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Enhancement of Polyhydroxybutyrate (PHB) Production using Organic Waste as Substrate

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Abstract: ***One of the greatest environmental issues we face today is the accumulation of non-degradable plastics. Plastic debris are discarded every year, everywhere polluting the nature. Polyhydroxybutyrate (PHB) is produced by joining of poly ß-hydroxybutyrate monomers by ester bonds. Bioplastics have numerous applications in different fields of life they are used in clothing industrial products, fluid containers, surgical materials. PHB is completely biodegradable and highly hydrophobic thermoplastic polyester. The aim of this study is to yield high and efficient PHB accumulation bacteria has been selected by quantification and extraction method. The culture medium and growth parameters for all the isolates were optimized for maximum production. The highest PHB production was observed with groundnut oil cake with 27% respectively and orange peel 23% respectively. The optimum PHB production condition were observed in 48h and 72h at 30°C. Authentication of PHB extract by Fourier transform–infrared (FTIR) by identifying its functional units as C-H, CH2, C=O, and C-O. The yield of PHB can further optimized by production parameters as substrates.***

Keywords: Polyhydroxybutyrate (PHB), agro-industrial waste, fourier-transform infrared spectroscopy (FTIR).

I. INTRODUCTION

The production of plastic requires four basic steps: the acquirement of raw material, synthesizing a basic Biopolymer, compounding the polymer into a usable fraction, and lastly, moulding or shaping the plastic. A large number of (PHA), only a few of them are employed for large scale production which includes: P(3HB); poly-(3-hydroxybutyrate-co-3 hydroxyvalerate) and poly-3-hydroxybutyrate-co-3-hydroxy hexanoate [2], [5], [6]. The current cost of PHB production is considerably more than that of synthetic plastics.

To overcome agro-industrial waste materials can be used to economize the production. Bioplastics are bio-based, biodegradable plastics almost similar properties to synthetic plastics. Biodegradation can be explained as a chemical process during which micro-organisms that present in the environment convert materials into natural substances such as waste carbon dioxide and compost. The term Bio based means material derived from plants (Biomass). Synthetic plastics remain in the environment for a long time as they are resistant to degradation. Bioplastics are made for variety of sources like polysaccharides, lipids, and also proteins. The selection of efficient carbon substrate is a key aspect, which validates the total cost of the final product. The unconventional approach is to choose renewable, economically reasonable and most readily available carbon substrates for both microbial growths and efficient PHB production. They often used for bags, trays, fruit and vegetable containers and blister foils, egg cartons, meat packaging, vegetables, and bottling for soft drinks and dairy products. These plastics are also used in non-disposable applications including mobile phones casings, carpet fibers insulation car interiors, fuel lines and plastic piping. New electro active bioplastics are being developed that can be used to carry electric current. In these zones, the goal is not biodegradability, but to make items from sustainable resources.

II. MATERIALS AND METHOD

A. Extraction and quantification

10 ml of overnight Culture was taken and centrifuged at 10,000 rpm for 15 min. The supernatant was discarded and the pellet was treated with 10 ml sodium hypochlorite and mixture was incubated at 30°C for 1h. The mixture was centrifuged at 5000 rpm for 15 min and then washed with distilled water, acetone, and methanol respectively. The pellet was dissolved in 10 ml Hot chloroform and filtered using whtmann filter paper and the powder were completely dried and weighed 1 ml were taken from the filtered chloroform solution and kept in boiling water bath until completely evaporated and 5 ml of sulphuric acid were added to the dried chloroform. Conversion of PHB into crotonic acid were observed by brown colour. PHB concentration were taken from the UV vis spectrophotometer, for the sharp peak absorbance were taken at 240 nm. The relative PHB accumulation by the different isolates were compared to help in identification of the best producer.

B. Pretreatment of Agricultural Residues

Locally collected (groundnut oil cake, sugarcane bagasse, and orange peel) were shredded into pieces, dried in oven for about one week for complete drying. And using mortar and pestle make it into a fine particles and added has a substrate for the isolates. To determine the particular species, bacterial DNA was isolated using 16s rRNA method. *Bacillus cereus* (isolate 1), *Bacillus endophyticus* (isolate 2), *Bacillus subtilis* (isolate 3), *Enterococcus durans* (isolate 4), *Bacillus sps* (isolate 5) and extraction was done by above methods.

C. Effect of agro-industrial waste on the production of PHB

Different waste substrates used as the carbon source (orange peel, sugarcane bagasse and groundnut). All the Argo-industrial waste is collected and then dried for a week. Then the peel is grinded Powderly and 2% of the peel is supplemented as the carbon source in the MSM broth medium and the isolates were inoculated.

D. FTIR (Fourier Transform Infra-red Analysis)

FTIR spectroscopy is a form of vibrational spectroscopy, the sample was irradiated with the infrared radiation from an infrared source, and absorption of the radiation stimulates vibrational motions by depositing quanta of energy into vibrational modes the functional groups of PHB were identified and compared with the standard references.

The IR spectrum of the sample represented the total chemical composition, because every chemical compound in the sample made its own distinct contribution to the absorbance spectrum. To determine the chemical structure of each component. The chloroform phase containing PHB were subjected to FTIR spectroscopic analysis in order to know the functional groups present in PHB 1 mg of the extracted sample of PHB was dissolved in 5 ml of chloroform and allowed to evaporate to get PHB powder, which was subjected to FTIR analysis using spectrophotometer. spectra were recorded in 4000 cm-1 to 450 cm-1 range.

III.RESULTS

Among the different carbon sources (sugarcane, orange peel, ground nut) used as substrate with varying concentrations and it is compared without substrate in this study. *Bacillus endophyticus* showed more production of (24% in groundnut, 34% in sugarcane bagasse and 27% in orange peel). The extracted PHB samples were identified for their functional groups through FTIR analysis. The functional groups were identified as, O-H alcohol groups, C-H methyl group, C=C alkene, C-O, C=O aldehyde.

A. Estimation of PHB from sugarcane bagasse, Groundnut oil cake, Orange peel

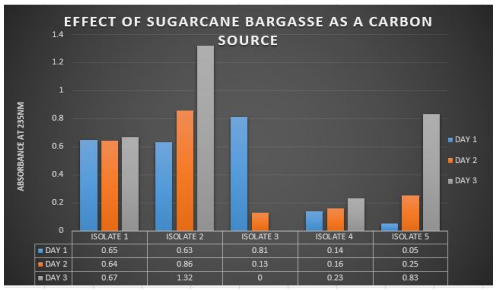

Fig 1. Yield of PHB with different concentrations of carbon source (sugarcane bagasse)

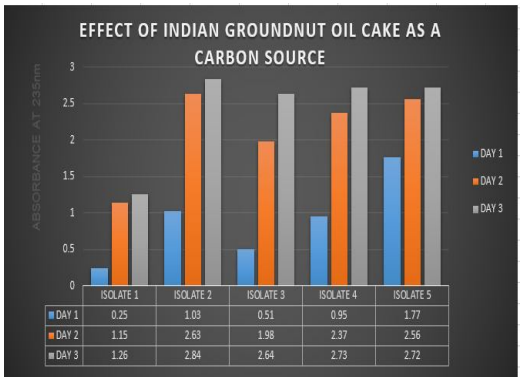

Fig. 2. Yield of PHB with different concentrations of carbon source (groundnut oil cake)

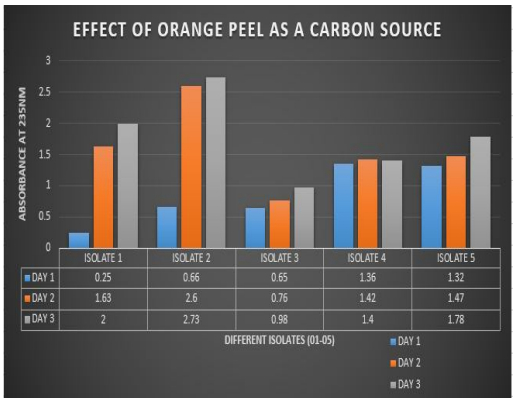

Fig. 3. Yield of PHB with different concentrations of carbon source (orange peel)

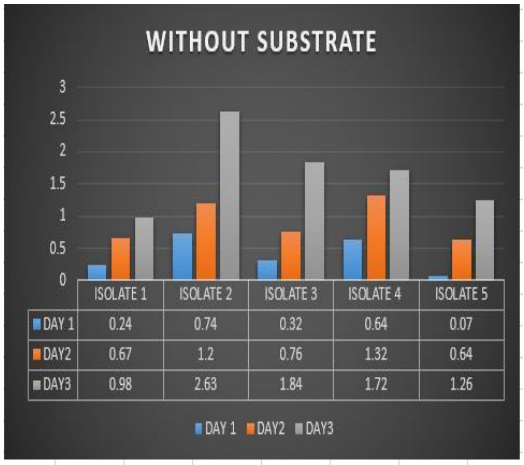

Fig. 4. Yield of PHB without substrate

B. FTIR Analysis

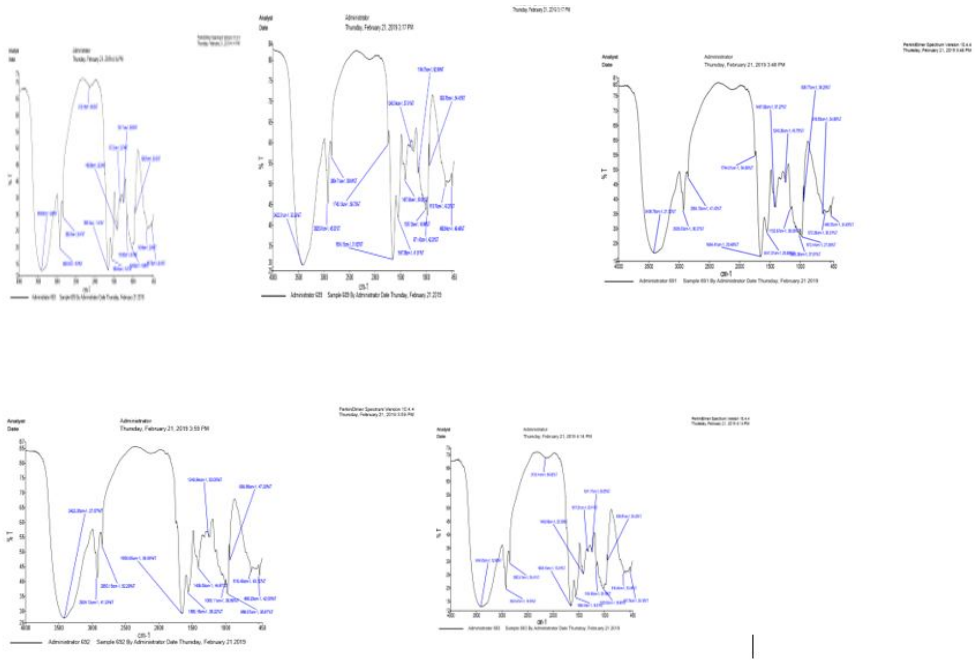

Fig. 4. FTIR results of isolates

TABLE I
FUNCTIONAL GROUPS OF FTIR ANALYSIS

FREQUENCY	FUNCTIONAL GROUP	INTENSITY	BOND
3423.29 cm^{-1}	Alcohol groups	Strong	O-H
2923.90 cm^{-1}	Methyl and Methylene	Strong stretching	C-H
1643.57 cm^{-1}	Alkene	Strong	C=C
1240.29 cm^{-1}	Alkyl ether	Varies	C-O
939.76 cm^{-1}	Methyl and methylene	Bending strong	C-H
1745.12 cm^{-1}	Aldehyde	Stretching strong	C=O

IV. DISCUSSIONS

Among the substrates used in the PHB production, groundnut oil cake showed higher production when compared with orange peel and sugarcane bagasse. This is because groundnut oil cake is rich in fat content. Nutrient limitation leads to increase in PHB production whereas high nitrogen state directed increase in biomass growth with no PHB production.

In this present study five bacterial species were identified for PHB production. Among the isolates, *Bacillus endophyticus* showed more production in 48h and 72h which is similar to the work of [22]. *Bacillus amyloliquefaciens* and *Nocardiopotens* using low cost raw materials like bran orangepeel, gives maximum production of PHB (16.5 μg/ml) and (26.8 μg/ml) which is reported in [17] showed similar results with the present study of *Bacillus endophyticus*.

Pre-treated sugarcane bagasse (56%) was the best cheap carbon source followed by corn cob (52% PHB) work done by [11] similar report was studied by the present study in which sugarcane showed 27% of yield. In the FTIR analysis results, the peaks at 3423cm−1 indicated stretching strong O-H bond created by the terminal OH groups found in isolates. The peaks at 2924/2925 and 2923/2924 cm−1 are assigned to C–H stretching methyl and methylene groups respectively. PHA marker bands allocated to carbonyl C= O stretches of the ester groups of FTIR peak 1745.12 cm-1.

V. CONCLUSION

To minimize the cost of Polyhydroxybutyrate production, different types of agro industrial waste was utilized as the source for the production of Polyhydroxybutyrate. The environmental safe, organic agro waste such as groundnut oil cake, orange peel, sugarcane bagasse was chosen as the substrate for the production of Polyhydroxybutyrate. And the conditions were followed by 24h, 48h, and 72h respectively. In which 48h and 72h showed more production in Isolate 2 (*Bacillus endophyticus*) average yield of -27%. further overall production was seen in groundnut oilcake than orange peel and sugarcane bagasse.

The isolated Polyhydroxy Butyrate was further confirmed by FTIR spectral analysis was performed and the functional groups C=O, -OH, CH2- groups were identified with the presence of alcohols, methylene and alkenes. Further statistical analysis of Annova were used for the substrates for each day in which $p<.05$ showed significant results. Hence it was concluded that *Bacillus endophyticus* species has served as the best producer among those isolates from the rhizospheric soils.

VI. ACKNOWLEDGMENT

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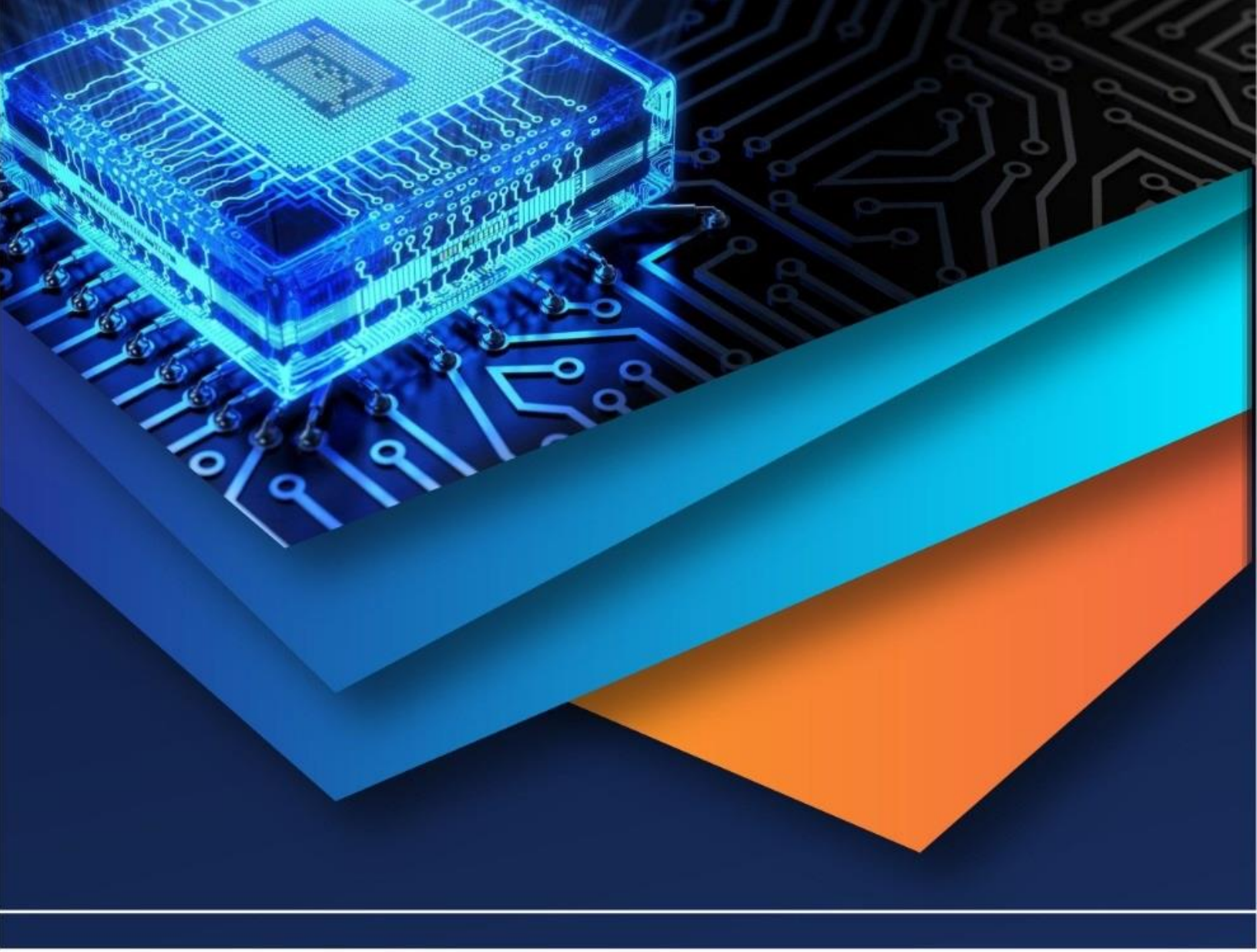
REFERENCES

[1] Aung, S. P. S., Shein, H. H. H., Aye, K. N., & Nwe, N. (2018). Environment-Friendly Biopolymers for Food Packaging: Starch, Protein, and Poly-lactic Acid (PLA). In Bio-based Materials for Food Packaging (pp. 173-195). Springer, Singapore.
[2] Asrar, J., Valentin, H. E., Berger, P. A., Tran, M., Padgette, S. R., & Garbow, J. R. (2002). Biosynthesis and properties of poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) polymers. Biomacromolecules, 3(5), 1006- 1012.
[3] Barrett, D. G., Lamb, B. M., & Yousaf, M. N. (2008). Microfluidic etching and oxime-based tailoring of biodegradable polyketoesters. Langmuir, 24(17), 9861-9867.
[4] Belal, E. B., & Farid, M. A. (2016). Production of Poly-β-hydroxybutyric acid (PHB) by Bacillus cereus. Int. J. Curr. Microbiol. Appl. Sci, 5, 442-460.
[5] Chanprateep, S. (2010). Current trends in biodegradable polyhydroxyalkanoates. Journal of bioscience and bioengineering, 110(6), 621-632.
[6] Chandani, N., Mazumder, P. B., & Bhattacharjee, A. (2018). Biosynthesis of biodegradable polymer by a potent soil bacterium from a stress-prone environment. Journal of Applied Biology & Biotechnology Vol, 6(2), 54-60.
[7] Costa, J. A. V., Moreira, J. B., Lucas, B. F., Braga, V. D. S., Cassuriaga, A. P. A., & Morais, M. G. D. (2018). Recent Advances and Future Perspectives of PHB Production by Cyanobacteria. Industrial Biotechnology, 14(5), 249-256.
[8] Ceyhan, N., & Ozdemir, G. (2011). Poly--hydroxybutyrate (PHB) production from domestic wastewater using Enterobacter aerogenes 12Bi strain. African Journal of Microbiology Research, 5(6), 690-702.
[9] DiGregorio, B. E. (2009). Biobased performance bioplastic: Mirel. Chemistry & biology, 16(1), 1-2.
[10] Elsayed, N. S., Aboshanab, K. M., Aboulwafa, M. M., & Hassouna, N. A. (2013). Optimization of bioplastic (poly--hydroxybutyrate) production by a promising Azomonas macrocytogenes bacterial isolate P173. African Journal of Microbiology Research, 7(43), 5025-5035.
[11] Getachew, A., & Woldesenbet, F. (2016). Production of biodegradable plastic by polyhydroxybutyrate (PHB) accumulating bacteria using low cost agricultural waste material. BMC research notes, 9(1), 509.
[12] Hoseinabadi, A., Rasooli, I., & Taran, M. (2015). Isolation and identification of poly β-hydroxybutyrate over-producing bacteria and optimization of production medium. Jundishapur journal of microbiology, 8(7).
[13] Hassan, M. A., Bakhiet, E. K., Ali, S. G., & Hussien, H. R. (2016). Production and characterization of polyhydroxybutyrate (PHB) produced by Bacillus sp. isolated from Egypt. J. App. Pharm. Sci, 6(4), 46-51.
[14] Haas, R., Jin, B., & Zepf, F. T. (2008). Production of poly (3-hydroxybutyrate) from waste potato starch. Bioscience, biotechnology, and biochemistry, 0712210692-0712210692.
[15] Jacquel, N., Tajima, K., Nakamura, N., Miyagawa, T., Pan, P., & Inoue, Y. (2009). Effect of orotic acid as a nucleating agent on the crystallization of bacterial poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) copolymers. Journal of applied polymer science, 114(2), 1287-1294.
[16] Kale, S. K., Deshmukh, A. G., Dudhare, M. S., & Patil, V. B. (2015). Microbial degradation of plastic: a review. J Biochem Technol, 6(2), 952-961.
[17] Mahitha, G., & Madhuri, R. J. (2016). Microbial polyhydroxybutyrate production by using cheap raw materials as substrates. Indian Journal of Pharmaceutical and Biological Research, 4(1), 57.
[18] Marjadi, D., & Dharaiya, N. (2014). Recovery and characterization of poly (3-Hydroxybutyric acid) synthesized in Staphylococcus epidermidis. African Journal of Environmental Science and Technology, 8(6), 319-329.
[19] Nehra, K., Chhabra, N., Sidhu, P., Lathwal, P., & Rana, J. S. (2017). Molecular identification and characterization of Poly-β-hydroxybutyrate (PHB) producing bacteria isolated from contaminated soils. Asian Journal of Microbiology, Biotechnology and Environmental Sciences, 17, 281-290.
[20] Nehra, K., Sidhu, P., & Rana, J. S. Molecular identification and characterization of Poly-β-hydroxybutyrate (PHB) producing bacteria isolated from bacillus.
[21] Nishida, H., & Tokiwa, Y. (1993). Distribution of poly (β-hydroxybutyrate) and poly (ε-caprolactone) aerobic degrading microorganisms in different environments. Journal of Environmental Polymer Degradation, 1(3), 227-233.
[22] Panigrahi, S., & Badveli, U. (2013). Screening, isolation and quantification of PHB-producing soil bacteria. Int. J. Eng. Sci. Inv, 2(9), 1-6.



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