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Biodegradation of Low-Density Polyethylene Plastic Using Marine Bacterial Consortium

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ABSTRACT

Recently, plastic wastes are considered a main environmental problem, and many bacterial isolates were tested to biodegrade them. The low-density polyethylene (LDPE) plastic sheets were tested to be degraded by a marine bacterial consortium. The potent marine plastic degrading isolates were biochemically identified as *Bacillus licheniformis*, *Bacillus subtilis*, and *Paenibacillus xylanilyticus* using the BIOLOG identification system. The identification of the most potent plastic-degrading bacterium was confirmed as *Bacillus licheniformis* FMMA using the 16S rRNA gene sequence. This bacterial consortium was physiologically adjusted as follows: pH 7, temperature 35°C, inoculum size 4ml/ 100ml ($1.0X10^7$CFU/ ml), and an incubation period of 30 days. It led to 34.1% plastic loss of weight. The mechanical properties (maximum force and the elongation% at break) of these treated LDPE plastic sheets showed 7.49N and 112.2%, respectively, compared to that of *B. licheniformis* FMMA, which showed a 25.5% plastic weight loss, with maximum force and elongation% at a breakpoint of 8.9N and 114.2%, respectively. In addition, the plastic biodegradation was also estimated through a scanning electron microscopy and Fourier transform-infrared (FTIR) spectroscopy, were a great reduction in the intensity of the -CH2 peak appeared at 2900cm^{-1}, and the disappearance of the -OH peak at 3500cm^{-1} was observed.

INTRODUCTION

Plastic polymers are a huge family of ethylene $(-CH_2-CH_2-)_n$ monomers that are utilized in packaging, electronics, and textiles **(Sridharan *et al.*, 2021)**. The annual production of plastic is expected to increase to more than 320 million tons, and this will be doubled by 2034 **(Geyer *et al.*, 2017)**. Massive amounts of polyethylene have been therefore growing up in the environment as a result of inappropriate disposal, causing major ecological issues. One of which is the microplastics, and PE present on the ocean's surface is estimated to be 2.5 million tons by 2050 **(Lebreton *et al.*, 2019)**.

Since PE waste produces stomach obstruction in fish, birds, and other aquatic and terrestrial animals, it is the main risk factor for marine animals in the aquatic ecosystem. At least 276 species are injured by plastic pollutants thrown into the aquatic environment; the most harmed species were determined to be seabirds (44%) and sea turtles (86%) **(Dey *et al.*, 2021)**.

PE trash is the primary hazardous factor in aquatic ecology due to polyethylene being uneasily degraded, it pollutes the environment by clogging drains and contaminating water, soil, and sewage pipelines. In the same way, plastic bags can clog the digestive tracts of numerous aquatic creatures, including fish and birds **(Venkatesh *et al.*, 2021)**.

Studies on microbial polyethylene degradation in the marine environment are quite limited **(Khandare *et al.*, 2021)**. However, at room temperature, LDPE is typically non-reactive; however, under aerobic culture conditions, they decompose to water and carbon dioxide as by-products, while anaerobically, they are decomposed to methane, water, and carbon dioxide. While in a controlled environment, the results of the oxidative breakdown of polyethylene were found to be different, as it produced hazardous compounds that are toxic for both people and the environment **(Rani *et al.*, 2022)**.

Therefore, this study aimed to find marine plastic degrading bacteria that can use LDPE plastic sheets as their only carbon source with great efficiency to avoid their accumulation as disposable wastes and stop the regular growth of this resistant polymer in the marine environment.

MATERIALS AND METHODS

1. Collection site and sampling process

Twenty seawater and sediment samples were collected from Al-Dabaa (Mediterranean Sea), Sidi Kerir (Mediterranean Sea), Al-Agamy (Mediterranean Sea), El-Max (Mediterranean Sea), Eastern Harbor (Mediterranean Sea), and Suez coast (Red Sea), Egypt, according to the standard methods published by the American Public Health Association press **(APHA, 2017)**.

2. LDPE plastic sheets preparation

The LDPE plastic bags were obtained from the local market and cut into similar plastic strips with dimensions of 10cm in length and 1.5cm in width, each weighing 200mg. They were then sterilized using 70% ethanol and dried under sterile conditions in a desiccator.

3. Determination of the plastic loss of weight percentage

The most widely used technique to calculate the plastic biodegradation efficiency was the weight-loss assay on LDPE sheets. In order to eradicate the colonized bacteria from the LDPE surface, the tested LDPE plastic sheets were treated with a 2% solution of sodium dodecyl sulfate (SDS) for two hours. Following this step, they were cleaned three

times with sterile distilled water and allowed to air dry in an oven set at 50°C until their weight remained constant **(Harshvardhan & Jha, 2013)**.

The loss of weight % was estimated according to **ASTM (2017)**, as follows:

Plastic loss of weight % = $(m_i - m_f / m_i)$ X 100% (where m_i is the initial plastic weight, and m_f is the final plastic weight).

4. Testing for the plastic mechanical properties

A universal testing machine, model 1195, from Instron (England) Zwick / Roell (1KN zwicki), was used to measure the changes in the mechanical properties (Maximum force and Elongation% at break) of the tested LDPE-plastic sheets. The test specimen type used was a rectangle, measuring 10cm in length and 1.5cm in width. The procedure was performed in accordance with the American Society for Testing and Materials ASTM-D882 (speed: 50mm/ min, gauge length: 12cm). The plastic sheets were checked for nicks, and their thickness was measured using a micrometer set to measure cross-sectional area with an accuracy of 0.01mm. An average value was then obtained. After washing with water and a small amount of detergent, the sheets were allowed to air dry in the room temperature. Following that, the sheets underwent tensile strength tests. The maximum load was divided by the initial cross-sectional area to determine the tensile strength at break. Additionally, the elongation at the moment of the specimen's rapture was divided by its initial gauge length, and then multiplied by 100 to determine the percentage elongation at break **(Yabannavar & Bartha, 1994)**.

5. Detection of plastic biodegradation process using Fourier transform-infrared (FTIR) analysis

The tested plastic sheets were submitted to an FT-IR spectroscope (BRUKER) at the National Research Center (NRC), Cairo, Egypt. The changes of functional groups and the transmission measurements were performed at room temperature in the frequency range of 500- 400cm^{-1} with 4cm^{-1} resolution. A vertex 80v FTIR spectrometer coupled with an infrared microscope (Hyperion 2000) with a 25x Cassegrain objective and a mercury cadmium telluride (MCT) detector was used.

6. Isolation of LDPE plastic marine degrading bacteria

A modified marine minimal culture medium of **Sivan *et al*. (2006)** was used for the isolation of plastic-degrading marine bacteria, where natural and filtered seawater was applied instead of the distilled water, and 1% nutrient broth was added for the enhancement of the bacterial growth. Some minerals were added as follows (g/L): NH_4NO_3 (1.0), $MgSO_4 \cdot 7H_2O$ (0.2), K_2HPO_4 (1.0), $CaCl_2 \cdot 2H_2O$ (0.1), KCl (0.15), yeast extract (0.1), with the addition of 1.0mg/ L of each of the following micronutrients: $FeSO_4 \cdot 6H_2O$, $ZnSO_4 \cdot 7H_2O$ and $MnSO_4$. The initial pH value was adjusted at 7.0, and ten sterile and pre-weighted plastic sheets were aseptically added per each 250mL Erlenmeyer conical flask. These flasks were separately inoculated with 1.0mL seawater or 1.0g sediment sample and incubated statically at 30°C for three months. After

incubation, the plastic strips from each tested sample were aseptically scratched and used for isolation and purification of the active plastic-degrading marine bacteria using a streaking plate technique. The purified bacterial isolates were maintained on a marine nutrient agar culture medium for further experiments.

7. Screening of LDPE degrading marine bacteria

The isolated bacterial colonies were tested according to their ability to degrade the LDPE plastic strips. Each bacterial isolate was grown in the presence of ten sterile and pre-weighted LDPE plastic sheets as their only carbon source and 100ml of the sterile culture medium. The bacterial inoculum for each flask was $1.0x10^7$ CFU/ mL. Then, the inoculated flasks were incubated under static conditions at 30°C for 30 days **(Gilan *et al.*, 2004)**.

8. Identification of plastic degrading marine bacteria

8.1. Biochemical identification technique

The biochemical identification process was carried out for the most active bacterial isolates as obtained from the previous experiment using the BIOLOG identification system GEN-III, Inc. Hayward GA94545, USA, version 5.2.1 presented at the Egyptian Microbial Culture Collection Network (EMCCN), NIOF, Alex., Egypt

8.2. DNA extraction

According to the manual instructions, the molecular identification was carried out using a 16S rRNA gene sequence at the Egyptian Microbial Culture Collection Network (EMCCN), NIOF, Alex., Egypt. The DNA was extracted from a pure culture using a Geneaid^{TM} kit (QIAGEN Inc., Germany) and a Genome DNA purification kit (Promegal). The preparations were analyzed on a 0.7% agarose gel, and then determined spectrophotometrically and underwent polymerase chain reaction (PCR) for sequence amplification.

8.3. PCR-amplification and sequencing of 16S rRNA gene

The amplification of the genomic DNA was carried out at EMCCN, NOIF, Alex., Egypt through a PCR process using FIREPol® master mix. The used universal 16S rRNA primers were F27: AGA GTT TGA TCC TGG CTC AG and R1492: GGT TAC CTT GTT ACG ACT T. The PCR thermal cycler conditions were: Initial denaturation at 94˚C for six minutes, then denaturation at 94˚C for 45 seconds, annealing at 56 ˚C for 45 seconds through 35 cycles, and extension at 72˚C for 1min. Final extension at 72˚C for 5min. The PCR products were purified using a QIAquick PCR Product extraction kit. (Qiagen, Valencia). The amplification of the genomic DNA was carried out at EMCCN, NOIF, Alexandria, Egypt, using a PCR process with FIREPol® master mix and BigDye Terminator. The used universal 16S rRNA primers were; F27: AGA GTT TGA TCC TGG CTC AG and R1492: GGT TAC CTT GTT ACG ACT T. The PCR thermal cycler conditions were: Initial denaturation at 94˚C for 6 minutes, then denaturation at 94˚C for 45 seconds, annealing at 56˚C for 45 seconds through 35 cycles, extension at 72˚C for 1 minute. Final extension was conducted at 72˚C for 5 minutes. The PCR

products were purified using a QIAquick PCR Product extraction kit (Qiagen, Valencia). Bigdye Terminator cycle sequencing kit V3.1 (Perkin-Elmer) was used for the sequence reaction. Then, it was purified using a Centrisep spin column. The DNA sequences were obtained by Applied Biosystems3130 genetic analyzer (HITACHI, Japan), and a BLAST® analysis (Basic Local Alignment Search Tool) **(Altschul *et al.*, 1990)** was initially performed to establish sequence identity to GenBank accessions. The phylogenetic tree was created by the MegAlign module version 12.1 **(Thompson *et al.*, 1994),** and phylogenetic analyses were done using maximum likelihood, neighbor-joining and maximum parsimony in MEGA6 (**Tamura *et al.*, 2013)**.

9. Detection of LDPE degradation using scanning electron microscope (SEM)

At the Central Lab., Faculty of Science, Alex. Univ., Egypt, the plastic sheets' surfaces were inspected using a scanning electron microscope (JSM-IT200SEM) to determine whether any structural alterations had occurred as a result of the biodegradation of LDPE plastic. Following a 30-day incubation period, three hours were spent incubating the treated and untreated LDPE.

Plastic sheets (blank) were placed in 4% glutaraldehyde at 4°C and pH 7.3. After rinsing them three times for ten minutes each with 0.05 M phosphate buffer, they were successively dehydrated in alcohol. The dehydrated LDPE sheets were subjected to a 40-second gold sputter coating using an ion sputter (JFC-1100E), dried, mounted, and subsequently analyzed using a JSM-IT200SEM **(Al-Salem *et al.*, 2019)**.

10. Microbial plastic degradation process using bacterial consortium

The effect of using the most active plastic-degrading marine bacterial consortium was studied. Practically, 1mL of each active bacterial suspension containing $1.0x10^7$ CFU was added to 100mL of the used sterile culture medium, with an initial pH equal to 7.0 and ten LDPE plastic strips as a sole carbon source. The incubation was carried out under static conditions for 30 days at 30°C.

11. Effect of different parameters on biodegradation process using bacterial consortium

The effect of different pH values, temperatures, and bacterial inoculum sizes on the plastic biodegradation process was estimated separately in triplicates by detecting the weight loss % and the changes in the mechanical properties (elongation %, and maximum force at break) of the tested LDPE plastic sheets.

11.1. Effect of different pH values on biodegradation process

The effect of different initial pH values 5, 6, 7, 8, and 9 on the plastic biodegradation process using the selected marine bacterial consortium was investigated. The culture media were adjusted using 1.0M NaOH or HCl. All flasks were inoculated with an initial inoculum size of 3mL (1.0×10^7 CFU/ mL) and incubated statically at 30°C for 30 days.

11.2. Effect of the inoculum size on plastic biodegradation process

Different inoculum sizes of 2, 3, 4, and 5ml (1.0 x 10^7 CFU/ ml) of the tested bacterial consortium were separately prepared using equal volumes (1:1:1) of each potent

plastic-degrading bacterial culture. The media were incubated statically for 30 days at 30°C, and the pH was adjusted as obtained from the previous experiment.

11.3. Effect of temperature on biodegradation process

The ability of the bacterial consortium to grow and degrade LDPE sheets was tested using different degrees of temperature ranging from 25 to 40℃. The media were incubated statically for 30 days at the most suitable pH value and initial inoculum size obtained from the previous experiments.

12. LDPE degradation analysis using gas chromatography-mass spectroscopy (GC-MS)

Thermo Trace 1300 GC combined with Thermo TSQ 8000 Triple Quadrupole MS, fitted with BP 5MS (30m × 0.25mm, 0.25µm) column, was used to detect the degraded LDPE by-products utilizing GC-MS (Perkin Elmer model: clarus 580/560S). The experiment was carried out using di-ethyl ether as a solvent and helium gas as a carrier **(Kyaw *et al.*, 2012)**. The infected culture was cleared of the LDPE leftovers. After that, the culture was centrifuged for 15 minutes at 5000rpm and -4°C. After discarding the cell pellet, the supernatant was extracted in a 250ml separating funnel using diethyl ether (1/1 vol). After the organic layer air-dried at room temperature to a total volume of 1mL, 1µL was injected into the GC-MS spectrophotometer. Estimates and identification of the discovered by-product compounds were made using the National Institute of Standards and Technology (NIST) library **(Shahnawaz *et al.*, 2016)**.

13. Statistical analysis

All experiments were performed in triplicates, and the least significant difference (L.S.D.) was calculated for each experiment through the F-test (ANOVA) for normally distributed quantitative variables to compare between more than two groups and post hoc test (Tukey) for pairwise comparisons using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Quantitative data were described using the mean value and its standard deviation. The significance of the obtained results was judged at the 5 and 1.0% levels, respectively.

RESULTS

1. Screening for microbial degradation of LDPE using weight loss measurements

The weight loss % measurements presented in Table (1) shows that the isolates coded with F_{18} and $F_{19,}$ followed by $F_{15,}$ were effective in the plastic biodegradation process. They reduced the weight of LDPE plastic sheets by 25.50 ± 1.90 %, 20.77 ± 2.56 % and 17.90 ± 2.25%, respectively.

2. LDPE-plastic degradation using FTIR

The FTIR spectra presented in Fig. (1) confirm the plastic degradation process by the most potent marine isolates F_{18}, $F_{19,}$ and F_{15}. They showed a reduction in the intensity of peaks that appeared at 3500 and 2900cm^{-1} which indicated: the removal of –OH

bounded compounds and the reduction in the CH_2 group, respectively. In addition, a reduction in the intensity of the peak appeared around 1465cm^{-1} in the LDPE sheets treated with the isolates F_{18} and F_{19}. Moreover, there was an appearance of a new peak around 1129cm^{-1} upon using the F_{18} marine isolate.

Table 1. Screening for plastic degrading marine bacterial isolates using plastic weight loss percentages as a biodegradation indicator

Collection site	Type of sample	Isolate code	Final plastic weight* (mg)	Weight loss%**
Al-dabaa	Sea water c	F_1	187	6.73^{gh} ±1.35
	Sediment	F_2	186	7.33^{gh} ± 1.90
Sidi kreer	Sediment	F_3	184	8.53^{fgh} ± 1.90
Al-agmy	Sea water	F_4	191	4.70^{h} ± 1.05
Al-max	Sediment	F_5	178	10.73^{defgh} ± 1.80
		F_6	185	7.63^{gh} ± 2.25
Eastern harbor	Sea water	F_7	167	16.50^{bcd} ± 2.95
	Sea water	F_8	170	15.47^{bcde} ± 0.96
	Sea water	F_9	184	8.33^{fgh} ± 3.16
	Sediment	F_{10}	182	9.30^{efgh} ± 2.36
	Sediment	F_{11}	177	11.57^{cdefg} ± 2.15
	Sediment	F_{12}	189	5.47^{gh} ± 1.36
	Sediment	F_{13}	181	9.43^{efgh} ± 2.22
	Sediment	F_{14}	183	8.47^{fgh} ± 2.15
Suez coast	Sediment	F_{15}	164	17.90^{bc} ± 2.25
	Sediment	F_{16}	183	8.37^{fgh} ± 1.35
	Sediment	F_{17}	181	9.37^{efgh} ± 2.25
	Sea water	F_{18}	149	25.50^{a} ± 1.90
	Sea water	F_{19}	158	20.77^{ab} ± 2.56

*The initial weight of the used LDPE plastic sheet was 200mg, **The percentages with different letters (a, b, c, d,….) are significantly different according to the L.S.D. at *P*> 0.05 = 6.4378 & L.S.D. at *P*> 0.01 =7.4601

3. LDPE plastic degradation using changes in plastic mechanical properties

The changes in the mechanical properties of the treated plastic strips using F_{18}, F_{19}, and F_{15} separately showed that the marine isolate F_{18} was the most efficient isolate where it reduced the maximum force and the elongation % at the breakpoint of the plastic sheets to 8.9N and 114.2%, respectively, compared to the untreated plastic sheets 18.5N and 176.1% (Table 2).

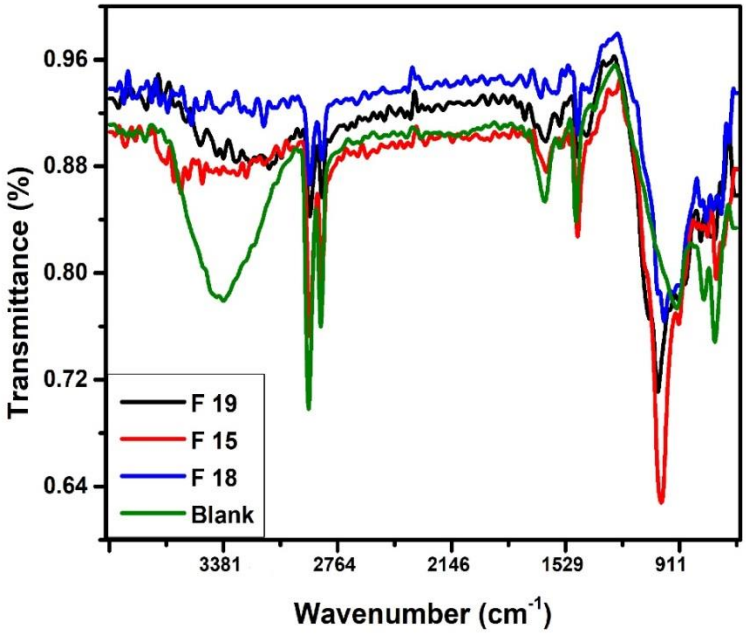

Fig. 1. FTIR spectra indicating the changes in the functional groups of LDPE plastic sheets after incubation for 30 days with the most potent biodegradable plastic marine isolates (F_{15}), (F_{18}) and (F_{19}) compared to the untreated LDPE plastic sheet (blank)

Table 2. Changes in mechanical properties of treated LDPE plastic sheets using the marine isolates F_{15}, F_{18} and F_{19} compared to untreated plastic sheets (blank)

Isolate code	Maximum force (N)	Elongation %
Blank	18.5	176.1
F_{18}	8.9	114.2
F_{19}	11.7	130.9
F_{15}	13.6	166.6

4. Biochemical Identification of promising plastic degrading marine bacteria

The most potent marine isolates F_{18}, F_{19} and F_{15} underwent a further microbiological purification process using a streaking technique, then they were biochemically identified as *B. licheniformis* FMMA, *Paenibacillus xylanilyticu,* and *Bacillus subtilis*, respectively, using the BIOLOG identification system.

5. Molecular identification process

The molecular identification was carried out for the most potent plastic degrading strain F_{18} using the 16S rRNA gene sequence. The obtained gene sequence was compared with the data present at the GenBank using the BLAST; it was found to be a new marine bacterial strain belonging to the *Bacillus licheniformis*, which was recorded as *Bacillus licheniformis* strain FMMA with an accession number MW980059.1 (Fig. 2).

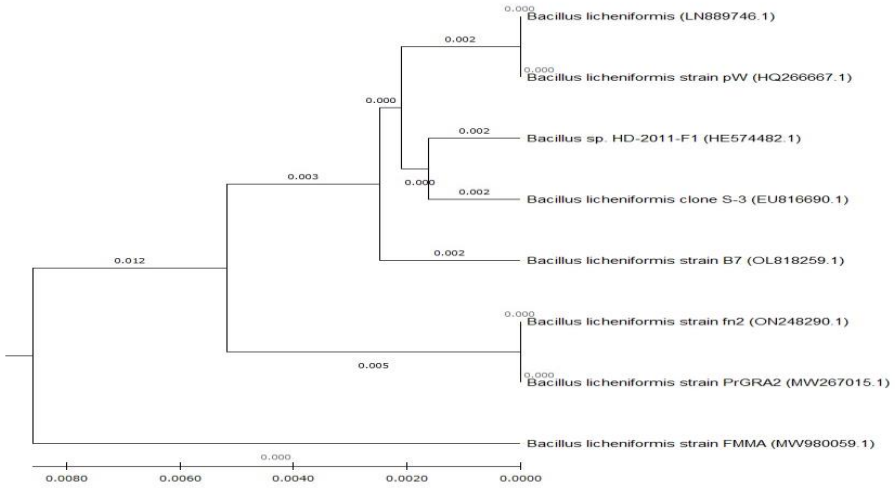

Fig. 2. Phylogenetic tree of the most potent plastic degrading marine bacterium *Bacillus licheniformis* strain FMMA using the 16SrRNA gene sequence for more related bacterial strains presented in the GenBank using MEGA 7 software

6. SEM analysis for LDPE sheets treated with *Bacillus licheniformis* FMMA

Surface morphological alternations on LDPE sheets were observed by SEM examination after 30 days of the treatment with marine *Bacillus licheniformis* FMMA. SEM images of the treated LDPE sheet showed surface degradation, fragility, cracked layer, and scratching in comparison with the untreated sheets which remained smooth, intact, and clear (Fig. 3).

7. Plastic degradation process using a marine bacterial consortium

The results in Table (3) and Fig. (4) display that the use of the bacterial consortium which is composed of *Bacillus licheniformis* FMMA, *Paenibacillus xylanilyticus,* and *B. subtilis* showed a significant plastic degradation process. Where a reduction in both the maximum force and the elongation % at the breakpoint was observed, they were 7.49N and 112.2%, respectively. Moreover, under the same

optimized culture conditions, the loss of the plastic weight % was increased to 34.1% compared to the use of the single culture of *Bacillus licheniformis* FMMA (25.5%).

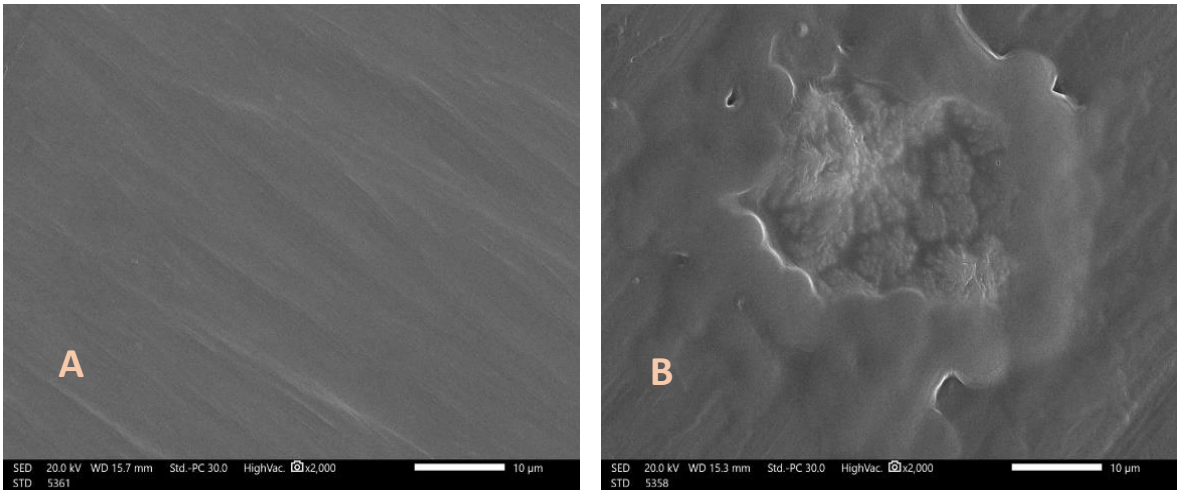

Fig. 3. (A) Micrographs showing SEM examination of untreated LDPE plastic sheet (blank) and **(B)** treated LDPE plastic sheet with marine *Bacillus licheniformis* FMMA after 30 days of incubation

Table 3. Changes in mechanical properties and weight loss % of tested LDPE plastic sheets using a marine bacterial consortium compared with *Bacillus licheniformis* FMMA and blank plastic sheets

Plastic marine bacterial treatment	Maximum force (N)	Elongation (%)	Weight loss (%)
The blank LDPE plastic sheet	18.50N	176.1	0.0
B. licheniformis FMMA	8.91	114.3	25.53
Marine bacterial Consortium*	7.49	112.20	34.1

*It composed of *B. licheniformis FMMA***,** *B. subtilis*, and *Paenibacillus xylanilyticus*.

8. Effect of different pH values on biodegradation process

The data presented in Fig. (5) show that the most effective pH value for the biodegradation process of LDPE sheets was 7.0, adding to the Wt. loss%, elongation% and maximum force at the breakpoints of 25.39± 2.0, 113.8± 2.0 and 8.85± 1.01, respectively. These values were highly significant compared to the values of the blank sheet. The calculated L.S.D. values at $P< 0.01$ were 4.195, 7.676 and 3.783, respectively.

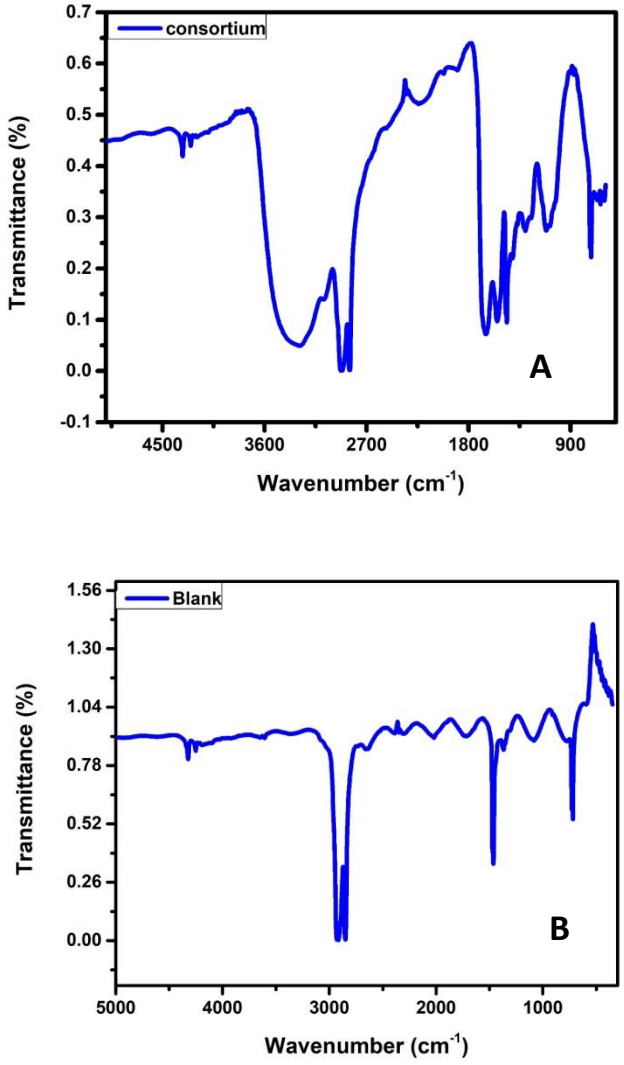

Fig. 4. FTIR spectra revealing the changes in functional groups of LDPE plastic sheets incubated at 35°C for 30 days using a marine bacterial consortium composed of **(A)** *B. licheniformis FMMA*, *B. subtilis* and *Paenibacillus xylanilyticus* compared to **(B)** blank sheets

9. Effect of different inoculum size on biodegradation process

The data presented in Fig. (6) reveal that the most effective inoculum size for the biodegradation process of LDPE sheets was 4ml, showing Wt. loss%, E%, and a maximum force at the breakpoints of 25.43± 3.01, 113.8± 3.05 and 8.86± 2.04, respectively. These values were highly significant compared with those of the blank sheet, which were 0.033± 0.033, 175.97± 0.42 and 18.33± 0.21, respectively. These data were statistically analyzed, showing L.S.D. values of 5.185, 7.902 and 6.749, respectively, at $P< 0.01$.

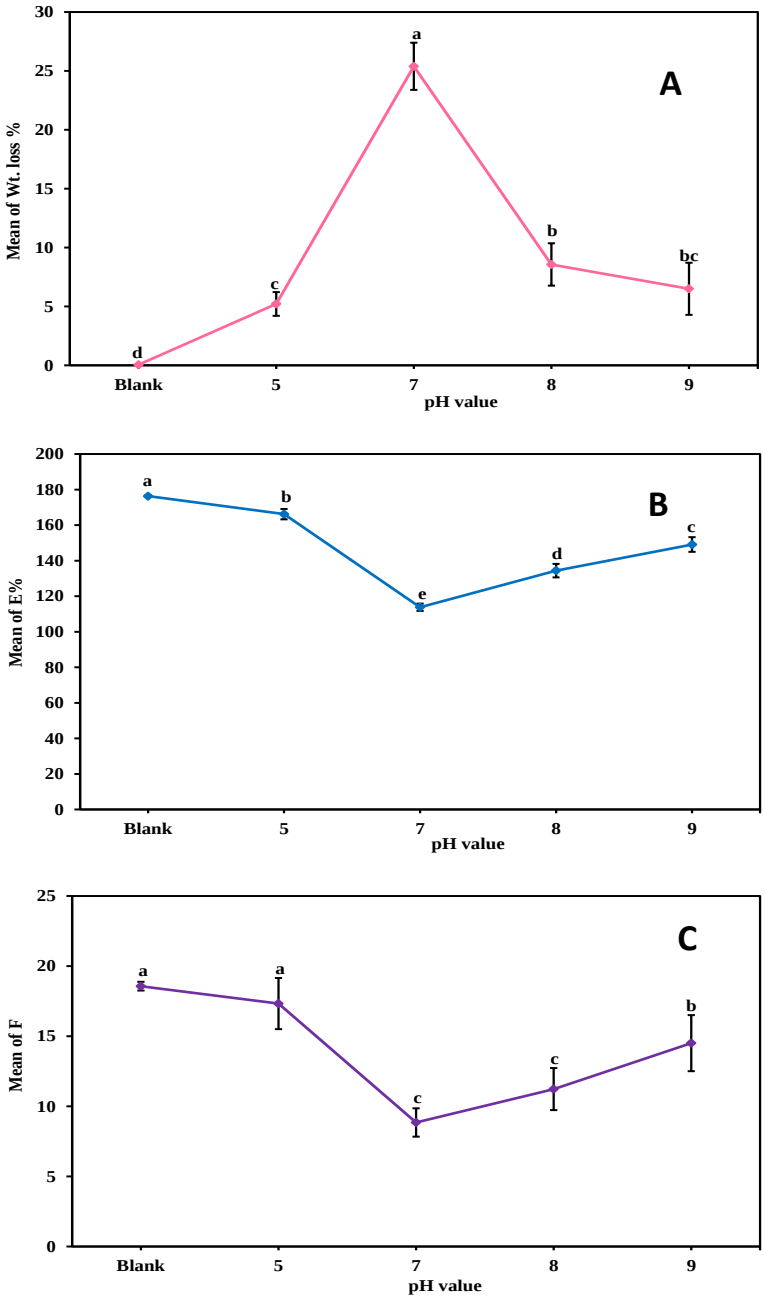

Fig. 5. Effect of different pH values on **(A)**Wt. loss %, **(B)** elongation% and **(C)** maximum force of degraded LDPE plastic sheets compared to untreated LDPE plastic sheets (blank). The values with different letters (a, b, c, d) means highly significant difference according to the L.S.D. at $P< 0.01$

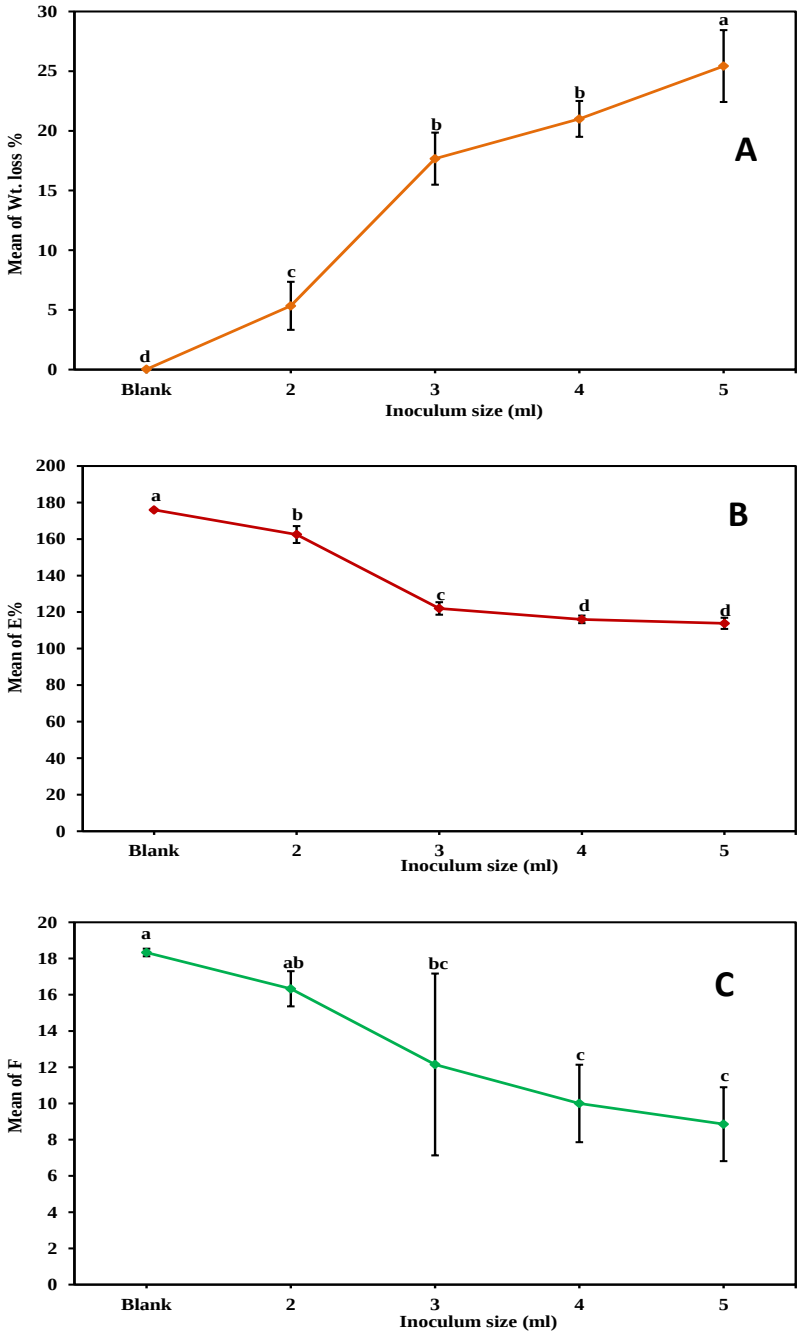

Fig. 6. Effect of different inoculum sizes (1.0X10^{7}CFU/ml) on **(A)** Wt. loss %, **(B)** elongation% at break point and **(C)** maximum force at break point of the degraded LDPE plastic sheets compared to the untreated LDPE plastic sheets (blank). The values with different letters (a, b, c, d) means highly significant difference according to the L.S.D. at $P< 0.01$

10. Effect of different temperature on biodegradation process

The data presented in Fig. (7) denote that the most effective temperature for the LDPE plastic biodegradation process using the marine bacterial consortium was 35°C, since the Wt. loss%, elongation% and maximum force at the breakpoint were 25.33± 0.91, 112.2± 1.96 and 7.13± 1.11, respectively, with a highly significant difference compared to the values of the blank sheets, 0.04± 0.04, 176.1± 0.10 and 18.47± 0.25, respectively. The L.S.D. at $P< 0.01$were 5.518, 5.0439 and 3.732, respectively.

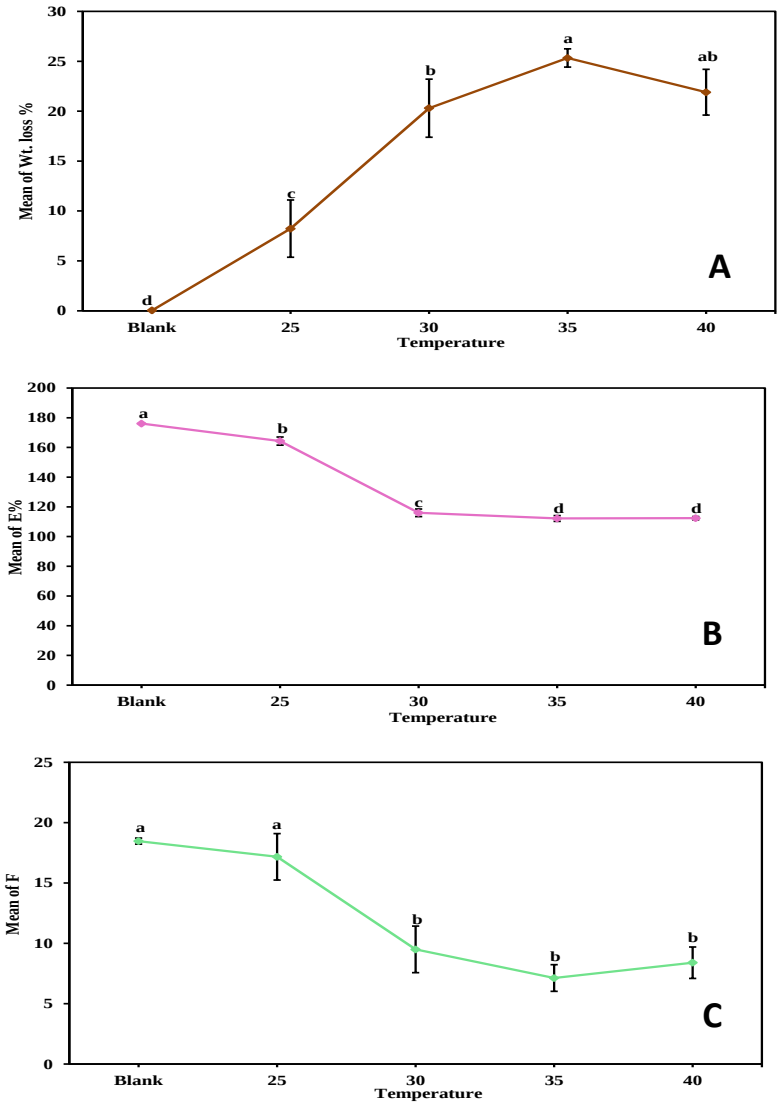

Fig. 7. Effect of different temperatures on **(A)** Wt. loss %, **(B)** elongation% at break point and **(C)** maximum force of degraded LDPE plastic sheets compared to untreated LDPE plastic sheets (blank). The values with different letters (a, b, c, and d) means highly significant difference according to the L.S.D. at $P< 0.01$

11. LDPE degraded by-products analysis using GC-MS

The GC-MS analysis was carried out on the samples taken from the culture medium after the incubation period of 30 days to recognize the by-products resulted from this biodegradation process. The data presented in Fig. (8) and Table (4) exhibit no highly toxic compounds for these metabolites according to their available biotoxicity information sheets, especially the cyclohexanol derivative which was the most obtained compound. Its area% was 42.4.

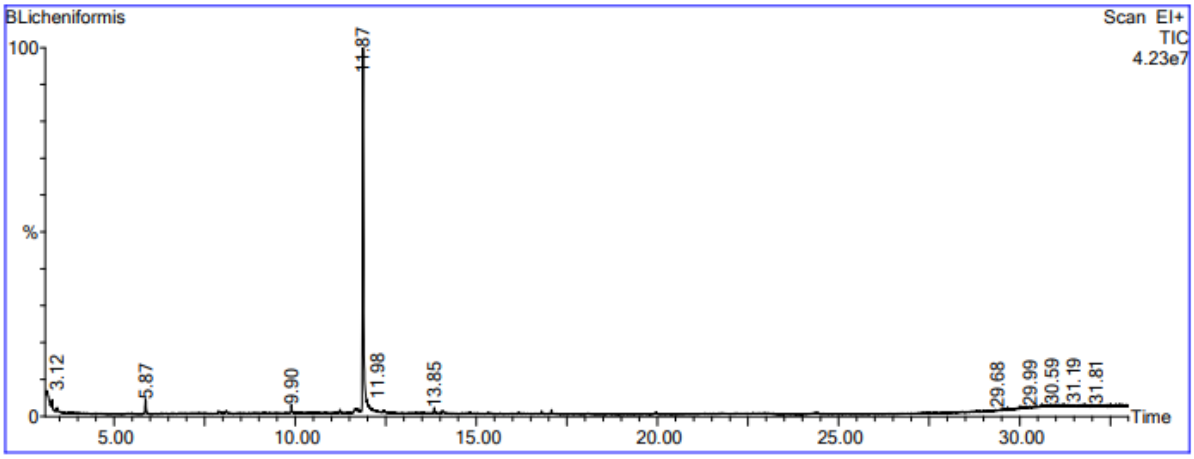

Fig. 8. GC–MS chromatograms for cell free filtrate of cultured marine bacterial consortium in presence of LDPE plastic sheets after 30 days of incubation

DISCUSSION

The most practical synthetic polymers are plastics, which are employed in a variety of applications. Plastic wastes have been produced quickly in the environment, leading to a number of environmental risks. Plastics take almost a thousand years to completely degrade. It was discovered that the most efficient way to reduce plastic pollution was through biodegradation **(Pathak & Navneet, 2017)**. Therefore, in this work, different seawater and sediment samples collected from Alexandria and Suez coasts, Egypt, were incubated for three months in the existence of LDPE plastic sheets. Then, 20 different bacterial strains were isolated, purified and, tested separately to degrade the LDPE plastic sheets as their only carbon source using the weight losses of LDPE plastic sheets as a remarkable method for estimating the biodegradation process. It was found that the isolates coded with F_{18}, F_{19} and F_{15} showed a great plastic loss of weight ranging from 18- 26% after one month of incubation. While, other authors who worked on the LDPE biodegradation processes found the *P. aeruginosa* strain ISJ14 led to only 6.5% LDPE degradation after two months of incubation **(Amodu *et al.*, 2016)**. In addition, *Brevibacillus borstelensis* strain 707 degraded by only 2.5% after one month of incubation **(Hadad *et al.*, 2005)**.

Table 4. Major degraded by-products of LDPE plastic sheets treated with marine bacterial consortium using GC-MS and their biotoxicity information sheets*

The estimated compound	RT	Area%	Biotoxicity*
Cyclohexanol, 5-methyl-2-(1-methylethyl) -, (1à,2á, 5à)-(ñ)-	11.872	42.384	not classified as toxic substance
Cyclobutane-1,1-dicarboxamide, n,n'-di-benzoyloxy	11.672	1.224	not classified as toxic substance
4-Ethylbenzoic acid, cyclopentyl ester	5.875	1.615	not classified as toxic substance
Sinapic acid	7.895	0.313	not classified as toxic substance
Pentanoic acid	9.901	0.937	0.6 mg/l
3-Methyl-4-isopropylphenol	13.848	0.555	870 mg/kg
Terpin hydrates	14.073	0.552	not classified as toxic substance

* https://www.fishersci.com/us/en/catalog/search/sdshome.html

These three marine bacterial isolates were identified as *Bacillus subtilis*, *Bacillus licheniformis* strain FMMA, and *Paenibacillus xylanilyticus.* The plastic biodegradation process was confirmed using FTIR spectroscopy; it was observed that the band appeared at 3100- 3600cm^{-1} in the untreated plastic sheets and underwent disappearance or reduction in the treated plastic sheets, which means the removal of -OH bounded compounds like; alcohol, hydroxy peroxide, and carboxylic acids by *Bacillus* sp. **(Ibiene *et al.*, 2013)**. In addition, the main peak appeared at 2900cm^{-1} resembling the CH_2 group, which is the backbone unit of the PE, it showed a reduction in its intensity in the LDPE plastic sheets treated with the marine *Bacillus subtilis*, *Bacillus licheniformis*, and *Paenibacillus xylanilyticus* compared to the untreated plastic sheets. Similar findings were documented when *Acinetobacter baumannii* biodegraded LDPE **(Pramila & Ramesh, 2015)**. Additionally, the use of the marine *Bacillus licheniformis* and *Paenibacillus xylanilyticus* showed a reduction in the intensity of the peaks that appeared at 1465 and 720– 724cm^{-1}, which means a bending deformation of the -CH bond and a rocking deformation of the =CH bond, respectively **(Mouallif *et al*., 2011)**. Moreover, an additional peak at 1129cm^{-1} was observed in the LDPE treated with *Bacillus licheniformis*

compared with the untreated LDPE plastic sheets which resembled the formation of C–O bond stretch **(Copinet *et al.*, 2004)**.

Devi *et al*. (2015) corroborated all of these findings, stating that the biodegradation of polyethylene began originally by an abiotic process. Dissolved oxygen caused the polymer chain to oxidize, forming carbonyl groups first, then carboxylic groups through β-oxidation, and ultimately entering the citric acid cycle to form CO_2 and H_2O.

Moreover, the changes in the mechanical properties of the tested LDPE plastic sheets using *B. subtilis*, *B. licheniformis*, and *Paenibacillus xylanilyticus* showed that the most potent isolate was *B. licheniformis*, where the maximum force and elongation % at the breakpoint of the LDPE plastic sheets were reduced by 51.8 and 35.2%, respectively, compared to the untreated plastic sheets under the optimized culture conditions. Similarly, **El-Naggar and Farag (2010**) showed the mechanical properties of polyethylene rice starch plastic sheets were changed through microbial treatment by *B. amyloliquefaciens*.

The most potent plastic degrading bacterium was genetically identified as *B. licheniformis* FMMA with accession number: MW980059.1, and its effect on the biodegradation of LDPE sheets was examined using the SEM. It showed significant cracks on the surface of the examined plastic sheets. Similarly, several researchers studied the microbial degradation mechanism of LDPE using the SEM and mentioned the bacteria utilized the oxidized region of the LDPE plastic sheets and solubilized it leading to pits formation on the surface. Moreover, they mentioned that this oxidation of LDPE occurred due to the microbial enzymatic activity which breaks the amorphous regions of LDPE **(Kunlere *et al.*, 2019)**. Meanwhile, a disintegrated surface of LDPE was noticed after treatment with *Pseudomonas fluorescens*, which indicated its strong adherence and LDPE utilization capacities **(Mukherjee *et al*., 2018)**. Moreover, the cracks and pits detected on the LDPE surface after the treatment with *Microbulbifer hydrolyticus* and *Streptomyces albogriseolus* confirmed the LDPE degradation **(Li *et al.*, 2018)**. Furthermore, several researchers studied the microbial degradation mechanism of LDPE and showed the bacteria utilized and solubilized the oxidized region of LDPE leading to pits formation on the surface of the examined LDPE **(Montazer *et al.*, 2018; Kunlere *et al*., 2019)**.

The plastic degradation process using the marine bacterial consortium composed of *B. subtilis, B. licheniformis* FMMA, and *Paenibacillus xylanilyticus* was very effective, where the weight loss% increased to 34.1% compared to that of the single culture. It showed 25% as a maximum plastic loss of weight after one month of incubation under the same optimized culture conditions. Similarly, **El-Sayed *et al*. (2021**) mentioned that a mixed fungal culture of *Aspergillus carbonarius* and *Aspergillus fumigatus* was effective in the LDPE degradation process, in the case of the mixed culture, the weight loss% of LDPE sheets was 5.01%, while the single culture of *A. carbonarius* was 3.8% and *A. fumigatus* was 2.267% under the same incubation conditions. Additionally, **Han *et al.***

(2020) demonstrated that the plastic degradation can be enhanced by the combined activities of *Arthrobacter* sp. and *Streptomyces* sp. in a mixed population. They formed thicker and more complex biofilms which had much greater effects on the hydrophobicity of plastic sheets and their surface chemistry led to higher rates of PE mineralization.

The effect of different parameters pH, inoculum size and temperature on the biodegradation process of LDPE sheets using the marine bacterial consortium was investigated, and it was found the optimum pH value was 7.0 where the Wt. loss% was 25.39%. Additionally, the elongation% and maximum force of the tested plastic sheets at the breakpoint were reduced by 35.46and 52.34 %, respectively, compared with the untreated sheet. Similarly, **Islami *et al*. (2019**) found the ability of *Thiobacillus* sp. and *Clostridium* sp. to degrade LDPE plastic was affected by the temperature and the pH, they decreased the weight of LDPE plastic by 2- 7% when 30°C and pH 7 were applied in the culture compared to the untreated plastic sheets.

Temperature is an important environmental component. It significantly impacts chemical and biochemical processes, as well as the taxonomic content and metabolic processes of microbial communities. Here, the optimum temperature ranged from 35 to 40°C, the Wt. loss% reached 25.33, E% was reduced by 36.1%, and maximum force at the breakpoint was reduced by 61.4% compared to the untreated plastic sheets. Similarly, **Mallseitty *et al*. (2023**) postulated that a temperature of 37°C, pH of 7.1 and inoculum volume of 4% v/v were found to be optimal conditions for the biodegradation of LDPE.

The GC–MS analysis of the obtained degraded byproducts showed a great formation of the cyclohexanol-derivative with an area of 42.4%. However, **Dangel *et al.* (1988)** mentioned that cyclohexanol had been proposed as an intermediate biodegradation metabolite, and about 60% of the formed cyclohexanol was completely oxidized to CO_2. Furthermore, they stated that one benefit of biological degradation is that, according to GC-MS analysis, the byproducts of degradation are not extremely toxic or harmful. Thus, the utilization of biological methods is a promising way for the degradation of environmentally harmful materials.

CONCLUSION

Nowadays, plastic wastes have become the main environmental problem due to the bad side effects of their removal from our ecosystem. Therefore, in this study, a great ability to degrade LDPE plastic sheets had been estimated by the isolated marine *B. subtilis*, *B. licheniformis* FMMA, and *Paenibacillus xylanilyticu* using the loss of plastic weight %, measuring the changes in plastic mechanical properties, FTIR, and SEM examinations. This promising marine bacterial consortium reduced the weight of the tested plastic sheets to 34.1%, changed the plastic mechanical properties where the maximum force and elongation % of treated LDPE sheets were reduced to 59.5 and 36.3%, respectively, compared to the untreated plastic sheets. In addition, the biotoxicity of the degraded LDPE by-products was studied using GC-MS, which showed no toxic

metabolites, and they were recommended as safe for the environment. Further studies are recommended to ensure the mechanism of this degradation process.

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