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Chemical and Biological Assay for The Degradation of Microwave Synthesized-Hyperbranched Poly (Urethane-Urea) By Local Bacterial Isolates

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Abstract

Ultra-fast recently Microwave-synthesized Hyperbranched Poly (urethane-urea) (MW-HBPU), is a polymer with a unique morphological structure and different applications in many fields as textile printing and coating. Because Polyurethanes (PUs) have excellent chemical and physical properties, their bio-decomposition and decay are difficult and time consuming. Therefore, MW-HBPU biodegradation by five bacterial isolates isolated from different local garbage sites has been studied. The highest activity was observed in three isolates (H1, H2 and H3), that were identified by morphology, physiology, biochemical and molecular biology (16S rRNA). *Achromobacter* sp. strain NRC2018, *Bacillus subtilis* strain NRC2018 and *Bordetella petrii* strain NRC2018, respectively. Carboxylesterase and acetylcholineesterase activity for the three strains were determined. *Achromobacter* sp. strain NRC 2018 showed high activity gives 91.2 and 526.4 U/ml for Carboxylesterase and acetylcholineesterase, respectively. The change in the characteristic functional groups reflected in the ATR-FTIR spectra of MW-HBPU after incubation with these bacteria showed attack at certain sites in the MW-HBPU structure and pointed to structural changes. SEM images clearly showed degradation in the MW-HBPU network. Additionally, a notable change in weight after incubation with isolated bacteria was also used to demonstrate the ability of these strains to effectively biodegrade MW-HBPU.

Keywords: Biodegradation; Bacteria; Identification; Hyperbranched polymers; Polyurethane

1. Introduction

Since human synthesize plastic of all its kinds, every second pass accumulates plastic waste causing significant environmental problems. The plastic waste represents approximately 30 % of the solid waste that hurts our planet. Polyurethane (PU) is one of the most important polymers in our daily life and is widespread in many medical and industrial applications [1, 2, 3]. Recently synthesized hyperbranched polymers possess super properties that make them more reactive than linear polymers, such as; compact molecular shape, lower viscosity and better solubility, and they possess many of reactive end groups. Because of the above-mentioned features, there are many applications of such polymers in characteristic fields, such as supra-molecular chemistry, material biomedical applications, photochemical molecular devices, conductive materials, coating additives and textile finishing [4, 5, 6].

Additionally, the application of advanced technologies such as microwave (MW) irradiation polymer synthesis (instead of thermal heating) gives brilliant features such as reaction rate enhancement, greatly reduced reaction time, higher control & selectivity, higher reaction yield, non-contact heating, homogeneous processes, reduction of side reactions greener products [7, 8,9]. The many PUs applications (foams, textile printing and finishing, automotive parts, coatings, varnishes, paints, adhesives etc.) are due to it brilliant chemical character and excellent abrasion and mechanical resistance, which make them highly resistant to microbial degradation [2, 10, 11, 12]. Balancing between manufacturing stable polymers that resist environmental conditions and minimizing their impact on the environment is a difficult challenge.

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The environmental problem appears with the accumulation of these polymers after their service has finished. With more clarity, these polymers have been designed to be stable and resistant to environmental degradation. The inevitable result of these structures is their very slow decay rate. It may take very long to degrade these large compounds into short chain hydrocarbons. Moreover, the disposal of these materials by burying them in certain places leads to a significant change in the pH of the surrounding environment. Groundwater changes followed by disturbances in the leaching of moisture and rain water make the land bare and unfertile [13, 14]. Furthermore, these materials also negatively impact human health and aquatic life.

The term biological degradation refers to the attack of living organisms such as bacteria, fungi and insects on a substrate which results in a lack of characteristic properties (changes in both mechanical properties and chemical structure). Fortunately, scientists have noted some microorganisms that produce significant degradation in the composition of some polymers under certain conditions that is faster than normal [15, 16]. The effect of microorganisms on attacked polymers can take three aspects: i) a biophysical effect (growth of organisms depending on the loss of mechanical resistance in the polymer), ii) a biochemical effect (bioactive compounds produced by microorganisms act on the polymer) and iii) direct enzymatic action. Carboxylic ester hydrolases are a large class of enzymes catalyzing the hydrolysis or synthesis of ester bonds [17]. Esterase are able to catalyze the hydrolysis or synthesis of a rather broad range of other substrates containing ester linkages, such as acylglycerols, aryl esters [18], and in some cases alcohol esters, cinnamylesters, xhantophyl esters [19], or synthetic polymers [20].

Therefore, the biodegradation of PU follows two different pathways: oxidative degradation and hydrolysis degradation [21]. Polyester is subject to hydrolytic degradation due to the presence of soft segments and ester group in their structural backbone [21]. However, polyether PUs is subject to oxidative degradation. The hydrolytic degradation of PU is carried out in three steps: i) hydrolysis of amide and ester links through the polymer chains, ii) both of molecular weight and viscosity has been decreased iii) final cleavage all polymer chains.

Moreover, few studies have addressed the ability of bacteria to biodegrade varieties of PU [1]. In contrast, many articles have clarified fungal abilities to biologically decompose polyester polyurethane. Additionally, the ability of some proteolytic enzymes on amino acid based polyurethane biodegradation has been found in special sites e.g. aromatic side chains. The aim of this work was to isolate and identify of microorganisms isolated from local soil at garbage site, to efficiently biodegrade MW synthesized hyperbranched poly (urethane-urea) (applied in textile finishing), also studies the chemical and morphological changes in the polymer structure.

2. Materials and Methods

2.1. Ultra-Fast synthesis of hyperbranched poly (urethane-urea) via microwave irradiation (MW-HBPU)

The synthesis of hyperbranched poly (urethane-urea) (MW-HBPU) was performed under microwave irradiation(MW) using a pulses multimode milestone MW reactor with an operating frequency of 2.45 GHz and maximum MW power of 1200 Watt. The synthesis process was conducted according to modification of procedure described elsewhere [12]. The synthesis of hyperbranched poly (urethane-urea) using MW irradiation accelerates the reaction rate by 24 times compared with the conventional heating method and without using the catalytic agent.

Sampling and bacterial isolation

Soil samples were collected during 2016 from plastic waste disposal sites from El Gharbia Government, Egypt. Soil samples were collected by divers in sterile tubes and kept in a refrigerator until managed in the lab. Soil samples (10 g) were transferred into sterilized saline solution (0.85 % NaCl, w/v) (90 ml) with MW-HBPU as a sole carbon sources to decrease the colony count in soil and sited in shaker incubator (150 rpm and 37 °C). After two- and four-weeks suspensions were plated for isolation of bacteria on mineral salt agar media (MSM) (g/l: K_2HPO_4 0.5, KH_2PO_4 0.04, NaCl 0.1, $CaCl_2{\cdot}2H_2O$ 0.002, $(NH4)_2SO_4$ 0.2, $MgSO_4.7\ H_2O$ 0.02, $FeSO_4$ 0.001) containing MW-HBPU (10 g/l) as a sole carbon source [22, 23]. The plates were prepared one day before plating and incubated at room temperature overnight to eliminate films of moisture on the agar surface. Then placed on static incubator at 37°C and examined for bacterial growth within one week to allow the development of slow growing bacteria. Isolates were then subjected to purification. Duplicate

agar plates were used for purification by streaking the inoculating plates. Single colonies were picked up and sub cultured on nutrient agar.

2.2. Biodegradation of MW-HBPU

2.2.1. Weight loss technique

The sheets for each formulation were tested for resistance to bacterial attack. Pre-weighed dried, autoclaved, surface sterilized and UV treated samples were exposed to bacterial culture in Petri dishes containing nutrient agar media followed by incubation of samples at 37°C. Triplicates were maintained for each type of hyper branched PU. After one week, samples were collected, washed thoroughly by water (distilled) then dried to room temperature until constant weight was reached. Samples were reweighted to determine the percentage of loss in samples weight using the following formula [22].

$$\textbf{Weight loss \%} = (m_i - m_f)/m_i$$

Where, m_i and m_f are the masses of the samples before and after before incubation respectively.

After that, the samples were dried to remove any residue that may interfere with the analysis process. After the incubation, three bacterial species, that were able to effectively utilize MW-HBPU as the sole carbon and/or nitrogen source (the isolates showed a maximum weight loss effect of over 50 %) under experimental conditions, were isolated. These isolates can affect polymer structure by attacking the polymer at sites where reactive groups are located (e.g., ester groups of the soft segments, urethane linkages, and amide groups) [1].

2.2.2. Analysis of changes in MW-HBPU

Mineral salt medium was prepared, containing (g/l: K_2HPO_4 0.5, KH_2PO_4 0.04, NaCl 0.1, $CaCl_2 \cdot 2H_2O$ 0.002, $(NH4)_2SO_4$ 0.2, $MgSO_4 \cdot 7\ H_2O$ 0.02, $FeSO_4$ 0.001), the prepared media was auto-claved at121°C for 15 min followed by cooling until reached to room temperature. Pre-weighed MW-HBPU samples were sterilized through exposing to UV ray (254 nm) for 15 min. After sterilization pro-cess, the samples were inoculated in the prepared medium in sterile conditions. For degradation study, (10^8 CFU/mL) of three days pre-culture bacterial isolates (H1, H2 and H3) were injected in conical flask (250 ml) having 100 ml MSM and10 g/l of MW-HBPU as a sole carbon sources, the cultures were incubated in an incubator shaker(37 °C and 100 rpm) for one week. Microorganisms development was observed using spectrophotometer (UV/Visible) through evaluating the absorbance optical density (at wavelength 600 nm) with respect to the control sample. The readings were taken at interval of seven days. At the end of the week MW-HBPU were recovered and washed for further analysis. The alterations in the morphology as well as the changes in the structure through chemical bonds modification of MW-HBPU as a result of incubation with isolated bacterial strains were analyzed by different techniques, such as SEM and ATR-FTIR spectroscopy [23].

a. Identification of screened strain

Morphological and physiological characterization

Bacterial strains were identified by morphological, physiological and biochemical experiments according to Bergey's Manual of Determinative Bacteriology [24]. The identification was confirmed by comparing morphology, Gram staining, substrates utilized as sole carbon sources and growing tests (catalyses, starch hydrolysis, Voges-Proskauer, simmon's citrate and nitrate reduction tests) at 37 ˚C and pH 7.

16SrDNA sequence identification

DNA extraction was carried out by a DNA extraction kit and DNA quality was evaluated on a 1.2 % agarose gel. A single band of high molecular weight DNA was observed. The forward primer was 5′-TCCGTAGGTGAACTTTGCGG-3′ and the reverse primer was 5′-TCCTCCGCTTATTGAT-ATGC-3′ [25]. The amplified PCR product was purified to remove contaminants. The forward and reverse DNA sequencing of PCR product was carried, followed by 35 amplification cycles at 94°C for 45 s, 55°C for 60 s and 72°C for 60 s. DNA fragments of approximately 988 bp were amplified. A positive control (*Escherichia coli* genomic DNA) and a negative control were performed for in the PCR. Sequencing was performed by using a Big Dye terminator cycle sequencing kit (Applied Biosystems, USA). Sequencing products were resolved on an Applied Biosystems model 3730XL automated DNA sequen-cing system. The forward and reverse 16S rDNA sequences obtained were checked for accurate base calling, assembled and analyzed using the BLAST available on the NCBI website (http://www.ncbi.-nlm.nih.gov) using the BLAST program [26]. The phylogenetic tree was constructed

using neighbor-joining tree method using the software MEGA7.

b. Determination of Carboxylesterase and acetylcholineesterase activity

Carboxylesterase and acetylcholineesterase were estimated in the crude homogenates. Carboxylesterase activity was determined using*P*-NPA as the substrate which forms nitrophenol compounds [27]. Reaction mixtures contained in 1ml: 2 mM*P*-nitrophenyl acetate dissolved in acetone, 80 mM phosphate buffer pH 7.5. Change in the absorbance was measured at 407 nm for 10 min. The assay was calibrated using *P*-nitrophenol. One unit of esterase enzyme activity was defined as 1µmoles of *P*-nitrophenol produced per hour under standard assay conditions. While, acetylcholineesterase determined by substrate [28]. The reaction mixture contained in 1ml: 60 mM Tris-HCl buffer, pH 8.5, 1mM AcSChI, 1 mM DTNB. The reaction mixtures were incubated at 37^{o}C for 1h and the absorbance was measured at 412 nm. One unit of AChE activity was defined as the amount of enzyme that hydrolyzes 1µmol of substrate per hour under standard assay conditions.

2.3. Assay of structure changes

2.3.1. ATR-FTIR spectroscopy analysis.

AT-FTIR spectra of MW-HBPU treated and untreated samples were done on JASCO FTIR 4100 spectrometer (Tokyo, Japan). The measurements were recorded in the range of 400– 4000 cm^{-1} Scanning electron microscopy (SEM) analysis [12].

SEM micrographs of samples were verified on a Quantum (FEG-250 microscope at a voltage of 10 kV). To avoid charging the samples are sputter-coated with gold before scanning [12].

3. Results and Discussion

3.1. Isolation of bacteria causing biodegradation

Biodegradation is the intentional degradation of polymer chains by microorganisms, which leads to changes in the polymer chain mechanical properties and chemical structures. Five bacterial isolates were isolated at selected medium using MW-HBPU as the carbon source for growth. These isolates were screened for their ability to biodegrade MW-HBPU. Polyester polyurethane (PU) was used as the sole carbon source in the isolation of Pseudomonas aeruginosa from soil and for the degradation of PU film pieces as reported by[23].

3.2. Biodegradation of MW-hyperbranched poly (urethane-urea) analyzed by weight loss technique

MW-HBPU was subjected to a weight loss technique for detecting the effects of different strains. The greatest effect was achieved by isolate H1 (66.32 %), followed by H2 (61.09 %), H3 (54.85 %), H4 (35.33 %) and the weakest one was H5 (30.51 %) (Table 1).This effect indicates their capacity to use MW-HBPU as the sole carbon source in their metabolism process. Bacillus cereus has been isolated from a local dumpsite for use in the biodegradation of polyethylene. Degradation was carried out using sterilized polyethylene and monitored by observing weight loss [29].

3.3. Identification of active isolates

Three isolates showed maximum weight losses in MW-HBPU (H1, H2 and H3) were identified according to their morphological, physiological and biochemical experiments (Table 2). Accordingly, it was to sequence 16S rDNA gene was amplified and sequenced, and the sequence has been placed in the GenBank database [30]. Phylogenetic trees based on the 16S rDNA sequence were built using the neighbor-joining method (Figures 1, 2 and 3). The nearly complete 16S rRNA gene sequences of strains were compared with the analogous sequences of other bacterial strains in the GenBank database through BLAST. BLAST results indicated that strain H1 was most similar to *Achromobacter* sp. strain and H2 was most similar to *Bacillus subtilis* strain, while, H3 was most similar to *Bordetella petrii* strain. Table (3) showed that the names and accession numbers of these strains. Phylogenetic trees were developed by the neighbour-joining algorithm (Figures 1, 2 and 3).

Table 1
Weight loss of MW-HBPU

Isolate no.	Initial weight (gm)	Final weight (gm)	Weight loss (gm)	Weight loss (%)
H1	0.1773	0.0597	0.1176	66.32
H 2	0.1784	0.0694	0.1090	61.09
H 3	0.4915	0.2219	0.2696	54.85
H 4	0.2870	0.1856	0.1014	35.33
H 5	0.3294	0.2289	0.1005	30.51

Table 2
Biochemical and physiological characteristics of bacterial isolates

Test	Microscopic examination		
Gram stain	H1	H2	H3
	Negative short rod	Positive bacilli	Negative short rod
	Culture characteristics &Appearance of the colony		
	H1	H2	H3
Colony surface	Smooth	smooth	smooth
Color	White	white	white
Elevation	Flat	Low convex	Low convex
Edge	undulate	entire	entire
Whole colony	Irregular	Circular	Circular
Pigmentation	Pink	no	no
Opacity of the bacterial colony	Opaque	translucent	translucent
	Biochemical tests		
	H1	H2	H3
Starch hydrolysis	+ve	-ve	-ve
Catalase test	+ve	+ve	+ve
Voges- proskauer test	-ve	-ve	-ve
Simmon citrate test	-ve	+ve	-ve
Nitrate reduction	+ve	-ve	-ve
Oxidase test	+ve	+ve	+ve
Glucose fermentation	-ve	-ve	-ve
Manitol fermentation	-ve	-ve	-ve

3.4. Carboxylesterase and acetylcholineesterase activity

It is well know that esterase enzyme play a major role in the biodegradation of polyurethane polymers [31]. Carboxylesterase and acetylcholineesterase activity were determined for the three strains. Table (4) showed that the maximum activity was *Achromobacter* sp. strain NRC 2018 which gives 91.2 and 526.4 U/ml for carboxylesterase and acetyl-cholineesterase, respectively. This agree with the highly weight loss percentage of *Achromobacter* sp. strain NRC 2018 in biodegradation of MW-HBPU.

Table 3
Names and accession numbers of bacterial isolates

Isolate	Name	Accession number
H1	*Achromobacter* sp. strain NRC 2018	MH410497
H2	*Bacillus subtilis* strain NRC 2018	MH410496
H3	*Bordetella petrii* strain NRC 2018	MH410499

3.5. Structure changes

3.5.1. ATR-FTIR analysis

The biodegradation of PUs occurs in two stages; in the foremost one, the polymer macromolecule is broken-down into smaller chains, followed by mineralization of polymer fragments. The degradation of PU polymers relies not only on the chemical structure but also on the nature of the degradation location (i.e. the existence of acidic, alkaline, aqueous, or oxidative conditions, the existence of enzymes). According to the literatures, polyester PUs are subject to hydrolytic decomposing due to the presence of ester group in both of urethane linkages and soft-segments [22, 32]. The capacity of microwave synthesized MW-HBPU to be degraded by soil bacteria has been studied. Infrared spectroscopy can be used as an effective tool for detecting and reviewing the chemical changes that may occur within polymer structures during degradation.

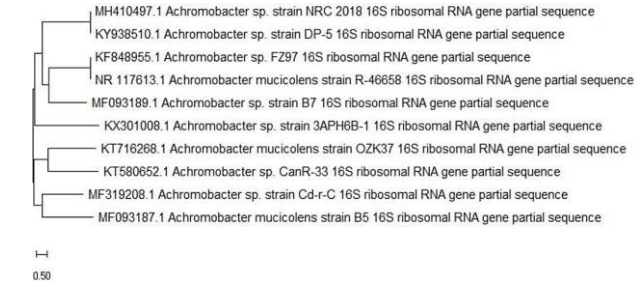

Fig. 1: Phylogenetic tree of *Achromobacter SP. NRC 2018.*

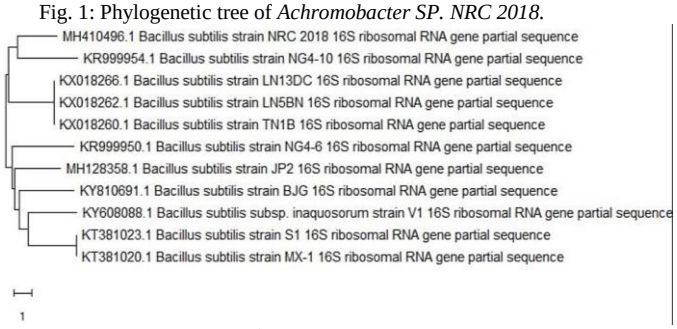

Fig. 2: Phylogenetic tree of *Bacillus Subtilis strain NRC2018.*

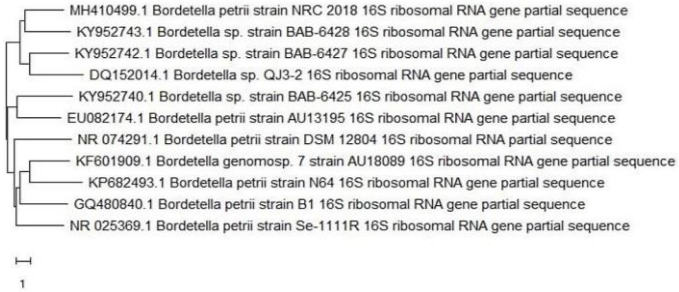

Fig. 3: Phylogenetic tree of *Bordetella petrii stain NRC 2018.*

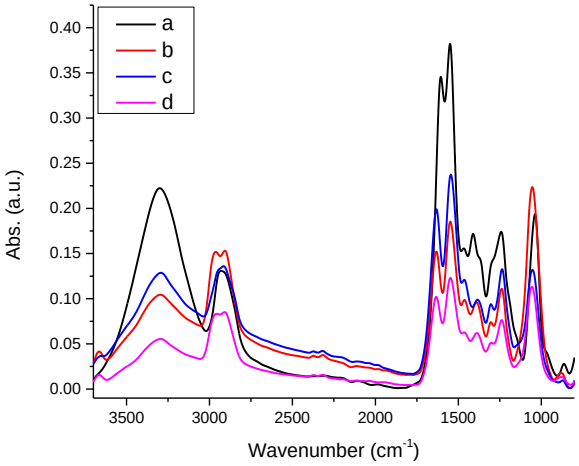

Fig. 4:(a) ATR-FTIR spectrum of control (MW-HBPU), (b) ATR-FTIR spectrum of MW-HBPU after incubation with *Bacillus subtilis* NRC2018, (c) ATR-FTIR spectrum of MW-HBPU after incubation with *Achromobacter* sp. NRC2018, (d) ATR –FTIR spectrum of MW-HBPU after incubation with Bordetella petrii NRC2018 after one week

ATR-FTIR spectra of control (polymer sample before incubation with bacteria) were taken as a control. The polymer samples were analyzed before and after being subjected to bacterial incubation. After one week of bacterial incubation, the incubated samples of MW-HBPU were washed successively by water (distilled), ethanol, and water again. Figure (4) showed ATR-FTIR spectra of MW-HBPU after incubation with *Bacillus subtilis NRC2018*, *Achromobacter sp. NRC2018* and *Bordetella petrii NRC2018* for one week.

In general, it is clear that, the incubation of MW-HBPU with all isolated bacteria has a significant effect on its structure. All the incubated samples exhibited a change in peak intensity (Figure 4). Both *Achromobacter* sp. NRC2018 and *Bordetella petrii* NRC2018 work by the same mechanism but with different activities, this may be explained by the different efficiency of bacteria in producing esterase (Carboxylesterase and acetylcholineesterase enzymes) which is shown in a table (4). AS well as, the spectra showed a decrease in the peak absorbance intensity for all degradation sites. However, in the case of *Bacillus subtilis* NRC2018, there was some variability in performance.

The literature states that, decrease in the absorbance of functional group (on IR spectra) indicate a weakening of that bond by the degradation process and occurrence of a physical change, while an increase in the peak absorbance intensity represent a morphology and chemical change in those bonds [31]. First, all treated samples showed a board band at 3300 cm^{-1} corresponding to overlapping str-OH and str-NH, which may be due to the formation of fragments with more O-H groups [23]. Additionally, the intensity of the band at 2940 cm^{-1}, which is attributed to str-C-H, increases, which can be explained by increases in the number of fragmented hydrocarbon molecules resulting from the degradation of the long-chain hydrocarbon polymer backbones to small chains such as methylene, methyl, amine or hydroxyl compounds. However, the intensity of this peak decreases in the *Bordetella petrii* NRC2018 incubations, which may be attributed to the cleavage of C-H-links [22].

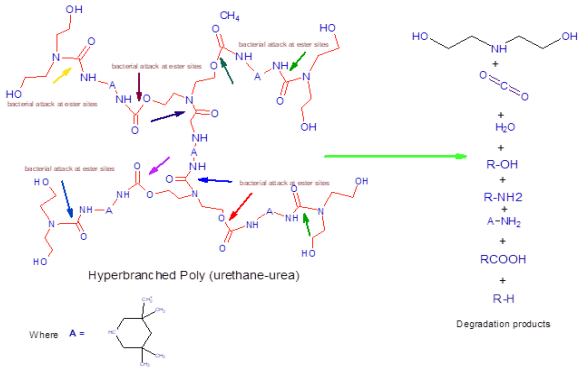

Fig. 5: Hypothetical degradation pathway of MW-HBPU by bacterial attack though ester hydrolysishe most important change observed in IR bands of attacked MW-HBPU is at the peaks of str-C=O (1607 cm-1), corresponding to NHCOO (urethane units):- str-C=O (1548 cm^{-1}), corresponding to NHCONH (ureido units) and (1055 cm^{-1}) corresponding to str-C-O-C.

The decrease in the intensity of those bands may attributed to the occurrence of chain scission in -RO-CO-NH_2 (urethane group) by attacking bacteria, resulting in free amines through ester hydrolysis (degradation of ester group) by bacterial attack [33, 34, 35]. The increase in the absorption of str-C-O-C in the case of *Bacillus subtilis* NRC2018 indicates the chemical change in the bond itself [31]. Hence, these results confirmed of attacks (chain scission) on urethane and ester linkages owing to the action of the isolated microorganisms as illustrated in figure 5 [22]. Therefore, we can expect the biodegradation of MW-HBPU occurs in two steps:-first, the microorganism is adsorbed onto the polymer surface, and second, the ester linkages are hydrolyzed. The hydrolysis occurs by dissociating polymer chains into smaller units, which leads to surface destruction. Extracellular enzymes from microorganism metabolism complete the hydrolysis by finalizing chain degradation.

The most interesting goal achieved in this research is the application of microwave irradiation in synthesis of HBPU, in a short time without using a toxic catalytic agent (DBTDL on the humans as well as microorganisms) consequently, enhances the degradation of the polymer by microorganisms [7, 36].

3.5.2. Scanning electron microscopy (SEM)

The morphological changes in MW-HBPU after incubation with isolated bacteria (*Bacillus subtilis* NRC2018, *Achromobacter* sp. NRC2018 and *Bordetella petrii* NRC2018) were investigated and detected by SEM (Figure 6). SEM images (Figure 6 a) represent the MW-HBPU polymer surface before incubation with microorganisms. The images show groups of sphere-shaped particles with a smooth surface and cluster shapes that are strongly linked to each other. These regular, uniform, homogenous round particles are characteristic features of dendrimer & hyperbranched polymers [37]. SEM images (Figure 6 b, c and d) represent the surface of MW-HBPU after incubation with *Bacillus subtilis* NRC2018, *Achromobacter* sp. NRC2018 and *Bordetella petrii* NRC2018, respectively. The surface of MW-HBPU after bacterial exposure showed considerable damage in the polymer network, with a porous structure, deep pits, and cracks [31]. Erosion regions were observed and increased in roughness and irregular morphology. It is also very clear that there is cracking in the polymer network, and an explosion in their round particles leads to the loss be regular and uniformity. The existence of holes in the attacked MW-HBPU is the focal point of microorganism's attack, initiating in the polymer layers cracking and aiding in degradation [22, 34]. The degradation starts from the polymer surface and progresses to the interior layers, increasing in the roughness of MW-HBPU outer surface as well as making it heterogeneous and irregular. The occurrence of all such damage in the images may strongly indicate degradation and decomposition in both network and polymer molecules. That degradation results from growth of bacterial isolates using MW-HBPU as a carbon and/or nitrogen source.

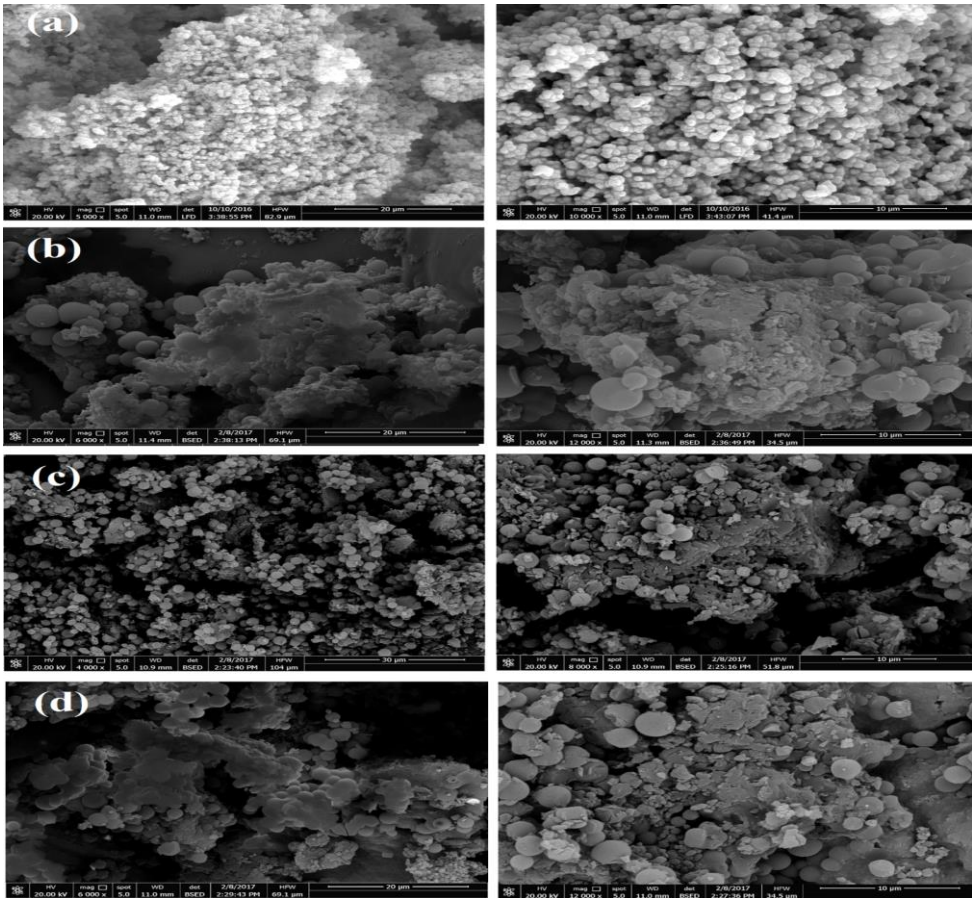

Fig. 6:(a) SEM images of control (MW-HBPU), (b) SEM images of MW-HBPU after incubation with *Bacillus subtilis* NRC2018, (c) SEM images of MW-HBPU after incubation with *Achromobacter* sp. NRC2018, (d) SEM images of MW-HBPU after incubation with Bordetella *petrii* NRC2018 after one week.

4. Conclusions

From these results, all of the chemical changes, occurring in microwave synthesized hyperbranched poly urethane–urea (MW-HBPU) structures during the bacterial incubation, confirm satisfactory biodegradation by bacterial strains attacking polymer chains. Additionally, all strains of isolated bacteria have the ability to cause effective biodegradation of MW-HBPU, but the greatest effect was observed in the case of *Achromobacter* sp. NRC2018 on the basis of esterase activity, weight loss results and ATR-FTIR spectra. Therefore, it is possible to conclude that, the three isolated bacterial strains can be used to induce effective MW-HBPU Biodegradation so as to avoid any problems that may be caused in the environment.

Conflict of interest

"There are no conflicts to declare".

Acknowledgments

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References

[1] Mahajan, N., and Gupta, P., New insights into the microbial degradation of polyurethanes. RSC Advances, 5(52), 41839-41854(2015).
[2] Christenson, E.M., Anderson, J.M., and Hiltner, A., Biodegradation mechanisms of polyurethane elastomers. Corrosion Engineering, Science and Technology, 42(4), 312-323(2007).
[3] Gaytán, I., Sánchez-Reyes, A., Burelo, M., Vargas-Suárez, M., Liachko, I., Press, M, and Loza-Tavera, H., Degradation of recalcitrant polyurethane and xenobiotic additives by a selected landfill microbial community and its biodegradative potential revealed by proximity ligation-based metagenomic analysis. Frontiers in microbiology, 10, 2986(2020).
[4] Vanjinathan, M., Shanavas, A., Raghavan, A., and Nasar, A.S., Synthesis and properties of hyperbranched polyurethanes, hyperbranched polyurethane copolymers with and without ether and ester groups using blocked isocyanate monomers. Journal of Polymer Science Part A: Polymer Chemistry, 45(17), 3877-3893(2007).
[5] Liu, J., Lin, L., Jia, X., Liu, R., Zhang, S., and Liu, X., Synthesis and properties of UV-curable hyperbranched polyurethane and its application in the negative-type photoresist. Journal of Wuhan University of Technology-Mater. Sci Ed., 29(1), 208-212(2014).
[6] Naik, R.B., Malvankar, N.G., Mahato, T.K., Ratna, D., and Hastak, R.S., Novel moisture-cured hyperbranched urethane alkyd resin for coating application. Journal of Coatings Technology and Research, 11(4), 575-586(2014).
[7] Hoogenboom, R., and Schubert, U.S., Microwave assisted polymer synthesis: Recent developments in a rapidly expanding field of research. Macromolecular Rapid Communicat-ions, 28(4), 368-386(2007).
[8] Hoogenboom, R., Schubert, U.S., and Wiesbrock, F., Microwave-assisted polymer synthesis (Eds.), Springer.Springer International Publishing AG Switzerland, p.1-9 (2016).
[9] Bogdal, D., Penczek, P., Pielichowski, J., and Prociak, A., Microwave assisted synthesis, cross-linking, and processing of polymeric materials. In Liquid chromatography/FTIR microspectroscopy/ microwave assisted synthesis, Springer, Berlin, Heidelberg, p.194-263(2003).
[10] Howard, G.T., Polyurethane biodegradation. In Microbial degradation of xenobiotics, Springer, Berlin, Heidelberg, p.371-394(2012).
[11] El-Molla, M. M., Haggag, K., and El-Shall, F. N., Shaker, N. O. and Alian, N. A., Use of novel synthesized aqueous binders for pigment printing of polyester fabrics. Indian Journal of Fibre & Textile Research, 38, 57-65(2013).
[12] Haggag, K.M., Hashem, A.I., and El Shall, F.N., Thermal Stability Enhancement of Cotton and Cotton Polyester Blend Fabrics by Hyper-branched Poly Urethane-Urea Treatment. Egyptian journal of chemistry, 60(5), 857-867(2017).
[13] Ishigaki, T., Sugano, W., Nakanishi, A., Tateda, M., Ike, M., and Fujita, M., The degradability of biodegradable plastics in aerobic and anaerobic waste landfill model reactors. Chemosphere, 54(3), 225-233(2004).
[14] Urgun-Demirtas, M., Singh, D., and Pagilla, K., Laboratory investigation of biodegradability of a polyurethane foam under anaerobic conditions. Polymer Degradation and Stability, 92(8), 1599-1610(2007).
[15] Cangemi, J.M., Santos, A.M.D., Neto, S., and Chierice, G.O., Biodegradation of polyurethane derived from castor oil. Polímeros, 18(3), 201-206(2008).
[16] Loredo-Treviño, A., García, G., Velasco-Téllez, A., Rodríguez-Herrera, R., and Aguilar, C.N., Polyurethane foam as substrate for fungal strains. Advances in Bioscience and Biotechnology, 2(2), 52(2011).
[17] Levisson, M., van der Oost, J., and Kengen, SW. Carboxylic ester hydrolases from hyperthermophiles. Extremophiles, 13, 567–581(2009).
[18] Du, L., Huo, Y., Ge, F., Yu, J., Li, W., Cheng, G., Yong, B., Zeng, L., and Huang, M. Purification and characterization of a novel extracellular cholesterol esterase from *Acineto-bacter* sp. J Basic Microb., 50, S30–S36(2010).
[19] Maeda, A., Mizuno, T., Bunya, M., Sugihara, S., Nakayama, D., Tsunasawa, S., Hirota, Y., and Sugihara, A. Characterization of novel cholesterol esterase from Trichoderma sp. AS59 with high ability to synthesize steryl esters. J Biosci Bioeng., 105, 341–349(2008).
[20] Barba Cedillo, V., Prieto, A., and Martnez, MJ., Potential of Ophiostoma piceae sterol esterase for biotechnologically relevant hydrolysis reactions. Bioengineered, 4, 249–253(2013).

[21] Stachelek, S.J., Alferiev, I., Choi, H., Chan, C.W., Zubiate, B., Sacks, M., and Levy, R.J., Prevention of oxidative degradation of polyurethane by covalent attachment of di-tert-butylphenol residues. Journal of Biomedical Materials Research Part A: An Official Journal of the Society for Biomaterials, the Japanese Society for Biomaterials, and the Australian Society for Biomaterials and the Korean Society for Biomaterials, 78(4), 653-661(2006).
[22] Oprea, S., and Doroftei, F., Biodegradation of polyurethane acrylate with acrylated epoxidized soybean oil blend elastomers by Chaetomium globosum. International Biodeterioration & Biodegradation, 65 (3), 533-538(2011).
[23] Shah, Z., Hasan, F., Krumholz, L., Akta, D.F., and Shah, A.A., Degradation of polyester polyurethane by newly isolated *Pseudomonas aeruginosa* strain MZA-85 and analysis of degradation products by GC–MS. International Biodeterioration & Biodegradation, 77 (3), 114-122(2013).
[24] Holt, J.G., Bergey's Manual of Systematic Bacteriology. Williams & Wilkins, Baltimore, 1130-1135(1986).
[25] Gardes, M., and Bruns, T.D., ITS primers with enhanced specificity for basidiomycete's application to the identification of mycorrhizae and rusts. Molecular ecology, 2(2), 113-118(1993).
[26] Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S., MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Molecular. Biol. Evolut., 28, 2731-2739(2011).
[27] Galliard, T. and Dennis, S., Esterase isozymes from Solanum tuber. Phytochemistry, 5, 727-733(1974).
[28] Ellman G. L., Courtney K. D., Andres V. Jr., and Featherstone R. M., A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7, 88 -95(1961).
[29] Sowmya, H.V., Ramalingappa, Krishnappa, M., Thippeswamy, B., Biodegradation of Polyethylene by *Bacillus cereus.* Advances in Polymer Science and Technology: An International Journal, 4(2), 28-32(2014).
[30] Huddleston, A.S., Cresswell, N., Neves, M.C., Beringer, J.E., Baumberg, S., Thomas, D.I., and Wellington, E.M., Molecular detection of streptomycin-producing streptomycetes in Brazilian soils. Applied and Environmental Microbiology, 63(4), 1288-1297(1997).
[31] Ozsagiroglu, E., Iyisan, B., and Guvenilir, Y.A., Biodegradation and characterization studies of different kinds of polyurethanes with several enzyme solutions, Polish Journal of Environmental Studies, 21, 1777-1782(2012).
[32] Das, S., Pandey, P., Mohanty, S., and Nayak, S.K. Evaluation of biodegradability of green polyurethane/silica composite synthesized from transesterified castor oil and palm oil-based isocyanate. International Biodeterioration & Biodegradation, 117, 278-288(2017).
[33] Russell, J.R., Huang, J., Anand, P., Kucera, K., Sandoval, A.G., Dantzler, K.W., and Marks, D.H., Biodegradation of polyester polyurethane by endophytic fungi. Appl. Environ. Microbiol., 77(17), 6076-6084(2011).
[34] Álvarez-Barragán, J., Domínguez-Malfavón, L., Vargas-Suárez, M., González-Hernández, R., Aguilar-Osorio, G., and Loza-Tavera, H., Biodegradative activities of selected environ-mental fungi on a polyester polyurethane varnish and polyether polyurethane foams. Appl. Environ. Microbiol., 82(17), 5225-5235(2016).
[35] Shah, Z., Gulzar, M., Hasan, F., Shah, A.A., Degradation of polyester polyurethane by an indigenously developed consortium of *Pseudomonas* and *Bacillus* species isolated from soil. Polymer Degradation and Stability, 134, 349-356(2016).
[36] Ballini, R., Eco-friendly synthesis of fine chemicals, RSC, 3, 280–293(2009).
[37] Veerapandian, S., Amudha, S., Suthanthiraraj, S.A., Rahman, M.A., and Nasar, A.S., Enhanced performance of a crystalline dye-sensitized solar cell based on polyurethane dendrimers, RSC Advances, 5: 31404-31409(2015).