

Studies on Degradation of synthetic polymer Nylon 6 and Nylon 6, 6 by *Pseudomonas aeruginosa* NCIM 2242

Chonde Sonal G¹., Chonde Sachin G². and Raut P. D¹.

¹Department of Environmental Science, Shivaji University Kolhapur, Maharashtra

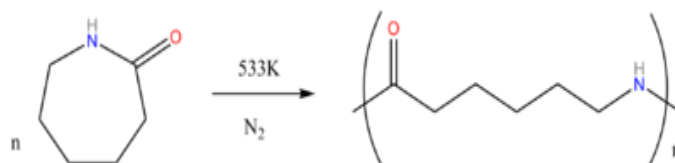
²Department of Chemistry, BAMU Aurangabad, Maharashtra

Abstract: Polymer is a chemical which is characterized by having extremely long molecules. This tends to give polymers useful properties such as flexibility and elasticity. Nylon is a generic designation for a family of synthetic polymers known generically as polyamides. It is a polymer having amide group. Present study has examined the feasibility of a bacteria *Pseudomonas aeruginosa* NCIM 2242 for actively degrading synthetic polymer Nylon 6 and Nylon 6, 6. For present experiment Nylon 6 and Nylon 6, 6 was the sole source of nitrogen in the medium. The analysis was carried out using I. R. spectroscopy and mechanical techniques. Nylon sheets were inserted in fermentation broth which was incubated on a rotary shaker at 30°C and 90 rpm for 6 month duration. Substantial degradation of Nylon 6 and Nylon 6, 6 was observed as the bacteria utilize nitrogen source from polymer. Weakening and breaking of polyamide bond have been confirmed by weakening in I. R. band. The degradation led to formation of new functional groups CH₃, CHO and COOH. It was observed that Nylon 6, 6 having more degradation than Nylon 6.

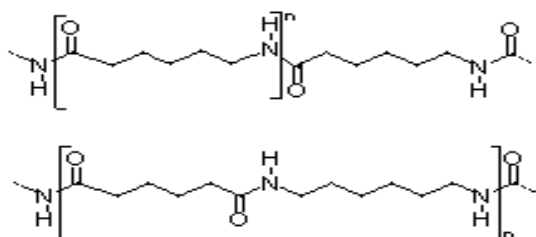
Keywords: Nylon 6 degradation, *Pseudomonas aeruginosa*, Plastic waste management.

I. Introduction

It is well known that the progress of human society was influenced by the development of man-made polymers. Polymer is a chemical which is characterized by having extremely long molecules. This tends to give polymers useful properties such as flexibility and elasticity. Research on degradable synthetic polymers begins in the early 1980's. The recognized lack of biodegradability of large volume of commercial polymers, particularly commodity plastics used in packaging (e.g. fast food), industry and agriculture, focused public attention on a potentially huge environmental accumulation and pollution problem that could persist for centuries.(Yüksel *et.al.*,2004). Biodegradation is therefore considered as a subset of degradation, which is defined here as any physical or chemical change in a material caused by any environmental factor, including light, heat, moisture, wind, chemical conditions or biological activity. One of the positive characteristics of plastic is the fact that it is durable. Unfortunately, this is not a positive characteristic when it comes to the environment. Polymers bearing recurring amide groups in their backbone are defined as polyamides. Polyamide types of polymer have been considered as a long standing existence. Therefore find, diversifies applications by all commodities. Nylon 6 and Nylon 6, 6 one of the polyamide polymers finds its application in the bristles, ropes, fabrics, fishing nets, automobile parts etc. Nylon 6 is a polymer obtained by ring – opening polymerization of ϵ caprolactam and has several commercial names including perlon, nylon and steelon. (Szostak *et. al.*, 2004).



Formation of Nylon 6 from ϵ caprolactam



Nylon 6 (above) has a structure similar to Nylon 6, 6 (below)

Biodegradation of polymers is seen as one of the solution for current plastic waste management problems. By definition biodegradable polymers are those that are degraded into carbon dioxide, water and biomass as a result of the action of living organism or enzymes. A degradation of polymers may proceed by one or more mechanisms, including microbial degradation in which microorganisms such as fungi and bacteria consume the material. The rate of biodegradation may vary substantially and depends on the molecular structure, morphology, surface area etc. (Ursa *et al.*, 2003). Among polymer category polyamide types of polymer have been considered as a long standing existence.

Therefore, find diversifies applications by all commodities. Polymers bearing recurring amide groups in their backbone are defined as polyamide. Aliphatic polyamides, such as Nylon 6, 6 and Nylon 6 have been produced industrially since the late 1930s and continue to be important materials. (Jozefa *et al.*, 2007). Due to its strong intermolecular cohesive force caused by hydrogen bonds between molecular chains of nylon, the rate of degradation is less compared to polyesters (Tomita *et al.*, 2003). Nylon 6 monomer is metabolized by numerous microorganisms, including the bacterial genera *Pseudomonas*, *Achromobacter* and *Corynebacterium* and fungal genera *Bjerkundera adusta*. (Mohee R. *et al.*, 2007) So far, several investigations on nylon degradation using microorganisms have been reported. Temita K. in 2003 reported thermophilic bacterium degradation of some kinds of nylon.

In this paper we report on nylon biodegradation using bacteria *Pseudomonas aeruginosa* NCIM 2242 in a submerged cultivation using nitrogenous nutrient broth as stimulator. The extent and nature of degradation of the polymer was evaluated using several physical and chemical measurements namely, weight reduction tests, Fourier Transform Infrared spectroscopy (FTIR).

II. Material and Methods

A. Materials

Commercial grade Nylon 6 was provided by Sigma Aldrich. The material is in the form of pellet. All chemicals and solvents used in all experiment were AR grade. A thin sheet of Nylon 6 was prepared from Nylon 6 pellets by melting and pressing the pellets of Nylon 6.

B. Selection of Microorganism

The bacterium selected in the study was a *Pseudomonas aeruginosa* NCIM 2242 that is well known for its biodegradation activity. The bacterial strain of *Pseudomonas aeruginosa* NCIM 2242 was obtained from the National Collection of Industrial Microorganisms (NCIM) NCL, Pune, Maharashtra, India.

C. Sterilization of the sample

The sample sheets were sterilized before they were inoculated into the test medium. The nylon sheets were dipped in absolute alcohol for a few hours. Washed with distilled water and later dried. No physical or chemical changes were observed in the sample sterilization treatment.

D. Submerged Cultivation Procedure

A sheets of Nylon 6 and Nylon 6, 6 were exposed to submerged cultivation process. Microbial degradation was performed in Erlenmeyer flasks in shaker condition. Each flask containing small sheet of Nylon 6 and Nylon 6, 6 and 100 ml of the liquid glucose – salt medium at a pH 6.25. The medium contained per liter of deionised water, 10 gm of glucose, 1 g KH_2PO_4 , 0.5 gm of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.5 gm of $(\text{NH}_4)_2\text{SO}_4$ and 0.1 gm of CaCl_2 . Aliquots (100 ml) of medium were poured into 500 ml Erlenmeyer flask and sterilized in an autoclave for 20 min at 121 °C and 1.2 atm, after cooling the medium was inoculated with 5 ml of the fungi spore suspension obtained by suspending spores from one agar slant tube in 20 ml sterile water. The fermentation broth was incubated on a rotary shaker at 30 °C and 90 rpm. Nylon 6 and Nylon 6, 6 was the sole source of nitrogen in the medium. Degradation was left to proceed for a period of six month.

III. Results and Discussion

Degradation of Nylon 6 and Nylon 6, 6 sheets in submerged cultivation using *Pseudomonas aeruginosa* NCIM 2242 was carried out six month. Parallel to samples inoculated with the fungus (biotic sample) blank experiment was carried out with Nylon 6 and Nylon 6, 6 sheets placed equally composed media without the bacterial culture (a biotic sample). Results presented in the following sections were obtained from two independent sets of degradation experiments.

A. Morphological Study

Degradation of sheets of Nylon 6 and Nylon 6,6 sheets was observed by measuring thickness of sheets and weight reduction of sheets as it is treated with selected bacteria. The color of sheets becomes brownish and surface morphology become rough when sheets are exposed to bacteria.

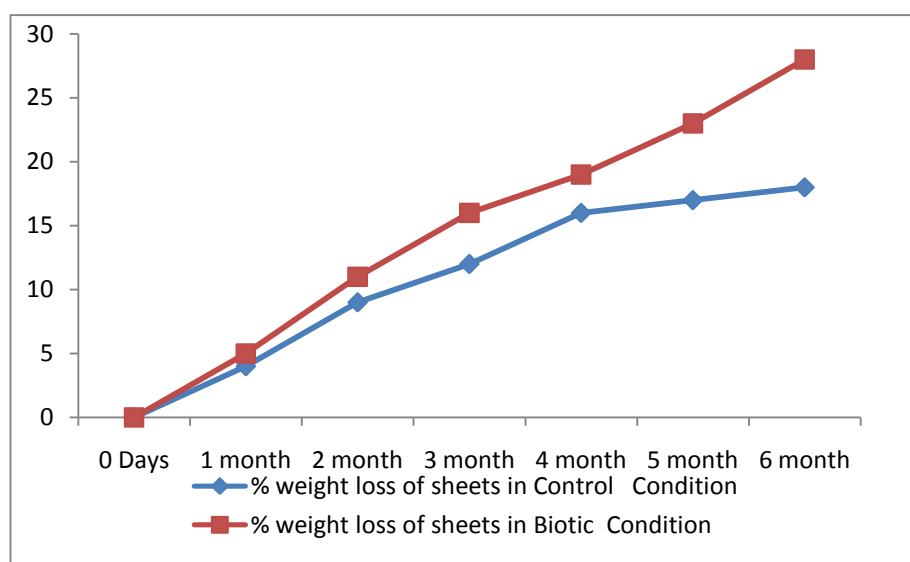
Table No. 1. Percentage (%) Weight loss of Nylon 6 by treating with *Pseudomonas aeruginosa* NCIM 2242

Sr. No.	Time Duration	% weight loss of sheets in Control Condition	% weight loss of sheets in Biotic Condition
1	0 Days	0	0
2	1 month	4	5
3	2 month	9	11
4	3 month	12	16
5	4 month	16	19
6	5 month	17	23
7	6 month	18	28

Table 2: Percentage (%) Weight loss of Nylon 6,6 by treating with bacteria *Pseudomonas aeruginosa* NCIM 2242.

Sr. No.	Time Duration	% weight loss of sheets in Control Condition	% weight loss of sheets in Biotic Condition
1	0 Days	0	0
2	1 month	2	6
3	2 month	6	10
4	3 month	8	18
5	4 month	11	25
6	5 month	13	28
7	6 month	14	31

Graph No. 1. Percentage (%) Weight loss of Nylon 6 by treating with *Pseudomonas aeruginosa* NCIM 2242



Graph No. 2. Percentage (%) Weight loss of Nylon 6, 6 by treating with *Pseudomonas aeruginosa* NCIM 2242.

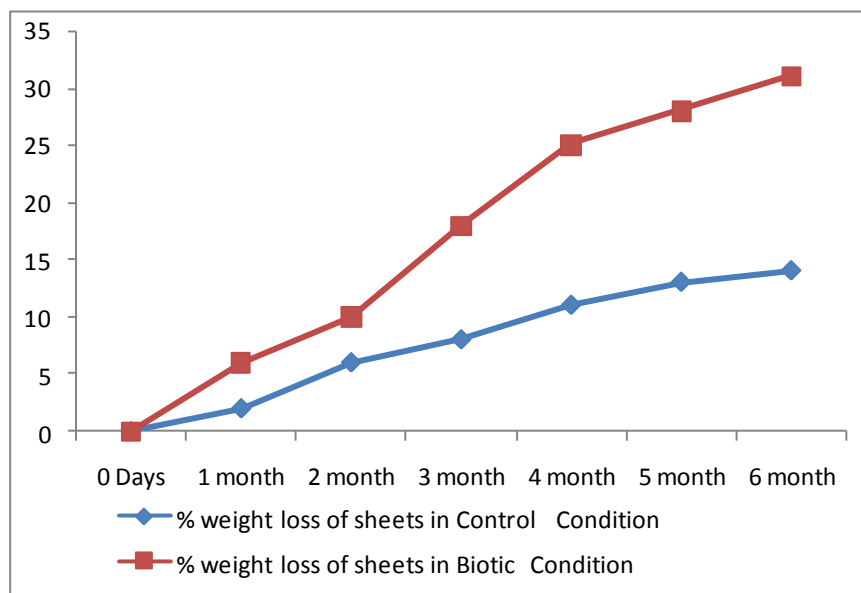


Table 3. Percentage (%) Thickness reduction of Nylon 6 by treating with *Pseudomonas aeruginosa* NCIM 2242

Sr. No.	Time Duration	Percentage (%) Thickness reduction Control	Percentage (%) Thickness reduction of Sample (Biotic)
1	0 days	0	0
2	1 Month	4	11
3	2 Month	10	20
4	3 Month	14	28
5	4 Month	19	37
6	5 Month	24	40
7	6 Month	30	46

Graph No. 3. Percentage (%) Thickness reduction of Nylon 6 by treating with fungi *Pseudomonas aeruginosa* NCIM 2242

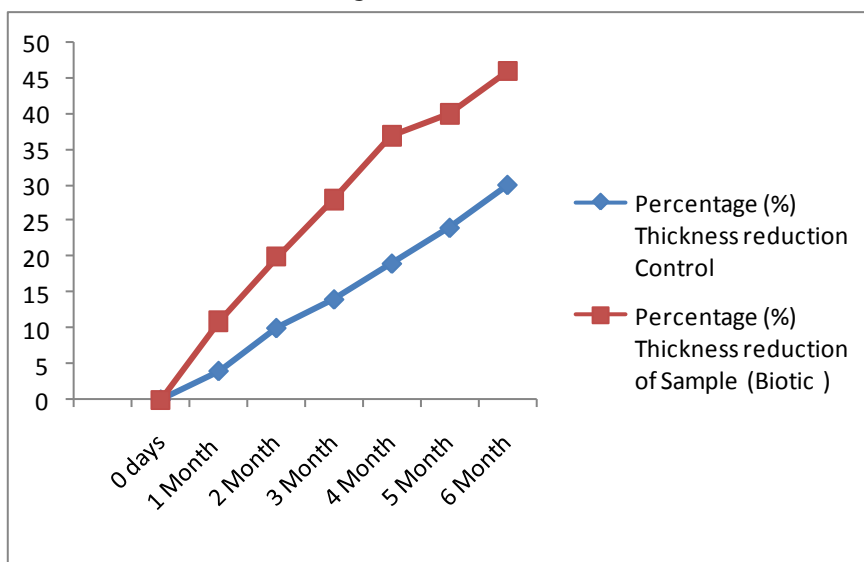
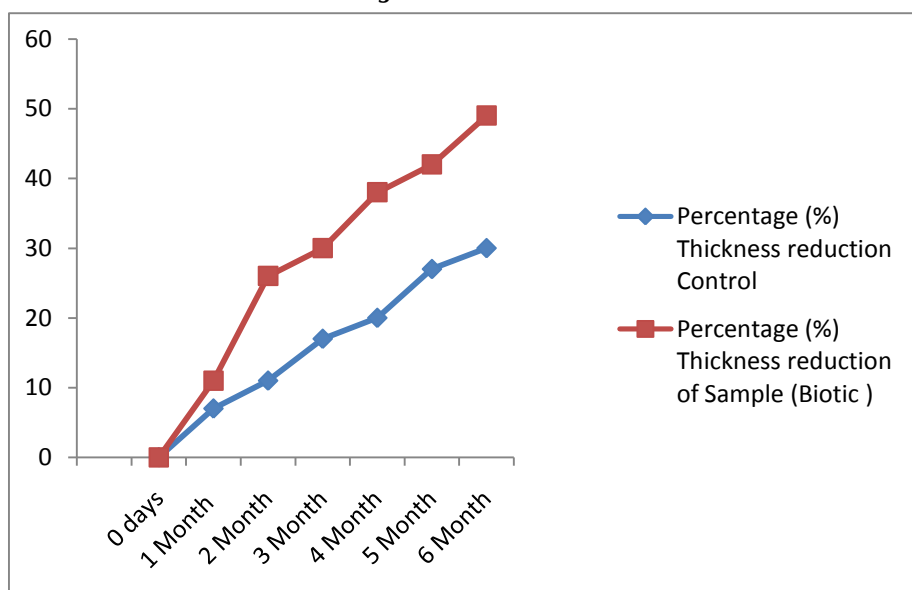


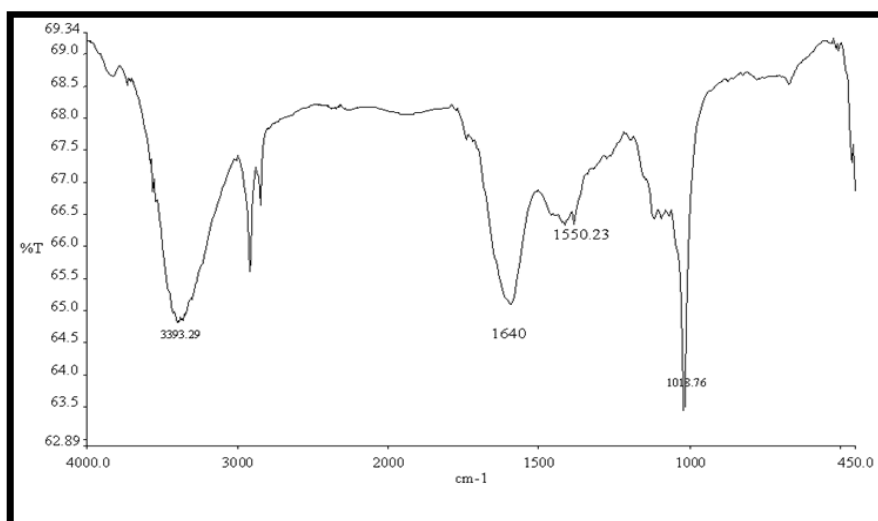
Table No. 4. Percentage (%) Thickness reduction of Nylon 6, 6 by treating with *Pseudomonas aeruginosa* NCIM 2242

Sr. No.	Time Duration	Percentage (%) Thickness reduction Control	Percentage (%) Thickness reduction of Sample (Biotic)
1	0 days	0	0
2	1 Month	7	11
3	2 Month	11	26
4	3 Month	17	30
5	4 Month	20	38
6	5 Month	27	42
7	6 Month	30	49

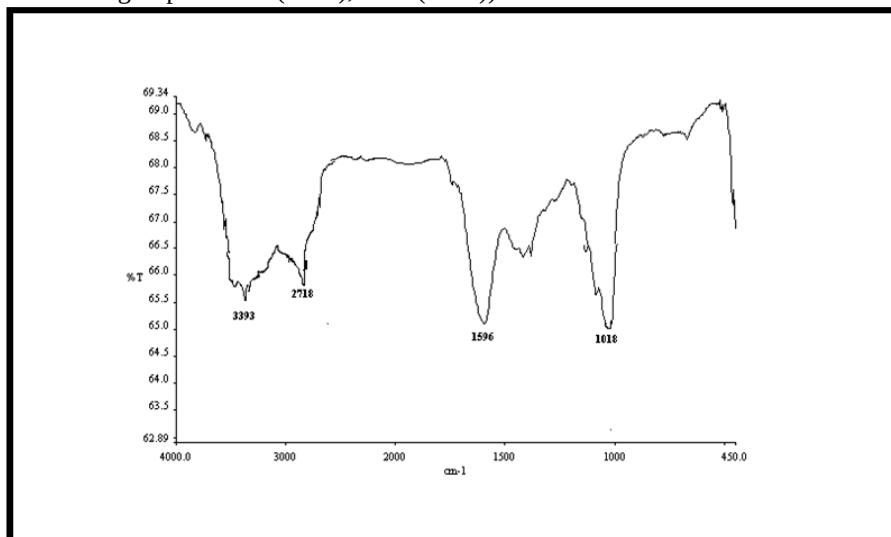
Graph No. 4. Percentage (%) Thickness reduction of Nylon 6 , 6 by treating with *Pseudomonas aeruginosa* NCIM 2242



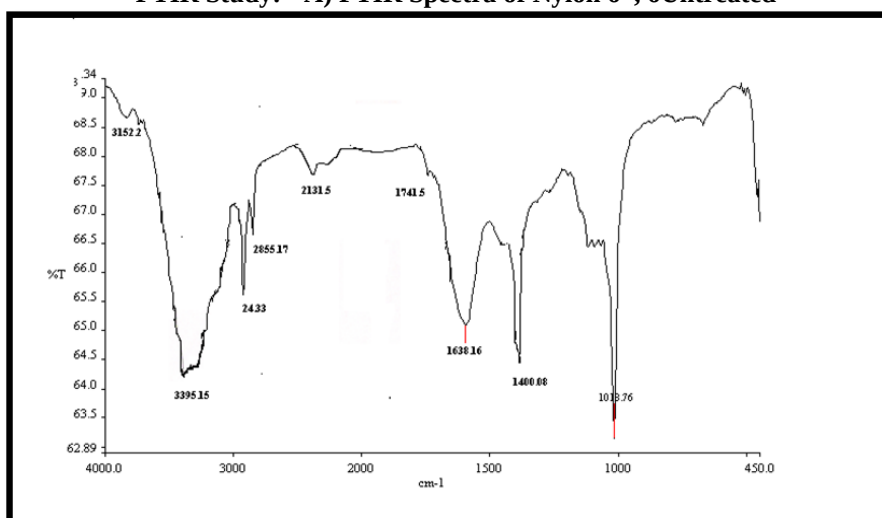
FTIR Study - A FTIR Spectra of Nylon 6 Untreated



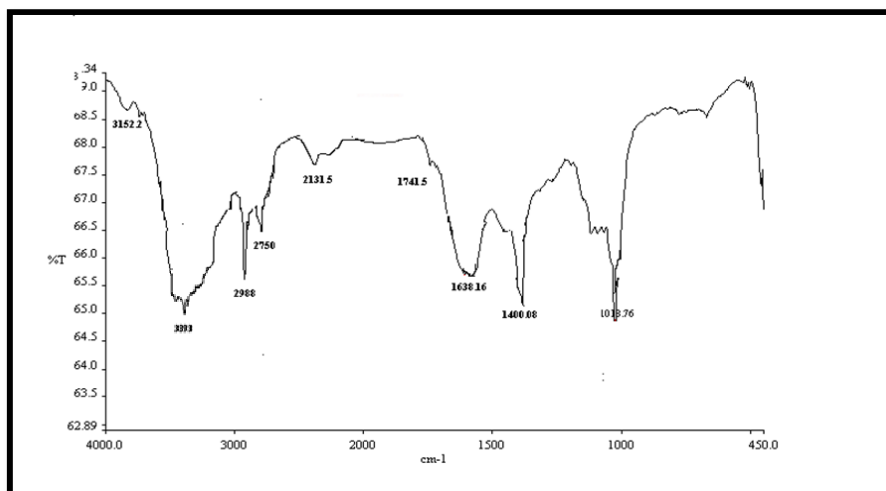
FTIR Spectra of Nylon 6 (B) *Pseudomonas aeruginosa* NCIM 2242 treated for 6 month (the strength of Characteristic bands of C (O) NH occurring around 3300, 1640 and 1550 cm^{-1} decreased after 90 days) Also shows formation of new groups as CH_3 (2922), CHO (2700)).



FTIR Study: - A) FTIR Spectra of Nylon 6 , 6Untreated



FTIR Spectra of Nylon 6 , 6(B) *Pseudomonas aeruginosa* NCIM 2242 treated for 6 month (the strength of Characteristic bands of C (O) NH occurring around 3300, 1640 and 1550 cm^{-1} decreased after 90 days) Also shows formation of new groups as CH_3 (2922), CHO (2700)).



B. Weight Loss

The partially degraded polymer sheets were removed from medium, washed with distilled water and dried. The amount of degradation was determined by studying weight loss of sheets. From Table no 1 which shows that the initial weight of the sample sheet was reduced very less within 15 days. The percentage weight loss observed in Nylon 6 within six month was 28% in biotic condition and 18% at control condition. Also the Nylon 6, 6 shows 31% weight loss at sample and 14% at control condition.

C. Decrease in thickness

Thickness of Nylon 6 sheets were observed by micrometer. The initial thickness of nylon sheet sample was 0.117 mm. Sheets which were treating with *Pseudomonas aeruginosa* NCIM 2242 show maximum thickness decrease Nylon 6, 6 as compare to Nylon 6. Nylon 6 sheets shows 46 percentage thickness reduction and Nylon 6, 6 shows 49 percentage thickness reduction.

D. FTIR Study of Nylon 6 :- (Fourier transforms Infrared Spectroscopy)

The functional groups of Nylon 6 mediated with selected bacteria *Pseudomonas aeruginosa* NCIM 2242 was determined by using FT-IR spectroscopy (at Room Temp.) The strength of characteristic bands of C (O) NH occurring around 3300, 1640, 1550 and 1018 cm⁻¹ decreased after six months. Formation of new groups like CH₃, CONH₂, CHO and COOH, may be formed due to hydrolysis and oxidation.

IV. Conclusion

Bacteria *Pseudomonas aeruginosa* NCIM 2242 mediated biodegradation of Nylon 6 and Nylon 6, 6 was studied. FTIR spectra for the polymer Nylon 6 and Nylon 6, 6 indicate formation of new groups such as CH₃, CHO and COOH. This group may be formed due to process of hydrolysis and oxidation (Chonde *et al.*, 2012). This may be caused due to cleavage of C-C bond in CH₂-CH₂ adjacent to Nitrogen atom, (Deguchi *et al.* 1997, Negoro *et al.*, 1992). Also the C=N stretching get weaker here. Degradation of polymer was also confirmed by weight loss of nylon sheets and decrease in thickness of nylon sheet. The color of sheets becomes brownish and surface morphology become rough when sheets are exposed to fungi which shows effect of enzymatic activity on Nylon 6. Therefore, the data indicates the potential for degrading recalcitrant polymer such as Nylon 6 by *Pseudomonas aeruginosa* NCIM 2242.

From the above set of results and observations it was concluded that, Nylon 6, 6 is more favored than Nylon 6, which could possibly be due to two reasons. Nylon 6, 6 is formed by the condensation of hexamethylene diamine and adipic acid while Nylon 6 is formed by the ring opening of caprolactum. So the repeat units of the former polymer has twice the number of C=N bond when compared to the repeat unit of the latter polymer. Since C-N bond are easily cleaved than the C-C bond more degradation is observed with the former (Chonde *et al.*, 2012). Cleavage of the bond would lead to the formation of CONH₂, CHO and COOH. Also presence of nitrogen in the repeats units of Nylon 6, 6 when compared to Nylon 6 makes the polymer more hydrophilic than the latter, leading to better interaction between the polymer and the organisms. The physical contact and surface growth are the prerequisite prior to degradation.

Acknowledgments

Authors are thankful to authorities of Shivaji University, Kolhapur and Department of Environmental Science, Shivaji University, Kolhapur and Department of CFC Shivaji University, Kolhapur for providing facilities to carry out work. Authors are also thankful to Sigma Aldrich for providing Nylon 6 and Nylon 6, 6 and NCIM for providing fungus culture.

V. References

1. Chonde Sonal. G, Chonde Sachin. G, Bhosale P. R, Nakade D. B, Raut P. D., Studies on degradation of synthetic polymer Nylon 6 by fungus *Trametes versicolor* NCIM 1086, *International Journal of Environmental Sciences*, 2, No.3:2435-2442, (2012).
2. Chonde Sonal. G, Chonde Sachin. G, Bhosale P. R, Raut P. D., Studies on degradation of synthetic polymer Nylon 6 by lignolytic fungus *Phanerochaete chrysosporium* NCIM 1073, *Journal of Environmental research and Development*, 6, No. 3A.:709-714, (2012).
3. Deguchi T., Massuki K., Nishida T., Nylon biodegradation by lignin- degrading fungi, *Applied Environmental Microbiology*, 63, 329-331 (1997).
4. Demetres Briassoulis , Cyril Dejean, Critical Review of Norms and Standards for Biodegradable Agricultural Plastics Part I, Biodegradation in Soil, *Journal of Polymer and Environment*, 18:384-400, (2010).
5. Gold M. H., Alle M., Molecular biology of the lignin degrading basidiomycete *phanerochaete chrysosporium*, *Microbiology review*, 57, 605-622, (1993).
6. Ioanna Kyrikou, Demetres Briassoulis, Biodegradation of Agricultural Plastic Films: A Critical Review, *Journal of Polymer and Environment*, 15:125-150, (2007).
7. Jozefa Friedrich, Polona zalar, martina Mohorcic, Ursula Klun, Andrej Krzan, Ability of fungi to degrade synthetic polymer nylon- 6, *Chemosphere*, 67, 2089-2095, (2007).
8. Julie Charles, G. R. Ramkumaar, S. Azhagiri and S. Gunasekaran, FTIR and Thermal Studies on Nylon – 66 and 30 % Glass Fibre Reinforced Nylon – 6, *E- Journal of Chemistry*, 6(1), 23-33, (2009).
9. Masayuki Shima, Biodegradation of plastics, *Current Opinion in Biotechnology*, 12:242-247 (2001).

10. Maria Rutkowska, Mariola Jastrzebska, Helena Janik, Biodegradation of polycaprolactone in sea water, *Reactive & Functional Polymers* 38, 27–30, (1998).
11. Negoro S., Biodegradation of Nylon oligomers, *Applied Microbiology*, 54, 461-466, (2000).
12. Mohee R., Unmar G., Determining biodegradability of plastic materials under controlled and natural composting environments, *Waste Management*, 27, 1486-1493, (2007).
13. Kumar Shristi, Hatha A.A.M. & Christi K.S., Diversity and effectiveness of tropical mangrove soil micro flora on the degradation of polythene carry bags, *Rev. Biol. Trop. Int. J. Trop. Biol.*, Vol. 55 (3-4): 777-786, September-December 2007.
14. Sudhakar M., Priyadarshini C., Doble Mukesh, P. Sriyutha Murthy, Venkatesan R., Marine bacteria mediated degradation of Nylon 6, 6 and 6, *International Biodeterioration and Biodegradation*, 60, 144-151, (2007).
15. Szostak – Kotwa, Biodeterioration of Textile, *Journal of Biodeterioration and Biodegradation*, 53, 165-170. (2004).
16. Tabassum Mumtaz, M.R. Khan, Mohd Ali Hassan, Study of environmental biodegradation of LDPE films in soil using optical and scanning electron microscopy *Micron* 41, 430–438, (2010).
17. Tomita K., Ikeda N., Ueno A., Isolation and Characterization of a thermophilic bacterium, *Geobacillus thermocatenulatus*, degrading nylon 12 and Nylon 6 6, *Biotechnology Letter* 25, 1743-1746, (2003).
18. Tomita K., Hyashi N., Ikeda N., Kikuchi Y., Isolation of a thermophilic bacterium degrading some nylons, *Polymer Degradation and Stability*, 81, 511-514, (2003).
19. Klun Ursa, Jozefa Friedrich, Andrej Krzan, Polyamide – 6 fibre degradation by lignolytic fungus, *Polymer Degradation and Stability*, 97, 99-104, (2003).
20. Yüksel Orhan,^a Jasna Hrenović,^b and Hanife Büyükgüngözü, Biodegradation of Plastic Compost bags under controlled soil conditions, *Acta Chim. Slov.* 2004, 51, 579–588.