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Biodegradation of Synthetic Polyesters (BTA and PCL) with Natural Flora in Soil Burial and Pure Cultures under Ambient Temperature

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Abstract: The aim of this study was to study the biodegradation of two synthetic polyesters, one aliphatic-aromatic (1, 4-butanediol, terephthalic-adipic acid, BTA) and the other aliphatic (poly (ϵ-caprolactone), PCL, under different soil types (canal shore soil, garden soil, compost and Peat moss, respectively), as well as using locally isolated cultures at ambient temperature. The results showed that the BTA films buried in canal shore and garden soil were degraded faster than that in the other soils. After six weeks about 90, 88 and 80% were degraded in garden, canal shore soil and compost respectively, while only 52% were degraded in Peat moss. On the other hand, 95 and 93% weight loss was obtained for PCL films buried for three weeks in canal shore and garden soil respectively. The Scanning Electron Microscope photos confirm the results of weight loss and revealed the presence of cracks and fungal growth on films buried in different soils. The results with pure cultures, especially with *Fusarium solani*, also confirmed the biodegradability of two polyesters under ambient temperature. Finally, it could be concluded that both synthetic polyester are degradable under ambient conditions.

Key words: *Fusarium solani*, microbial degradation, polyesters, scanning electron microscope

INTRODUCTION

Synthetic polymers are a group of materials that surrounds us every day of our life. Because of their mechanical properties they are able to possess various forms and serve in many functions, therefore, it is almost impossible to image just one day without them. On the other hand, the dark side of their expansion is their impact on environment. Because of their resistance against action of microorganisms and other environmental factors, half-life of synthetic materials is expected to be tens maybe hundreds of years. Thus, accumulation of solid waste constituting of synthetic polymers has been recently found to be serious environmental issue (Obruca, 2010).

Production and expenditure of plastics materials grows an average of 9% per year. Over the 60 years, production has grown from 1.5 million tons in 1950 to 245 million tons in 2008 (Dacko *et al*., 2008).

Egypt generated about 16 million tons of Municipal Solid Waste (MSW) in 2006, growing by about 3.4% per year and plastics amount post consumer waste (PCW) reaches about 970 thousands MT/year which equal to about 6% of the total MSW (Plastics Technology Center, 2008).

It is necessary to find a solution and prevent accumulation of thousands tons of solid waste every year. Biodegradable plastics opened the way for new waste management strategies since these materials are designed to degrade under environmental conditions or in municipal and industrial biological waste treatment facilities. Most of the plastics on the market, claimed to be biodegradable, are based on synthetic and microbial polyesters (Witt *et al*., 1997; Eubeler *et al*., 2010; Nowak *et al*., 2011a). Polyesters are potentially biodegradable due to the hydrolyzable ester bonds. In addition, they combine several properties that make them attractive candidates for various industrial applications. Compared with most aliphatic polyesters, aromatic polyesters, such as poly (ethylene terephthalate) and poly (butylene terephthalate), have excellent material properties. However, it is now considered that their susceptibility to microbial attack is negligible (Ki and Park, 2001; Müller *et al*., 2001; Nowak *et al*., 2011b). Therefore, to increase the biodegradability of aromatic polyesters, some studied focused on the synthesis of aliphatic-aromatic copolyesters (Müller *et al*., 2001) or incorporation of aliphatic dicarboxylic acids or polyethylene glycol in polyester chains which greatly enhance the degradation rate. The combinations of terephthalic acid (30 to 60%), adipic acid and 1, 4-butandiol (BTA-polymer turned out to be the most appropriate aliphatic-aromatic combination, with regard to the material properties (flexible films, melting point from 90-140°C) (Witt *et al*., 1995). The degradation of this co-polyester has been

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investigated in compost environments or with compost isolates (Kleeberg *et al*., 1998; Witt *et al*., 2001). The experiments with compost isolates were all performed at elevated temperatures with *Thermobifida fusca* (known previously as *Thermomonospora fusca*) and a hydrolase isolated from this bacterial strain. Results from these studies demonstrated rapid degradation of the co-polyester, with essentially complete degradation having been achieved in 21 days in the presence of a readily available carbon source.

Degradation of synthetic co-polyester was also studied with pure culture at moderate environmental conditions (Trinh *et al*., 2008). The author reported that the degradation rate was slower than that obtained at elevated temperature.

Another example of polyester, PCL, a synthetic aliphatic linear polyester with an almost 50% crystallinity, is biologically degradable and consists of 6-hydroxyhexanoates. Generally, the environmental degradation of PCL appears to occur by the action of bacteria that are widely distributed in the ecosystem (Mergaert and Swings, 1996; Suyama *et al*., 1998; Fukushima *et al*., 2010). *Pseudomonas* lipase (PS lipase) has been reported for the degradation of PCL and its copolymer (He *et al*., 2003; Li *et al*., 2003). Highly crystallized PCL degraded completely in 4 days in the presence of *Pseudomonas* lipase (Gan *et al*., 1999) in contrast to hydrolytic degradation lasting several years (Li *et al*., 2002).

The aim of this research is concentrated on biodegradation of two synthetic polyesters, one aliphatic-aromatic and the other aliphatic (BTA and PCL, respectively) in soil burial with the natural flora, as well as with pure isolated culture at ambient temperature (ca 30°C), because most of the polyester wastes will be disposed more at ambient conditions.

MATERIALS AND METHODS

Plastic materials*:* The aliphatic-aromatic copolyester (1, 4-butanediol, terephthalic acid and adipic acid, BTA (Trade name, Ecoflex, (BASF, 2007)) and poly (ϵ-caprolactone), PCL. Both types were provided by the Lab of Prof. Deckwer and Dr. Müller, Gesellschaft fuer Biotechnologische Forschungen (GBF, Braunschweig, Germany).

Source and analysis of soils: Four local types of soils were used in this study (canal shore soil, garden soil, compost and Peat moss, respectively). The physico-chemical composition of soils was determined by the central Lab, Faculty of Agriculture, Alexandria University.

Soil burial: Polymer films (BTA or PCL) of about 1.0×1.0 cm with initial weight of 0.1 g were buried in plastic containers (pots) 20 cm diameter and 18 cm height containing about 150 g of different types of soils (one at a time). The films were hanged with fishnet thread for easy follow up. Moisture content of soil was maintained around 60% by the addition of water. All the containers were incubated at ambient temperature (ca 30°C). Control film samples were incubated under the same condition in soil, which is sterilized by autoclaving. The experiments of this work were conducted in 2009 in microbiology Laboratory, Botany and Microbiology Department, Faculty of Science, Alexandria University, Egypt.

Medium for isolation of polyester degrading microorganisms: Mineral Salt Vitamin medium (MSV) according to Gouda *et al*. (2002) was used for isolation of polyester degrading microorganisms and evaluation of polyester degradation with pure isolates.

Preparation of clear zone plates with BTA and PCL polyester*:* The suitable method to prepare turbid agar plates is that the polyester (0.1-0.2 g) was dissolved in 3 mL methylene chloride then completed to 10 mL with distilled water and the solution was emulsified by sonication. The emulsion was then added to 250 mL Erlenmeyer flask containing 100 mL of buffered MSV medium with 2.0 g agar then stirred continuously while heating for at least 30 min to evaporate the solvent completely. pH was adjusted to 7.0 and the medium was autoclaved resulting in homogenous opaque plates where it was poured into Petri dishes (9 cm in diameter) with about 15 mL/plate (Augusta *et al*., 1993).

Evaluation of polyester degradation by:

Weight loss of polyester films buried in soil: As a quantitative measure of polyester depolymerization, the determination of the weight loss of polymer films buried in the soil or added to MSV medium inoculated with pure fungal isolates was used. After different incubation periods, the pre-weighed films were removed from soil or medium, brushed softly, washed with distilled water several times to clean off the soil particles and fungal mycelia then dried at room temperature to constant weight (Kleeberg *et al*., 1998).

Estimation of clear zone: The pure strains were inoculated on 9 cm diameter Petri-dishes containing MSV medium with the polyester. The gradual increase in colony and clear zone size was measured in centimetres with a ruler every day for 8 days.

Scanning Electron Microscope (SEM) of the films: The surface microstructure of polyester films as well as changes due to degradation was examined using SEM. Polymer pieces which have been buried in soil were taken after different incubation periods. The plastic films were prepared according to Lee *et al.* (1994) and Arévalo-Niño *et al.* (1996) and examined by a Joel SEM (JSM-5300

Table 1: Physicochemical composition of the soil samples

Sample	pH	EC* (dS/m)	Organic matter (%)	Organic carbon (%)	Nitrogen (%)	C/N ratio
Canal shore	6.88	0.37	3.9	2.27	0.48	4.73
Garden	6.84	0.98	3.2	1.86	0.45	4.13
Compost	7.01	2.41	6.5	3.78	1.1	3.44
Peat moss	4.79	0.11	44.1	25.63	1.23	20.84

*EC: Electrical conductivity

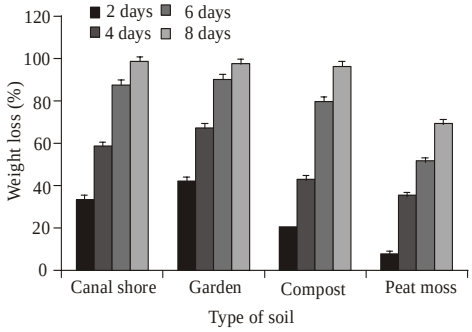

Fig. 1: Weight loss (%) of BTA films buried in different types of soil after different incubation periods. The weight loss in control samples after 8 weeks was about 0.2%

LV-Japan) in the Central laboratory, Faculty of Science, Alexandria University.

Statistical analysis: Each experiment was performed in duplicate and the value is the mean of the two replicates. The Standard error was calculated with Microsoft Excel 2003.

RESULTS AND DISCUSSION

Soil characterization: The main aim of this research is the study of the degradability of two synthetic polyesters in different types of soil under laboratory conditions. For this purpose, it is essential to characterize the physicochemical features of the soils (Table 1). The results revealed that canal shore and garden soil have the lowest organic matter (3.9 and 3.2%, respectively), followed by compost (6.5%). On the other hand, peat moss has the highest organic matter content (44.1%). The pH of canal shore, garden and compost was recorded to be neutral, while for peat moss was found to be in the acidic range (4.8).

Characteristic of BTA films after biodegradation in soil burial:

Weight loss: Usually, enzymatic degradation of plastics is a surface erosion process, because enzymes are not able to penetrate the polymer. Thus the weight loss can be used to measure the enzymatic cleavage of the polymer. The weight loss results presented in Fig. (1) shows that the films buried in canal shore and garden soil, as well as in compost were degraded faster than that in peat moss. After six weeks about 90, 88 and 80%, were degraded in garden, canal shore soil, and in compost respectively, while only 52% were degraded in peat moss. Increasing the degradation time (8 weeks) lead to about complete degradation of the films buried in canal shore and garden soil and 95% for the films buried in compost. The results obtained revealed that the type of soil and the incubation time had a great effect on the biodegradation of BTA. These data shows that the rate of degradation at moderate temperature was slower than that reported by Witt *et al.* (2001), which showed complete biodegradation of the same copolyester after three weeks by *Thermomonospora fusca* at elevated temperatures. Lotto *et al.* (2004) reported that both abiotic and biotic rates of degradation increase with an increase in temperature. On the other hand, the results of this study was better than that obtained by Mostafa *et al*. (2010) who studied the biodegradation of different commercial bioplastics at moderate temperature under different soil types and reported more than 30% weight losses of Ecoflex and Bioflex after three months. Although peat moss had the highest organic matter, 44% (Table 1), it was found that the films buried in peat moss showed only 69% weight loss after eight weeks. This may be due to the very high C/N ratio (20.8) which is not suitable for the growth of most microorganisms. Nowak *et al.* (2011a) obtained the maximum degradation of polyethylene films in soil with organic matter content of 7.2%.

Scanning electron microscope*:* The changes in the surface microstructure of the films were examined using scanning electron microscope. Figures of SEM show different kinds of surface alternations on basis of soil type and time of incubation. Abiotic controls were used to establish baseline degradation for comparison.

The surface of the film buried for four weeks in canal shore and garden soil revealed the presence of cracks and fungal growth in the cracks (Fig. 2A, B). Increasing the degradation time increased the cracks. It was also observed that the abiotic controls show no obvious changes. Kawai (1995) suggested that the strong pressure caused by growth of the roots of fungi leads to such crack formation in the case of biodegradation in soils. The SEM pictures shows that the degradation began with microbial growth on the films which mainly fungi. This is in agreement with the early observation that the primary step of the degradative attack of plastic materials occurs by colonization of filamentous microorganisms (Chiellini *et al*., 1996). The SEM pictures (Fig. 2C) of the films

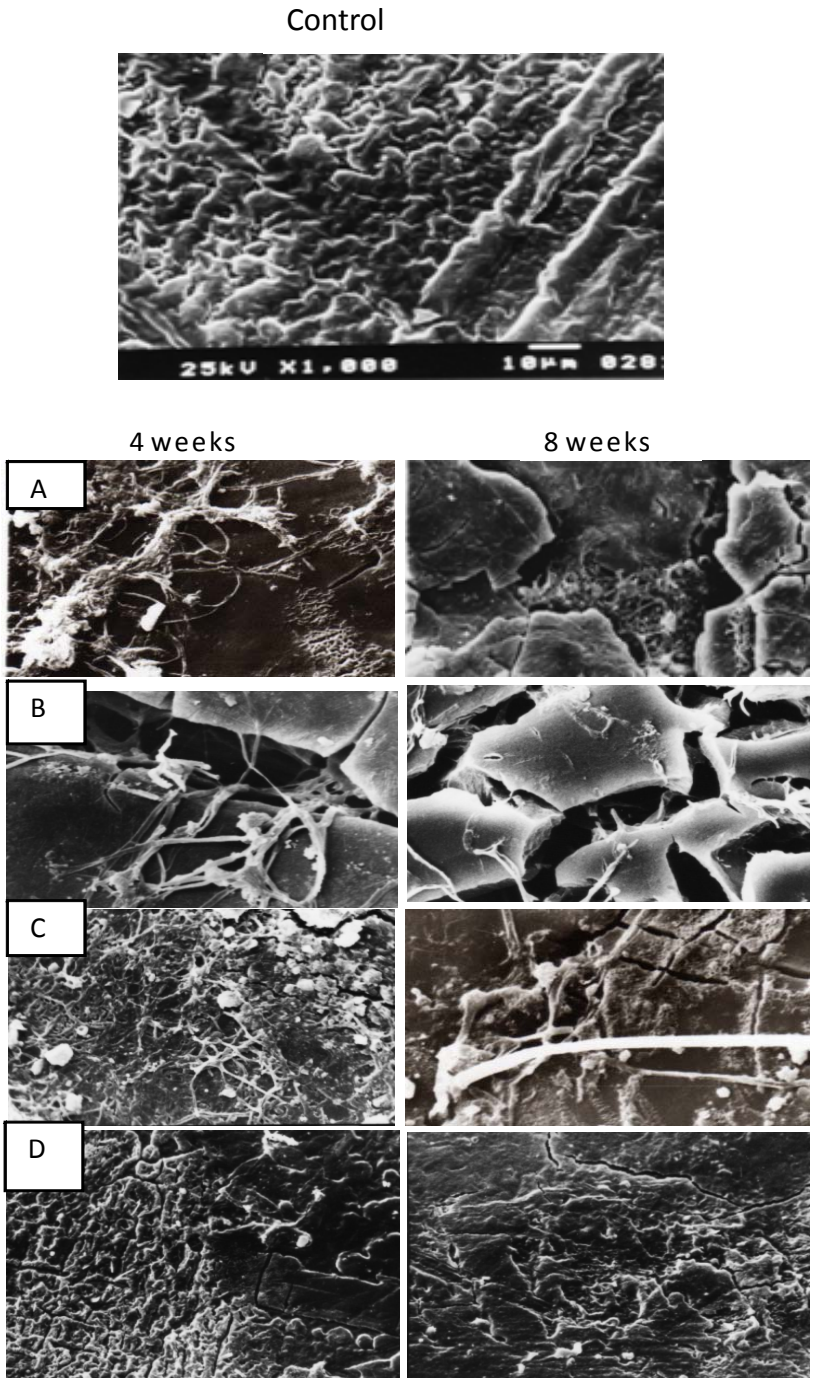

Fig. 2: SEM of BTA films buried in soils for different periods (x 1000): (A) Canal shore soil, (B) Garden soil, (C) Compost and (D) Peat moss

buried in compost revealed the presence of fungal mycelia on the film surface, in addition to some cracks after four weeks. After eight weeks the number of cracks was increased, however the fungal mycelia decreased. On the other hand, the BTA films buried in peat moss (Fig. 2D) showed less cracks in the films as well as less fungal growth after the same incubation time (8 weeks). Nowak *et al.* (2011a) reported the presence of small single hollows on the surface of modified polyethylene films buried in waste coal soil for 75 days.

Table 2: Total fungal colonies, BTA and PCL degrading fungi

Soil type	Total fungal count (colony/g soil)	BTA- degrading fungi (colony/g soil)	BTA degrading fungi (%)	PCL- degrading fungi (colony/g soil)	PCL degrading fungi (#)
Garden soil	6×10^4	17×10^2	2.8	21×10^2	3.5
Canal shore soil	31×10^4	22×10^2	0.71	35×10^2	1.1

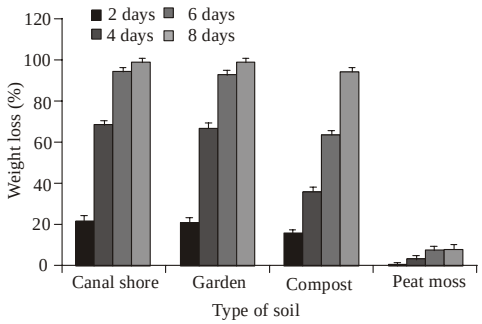

Fig. 3: Weight loss (%) of PCL films buried in different types of soil after different incubation periods. The weight loss in control samples after 8 weeks was about 0.5%

Characteristics of PCL films after biodegradation in soil burial:

Weight loss: The results obtained in this work with PCL films buried in the soils under test revealed that PCL was degraded faster than BTA due to its aliphatic nature (Fig. 3). About 100% of the added polymer was degraded in garden and canal shore soil after four weeks, while only 8% was degraded in peat moss after the same time which might be due to the unbalanced C/N ratio as reported previously for BTA. These results was better than that obtained by Chiellini *et al.* (1996) who reported only 25-30% weight loss in PCL films buried in soil after three months in presence of filamentous fungi as the biological agents. Fukushima *et al.* (2010) also reported that the PCL films buried in compost was almost completely degraded after 8 weeks, which is twice the time used in the present study.

Scanning electron microscope: The results of SEM for PCL films buried in canal shore soil for 1 week revealed the presence of many long thin cracks with fungal mycelia distributed on the film surface (Fig. 4A). On the other hand, the surface of films buried in garden soil, was eroded and comprised numerous irregular pits in addition to the fungal growth with clear fungal head. Increasing the incubation time increased the size of the pits, while the fungal mycelia decreased, this means that bacteria may also involved in the degradation (Fig. 4B). On the other hand, the SEM of the PCL films buried in compost for 1-week shows small cracks that increased with increasing the time, while the films buried in peat moss showed no obvious changes on the film surface with absence of fungal growth and appearance of few rod-shaped bacterial cells (Fig. 4C, D). Ikada (1999) reported some cracks on PCL films buried in mountain soil for 50 days. Our results are in contrary with several studies reported that the environmental degradation of PCL appears to occur by the action of bacteria that are widely distributed in the ecosystem (Mergaert and Swings, 1996; Suyama *et al*., 1998). On the other hand, the results are in agreement with Tokiwa (1995) who found that PCL and polyethylene adipate (PEA) can be decomposed almost completely by soil fungi such as *Penicillium* sp.

Number of polyesters-degrading fungi in some tested soil samples: From the previously described results, SEM pictures revealed that fungi were the most dominant microorganisms in degradation. In order to determine the number of polyester degrading fungi, the clear zone method using agar plate containing emulsified BTA or PCL was used to estimate the number in garden and canal shore soil, which show the highest degradation ability of the plastic films under test. The results of the present study revealed that the percentage of BTA degrading fungi was about 0.71% of the total fungal count in canal shore soil and 2.8% in garden soil, while the percentage of PCL degrading fungi was 1.1 and 3.5% of the total fungal count in canal shore and garden soil respectively (Table 2). Nishida and Tokiwa (1993a) reported that the percentage of PHB and PCL-degrading microorganisms in different environments was in the range of about 0.1-10% of the total count (including fungi and bacteria). The distribution and abundance of PCL-, PHB-, PTMS, and poly (propiolactone) (PPL)-degrading microorganisms in the environment have been also investigated by other authors (Nishida and Tokiwa 1993b; Pranamuda *et al*., 1995). Ecological studies on the diversity and abundance of such microorganisms in the natural environment are one of the essential elements for evaluating the biodegradability of polyesters. In this work seven fungal isolates which have the ability to degrade BTA were isolated in pure cultures, two isolates from canal shore soil, four isolates from garden soil and one isolate from compost. Although the number of PCL-degrading fungi was higher than the number of BTA-degrading fungi, only three PCL-degrading isolates were obtained in pure form.

Biodegradation of different forms of BTA by pure isolates:

BTA films: The ability of pure isolates to degrade BTA films were tested in liquid medium with the polyester as

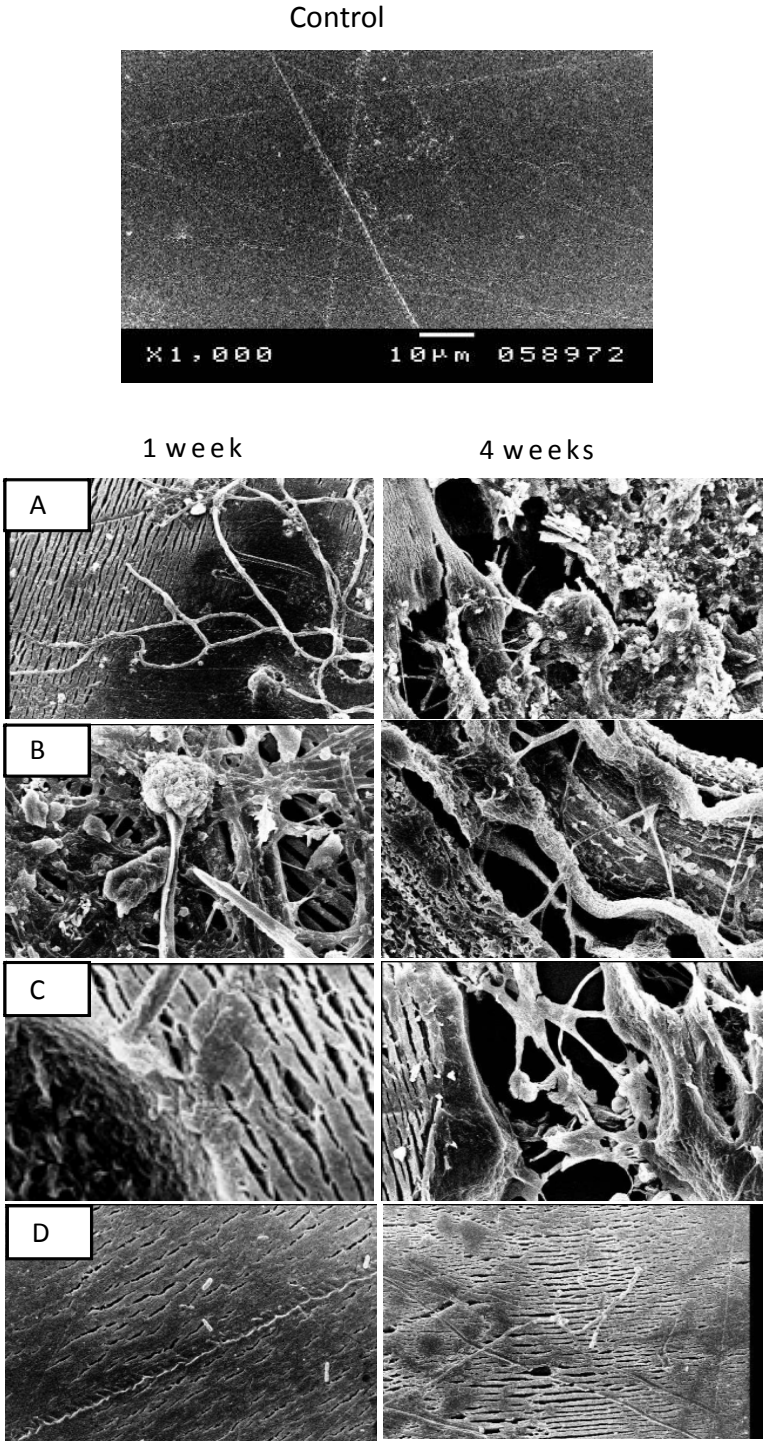

Fig. 4: SEM of PCL films buried in soils for different Periods (x1000): (A) Canal shore soil, (B) Garden soil, (C) Compost, (D) Peat moss

the sole carbon source incubated at 30°C for 15 days. The results revealed that only 0.05 mg weight loss was observed in control (without inoculation), which may be due the abiotic hydrolysis in the medium (Table 3). Maximum weight loss (9.8 mg) was obtained with isolate 3, while the lowest loss (0.5 mg) was recorded for isolate

Table 3: Weight loss of BTA films in liquid medium inoculated with pure fungal isolates for 15 days

Isolates	Final weight* (mg)	Weight loss (mg)	Weight loss (%)
Control**	49.95	0.05	0.1
Isolate 1	47.0	3	6.0
Isolate 2	47.2	2.8	5.6
Isolate 3***	40.2	9.8	19.6
Isolate 4	46.2	3.8	7.6
Isolate 5	47.2	2.8	5.6
Isolate 6	49.5	0.5	1.0
Isolate 7	46.9	3.1	6.2

*: Initial weight 50 mg; **: BTA film added to the medium without inoculation; ***: Isolate 3 was identified as *Fusarium solani*

Table 4: Weight loss of PCL films in liquid medium inoculated with pure fungal isolates for 15 days

Isolates	Final weight* (mg)	Weight loss (mg)	Weight loss (%)
Control**	24.8	0.2	0.8
Isolate 1	20	5	20
Isolate 2	18	7	28
Isolate 3***	14	11	44

*: Initial weight 25 mg; **: PCL film added to the medium without inoculation; ***: Isolate 3 was identified as *Fusarium solani*

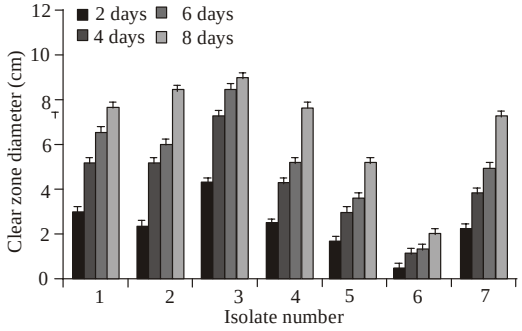

Fig. 5: Clear zone diameter (cm) of BTA plates inoculated with pure isolates after different incubation time (BTA powder was added at 100 mg/100 mL medium). Isolate 3 was identified as *Fusarium solani*

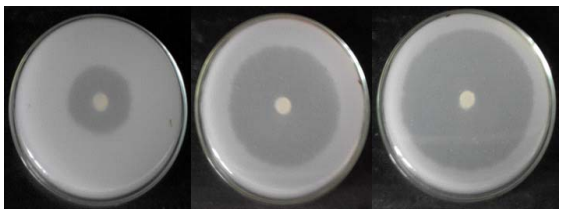

Fig. 6: Photograph of clear zone formed by *Fusarium solani* after different incubation time (2, 4 and 6 days, respectively)

6. The most efficient fungus (Isolate 3) was identified as *Fusarium solani* by Mycological centre, Assiut University, Assiut, Egypt and deposited in the same centre under the number AUMC 5098. Trinh *et al*. (2008) studied the biodegradation of Ecoflex with pure culture of fungi and yeasts at 30°C and revealed weight loss in the

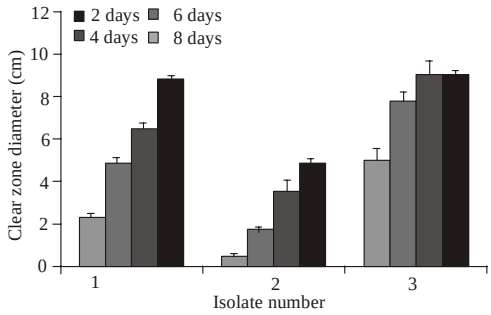

Fig. 7: Clear zone diameter (cm) of PCL plates inoculated with pure isolates after different incubation time (PCL powder was added at 100mg/100mL medium). Isolate 3 was identified as *Fusarium solani*.

films ranged from 0.0 to 1.9 mg with no visual degradation in the films, while visual degradation was observed with two bacterial strains of *B. subtilis*.

BTA powder: The biodegradation of BTA powder was evaluated by clear zone determination under the same condition used for BTA films. The results demonstrated in Fig. 5 showed that the powder was degraded faster than the films and degradation can be observed after two days. Maximum degradation value (9 cm) was recorded after eight days for isolate 3, while isolate 6 showed the lowest degradation ability, which confirms the weight loss results of the BTA films. The results are in agreement with that observed by Pranamuda *et al.* (1995) who reported that the nanoparticles with large surface area are degraded faster than the other form of polyester such as films. It was also reported that the specific surface area of the specimens affect the biodegradation results because of the different contact area between microbes and the specimens (Yang *et al*., 2005). Figure 6 showed the clear zone formation with incubation time due to degradation of BTA powder by *Fusarium solani*.

Biodegradation of different forms of PCL by pure isolates:

PCL films: The results presented in Table 4 revealed that the maximum weight loss (11 mg) was obtained after 15 days with isolate 3 (*Fusarium solani*), while the minimum value (5 mg) was recorded for isolate 1, which is little better than the degradation of BTA films with *Fusarium solani*.

PCL powder: The biodegradation of PCL powder was evaluated by clear zone method. The results showed that the maximum degradation (9 cm) was obtained after six days by isolate 3 (*Fusarium solani*), while the minimum (4.8 cm) was recorded by isolate 2 after 8 days

(Fig. 7). Herzog *et al*. (2006) demonstrated that aliphatic polyester nanoparticles suspension was totally hydrolysed with Candida lipase within some minutes in comparison with some hours to many days with films.

CONCLUSION

The results of the present study concluded that the aromatic-aliphatic copolyester (BTA) and aliphatic polyester (PCL) are readily biodegraded in different environments under mesophlic conditions and that the biological agent was mainly fungi. The degradation was evaluated with different methods including clear zone, weight loss and microscopic observation using SEM and all the results prove the degradability of the polyesters under test.

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REFERENCES

Arévalo-Niño, K., C.F. Sandoval, L.J. Galan, S.H. Imam, S.H. Gordon and R.V. Greene, 1996. Starch-based extruded plastic films and evaluation of their biodegradable properties. Biodegrad., 7: 231-237.

Augusta, J., R.J. Müller and H. Widdecke, 1993. A rapid evaluation plate-test for the biodegradability of plastics. Appl. Microbiol. Biotechnol., 39: 673-678.

BASF Company, 2007. Production Information. Ecoflex® Brochure, Retrieved from: (www.basf-ag/ecoflex)

Chiellini, E., A. Corti, A. Giovannini, P. Narducci, A.M. Paparella and R. Solaro, 1996. Evaluation of Biodegradability of Poly (ϵ-Caprolactone)/Poly (ethylene Terephthalate) Blends. J. Environ. Polym. Degrad., 4: 37-50.

Dacko, P., J. Rydz, W. Sikorska, M. Sobota and M. Kowalczuk, 2008. Industrial composting of polymer- based materials produced from recycled materials. Problemy Ekologii, 67: 39-42.

Eubeler, J.P., M. Bernhard and T.P. Knepper, 2010. Environmental biodegradation of synthetic polymers. II. Biodegradation of different polymer groups. Trends Anal. Chem. TRAC., 29: 84-100.

Fukushima, K., C. Abbate, D. Tabuani, M. Gennari, P. Rizzarelli and G. Camino, 2010. Biodegradation trend of poly(ϵ-caprolactone) and nanocomposites. Mater. Sci. Eng. C., 30: 566-574.

Gan, Z., D. Yu, Z. Zhong, Q. Liang and X. Jing, 1999. Enzymatic degradation of poly(ϵ-caprolactone) /poly (DL-lactide) blends in phosphate buffer solution. Polymer., 40: 2859-2862.

Gouda, M.K., I. Kleeberg, J. van den Heuvel, R.J. Müller and W.D. Deckwer, 2002. Production of a Polyester Degrading Extracellular Hydrolase from *Thermomonospora fusca*. Biotechnol. Prog., 18: 927-934.

He, F., S.M. Li, M. Vert and R.X. Zhuo, 2003. Enzyme-catalyzed polymerization and degradation of copolymers prepared from e-caprolactone and poly (ethylene glycol). Polymer, 44: 5145-5153.

Herzog, K., R.J. Müller and W.D. Deckwer, 2006. Mechanism and kinetics of the enzymatic hydrolysis of polyester nanoparticles by lipases. Polym. Degrad. Stab., 91: 2486-2498.

Ikada, E., 1999. Electron Microscope Observation of Biodegradation of Polymers. J. Environ. Polym. Degrad., 7: 197-201.

Kawai, F., 1995. Breakdown of Plastics and Polymers by Microorganisms. Adv. Biochem. Eng. Biotechnol., 52: 152-194.

Ki, H.C. and O.O. Park, 2001. Synthesis, characterization and biodegradability of the biodegradable aliphatic-aromatic random copolyesters. Polymer, 42: 1849-1860.

Kleeberg, I., C. Hetz, R.M. Kroppenstedt, R.J. Müller and W.D. Deckwer, 1998. Biodegradation of aliphatic-aromatic copolyesters by *Thermomonospora fusca* and other thermophilic compost isolates. Appl. Environ. Microbiol., 64(5): 1731-1735.

Lee, S.Y., K.M. Lee, H.N. Chan and A. Steinbüchel, 1994. Comparison of recombinant *Escherichia coli* strains for synthesis and accumulation of poly-(3-hydroxybutyric acid) and morphological changes. Biotechnol. Bioeng., 44: 1337-1347.

Li, S., H. Garreau, B. Pauvert, J. McGrath, A. Toniolo and M. Vert, 2002. Enzymatic degradation of block copolymerrs prepared from ϵ-caprolactone and poly (ethylene glycol). Biomacromolecules, 3: 525-530.

Li, S., L. Liu, H. Garreau and M. Vert, 2003. Lipase-catalyzed biodegradation of poly (ϵ-caprolactone) blended with various polylactide-based polymers. Biomacromolecules, 4: 372-377.

Lotto, N.T., M.R. Cail, C.G.F. Guedes and D.S. Rosa, 2004. The effect of temperature on biodegradation test. Mater. Sci. Eng. C, 24*:* 659-662.

Mergaert, J. and J. Swings, 1996. Biodiversity of microorganisms that degrade bacterial and synthetic polyesters. J. Ind. Microbiol., 17: 463-469.

Mostafa, H.M., H. Sourell and F.J. Bockisch, 2010. The mechanical properties of some bioplastics under different soil types for use as a biodegradable drip tubes. Agri. Eng. Inter.: The CIGR Ejournal. Manuscript 1497, Vol. 12.

Müller, R.J., I. Kleeberg and W.D. Deckwer, 2001. Biodegradadation of polyesters containing aromatic constituents. J. Biotechnol., 86: 87-95.

Nishida, H. and Y. Tokiwa, 1993a. Distribution of Poly (β-hydroxybutyrate) and Poly (ϵ-caprolactone) Aerobic Degrading Microorganisms in Different Environments. J. Environ. Polym. Degrad., 1: 227-233.

Nishida, H. and Y. Tokiwa, 1993b. Distribution of Poly (β-hydroxybutyrate) degrading anaerobic bacteria. Jap. Polymer Sci. Technol., 50: 745-746.

Nowak, B., J. Pajak, M. Drozd-Bratkowicz and G. Rymarz, 2011b. Microorganisms participating in the biodegradation of modified polyethylene films in different soils under laboratory conditions. Int. Biodeter. Biodegrad., 3: 1-11.

Nowak, B., J. Pajak, S. qabuzek, G. Rymarz and E. Talik, 2011a. Biodegradation of poly (ethylene terephthalate) modified with polyester ionolle by *Penicillium funiculosum*. Polimery, 56: 35-44.

Obruca, S., 2010. Controlled production and degradation of selected biomaterials. Ph.D. Thesis, Brno University of Technology, Faculty of Chemistry, Department of Food Chemistry and Biotechnology, Czech Republic.

Plastics Technology Center, 2008. Assessment of plastic recycling sector in Egypt.

Pranamuda, H., Y. Tokiwa and H. Tanaka, 1995. Microbial degradation of an aliphatic polyester with a high melting point Poly(tetramethylene succinate). Appl. Environ. Microbiol., 61: 1828-1832.

Suyama, T., Y. Tokiwa, P. Ouichanpagdee, T. Kanagawa and Y. Kamagata, 1998. Phylogenetic Affliation of Soil Bacteria that Degrade Aliphatic Polyesters Available Commercially as Biodegradable Plastics. Appl. Environ. Microbiol., 64: 5008-5011.

Tokiwa, Y., 1995. Handbook of Biodegradable Plastics Part II. NTS Pub, Tokyo, pp: 454.

Trinh, T.F., D.G. Cooper, M. Maric and J.A. Nicell, 2008. Biodegradation of a synthetic co-polyester by aerobic mesophilic microorganisms. Polym. Degrad. Stab., 93: 1479-1485.

Witt, U., T. Einig, M. Yamamoto, I. Kleeberg, W.D. Deckwer and R.J. Müller, 2001. Biodegradation of aliphatic-aromatic copolyesters: Evaluation of the final biodegradability and ecotoxicological impact of degradation intermediates. Chemosphere, 44(2): 289-299.

Witt, U., R.J. Müller and W.D. Deckwer, 1995. Biodegradadation of polyester copolymers containing aromatic compounds. JMS Pure Appl. Chem., 32: 851-856.

Witt, U., R.J. Müller and J. Klein, 1997. Biologisch abbaubare Polymer-Status und Perspektiven, Report of the Franz-Patat-Zentrum, Braunschweig, Germany, ISBN: 3-00- 001529-9.

Yang, H.S., J.S. Yoon and M.N. Kim, 2005. Dependence of biodegradability of plastics in compost on the shape of specimens. Polym. Degrad. Stab., 87: 131-135.