International Journal of Current Science Research
www.drbgrpublications.in

Biodegradation of Super Absorbent Polymers (SAP) from Sanitary napkins in *invitro* conditions

[1]Subashini Devi P, [1]Shenbaga Priya M, [1]Logeshwaran K.S and [2*]Kannan M

[1]*PG & Research Department of Microbiology, V.H.N.Senthikumara Nadar College, (Autonomous) Virudhunagar, Tamilnadu*

[2]*Department of Zoology, V.H.N.Senthikumara Nadar College, (Autonomous) Virudhunagar, Tamilnadu*

[*]*Corresponding author email: kannan.m@vhnsnc.edu.in*

Abstract

The disposal of sanitary napkins poses significant environmental challenges due to their non-biodegradable components such as polyacrylate, polyethylene and polypropylene. These substances lead to ongoing waste buildup in landfills and disposal sites. This research focused on creating a microbial formulation that includes particular bacterial isolates to improve the biodegradation of sanitary napkins. Soil samples were gathered from a waste disposal site and underwent microbial isolation techniques. The bacterial isolates like Bacillus subtilis, Staphylococcus aureus, and Pseudomonas sp., are normally found in incinerator dump site soil. These findings indicate that these specific strains have powerful enzymatic systems capable of degrading the intricate polymers found in sanitary napkins. To access the practical relevance of these results, research on soil degradation were conducted under controlled conditions. The fragments of sanitary napkins were interred in these soils, and their decomposition was observed over time. Among the bacterial isolates, Pseudomonas aeruginosa demonstrated the highest degradation rate of sanitary napkins. These results suggest that these particular strains posses' potent enzymatic systems capable of breaking down the complex polymers present in sanitary napkins. Based on these observations, lyophilized preparations of the effective bacterial isolates were developed. These formulations have the potential to be marketed as biodegradation agents to tackle sanitary napkin waste, thus reducing the environmental pollution linked to their disposal. Utilizing these biological solutions provides a sustainable method for handling sanitary waste and diminishing the environmental impact of these commonly used personal hygiene items.

Keywords: Sanitary Napkin, Biodegradation, Pseudomonas aeruginosa, Polymer Degrading Enzymes, Waste management

Introduction

Menstruation is a natural occurrence, yet it remains a taboo in Indian culture, often viewed as impure and unclean. Menstrual waste refers to the refuse produced by the female reproductive system. The menstrual cycle comprises three stages: the follicular phase, the ovulation phase, and the luteal phase. Approximately 70% of women residing in urban India utilize sanitary napkins, in contrast 48% of women in rural areas. Each year, around 12.3 billion disposable sanitary pads are produced, and the disposal of these plastic items has become a significant issue. According to the Menstrual Health Alliance of India, a single sanitary pad may take anywhere from 500 to 800 years to breakdown because the plastic used is non-biodegradable, which can lead to environmental hazards. Sanitary napkins are utilized by 36% of menstruating females, with the majority being composed of 90% plastic; each pad is equivalent to four plastic bags. Proper disposal methods for used menstrual products remain inadequate worldwide.

Most countries have developed similar techniques to handle their fecal and urinary waste; however, the absence of menstrual management practices worldwide leads to many women to dispose of their sanitary pads in domestic solid wastes or garbage bins. Toilet facilities in India often lack appropriate bins for disposing of sanitary pads and do not provide hand washing stations for maintaining menstrual hygiene. While many modern disposable menstrual products are utilized in urban areas, they are often disposed of by flushing them down toilets or throwing them into dustbins. In rural regions, pit latrines are constructed, and once they are full, they are covered with soil while a new pit is dug; this method, however, is not feasible in urban areas due to space constraints. (Bhagwan *et al*, 2008). According to a survey, women and adolescents keep their used menstruation napkins from decomposing by wrapping them in polythene bags and discarding them in pit latrines. These days, manufactured sanitary pads composed of highly absorbent polymers like polyacrylate are used by women and girls. These pads swell up and get saturated with liquid when flushed in toilets, which causes sewage backflow and poses a major health risk. The commercial sanitary napkins adhesive wings and perforated plastic layers are difficult for soil microbes to breakdown.

Sewage system clogs are a global problem. This is related to the flushing of menstruation products in toilets. Nowadays, most women and girls prefer deodorized sanitary napkins, which include chemicals like bleaching and organ chlorines when buried in soil.

Flushing menstruation items in toilets are a major contributor to sewage system blockages system blockages, which is a global issue. Deodorized feminine hygiene products contain bleaching chemicals such as organo chlorines, which can disrupt soil microflora and take longer to decompose. Used sanitary napkins obstructed the drainage system, forcing conservancy staff to manually clean it without protective protection. It causes employees to reveal harmful chemical and pathogen.

Water bodies become contaminated when people who live next to rivers dump their menstrual discharge into them. These blood-soaked materials served as havens for harmful microorganisms and pathogens (Shoemaker *et al*, 2008). Sanitary goods drenched in soil blood can contaminate with HIV and Hepatitis infected women and girls, who can live in soil for up to six months. The best way to get rid of menstrual waste is to incinerate it, however burning pads emits toxic gases that are bad for the environment and human health. When inorganic materials are burned, harmful and cancer-causing substances like dioxins are released into the atmosphere.

In a number of ways, polymeric material offers heterotrophic microorganisms, such as bacteria and fungi, a high potential source of carbon and energy in a number of ways. Polymeric materials are biodegraded by natural microorganisms such as bacteria and fungi by enzymatic action into microorganism metabolic products (eg.H_2O, CO_2, CH4 biomass etc.). The end effect is a reduction in molecular weight and a loss of structural integrity. Microbial agents that contribute to the deteriorating process by forming biofilms. This review's primary goal was to provide an overview of menstrual waste and fungi, a high potential source of carbon napkins, in contrast 48% of women in rural areas.

The different kinds of incinerator dumpsite soil samples were employed in the soil degradation technique. Wherein the deterioration of torn sanitary pads can be examined. By using an in-vitro technique, it is possible to separate the organisms that breakdown polymers from the soil, screen them, and then cultivate them alongside shredded sanitary pads to see how they breakdown. The organisms used are determined by the enzyme needed to breakdown sanitary pads. Protease, amylase, esterase, or lipase, alkaline phosphatase and urease are the enzymes that breakdown sanitary pads (Swapnil *et al*, 2015).

Several microorganisms including *Bacillus subtilis, Pseudomonas aeruginosa*, and *Staphylococcus aureus* have been linked to the biological degradation of sanitary napkins

based on the enzymes they produce. The weight of the sanitary pads can be used to calculate the rate of degradation (Singh *et al.*, 2012). Degradation rate is equal to the sanitary pad's initial weight less its final weight. It provides an overview of the current necessity of managing menstrual hygiene. The document also includes an assessment of the existing knowledge in the areas of public health, water and sanitation, and solid waste management.

Materials and Methods

Collection of soil samples and sanitary pads

- The soil samples were collected from incinerator dumpsite soil in two different regions under environmental condition.
- Two different types of sanitary pads are collected from stationary shop.

Degradation of sanitary pads in soil

The sanitary pads were sieved into tiny pieces. After that, the sanitary pad pieces' initial weight was measured and they were added to the sand container. After a predetermined amount of time, the shredded pads were taken out of the ground, cleaned with distilled water once more, and dried and their weight was recorded. The samples were biodegraded; the weight loss of the sample was measured.

Isolation of microorganisms from soil

A 1gm of soil sample was taken from the incinerator dump site was mixed with 10 ml of saline. Eight sterile test tubes were filled with 9ml of saline each. Soil sample (1ml) was put serially to each tube. The samples were dispersed on the appropriate media using the spread plate method. Plating media included nutrient agar for bacterial growth. Nutrient agar plates were incubated at 37°C for 24 hours. After 24 hours of incubation the colonies are observed. The isolated colonies are streaked on king's B medium for bacterial isolates like *Pseudomonas sp*. The plates are incubated at 37°C for 24 hours.

Identification of microorganisms

The suspected colonies were inoculated in a nutrient broth with the help of sterile loop and incubated for 24 hours at 37°C.Then the preliminary test was done to identify the organisms. The test including gram staining, biochemical tests are used.

Gram staining

Based on their cell walls, gram positive and gram negative bacteria can be distinguished using the Gram staining procedure. This four-step process makes a bacterial cell stand out against its surroundings by using specific dyes.

Biochemical test

These assays were conducted to confirm the presence of microorganisms. The tests include indole, methyl red, Voges-Proskauer, citrate, urease, as well as oxidative and fermentative testing, and the triple sugar iron test.

Indole test

The capacity of certain microorganisms to generate indole can help differentiate members of the organisms. The presence of indole can be identified with Kovac's reagent, which produces a cherry red color in the presence of indole. If there is no color change, it indicates that tryptophan in the medium was not hydrolyzed, meaning the organism is indole negative.

Methyl red test

The methyl red test serves to differentiate gram-negative bacteria within the Enterobacteriaceae family; a bright color symbolizes a positive result, while yellow or orange indicates a negative one. In the absence of glucose or lactose, some microorganisms utilize citrate as a carbon source, which is dependent on the presence of citrate permease. A positive result is indicated by a blue coloration of the medium, while a green coloration or no color change signifies a negative result.

Urease test

A urease test is a biochemical examination that identifies bacteria capable of decomposing urea into ammonia and carbon dioxide. It is useful for detecting Helicobacter pylori, distinguishing between different yeasts, and identifying organisms that produce urease. Further the organisms confirmed by molecular biology techniques like sequencing.

Degradation of sanitary pads in *invitro* conditions

The microorganism involved in the degradation process was extracted and identified. After that, the necessary medium for the degradation process was determined. For bacterial cultures, nutrient broth or trypticase soy broth was used. The bacterial isolates were

individually introduced with liquid medium along with shredded pads. The conical flask was then placed in an incubator with periodic shaking (Lee *et al*., 1991). At specified intervals, the shredded pads were aseptically removed, washed, dried, and weighed to evaluate the degradation rate. The degradation rate can be evaluated by weighing the samples at consistent intervals (every weekend). The monthly degradation rate can be calculated as an average, with results recorded for each month.

The monthly degradation rate = weight measured at each weekend / number of weeks. Based on the degradation rate, the active degraders were freeze-dried and introduced into the soil to improve the degradation process. The effectiveness of the degradation can be explored further.

Result Interpretation

Isolation of microorganisms from soil

After 24 hours of incubation the colonies are observed from the nutrient agar plate by using spread plate technique. Observed colonies are streaked on to King's B medium for the bacterial growth of *Pseudomonas sp*. The colonies are observed after 24 hours of incubation. Further the organisms are confirmed by preliminary tests and biochemical tests.

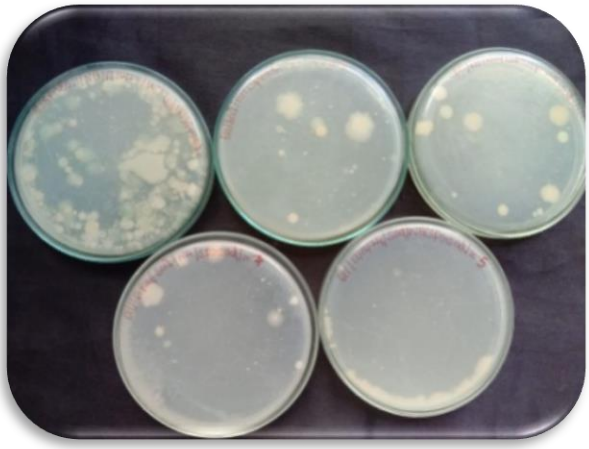

Fig: 1 Isolation of microorganisms from soil

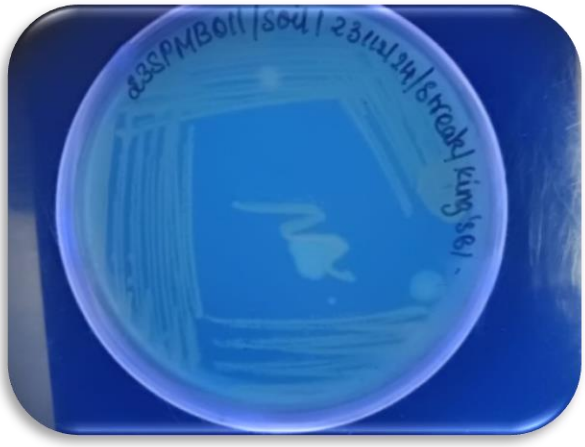

Fig: 2 King's **B** medium plate

Degradation of sanitary pads from soil sample

The shredded pads are sieved and measured. After some intervals the pads are weighed:

Table: 1 Degradation rate of sanitary napkins in soil

Material	Dry weight	Wet weight
Sample 1(Gel)	0.34	0.30
Sample 2 (Cotton)	0.27	0.21

Fig: 3 Degradation of napkins from soil

Gram staining

The organisms was confirmed by morphological characterization as gram negative bacteria *Pseudomonas sp*.

Biochemical test

The organisms are further confirmed by biochemical test. These test are indicated the presence of *Pseudomonas sp*.

Table: 2 Result of biochemical test

Biochemical test	Result
Indole	Negative
Citrate	Negative
Methyl red	Negative
Voges proskaeur	Negative
Triple sugar iron	Negative
Urease	Positive

Fig: 4 Biochemical test

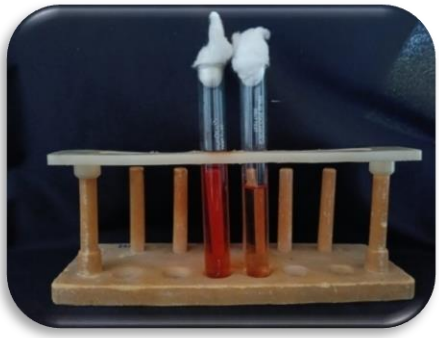

Fig: 5 Urease test

Urease test gives positive result therefore the suspected strains are confirmed it may be a *Pseudomonas aeruginosa.* Normally, most of the pseudomonas species utilize citrate and gives citrate positive result but *aeruginosa* not utilize citrate. Further the organisms are confirmed by molecular biology technique.

Invitro degradation of sanitary pads:

The bacterial isolates are inoculated in tryptic soy broth or nutrient broth along with shredded pads. Shake flask incubation was done at 37^{o}c. After some intervals the degradation rate were calculated.

Fig: 6 Before Degradation of napkins from organisms

Discussion

Based on the literature, (Mishra *et al*., 2012) indicated that bamboo fibre was more absorbent than cotton. However, this experiment demonstrated that bamboo fibre in a non-woven form was nine times more absorbent than cotton and nearly twice as absorbent as a standard sanitary pad. The exceptional absorbency of the bamboo wadding results from the

distinctive structure of the bamboo fiber. Bamboo fibers consist of a distinct cellulose structure that varies from that found in other materials. Although all cellulose sugar molecules have the ability to disrupt a liquid's surface tension and facilitate the liquid's absorption into spaces and within fibres, the crystalline and hierarchical structure of bamboo cellulose is distinct, enhancing the fabric's absorbency. Bamboo possesses effective moisture management properties, categorizing it as a water-resistant fabric with a limited spread area, according to (Amran *et al*., 2015). A recent investigation revealed that bamboo fiber, when used as an absorbent core in a conventional sanitary napkin design, absorbs and wicks water 3-4 times more effectively than cotton and minimizes odor due to its numerous micro-holes and micro-gaps (Barman *et al*., 2017). Consequently, utilizing bamboo fibers as the main component of sanitary pads is a favorable option when compared to SAP, and additionally, it is naturally biodegradable. Moreover, bamboo fiber in wadding form, as explored in the current study, is easier to use and less expensive compared to commercial sanitary pads that contain bamboo fiber. For bamboo wadding to be used as a sanitary pad, there are concerns that need to be addressed regarding its potential scaling and production, which might hinder widespread adoption.

That said, a different option for extensive commercialization is a small-scale, handicraft-based approach that includes the users who will utilize it. In this method, non-governmental organizations (NGOs) can take the forefront, as NGOs in low- and middle-income nations are significantly contributing to socio-economic advancements. For case, Goonj. (Sanjeev *et al.*, 2011) an Indian NGO in New Delhi, presently collect civic supernumerary fabrics, also wet dry and cut them into pads which are packed and distributed via mate grassroots. It possible to involve similar NGOs to distribute low- cost bamboo filling to replace lower spongy fat fabrics, with user’s hand cutting the fabric to give the needed shape. It's also possible to involve similar NGOs to train- up original communities to make bamboo filling from shops. This will reduce the cost, make it readily available among academy girls and women and could have a significant transformative effect. Also, bamboo filling is re-usable in nature.

Conclusion

Environmental contamination is one of the most serious issues that our country faces. This is due to two factors: The primary factor is the use of non-biodegradable materials materials, and secondly, there isn't a sufficient system in place. Disposal of rubbish that has been deposited in environment they are recalcitrant in the environment due to their non-

degradable nature. When waste items are put into soil, microorganisms in the soil use their metabolic processes to try to decompose those non-degradable materials, which are then used as a carbon or energy source by the organisms for further metabolism. However, this is not always the case, which is why pollution happens. Many different types of waste are produced, but domestic waste accounts for the majority of the waste. Sanitary Pads, which are constructed of synthetic polymers and are non-biodegradable, are the most common trash found in residential areas. They are non-degradable due to their makeup, which causes a slew of health problems. The goal of this research is to use microorganisms that naturally reside in soil to decompose sanitary pads. The bacterial and isolates used in this investigation were obtained from the soil of a dumpsite. *Bacillus, Pseudomonas aeruginosa, Escherichia coli,* was among the microorganisms isolated, when comparing the findings of both in vitro and soil degradation studies, in vitro degradation produced the most productive results. The bacterial strain *Pseudomonas sp* in vitro degradation and were also determined to be the most efficient degraders. Both bacterial and fungal isolates were lyophilized and put to the soil to carry out the quicker degradation process, and their efficiency in soil can be further researched based on the degradation.

References

Bhagwan J.N., Still D., Buckley C and Foxon K., (2008). Challenges with up-scaling dry sanitation technologies, *Water Science and Technology*, 58:1:21-27.

Gauri Singh., Ashok Kumar Singh and Kalpana Bhatt, (2016.). Biodegradation of polythenes by Bacteria isolated from soil. *International Journal of Research and Development in Pharmacy and Life Science* ISSN (P): 2393-932X, ISSN (E): 22780238.

Gnanavel G., Mohana Jeya Valli V.P., Thirumarimurugan M., and Kannadasan, T., (2012). Degradation of Plastics Using Microorganisms. *International Journal of Pharmaceutical and Chemical Sciences*. ISSN 2277-5505.

Gu J.D., Ford T.E., Mitton D.B., and Mitchell R., (2000). Microbial degradation and deterioration of polymeric materials. Revive RW (eds) In: Uhlig Corrosion Handbook. s John Wiley and Sons, New York.

James D., Stahl, Michael, D., Cameron, Joachim Haselbach., and Stephen D., Aust, (2000). Biodegradation of Super Absorbent Polymers in Soil. *ESPR-Environmental. Science & Pollution. Res*. 7 (2): 83-88.

Kumar, S., Das, M.L., Rebecca, J., and Sharmila, S., (2013). Isolation and Identification of LDPE Degrading Fungi Municipal Solid Waste. *J.Chem.Pharm.Res*.5 (3): 78-81.

Lee, B., Pometto, A.L., Fratzke, A. and Bailey, T.B. Biodegradation of degradable plastic polyethylene by Phanerochaete and Streptomyces species. *Appl. Environ. Microbial*. (1991). 57: 678-685.

Mahalakshmi, V., Siddiq, A., and Andrew, S.N., (2012). Analysis of Polyethylene Degrading capabilities of Microorganisms isolated from Compost Soil. *Int. J.Pharm. Biol Sci Arch*. 3 (5): 1190-1196.

Nayak Priyanka and Tiwari Archana 2011.Biodegradability of Polythene and Plastic by the Help of Microorganism- A Way for Brighter Future. *J Environmental Analytic Toxicol*. 1: 4.

Nayak, P. and Tiwari, A. (2011). Biodegradation of polythene and plastic by the help of microbial tools: A recent approach (2011). IJBAR. 2 (9): 344-355.

Raziyafathima, M., Praseetha, P.K. and Rimal Issac, R.S. (2016). Microbial Degradation of plastic waste. *Journal of Pharmaceutical, Chemical and Biological Science* ISSN: 2348-7658.

Shah, A.A., and Fariha, H., (2008). Biological degradation of plastics: A Comprehensive review. Biotechnology Advances. 26: 246-265.

Shoemaker D., "Proper Procedure for sanitary napkin disposal", Cleaning and Maintaining Management, 2008: 45(4):33-37.

Sikha Dey., Ashok Kumar Singh and Gauri Singh., (2016). Biodegradation Ability of Bacteria on Plastic and Thermocol Cups. *European J. of Biomedical & Pharmaceutical Sci.* ISSN 2349-8870 3(10): 272-277.