J. Agric.Chem.and Biotechn., Mansoura Univ.Vol. 6 (11) : 461 - 472 , 2015

EVALUATION OF BIODEGRADABILITY OF POLYLACTIC ACID FILMS IN THE SOIL

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ABSTRACT

A pot experiment was carried out at Agriculture Faculty, Benha University, Qalubia Governorate, Egypt, during April to August in 2014 to evaluate biodegradability of two types of biodegradable plastic films produced from polylactic acid (Bi-OPL® and Ecovio®) compared with nondegradable one produced from polyethylene that incubated in the soil for five months. As well as, the effects of degradation compounds on microbial activity were debated. The obtained data revealed increasing amount of evolved carbon dioxide, microbial biomass and dehydrogenase activity from the soil amended with degradable plastic by increasing incubation period. There are significantly increase in the previous parameters in the soil amended with degradable plastic compared with nondegradable one. Bi-OPL® films that is produced from pure polylactic acid has higher weight loss and lower tensile strength compared with Ecovio® which consists of 45% polylactic acid (PLA) and 55% Ecoflex. While, polyethylene films have slight decrease in weight during incubation periods. It worthy to reported that increasing weight loss values and decreasing tensile strength values of degradable plastic films were related to the higher values of CO_2 evolution, microbial biomass and dehydrogenase activity. Accordingly, it may be depended on the previous parameters to evaluate biodegradability of PLA.

Keywords: polylactic acid polymer, biodegradation, microbial biomass, dehydrogenase, CO_2 evolution, weight loss and tensile strength.

INTRODUCTION

An increased environmental damage was noticed recently due to the amount of plastic wastes persisting many years after disposal. A partial solution is to replace conventional, petrochemical commodity plastics with biodegradable substitutes (Iovino *et al.*, 2008)

Biodegradable plastics have been developed rapidly owing to rising petroleum prices and many environmental concerns related to plastic pollution. Increasingly, reduction of carbon dioxide emissions has become another reason for promoting bio-based plastics (Gross and Kalra, 2002). Such a polymer is polylactic acid (PLA), a biodegradable and bioabsorbable, renewably derived polyester that has attracted attention in response to increasing concerns about the environmental effects of disposal of non-biodegradable plastics(Auras *et al.,* 2010 and Lim *et al.,* 2008). Its low toxicity, along with its environmentally benign characteristics, has made PLA an ideal material for food packaging and for other consumer products (Ren, 2010). Recently, new techniques which allow economical production of high molecular weight PLA polymer have broadened its uses.

Among all degradable polymers, PLA plays a key-role because of its highly hydrolysable ester bonds, making this polymer highly subject to degradation in a humid environment. Degradation of these polyesters in the soil burial test depending on the distribution of polyester-degrading microorganisms. PLA-degrading microorganisms are not widely distributed in the natural environment and thus, PLA is less susceptible to microbial attack in the natural environment than other microbial and synthetic aliphatic polyesters. For these reasons, it is very important to study the biodegradation of PLA and to develop treatment processes for plastic waste containing PLA. (Ren, 2010)

In general, there are two types of degradation of plastic materials, surface degradation (erosion) and bulk degradation, depending on the main degradation site. Surface degradation may occur when catalytic molecules such as an alkaline catalyst or enzyme, exclusively act on the surface of the plastics or water molecules cannot diffuse into the bulk layer. During surface degradation, the sample weight may decrease soon after the beginning of degradation. Changes in the mechanical properties of the sample are dependent on the shape of the sample, thickness and crystallinity (Smith, 2005)

PLA polymer film was degraded in abiotic and biotic environments to understand the role of microbes in the degradation process of lactic acid based polymers. CO_2 evolved, weight loss, and molecular weights were measured to evaluate the extent of degradation. (Agarwal *et al.,* 1998 and Rudeekit *et al.,* 2008). In most applications that use films or fibers in contact with the soil, loss in tensile properties is the most relevant practical criterion to characterize its degradation (Mostafa *et al.,* 2010 and Orhan *et al.,* 2004). The polymer carbon is converted by microorganisms into biomass carbon, carbon dioxide, water soluble by-products and polymer carbon which is not yet degraded (Copinet *et al.*, 2011).

The aim of this study is to evaluate biodegradability of two types of polylactic acid (biodegradable plastic films) that are used in agricultural mulch and compost bags compared to nondegradable one in soil under incubation periods. Moreover, it contributes to estimate the material stability, life expectancy and effects of degradation compounds on microbial activity.

MATERIALS AND METHODS

The biodegradability of three plastic types were studied in pot experiment in greenhouse at Agriculture Faculty, Benha University, Qalubia Governorate, Egypt, during April to August 2014. Two types of the plastic film are biodegradable (produced from commercial bioplastics available on the markets (Ecovio and Bi-OPL) and the third type is non-biodegradable one (produced from polypropylene plastics available on the local market). Three types of the tested plastic films with thickness 0.036 mm were cut into slides with dimension 60 mm× 22 mm.

The tested polymers

- Ecovio®, a biodegradable polymer consists of 45% polylactic acid and

55% Ecoflex (aliphatic-aromatic copolyester based on the monomers 1, 4-butanediol, adipic acid and terephthalic acid) for film extrusion (BASF, 2010).
- Bi-OPL® is produced from polylactic acid only (PLA is made of degradable materials and compostable in accordance with DIN EN 13432 (Oerlemansplastics, 2008).
- Non-degradable plastic is produced from low density polyethylene (LDPE).

These materials have been developed for conversion to flexible films using a blown film or cast film process. Typical applications are agricultural films for mulching and compost bags.

Experimental soil

An agricultural soil was collected in the area of Moshtohor, Tukh, Qalubia, Egypt. Its moisture was corrected to 60% of the maximum water retention capacity, keeping its original characteristics and submitting all samples to the same moisture level. Physical and chemical analyses of the soil (Table 1) were carried out according to Page *et al*., (1982).

Table 1. Physical and chemical analyses of the experimental soil.

Particle size distribution				Textural class	pH	E.C. (ds/m)	Organic matter %	Soluble cations meq / l				Soluble anions meq / l				Total and available macronutrients (%)					
																N		P		K	
Coarse sand %	Fine sand %	Silt %	Clay %					Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	$CO_3^{=}$	Cl^{-}	$SO_4^{=}$	total	available	total	available	total	available
6.59	27.64	12.60	53.17	Clay	8.03	2.02	2.12	12.1	5.8	0.44	1.86	4.40	0	11.2	4.6	0.173	0.0053	0.0561	0.0130	0.35	0.161

Experimental design

Plastic film pieces (60 mm × 22 mm × 0.036 mm) of both biodegradable and nondegradable materials were prepared and weighed. Sixty pot with 20 cm diameter were filled with soil. Compost is primarily used as a soil conditioner with rate of 20 m^3/fed for all treatments The physical and chemical properties of the used cattle manure and herbal plants residues (50: 50) are: pH 7.6, Electrical conductivity (EC) 3.1 dS m^{-1}, total organic matter values 32.7 ،% bulk density 0.625 g cm-3. The moisture content 23.50 %, water holding capacity value 3.7 g water/g dry and the porosity 62.67% (Khater, 2012). The pots divide into four groups. The first group were left without treatment (control). While the 2nd, 3rd and 4th groups amended with nondegradable, Ecovio® and Bi-OPL® plastic film pieces, respectively with rate of 1% (w/w). All of the pots were kept under greenhouse condition and each of them was irrigated every week to adjust the humidity to 60% of soil holding capacity.

Analytical procedures

The pieces of plastic films were tested at the beginning of April and were retrieved at the 30th day of May, June, July and August of incubation, and were gently rinsed with water to remove the soil particles and were air-dried for 24 h, photographed and weighed. Weight losses percentage and tensile strength (TS) were measured. TS was measured with a tensile testing machine (Daiei Kagaku – ArimotoKigyo Co., Ltd. Japan). Weight losses for the materials were measured according to Mostafa & Abdel-rahman, (2014) by the following equation:

$$\text{Weight losses } (\%) = \frac{(W_1 - W_2)}{W_1} \times 100$$

Where: W_1 and W_2 are the films weight before and after treatment respectively.

Microbial activities

Dehydrogenase (DH) activity, soil respiration (as mg $CO_2.g^{-1}dm.24h^{-1}$) and biomass-C· (mg. 100 g^{-1} dm) were measured using the methods described by Schinner *et al.,* (1996) in soil surrounded to plastic pieces for each treatment.

Statistical analysis

The data were statistically analyzed according to the procedures outlined by Gomez and Gomez (1984). For comparison between means, Duncan's multiple range test was used (Duncan , 1955). Means followed by the same alphabetical letters were not significantly different at 5% level of significance.

RESULTS AND DISCUSSION

Soil respiration

Soil respiration results from the degradation of organic matter. This soil biological activity consists of numerous individual activities; the formation of CO_2 is the last step of carbon mineralization. Addition of organic matter observe a change in the soil respiration due to more rapid growth and a higher mineralization of the microorganisms. CO_2 evolution from a soil is thus a measure of the total soil biological activity.

Data in Table 2 showed the amount of carbon dioxide with mg $CO_2.g^{-1}$ $dm.24h^{-1}$ that evolved from soil due to microbial activity. In the first month of incubation, there are significant increase in CO_2 evolution from soil that amended with biodegradable plastics compared with that amended with nondegradable one. While there are not any significant differences between soil amended with nondegradable plastic compared with control. In case of soil amended with biodegradable plastic, the amount of carbon dioxide that evolution from soil increased by increasing incubation period. The amount of evolved CO_2 from soil that amended with degradable plastic (Bi-OPL®) was higher than that amended with degradable plastic (Ecovio®). Generally, the highest records of evolved CO_2 were recorded with soil amended with degradable plastic (Bi-OPL®).

Table 2 Soil respiration (mg CO2. g^{-1} dm.$24h^{-1}$) in soil amended with different types of plastic during incubation periods.

Treatments	mg CO_2.g^{-1} dm.$24h^{-1}$			
	1	2	4	5 months
Control	0.145^{g}	0.061^{j}	0.070^{i}	0.075^{h}
Soil amended with non-degradable plastic	0.146^{g}	0.055^{k}	0.069^{i}	0.077^{h}
Soil amended with degradable plastic (Ecovio®)	0.155^{f}	0.159^{e}	0.178^{c}	0.180^{bc}
Soil amended with degradable plastic (Bi-OPL®)	0.158^{ef}	0.170^{d}	0.182^{ab}	0.185^{a}

The degree of biodegradable plastic decomposition after a certain time was first determined as the difference between the quantity of CO_2 that had evolved in the presence of the biodegradable plastic and the quantity that evolved in the absence of the biodegradable plastic (Ohtaki and Nakasaki, 2000). Carbon dioxide evolution was found to be a useful screening tool for plastic film biodegradation (Yabannavar and Bartha, 1994)

From data in Table 2 it can noticed that the decomposition of degradable plastic does not started in earliest stage and evolved CO_2 after the first month may be due to decomposition of organic matter that exist in soil. It is well known that when a microorganism is incubated in the presence of two or more substrates، the substrates will be degraded in the order of their ease of degradation (e.g., catabolic repression).We call this period from the start of composting to the start of plastic degradation the lag time (Ohtaki and Nakasaki, 2000). Increasing amount of evolved carbon dioxide from soil amended with degradable plastic by increasing incubation period may be attributed to increase decomposition rate of degradable plastic. The PLA undergoes a hydrolytic degradation process, causing a decrease of the polymer molecular weight. The portion of the polymer chains was broken down into small fraction (or oligomers) with low molecular weight in a biodegradation phase (14 to 96 days). The low molecular weight oligomers are consumed by microorganisms to evolve carbon dioxide. (Rudeekit *et al*., 2008)

Dehydrogenase activity

Table 3 show dehydrogenase activity in soil amended with different types of plastic films. Data showed significantly increase in dehydrogenase activity in the soil that amended with degradable plastic than that amended with nondegradable one during all incubation periods. The highest values of dehydrogenase activity were recorded with soil amended with degradable plastic (Bi-OPL®). It could be also noticed decreasing dehydrogenase activity values during the 1^{st} and 2^{nd} months of incubation than control, while they increase again in 4^{th} and 5^{th} months.

The dehydrogenase activity of a soil result from the activity of different dehydrogenases, which are an important component of the enzyme system of all microorganisms (enzymes of respiratory metabolism, citrate cycle, and nitrogen metabolism). Dehydrogenase activity is thus an indicator of biological redox-systems, and can be taken as a measure for the intensity of microbial metabolism in soil (Tabatabai, 1982).

The role of dehydrogenases may be achieved after polymer degradation by hydrolysis or enzymatic process. Where PLA is more easily degraded by hydrolysis rather than enzymatic degradation, and its degradation products can be totally digested by microorganisms such as fungi or bacteria (Corrêa *et al.,* 2008 and Nampoothiri *et al.,* 2010)

Biodegradation is a process whereby bacteria, fungi, yeasts and their enzymes consume the polymer leading to significant changes in the material's chemical structure. In essence, biodegradable plastics should break down cleanly, in a defined time period, to simple molecules found in the environment such as carbon dioxide and water. Biodegradation for limited periods is a reasonable target for the complete assimilation and disappearance of an article leaving no toxic or environmentally harmful residue (Chandra and Rustgi, 1998)

Table 3 Dehydrogenase activity in soil amended with different types of plastic during incubation periods.

Treatments	Dehydrogenase activity (µg TPF.g^{-1}dm.24h^{-1})			
	1	2	4	5 months
Control	27.16^{bcd}	16.93^{e}	14.98^{f}	14.98^{f}
Soil amended with non-degradable plastic	26.30^{d}	16.02^{ef}	16.17^{ef}	17.72^{e}
Soil amended with degradable plastic (Ecovio®)	26.89^{cd}	27.06^{cd}	27.67^{abcd}	27.87^{abcd}
Soil amended with degradable plastic (Bi-OPL®)	27.89^{abcd}	28.55^{abc}	28.98^{ab}	29.40^{a}

Microbial biomass-c

Microbial biomass-c in soil amended with different types of plastic was tabulated in Table 4. There are significantly increase in microbial biomass-c of soil amended with degradable plastic compared with that amended with nondegradable one. Soil amended with degradable plastic (Bi-OPL®) have higher values of microbial biomass-c than that amended with degradable plastic (Ecovio®). These results confirmed that degradable plastic (Bi-OPL®) was able to degrade by soil microorganisms more than Ecovio® during the incubation periods. Abovementioned results interpreted increasing of the dehydrogenase activity in Table 3 in the soil that amended with degradable plastic than that amended with nondegradable one. On another side data confirmed importance of microbial biomass-c as indicator to evaluate biodegradability of polymers.

The proportion of carbon biomass in the soil has been found to be 1-3 % of the organic carbon (Sparling,1985). Investigations on cultivated soils showed that the proportion of the metabolically active microbial biomass is 1-5 and 2-8% of the organic matter in arable and grassland soils, respectively (Beck *et al.,* 1992).

Dominant groups of microorganisms and the degradative pathways associated with PLA degradation are often determined by the environmental conditions. When O_2 is available, aerobic microorganisms are mostly

responsible for destruction of complex materials, with microbial biomass, CO_2, and H_2O as the final products. In contrast, under anoxic conditions, anaerobic consortia of microorganisms are responsible for polymer deterioration. The primary products will be microbial biomass, CO_2, CH_4 and H_2O under methanogenic (anaerobic) conditions (Barlaz *et al.,* 1989). It is important to note that the degradation of polymer can rarely be complete because a small portion of the polymer will be incorporated into microbial biomass, humus and other natural products (Alexander, 1977; Atlas and Bartha, 1997 and Narayan, 1993).

Table 4. Microbial biomass-C in soil amended with different type of plastic during incubation periods.

Treatments	mg biomass-C· 100 g^{-1} dm			
	1	2	4	5 months
Control	1.65^{j}	1.00^{m}	0.98^{n}	0.89^{p}
Soil amended with non-degradable plastic	1.71^{i}	0.96^{o}	1.07^{k}	1.06^{l}
Soil amended with degradable plastic (Ecovio®)	7.22^{h}	7.38^{g}	7.45^{f}	7.60^{e}
Soil amended with degradable plastic (Bi-OPL®)	12.54^{d}	12.88^{c}	12.96^{b}	13.57^{a}

Weight loss % and tensile strength (MPa) of plastic films during incubation periods

Another frequently method used for estimation biodegradation of polymer is measurement of weight loss and tensile strength which illustrated in Fig 1. The higher weight losses were related to the higher concentration of polylactic acid content in degradable plastic. So degradable plastic (Bi-OPL®) that contain 100% PLA recorded the highest values of weight loss. The weight loss % degradable plastic increases with the increase in incubation periods. Compared to degradable plastic, polyethylene films have incommodiously decrease in weight.

Weight-loss degradability strongly depended on the specific types of biodegradable plastic and degrading microorganisms. Regarding values of tensile strength obtained data showed the same trend with a notable decrease until the 5th month with degradable plastic compared with nondegradable one. At the end of the experiment, more reduction in weight-loss was noticed with ECOVIO® and BIO-OPL® (10.5 and 9.8, respectively). The losses of tensile strength were slight in first three months but it increased at the end of the experiment. According to the photographic observation Fig. 2, all degradable plastic became more brittle than before, in addition, some cracks appeared while that is on change appeared on nondegradable one until the end of the experiment. Results in Fig 1, 2 indicate that biodegradable plastic was not decomposed in the earliest stage of incubation periods and the ultimate degradability of the biodegradable plastic tends to begin to raise after four months of incubation under soil condition. These results show that PLA-degrading microorganisms are not widely distributed in the natural environment and thus, PLA is less susceptible to microbial attack in the natural environment than other microbial and synthetic aliphatic

polyesters. For the above reasons, it is very important to study the biodegradation of PLA and to develop treatment processes for plastic waste containing PLA (Ohtaki and Nakasaki, 2000).

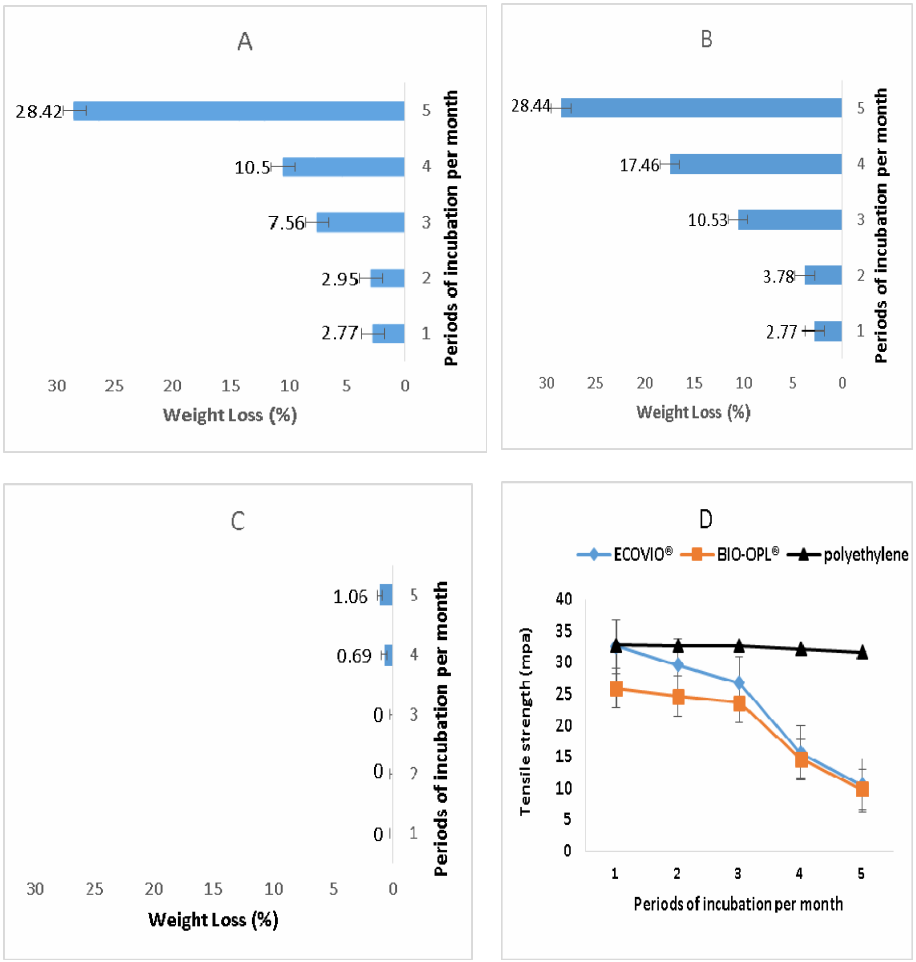

Fig 1. Weight Loss (%) and tensile strength (MPa) of plastic films in Soil during incubation periods.

a- Weight Loss (%) of ECOVIO®.
b- Weight Loss (%) of BIO-OPL®.
c- Weight Loss (%) of non-degradable plastic.
d- Tensile strength (MPa) of different types of plastic.

The degradability of the biodegradable plastics used in the present study may be changed by changing soil conditions. So the effects of composting conditions on the degradability of biodegradable plastic should be performed in the future.

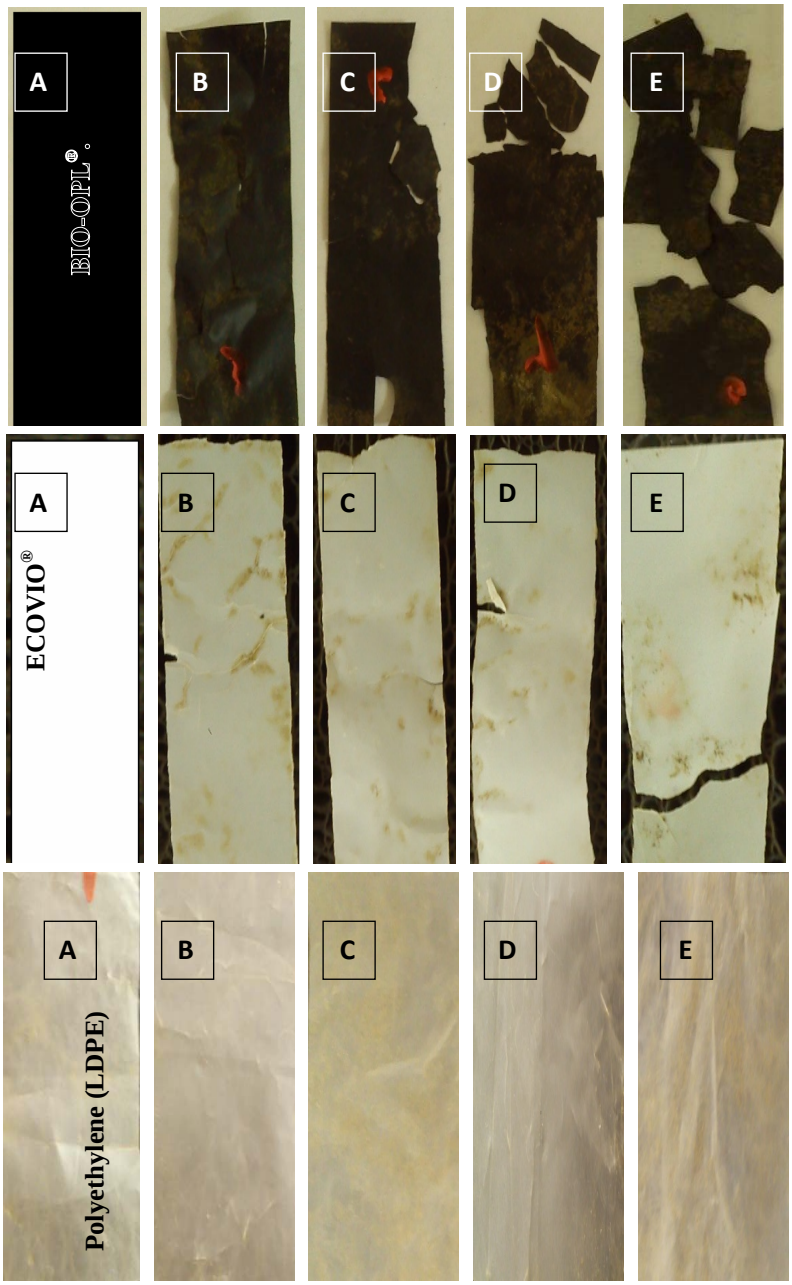

Fig2. Photographical comparison between the different types of plastic films before and after incubation periods showing the cracks on ECOVIO® and Bi-OPL films.

A: original plastic film, B: After one month, C: After two month, D: After four month and E: After five month

It worthy to reported that increasing weight loss values and decreasing tensile strength values of degradable plastic films were related to the higher values of CO_2 evolution, microbial biomass and dehydrogenase activity. Accordingly, it may be depended on previous parameters to evaluate biodegradability of PLA. This results is agreement with (Agarwal *et al.,* 1998 and Rudeekit *et al.,* 2008) who reported that PLA polymer film was degraded in abiotic and biotic environments to understand the role of microbes in the degradation process of lactic acid based polymers. CO_2 evolution, weight loss, and molecular weights were measured to evaluate the extent of degradation. The polymer carbon is converted by microorganisms into biomass carbon, carbon dioxide, water soluble by-products and polymer carbon which is not yet degraded (Copinet *et al.,* 2011). Regarding the role of tensile strength to evaluate degradability of polymers (Mostafa *et al.,* 2010 and Orhan *et al.,* 2004) reported that loss in tensile properties is the most relevant practical criterion to characterize degradation of polymer films or fibers that contact with the soil**.**

CONCLUSION

From the obtained data, it could be concluded that some microbial activities such as amount of evolved carbon dioxide, microbial biomass and dehydrogenase activity in the soil amended with degradable plastic increased by increasing incubation period. The higher weight loss % was related to the higher concentration of polylactic acid in degradable plastic. Compared to degradable plastic, polyethylene films have incommodiously decrease in weight. It worthy to reported that the higher weight loss of degradable plastic films were related to the higher values of CO_2 evolution, microbial biomass and dehydrogenase activity. Accordingly, it may be depended on previous parameters to evaluate biodegradability of PLA

Acknowledgements

The authors would like to present deep gratefulness and appreciation to Banha University, Egypt (www.bu.edu.eg) for giving them the opportunity to fulfil this study

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تقييم التحلل البيولوجي لشرائح البلاستيك الحيوي المصنوعة من معقد حمض اللاكتيك في التربة

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قسم النبات الزراعي، فرع الميكروبيولوجيا الزراعية، كلية الزراعة، جامعة بنها، مصر.

أجريت تجربة اصص داخل صوبة قسم النبات فرع الميكروبيولوجي بكلية الزراعة، جامعة بنها، خلال الفترة من أبريل وحتي أغسطس لعام ٢٠١٤ لتقييم التحلل البيولوجي لنوعين من شرائح البلاستيك الحيوي المصنوعة من معقد حمض اللاكتيك مقارنة بنظيرتها المنتجه من البولي ايثلين المقاوم للتحلل البيولوجي وقد وضعت الشرائح في التربة لمدة خمسة أشهر. وكذلك تم مناقشة تأثير نواتج التحلل البيولوجي للمواد السابقة على النشاط الميكروبي داخل التربة. وقد كشفت النتائج المتحصل عليها زيادة كمية ثاني أكسيد الكربون المتصاعد، والكتلة الحيوية الميكروبية ونشاط إنزيم الديهيدروجينيز في التربة المضاف اليها البلاستيك القابل للتحلل والمصنوعة من معقد حمض اللاكتيك بزيادة فترة التحضين. وقد زات هذه القياسات زيادة معنوية في التربة المضاف اليها البلاستيك القابل للتحلل مقارنة بالتربة المضاف اليها البلاسيتك الغير قابل للتحلل الحيوي(شرائح البولي ايثلين). وقد اوضحت النتائج ان شرائح البلاستيك الحيوي من النوع BiOPL® المنتج من معقد حمض اللاكتيك بنسبة نقاوة ١٠٠% حصلت علي أعلي قيم لفقدالوزن وأقل قيم لمقاومة الشد مقارنة مع شرائح البلاستيك الحيوي من النوع Ecovio® المنتج من معقد حمض اللاكتيك بنسبة ٤٥٪ و معقد Ecoflex بنسبة ٥٥٪. في حين ان، شرائح البولي إثيلين حصلت علي نقص طفيف في الوزن خلال فترات التحضين. و من الجدير بالذكر أن هناك ارتباط بين زيادة قيم فقدان الوزن وخفض قيم مقاومة الشد للشرائح البلاستيكية القابلة للتحلل من ناحية وزيادة كمية ثاني أكسيد الكربون المتصاعدة من التربة، والكتلة الحيوية الميكروبية ونشاط انزيم الديهيدروجينيز. وبناء على ذلك، فإنه يمكن أن يعتمد على القياسات السابقة لتقييم التحلل البيولوجي لشرائح البلاستيك الحيوي المصنوعة من معقد حمض اللاكتيك.