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BIODEGRADATION OF DIFFERENT STARCH-POLYETHYLENE PLASTIC FILMS BY PSEUDOMONAS STUTZERI AND PAENIBACILLUS POLYMYXA.

By

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ABSTRACT

Ten types of starch-polyethylene plastic films, with no additives like prooxidants or photooxidants were provided by plastic Development Center, Alexandria, Egypt. These films were incubated in marine sediment (collected from eastern harbour, Alexandria) and compost (provided from the manufacture site, Abees, Alexandria). Two bacterial strains were isolated and identified as ***Pseudomonas stutzeri*** *and* ***Paenibacillus polymyxa****. Both represented the major bacterial growth present on the tested plastic films after incubation on marine sediment and compost. The plastic biodegradability was investigated by weight loss, tensile strength loss and changes in percent elongation, after 40, 70 and 120 days of incubation. The results of the weight loss were inconclusive, while tensile strength loss and changes in percent elongation showed a highly significant reduction ($P < 0.01$) compared with non-incubated zero time control or with the untreated -polyethylene control (PE).*

INTRODUCTION

Plastics developed during the last five decades are characteristically inert and resistant to microbial attack (Johnson, ***et al.***, 1993), they accumulate in the environment at a rate of 25 million tons per year all over the world (Lee, ***et al.***, 1991). So, there is a growing interest in the development of degradable plastics to enhance the biodegradability of the plastics in land fills and composts, such as polylactides, poly (3 hydroxy – butyrate – 3 – hydroxy valerate), ethylene – carbon monoxide polymers, vinyl ketone copolymers and starch – filled polyethylene (Scott, 1990). Three types of degradation of polyethylene in these degradable starch – polyethylene polymers occur by different molecular mechanisms including chemical degradation , photodegradation and biological degradation. The first two mechanisms are dependent on using pro-oxidants and photo-oxidants additives in the manufacturing process of the polyethylene plastic films, causing the material to loss some of its physical properties, to become oxidized and possibly, to become more accessible to microbial biodegradation (Cornell, *et al.*, 1984; Chanda and Roy, 1986 ; David, *et al.*, 1992). The biodegradability of various plastic films has been investigated by different analytical approaches and parameters, including weight loss, tensile strength loss, changes in elongation percent, changes in polymer molecular weight distribution (Lee, *et al.*, 1991 and Johnson, *et al.*, 1993), spectroscopic determinations (Cacciari, *et al.*, 1993) and the release of $^{14}CO_2$ from ^{14}C-labelled polymers (Albertsoon, *et al.*, 1987 ; Albertsoon, and Karlsson, 1988).

In this paper, we studied the biological degradation of ten different starch – polyethylene plastic films, without attention to any chemical or physical pretreatments, by two local bacterial strains ***Pseudomonas stutzeri*** and ***Paenibacillus polymyxa***, under a static condition and with no additional carbon source. The plastic biodegradability was determined by loss of weight, loss of tensile strength and changes in elongation percent. The study was carried out in order to keep our aquatic and terrestrial environment free from plastic accumulation hazards.

MATERIALS AND METHODS

MICROORGANISMS

Two local bacterial strains including ***Pseudomonas stutzeri*** and ***Paenibacillus polymyxa*** were isolated from two different local sites, a marine sediment sample (from the Eastern Harbor of Alexandria) and a compost sample (from municipal yard waste compost site, Abees, Alexandria). The identification of the degrading bacteria was made by Micro Check Inc., Microbial Analysis Lab., Vermont, USA.

STARCH DEGRADATION TEST

The starch – degrading ability of these bacterial isolates was detected by streaking them separately on starch-nitrate agar plates , containing 1% (wt/vol) native corn starch; $NaNO_3$ 2.0 g/l; K_2HPO_4 1.0 g/l; Mg SO_4. $7H_2O$ 0.5 g/l; KCl 0.5 g/l and Fe SO_4. $7H_2O$ 0.001 g/l. The pH was adjusted at 7.0 . The plates wear incubated at 37°C for 24 -72 h. Starch hydrolysis was confirmed by flooding the incubated plates with iodine (Gerhardt, *et al.*, 1981). The cultures were maintained on the former medium at 4°C.

PLASTIC STRIPS PREPARATION

The plastic films used in this study were obtained from the Plastic Development Center, Alexandria, Egypt. The polyethylene was treated using different types of starch, rice starch with 2.5% ($PE\text{-}R_{2.5}$) and 5% ($PE\text{-}R_5$), corn starch with 2% ($PE\text{-}C_2$), 3% ($PE\text{-}C_3$) and 4% ($PE\text{-}C_4$) and potato starch with 2% ($PE\text{-}P_2$), 3% ($PE\text{-}P_3$), 4% ($PE\text{-}P_4$) and 10% ($PE\text{-}P_{10}$). The tested plastic materials were compared with untreated control (starch free-polyethylene PE). The strips were cut in a transverse direction to the blowing direction of the film, with 1.5cm in width, 10cm in length and the thickness was 0.02 – 0.05mm.

CULTURING AND INCUBATION OF THE FILM

The prepared strips of each tested plastic type were washed with distilled water and transferred to sterile petri-dishes (15cm in diameter), containing 50gm of marine sediment or compost samples. simulating some of the environmental conditions in which microorganisms may degrade the tested plastic films. The petri – dishes were incubated in dark, at room temperature for a period of four months, and 10-15ml of sea water (for the sediment samples) or of tap water (for the compost samples) were added each five days to avoid dryness.

ISOLATION AND PURIFICATION PROCESSES

For detecting the bacteria involved in the biodegradation of the tested plastic strips. The isolation process was carried out from the most degraded plastic strips in both sediment and compost plates, while the purification process was carried out using striking technique on different media, nutrient agar (peptone, 5 g/l; beef- extract, 3 g/l; agar-agar 20 g/l). minimal medium $(NH_4)_2$ SO_4, 1 g/l; KH_2 PO_4, 1 g/l; Mg SO_4, 0.5 g/l; Fe SO_4, 0.001 g/l; 0.1 g/l; agar-agar, 20 g/l) and starch – nitrate agar (as mentioned above). one gm of small cut pieces of starch – polyethylene plastic films were added as a carbon source to each 100 ml medium prepared .

FILM HARVEST AND LOSS WEIGHT DETERMINATION:

The incubated plastic strips were aseptically harvested after 40,70 and 120 days of incubation five strips in each. these strips were washed with sterile distilled water for five times. Then transferred to a standing 70% (vol / vol) ethanol solution, and left for 30 min. Each strip was placed into a pre-weighed sterile petri dish (5cm in diameter), and dried at 45-50°C overnight, allowed to equilibrate to the room temperature and re-weighed to $\pm$ 0.1 mg accuracy, then the weight of each strip was determined with a comparison to the starch free-polyethylene PE (untreated control) strips as well as to the corresponding non-incubated plastic strips (zero time control), (Johnson, ***et al.***, 1993).

TENSILE STRENGTH AND PERCENT ELONGATION DETERMINATIONS:

For determining the changes in tensile strength and percent elongation a Zwick / Z 2.5 N Universal Testing Machine was used (Fig. 1). Five strips of the tested plastic film were retrieved separately after 40, 70 and 120 days of incubation, washed with water and mild detergent and left for air drying at room temperature. The strips were subjected to tensile strength tests at 50 mm/min and the a 5 cm gap, using ASTM D882 standard test method for tensile properties of thin plastic sheeting (Yabannavar and Bartha, 1994).

STATISTICAL ANALYSIS

The design was completely randomized with five replications , the analysis of variance computed according to Steel and Torrie ,1980.

The statistical analysis was done by factor (polyethylene treatment "factor A" were applied in factorial combination of T_0 ,T_1 ,T_2 and T_3 incubation periods "factor B"), ANOVA test , "F" test and L.S.D. (Least Significant Difference) procedures available within the MSTAT-C software package (version 1.4 1995). Histogram graphics were produced using Harvard graphics software (HG version 4 1995).

Fig. (1) : A photograph shows the Zwick Universal Testing Machine used to measure the changes in the tensile strength and the elongation percent of the plastic films.

RESULTS

The isolation and the purification processes of bacteria from the degraded plastic strips indicated that two bacterial species ***Pseudomonas stutzeri*** and ***Paenibacillus polymyxa*** were present as common distributed species on the most degraded plastic strips and involved in the degradation process. The identification process of the isolated strains was carried out using FAME analysis (Fatty Acid Methyl Ester Analysis) through a gas liquid chromatography. The ***P. stutzeri*** is a Gram (-), rod shape, motile, non endospore former and able to utilize starch, while the ***P. polymyxa*** is a Gram (+), rod shape, motile, endospore former bacterium and able to utilize starch, gelatine and casein (Sneath, ***et al.***,1986) These two bacterial species showed a great adaptation to grow on the tested starch – polyethylene plastic films in the sediment and compost samples without any addition of carbon or nitrogen sources.

Slight losses of 3.5% and 5% of the original weight of PE-P_2, PE-P_3 after 70 days and 6% of PE-P_4 after 120 days were observed in the sediment samples. While in the compost samples PE-C_3 and PE-P_{10} strips showed 4 and 5% loss after 70 days, also PE-P_4 showed 6% loss after an incubation period of 120 days.

TENSILE STRENGTH DETERMINATIONS

The histograms (Fig. 2 "A and B"), showed that the maximum reduction in the tensile strength of the polyethylene plastic strips incubated in compost sample were 55% and 47% obtained for polyethylene strips treated with 4% corn starch and 4% potato starch, respectively. In the sediment sample the reduction percent was 56.3% and 54.8% in case of 4% corn starch and 2.5% rice starch respectively compared with the corresponding non-incubated strips .On the other hand, 2% and 3% corn starch sample showed an increase in tensile strength of 4.5% and 21.3%, respectively.

Statistical analysis for different compost samples (Table 1), showed that a highly significant decrease ($P < 0.01$) in tensile strength occurred in all tested starch treatments except in case of 2.5% rice starch where no significant change was obtained in the manufactured biodegradable polyethylene plastic films, compared with starch free- polyethylene strips (untreated control) or with the corresponding non-incubated strips (zero time control).

Table (1): Biodegradation of starch-polyethylene plastic films in compost sample at different incubation periods.

Type Starch	Starch Conc. (%)	Tensile Strength (Kg/cm^2)					Elongation (%)				
		T_0	T_1	T_2	T_3	Mean	E_0	E_1	E_2	E_3	Mean
Control	0	275.2	298.2	282.4	281.6	284.4^{a*}	233.0	231.9	208.6	201.5	218.7^{a}
Rice starch	2.5	310.1	305.8	263.5	240.6	280.0^{ab}	191.4	230.9	99.0	131.7	163.3^{b}
	5	238.6	203.4	172.4	162.1	194.2^{cd}	280.2	166.0	129.5	152.2	182.0^{b}
Corn starch	2	302.4	212.4	190.7	180.8	221.6^{bc}	209.3	250.5	162.5	143.3	191.4^{ab}
	3	227.9	209.4	177.0	145.5	189.9^{cd}	195.6	77.9	38.5	28.4	85.1^{cd}
	4	239.1	114.8	133.9	107.7	148.9^{d}	141.2	87.7	54.2	41.9	81.3^{cd}
Potato starch	2	225.6	162.2	138.5	158.7	171.3^{cd}	127.4	71.4	67.5	81.6	86.9^{cd}
	3	220.9	180.1	157.4	138.3	174.2^{cd}	113.0	101.0	94.5	81.1	97.4^{c}
	4	213.1	178.9	125.7	113.0	157.7^{d}	106.4	95.1	90.2	75.2	91.7^{cd}
	10	166.6	159.5	114.3	104.9	136.4^{d}	114.0	56.7	35.2	32.7	59.7^{d}
Mean		241.9^{a**}	202.5^{b}	175.6^{bc}	163.3^{c}		171.2^{a}	136.9^{b}	98.0^{c}	97.0^{c}	

T_0, E_0, = non-incubated strips, T_1, E_1 = incubated strips for 40 days, T_2, E_2 = incubated strips for 70 days, T_3, E_3 = incubated strips for 120 days, (each value is a mean value for five measured strips).

Means followed by the same letter are not significant but different letters are highly significant ($P < 0.01$).

* L.S.D. $_{0.01}$ for starch-polyethylene strips (tensile strength T and elongation % E) = 61.01 and 37.66 respectively.

** L.S.D. $_{0.01}$ for incubation period (tensile strength T and elongation % E) = 27.28 and 16.84 respectively.

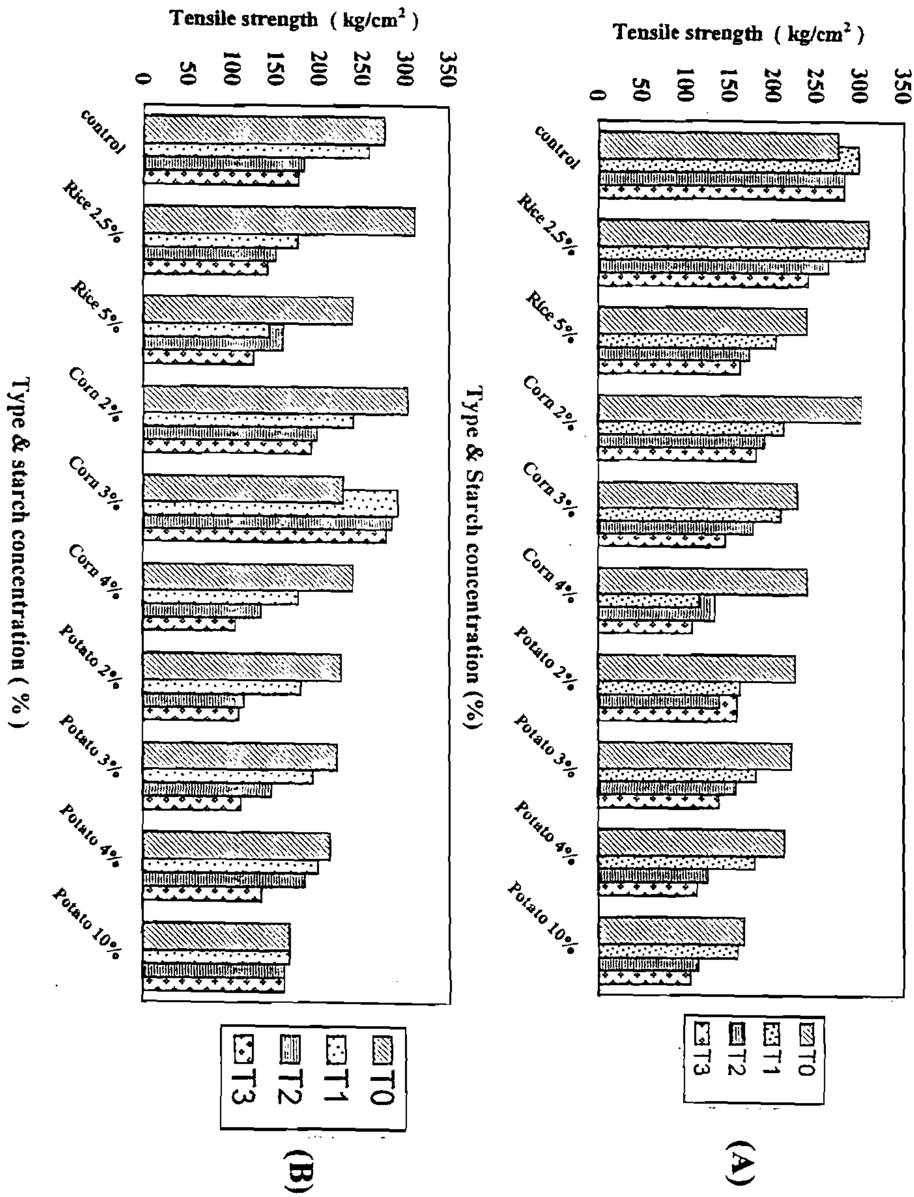

Figure (2) : Changes in tensile strength after 0 (T_0),40 (T_1),70 (T_2) and 120 (T_3) days of incubation in compost (A) and in sediment (B) samples.The control is starch free-polyethylene plastic strips.Eash bar value represents the meam of five replicates.

While the statistical analysis of the sediment samples (Table 2), indicated that all treatments showed a highly significant reduction ($P < 0.01$) in tensile strength of the tested strips compared with starch free-polyethylene control or with zero time control except in 2.5% rice starch. 2% corn starch and 10% potato starch no significant difference was obtained.

ELONGATION PERCENT CHANGES

The reduction in the elongation percent values was clearly detected along the incubation period in both compost and sediment samples in this study (Fig. 3 "A and B"). It was found that the elongation percent reduction in compost sample ranged from 28.2%, as in 3% potato starch, to 85.5% in case of 3% corn starch . While in the sediment sample the reduction in percent elongation reached to 78.8%, in 3% potato starch, further more the zero starch control showed 31.1% reduction compared with the corresponding zero time control (non–incubated control).

From the data of the compost sample (Table 1) a highly significant reduction ($P < 0.01$) in percent elongation was observed in all studied starch – polyethylene films except the 2% corn starch sample which showed no significant change compared with starch free- polyethylene and with non-incubated controls. Similarly, in the sediment sample all types of the tested starch polyethylene plastic films showed a highly significant reduction ($P< 0.01$) in percent elongation except in the case of 2.5% rice starch and 2% corn starch which showed no significant reduction in the percent elongation compared with zero – starch and non-incubated controls.

DISCUSSION

From this study it was showed that the accumulation of the plastic wastes be reduced or eliminated in both terrestrial and aquatic environments using these isolated bacteria ***Peanibacillus polymyxa*** and ***Pseudomonas stutzeri***; respectively. One of the advantage of making a comparison with zero starch control (starch free- polyethylene strips) is the ability to identify what effect is due to the addition of different starch types in manufacturing the biodegradable plastic films. However, the comparison of these tested plastic strips with the corresponding non-inoculated controls for each of the different starch type tested give a good indication for the microbial effect on the degradation process, table (1 & 2).

Table (2): Biodegradation of starch-polyethylene plastic films in sediment sample at different incubation periods.

Type Starch	Starch Conc. (%)	Tensile Strength (Kg/cm^2)					Elongation (%)				
		T_0	T_1	T_2	T_3	Mean	E_0	E_1	E_2	E_3	Mean
Control	0	275.4	257.8	181.8	176.0	222.7^{bc*}	233.0	184.4	165.7	160.6	185.9^{b}
Rice starch	2.5	310.1	174.8	149.4	140.1	193.6^{cd}	191.4	194.1	168.3	166.5	180.1^{b}
	5	238.6	142.4	158.8	124.9	166.2^{de}	280.2	276.5	237.2	195.2	247.3^{a}
Corn starch	2	302.4	239.8	196.9	191.1	232.6^{b}	209.3	193.1	138.3	123.2	165.9^{bc}
	3	227.9	290.8	284.3	277.7	270.2^{a}	195.6	155.9	132.2	114.0	149.4^{c}
	4	239.1	176.3	133.1	104.6	163.3^{e}	141.2	108.5	62.7	56.8	92.3^{d}
Potato starch	2	225.6	179.0	113.7	108.1	156.5^{e}	127.6	70.9	57.9	61.0	79.3^{de}
	3	220.9	192.4	144.9	110.8	160.2^{e}	113.0	43.0	23.9	25.9	51.5^{f}
	4	213.1	198.3	183.6	134.3	182.3^{de}	106.4	60.9	56.0	40.2	65.9^{ef}
	10	166.6	166.2	160.5	161.0	168.6^{de}	114.0	43.9	42.8	54.3	63.7^{ef}
Mean		241.9^{a**}	201.8^{b}	170.6^{c}	152.9^{c}		171.1^{a}	133.1^{b}	108.5^{c}	99.8^{c}	

T_0, E_0, = un-incubated strips, T_1, E_1 = incubated strips for 40 days, T_2, E_2 = incubated strips for 70 days, T_3, E_3 = incubated strips for 120 days (each value is a mean value for five measured strips).

Means followed by the same letter are not significant but different letters are highly significant ($P < 0.01$).

* L.S.D. $_{0.01}$ for starch-polyethylene strips (tensile strength T and elongation % E) = 30.24 and 22.73 respectively.

** L.S.D. $_{0.01}$ for incubation period (tensile strength T and elongation % E) = 19.13 and 14.38 respectively.

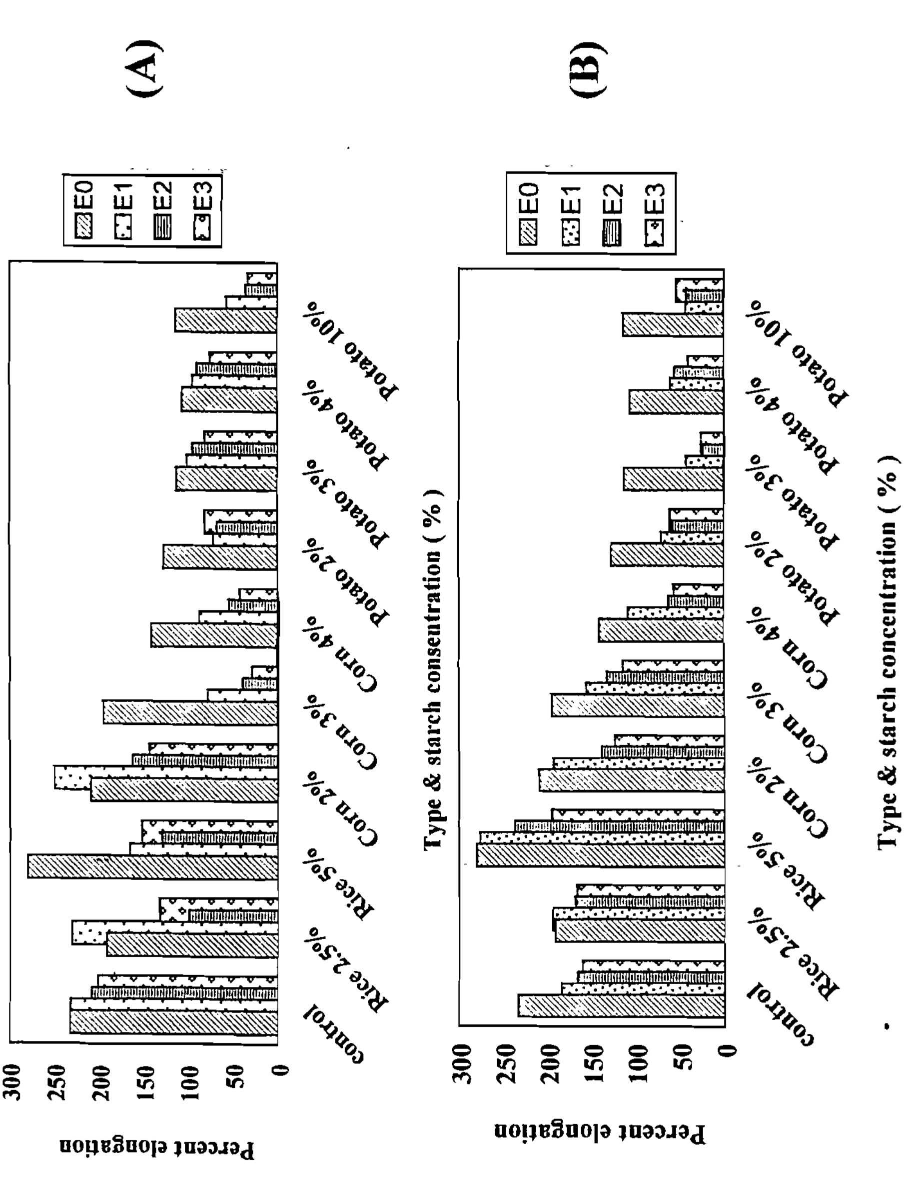

Figure (3) : Changes in elongation percent after 0 (E_0),40 (E_1), 70 (E_2) and 120 (E_3) days of incubation in compost (A) and in sediment (B) samples.The control is starch free-polyethylene plastic strips.Eash bar value represents the mean of five replicates.

Results obtained in this study reveled that the weight loss determinations were inconclusive and it may be due to the bacterial adhesion on the tested plastic films .

Lee, *et al.* 1991, reported that some lignocellulose-degrading bacterial strains (***Streptomyces viridosporus*** , ***Streptomyces badius*** and ***Streptomyces setonii***) showed a reduction in elongation percent values of starch-polyethylene plastic strips and our results were in accordance with that. However, the bacterium ***Paenibacillus polymyxa*** isolated from the compost showed higher reduction in elongation percent (85.5% as in 3% corn starch-polyethylene plastic films) compared with the bacterium ***Pseudomonas stutzeri*** (78.8% as in 3% potato starch-polyethylene plastic films) under the same conditions of incubation. Also, the last bacterium can reduce the elongation percent (31.1%) of the starch free-polyethylene plastic films

The elongation percent increased during the incubation of the plastic strips with 2.5% rice starch in compost sample, or with 10% potato starch in sediment sample treatments. The possible explanation for this unexpected behavior is that the removal of starch granules promotes a slippage of polyethylene strands past each other, thus elongation may occured. Also, the variation in the results of tensile strength or elongation percent may be due to the heterogeneity in starch granules distribution or to instrument malfunctions (Yabannavar and Bartha , 1994) .

The use of starch as a filler in various plastic manufactured items could be considered as a strategy to drive co-metabolic processes (Reich and Bartha, 1977). Moreover, the filler can enhance the adhesion of bacteria to plastic films and thus increase the susceptibility of the polymer to microbial attack (Cacciari, ***et al.***, 1991; Imam and Gould, 1990). So, it was necessary to make a comparison between the different types of manufactured starch –polyethylene available as well as their concentrations that will favour the reduction in the elongation percent and the tensile strength of the tested polyethylene plastic films.

In conclusion, results obtained in this study clearly indicated that the addition of 3.0% corn starch or 3 0 % potato starch in manufacturing of the used polyethylene plastic strips will enhance the biodegradation of these plastic types, by ***Paenibacillus polymyxa*** or ***Pseudomonas stutzeri***, respectively. Of course, this will help to keep our terrestrial and aquatic environment free from plastic accumulation .

A further study could be done to determine the activity of the enzymes involved in the biodegradation process. also the adhesion percent of these degrading bacteria on the tested plastic films could be determined .

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