved. After sterilization, the flasks were inoculated with 1ml v/v 18hrs. old (OD = 0.5) of *P. fluorescens, P. aeruginosa and Acinetobacter ursingii* with control (medium without inculation). The degradation ability of these bacteria was determined by monitoring the growth density of the liquid culture at 600nm using UV-spectrophotometer (Model SP-3000 Plus; Kapri *et al.,* 2010).

Dissolved $CO_2$ was evolved as a result of LDPE degradation and determined volumetrically by alkalinity method. Sample (25 ml of culture) was taken in a conical flask and 0.05 ml of 0.1 N sodium thiosulphate solutions was added, then 2 drops of methyl orange indicator was added, and then titrated against 0.02N sodium hydroxide solution until the color was changed from orange red to yellow. Two drops of phenolphthalein indicator was added and titration continued till a pink color developed (kumar *et al.,* 2013). Volumes of the titrant used were recorded and the amount of $CO_2$ calculated according to the equation:

$$\text{Dissolve } CO_2\,(mg/l) = \frac{A \times B \times 50 \times 1000}{V}$$

Where; A = ml of NaOH titrant; B = normality of NaOH; V = ml of the sample

**Optimization of polyethylene biodegradation**

**Effect of Temperature**

Twenty five ml of liquid MSM with one pieces of LDPE strip (1cm X 5cm) was prepared and dispensed in 100 ml Erlenmeyer flasks containing (pH 7), at different temperatures 25, 30, 35 and 40 °C for 15 days (samples were taken after 5 days), to determine the effect of temperature on the ability of *bacterial isolates* to degrade LDPE. Optimal temperature was subsequently employed, depending on the growth density measurement, and amount of dissolved $CO_2$.

**Effect of pH**

To determine the effect of pH on the ability of *P. fluorescens, P. aeruginosa and Acinetobacter ursingii* to utilize LDPE as a sole source of carbon and energy, MSM with one piece of LDPE at different pH values (5, 6, 7, 8 and 9), were prepared, then the cultures were incubated in a shaker incubator (150 rpm) (Model IS-971R) at 30°C for 15 days. After the incubation, the bacterial growth (OD) at 600 nm and amount of dissolved $CO_2$ were measurement.

**Effect of incubation period**

MSM (pH7) supplemented with one piece of LDPE strip was prepared and dispensed in 100 ml Erlenmeyer flasks containing 25ml. Then the flasks were inoculated with 1ml bacterial isolates and incubated in shaker incubator, (150rpm) at 30°C for a periods (0, 5, 10, 15, 20, 25 and 30 days). After the end of incubation, the bacterial growth at 600 nm, amount of dissolved $CO_2$ and the changes in transmission spectrum by FTIR techniques were determined to any relative degradation of LDPE strip in each studied time.

## Results and Discussion

The use of biodegradation offers a cheap method for recycling wastes efficiently and, when optimized, at a faster rate than under natural conditions (Tokiwa *et al.*, 2009).

**Temperature**

The cultures of *P. fluorescens, P. aeruginosa* and *A. ursingii* were grown and incubated at different temperatures (25, 30, 35 and 40°C). Results presented in Figure (1) were pointed out that the highest optical density (OD) of bacterial growth was shown at 30°C reached 0.332, 0.262 and 0.236, respectively, while the optical density of bacterial growth at 35°C was 0.332, 0.264 and 0.238 in liquid MSM pH 7 after fifteen days of incubation.

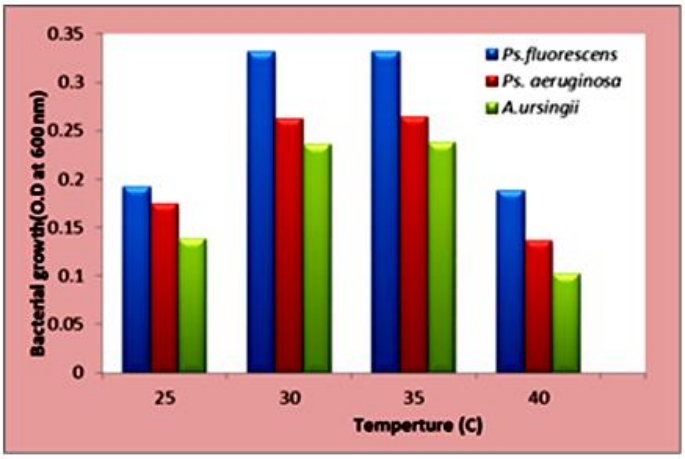



**Figure 1.** Effect of temperature on bacterial growth of *P. fluorescens, P. aeruginosa and A. ursingii* at pH 7in a shaker incubator (150rpm) for 15 days.

*P. fluorescens* showed the maximum growth rate in MSM ( pH 7.0) with LDPE strip after 15 days of incubation at 30˚C and 35˚C was significantly different at ($p<0.05$) than those of *P. aeruginosa* and *A. ursingii* with no significant difference between the latter two isolates. Results also revealed gradual decrease in bacterial growth for all bacterial isolates with increasing temperature, this is due to the accumulation of metabolites resulting from oxidation processes produced by bacterial isolates, or to a lack of oxygen and nutrients as suggested by (Bishnoi *et al*., 2009).

Results of bacterial growth recorded in the previous study of Jailawi *et al*.,( 2015) showed that the optical density of *P.putida* growth was 0.3 and 0.36 after three and seven days of incubation at 37˚C. Burd, (2008) revealed that (polyethylene) PE film degradation significantly increased at 30˚C for 6 weeks *by Sphingomonas sp.* and *Pseudomonas sp*. Skariyachan *et al.,* (2015) indicated that *Pseudomonas* spp was grown optimally at 37˚C in pH 9 and showed 35-40% of plastic weight reduction over 120 days. High temperature (40˚C) was found to enhance degradation rate of PE more effectively by 24-28% compared to temperature of 30˚C (18-21%) (Kumari *et al.,* 2009).

Figure (2) shows that *P. fluorescens* gave highest value of dissolved $CO_2$ generated from the degradation of LDPE film 1.16 and 1.17 g/ l at 30˚C and 35˚C, respectevily, with significant differences ($p<0.05$), compared with other two isolates which had (1.02, 1.05 g/ l) for *P. aeruginosa* at 30˚C and 35˚C, respectevily, and (0.93, 0.98 g/ l) for *A. ursingii* at 30˚C and 35˚C, respectively.

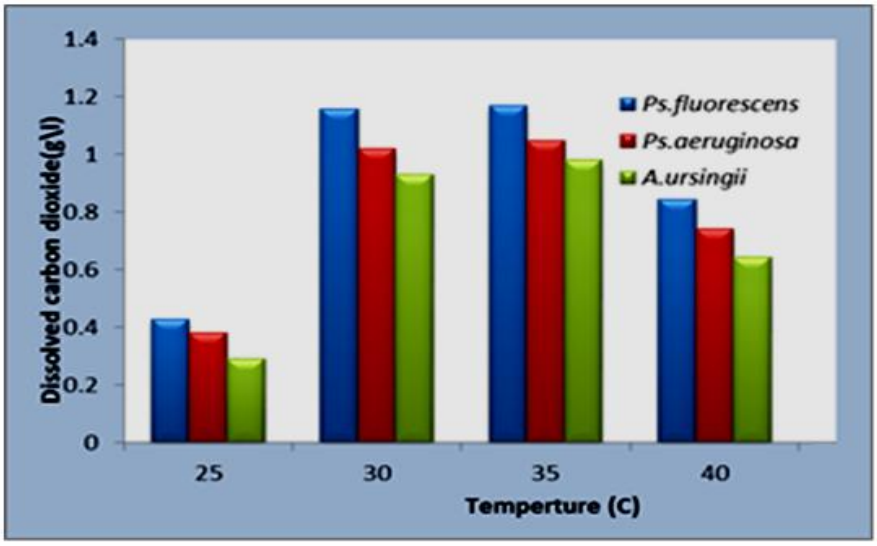



**Figure 2.** Effect of temperature on $CO_2$ generation by *P. fluorescens*, *P. aeruginosa* and *A. ursingii* at pH 7 in a shaker incubator (150rpm) for 15 days

**The effect of pH**

The obtained results, in Figure (3) showed that the optimum growth was occurred at pH 7 for all bacterial isolates after 15 days of incubation, 150rpm at 30˚C.

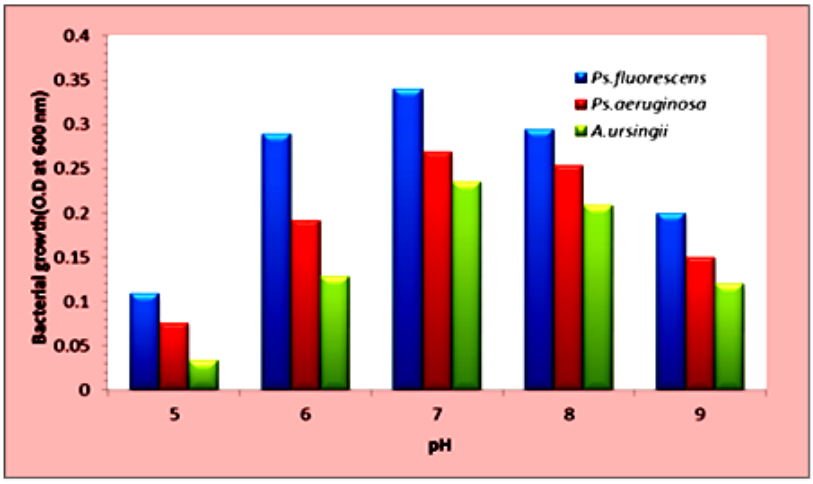



**Figure 3**. Effect of pH on bacterial growth of *P. fluorescens, P. aeruginosa and A. ursingii* at 30°C (150rpm,) for 15 days.

*P. fluorescens* had highest growth rats (0.31 at 600 nm) which was significantly different ($p<0.05$) from those of *P. aeruginos*a and *A. ursingii* which gave a growth rate of 0.23 and 0.212, respectively. However, bacterial growth was decreased at lower and higher pH values compared with growth at pH 7. Al-Jailawi *et al.*, (2015) found that pH 6.5 was suitable for the growth of *P. putida* grown in medium containing 0.5% of LDPE. Optimum pH required to degrade LDPE was 8 by *Alcaligens* sp and *Methylobacillus* sp. (Hima *et al.*, 2014). While suitable pH for LDPE film degradation in soil by *Lysinibacillus xylanilyticus* was found to be 7.5 (Esmaeili *et al.*, 2013).

The most common end products of polyethylene degradation were $CO_2$, $CH_4$, and/or $H_2O$. Percentage of biodegradation is the evolution of $CO_2$ during depolymerization in which polymer is first converted to monomers by breaking the links and then to simpler compounds to be assimilated into the living cells (Merina and Santosh, 2014). *P. fluorescens* gave highest value of $CO_2$ evolution from the degradation of LDPE film 1.16 g/ l with significant differences ($p<0.05$) compared with other two isolates that showed 1.02 and 0.93 g/ l for *P. aeruginosa* and *A. ursingii***,** respectively (Figure 4).

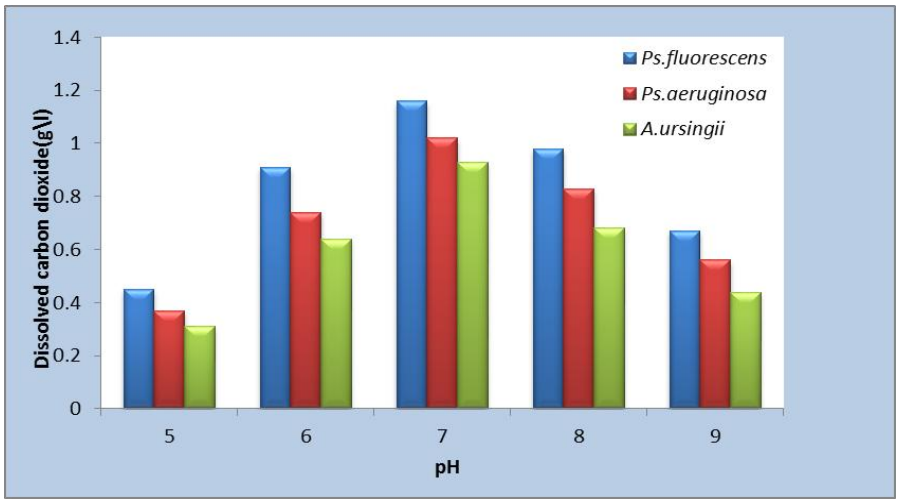



**Figure 4**. Effect of pH on values of dissolved carbon dioxide g/l realesed from LDPE degradation by *P. fluorescens, P aeruginosa* and *A. ursingii* at 30˚C in a shaker incubator (150rpm,) for 15 days**.**

The biodegradation values for pure LDPE films without oxidation pre-treatment in the SMP (Soil + Selected Microorganisms + non-UV-irradiated PE) treatment was 15.8% at 30°C, for 126 days by *Lysinibacillus xylanilyticus* at pH value of 7.5 (Esmaeili *et al.,* (2013). This pH was found to be near optimal for hydrocarbon biodegradation and it was assumed that this pH would also favor the biodegradation of plastic materials (Yabannavar & Bartha, 1994).

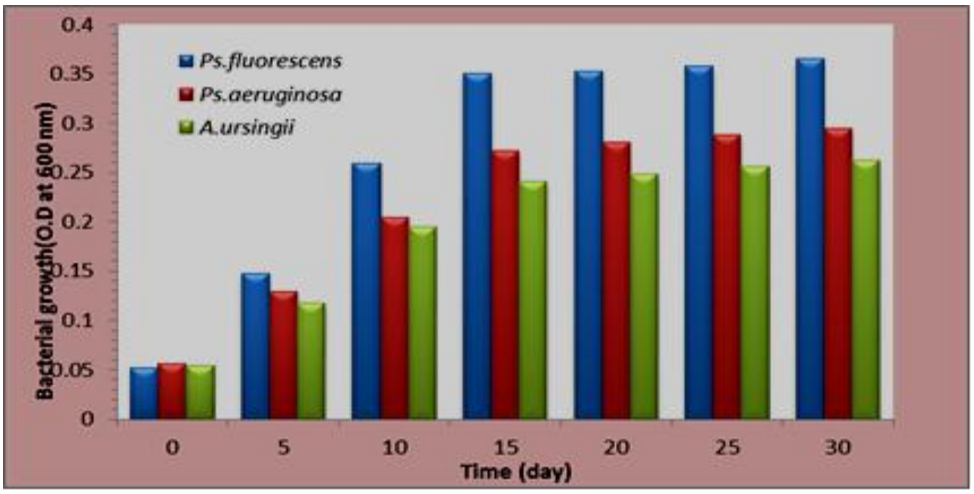



**Figure 5**. Effect of different incubation period on bacterial growth of *P. fluorescens*, *P. aeruginosa* and *Acinetobacter ursingii* at pH7, 30°C, and 150 rpm.for 30 days.

**The effect of incubation period**

Incubation period is one of the most important factor that affect biodegradation processes (Atlas, 1981). The results given in figure (5) revealed that the best period for LDPE biodegradation was significant at 15 days ($p<0.05$). The results of *P. fluorescens* isolate was significantly the best for LDPE degradation, which gave growth rate (OD= 0.351) after 15 days of incubation than both *P. aeruginosa* (0.272) and *A. ursingii* (0.241) isolates.

The amount of dissolved $CO_2$ evolved was determined (figure 6). The results explaining there were significant differences ($p<0.05$) in dissolved $CO_2$ evolution between the *P. fluorescens*, *P. aeruginosa* and *A. ursingii* isolates. These isolates, however showed a slight and gradual increase in dissolved $CO_2$ generation during incubation periods and the *P. fluorescens* treatment demonstrated the highest amount of dissolved $CO_2$ production reached 1.18g/l after 15 days of incubation, while the dissolved $CO_2$ levels reached 1.05g/l and 0.95g/l for *P. aeruginosa* and *A. ursingii,* respectively, due to the utilization of LDPE film as the carbon source.

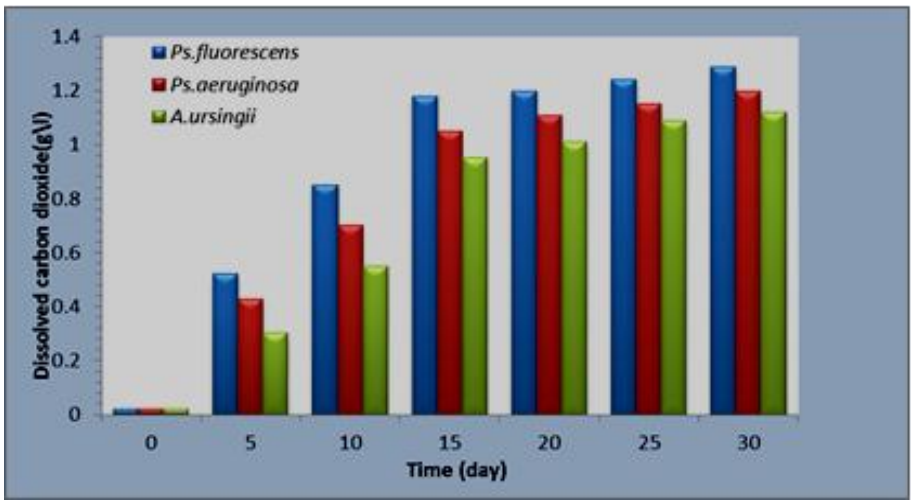
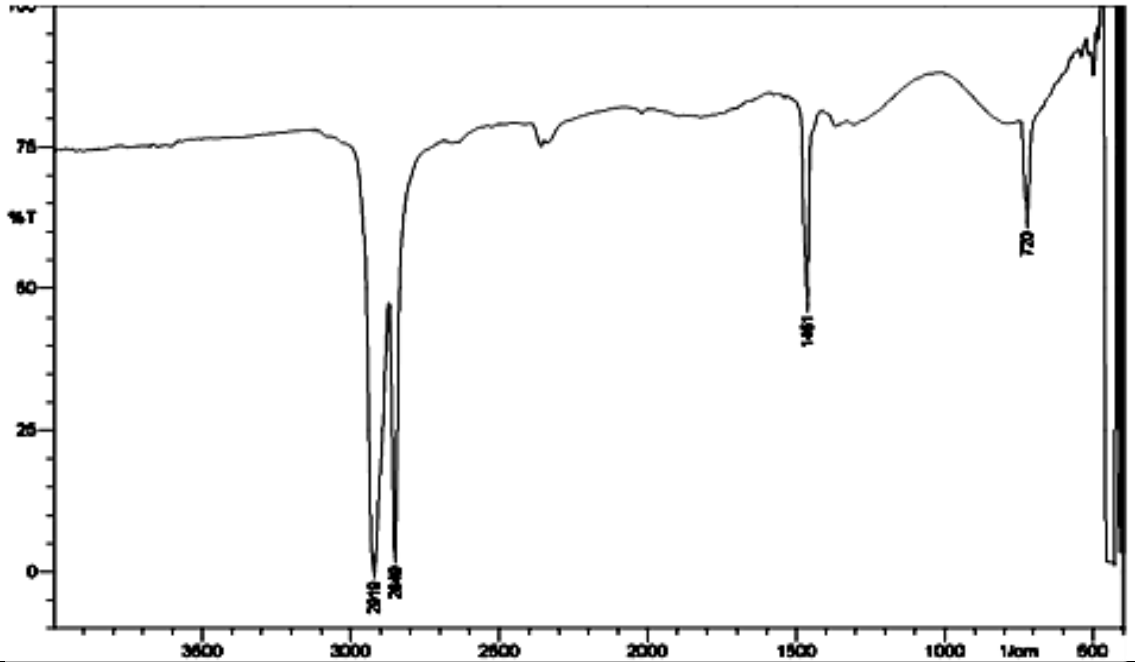



**Figure 6.** Effect of different incubation period on dissolved carbon dioxide (g/l) production during biodegradation of LDPE by *P. fluorescens*, *P. aeruginosa* and *A. ursingii* at 30°C, pH7, for 30 days.

All previous works did not take into account the dissolved $CO_2$ during Sturm test evaluation (Ruffo *et al*., 1984).). Shah *et al*. (2007) performed this test with a consortium of bacterial and fungal isolates, and reported a $CO_2$ concentration of 1.85 g/ l for 4 weeks. A study done by Muhammad *et al.*, (2009) reported a concentration of about 10 g/l of $CO_2$ after a period of 30 days. Shah *et al.* (2009) also reported $CO_2$ concentration of about 1.85 g/ l after a 30 day period of growth of *Fusarium* sp. on LDPE films.

Polyethylene degradation was confirmed by using Fourier Transform Infrared (FTIR) Spectroscopy to measure the changes in the spectra of the LDPE strips or in the intensity of the bands before and after treated with the bacterial isolates. A percentage of LDPE biodegradation was detected depending on the change in the intensity of 2919 $cm^{-1}$ band , which represent C–H stretching vibration of functional group supporting the conformational changes on polymer surface (Das & Kumar, 2015).

The *P. fluorescens* showed maximum percentage of LDPE degradation 40.47%(equation 1) as shown in Figure 7 when it inoculated in liquid MSM, pH 7 and incubated at 30°C for 15 days, compared with *P. aeruginosa* and *A. ursingii* which showed 35.89% (Eq. 2) and 30.55% (Eq. 3) as in Figures 8 and 9 for LDPE biodegradation under the same conditions, respectively.



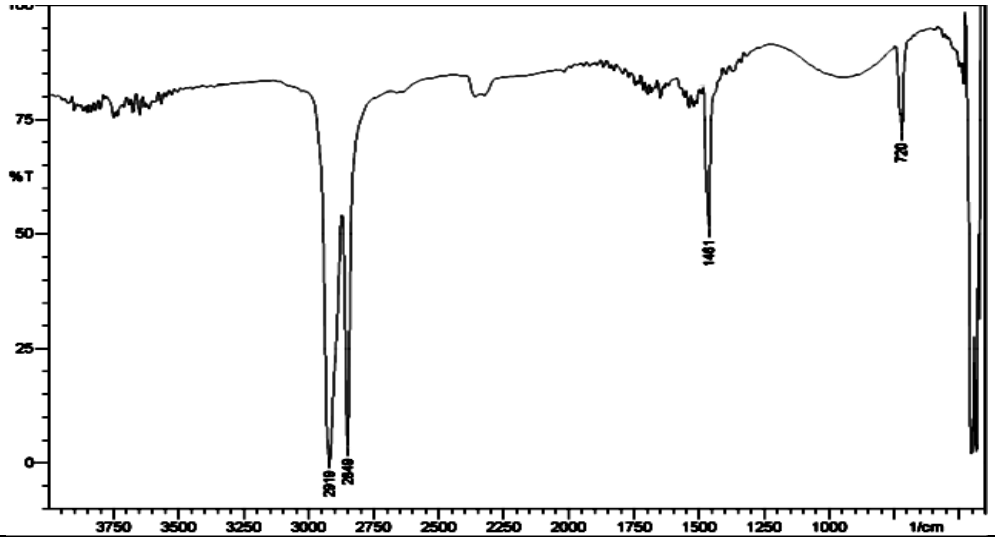

| Peak Number | Begin [1/cm] | Maximum[1/cm] | End [1/cm] | Width [1/cm] | Height [%T] | Absolute Height[%T] | Peak Area | Peak Area (Abs.Sum) | Peak Area (Algebr.Sum) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 577.44 | 720.16 | 743.3 | 165.86 | -18.9 | 62.1 | -589.47 | 589.47 | -589.47 |
| **2** | 1414.47 | 1460.76 | 1530.19 | 115.72 | -35.06 | 47.46 | -756.92 | 756.92 | -756.92 |
| **3** | 2683.52 | 2849.39 | 2868.67 | 185.15 | -73.79 | 3.09 | -2478.35 | 2536.3 | -2536.3 |
| **4** | 2868.67 | 2918.82 | 3119.4 | 250.72 | -76.77 | 0.42 | -4955.73 | 5013.68 | -5013.68 |

**Figure 7.** FTIR spectrum for LDPE film in liquid MSM, pH 7 incubated with *P. fluorescens* isolate after 15days, at 30°C,in a shaker incubator(150 rpm) after 15 days.

$$\%\ \text{of C} - \text{H group biodegradation} = \frac{\text{T value of Shw43 isolate} - \text{T value of positive control}}{\text{T value of Shw43 isolate}} \times 100$$

(Herzberg, 1945)

$$\%\ \text{of C} - \text{H group biodegradation} = \frac{0.42 - 0.25}{0.42} \times 100 = 40.47\ldots\ldots\ldots 1$$

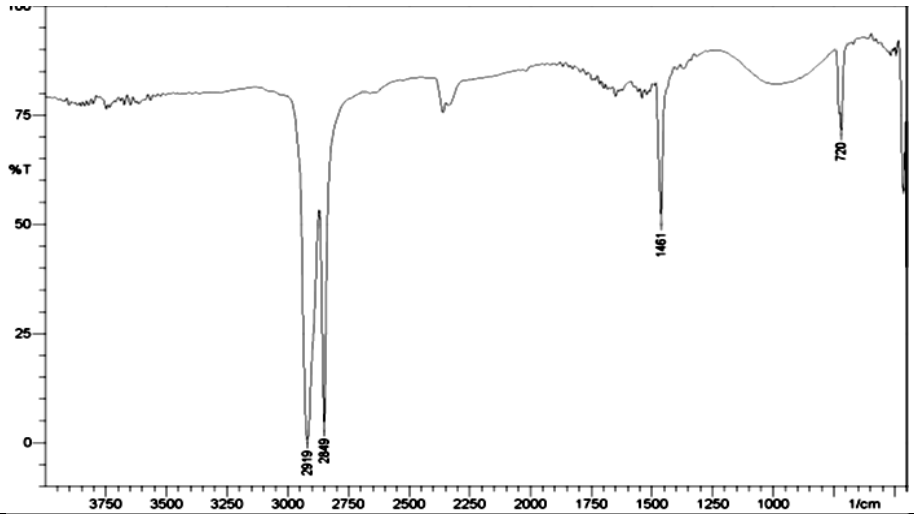



| Peak Nu. | Begin [1/cm] | Max. [1/cm] | End [1/cm] | Width [1/cm] | Height [%T] | Absolute Height[%T] | Peak Area | Peak Area(Abs .Sum) | Peak Area (Algebr. Sum) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 673.87 | 720.16 | 743.3 | 69.43 | -19.66 | 72.08 | -386.69 | 386.69 | -386.69 |
| **2** | 1410.61 | 1460.76 | 1483.9 | 73.29 | -32.56 | 50.78 | -695.18 | 695.18 | -695.18 |
| **3** | 2691.24 | 2849.39 | 2868.67 | 177.44 | -78.2 | 3.13 | -2387.01 | 2440.24 | -2440.24 |
| **4** | 2668.67 | 2918.82 | 3019.11 | 150.43 | -80.95 | 0.39 | -4720.34 | 4773.57 | -4773.57 |

**Figure 8**. FTIR spectrum for LDPE film in liquid MSM, pH 7 incubated with *P. aeruginosa* isolates after 15days, 150 rpm at 30°C.

$$\%\ \text{of C} - \text{H group biodegradation} = \frac{\text{T value of Tw53 isolate} - \text{T value of positive control}}{\text{T value of Tw53 isolate}} \times 100$$

$$\%\ \text{of C} - \text{H group biodegradation} = \frac{0.39 - 0.25}{0.39} \times 100 = 35.89\ldots\ldots\ldots..2$$




| Peak Nu. | Begin [1/cm] | Max. [1/cm] | End [1/cm] | Width [1/cm] | Height [%T] | Absolute Height[%T] | Peak Area | Peak Area (Abs. Sum) | Peak Area (Algebr. Sum) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | 693.15 | 720.16 | 743.3 | 50.14 | -19.64 | 70.93 | -372.38 | 372.38 | -372.38 |
| **2** | 1410.61 | 1460.76 | 1483.9 | 73.29 | -33.34 | 50.24 | -683.41 | 683.41 | -683.41 |
| **3** | 2687.38 | 2849.39 | 2868.67 | 181.29 | -76.92 | 3.12 | -2337.31 | 2389.51 | -2389.51 |
| **4** | 2868.67 | 2918.82 | 3019.11 | 150.43 | -79.53 | 0.36 | -4636.96 | 4689.17 | -4689.17 |

**Figure 9**. FTIR spectrum for LDPE film in liquid MSM, pH 7 incubated with *A. ursingii* isolates after 15days, 150 rpm at 30°C.

$$\% \text{ of } C-H \text{ group biodegradation} = \frac{\text{T value of Mw51 isolate} - \text{T value of positive control}}{\text{T value of Mw51 isolate}} \times 100$$

$$\% \text{ of } C-H \text{ group biodegradation} = \frac{0.36-0.25}{0.36} \times 100 = 30.55\ldots\ldots\ldots 3$$

The microorganisms get attached to the surface; it starts to grow by using the polymer as the carbon source. In the primary degradation, the main chain cleaves leading to the formation of low-molecular weight fragments (oligomers), dimers or monomers (Vasile, 1993). The degradation is due to the extracellular enzyme secreted by the microorganism. These low molecular weight compounds are further utilized by the microorganisms as carbon and energy sources. (Narayan, 2006; Usha *et al*., 2011).

## Conclusion

The optimal conditions for the LDPE biodegradation by selected isolates (*Pseudomonas fluorescens*, *Pseudomonas aeruginosa* and *Acinetobacter ursingii*) were after 15 days of incubation in MSM, pH 7 at 30°C.

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