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Promising biodegradable composite derived from corn straw fiber and waste Polyethylene

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Abstract

Many efforts have been done to overcome of the environmental pollution problems especially waste plastic materials. So, we try to go through this point of view via studying the availability of using waste agriculture material, represented as corn straw (MS), with waste polyethylene (W-PE) as main components to represent a biodegradable compound for agriculture uses. In this study incorporation of different ratios of MS (~100 micron) (10-40%) and W-PE (45-75%) with weight ratios of (5%) for malic anhydride (MA), and each of bentonite (B), diammonium phosphate (DAP), hydrolyzed starch (HSt) and polyvinyl alcohol (PVA) has been constructed. In other incorporations PVA is replaced by chitosan (Ch). Also another trail was done by replacing PVA and HSt with chitosan with weight ratio of (5%). The admixture components were formulated using Parbener mixer at temperature of 150ºC, for 10 minutes, at shear rate of 30 rpm. The obtained compounds were subjected to biodegradability study using cellulase enzyme from fungal local isolate (*Aspergillus oryzae*) as well as commercial cellulase from *Aspergillus niger* (Sigma). Moreover, the compounds before and after biodegradation were characterized using FTIR, and SEM. The biodegradation studies, using cellulases from two different sources, showed that the presence of chitosan (5%) or chitosan (2.5%) with starch (2.5%) speed up the process of biodegradability with achievement of about 95% more other compounds without chitosan. This study verify two goals, firstly pollution prevention for waste plastic materials and secondly, biodegradable composites could be used in different applications.

Keywords: Waste polyethylene, corn straw, biodegradation, chitosan, cellulases, *Aspergillus oryzae*

Introduction

Plastic waste is one of the most important problems that require the effective contribution of industry, government and the public. Therefore, remediation of environmental pollution arises from recycling and reprocessing of plastic waste [1]. Plastic waste generation all over the world is about 57 million tons [2]. Polyethylene is the most abundant commercially generated synthetic polymers. The world produces Low Density Polyethylene (LDPE) estimated by about 60 % of the total plastics production. Plastic bags produced from LDPE are the most commonly found solid waste. LDPE has different properties such as: its excellent chemical resistance, ductility and high toughness, low water vapor permeability, as well as very low water absorption. On the other hand, due to the high hydrophobicity and long carbon chains of polyethylene, it has very resistant to biodegradation [3].

Many researches clarified that the incorporation of polysaccharides biopolymer as starch, chitosan and nanocellulose to polyethylene increase bio-degradability of the obtained composite [4-7]. Composite materials are incorporation of two or more components, one of which is present in the matrix phase, and the another could be in particle or fiber type [8,9]. Straws derived from cellulose, hemicellulose, and lignin are another waste materials which accelerate biodegradation process [10-12]. Another work proved that when the amount of cellulose (10-40wt %) of 10 micron in size was incorporated into polyethylene matrix by using maleic anhydride as coupling agent produce a

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composite has susceptibility for biodegradation [13]. Currently, the most commonly used biopolymer for incorporation into polyethylene is starch. Granular starch mixed with PE together with an unsaturated polymer, a thermal stabilizer, and a transition metal give a material with increased susceptibility to photo-oxidation, thermolysis, and biodegradation [14-17]. Cellulosic straw is also considered as one of the most important component used with PE to give biodegradable polymer composite. The components of cellulosic straw are mainly cellulose with other ingredients such as hemicellulose, and lignin [18-20]. Biodegradation, an eco-friendly technique of degradation, is the process by which organic materials are decomposed or broken down into smaller compounds, including CO_2 and H_2O, by microbial action [21]. Cellulase enzyme, from Aspergillus niger, was used to degrade Polyviynl alcohol (PVA)/ starch/carboxymethyl cellulose (CMC) and study the effect of sodium montmorillonite (MMT-Na) clay content on the rate and extent of enzyme hydrolysis [22]. It has been found that MMT-Na when added to the blend decrease the rate of glucose release (enzyme activity). The changes in the blend due to cellulase degradation were demonstrated by measuring the weight loss, water absorbing capacity, sugar release during biodegradation in addition to UV-spectroscopy. Total sugar released were measured using dinitrosalicylic acid (DNS).

The objective of this study is to investigate the biodegradability of composite derived from LDPE and corn straw fiber in presence of MA as compatibilizing agent and variable additives such as chitosan, hydrolysed starch, diammonium phosphate, PVA as well as bentonite. Characterization for the prepared composites before and after biodegradability was carried out using FTIR and SEM.

Experimental

Materials

Polyethylene waste (PE-W) was obtained from EL- Mokatam waste Collection area. PE-W was obtained as granules. Maleic anhydride and diammonium phosphate supplied by Sigma Aldrich were used. Bentonite was received from Sigma-Aldrich chemical company (USA). Chitosan of molecular weight (~ 480,000Da) and degree of deacetylation of about 79.0%, supplies by Alfa Company. Polyvinyl alcohol (PVA) having molecular weight ~ [115000 Da] and degree of hydrolysis of about 98% Oxford Company was used. Hydrolyzed corn starch (HSt) was prepared using 0.5N HCl at 60°C for one hour. The estimated copper number for prepared HSt was 0.54 **[23]**.

Preparation of corn straw powder

Corn straw waste (CS-W) was collected from corn plantation located at El- Badrashin(Giza Egypt). The collected CS-W was dried at ambient condition, crushed and ground into fine powder using available grinder. The fine CS powder was sieved using 100 μm sieve to obtain in average particle size of upto 100μm.

Composites procedure

The PE-W/CS-W composites in presence of different added ingredients were prepared using in a Brabander plasticorder at rotor speed of 30 rpm. The temperature of mixing was 150°C. The formulation of the composites was listed in Table (1). The mixing process of the composites as following: i) charging the compounding chamber with PE-W for **5** minutes until it completely melted, ii) adding the other composite ingredients (MA, B, DAP, HSt, Ch, and/or PVA) for **2** minutes, iii) inserting of the CS-W powder into melted PE-W containing the specified ingredients and mixed for **3** minutes. The total mixing time involved was **10** minutes.

TABLE 1. Formulation of the different prepared composites

Code	PE-W (%)	CS-W (%)	M.A (%)	B (%)	DAP (%)	HSt. (%)	PVA (%)	Ch (%)
Comp-I	**75**	**10**	**5**	**2.5**	**2.5**	**2.5**	**2.5**	**-**
Comp-II	**65**	**20**	**5**	**2.5**	**2.5**	**2.5**	**2.5**	**-**
Comp-III	**55**	**30**	**5**	**2.5**	**2.5**	**2.5**	**2.5**	**-**
Comp-IV	**45**	**40**	**5**	**2.5**	**2.5**	**2.5**	**2.5**	**-**
Comp-V	**45**	**40**	**5**	**2.5**	**2.5**	-	-	**5**
Comp-VI	**45**	**40**	5	**2.5**	**2.5**	**2.5**	-	**2.5**

Characterization

FTIR

Fourier transform infrared (FTIR) spectroscopy was measured using a Nicolet 380 spectrophotometer (Thermo Scientific).

SEM

Scanning electron microscope (SEM) for the studied samples was taken using (Joel GM4200, Quanta 200, Holland). The sample surfaces were firstly coated with a gold thin layer under vacuum. All samples were studied at an accelerating voltage of 20 kV.

Biodegradation studies

Materials

Cellulase enzyme (from *Aspergillus niger*) was purchased from Sigma, the other chemical used in this part are analytical grade.

Isolation of cellulase producing fungus

Soil sample, obtained from Dekernis city, Mansoura governate in 1st of October 2020, was used for the isolation of cellulase-producing fungus. Samples were taken at 10cm depth from soil surface. Samples were sieved and air dried at room temperature. After drying, samples were kept at 4°C until used. The fungi were isolated from the soil by serial dilution protocol. 100 microliter from each dilution up to dilution 10^{-3} were used to inoculate potato dextrose agar (PDA) medium. The plates were incubated at the proper temperature 28°C ± 2°C until the appearance of growth (2-7days). Plate streak technique was used to purify the appearing fungal colonies. Rose Bengal at a concentration of 30mg/l was used to control the growth of fungi and neomycin at a concentration of 50mg/l was used to inhibit the bacterial growth **[24&25].** The isolated fungi were maintained on PDA slants.

Identification of cellulase producing fungus

The isolated fungus (MHN) was identified by the molecular protocol 18SrRNA The primers ITS2 (GCTGCGTTCTTCATCGATGC) and ITS3 (GCATCGATGAAGAACGCAGC) applied for PCR amplification but ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) were applied for sequencing. The sequence data was matched with the previous work through Blast search.

Cellulase production medium

Cellulase production

The fungus was cultivated on Vogel's medium (g/100ml): $Na_3C_6H_5O_7$, 0.25; KH_2HPO_4, 0.50; ammonium nitrate (0.20), ammonium sulphate (0.40), magnesium sulphate.$7H_2O$ (0.02), peptone (0.10),yeast extract (0.20); and cellulose (1), sodium hydroxide and hydrochloric acid (0.1M) were used to adjust the pH to around 6. Fungal spore suspension was used to inoculate the mentioned medium and the inoculated medium was incubated in rotary shaker (150rpm) at 30ºC **[26]**. Mycelia were removed by centrifugation at 5000rpm (2236g) at 4ºC/. The culture filtrate is considered as the enzyme source **[26]**.

Cellulase assay

Cellulase was assayed as described by **[27]**.50 mg of cellulose was mixed with 1ml of citrate buffer (0.05M, pH 4.8) at 50ºC for 1 hour. Reducing sugars were determined as described by Miller **[28]** as glucose. One cellulase unit of enzyme is defined as the enzyme amount that liberate 1 micromole of reducing sugars, expressed as glucose, per min. under specified condition.

Results and Discussion

Fungal identification

The DNA sequence of MHN isolate was applied to BLAST search. MHN isolate exhibited 100% similarity to Aspergillus oryzae isolate LNBK (accession number: MT071405.1). The phylogenic tree of the fungal isolate was also constructed (Fig. 1). Based on the above identification techniques, our local terrestrial fungal isolate was identified as *Aspergillus oryzae* MHN-EGY). The traditional methods (The morphological and biochemical evaluations) of identifying fungi are known as hard and taking much times methods and required extremely knowledgeable and clever professionals **[29]**. Identification by the molecular technique, by DNA sequence, is an accurate, reproducible and fast means for identification and widely used **[30-32].** Cellulase-producing fungi were considered by using the molecular technique (18SrRNA) as an exact method for identification **[33&34].**

The effect of cellulase from different sources on cellulose blend

The ability of prepared PE-W/CS-W blends to be degraded were assessed by measuring their ability to be dissociated by cellulase enzyme produced from the fungal isolate *Aspergillus oryzae* MNH-EGY as well as the commercial cellulose (Sigma) compared to cellulose as standard substrate. Cellulase enzyme cut β-1,4-glycosydic linkages of cellulose chains to enable further degradation of the polymer. Many factors were found to affect the efficiency of enzymes activity, i.e., temperature, pH, metal ions and the structure of both enzyme and the substrate **[25]**. In Table II samples V and VI exhibited better degradation compared to cellulose standard. Samples I,II, and III had moderate degradation whereas sample IV had lower degradation.

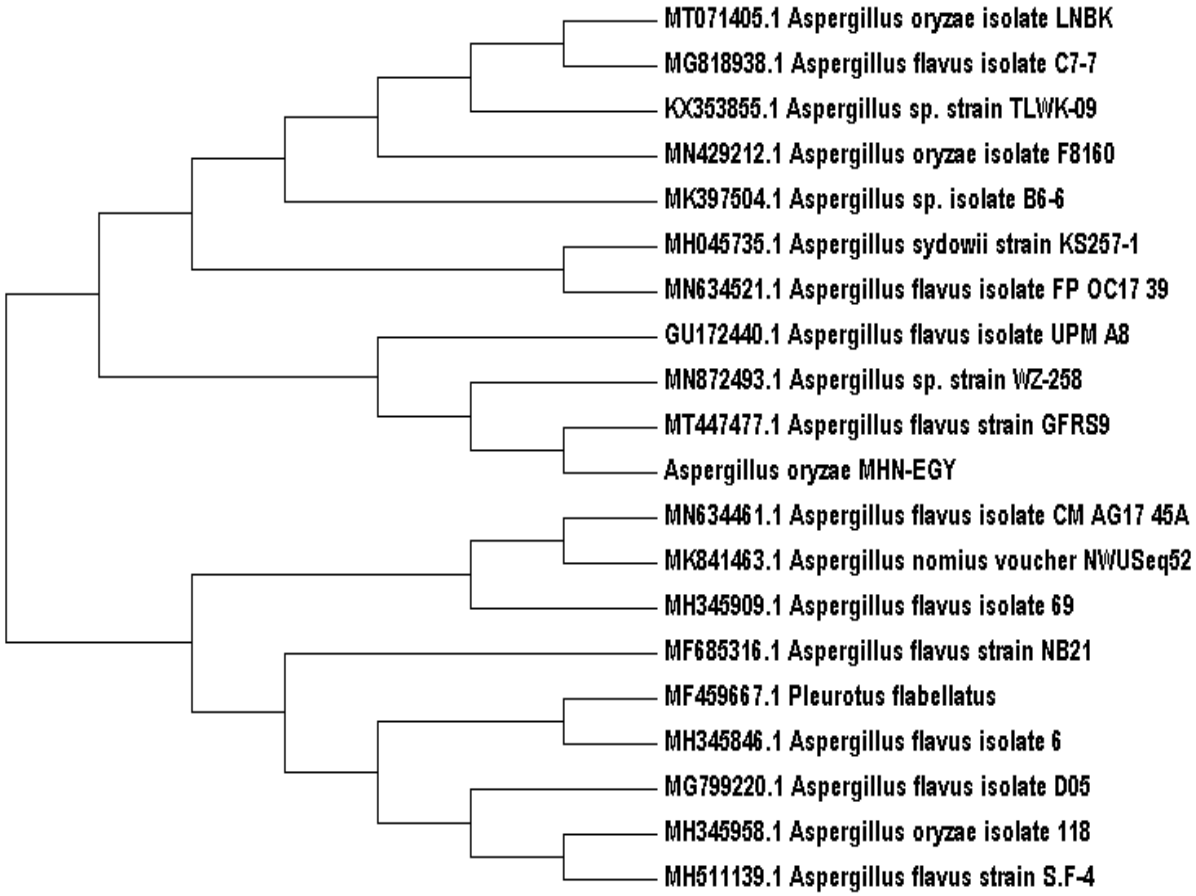


Fig.1. Phylogenetic trees viewing relationship of *Aspergillus oryzae* MNH-EGY) to related fungi recovered from GenBank based on their sequence homologies of 18S rRNA.

TABLE 2. Cellulase activity against different samples and cellulose (standard) using two enzyme sources (fungal and commercial) as indication of samples biodegradability

Sample code	Cellulase (fungal) activity (U/ml)	% to cellulose with fungal cellulase	Cellulase (Commercial) activity (U/ml)	% to cellulose with commercial cellulose
Comp-I	0.43	70.49	0.73	75.26
Comp-II	0.45	73.33	0.67	69.07
Comp-III	0.37	60.66	0.76	78.35
Comp-IV	0.32	52.46	0.40	41.24
Comp-V	0.58	95.08	0.59	60.82
Comp-VI	0.52	85.25	0.76	78.35
Cellulose	0.61	100	0.97	100

FT-IR analysis

FTIR spectra for the studied composite (comp-VI) before and after biological treatment are shown in Figure (2) in the range of 4000–400 cm^{-1}. Firstly, FTIR for the composites before biological treatment show characteristics absorbance bands for PE which are located at: 2914 cm^{-1}, 2847 cm^{-1}, 1470 cm^{-1}& 718 cm^{-1}**.** The peak bands at 1470 cm^{-1}&718 cm^{-1} are used to identify and quantify the presence of PE **[35]**. The presence of characteristic peak bands at the range of 3500–3250 cm^{-1}, 1475–1450 cm^{-1}, 1275–1070 cm^{-1}, and 1200–1000 cm^{-1}, representing

O–H stretching, $–CH_2–$ deformation, C–O–C stretching as well as C–O–H stretching, respectively, demonstrates the existing of D-glucose units for each of cellulose fiber and hydrolyzed starch **[36]**. The peak position of 1720-1750 cm^{-1}, assigned for C= O stretching and appeared from ester group formation between the Cellulosic material and MAH **[37].** Also, the presences of characteristic peak bands at 1150 cm^{-1}, 1080cm-1, are characteristic N-H and $NH_2$for chitosan **[38]**. Meanwhile, the peak position at 1021 - 1050 cm^{-1}, indicating of Si–O–C stretching was found, 2150-2360 cm^{-1} represented Si-H, which verify the presence of bentonite clay **[39]. A**nother peak at 1200-1380 cm^{-1} represented to the phosphate P=O function group **[36]**, demonstrating of new chemical bond formed by the addition of additive sand compatibilizer.

Secondly, FTIR analysis clarified that the bacterium-treated PE (composite VI*) formed higher intensity absorbance bands for certain groups such as hydroxyl groups (3100-3620 cm^{-1}) and carbonyl groups (1650 cm^{-1}), indicating the oxidation on the surface to create either hydroxyl or carbonyl groups. This is in accordance with previous studies **[40&41]** which mentioned that the appearance of hydroxyl and carbonyl groups gave the evidence of bio-oxidation of PE and basically promoted the degradation of PE. In addition, there is a decrease in the intensity of the characteristic peaks for PE located at: 2914 cm^{-1} and 2847 cm^{-1}. This finding may indicate that in our study, the degradation of PE was carried out along the PE surface.

Morphological Properties:

Morphological properties of composite polyethylene and cellulose explain the unfortunate phase distribution between cellulose and polyethylene due to the difference in polarity as shown in Figure (3 (a-f)). For the compatibility of cellulose with LDPE and to assure homogeneity in the blend at micro-scale level, it is needful to minimize the interfacial tension between the two components. This can be done via chemical modification of LDPE and/ also via utilization of the compatibilizer such as maleic anhydride containing groups able to make hydrogen bond with cellulose hydroxyls.

Figure (3) clarified that increasing cellulose content from 10% up to 40% in the composite composition was accompanied by increasing amorphous region which was attacked first by enzyme and this subsequently followed by increasing Microbial content. Copm.I having low cellulose content (10%), causing a high compacted of component, less wetted so the grown microorganisms would'nt ability to grow, so degradation take more time. Comp.II having cellulose content 20% show slightly dispersion of cellulose with PE, which give availability of microorganisms to spread all over composite and attack pores as slightly wetted so degradation will be accelerated. Comp.III with cellulose content (30%), show more dispersion of cellulose with PE. Comp.IV, V &VI having cellulose content (40%) show highly Fabricated, dispersion, randomly rearmament and highly wetted so degradation will be take less time.

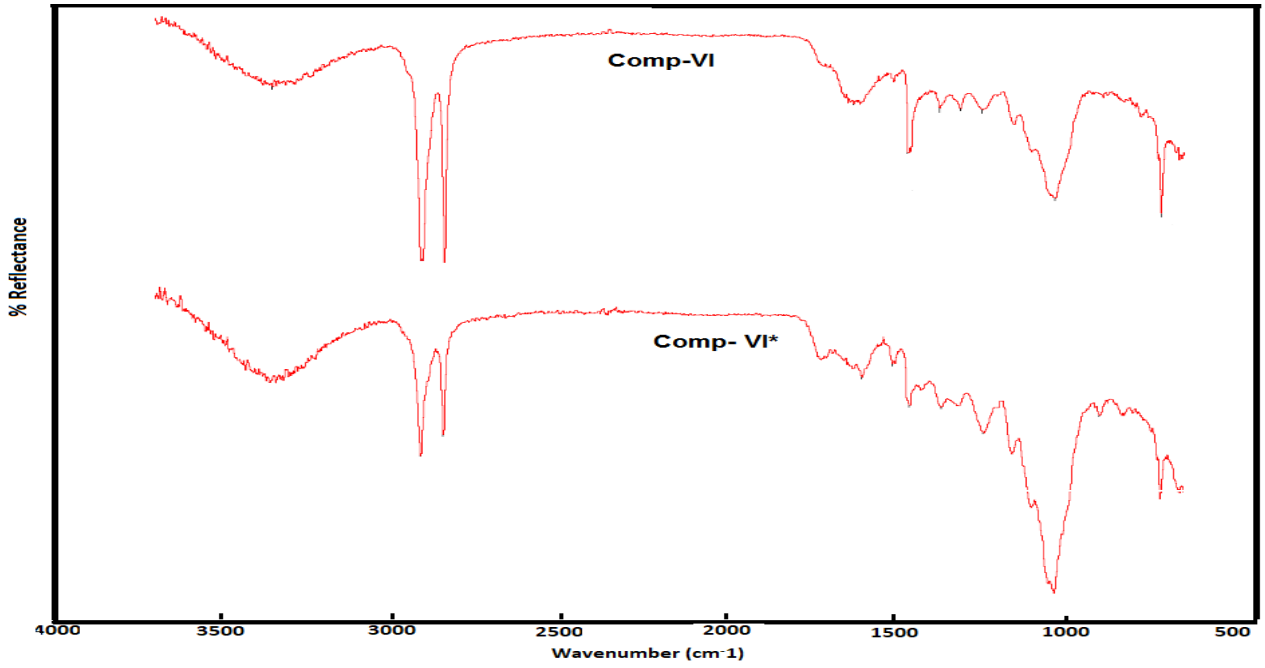
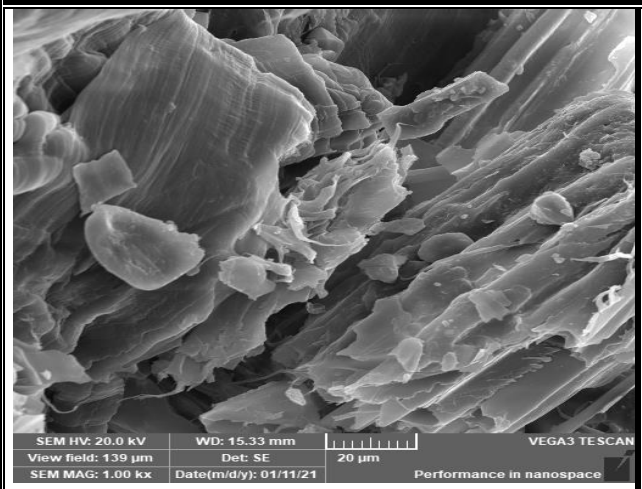


Fig.2. FTIR for the composites before biodegradation (VI) and after biodegradation (VI)

Before culture of microorganisms	After culture of microorganisms



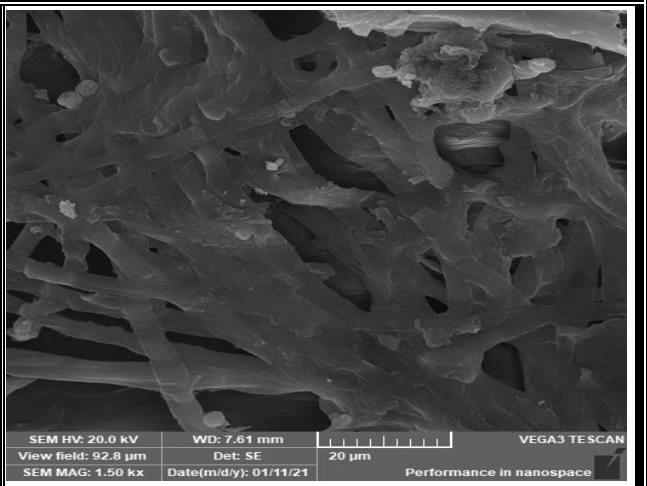
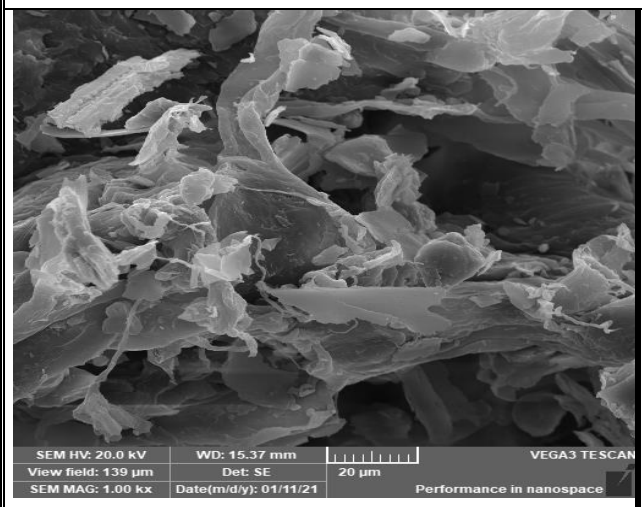


Fig.3a. Morphological properties of Comp-I before and after culture of microorganisms



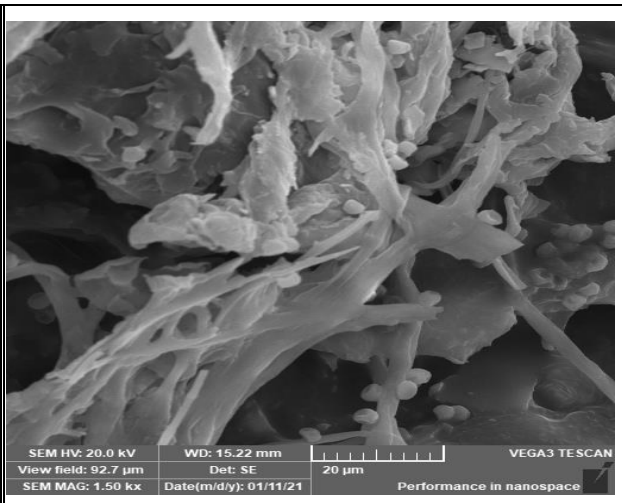


Fig.3b. Morphological properties of Comp-II before and after culture of microorganisms

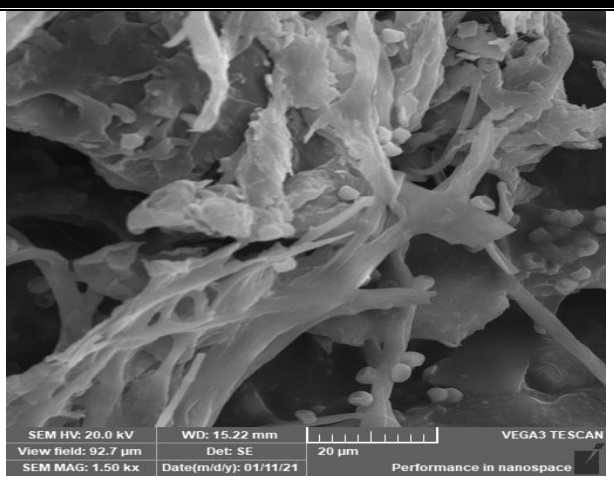


Fig.3c. Morphological properties of Comp-III before and after culture of microorganisms

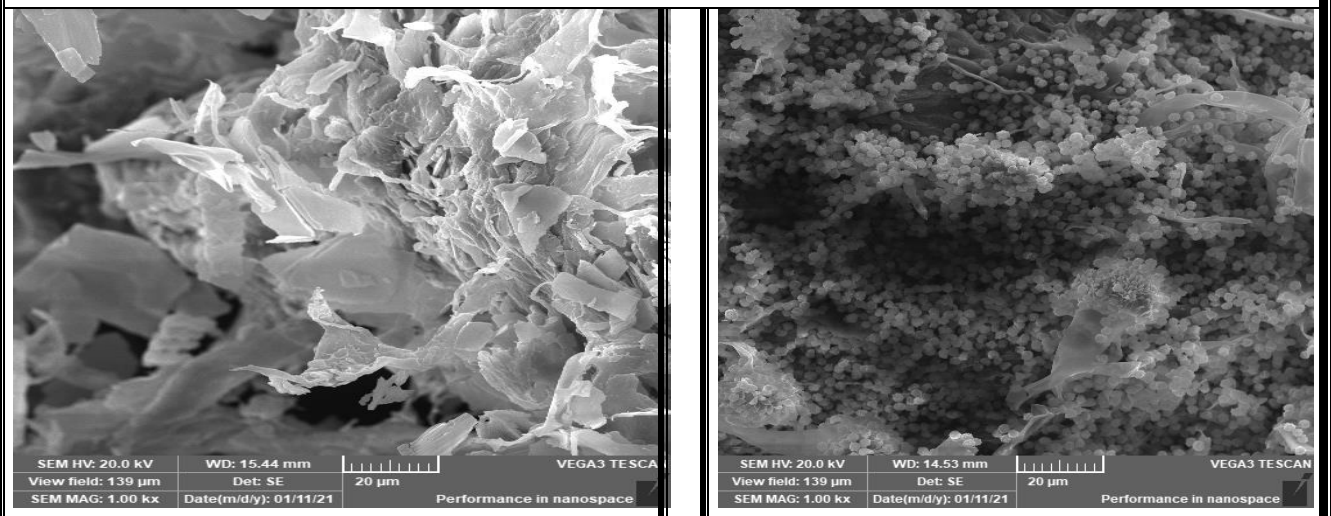


Fig.3.d. Morphological properties of Comp-IV before and after culture of microorganisms

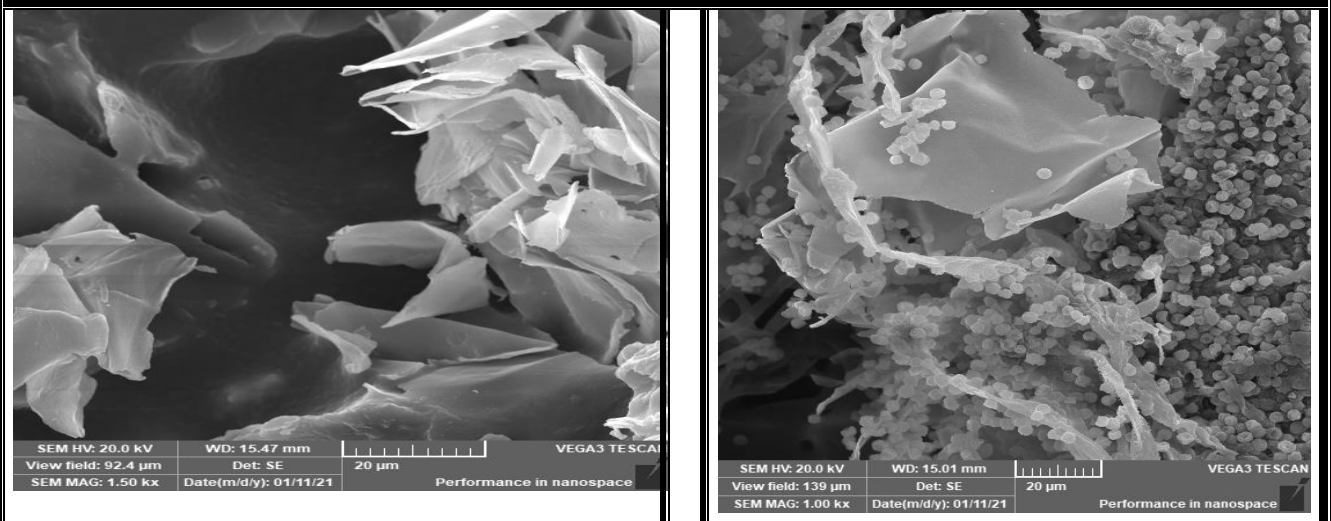


Fig.3e. Morphological properties of Comp-V before and after culture of microorganisms

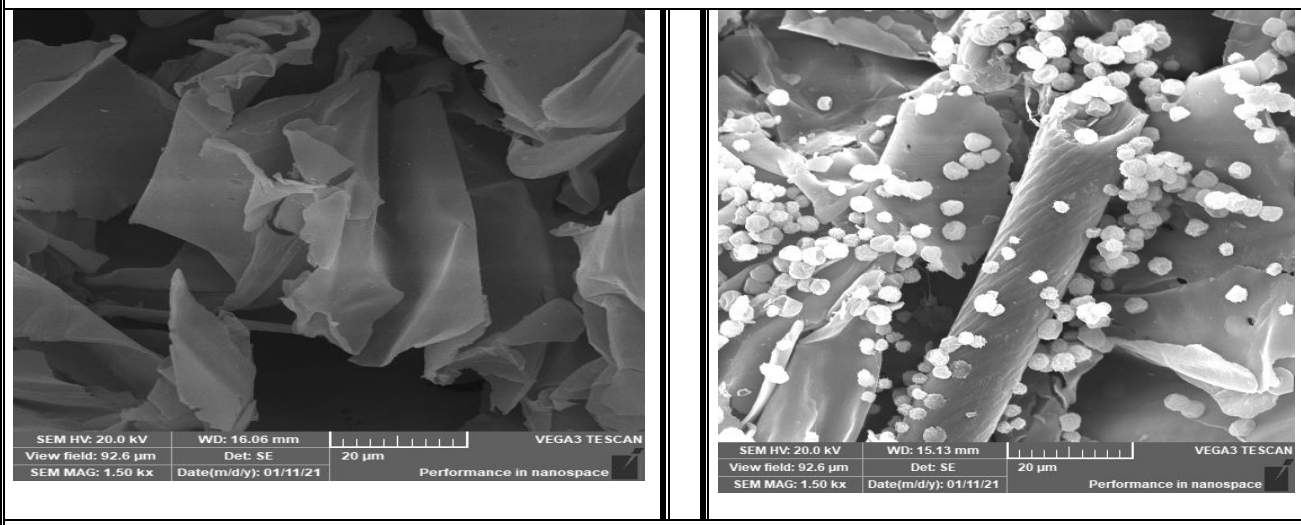


Fig.3f. Morphological properties of Comp-VI before and after culture of microorganisms

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مركب مبشر قابل للتحلل الحيوي مشتق من ألياف قش الذرة ونفايات البولي إيثيلين

تم بذل العديد من الجهود للتغلب على مشاكل التلوث البيئي وخاصة النفايات البلاستيكية. لذلك ، نحاول أن نتطرق إلى وجهة النظر هذه من خلال دراسة مدى توفر استخدام مواد زراعة النفايات، ممثلة بقش الذرة، مع نفايات البولي إيثيلين كمكونات رئيسية لتمثيل مركب قابل للتحلل للاستخدامات الزراعية. تم في هذه الدراسة تضمين نسب مختلفة قش الارز (10-40%) بحجم جزيئات حوالي 100 ميكرون و نفايات البولي إيثيلين بنسب تراوحت بين 45-75% بالاضافة الي نسبة وزن 5٪ لانهيدريد الماليك وايضا تم اضافة نسبه 2.5% لكل من البنتونايت ، فوسفات الأمونيوم، النشا المتحلل، كحول البولي فينيل. كما في عمليات دمج أخرى ، يتم استبدال وكحول البولي فينيل بالكيتوزان. وايضا تم عمل مسار آخر باستبدال المتحلل كحول البولي فينيل و النشا المتحلل بالكيتوزان بنسبة وزن (5٪). تمت صياغة مكونات المزيج باستخدام خلاط Parbender عند درجة حرارة 150 درجة مئوية ، لمدة 10 دقائق ، بمعدل قص 30 دورة في الدقيقة. تم عمل دراسة التحلل الحيوي باستخدام انزيم السليولاز من العزلة الفطرية الموضعية Aspergillus oryzae وكذلك السليولاز التجاري من *Aspergillus niger*. علاوة على ذلك، تم تمييز المركبات قبل وبعد التحلل البيولوجي باستخدام FTIR و SEM. أظهرت دراسات التحلل البيولوجي ، باستخدام سليلاز من مصدرين مختلفين ، أن وجود الكيتوزان (5٪) أو الكيتوزان (2.5٪) مع النشا (2.5٪) يسرع من عملية التحلل البيولوجي مع تحقيق 95٪ أكثر من المركبات الأخرى بدون الكيتوزان. . تتحقق هذه الدراسة من هدفين ، أولاً ، منع التلوث لمخلفات المواد البلاستيكية ، وثانيًا ، يمكن استخدام المركبات القابلة للتحلل في تطبيقات مختلفة