World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 7.409

STUDY ON BIODEGRADATION OF NATURAL RUBBER BY FUNGAL CONSORTIUM AND ENZYMES RESPONSIBLE FOR BIODEGRADATION

Nayanashree G., Sowmya H. V., Thippeswamy B.*

Dept. of P.G. Studies and Research in Microbiology, Bio-Science Complex, Jnanasahyadri, Kuvempu University, Shankaraghatta-577451, Shivamogga.(Dist.), Karnataka, INDIA.

***Corresponding Author: Thippeswamy B.**
Dept. of P.G. Studies and Research in Microbiology, Bio-Science Complex, Jnanasahyadri, Kuvempu University, Shankaraghatta-577451, Shivamogga.(Dist.), Karnataka, INDIA.
DOI: https://doi.org/10.5281/zenodo.17224769

Article Received on 11/8/2025 **Article Revised on 31/08/2025** **Article Accepted on 21/09/2025**

INTRODUCTION

Natural rubber is a biopolymer which accumulates in environment after the usage of rubber product. Subjecting the waste rubber product to biodegradation is one of the solutions to this problem which is carried out by various microorganisms and enzymes secreted by microorganisms.

As natural rubber is a complex biopolymer due to the cross linkage present in natural rubber is not so easy to degrade rubber, if a single microorganism is used for degradation and may take a very long time for degradation. So instead of single microorganism if combination of microorganism or microbial consortium is used for degradation it will give better result.

Microbial consortium is group of different species of microorganisms that act together as a community. In a consortium, each species may bring unique sets of enzymes or metabolic pathways (Paliwal *et al.*, 2012).

The present study was taken to isolate the natural rubber degrading fungi from the soil, and also to develop microbial consortium which can be used to degrade the rubber waste. In the present study, an attempt was made to study about the enzymes produced by *Trichoderma harzianum*, *Aspergillus niger* and *Verticillium* sp. to degrade natural rubber.

MATERIALS AND METHODS

For the isolation of fungi which were able to degrade natural rubber, the soil sample was collected from a local land fill of Shivamogga district and brought to the laboratory, along with this natural rubber latex and natural rubber sheet samples were collected from rubber processing unit and then it was brought to the laboratory and preserved in the refrigerator at 4°C for further use.

Isolation of natural rubber degrading fungi

For the isolation of natural rubber degrading fungi soil burial method was followed. Previously weighed Natural rubber discs were dumped in the soil and left for a period of six months of time interval. These natural rubber discs were removed at regular time interval and weight loss was recorded. For the isolation of natural rubber degrading fungi soil sample and natural rubber samples were plated on the potato dextrose agar media and kept for incubation at room temperature at 27±2ºC for 3 to 4 days (Tsuchi *et al.*, 1996). After incubation period, fungi were identified by staining and based on their microscopic and macroscopic characters using standard manuals (Ellis, 1971 and 1976; Pitt, 1979; Domsch *et al.*, 1980; Subramanian, 1983; Ellis, 1997; Gilman, 2001; Nagamani *et al.*, 2006).

Plate assay for the screening of fungi capable of degrading natural rubber

For the screening of natural rubber degrading fungi pure culture isolates were directly inoculated on the sterilized, pre weighed natural rubber discs and then kept for incubation for 2 months. After a time interval of 2 months natural rubber sample inoculated with organisms were washed thoroughly, dried at 50°C in hot air oven for 24 hours and final weight was recorded (Borel *et al.*,1981).

Degradation of sterilized natural rubber by using microbial consortium

Use of single microorganisms for degradation gave considerably less weight loss, so combination of microorganisms was used for rubber degradation.

Degradation of rubber by using microbial consortium was carried out by combining different microorganisms isolated. Then this consortium was inoculated into the conical flask containing MSM and pre-weighed discs of

sterilized natural rubber. Then these flasks were incubated for a period of 2 months on rotary shaker at 150 rpm in room temperature. Triplicates were maintained. After 2 month of incubation, the natural rubber discs were collected, washed thoroughly using distilled water, dried in hot air oven at 50°C overnight and then weighed for final weight, then these discs were observed under SEM to record physical changes and FTIR was carried out to study the products released in degradation (Satlewal *et al.*, 2008).

Confirmation of natural rubber degradation by staining with Schiff's reagent

Evidence for degradation and mineralization of *cis*-1,4-polyisoprene rubber hydrocarbon chain was obtained by staining treated natural rubber discs with Schiff's reagent. In a tightly stopper bottle, 10 ml of fuchsin reagent was added to a sample and kept for incubation for 10-30 minutes at room temperature. After 10-30 minutes excess amount of the reagent was discarded and 10ml of the sulfite solution was added in order to suppress nonspecific reaction of untreated sample (Berekka *et al.*, 2000).

Confirmation of natural rubber degradation by Scanning Electron Microscopy (SEM)

Evidence for degradation and mineralization of *cis*-1,4-polyisoprene natural rubber hydrocarbon chain was obtained by observing the natural rubber discs under SEM. For the observation natural rubber discs buried in the soil and present in the MSM, which were subjected for degradation were observed under field emission-scanning electron microscopy (FEI-SIRION, Eindhoven, Netherland) (Lions *et al.*, 2000).

Confirmation of natural rubber degradation by Fourier Transform Infrared Spectroscopy (FTIR)

Chemical changes that arose directly on the natural rubber surface as result of the degradation process were determined using FTIR spectroscopy. NICOLET 380 FTIR spectrophotometer from Thermo Fisher Scientific, France was used which gives transmittance spectra in IR range 4000 to 400 nm. (Roy *et al.*, 2005).

Characterization of enzymes responsible for biodegradation of natural rubber

It was studied that laccase and manganese peroxidase enzymes were responsible for the natural rubber degradation.

Screening for Laccase and Manganese peroxidase enzyme

Screening for laccase enzyme produced by *Trichoderma harzianum*, *Aspergillus niger* and *Verticillium* sp. was done on plates containing following composition (g/l): 3.0 peptone, 10.0 glucose, 0.6 KH_2PO_4, 0.001 $ZnSO_4$, 0.4 K_2HPO_4, 0.0005 $FeSO_4$, 0.05 $MnSO_4$, 0.5 $MgSO_4$, 20.0 Agar (pH-6) supplemented with 0.02% guaiacol. *Trichoderma harzianum*, *Aspergillus niger* and *Verticillium* sp. were inoculated into plates and the plates were incubated at 30°C for 7 days. Laccase activity was visualized on plates containing 0.02% guaiacol, since laccase catalyzes the oxidative polymerization of guaiacol to form reddish brown zones in the medium (Viswanath *et. al*, 2008).

For the screening of manganese peroxidase enzyme producing organisms H_2O_2 was added to the laccase screening media.

Mass production of enzyme by submerged fermentation

Pure cultures of *Trichoderma harzianum*, *Aspergillus niger* and *Verticillium* sp. was inoculated to submerged state fermentation medium for the production of extracellular enzymes by using MSM media and was maintained at the incubation temperature of 27±2ºC for 3 months (Shraddha *et al.*, 2011).

Determination of Laccase and Manganese peroxidase enzyme activity by using Spectrophotometer

Guaiacol (2mM) in sodium acetate buffer (10mM pH 5.0) was used as substrate. The reaction mixture contained 3ml 10mM acetate buffer of pH 5, 1ml guaiacol and 1ml enzyme source and for blank 1ml of distilled water used instead of enzyme source. The mixture was incubated at 30ºC for 15minutes and absorbance was read at 450nm blank using UV spectrophotometer (Papinutti *et al.*, 2006). Manganese peroxidase enzyme activity was calculated by following laccase enzyme activity determination procedure, but for the reaction mixture 1 ml of H_2O_2 was added and incubated.

RESULTS

Isolation of natural rubber degrading fungi

Rubber samples and the soil sample of 2, 4 and 6 months were plated on the potato dextrose agar medium, different fungi such as *Trichoderma harzianum*, *Aspergillus niger* and *Verticillium* sp. were isolated and recorded. Weight loss was also observed in all the rubber samples, which was removed at different time interval (Table 1).

Table 1: Weight loss of Natural rubber by soil burial method.

Sl.No.	Number of months	Initial weight (g)	Final Weight (g)	Weight loss (g)	Weight loss (%)
1.	2	3	2.84	0.16±0.01*	5.3
2.	4	3	2.63	0.37±0.04*	12.3
3.	6	3	2.12	0.88±0.01*	29.3

*Standard error, where n=3.

Plate assay for the screening of fungi capable of degrading natural rubber

In plate assay weight loss was observed in *Trichoderma harzianum*, *Aspergillus niger* and *Verticillium* sp. inoculated natural rubber discs. *Trichoderma harzianum* showed 30.2%, *Aspergillus niger* showed 25.9% and *Verticillium* sp. showed 15.5% weight loss.

Degradation of surface sterilized natural rubber by using microbial consortium

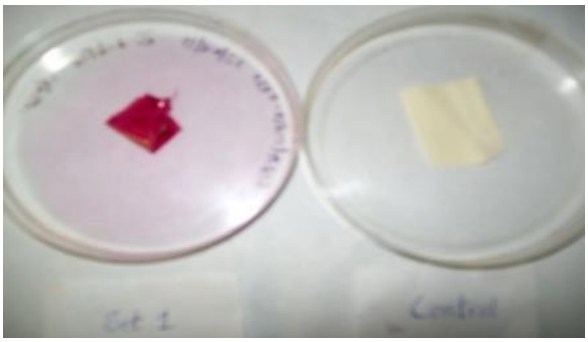
Combination of more than one organism was used to enhance the natural rubber degradation. Dominant microorganisms showed growth first then it was followed by the growth of other ressive microorganism and percentage of degradation was more in this method compared use of single microorganism.

Table 2: Weight loss of natural rubber treated with microbial consortium.

Set of Organism	Initial weight (g)	Final Weight (g)	Weight loss (g)	Weight loss (%)
Consortium - Trichoderma harzianum, Aspergillus niger and Verticillium sp.	3	1.35	1.65±0.017	55.0

Confirmation of natural rubber degradation by Schiff's staining

Consortium treated surface sterilized natural rubber discs were subjected for Schiff's staining, all the rubber discs turned to purple colour and there was no colour formation in the control. Formation of purple colour in the treated sample was due to the presence of aldehyde and ketone group which was produced as a result of degradation of cis-1,4-polyisoprene units by the action of microorganisms (Fig. 1).

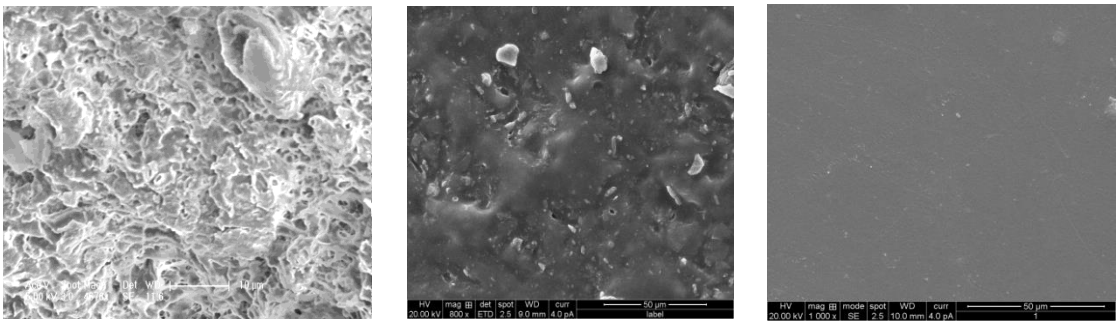


Consortium (Th, An, Ve)

Fig. 1: Confirmation of natural rubber degradation by Schiff's staining.

Confirmation of rubber Degradation by Scanning Electron Microscopy (SEM)

Surface sterilized natural rubber discs, which were inoculated with microbial consortium were observed under scanning electron microscope. Biofilm formation, complete disintegration of the rubber disc material and formation of cavities due colonization of microorganisms were also observed (Fig.2).



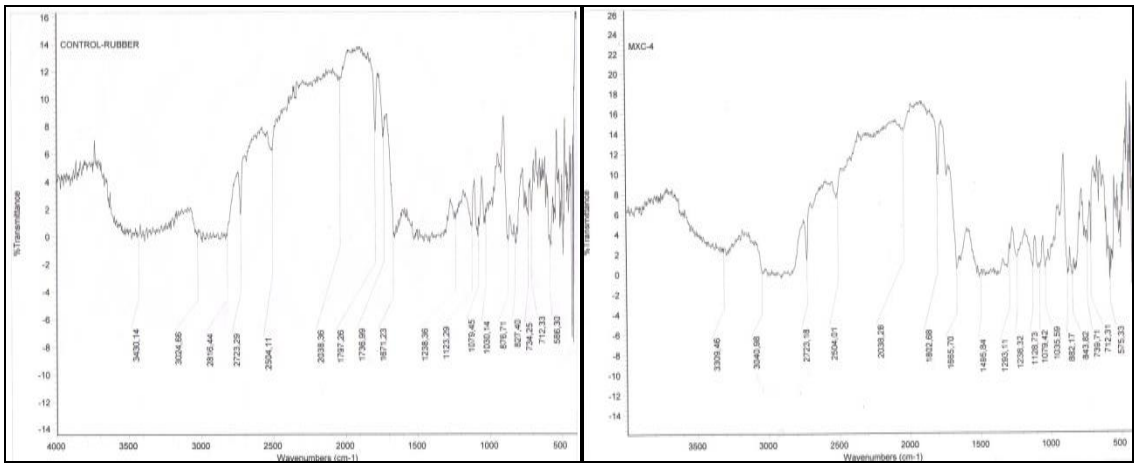
Buried in soil *Trichoderma harzianum* (Th), **Consortium(Th, An, Ve) *Aspergillus niger* (An)** **Control *Verticillium* sp.(Ve)**

Fig. 2. Confirmation of rubber Degradation by Scanning Electron Microscopy (SEM).

Confirmation of rubber degradation by Fourier Transform Infrared Spectroscopy (FTIR)

Natural rubber discs, which were treated by microbial consortium were subjected for FTIR studies peaks were observed at the wave length between 2725.89 cm^{-1} and 1662.34 cm^{-1} having H–C=O:C–H stretch and C=O stretch which indicates the presence of aldehydes and ketones, released as a result of natural rubber degradation in the treated sample. Presence of these aldehyde and ketone group confirms natural rubber degradation. Peaks

showing the presence of aldehyde and ketone are absent in control (Fig. 3).



Control **Treated with consortium**

Fig. 3: FTIR spectrum of natural rubber.

Enzymatic studies of natural rubber degradation

It was studied that laccase and manganese peroxidase enzymes were responsible for the rubber degradation.

Screening for Laccase and Manganese peroxidase enzymes

Trichoderma harzianum, Aspergillus niger and *Verticillium* sp. was inoculated on the laccase and manganese peroxidase medium, there was a formation of reddish brown colour around the colonies, as laccase and manganese peroxidase catalyzes the oxidative polymerization of guaiacol to form reddish brown zone. *Trichoderma harzianum, Aspergillus niger* and *Verticillium* sp. which showed positive result for rubber degradation also showed positive result for laccase and manganese peroxidase enzyme screening.

Spectrophotometrical analysis of Laccase and Manganese peroxidase enzyme activity

Trichoderma harzianum, Aspergillus niger and *Verticillium* sp., showed more manganese peroxidase activity compared to laccase activity. Maximum activity of both laccase and manganese peroxidase enzyme activity was maximum in 10th week (Table 3, 4 and Fig.4, 5).

Table 3: Laccase enzyme activity in IU.

Name of organism	1st week	2nd week	3rd week	4th week	5th week	6th week	7th Week	8th week	9th week	10th week	11th week	12th week
Trichoderma harzianum	0	0	0.0037 ±0.0004	0.0066 ±0.0002	0.0105 ±0.0003	0.0142 ±0.0001	0.0172 ±0.0004	0.0197 ±0.0002	0.0226 ±0.0001	0.0259± 0.0003	0.0201± 0.0001	0.0159± 0.0003
Aspergillus niger	0	0	0.0015 ±0.0001	0.0032 ±0.0004	0.0054 ±0.0003	0.0081± 0.0003	0.0109 ±0.0004	0.0128 ±0.0001	0.0144 ±0.0003	0.0163± 0.0003	0.0145 ±0.0002	0.0116 ±0.0003
Verticillium sp.	0	0	0.0013 ±0.0001	0.0031 ±0.0003	0.0051± 0.0004	0.0066 ±0.0004	0.0093± 0.0003	0.0113± 0.0001	0.0122 ±0.0004	0.0141± 0.0001	0.0125± 0.0001	0.0105 ±0.0002

± = Standard error, where n=3

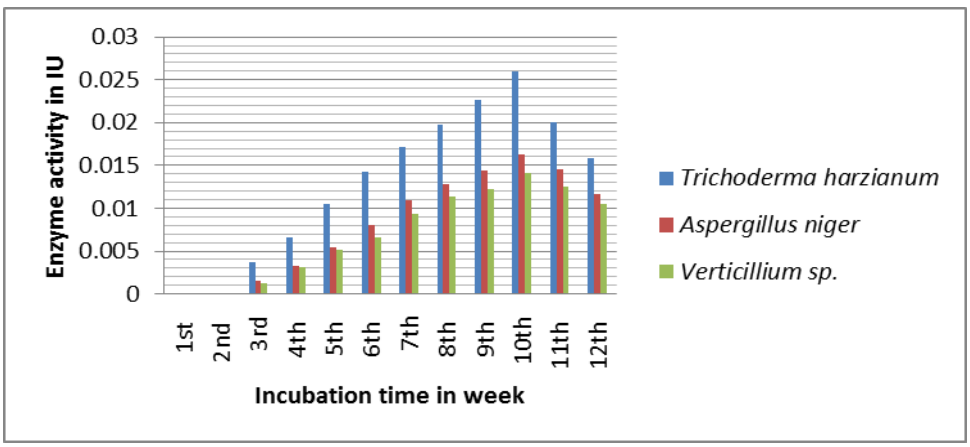


Fig. 4: Laccase enzyme activity in IU.

Table 4: Manganese peroxidase enzyme activity in IU.

Name of organism	1st week	2nd week	3rd week	4th week	5th week	6th week	7th Week	8th week	9th week	10th week	11th week	12th week
Trichoderma harzianum	0	0	0.0079 ±0.0002	0.0114 ±0.0003	0.0134 ±0.0001	0.0169 ±0.0003	0.0198 ±0.0003	0.0224 ±0.0002	0.0251 ±0.0004	0.0275± 0.0004	0.0255 ±0.0003	0.0210 ±0.0003
Aspergillus niger	0	0	0.0018 ±0.0004	0.0037 ±0.0001	0.0060 ±0.0003	0.0088 ±0.0001	0.0116 ±0.0001	0.0133 ±0.0001	0.0149 ±0.0003	0.0182 ±0.0001	0.0152 ±0.0001	0.0116 ±0.0003
Verticillium sp.	0	0	0.0015 ±0.0002	0.0032 ±0.0004	0.0053 ±0.0001	0.0078 ±0.0002	0.0094 ±0.0002	0.010 5 ±0.0004	0.0152 ±0.0002	0.0167 ±0.0002	0.0129 ±0.0004	0.0104 ±0.0002

± = Standard error, where n=3

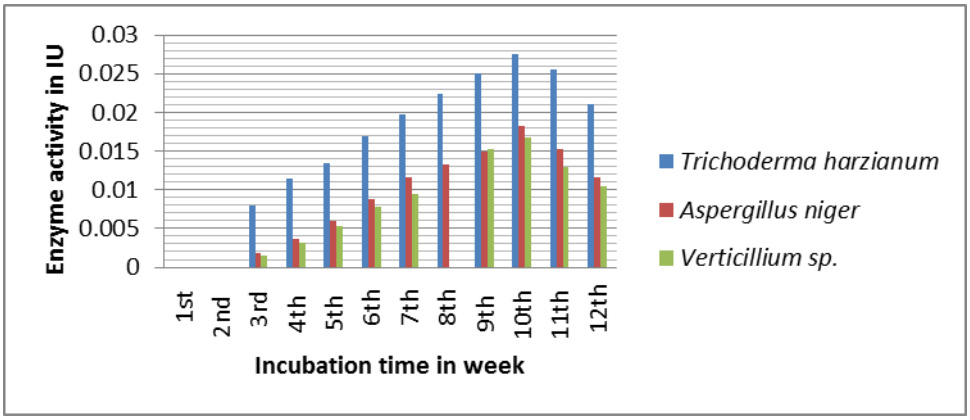


Fig. 5: Manganese peroxidase enzyme activity in IU.

DISCUSSION

Natural rubber (NR) or *cis*-1,4poly-isoprene is one of the most important biopolymers. Due to its unique property it is extensively used worldwide for the manufacture of many products. But after the usage of these products its disposal is one of the big solid waste problems. As natural rubber is a complex biopolymer due to the cross linkage present in natural rubber which is not so easy to degrade rubber if a single microorganism is used for degradation and may take a very long time for degradation. So instead of single microorganism if combination of microorganism or microbial consortium is used for degradation it will give better result. Microbial consortium is group of different species of microorganisms that act together as a community. In present work consortium of microorganism was used to degrade rubber more effectively compared to single organism. In this study proper selection of the consortium was done by grouping different microorganisms and some micronutrients were supplied for the initiation of growth of the microorganism and natural rubber was used as sole source of carbon.

Microbial consortium of *Trichoderma harzianum, Aspergillus niger* and *Verticillium* sp., showed maximum weight loss compared to single organism. SEM and FTIR was performed to the treated rubber sample to confirm degradation. Similar attempts were made by several other scientists to degrade complicated biopolymers such as rubber, plastic and oil fills.

Berekaa *et al.,* (2000) conducted similar work and tested the biodegrading ability of different bacteria belonging to the genera *Gordonia* (strains Kb2, Kd2 and VH2), *Mycobacterium, Micromonospora* and *Pseudomonas*. All strains were able to use natural rubber (NR) as well as NR latex gloves as sole carbon source.

Similar study was carried out by Tokiwa *et al.,* (1999) forty-seven percent of a tire tread strip with a natural rubber content of 100 phr (parts per hundred of rubber) was completely mineralized by a mutant strain, Rc, of the rubber-degrading organism, *Nocardia* sp. Strain 835A.

Gilbert *et al.,* (2003) showed that by combining metabolically complementary bacteria in a consortium for xenobiotic bioremediation is an alternative to constructing a complete degradative pathway in a single microorganism

Similarly the consortium was developed from the indigenous microflora enriched under artificial soil conditions with polyethylene (LDPE) pieces. The isolates of this consortium have earlier been reported to degrade a variety of polymers like LDPE (non-poronized and poronized), HDPE, epoxy and epoxy silicone blends as components of different consortia (Satlewal *et al.,* 2008).

CONCLUSION

Natural rubber (NR) or *cis*-1,4poly-isoprene is one of the most important biopolymers. Due to its unique property it is extensively used worldwide for the manufacture of many products. But after the usage of these products its disposal is one of the big solid waste problems. One of the solutions to this problem is subjecting rubber products to degradation by microorganism. As natural

rubber is a complex biopolymer due to the cross linkage present in natural rubber is not so easy to degrade rubber, if a single microorganism is used for degradation and may take a very long time for degradation. So instead of single microorganism if combination of microorganism or microbial consortium is used for degradation it will give better result.

Combination of microorganisms was used for the degradation of rubber by using microbial consortium combination of three different microorganisms. It showed maximum weight loss of 55% compared to the use of single microorganism.

ACKNOWLEDGMENT

Authors are grateful to department of microbiology for providing facility to conduct all the experimental works.

Conflict of Interest

Authors do not have any conflict of interest.

REFERENCE

1. Berekaa, M.M., Lions, A., Reichelt, R., Keller, U. and Steinbuchel, A. Effect of pretreatment of rubber material on its Biodegradability by various rubber degrading Bacteria, *FEMS Microbiology Letters*, 2000; 184: 199-206. .
2. Borel, M., Kergomard, A., and Renard, F. Degradation of Natural rubber by fungi imperfecti, *Agricultural Biology and Chemistry*, 1981; 46: 877-881.
3. Domsch, K.H., Gams, W. and Anderson, T.H. Compendium of soil fungi. Vol 1. Academic Press Inc. New York, 1980; 1-859.
4. Ellis, M.B. Dematiaceous hyphomycetes. Kew: Common wealth mycological institute. England, 1971; 1-608.
5. Ellis, M.B. More Dematiaceous hyphomycetes. Kew: Common wealth mycological institute. England, 1976; 1-507.
6. Ellis, M.B. and Ellis, J.P. Microfungi on Land plants: An Identification Handbook. Richmond Publishers, London: Croom Helm, 1997; 868.
7. Gilman, J.C. A manual of soil fungi. 2nd edition. Biotechnology Books. New Delhi, 2001; 1-392.
8. Gilbert, E.S., Walker, A.W. and Keasling, D. J., A Constructed Microbial Consortium for Biodegradation of the Organophosphorus Insecticide Parathion, *Applied Microbiology Biotechnology*, 2003; 61: 77–81.
9. Lions, A., Rudolf Reichelt, R., Keller, U. and Steinbuchel, A. A Gram negative bacterium identified as *Pseudomonas aeruginosa*, AL98, is a potent degrader of natural rubber and synthetic cis-1,4-polyisoprene, *FEMS Microbiology Letters*, 2000; 182: 155-161.
10. Nagamani, A., Kunwar, I.K. and Manoharachary, C. *Handbook of Soil Fungi.* I. K. International Pvt. Ltd. New Delhi, 2006; 1-477.
11. Paliwal, V., Puranik, S. and Purohit, H.J. Integrated perspective for effective bioremediation. *Applied Biochemistry and Biotechnology*, 2012; 166: 903-924.
12. Papinutti, L., and Martinez, J. M., Production and Characterization of Laccase and Manganese peroxidase from the ligninolytic fungus *Fomes sclerodermeus, Journal of technology and biotechnology*, 2006; 81: 1064-1070.
13. Pitt, J.I. The genus *Penicillium* and its teleomorphic states *Eupencillium* and *Talaromyces.* Academic Press Inc, Ltd. London, 1979; 1-634.
14. Roy, V.R., Das, M., Banrjee, R. and Bhowmick, A. Comparative studies on rubber biodegradation through Solid state and Submerged fermentation, *Process Biochemistry*, 2005; 42: 181-186.
15. Satlewal, A, Ravindra, S, Mgh, Z, Yogesh, S, and Reeta, G. Comparative Biodegradation of HDPE and LDPE Using an Indigenously Developed Microbial Consortium, *Journal of Microbiology and Biotechnology*, 2008; 18: 477–482.
16. Shraddha, Shekher, R., Sehgal, S., Kamtania, M., and Kumar, A., Laccase microbial source Production, Purification, Potential biotechnological application, *Enzyme Research*, 2011; 1-11.
17. Subramanian, C.V. Hyphomycetes, taxonomy and biology. London. New York: Academic Press, 1983; 410-461.
18. Shah, A.A., Hasan, F., Hameed, A and Ahmed, S. Biological degradation of Plastics: A comprehensive review. *Biotechnology Advances*, 2008; 26: 246 265.
19. Viswanath, B., Chandra, M. S., Pallavi, H., Reddy, Screening and Assessment of Laccase Producing Fungi isolated from different environmental samples, *African Journal of Biotechnology*, 2008; 7: 1129-1133.
20. Tsuchii, A. and Tokiwa, Y. Colonization and disintegration of Tire Rubber by a colonial Mutant of *Nocardia*, *Journal of Bioscience and Bioengineering*, 1999; 87: 542-544.
21. Tsuchii, A., Takeda, K., Suzuki, T. and Tokiwa, Y. Colonization and degradation of rubber pieces by *Nocardia* species, *Biodegradation Journal*, 1996; 7: 41-48.
22. Tsuchii, A., Takeda, K., Suzuki, T and Tokiwa, Y, *Biodegradation Journal*, 1996; 7: 41-48.