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Original Article

BIOREMEDIATION OF SANITARY NAPKIN BY CELLULOSE-DEGRADING BACTERIA

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ABSTRACT

Objective: This research aims to isolate cellulase-producing microbes from ruminants and investigate their potential for bioremediation of organic wastes, like sanitary napkins. Organic waste management is a critical environmental challenge, and bioremediation offers a sustainable approach for waste treatment. Ruminant animals possess a unique microbial population in their digestive systems that can efficiently degrade cellulose, a major component of sanitary napkins.

Methods: In this study, samples of garden soil, cow dung, buffalo dung, and dumping yard soil were collected and screened for cellulase-producing microbes using Carboxy Methyl Cellulose (CMC) agar medium. Subsequently, the cellulase-producing microbes were employed in the whattman filter paper degradation and their capacity to degrade the cellulose in it by performing a DNSA assay. Furthermore, these isolates were employed in the bioremediation process to degrade sanitary napkins. Thereafter, we prepared various consortia of the isolates to check if it led to better degradation of sanitary napkins.

Results: The results demonstrated the successful isolation of cellulase-producing microbes from all the samples using CMC agar medium and were labeled as Isolates 1, 2, 3, 4, G, and D. In the filter paper degradation assay, isolate 3 produced the highest amount of reducing sugar from 0.1 g of cellulose, followed by isolate G, indicating the highest cellulase or FPase activity among all isolates. Additionally, these isolates exhibited promising potential for the degradation of sanitary napkins. Tube with isolate 3 had the highest concentration of reducing sugar and the lowest dry weight of sanitary napkin, followed by isolate G. Isolates 3 and G showed promising results as compared to the other isolates, but isolated 3 had an antagonistic effect when it was used with other isolates in the consortium. In contrast, isolate G showed synergistic effects in the consortium, and G+D showed the highest degradation of sanitary napkins.

Conclusion: This research contributes a microbial-based bioremediation approach to the development of sustainable and environmentally friendly strategies for waste management.

Keywords: Carboxy methyl cellulose (CMC), Cellulase-producing bacteria, Consortium, Bioremediation, Sanitary napkin

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INTRODUCTION

Over the years, consumer demand for disposable Personal Care Products (PCPs) has increased and is expected to remain strong over the next few years [1]. These rapidly discarded hygiene and sanitary products include wet wipes, sanitary napkins, tampons, incontinence products, panties, diapers, and cosmetic pads. Sanitary materials, such as pads and diapers, are primarily composed of cellulose and non-degradable synthetic elements, including superabsorbent gel, to collect fluids [2]. In low-income urban settlements, these materials are typically discarded in open areas along with other household waste. Despite the benefits these sanitary materials provide, their disposal methods have raised significant concerns, especially in residential areas, where they frequently remain uncollected for extended periods.

Several methods, such as landfilling and incineration, have been successfully employed to remove sanitary pads and diapers from the environment. However, there are three major concerns: (a) sanitary materials in landfills do not readily degrade; (b) sites filled with used sanitary materials become unproductive, rendering them unsuitable for cultivation, grazing, or recreation; and (c) incineration causes environmental pollution by releasing dioxins and noxious gases [3]. These gases have been reported to negatively impact human health, particularly in pregnant women, potentially causing birth defects, miscarriages, and genetic interference [4]. Therefore, there is a need to develop a new and effective method for degrading used sanitary materials that poses less risk to human health and the environment.

Cellulose is a crystalline polymer, which is an unusual characteristic among biopolymers. The cellulose chains in the crystals are stiffened by inter-and intra-chain hydrogen bonds, while the adjacent sheets that lie over one another are held together by weak Van der Waals forces [5]. In nature, cellulose is rarely found in a nearly pure state; more commonly, the cellulose fibers are embedded in a matrix of other structural biopolymers, primarily hemicelluloses and lignin.

Microbial degradation of lignocellulosic waste and the resulting downstream products is achieved through the concerted action of several enzymes, with cellulases being the most prominent. These enzymes, produced by various microorganisms, include several different classifications. Cellulases hydrolyze cellulose (β-1,4-D-glucan linkages) and primarily produce glucose, cellobiose, and cello-oligosaccharides. The three major types of cellulase enzymes are cello-biohydrolase, endo-β1,4-glucanase, and β-glucosidase [6].

Microbial cellulolytic enzymes, responsible for breaking down cellulose, are abundantly found in diverse and extreme environments across the globe [7]. These enzymes are produced by a variety of microorganisms, including fungi, bacteria, and actinomycetes. Notable environments where these enzymes are prevalent include the rumen of ruminant animals, marine and saltwater habitats, soil, insects, and termite intestines. This research underscores the remarkable diversity of cellulolytic microorganisms and their enzymes, highlighting their roles in various environments and suggesting potential applications in industries such as bioremediation and waste management [8].

The diet of ruminant animals, such as cows and sheep, is predominantly composed of cellulose. Cellulose is essential for the fermentation process that takes place in the rumen, a specialized chamber in the animal's digestive system [9]. The microbial population in the rumen produces enzymes that break down cellulose, enabling ruminants to digest and utilize cellulose-rich feed more effectively. Consequently, these animals can access the nutrients and energy stored in cellulose, which would otherwise be

indigestible. It is estimated that the rumen microbiota globally is responsible for degrading billions of tons of cellulosic materials.

In the rumen microbiota, both Gram-negative and Gram-positive bacteria are abundant. The most common cellulolytic bacteria include *Ruminococcus flavefaciens, Fibrobacter succinogenes,* and *Ruminococcus albus*, which produce enzymes capable of breaking down crystalline cellulose [10]. Besides these bacteria, other fiber-degrading bacteria are also present in the rumen. These include *Clostridium lochheadii, Clostridium longisporum, Eubacterium ruminantium, Butyrivibrio fibrosolvens, Eubacterium cellulosolvens,* and *Prevotella ruminicola* [11]. These bacteria play a crucial role in breaking down the fibers in the animal's diet.

Biodegradation is the biologically catalyzed process that breaks down materials into simpler substances, encompassing biodeterioration, which results from factors such as moisture, oxygen, UV light, and microbial activity [12]. For disposable PCPs, this phenomenon has been scarcely studied. Most research focuses on the presence and persistence of these products during wastewater treatment processes and in aquatic environments. Without an established safe disposal method, the disposal of these PCPs, which contain a wide range of chemical components, could lead to various environmental issues. Hence, we proposed to use such enzyme producers in the bioremediation of discarded sanitary napkins.

MATERIALS AND METHODS

Isolation of cellulase-producing microbes

Cow dung and buffalo dung samples were collected from local stables at Sus Gaon, Pune. The samples were 3 d old at the time of use. Garden soil was collected from the Orange Tree Society, Sus Pune. Dumping yard soil was collected from Chinchwad Gaon, Pune. For the isolation of cellulase-producing microbes, CMC agar medium was prepared, where CMC acts as a substrate for enzyme production.

Samples were serially diluted up to 10^5 in sterile saline. 100 µl of the last three dilutions was spread aseptically on sterile CMC media plates and incubated at 37 °C overnight. Cellulase-producing microbes use CMC as their carbon source. A clear zone around the colony indicated the hydrolysis of CMC, which was observed by flooding the plate with Gram's iodine. Before flooding, the individual colonies were secured on a fresh CMC plate [13].

Screening of cellulase-producing capacity of the isolates

Mineral media with cellulose filter paper as the sole carbon source was used for screening the cellulase-producing capacity of the isolates [14]. 0.1 g of cut-out filter paper was weighed, added to the bumper tube containing 10 ml of mineral media, and autoclaved.

Isolated colonies were inoculated in nutrient broth (5 ml), and the tubes were incubated overnight at 37 °C. 1 ml of this overnight-grown culture was inoculated in the filter paper medium (10 ml) in bumper tubes. The tubes were incubated at 37 °C for 3 d. Visible turbidity in the filter paper tubes was an indication of cellulose degradation. Qualitative analysis for cellulose hydrolysis was performed using the DNSA (3, 5-DiNitro Salicylic Acid) assay. Briefly, 1 ml of filter paper containing media showing turbidity was centrifuged, and the enzyme produced by the organisms in the supernatant was estimated by the DNSA method using CMC as substrate. Enzyme and substrate were incubated for 30 min at 37 °C, followed by the addition of 1 ml of DNSA and boiling the tubes in a water bath for 10 min. 7.5 ml of distilled water was then added to make up the volume to 10 ml.

DNSA was added to known concentrations of glucose (200–2000 µg), and the same procedure as mentioned above was followed. In both cases, optical density was measured at 540 nm using a colorimeter. A glucose standard graph was plotted and used to calculate the amount of reducing sugar produced in the test samples.

Bioremediation of sanitary napkins

Mineral media containing sanitary napkins and blood was used to check the bioremediation capacity of the isolates. Sanitary napkins (Whisper Choice ultra-XL) with only the middle part and not the wings were cut into very tiny pieces. 0.1 g of these pieces was weighed and added to 10 ml of mineral media. This medium was then autoclaved, and 5% blood was added to the medium in each tube. The inoculum was prepared as mentioned above. 1 ml of this overnight-grown culture was added to the sanitary napkin medium. These tubes were then incubated at 37 °C. To check the degradation of sanitary napkin, the DNSA method and dry weight assay were used. Readings were taken on days 2 and 4 [15].

Bioremediation of sanitary napkins using consortium of isolates

The isolates that were able to degrade the sanitary napkin were used to make different consortia to check if it led to better degradation of sanitary napkins. Sterile mineral media (10 ml) with 0.1 g of tiny pieces of sanitary napkin and 5% blood was inoculated with the consortium as mentioned in table 1. Another set of similar tubes containing the media and 0.1 g of tiny pieces of sanitary napkin without blood was also inoculated with the consortium. These tubes were incubated at 37 °C for six days. The degradation of sanitary napkins was determined by the DNSA method on days 3 and 6 for both sets (tubes with and without blood). After 3 d of incubation, the culture tubes were filtered through a pre-weighed filter paper; the filter paper, along with the sanitary napkin, was kept in a hot air oven for 1 hour at 50 °C. After complete drying, the filter paper was weighed again to determine the decrease in the weight of sanitary napkin due to cellulose degradation [16].

Table 1: Design of consortium of isolates

S. No.	Consortium
1	1+2
2	1+3
3	1+4
4	1+G
5	1+D
6	3+2
7	3+4
8	3+G
9	3+D
10	G+2
11	G+4
12	G+D
13	1+2+3+4
14	1+3+G+D
15	1+2+3+4+G+D

RESULTS

Isolation of cellulase-producing microbes

For the isolation of cellulase-producing microbes from cow dung, buffalo dung, dumping yard, and garden soil samples, CMC media was used. The six different colonies showing zones of hydrolysis after flooding the plates with Gram's iodine from all four samples were selected for further studies and labeled as isolates 1, 2, 3, 4, G, and D.

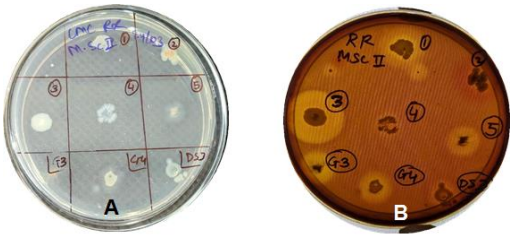
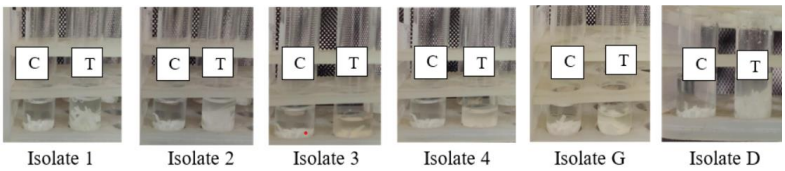


Fig. 1: Isolated colonies were flooded with Gram's iodine to measure zone of hydrolysis; A) Colonies on CMC plate before addition of Gram's iodine B) Zone of hydrolysis observed after flooding with Gram's iodine

Screening of cellulase producing capacity of the isolates

The zone of hydrolysis observed around the colonies served as a qualitative measure of the cellulase enzyme produced by the isolates. The quantitative analysis of the cellulose degradation efficiency of isolates was determined by checking Whattman filter paper degradation. Visible turbidity observed after 3 d of incubation at 37 ℃ was an indication of degradation of the filter paper (fig. 2). The DNSA method was used for estimating the amount of reducing sugar produced after the breakdown of the cellulose. The ODs and their respective concentrations calculated from the glucose standard graph are depicted in table 2. 0.1 g cellulose was thus broken down to 1980 µg reducing sugar by isolate 3, followed by 1950 µg production by isolate G, indicating the highest cellulase or FPase activity among all other isolates.



Fig, 2: Whattman Filter paper degradation assay. C=control; T=test

Table 2: Reducing sugar produced by the isolates after degradation of the Whattman Filter paper (DNSA assay)

Isolates	O. D at 540 nm	Concentration (µg)
1	0.79	1960
2	0.67	1870
3	0.82	1980
4	0.72	1920
G	0.77	1950
D	0.65	1860

Table 3: Concentration and dry weight of sanitary napkins after bioremediation

Isolates	Day 2 O. D at 540 nm	Day 4 O. D at 540 nm	Concentration (µg)	Dry weight of sanitary napkins (g)
1	0.13	0.26	645	0.06
2	0.06	0.22	545	0.07
3	0.15	0.36	893	0.04
4	0.02	0.16	396	0.08
G	0.13	0.35	868	0.05
D	0.01	0.16	396	0.08

Bioremediation of sanitary napkins

To check the sanitary napkin degradation ability of the isolates, they were inoculated in mineral medium with 0.1 g of sanitary napkin as the sole carbon source. Visible turbidity in tubes was an indication of sanitary napkin degradation. The DNSA assay was used for the estimation of reducing sugars formed after degradation of the cellulose present in the sanitary napkin. It was observed that reducing sugar concentration increased significantly as the incubation time increased, which indicates that isolates were able to degrade sanitary napkins. Furthermore, a decrease in the dry weight of sanitary napkin also confirmed the degradation of sanitary napkins, as shown in table 3. It was observed that tube with isolate 3 had the highest concentration of reducing sugar as well as the lowest dry weight of sanitary napkin, followed by isolate G.

Table 4: Concentration and dry weight of sanitary napkins after bioremediation with 5% blood by using consortium of isolates

Consortium	Day 3 reading (With Blood)	Day 6 reading (With Blood)	Concentration of reducing sugar Day 6 (µg)	Dry weight (With Blood) (g)
1+2	0.19	0.33	818	0.05
1+3	0.23	0.34	843	0.05
1+4	0.19	0.29	719	0.06
1+G	0.27	0.35	868	0.05
1+D	0.24	0.32	793	0.05
3+2	0.14	0.27	669	0.06
3+4	O.13	0.22	545	0.07
3+G	0.22	0.27	669	0.07
3+D	0.13	0.27	669	0.07
G+2	0.16	0.24	595	0.07
G+4	0.11	0.26	645	0.07
G+D	0.26	0.38	942	0.03
1+2+3+4	0.19	0.27	669	0.07
1+3+G+D	0.21	0.29	719	0.05
1+2+3+4+G+D	0.22	0.21	521	0.08

Bioremediation of sanitary napkin using consortium of isolates

Consortiums of the isolates were prepared to check if it enhanced their degradation activity. The same protocol as mentioned above was followed, with the difference that in this assay, instead of a single isolate, combinations of isolates with an equal number of cells matched through the McFarland method, was performed. The DNSA assay was performed on days 3 and 6. It was observed that isolate 3 performed better individually but not in the consortium. Other isolates, like isolates 2 and 4, performed better in the consortium.

The highest concentration of reducing sugar was obtained by strain 1+G after 2 d, but after two more days of incubation, strain G+D showed better degradation of sanitary napkins. Strain G proved to be working better in consortium with other isolates, especially with strain D, whereas isolate 3 does not work better with other organisms. The assay set in which blood was not used showed diminished degradation (table 5), indicating that blood is a major component for cellulose degradation. Further, the dry weight assay of the sanitary napkins also supported these observations that are mentioned in table 4. A similar pattern was observed in the dry weight assay, where strain G+D showed a significant decrease in both with and without blood. Strain G proved to be working better in consortium with other isolates, especially with strain D, whereas isolate 3 showed antagonistic effect with other isolates.

Table 5: Concentration and dry weight of sanitary napkins after bioremediation without blood by using consortium of isolates

Consortium	Day 3 reading (Without Blood)	Day 6 reading (Without Blood)	Concentration of reducing Sugar Day 6 (µg)	Dry weight (Without Blood) (g)
1+2	0.01	0.02	49	0.09
1+3	0.02	0.03	74	0.09
1+4	0	0.02	49	0.1
1+G	0	0.08	198	0.08
1+D	0.03	0.07	173	0.08
3+2	0.01	0.02	49	0.09
3+4	0.02	0.03	74	0.1
3+G	0.01	0.03	74	0.1
3+D	0.01	0.01	24	0.1
G+2	0.01	0.08	198	0.08
G+4	0.02	0.06	148	0.08
G+D	0.03	0.04	99	0.09
1+2+3+4	0.01	0.03	74	0.09
1+3+G+D	0.03	0.04	99	0.08
1+2+3+4+G+D	0.03	0.05	124	0.08

DISCUSSION

Cellulases have broad commercial applications because they convert lignocellulosic biomass into simple monosaccharides via enzymatic degradation, which can then be used to manufacture several valuable products in the industry [17]. Furthermore, cellulase can be used for many other applications, including waste management, pigment extraction, and bioactive molecules extraction from plant materials [18], along with bioremediation, as discussed in this study.

In this study, cellulase enzyme was produced by microorganisms isolated from cow dung, dumping yard, and garden soil samples. Isolate 3 and G proved to be promising in degrading the CMC, filter paper, and sanitary napkins. Another group studied CMC degradation, and comparable outcomes were documented for *Acinetobacter anitratus* and *Branhamella* sp. cultivated on a basic salt medium, both using glucose and CMC as their exclusive carbon sources. The cellulase-degrading enzymes of *A. anitratus* and *Branhamella* sp. were quantitatively identified by [19] and showed to be maximum for CMC. The results of present study were also consistent with prior studies that described the degradation of amorphous substrates, such as CMC, by strains of *Bacillus licheniformis* [20].

The consortium studied here identified that isolate 3 was not able to perform in the presence of other isolates, but isolate G could do well in a synergistic manner with other isolates. Similarly, the statistics for synergetic cellulose degradation found in four sets of mixed cultures were only 23.5%, 26.3%, 19.4%, and 24.5%, respectively, according to a study published by lu *et al.* [18]. On the same lines, according to Bichet-Hebe *et al.* [21], using a gravimetric technique, the rates of paper degradation for mixed bacterial populations varied from 31 to 60% after 10 d, with some mixtures showing antagonistic effects and some showing synergistic effects.

The degradation of the sanitary napkins was studied by a DNSA assay and a dry weight assay. Consortia of the isolated bacteria were used to degrade the sanitary pad, which was cut into tiny pieces. G+D showed the most promising results. These two isolates could synergistically break down 0.1 g of sanitary napkin to 0.03 g after two days and produce 942 µg of reducing sugar. These findings were equated with those of a prior study conducted by Nanda *et al.* [22] on the biodegradation of polymers assessed based on weight loss.

CONCLUSION

We aimed at isolating cellulase-producing microbes from ruminants and checking their cellulase activity. In the screening assays, it was confirmed that our isolates did show cellulase activity, both with respect to the zone of hydrolysis and in terms of the estimation of reducing sugars as well.

We also checked their ability to degrade filter paper; these isolates could not completely degrade the filter paper in 48 h but showed promising loss in the weight of degraded paper.

Additionally, we checked their ability to degrade sanitary napkins; most of our isolates did degrade the sanitary napkins; some strains worked better alone; some of the isolates showed better activity when they were in a consortium.

Sanitary napkins are mostly released in water bodies or are incinerated; both of these modes of disposal cause water and air pollution, making them more and more hazardous to the environment. With this concept in mind, we isolated bacteria that were able to degrade the sanitary napkins. Further modifications in our isolated strains, like genetic modifications, can lead to even better degradation efficiency.

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AUTHORS CONTRIBUTIONS

Experimental design, guidance, supervision and review work for the research was done by Dr. Suneeta Panicker. Experimental work, development and optimization of the formulations, interpretation of result and writing of this manuscript was done by Ms. Radha Kundaliya and Ms. Ruchita lohakane. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Mango P. The future of global nonwoven wipes market forecasts to 2023. In: 57th Dornbirn global fiber congress: Dornbirn, Austria; 2018.
2. Reyes NJ, Geronimo FK, Yano KA, Guerra HB, Kim LH. Pharmaceutical and personal care products in different matrices: occurrence, pathways, and treatment processes. Water. 2021;13(9):1159. doi: 10.3390/w13091159.

3. Kjellen M, Pensulo C, Nordquist P, Fogde M. Global review of sanitation system trends and interactions with menstrual management practices [SEI Project report]: Stockholm. Sweden; 2012.
4. Siddiqua A, Hahladakis JN, Al-Attiya WA. An overview of the environmental pollution and health effects associated with waste landfilling and open dumping. Environ Sci Pollut Res Int. 2022;29(39):58514-36. doi: 10.1007/s11356-022-21578-z, PMID 35778661.
5. Rajinipriya M, Nagalakshmaiah M, Robert M, Elkoun S. Importance of agricultural and industrial waste in the field of nanocellulose and recent industrial developments of wood based nanocellulose: a review. ACS Sustainable Chem Eng. 2018;6(3):2807-28. doi: 10.1021/acssuschemeng.7b03437.
6. Neelamegam A, Rajeswari M, Thangavel B. β-glucanases: role, applications and recent developments. Endocrinologist Microbial Enzymes in Bioconversions of Biomass. 2016;1,4:37-45.
7. Dienes D, Egyhazi A, Reczey K. Treatment of recycled fiber with Trichoderma cellulases. Ind Crops Prod. 2004;20(1):11-21. doi: 10.1016/j.indcrop.2003.12.009.
8. Narendrakumar G, Nmd S, PP, Tv P. Analysis of gut flora from damp wood termites (*trinervitermes* spp.) and extraction, characterization of cellulase from the isolate. Asian J Pharm Clin Res. 2017;10(6):233-6. doi: 10.22159/ajpcr.2017.v10i6.17565.
9. Das KC, Qin W. Isolation and characterization of superior rumen bacteria of cattle (Bos taurus) and potential application in animal feedstuff. Open J Anim Sci. 2012;02(4):224-8. doi: 10.4236/ojas.2012.24031.
10. Jindou S, Borovok I, Rincon MT, Flint HJ, Antonopoulos DA, Berg ME. Conservation and divergence in cellulosome architecture between two strains of *Ruminococcus flavefaciens*. J Bacteriol. 2006;188(22):7971-6. doi: 10.1128/JB.00973-06, PMID 16997963.
11. Nyonyo T, Shinkai T, Mitsumori M. Improved culturability of cellulolytic rumen bacteria and phylogenetic diversity of culturable cellulolytic and xylanolytic bacteria newly isolated from the bovine rumen. FEMS Microbiol Ecol. 2014;88(3):528-37. doi: 10.1111/1574-6941.12318, PMID 24612331.
12. Arshad K, Skrifvars M, Vivod V, Volmajer Valh JV, Voncina B. Biodegradation of natural textile materials in soil. Tekstilec. 2014;57(2):118-32. doi: 10.14502/Tekstilec2014.57.118-132.
13. Hema JN, Shobha SSD. Isolation and characterization of cellulose-degrading bacteria from decomposing plant matter. Int J Pharm Pharm Sci. 2023;15(4):22-7.
14. Gupta P, Samant K, Sahu A. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. Int J Microbiol. 2012;2012:578925. doi: 10.1155/2012/578925, PMID 22315612.
15. Gayathri B, Gayathri V. Formulation of bacterial and fungal isolates for the degradation of sanitary napkins. Asian J Microbiol Biotechnol Environ Sci. 2018;20(2):S230-6.
16. Patel AK, Singhania RR, Sim SJ, Pandey A. Thermostable cellulases: current status and perspectives. Bioresour Technol. 2019;279:385-92. doi: 10.1016/j.biortech.2019.01.049, PMID 30685132.
17. Ajeje SB, Hu Y, Song G, Peter SB, Afful RG, Sun F. Thermostable cellulases/xylanases from thermophilic and hyperthermophilic microorganisms: current perspective. Front Bioeng Biotechnol. 2021;9:794304. doi: 10.3389/fbioe.2021.794304, PMID 34976981.
18. Ekperigin MM. Preliminary studies of cellulase production by *Acinetobacter anitratus* and *Branhamella* sp. Afr J Biotechnol. 2007;6(1):28-33.
19. Fujimoto N. Bacillus licheniformis bearing a high cellulose-degrading activity, which was isolated as a heat-resistant and micro-aerophilic microorganism from bovine rumen. TOBIOTJ. 2011;5(1):7-13. doi: 10.2174/1874070701105010007.
20. Lu WJ, Wang HT, Nie YF, Wang ZC, Huang DY, Qiu XY. Effect of inoculating flower stalks and vegetable waste with ligno-cellulolytic microorganisms on the composting process. J Environ Sci Health B. 2004;39(5-6):871-87. doi: 10.1081/LESB-200030896, PMID 15620093.
21. Bichet Hebe I, Pourcher AM, Sutra L, Comel C, Moguedet G. Detection of a whitening fluorescent agent as an indicator of white paper biodegradation: a new approach to study the kinetics of cellulose hydrolysis by mixed cultures. J Microbiol Methods. 1999;37(2):101-9. doi: 10.1016/s0167-7012(99)00043-3, PMID 10445310.
22. Nanda S, Sahu S, Abraham J. Studies on the biodegradation of natural and synthetic polyethylene by Pseudomonas sp. J Appl Sci Environ Manag. 2010;14(2):57-60.