International Journal of Ecology and Environmental Sciences
www.ecologyjournal.in
Online ISSN: 2664-7133, Print ISSN: 2664-7125
Received: 25-05-2022, Accepted: 10-06-2022, Published: 27-06-2022
Volume 4, Issue 2, 2022, Page No. 69-74

In vitro microbial degradation of sanitary napkins

R Ellammal[1], J Ishwarya[2], S Agila[2]
[1] Research Scholar, Department of Microbiology, Kamban college of Arts and Science for women, Tiruvannamalai, Tamil Nadu, India
[2] Assistant Professor, Department of Microbiology, Kamban College of Arts and Science for women, Tiruvannamalai, Tamil Nadu, India

Abstract
The present investigation aimed to degrade the sanitary napkins by microorganisms and its enzymatic activity. The invitro microbial degradation were performed under the controlled condition and soil degradation under the controlled environmental condition. The higher grade of degradation efficiency found on the organisms like Bacillus, Pseudomonas, Aspergillus and Fusarium. Lyophilized organisms were used to degrade the used napkinson dumpyards to prevent the environmental pollution impacts.

Keywords: sanitary napkins, microorganisms, organisms

Introduction

Menstruation is a natural process but it is still a taboo in Indian society as it is considered unclean and dirty. Menstruation wastes are waste generated by a female reproductive organ. The menstrual cycle has three phases, that is follicular phase Ovulation phase and luteal phase.

Nearly 70% of women living in urban India use sanitary Napkins compared to 48% women living in rural India. Roughly 12.3billion disposable sanitary pads are generated in every year. The disposal of such plastic pads have become a huge concern.

According to menstrual health alliance of India, one sanitary pad could take 500 to 800 years to decompose the plastic used is non-biodegradable and lead to hazards. Sanitary napkins are used by 36% of the menstruating females, most of the Napkins are 90% plastic, each of Napkin has equivalent to four plastic bags.

Appropriate disposal of used menstrual material is still lacking in worldwide. Most countries developed same techniques to manage their fecal and urinary wastes but the lack of menstrual management practices in world, most of the women dispose of their sanitary napkins into domestic solid wastes or garbage bins. Toilet facilities in India lack bins for the disposal of sanitary pads and hand washing facilities for handle menstrual hygiene. In urban areas, many modern disposable menstrual products were used, they were disposed by flushing in toilets and throwing in dustbins.

In rural areas, pit latrines made and once full they were covered with soil and new pit dugged but due to space limitations this was not practiced in urban areas (J.N. Bhagwan *et al*.,2008) [9]. There was a report that women and girls wrap their used menstrual napkins and packed in polythene bags before disposing in pit latrines which prevents them from decomposition.

Nowadays, women/girls use commercial sanitary pads which are made up of super absorptive materials like polyacrylate. These pads are when flushed in the toilets they get saturated with liquid and swell up, thus results in sewage backflow, a cause serious health hazard. The adhesive wings and the perforated plastic layers in the commercial sanitary napkins are not easily degradable by the microbies of soil.

Sewage system blockages are the global problem. It is due to flushing of menstrual products in toilets. Nowadays most of women/girls prefer deodorised sanitary napkins, which made of chemicals like bleaching that is organo chlorines when buried on the soil. Blockage of sewage system is a global problem and major contributing factor is flushing of menstrual products in toilets. Deodorised sanitary products used by women/girls contain chemicals used in bleaching such as organo chlorines which when buried in the soil and it disturb the soil micro flora and decomposition takes time. The drainage system were clogged by the used sanitary napkins which has to be unblocked (or) cleaned by conservancy workers manually by their hands without proper protection. It leads the workers to expose with harmful chemical and pathogen.

People living alongside river banks throw menstrual waste into water bodies which contaminate them. These materials soaked with blood were breeding places for germs and pathogenic microbes (D. Shoemaker, 2008) [49].

Sanitary products soaked with blood of an soil may contaminate with the Hepatitis and HIV infected women/girl, which retain soil and live up to six months in soil. Incineration is the best technique to dispose the menstrual waste but burning of pads releases harmful gasses that affects health and environment. Burning of inorganic material releases chemicals like dioxins which are toxic and carcinogenic to the nature.

Domestic wastes has Feminine sanitary napkins which are usually considered as residual waste, those are not recyclable and, therefore, goes to dumpsites and landfills. The sanitary pads are made up of chemicals like polyacrylate (absorbent polymer gel), polyethylene, polypropylene, propylene glycol. Polymers materials have wide influence due to their high stability, mechanical and thermal properties. They have distinctive chemical composition, physical properties and their applications. Synthetic polymers have great hydrophobic level and heavy molecular weight. Polymeric materials have high potential source of carbon and energy for heterotrophic microorganisms including bacteria and fungi in several ways.

Biodegradation of a polymeric materials brought by the natural action microorganisms such as bacteria and fungi via enzymatic action into metabolic products of microorganisms (eg, H_2O,CO_2, CH4 biomass etc. The ultimate result is the loss of structural integrity and as a result of decrease in molecular weight. Microorganisms which are involved in the degradation or deterioration process by biofilms formation.

The main objective of this review was to summarize the concern and possible methods of menstrual waste management in low-income countries. The aim of the study understanding the menstrual practices, product design, demands, and disposal strategies. It includes a summary of both the existing need for menstrual hygiene and its management. In addition it has an analysis of the current knowledge in the fields of public health, water and sanitation, and solid waste management.

Material and Methodology

Collection of Soil

Soil collected from different environment for the degradation under the invitro condition.

Degradation of Sanitary Pads

The sanitary pads were sieved into small pieces. The pads were weighted and measured then inoculated into the sand container. After specific time period, shredded pads were removed from soil and completely rinsed with distilled water, dried and again weighted and measured. Biodegradation of sample measured by weight loss of sample.

Isolation of Micro Organisms From Soil

1gm of soil sample taken and mixed with 10ml of saline then serially diluted. Then they were placed on the spread plate technique with Nutrient agar, Nutrient broth and Sabouraud's dextrose agar(SDA). The nutrient agar plates were incubated at 37 $^{\circ}$c for 24 hours and SDA plates were incubated at room temperature for 3-4 days.

Identification of bacterial isolates

Sterile peptone water was inoculated with the colonies isolated from the nutrient agar and incubated for 24 hours at 37°C. Then prelimary test were made to determine the characterization of those isolates, Gram staining motility and biochemical test were done.

Identification of Fungal Isolates

Sterile saline used for wet mount technique and LPCB for the identification of fungal isolates.

Screening of Bacteria For Degradation

Mineral salt media [MSM] was used to screen the degradation of sanitary pads with little modifications. The media has all the nutrients except carbon source, necessary for the bacterial growth. The bacterial isolates were screened for the ability to use polypropylene as the carbon source for growth which is present on the sanitary pad. 0.54g of polypropylene polymer granules [sterilized] were kept at different locations into sterilized MSM prior to solidification. The polymer granules become stacked to agar after solidification. Then the medium seeded with each of the isolated bacterial isolates and incubated for 4 to 8 days at 37°C. Controls sets were maintained without polymer and the media was observed in regular intervals for growth.

Microbial Formulation

Bacteria isolated and identified as polypropylene component of sanitary pad degradation microbes were formulated for biodegrading potential. The isolates were grown on freshly prepared brain heart infusion agar to obtain pure cultures at 33°C for 24 hours. The pure cultures were inoculated into brain heart infusion agar and grown to a stationary phase in rotating shaker at 29°C at 150rpm. Individual suspension were pooled in equal proportion to setup inoculums for the biodegradability experiment.

In Vitro Degradation of Sanitary Pads

The trypticase soy broth prepared for the bacterial isolates and potato dextrose broth for the fungal isolates. The bacterial isolates was inoculated into the liquid medium individualy, fungal isolates inoculated into potato dextrose broth, both were shredded pads. Then the conical flask was incubated in rotary shaker. At regular intervals, shredded pads were isolated aseptically washed and dried then weighted to test the degradation rate.

The rate of degradation can be measured by weight at every three days intervals. The rate of degradation for month noted as average and results were recorded.

$$\text{Rate of degradation (month)} = \text{weight measured for every 3 days / no. of days}$$

Based on the rate of degradation, the active bacterial and the fungal degraders were lyophilized and implemented into soil for enhancement of degradation process.

Degradation of Residual Napkins By Dry Weight

The sanitary napkins used for the studies were recovered from respective culture media, washed with 2% SDS and rinsed with autoclaved distilled water. The strips were then dried overnight at 60° C before weighing for the determining the loss in weight of the sanitary napkins using the following formula.

$$\text{Weight Loss (\%)} = \frac{\text{Initial Weight} - \text{Final Weight X 100}}{\text{Initial Weight}}$$

Spectroscopic Analysis by FTIR

The changes in chemical structure of the Polypropylene present in the napkin which include alterations in their bonds of chemical and other changes in their bonds of chemical and other changes in different functional groups were analyzed using FTIR spectroscopy. The polypropylene napkins subjected to degradation by microbes was analyzed with different sampling intervals.

Result and Discussion

A sanitary pad could take 500-800 years to decompose as the plastic used in non biodegradable and can lead to health and environmental hazards. You roll it, chuck it, sparing a little through on what will happen to sanitary pads menstruation in India, out of which approximately 121 million use disposable sanitary napkin. According to WHO report nearly 70% of women living in urban India use sanitary pads compared to 48% women rural India. Over 12.3 billion disposable sanitary pads are generated every year. The disposal of such plastic pads have become a huge concern.

The main objective of the review was to summarize the concern and possible methods of menstrual waste management in low – income countries. The review article was aimed at understanding the menstrual practice, product design, demands and disposal strategies. It includes a summary of both the existing need for menstrual hygeine and management, and an analysis of the current knowledge in the fields of the pubilc health, water and sanitation and solid waste management.

The bacterial isolates confrimed with preliminary tests like staining and biochemical tests & were inoculated into the trypticase soy broth with shredded pads of 1gm for each isolates and shake flask incubation method. The fungal isolates confirmed with LPCB and colony morphological characterization were inoculated in sabourad's dextrose broth with 1gm of shredded pads, incubated at room temperature. Weight loss determination was made in regular interval. The degradation rate were obtained by measuring the pad

Table 1: In vitro Degradation of Sanitary Napkins By Bacteria and Fungi

Week	*Bacillus*	*Pseudomonas*	*Staphylococcus*	*Klebsiella*	*Clostridium*	*Aspergillus*	*Fusarium*	*Penicillium*	*Rhizopus*
Initial weight of Napkins (g)	1.51	1.51	1.51	1.51	1.51	1.51	1.51	1.51	1.51
1	0.90	0.65	0.70	0.79	0.81	0.83	0.56	0.76	0.70
2	0.65	0.58	0.52	0.58	0.71	0.76	0.53	0.65	0.60
3	0.59	0.51	0.59	0.63	0.79	0.69	0.45	0.55	0.54
4	0.46	0.44	0.48	0.52	0.60	0.45	0.40	0.35	0.58

Based on the study, found that Bacillus & Pseudomonas shows efficiently degradation of sanitary napkin. Among the fungal isolates *Aspergillus* and *Fusarium* shows efficient degradation of sanitary napkin. The result has confirmed that the *Bacillus* and *Pseudomonas* are the commonest soil borne isolate have the highest efficient in biodegradation than other organisms. *Bacillus* shows 30-35% of weight loss of pads and *Pseudomonas* show 45% of weight loss.

Table 2: Mass Reduction Efficiency of Bacterial and Fungal Isolates

Reduction efficiency	Bacteria			Fungi		
	Control	*Bacillus*	*Pseudomonas*	Control	*Aspergillus*	*Fusarium*
Initial mass	0.64	0.64	0.64	0.54	0.54	0.54
Final mass	0.64	0.38	0.48	0.54	0.47	0.48
Percentage	0	12	11	0	12	11

The results of fungal isolates has confirmed that *Fusarium* and *Aspergillus* are the commonest fungi present on rotten soil waste have the great efficient biodegradation than from the other fungal isolates. It shows 35 to 45% of weight loss in napkins.

The samples undergo biotic degradation for starting from 5th day to 30th days. The weight loss of the samples slightly increase during that period which contributed to 31%, 39% and 46%. The samples that contained polyprophylene acted as a carbon sources where microbes can growth by absorbing the carbon sources due to it saprotrophic characteristics. Molecule structure of polyprophylene are types of plastic that have similar molecules with lignin. The degradation using green polyprophylene as a sample where the weight loss result for biotic degradation using Microbes after undergoes 30 days degradations. It proved that Microbes could degrade the samples that mostly made up from PP without using any prior physical treatment such as exposure to the sunlight. However, the fungi growth phase on sanitary pads takes a longer time and slower degradation rate as resulted a lower weight loss. It is due to the adhesive tape on the surface of the sanitary pads, and those types of material uneasy to degrade.

FTIR Analysis

Table 3

S. No	Incubation Period	Band Position Control / Test Sample	Function Group Involved
1	1 WEEK	459-458.1	C-I
2	2 WEEK	1703.92-1705.34	C=O,C=C,C=N
3	3 WEEK	1378.18-1373.36	CH cellulose lignin and = - CH_3
4	4 WEEK	1450.52-1449.55	CH_3

In soil degradation under environment condition, the degradation rate was found higher in soil collected near dump yard contaminate with sewage. This is due to most of microorganism present in the sewage environment were able to degrade the source present there. All the organisms were involved in the process of natural biodegradation of sanitary napkins.

Conclusion

Environment pollution is one of the major problem of our nation. Due to usage of non-degradable waste dumped in soil, the microorganisms in soil by their metabolic activities they try to degrade those non-degradable material, they utilize the energy source from those material for their metabolism, but this is impossible for the pollution occurs.

The non-degradable waste like pads made many health complication. In this study the biodegradation of sanitary pads with help of soil borne microorganisms. The bacterial and fungal isolates were found to more effective in degradation. From the result obtained through this work this the bacterial and fungal isolates were lyophilized and applied to the soil for the enhancement and speed up of the natural biodegradation process and their efficiency in soil.

References

1. Amata RL, Otipa MJ, Waiganjo M, Wabule M, Thuranira EG, Erbaugh M *et al*. Incidence, prevalence and severity of passion fruitfungal diseases in major production regions of Kenya. *Journal of Applied Biosciences*,2014:20:1146–1152.
2. Ambarwati A, Sembiring L, Soegihardjo C. Antibiotic produced by *Streptomycetes* associated with rhizosphere of purple nut sedge (*Cyperus rotundus* L.) in Surakarta, Indonesia. *African journal of microbiology research*,2012:6(1):52-57.
3. Anthony L, Andrady, Mike A Neal. Applications and societal benefits of plastics, Phil. Trans. R. Soc. B,2009:364:1977-1984.
4. Aouar L, Lerat S, Ouffroukh A, Boulahrouf A, Beaulieu C. Taxonomic identification of rhizospheric actinobacteria isolated from Algerian semi-arid soil exhibiting antagonistic activities against plant fungal pathogens. *Canadian Journal of Plant Pathology*,2012:34(2):165-176.
5. Artham T, Doble M. Biodegradation of Aliphatic and Aromatic Polycarbonates. Macromol Biosci,2008:8:14-24.
6. Ariba BM, Varalakshmi B, Umamagheswari K. Biodegradation of polythene bag using bacteria isolated from soil. *International journal of current microbiology and applied sciences*,2015:4:674-680.
7. Arugula P, Paramasivam SK, Kanuri N, Srirangam A, Vemuluri M. Perception on use of sanitary napkins among students in Khammam locality: a survey. Indian J Pharm Pract,2017:10(2):133.
8. Ashley R, Blackwood DN, Souter *et al*, "sustainable disposal of domestic sanitarywaste*,"Journal of environmental Engineering*,2005:113(2):206-215.
9. Bhagwan JN, Still D, Buckley C, Foxon K. "Challenges with up-scaling dry sanitation technologies,"*Water Science and Technology*,2008:58:1:21-27.

10. Barman A, Katkar PM, Asagekar SD. Natural and sustainable raw materials for sanitary napkin. Man Made Textiles India,2018:46(12):408-411.
11. Barnes DK, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. Philos Trans R Soc Lond B *Biol Sci*,2009:364:1985-1998.
12. Bhardwaj H, Gupta R, Tiwari A. Microbial Population Associated With Plastic Degradation, Scientific Reports,2012:5:272-274.
13. Bikiaris D, Aburto J, Alric I, Borredon E, Botev M *et al*. Mechanical properties and biodegradability of LDPE blends with fatty-acid esters of amylase and starch. J *Appl Polym Sci*,1999:71: 089-1100.
14. Das MP, Kumar S, Rebecca JL, Sharmila S. Isolation and identification of LDPE degrading fungi from municipal solid waste. *Journal of chemical and pharmaceutical research*,2013:5:78-81.
15. Devadass BJ, Paulraj MG, Ignacimuthu S, Theoder PAS, Dhabi NAA. Antimicrobial activity of soil actinomycetes isolated from Western Ghats in Tamil Nadu, India. *Journal of bacteriology and mycology*,2016:3(2):00059-00065.
16. Gnanavel G, Valli V, Thirumarimurugan M, Kannadasan T. Degradation of plastics using microorganisms. *International journal of pharmaceutical and chemical sciences*,2012:1:691-694.
17. Gu J-D. Microbiological deterioration and degradation of synthetic polymeric materials: Recent research advances. *International Biodeterioration & Biodegradation*,2003:52(2):69-91. doi:10.1016/S0964-8305(02)00177-4.
18. Hadad D, Geresh S, Sivan A. Biodegradation of polyethylene by the thermophilic bacterium Brevibacillus borstelensis. J *Appl Microbiol*,2005:98:1093-1100.
19. Hao J, Wang J, Zhao W, Ding L, Gao E, Yuan W. Effect of bisphenol a exposure on sex hormone level in occupational women. Wei Sheng Yan Jiu,2011:40(3):312-214.
20. Jayasiri HB, Purushothman CS, Vennila A. Plastic litter accumulation on high-water strandline of urban beaches in Mumbai, India. Environ Monit Assess,2013:185:7709-7719.
21. Jiang Y, Chen X, Lou K, Mao P. Cultivable soil actinomycete communities in some areas of western China. *Academia Journal of biotechnology*,2013:1(1):1-13.
22. Kathiresan K. Polythene and plastics-degrading microbes from the mangrove soil. *Rev. Biol. Trop*,2003:51:3-4.
23. Krueger MC, Hofmann U, Moeder M, Schlosser D. *PLOS ONE*, 10(7), e0131773. Kumar SS, Raut S (2015) *J Env Chem Eng*,2015:3:462-473.
24. Kumari NA, Kumari P, Murthy NS. A Novel mathematical approach for optimization of plastic degradation. *International journal of engineering trends and Technology*,2013:4(8):3539-3542.
25. Kumar V, Bisht G, Gusain O. Terrestrial actinomycetes from diverse locations of Uttarakhnad, India: Isolation and screening for their antibacterial activity. *Iranian journal of microbiology*,2013:5(3):299-308.
26. Laycock B, Halley P, Pratt S, Werker A, Lant P. The chemomechanical properties of microbial polyhydroxyalkanoates. *Progress in polymer science*,2013:38:536-583.
27. Mangamuri UK, Muvva V, Poda S, Kamma S. Isolation, identification and molecular characterization of rare actinomycetes from mangrove ecosystem of Nizampatnam. *Malays Journal of microbiology*,2013:8(2):83-91.
28. Manikkam R, Venugopal G, Ramasamy B, Kumar V. Effect of critical medium components and culture conditions on antitubercular pigment production from novel *sciences*,2015:5(6):15-9.*Streptomyces* sp. D25 isolated from Thar desert, Rajasthan. *Journal of applied pharmaceutical.*
29. Manjunath NS, Rangaswamy BE. Development of biodegradableeco- friendly and cost effective sanitary napkin. Bapuji institute of engineering and technology, Davangere. Project reference no. 41S-BE-2798.
30. Muhamad WNAW, Othman R, Shaharuddin RI, Irani MS. *Adv Environ Biol*,2015:9:8-14.
31. Muthu SS. Roadmap to sustainable textiles and clothing: Environmental and social aspects of textiles and clothing supply chain. NY, USA: Springer; 2014.
32. Nigel J. Mills, Plastics. Third Edition Ch01, 2005, 1-20.
33. Premraj R, Mukesh CD. Biodegradation of polymer. *Indian J Biotech*,2005:4:186-193.
34. Pilz H, Brandt B, Fehringer R. The impact of plastics on life cycle energy consumption and greenhouse gas emissions in Europe, summary report. Vienna, Austria: Denkstatt GmbH, 2010.
35. Priya T, Adria H, Salman AM, Haris S, Usman S, Kalim M. Role of microbes in degradation of synthetic plastics and manufacture of bioplastics. *Journal of chemical and pharmaceutical research*,2016:8(3):211-216.
36. Priyanka N, Archana T. Biodegradability of polythene and plastic by the help of microorganism: A way for brighter future. *J Environ* and *Toxicol*,2011:1(4):1000111. doi: 10.4172/2161- 0525.1000111.
37. Qais YMA, Maher A, Ola A, Abdullah Y. "New Records of *Streptomyces* and Non *Streptomyces* Actinomycetes Isolated from Soils Surrounding Sana'a High Mountain". *International journal of research in pharmacy and biosciences*,2016:3(I3):19-31.
38. Rajput A, Ramachandran M, Gotmare VD, Raichurkar PP. Recent bioactive materials for development of eco-friendly dippers: an overview. *J Pharm Sci Res*,2017:9(10):1844-1848
39. Ravi RK, Vasantba JJ. Isolation of Actinomycetes: A complete approach. *International journal of current microbiology applied sciences*,2016:5(5):606-618.

40. Richard C. Thompson, Charles J. Moore, Frederick S. vom Saal and Shanna H. Swan, 2009. Plastics, the environment and human health: current consensus and future trends, Phil. Trans. R. Soc. B, 364: 2153-2166.
41. Rohindra DR, Preeti PS, Khurma JR. Biodegradation study of poly (e-caprolactone)/poly(vinyl butyral) blends. South Pac. *J. Nat. Sci*,2003:21:47-49.
42. Rowe L, Howard GT. Growth of *Bacillus* subtilis on polyurethane and the purification and characterization of a polyurethanase-lipase enzyme. Int Biodeterior Biodegradation,2002:50:33-40.
43. Rujnic-Sokele M, Pilipovic A. *Waste Manag Res*,2017:35(2):132-140.
Russell JR, Huang J, Anand P, Kucera K, Sandoval AG *et al*. Biodegradation of polyester polyurethane by endophytic fungi. *Appl Environ Microbiol*,2011:77:6076-6084.
44. Saminathan P, Sripriya A, Nalini K, Sivakumar T, Thangapandian V. *J Adv Bot Zool*,2014:1(3):34-38.
45. Saker R, Bouras N, Meklat A, Zitouni A, Schumann P, Spröer C. *Prauserellaisguenensis* sp. Nov. A halophilic actinomycete isolated from desert soil. *International journal of systematic evolution in microbiology*,2015:65:1598-603.
46. Sharma P, Kalita MC, Thakur D. Broad spectrum antimicrobial activity of forest-derived soil Actinomycete, *Nocardia sp*. PB-52. *Frontiers in microbiology*,2016:7:347-350.
47. Shah AA, Fariha H. Biological degradation of plastics: A Comprehensive review. *Biotechnology Advances*,2008:26:246-265.
48. Shristi Kumar K, Hatha AAM, Christi KS. Diversity and effectiveness of tropical mangrove soil microflora on the degradation of polythene carry bags. *Int. J. Trop. Biol*,2007:55:777-786.
49. Shoemaker D. "Proper Procedure for sanitary napkin disposal", *Cleaning and Maintaining Mangement*,2008:45(4):33-37.
50. Sivan A. New perspectives in plastic biodegradation. *Curr Opin Biotechnol*,2011:22(3):422-426. doi: 10.1016/j.copbio.2011.01.013.
51. Shimao M. Biodegradation of Plastics. Curr Opin Biotechnol,2001:12:242-247.
52. Siddiquee M, Helali M, Gafur A, Chakraborty S. Investigation of an Optimum Method of Biodegradation Process for Jute Polymer Composite. *American journal of engineering research*,2014:3(1):200-206.
53. Sreedevi S. Solid Waste Generation and its Management- A case study. *International research journal of environmental sciences*,2015:4(1):90-93.
54. Usha R, Sangeetha T, Palaniswamy M. Screening of Polyethylene degrading Microorganisms from Garbage Soil. Libyan Agric Res Cen *J Intl*,*2011*:2(4):200-204.
55. Vatseldutt, Anbuselvi S. Isolation and Characterization of Polythene Degrading Bacteria from Polythene Dumped Garbage. *Int J Pharm*,2014:25(2):205-206.
56. Webb JS, Nixon M, Eastwood IM, Greenhalgh M, Robson GD, Handley PS. Fungal colonization and Biodeterioration of plasticized polyvinylchloride *Appl. Environ. Microbiol*,2000:66:3194-3200.
57. Yang H-S, Yoon J-S, Kim M-N. Dependence of biodegradability of plastics in compost on the shape of specimens. Polymer Degradation and Stability,2005:87:131-135.