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Biodegradation of Oil-Based Plastic Wastes by Bacteria Isolated from Fish Breeding Tanks

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Abstract

Several studies have shown that certain microbes, mainly bacteria may have the ability to digest plastic wastes. The goal of this study was to see how well *Bacillus subtilis*, *Staphylococcus lentus*, *Aeromonas hydrophila*, *Sphingomonas paucimobilis* and *Kocuria paedia* degrade three kinds of oil-based plastics: low-density polyethylene (LDPE), polystyrene (PS) and polyvinylchloride (PVC) polymer sheets. The experiment was conducted for 30 days under laboratory conditions with occasional shaking at 180 rpm and 32°C. Biodegradation was measured in terms of weight loss. According to IR Spectroscopy, the C-H stretch band at 2920cm⁻¹ improved as a result of bacterial degradation of polyethylene. The most affected polymers were LDPE and PVC films. While PS films were the least affected polymers. *B. subtilis* was shown to be the most successful of the five bacterial species, whereas *K. paedia* was determined to be the least effective.

Keywords: Polymers waste, Environmental pollution, Polyvinylchloride, polystyrene, Polyethylene, Degradation of plastics

التحلل الحيوي للنفايات البلاستيكية ذات الأساس البترولي بواسطة البكتيريا المعزولة من أحواض تربية الأسماك

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الخلاصة:

أظهرت العديد من الدراسات أن بعض الميكروبات وخاصة البكتيريا لها دور كبير في تحلل بعض الفضلات البلاستيكية عن طريق هضمها ، لذلك كان الهدف من هذه الدراسة هو معرفة مدى نجاح بعض أنواع البكتيريا وهي *Aeromonas hydrophila* ، *Staphylococcus lentus*، *Bacillus subtilis* ، *Kocuria paedia* و *Sphingomonas paucimobilis* في تحلل ثلاثة أنواع من البلاستيك المصنع من المشتقات النفطية وهي: البولي إيثيلين منخفض الكثافة (LDPE) ،البولي ستايرين (PS) والبولي فينيل كلوريد (PVC) . أجريت التجربة لمدة 30 يوماً في ظروف مختبرية مع اهتزاز لمكونات التجربة عند 180 دورة في الدقيقة و 32 درجة مئوية. تم قياس التحلل الحيوي من حيث فقدان الوزن . وفقاً لمطيفية الأشعة تحت الحمراء FTIR، الحزمة العائدة للأصرة C-H عند 2920 سم⁻¹ أثبتت التحلل البكتيري للبولي أثيلين واطيء الكثافة. كانت البوليمرات الأكثر تأثراً هي البولي إيثيلين منخفض الكثافة، بينما كانت البوليمرات الأقل تأثراً هي أغشية البولي ستايرين PS ، وقد تبين أن بكتيريا *B. subtilis* هي أفضل الأنواع الخمسة من حيث قدرتها على تحليل الفضلات البلاستيكية، في حين وحسب النتائج المستحصل عليها فقد تم تحديد *K. paedia* على أنها الأقل فعالية في تحلل البوليمرات المستخدمة في هذه الدراسة.

1. Introduction

Ease of plastics utility and applicability has made them an essential part of daily life. Plastics are widely used in the household as well as for industrial purposes. Plastics are widely accepted because of their cost-effectiveness, superior barrier qualities, bio-inertness and them being lightweight. Over the previous half-century, plastics industry has risen exponentially, reaching a production of 322 million tons in 2015 worldwide [1]. Approximately 350 to 400 million tons of synthetic polymers are manufactured worldwide each year.

A number of cost-effective choices for plastic manufacture include polyethylene terephthalate (PET), polyvinyl chloride (PVC) and polyethylene terephthalate (PP) [2]. The problem is that these polymer products are typically made up of non-biodegradable plastics. Due to unmanaged garbage disposal, a substantial amount of plastic has entered the ecosystem, and its widespread use has put the environment in danger. A lack of adequate plastic waste management and not well-informed public approach to a proper management of this waste stream are the key reasons behind this problem [3]. Oil-based plastics have the disadvantage of degrading more slowly due to abiotic environmental conditions (such as ultraviolet light, high temperatures and physical stress). During biodegradation, plastic trash is not entirely broken down by microbes or digested by other organisms (biotic factors). Some of the driving factors behind the lack of a suitable functional group are high molecular weight (MW), hydrophobicity and crystallinity. Certain microorganisms can help with plastic waste material fragmentation through the enzymatic activity of microbes and breaking of chemical bonds [4].

Several bacteria, particularly from the genus *Bacillus* [5-6] and fungi like *Aspergillus* spp. [7], have been observed to exhibit promising results for biodegradation of oil-based plastics. In one of the previous approaches, [6] the biosurfactant production ability of *B. subtilis* (B30) to enhance oil recovery was evaluated. However, employing microbially synthesized biosurfactants for polymer degradation has not yet been adequately studied. Therefore, the present study aimed to showcase the ability of selected local bacterial isolates to degrade oil-based plastic wastes and to provide an eco-friendly approach towards removing these toxic waste materials.

2. Material and Method**2.1 Polymer Sample Collection**

Study samples were exposed to low-density polyethylene (LDPE), PS and PVC films, all of which were made up of three different polymers (derived from waste). They were all made in the 2cm × 2cm format and were then exposed to UV light for 72 hours [8]. The experiments were carried out in marine bacteriology lab, Marine Science Centre, University of Basra, Basra, Iraq.

2.2. Bacteria and growth conditions

In this study, *Bacillus subtilis*, *Staphylococcus lentus*, *Aeromonium hydrophilic*, *Sphingomonas paucimobilis* and *Kocuria paedia* were all included as possible study subjects. These bacteria were isolated from fish breeding tanks in Marine Science Centre, Basra University, Basra, Iraq. They were identified through VITEK® 2 BCL card (bioMérieux, France) [9-10]. At 32°C, bacterial species were incubated for 24 hours in a nutrient broth [11].

2.3. Biosurfactant Output

The production of biosurfactants verification was done using Vipulanandan and Ren's method [12]. Each bacterial species was injected with a fresh nutrient medium and cultured at 32°C for 24 hours to create biosurfactants. Olive oil, 30 ml/L, was added to water to promote bacterial growth. For three and seven days, conical flasks were placed in a shaking incubator at 180 rpm and 32°C (measurements were made after three and seven days).

2.4. Estimation of Biosurfactants

The estimation of biosurfactants was carried out through a screening test [13]. In an oil-spreading experiment, a thin oil layer was created in a petri dish using 10 L of crude oil and 40 ml of distilled water. Ten liters of crop culture or crop culture supernatant were deposited gently in the middle of the oil sheet. Because of the biosurfactant, oil moved to a more open area.

2.5. Quantification of Biosurfactants

Biosurfactant generated by various bacterial species was quantified using Biuret test [14]. Reagent kits: Bacterial biosurfactant was estimated using a total protein measurement kit from the manufacturer: BIOLABO SAS, Les Hautes Rives 02160, Maizy, France. The colorimetric kit for total protein assay was purchased from commercial laboratories. In order to determine the total protein content, we used the following equation [8]:

$$\text{Total protein} = \frac{\text{sample absorbance}}{\text{standard absorbance}} \times 60$$

2.6. Biosurfactant Extraction

Biosurfactants were extracted using the acid precipitation method [15]. The cultures were centrifuged at 4000 rpm for 30 minutes at room temperature. The pH of the supernatant was changed to 2 by adding 1 M H₂SO₄ to it (acid was added until the pH reached 2). It was diluted with chloroform and ethanol in a 2:1 ratio. These combinations were agitated vigorously and were left to dry overnight to ensure adequate mixing.

2.7. Setting Up an Experiment

3.5L of the mineral salt medium which contained 1litre of NaNO₃ (2 gram), MgSO₄ (0.5 gram), KH₂PO₄ (0.14 grams), K₂HPO₄ (1 kilogram), yeast extract (0.02 grams) and water was placed in 20 conical flasks, which were then shaken for 30 days. Tests were conducted to determine the initial weight of polymer films. Proportionate combinations of polymer film, microorganisms and biosurfactants were used to inoculate the conical flasks. For 30 days, the experiments were incubated at room temperature with 180 rpm shaking at 32°C [16].

Combinations formulated were: UP + B1; UP + B1 + BS; TP + B1; TP + B1 + BS

where,

UP=Untreated PE film;
 TP= Treated Polymer film;
 B1= Bacterial species;
 BS= Biosurfactant.

2.8. Scanning Electron Microscope with Field Emission (FESEM)

Field emission scanning electron microscopy (FESEM) was used to observe the structural morphology of these three polymers [Mod. T did the scan]. On display at the Beam Star Laboratories in Iran were Mirall (made in the Czech Republic). According to Dang *et al.*'s recommendations [17], a thin layer of platinum was applied to their surface to aid in electrical conductivity.

3. Results and Discussion

Many environmental issues, such as high and low-density polyethylene, are associated with the most prevalent synthetic plastics. Bacteria, fungi and other microorganisms play an essential part in the breakdown of synthetic polymers in their natural habitats.

3.1. Pretreatment with UV

Following the UV treatment, the films were subjected to gravimetric analysis. Table I summarizes the findings. The weight reduction, examined after pretreatment, was not determined to be necessary, as can be seen in the table.

Weight loss = (Polymer film weight before UV-Polymer film weight after UV).

Table I: Gravimetric analysis of the films

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Polymer type	Before UV (g)	After UV (g)	Weight loss (g)
LDPE (P1)	0.0058	0.0056	0.0002
	0.0058	0.0057	0.0001
	0.0056	0.0055	0.0001
	0.0057	0.0056	0.0001
	0.0056	0.0054	0.0002
PVC (P2)	0.078	0.077	0.001
	0.076	0.076	0.000
	0.077	0.077	0.000
	0.078	0.078	0.000
	0.078	0.078	0.000
PS (P3)	0.097	0.097	0.000
	0.096	0.096	0.000
	0.097	0.097	0.000
	0.096	0.096	0.000
	0.097	0.097	0.000

3.2. Estimation of Biosurfactants

The presence of biosurfactants was confirmed by adding the produced supernatant to an oil layer spread over water. The results are shown in Figure 1. These results were in agreement with [18] who proved that the degree of polymer degradation is proportional to the amount of UV light irradiation time. As well as these results were in agreement with Montazer *et al.* [19] who stated that a combination of photo-oxidation generated by UV exposure and biodegradation with new bacteria, could increase plastic disintegration in a natural and feasible way with no negative environmental consequences.



Figure 1: Clear zone formation of biosurfactant

3.3. Biosurfactants Quantification

Table 2 represents the estimation of biosurfactants after three days of incubation by different bacteria employed in the study. It can be seen that among five used bacterial species, *B. subtilis* was the largest total protein producer (133.38 $\mu\text{g/l}$) followed by *S. lentus* (115.80 $\mu\text{g/l}$). Whereas *Sphingomonas paucimobilis* was found to be the least total protein producer (49.25 $\mu\text{g/l}$). More or less, a similar pattern was observed after seven days of incubation as represented in **Table 3**. The only difference found this time was that *K. paedia* was found to be the least total protein producer (72.88 $\mu\text{g/l}$) instead of *Sphingomonas paucimobilis*. These results were in agreement with Al-Wahaibia *et al.* [6] who pointed out that *B. subtilis* developed a powerful biosurfactant (lipopeptide comparable to Surfactin) that is quite stable under hard conditions and provides stable emulsions with a wide range of hydrocarbons.

Table 2: Biosurfactant estimation after 3 days of incubation

Bacterial species	Absorbance	Total proteins $\mu\text{g/l}$
	Blank: 0.133	
	Standard : 0.843	
<i>B. subtilis</i>	1.874	133.38
<i>S. lentus</i>	1.627	115.80
<i>K.paedia</i>	0.692	65.83
<i>A. hydrophila</i>	0.732	52.09
<i>S. paucimobilis</i>	0.925	49.25

Table 3: Biosurfactant estimation after 7 days of incubation

Bacterial species	Absorbance	Total proteins $\mu\text{g/l}$
	Blank: 0.133	
	Standard : 0.843	
<i>B. subtilis</i>	2.213	157.50
<i>S. lentus</i>	1.823	129.75
<i>K.paedia</i>	1.024	72.88
<i>A. hydrophila</i>	0.732	93.80
<i>S. paucimobilis</i>	1.554	110.60

3.4. Analysis of Polymer Film by Gravimetric Method

Polymer films were weighed after 30 days of incubation. The gravimetric analysis findings are shown in Table 4.

Table 4: Gravimetric analysis of olymer films after 30 days of incubation

Bacterial Species	Polymer Type					
	P1	P1*	P2	P2*	P3	P3*
<i>B. subtilis</i>	0.0050	0.0043	0.074	0.072	0.093	0.094
<i>B. subtilis</i> + BS	0.0048	0.0040	0.073	0.070	0.092	0.091
<i>S. lentus</i>	0.0053	0.0050	0.075	0.060	0.094	0.094
<i>S. lentus</i> + BS	0.0051	0.0048	0.078	0.077	0.093	0.093
<i>K.paedia</i>	0.0056	0.0051	0.078	0.076	0.097	0.097
<i>K.paedia</i> + BS	0.0054	0.0047	0.077	0.074	0.097	0.096
<i>A. hydrophila</i>	0.0055	0.0051	0.076	0.070	0.096	0.095
<i>A. hydrophila</i> + BS	0.0052	0.0048	0.075	0.070	0.095	0.095
<i>S. paucimobilis</i>	0.0054	0.0050	0.076	0.070	0.097	0.097
<i>S. paucimobilis</i> + BS	0.0052	0.0044	0.073	0.070	0.096	0.096

- P1*, P2* and P3* referred to the weight of the three polymers after 30 days of incubation
- The initial weights of the three polymers were 0.0058, 0.078, 0.097g, respectively

The treated polymers had a more significant weight loss than the untreated polymers. Due to UV light role in polymer oxidation, bacteria were found to have an easier time breaking down the material. It has also been observed that degradation aids in the adhesion of microorganisms to polymer films by supporting the degradation process. That is precisely what Moore [16] found: polymers become brittle and finally break apart due to the hydrolytic qualities of seawater, oxidative conditions in the atmosphere and ultraviolet (UVB) radiation. Consistent with Danso *et al.* [2], microorganisms biodegradation performance is related to crucial parameters like molecular weight and polymer crystallinity.

Outside of the cell, exo-enzymes (the enzymes that break down polymers) have a wide range of reactions, from oxidative to hydrolytic and are found in abundance. Exo-enzymes can break down many polymers into simpler building blocks which the microbial cell can then use to carry out the degradation process. Polymer reaction to a depolymerization agent is called depolymerization. Following Dang *et al.* [17], this study found that *Bacillus subtilis* was the most affected bacterial species.

3.5. Scanning Electron Microscopy.

Distorted structures of the treated polymers were discovered using FESEM. At magnification ranging from 50 to 200 kx. FESEM pictures of polymers treated with *B. subtilis* (biosurfactant or not) were taken. Despite this, images with a 200kx magnification were selected (**Figure 2 a-f**).

The surface degradation of polymers treated with *Bacillus subtilis*, with or without biosurfactant, was more significant with holes, cracks and disintegration. The biosurfactant interaction with the polymers reduced polymer dimensions, thus accelerating their natural breakdown. *Bacillus subtilis* was seen to break down the polymers into smaller molecules. As Raaman and *et al.* [20] discovered that the plastic breakdown began only after *Bacillus* colonized polymers as the sole carbon source that lead to their degradation. According to Patnaik [21], the biosurfactants produced by bacteria can be further tailored to aid in the

bioremediation of highly contaminated industrial effluents containing aliphatic and polyaromatic hydrocarbons to improve solubilization and degradation.

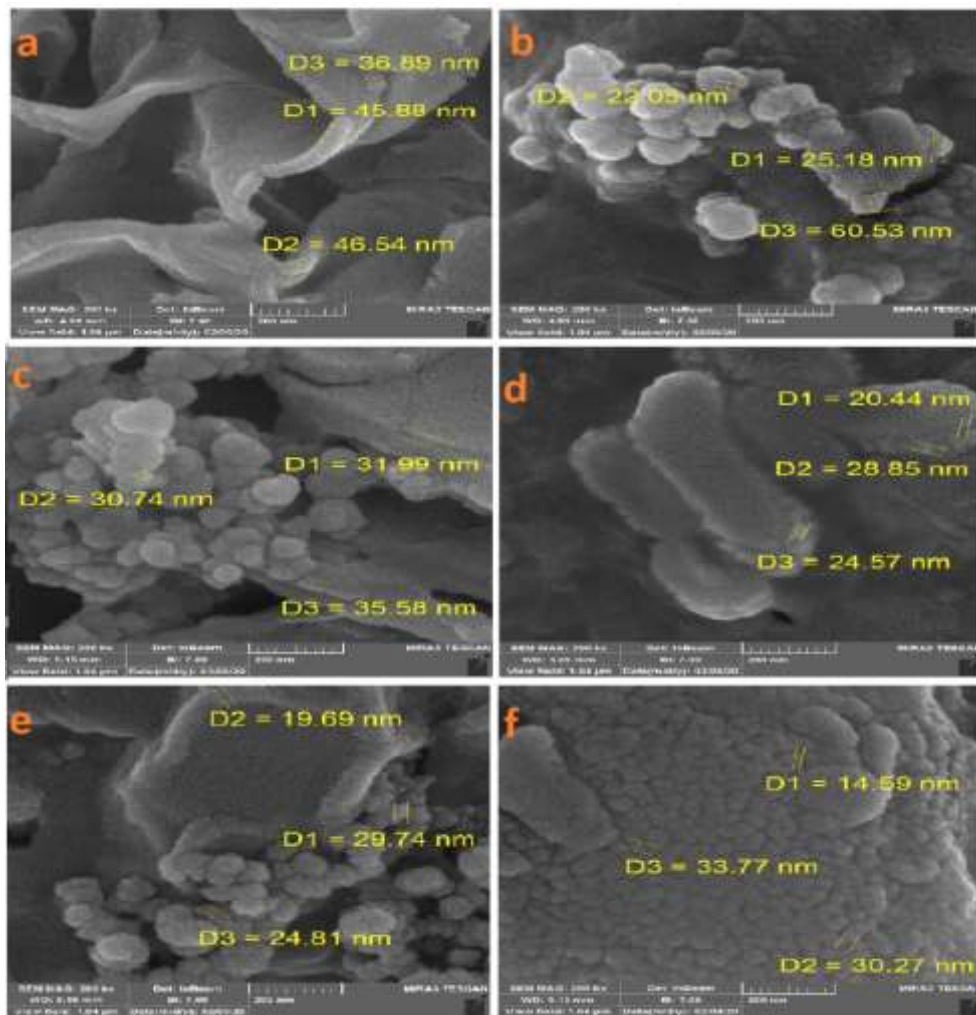


Figure 2: FESEM image of (a) treated LDPE with *Bacillus subtilis*, (b) treated LDPE with *Bacillus subtilis* and biosurfactant, (c) treated PVC with *Bacillus subtilis*, (d) treated PVC with *Bacillus subtilis* and biosurfactant, (e) treated PS with *Bacillus subtilis*, (f) treated PS with *Bacillus subtilis* and biosurfactant

3.6. Fourier Transform InfraRed (FTIR) Spectroscopic Analysis of Polymers Films

These polymeric films were treated with biosurfactants, as seen in FTIR spectroscopy in Figures 3, 4 and 5. (PE, PVC, and PS). Figures 3a, 4a and 5a show that the films, without the addition of biosurfactants PE, PVC and PS, displayed more considerable intensity peaks than the films with the addition of biosurfactants (Figures 5a, 3b, 4b and 5b). The greater the peak intensity, the greater was the relationship. For example, central band C-H stretch of 2920 cm^{-1} was visible (Table 5). Polyethylene's C-H stretch band at 2920 cm^{-1} grew significantly due to bacterial deterioration, as demonstrated by IR spectroscopy (Figure 3c).

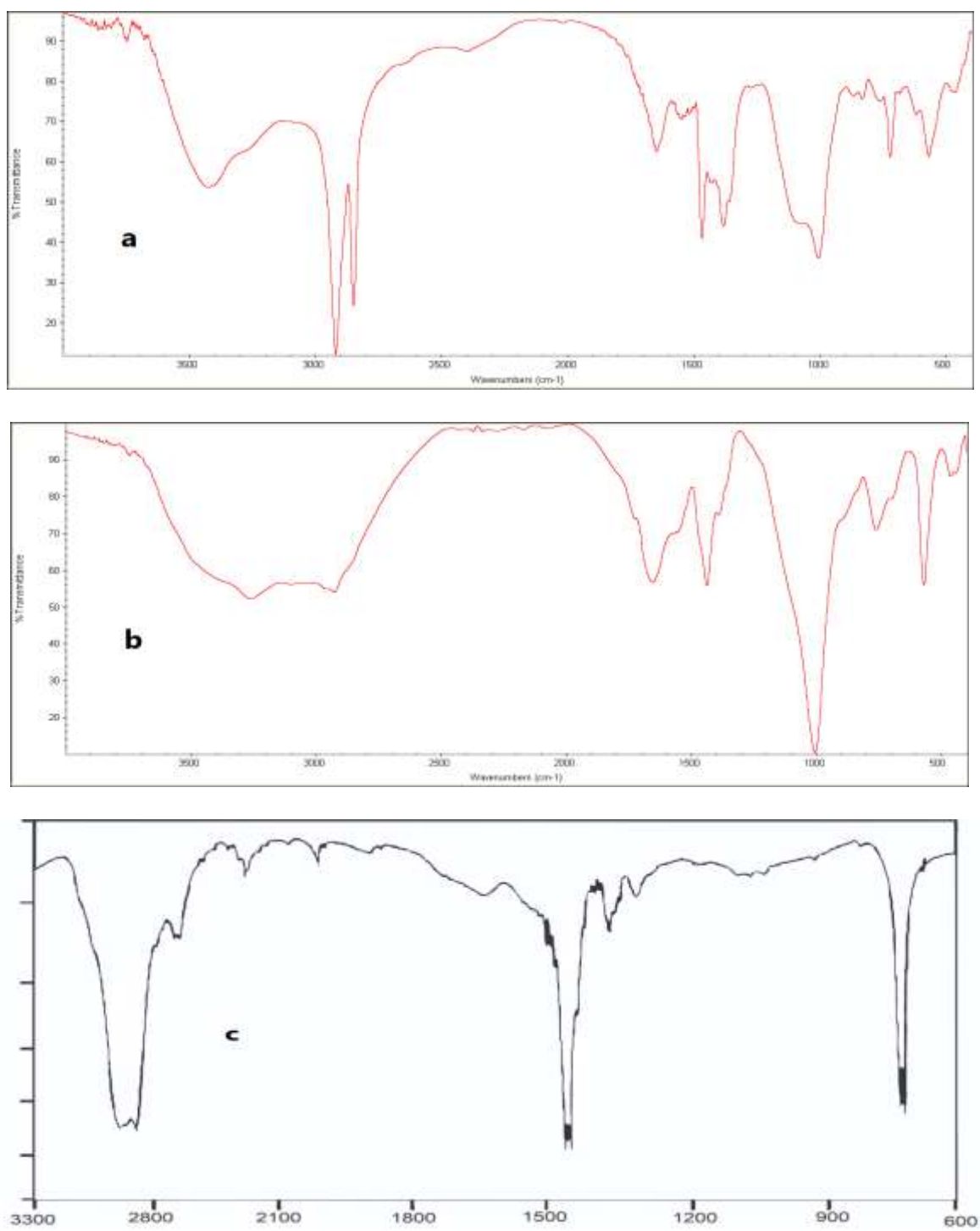


Figure 3: FTIR spectrum of PE (a) without addition of biosurfactant (b) with the addition of biosurfactant (c) virgin PE

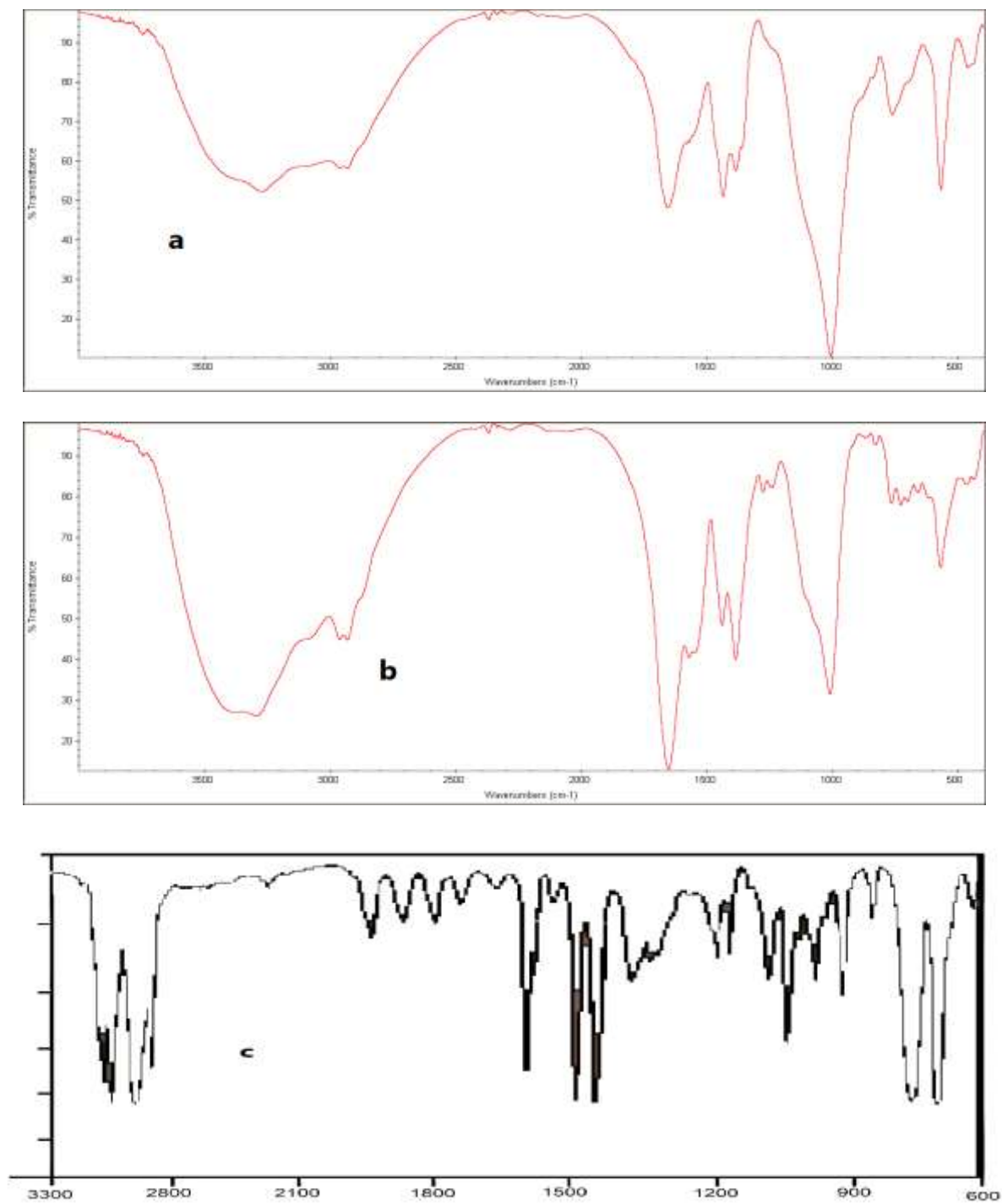


Figure 4: FTIR spectrum of PVC (a) without addition of biosurfactant (b) with the addition of biosurfactant (c) virgin PVC

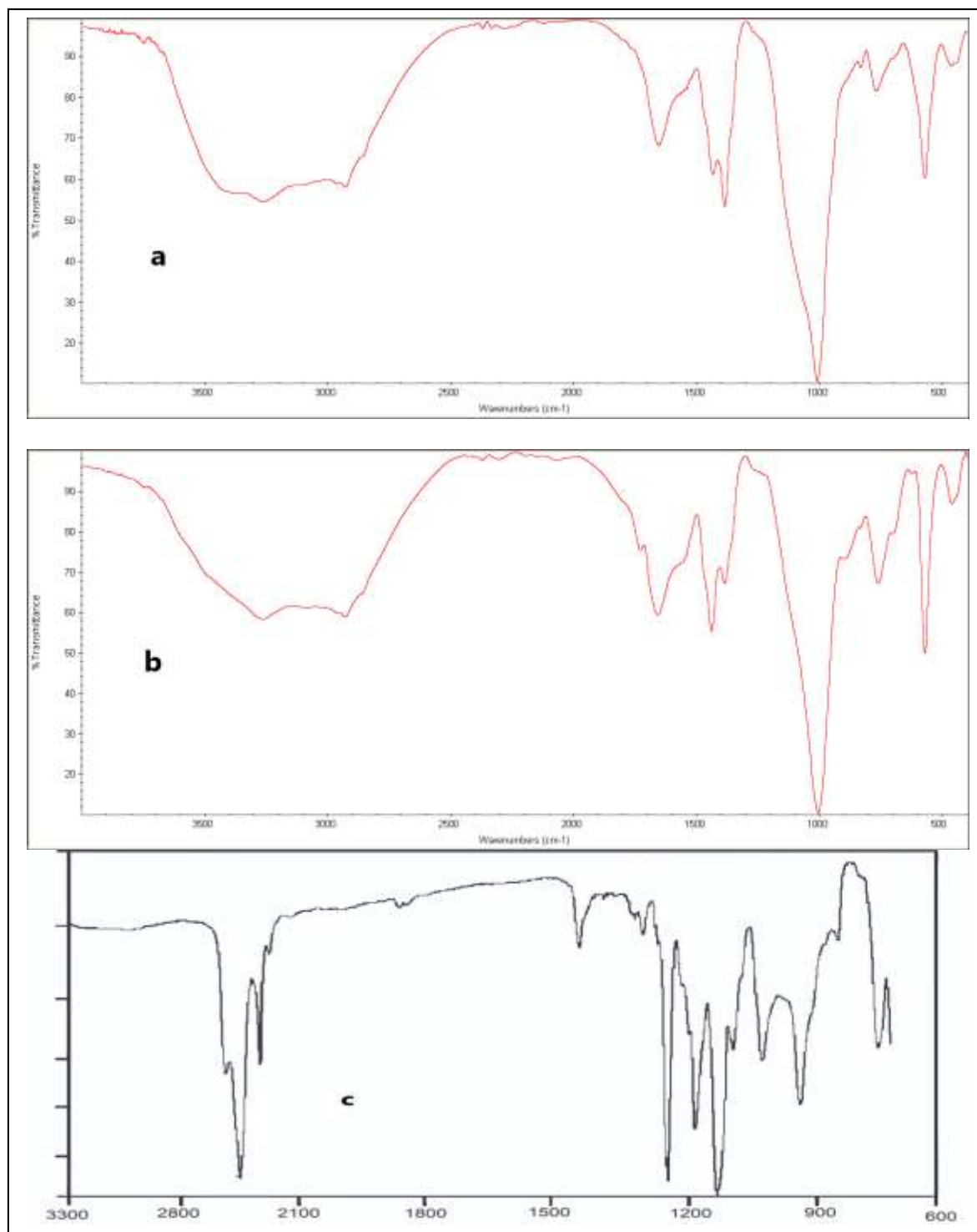


Figure 5: FTIR spectrum of PS (a) without addition of biosurfactant (b) with the addition of biosurfactant (c) virgin PS

Table 4: The important characterization peaks in FTIR

IR Peak No.	Wave Number (cm ⁻¹)	Bond Functional Group
1	3200-3500	O-H Stretch Alcohols, Carboxylic acid
2	3050-3150	-C-H Stretch Aromatic
3	3000-2850	-C-H Stretch Alkanes
4	2830-2700	H-C=O: C-H stretch aldehydes
5	1710-1650	-C=O Stretch Ketones, Aldehydes
6	1470-1450	-C-H Bend alkanes
7	1200-1000	-C-O Stretch Alcohols, Carboxylic acid, ester, ethers
8	850-600	=C-H Bend alkenes

The biodegradation of all polymeric films provided ketone, aldehyde, carboxylic acids and alcohols due to FTIR. After biodegradation, the carbonyl absorption band at (3200-3500) cm⁻¹ of all polymers rose dramatically due to the oxidation of polyethylene moieties during UV treatment. The above results were identical to many previous studies dealing with the biodegradation of the polymers [22-24].

4. Conclusion

This research studied the biodegradation of three polymeric films by five different bacterial species. The physical pretreatment method of UV irradiation was chosen because of its ability to boost microorganisms ability to ingest polymer films. Biosurfactant amphiphilic design was responsible for the microbes that attached to hydrophobic surfaces. As a result, bacteria could use polymer as a carbon source at a faster rate when biosurfactants were added to polymer films. Researchers have discovered that bacteria may obtain energy from polymer. The thinner the polymer coating, the faster is its degradation, hence resulting in a more significant weight loss. This study showed bacteria isolated from Iraqi environment, especially *B. subtilis*, can be used as eco-friendly tool to decompose the harmful plastic wastes instead of the accumulating huge quantities of these wastes. This research also revealed that bacteria and enzymes in the micro-ecosystem break down plastics in composted trash. Biodegradable plastic polymers can produce eco-friendly materials, but there is a difference between the three kinds of biodegradable polymers.

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