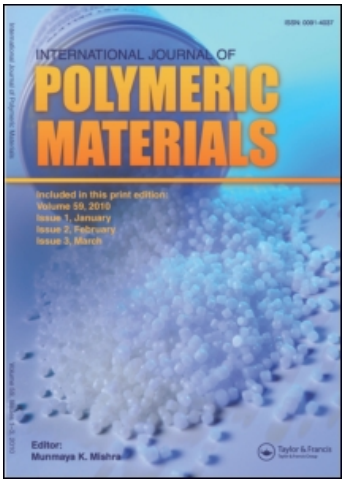

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Biodegradation Study on the Starch-LDPE Film by Soil Micro-organism

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Degradation of low density polyethylene (LDPE) film containing starch as the biodegradable additive has been studied by isolating a soil microbe identified as Pseudomonous species. The degradation of the film was monitored by mechanical property and the surface starch concentration by UV-spectrophotometry at different intervals of time. The degradation depends on the accessibility of starch *i.e.*, the carbon source in the starch-LDPE film. By adding external carbon source like, monosaccharide and disaccharide sugars some changes in the rate of biodegradation was observed. The ultimate fall in tensile strength was higher for the film when exposed to a nutrient medium without any external carbon source. Mostly the added sugar helps in the growth of micro-organisms. Among the various external carbon sources, maltose was found to be the best. In case of maltose the loss of tensile strength was 50% in 58 days, but the film without additional sugar showed a fall in tensile strength of 48% in the same period.

Keywords: Biodegradable film; starch; LDPE; microbes

INTRODUCTION

Pollution by waste polymeric materials creates a great problem because an appreciable percentage of municipal waste consists of

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polymeric substances. This is due to the fact that synthetic polymers, *e.g.*, LDPE, do not rust or not. The disposal by degradation of these polymeric wastes to harmless and useful compounds is a challenging task to combat pollution [1]. The extent of degradation of polymers depends on the nature of chemical processes involved. Again, the degradation of polymers may be carried out by photochemical, thermal and biodegradation process. Out of these processes biodegradation is much more simple, economic and occurs in actual conditions of waste disposal [2, 3].

The main attacking agents in biodegradation are micro-organisms *e.g.*, actinomycetes, fungi, bacteria *etc.*, which are widespread in soil, water and air. Carbon, hydrogen, oxygen, nitrogen are the basic nutritive elements for their growth and metabolic activities. Biodegradable polymers provide the nutrient source for these micro-organisms.

Virgin synthetic polymers are generally resistant to biodegradation due to their limited water absorption, (*e.g.*, LDPE shows water absorption $< 0.2\%$) [4], their special chemical structure, and specially by the presence of a large variety of additives like pigments, plasticizers, fillers, antioxidants, flame retardants *etc.*, some of which prove toxic to microbes.

Natural polymers *e.g.*, natural rubber, starch, cellulose, gums *etc.*, on the other hand, are biodegradable. Starch is one of the most abundant and low cost natural polymers present in plants. Effective incorporation of starch would extend the use of LDPE as well as confer biodegradability on the LDPE film.

The microbial assimilation of the natural component such as starch in the LDPE-starch blend is expected to cause both a failure of the physico-mechanical properties of the polymer blend and facilitate the bio-chemical action of the exposed surface of the blend film [5]. Goheen *et al.* [6] reported the degradation of starch based polyethylene film in the soil and monitored the degradation by estimation of the removal of starch and chemical changes of the matrix using FTIR spectroscopy. From spectral data it was evident that the starch was effectively removed from the starch-LDPE matrix over a period of 8 months under soil burial. The degradation was same in different soils, although they contained different amounts of organic matter.

Although IR analysis shows the effective removal of starch, it did not show the significant change in the polyethylene matrix. In the IR spectra the removal of starch from LDPE matrix is related to the decrease in the area of the C—O stretching band. From their study it was evident that the starch was effectively removed from the starch-LDPE matrix, but the LDPE matrix remained unchanged over a period of 8 months under soil burial.

Sung *et al.* [7] reported the degradation of the starch-LDPE film incorporating some prooxidant additives [8, 9] in the formulation. These pro-oxidants promote the oxidative degradation of the polyolefin. The accelerated biodegradation of starch in the film was investigated using some enzymes. The oxidative degradation of polyethylene was done by incubating the enzyme treated starch-polyethylene film in a forced air oven at 70°C. The progress of degradation was monitored by measuring physical and chemical changes. The films in the absence of the pro-oxidant additive did not show any change in the physical property significantly. Albertsson *et al.* [10] also reported the degradation of starch-LDPE films containing some transition metal salt as a pro-oxidant in the film formulation with bacteria or fungi in aqueous medium at ambient temperature. Regarding mechanism of degradation they proposed that the salt-water medium containing trace elements triggered auto-oxidation of the pro-oxidant through decomposition of hydroperoxide of the salt which catalyzed the auto oxidation of the pro-oxidant as well as auto-oxidation of the LDPE matrix.

We have developed first a laboratory scale biodegradable film [11], and later we successfully completed commercial trial production of a LDPE-starch based film using a modified formulation and procedure with the help of Indian Petrochemicals Corporation Ltd. [12], in which starch is blended with LDPE by using a novel proprietory coupling system. In this communication the biodegradability of the LDPE-starch film has been studied by isolating a soil microbe identified as Pseudomonous species. The properties of the film after degradation were also compared with those of the control set. We have also tried recently to modify the film for application in other areas of commercial application [13, 14].

EXPERIMENTAL

Micro-organism

Pseudomonas species (Sp.) isolated from the local soil of IIT campus, Kharagpur was maintained in a 2% nutrient agar slant.

Chemicals

The chemicals used for this study were all analytical grade.

Starch-based low density polyethylene film has been developed in collaboration with IPCL, Baroda and used as such. Sodium nitrate (SD, India), potassium chloride (SD, India), magnesium sulfate (E. Merck, India), potassium di-hydrogen orthophosphate (E. Merck, India), glucose (E. Merck, India), raffinose (E. Mark, India), maltose (LOBA, India), sucrose (E. Merck, India), urea (SD, India), fructose (E. Merck, India) were used as received.

DEGRADATION PROCEDURE

The study on biodegradation of starch-based films was carried out by three different experiments.

Experiment 1

The biodegradation of the starch-based films was performed in different petriplates by varying the different concentrations of carbon and nitrogen sources. The effect of carbon and nitrogen sources on biodegradation of the films was studied as follows:

1A: Effect of Carbon Sources on Biodegradation

A nutrient medium (Czapek dox) was selected which has got the following composition: 2.5 g/L $NaNO_3$, 0.5 g/L KCl, 0.5 g/L $MgSO_4$, 1 g/L KH_2PO_4. A small piece (40 mm $\times$ 35 mm) of the starch-based film and 10 ml nutrient solution were taken in each petriplate. To

evaluate the effect of additional carbon source on biodegradation, 1% of the following sugars like glucose, fructose, raffinose, maltose, mannose, manitol, lactose, sucrose, cellobiose, ribose and xylose were added individually in different petriplates respectively. The effect was compared with a control where no additional carbon source was added except film sample and 10 mL nutrient medium. Finally, all these petriplates were inoculated with the isolated microorganism and kept in the humidifier cabinet for 1 month at 32°C, 93% relative humidity and 5.5 pH. The growth of the micro-organism was studied.

1B: Effect of Nitrogen Sources on Biodegradation

Exactly in the same way another set of experiment was designed where only different nitrogen sources (1%) were used keeping all other parameters constant as mentioned earlier. Different nitrogen sources selected for this study were urea, $NaNO_3$, NH_4Cl, ammonium oxalate. $Mg(NO_3)_2$, casein and glycine. After inoculation the petriplates were kept in the humidity cabinet at 32°C, 93% relative humidity and 5.5 pH.

Experiment 2

The identification of the soil bacteria size, shape and the growth was studied under microscope, which are responsible for the degradation of the film [15].

Experiment 3

The assessment of biodegradation and comparison of the starch based plastic films were done by measuring the standard physical property such as tensile strength, or by spectrophotometric analysis of the starch content of the film *etc.* The composition of the four connical flasks containing the better carbon sources and soil are shown in Table I. These four connical flasks were inoculated with the bacterium and incubated at 32°C, 93% humidity and 5.5 pH for 6 weeks during which the samples were removed at different intervals for analysis.

TABLE I Composition of different conical flask for biodegradation study

Connical Flask No.	*Wt. of the film sample taken (g)*	*Nutrient Medium added (mL)**	*Soil added (g)*	*Sugar added (g)*
1	1.44	100	10	–
2	1.42	100	–	–
3	1.44	100	–	1[a]
4	1.44	100	–	1[b]

* The composition of the neutrient medium is same as stated in the Experimental Section, Experiment 1.
[a] Raffinose;
[b] Maltose.
Inoculum concentration for each experiment is 2.5×10^8 per ml.

TENSILE STRENGTH MEASUREMENT

The tensile strength of the film samples was measured in an Instron Universal Testing Machine (Instron, Table Model TM-M), as per ASTM D638-71 at different time intervals.

SPECTROPHOTOMETRIC ANALYSIS

The starch contents on the surface of the film at different time intervals were measured by measuring the optical density of the starch-iodine complex spectrophotometrically. The details of the procedure have been reported elsewhere [16].

RESULTS AND DISCUSSION

The growth of bacteria/fungus in different petriplates for Experiment 1A is presented in Table II. The growth of bacteria/fungus in different petriplates for Experiment 1B is presented in Table III.

The most common method for assessing the degradation of plastics materials is by measuring the changes in their mechanical properties. The change in tensile strength of the starch-LDPE film under different experimental conditions is presented in Figure 1.

It is evident that the initial tensile strength of the starch based films reduced to half within 30 days of inoculation with pseudomonous species bacteria in presence of the nutrient Czapek dox solution. Sung *et al.* [7] also reported that the starch-LDPE films containing only some pro-oxidant additives undergo reduction in tensile strength to

TABLE II The effect of additional sugar (carbon source) in the film and Czapek dox medium on the growth of bacteria/fungus

Added C-source sugar	*Growth of bacteria*	*Growth of fungus*
Glucose		+ + +
Fructose		+ + +
Raffinose	+	+ +
Maltose		+ + +
Mannose		+ + +
Manitol	+ +	+ +
Lactose	+ +	
Sucrose	+ +	
Cellobiose	+ +	+
Ribose	+ +	
Xylose	+ +	
Controlled biodegradable film	+ +	

+ + + vigorous, + + moderate, + slow.

TABLE III The effect of additional nitrogen source in the film and Czapek dox medium on the growth of bacteria/fungus

Added N-source	*Growth of bacteria*	*Growth of fungus*
$NaNO_3$		
Urea	+	+
Ammonium	+ +	
Oxalate		
NH_4Cl	+	
$Mg(NO_3)_2$		+ + +
Casein	+ +	
Glycine	+	

+ + + vigorous, + + moderate, + slow.

half of the initial value after 30 days of heat treatment at 70°C. But the films without pro-oxidant did not change mechanical property under the same treatment. The presence of the pro-oxidant in the film catalyses the oxidative degradation of polyethylene at 70°C. The film thus degraded was subjected to hydrolysis by enzyme Bacillus sp. α-amylose for degradation of the starch content of the film. Actually they study the overall mechanism of biodegradation. But our study is different; here the mixed cultured bacteria of pseudomonous species are responsible for effective degradation of starch in the starch-LDPE film. The degradation of starch weakens the bond strength in the polyethylene chain at different sites. These overall degradation effects are responsible for lowering of the tensile strength.

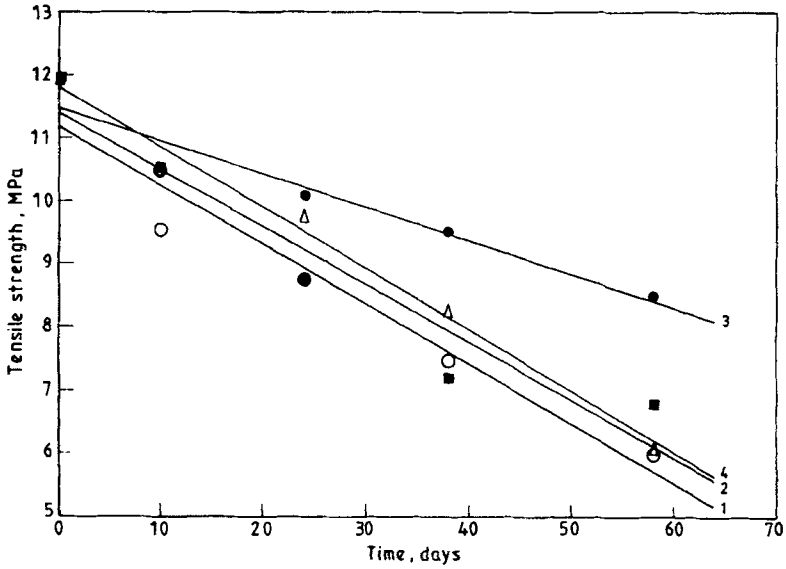

FIGURE 1 The tensile strength of the starch-LDPE films at different incubation period at room temperature (30°C) under different conditions: Curve 1. Control; Curve 2. in presence of maltose; Curve 3. in the soil, and Curve 4. in presence of raffinose. The standard deviation values (σ) for Curve 1 (0.713), for Curve 2 (0.635), for Curve 3 (0.287) and for Curve 4 (0.392).

Carbon, hydrogen, and oxygen are the essential elements for the growth as well as metabolic activities of bacteria which may degrade the starch in the LDPE-starch film at a faster rate. So we investigate the degradation effect by adding some external sugar. From our experiment it has been found that although the bacterial growth is maximum with raffinose and maltose sugar (Fig. 2), the rate of decrease of tensile strength is not sharp in these cases. It is possible that the bacterium grows at a faster rate with the available added sugar without hydrolyzing the starch present in the LDPE matrix [15].

The growth of bacteria is very much dependent on temperature and concentration [17]. In order to observe the effect of temperature and concentration of the bacterium on the bacterial growth in presence of the starch-LDPE film and Czapek dox nutrient medium, we have measured the growth of bacteria by monitoring the optical density [17]

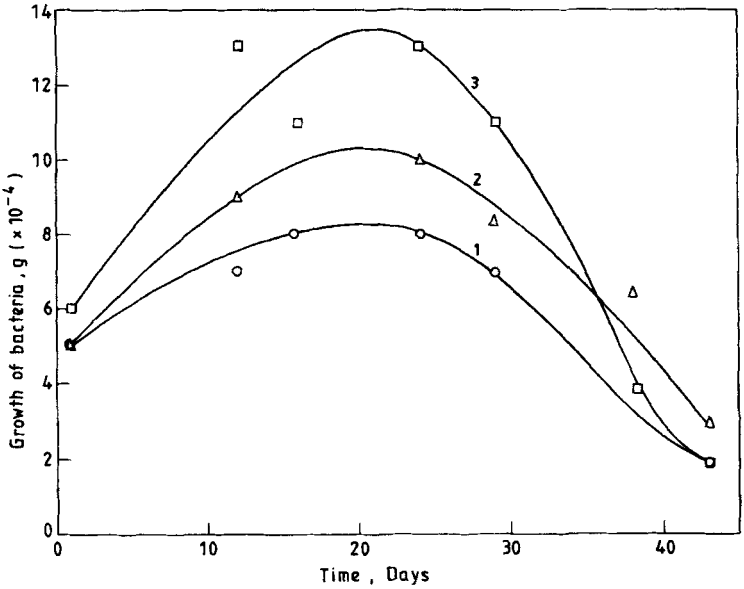

FIGURE 2 The growth of bacteria on the starch-LDPE film under different experimental conditions: Curve 1. in absence of sugar, Curve 2. in presence of maltose, and Curve 3. in presence of raffinose.

of the solution at different intervals of time and at different temperatures. The plot of optical density against time interval and at different temperatures is presented in Figure 3.

The growth of the bacterium at different concentrations of bacterium inoculation was also monitored by gravimetric analysis. The plot of bacterial growth by dry weight against time interval at different inoculum concentrations is presented in Figure 4.

The growth of bacterial population is normally limited either by the exhaustion of available nutrient or by the accumulation of toxic products of metabolism. Here the Czapek dox solution and the starch present in the film are the only nutrient medium for the bacterium. Moreover, the starch is not readily available as it is present in the LDPE matrix. So the bacterial population in the stationary phase is very low. As a result, the bacterial cells are held in a nongrowing state and eventually die *i.e.*, the death phase is the most prominant in this case.

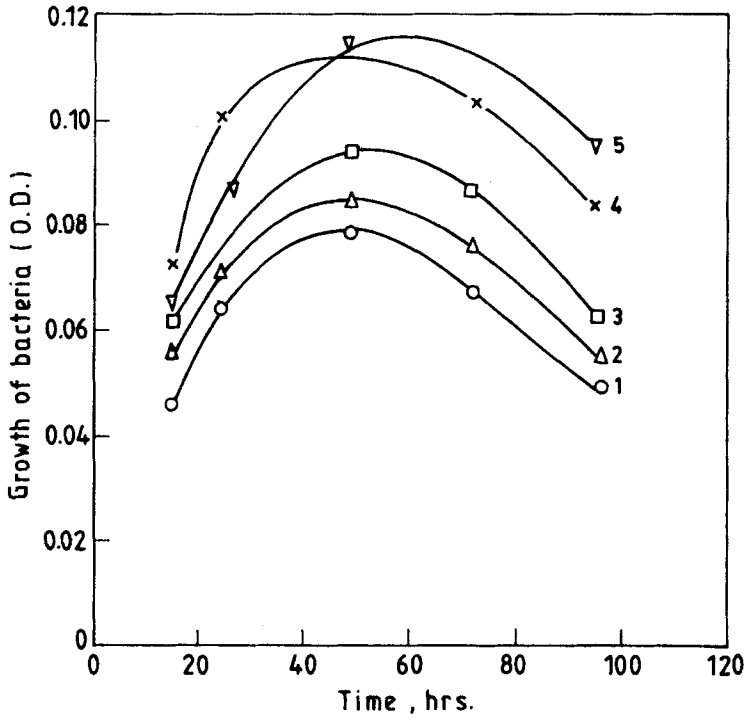

FIGURE 3 The growth of bacteria on the starch-LDPE film under different temperature: Curve 1. for 20°C, Curve 2. for 25°C, Curve 3. for 45°C, Curve 4. for 30°C and Curve 5. for 37°C.

The change in the starch content on the surface of the film during biodegradation is monitored by measuring the optical density of the starch-iodine complex. The variation of starch content in the film with time is presented in Figure 5. The starch content in the film decreases initially at a very faster rate and attains almost a constant value after 24 days. Here initiation for degradation by microbial agents in the soil occurs within 8 to 10 days.

Goheen *et al.* [6] reported that the starch is removed initially from the blends of starch-LDPE film at a faster rate by IR-analysis. Albertsson *et al.* [10] also reported that the starch is removed initially at a faster rate. Finally, they conclude that the starch was consumed upto 48% during the first year when inoculated with bacteria in aqueous media. In the starch-LDPE film most of the starch particles

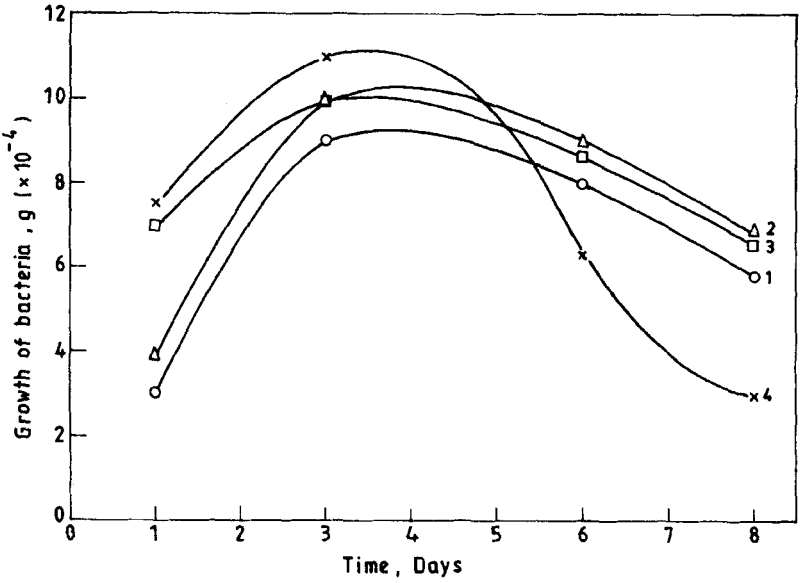

FIGURE 4 The growth of bacteria on the starch-LDPE film under different dose of bacteria. Inoculum concentration 2.5×10^8 per ml: Curve 1. for 1 ml, Curve 2. for 2 ml, Curve 3. for 3 ml and Curve 4. for 4 ml.

are either embedded in or is completely covered with polyethylene leaving only a few uncoated reactive sites. They are responsible for the attack of microorganisms. After 24 days the starch molecules present on the surface of the film are almost completely hydrolyzed by the soil bacteria.

CONCLUSION

The most common test for assessing biodegradability of polymers is the soil burial test. Though this technique is much more simple but there are some limitations to its reproducibility due to climatic factors and lack of the control over microbe populations in the soil. An attempt has been made to establish a systematic study on biodegradation of the plastics film under controlled environment. The results obtained are quite encouraging and the initiation for degradation by

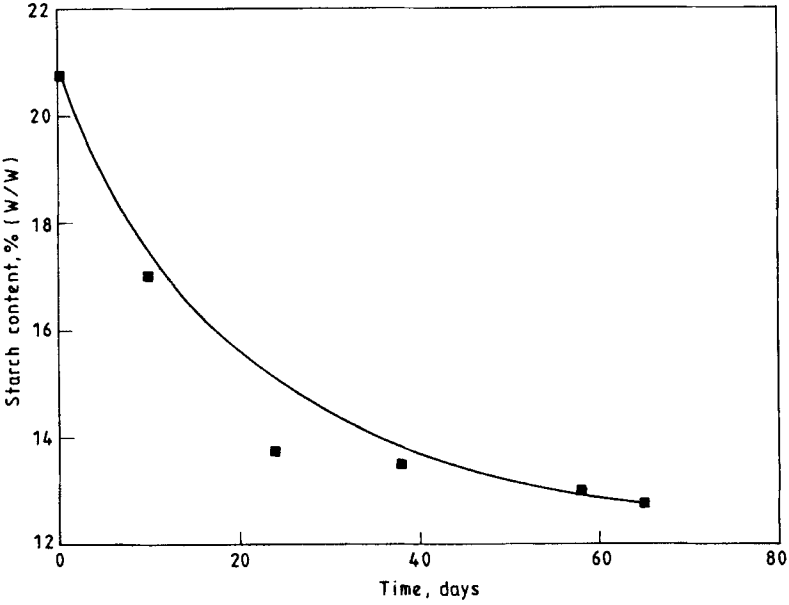

FIGURE 5 The variation of starch content in the film in presence of pseudomonous species bacteria and nutrient Czapek dox medium at different incubation period.

microbial agents in the soil occurs within 8 to 10 days. This has not been reported earlier.

References

[1] Kumar, G. S., Kalpagam, V. and Nandi, U. S. (1982–83), *J. Macromol. Sci. Rev. Macromol. Chem. Phys.*, **C22**(2), 225.
[2] Huang, S. J. (1985). in '*Ency. Polym. Sci. Eng.*', 2nd edn. Vol. 2, Mark, H. F. and Kroschwitz, J. L. (Eds.), Wiley, New York, pp. 220–243.
[3] Huang, S. J. (1989). in 'Biodegradation, in *Comprehensive Polymer Science*', **6**, Allen, G. and Bevington, J. C. (Eds.), Pergamon Press, Oxford, pp. 597–606.
[4] Harper, C. A. (Ed.), (1975). 'Handbook of Plastics and Elastomers', Mc. Graw Hill Book Company, New York, pp. 1–26.
[5] Kohan, M. I. (1973). Nylon Plastics, Wiley and Sons, New York.
[6] Goheen, S. M. and Wool, R. P. (1991). *J. Appl. Polym. Sci.*, **42**, 2691.
[7] Sung, W. and Nikolov, Z. L. (1992). *Ind. Eng. Chem. Res.*, **31**, 2332.
[8] Griffin, G. J. L., Biodegradable synthetic resin sheet material containing starch and a fatty acid material, U.S. Pat. No. 4016117.
[9] Griffin, G. J. L., Synthetic resin sheet material, U.S. Pat. No. 4021388.
[10] Albertsson, C., Barenstedt, C. and Karlsson, S. (1994). *J. Appl. Polym. Sci.*, **51**, 1097.

[11] Nanda, S. K., Chakravorty, A. and Maiti, S. (1990). *J. Polym. Mater.*, **7**, 331.
[12] Maiti, S. and Jana, T. (1994). Development of Biodegradable Film, Technical Report, IPCL, Baroda, India.
[13] Jana, T. and Maiti, S. (1997). *J. Polym. Mater.*, **14**, 367.
[14] Jana, T. and Maiti, S., *Angew. Makromol. Chem.* (Communicated).
[15] Talaro, K. and Talaro, A. (1993). Foundation in Microbiology, W.C.B. Publishers, New York, pp. 1–94.
[16] Jana, T. and Maiti, S., *Indian J. Chem.* (Communicated).
[17] Stainer, R. Y., Ingraham, J. L., Wheelis, M. L. and Painter, P. R. (1987). General Microbiology, Macmillan, Hong Kong, pp. 183–189.