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Acceptance Letter

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Title:

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

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Your Sincerely

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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 digest plastic wastes. The goal of this study was to see how well Bacillus subtilis, Staphylococcus lentus, Aeromonas hydrophila, Sphingomonas paucimobilis, and Kocuria paedia degraded 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. In terms of weight loss, biodegradation was measured. 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 films PVC films, while the least affected polymers were PS films. 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 البايولوجي 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 البكتريا وهي Bacillus subtilis و Staphylococcus lentus و Aeromonas hydrophila و Sphingomonas paucimobilis و Kocuria paedia في تحلل ثلاثة أنواع من البلاستيك المصنع من المشتقات النفطية وهي: البولي إثيلين منخفض الكثافة (LDPE) والبولي ستايرين (PS) والبولي فينيل كلوريد(PVC) . أجريت التجربة

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لمدة 30 يومًا في ظروف مختبرية مع اهتزاز لمكونات التجربة عند 180 دورة في الدقيقة و 32 درجة مئوية. وتم قياس التحلل البايلوجي من حيث فقدان الوزن . وفقًا لمطيافية الأشعة تحت الحمراء FTIR، الحزمة العائدة للآصرة C-H عند 2920 سم $^{-1}$ أثبتت التحلل البكتيري للبولي أثيلين واطيء الكثافة. كانت البوليمرات الأكثر تأثراً هي البولي إثيلين منخفض الكثافة، بينما كانت البوليمرات الأقل تـأثرًا هي أغشية البولي ستايرين PS وقد تبين أن بكتيريا B. subtilis هي أنجح الأنواع الخمسة من البكتريا من حيث قدرتها على تحلل 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

Plastics' ease of utility and applicability have made them an essential part of human life. Plastics are widely used for household activities as well as industrial purposes. Plastics are widely accepted because of their cost-effectiveness, superior barrier qualities, bio-inertness, and lightweight. Over the previous half-century, the plastics sector has risen exponentially, reaching 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 of non-biodegradable plastics. As a result of unmanaged garbage disposal, a substantial amount of plastic has entered the ecosystem. As a result of the widespread use of plastics, the environment is in danger. A lack of adequate plastic waste management and a well-informed public approach to the proper management of this waste stream are the key reasons behind this [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). When 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 the breaking of chemical bonds [4].

Several bacteria, particularly from the genus *Bacillus* [5-6] and fungi like *Aspergillus* sp. [7] have observed promising results for biodegradation of oil-based plastics. In one of the previous approaches, [6**]** evaluated the biosurfactant production ability of B. subtilis B30 to enhance oil recovery. However, employing microbially synthesized biosurfactants for polymer degradation is not 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 of three different polymers (derived from waste). They were all made in the 2cm × 2cm format and exposed to UV light for 72 hours[8]. The experiments were carried in marine bacteriology lab, Marine Science Centre , University of Basrah, Basrah, 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 nutrient broth [11].

2.3. Biosurfactant Output

Verifying the production of biosurfactants 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 the 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 two periods three days 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

The biosurfactant generated by various bacterial species was quantified using the Biuret Test [14]. Reagent kits: Bacterial biosurfactant was estimated using a total protein measurement kit from of 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]:

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

2.6. Biosurfactant Extraction

Biosurfactants were extracted using the acid precipitation method [15]. The cultured 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 (the acid has been added until the pH reached 2). It was diluted with chloroform and ethanol in a 2:1 ratio. These combinations were agitated vigorously and left to dry overnight to ensure adequate mixing.

2.7. Setting Up an Experiment

3.5L of the mineral salt medium which contains in 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 into 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 does a scan. On display at the Beam Star Laboratories in Iran are 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 are associated with the most prevalent synthetic plastics, such as high- and low-density polyethylene. Bacteria and 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 shown.

Weight loss = (Polymer Film Weight before UV-Polymer Film Weight after UV).

Table-I: Gravimetric analysis of the films

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-I. These results were in agreement with [18] who indicated that It proves 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-I: Clear zone formation of biosurfactant

3.3. Biosurfactants quantification

Table-II 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 µg/l) followed by *S. lentus* (115.80 µg/l), while *Sphingomonas paucimobilis* was found to be the least total protein producer (49.25 µg/l). More or less, a similar pattern was observed after seven days of incubation as represented in **Table-III**; the only difference was that *K. paedia* was found to be the least total protein producer (72.88 µg/l) instead of *Sphingomonas paucimobilis*, , these results were in agreement with Al-Wahaibia *et al*. [6] who pointed that *B. subtilis* developed a powerful biosurfactant (lipopep-tide comparable to Surfactin) that is quite stable under hard conditions; provides stable emulsions with a wide range of hydrocarbons.

Table-II: Biosurfactant estimation after 3 days of incubation

Bacterial species	Absorbance	Total proteins µg/ l
	Blank: 0.133	
	Standard : 0.843	
B. subtilis	1.874	133. 38
S. lentus	1.627	115.80
K.paedia	0.692	65.83
A. hydrophila	0.732	52.09
S. paucimobilis	0.925	49.25

Table-III: Biosurfactant estimation after 7 days of incubation

Bacterial species	Absorbance	Total proteins µg/ l.
	Blank: 0.133	
	Standard : 0.843	
B. subtilis	2.213	157. 50
S. lentus	1.823	129.75
K.paedia	1.024	72.88
A. hydrophila	0.732	93.80
S. paucimobilis	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 IV.

Table-IV: Gravimetric analysis of Polymer films after 30 days of incubation

Bacterial species	Polymer type					
	P1	P1*	P2	P2*	P3	P3*
B. subtilis	0.0050	0.0043	0.074	0.072	0.093	0.094
B. subtilis + BS	0.0048	0.0040	0.073	0.070	0.092	0.091
S. lentus	0.0053	0.0050	0.075	0.060	0.094	0.094
S. lentus + BS	0.0051	0.0048	0.078	0.077	0.093	0.093
K.paedia	0.0056	0.0051	0.078	0.076	0.097	0.097
K.paedia + BS	0.0054	0.0047	0.077	0.074	0.097	0.096
A. hydrophila	0.0055	0.0051	0.076	0.070	0.096	0.095
A. hydrophila + BS	0.0052	0.0048	0.075	0.070	0.095	0.095
S. paucimobilis	0.0054	0.0050	0.076	0.070	0.097	0.097
S. paucimobilis + 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's role in polymer oxidation, bacteria 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. When a polymer reacts to a depolymerization agent, this is called depolymerization. Following Dang *et al.* [17], this study found that Bacillus subtilis was the most affected bacterial species. Various enzymes, including CMC, chitinase, xylanase, protease, and high-temperature lipase.

3.5. Scanning Electron Microscopy.

The treated polymers' distorted structures were discovered using FESEM. At magnifications 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: IIa-f**).

The surface degradation of polymers treated with *Bacillus subtilis* (with or without biosurfactant) was more significant with holes, cracks, disintegration, and holes. The biosurfactant's interaction with the polymers reduced polymer dimensions, accelerating their natural breakdown. Bacillus subtilis was shown to break down the polymers into smaller molecules. As Raaman and *et al.*,[20] discovered, plastic breakdown began only after Bacillus colonized polymers as the sole carbon source and began degrading them. 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 improve solubilization and degradation.

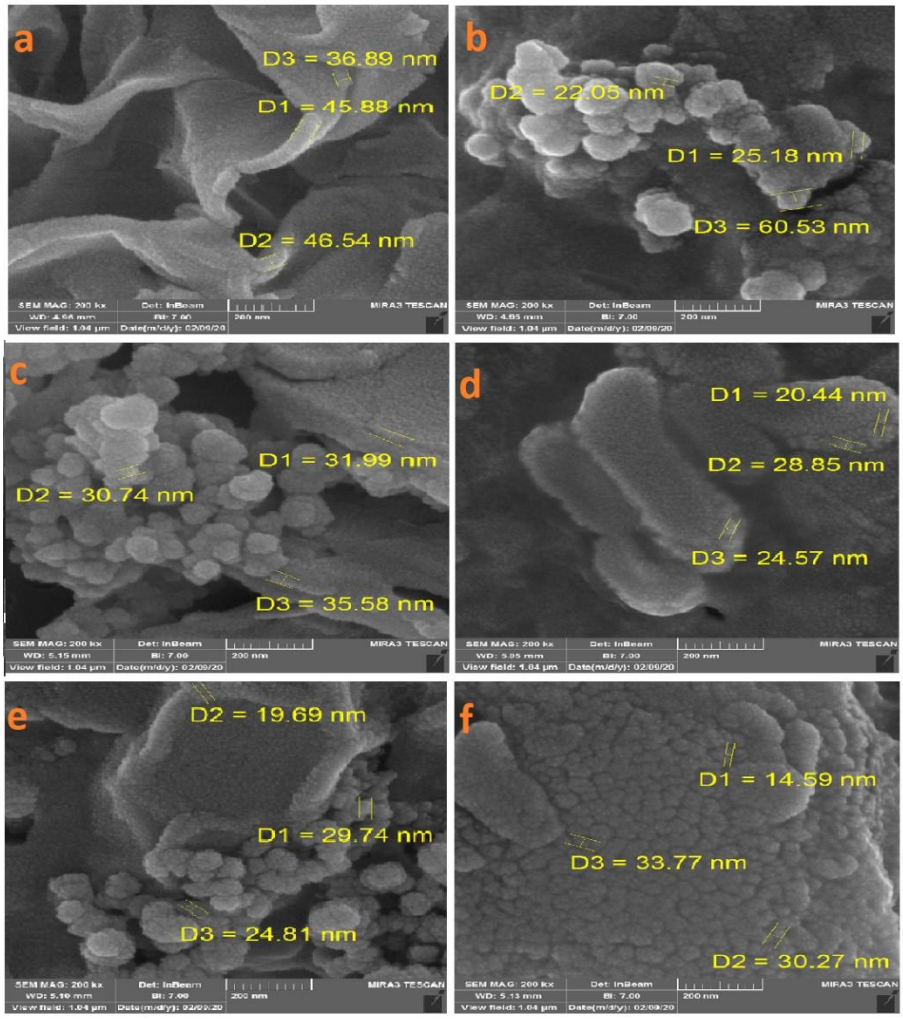

Figure-II FESEM image of **(a)** *treated* LDPE with *Bacillus subtilis*, **(b)** *treated* LDPE with *Bacillus subtilis* and biosurfactant, **(c)** *treated* PVC with *Bacillus subtilis*, **(d)** *treated* PVC with *Bacillus subtilis* and biosurfactant, **(e)** *treated* PS with *Bacillus subtilis*,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

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

These polymeric films have been treated with biosurfactants, as seen in FTIR spectroscopy in Figures III, IV, and V. (PE, PVC, and PS). Figure-IIIa, Figure-IVa, and Figure-Va reveal that the films without the addition of biosurfactant (PE, PVC, and PS) display more considerable intensity peaks than the films with the addition of biosurfactants (Figure-Va) (Figure-IIIb, Figure-IVb, and Figure-Vb). The greater the peak intensity, the greater the relationship. For example, the central band's C-H stretch of 2920 cm-1 was visible (Table-V). Polyethylene's C-H stretch band at 2920cm-1 grew significantly due to bacterial deterioration, as demonstrated by IR spectroscopy (Figure-IIIc).

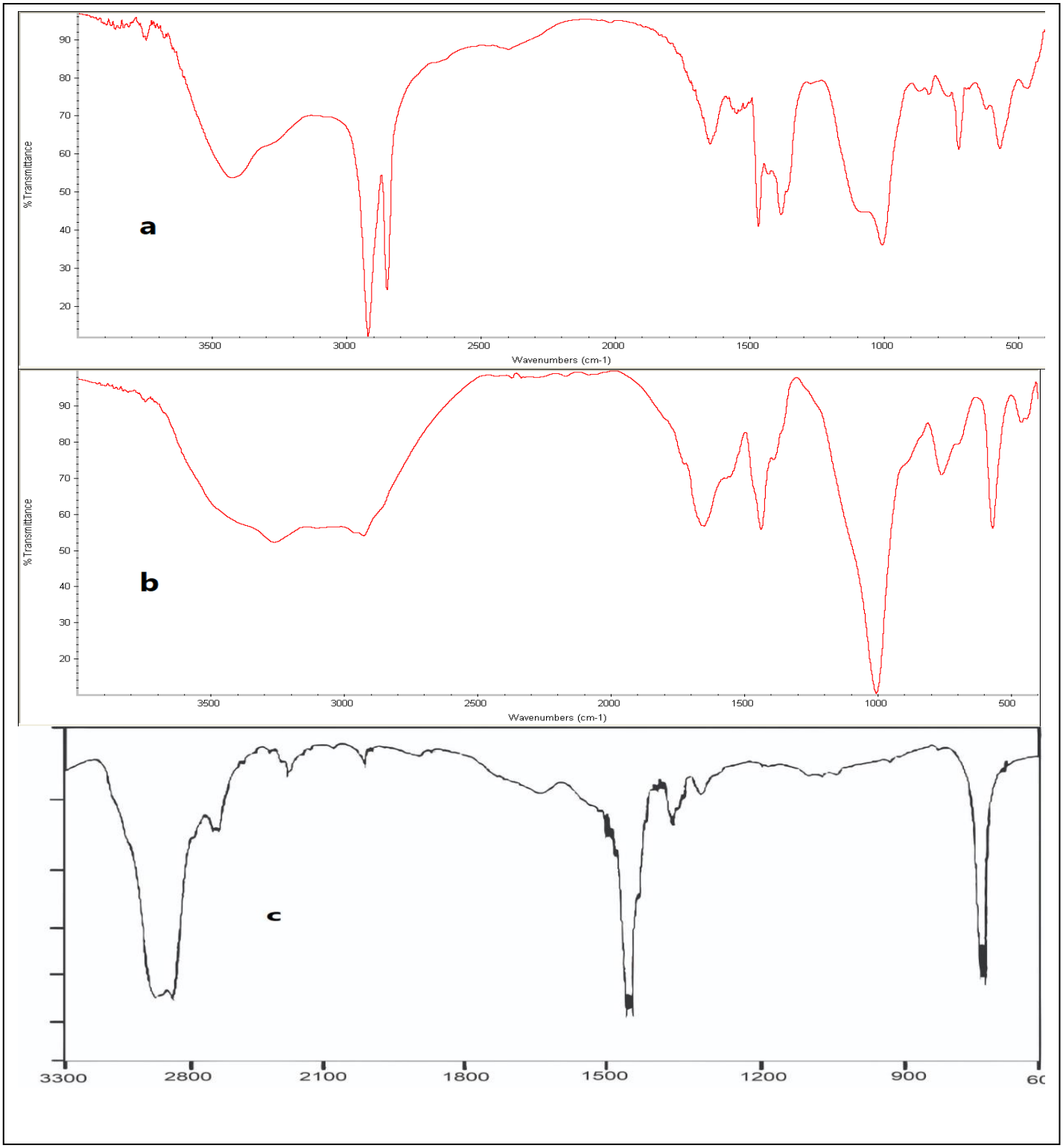

Figure-III:FTIR spectrum of PE(a) without addition of biosurfactant(b) with the addition of biosurfactant(c) virgin PE

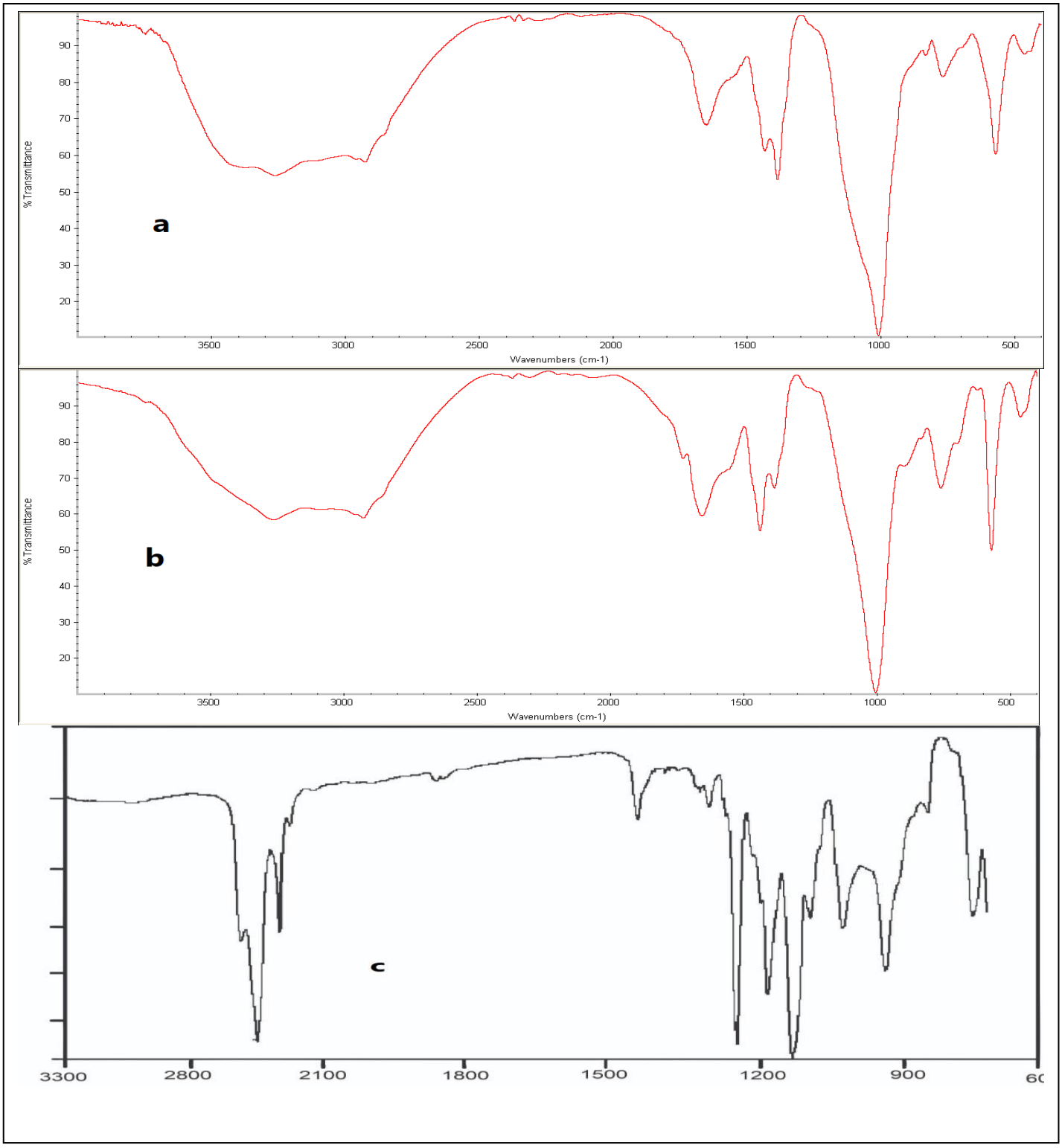

Figure-IV: FTIR spectrum of PVC (a) without addition of biosurfactant(b) with the addition of biosurfactant(c) virgin PVC

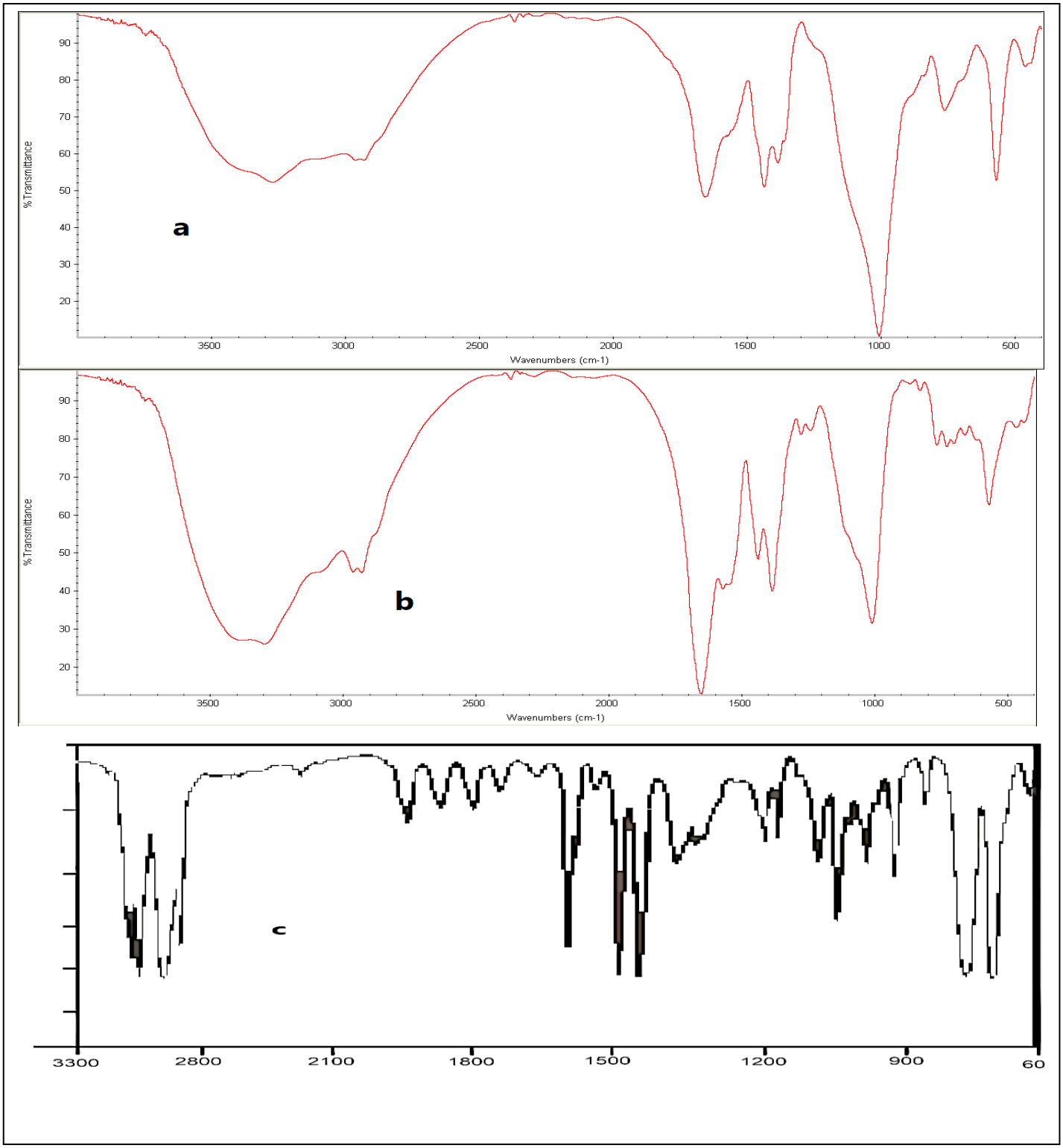

Figure-V: FTIR spectrum of PS (a) without addition of biosurfactant(b) with the addition of biosurfactant(c) virgin PS

Table-IV: 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 study of the biodegradation of the polymers in this study.[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. When microbes were attached to hydrophobic surfaces, the biosurfactant's amphiphilic design was responsible. 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 it degraded, 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 insteade of the accumulation of huge quantities of these wastes. Also this research revealed that bacteria and enzymes in the micro-ecosystem to break down plastics in composted trash. Biodegradable plastic polymers can produce eco-friendly materials, but there is a gap between the three kinds of biodegradable polymers.

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