POTENTIAL UTILIZATION OF THE UNDERUTILIZED FOOD STUFF PALMYRA HAUSTORIUM AS THE SOLE CARBON SOURCE FOR THE SYNTHESIS OF BIOPOLYMER POLY HYDROXY BUTYURATE (PHB) USING *BACILLUS MEGATERIUM* MTCC 6544

[1]N. Thulasi Raman*
Register Number: 12158
Research Scholar
Manonmaniam Sundaranar University
Sri Paramakalyani Centre of Excellence in Environmental Sciences
Alwarkurichi, Tamil Nadu, India – 627 412

[2]A.G.Murugesan
Professor
Research Supervisor
Manonmaniam Sundaranar University
Sri Paramakalyani Centre of Excellence in Environmental Sciences
Alwarkurichi, Tamil Nadu, India – 627 412
*Corresponding author
*Corresponding author E mail: thulasiraman.ntr@gmail.com

Abstract:

Bioplastics provide voluminous rewards compared to conventional plastics derived from fossil fuels. Among the principal advantages of bioplastics are the following: Bioplastics are made from plant-based materials such as potato starch, sugarcane, maize, and other renewable resources. When comparing standard plastics to bioplastics, the former often have a lower carbon footprint. Biodegradable or compostable, bioplastics are designed to disintegrate over time into their constituent parts. This might help reduce environmental contamination and the long-term impacts of plastic waste. A class of biopolymers known as polyhydroxybutyrates (PHBs) is produced by different microbial species. PHB is not made of petroleum-based chemicals, like produced plastics, and is recyclable and biocompatible. PHBs have innovative applications in packaging, nanotechnology, medicine, and agriculture, among other areas. PHB is produced using a range of feed sources, such as dairy waste, food industry waste, and agricultural waste. Right now, PHB characterization and production from Palmyra haustorium which is an underutilised carbohydrate containing substrate. The ability of *Bacillus megaterium* to synthesize PHB under the right circumstances and with the right amount of nutrients. In order to increase the cellulose content by 84.20 percent, the physical and chemical characteristics of the raw substrate Palmyra haustorium were investigated. The optimal conditions for PHB formation are 35°C and pH 7. The highest temperature, as

determined by thermo gravimetric analysis, is 259.8°C. The thermal deterioration range is 287°C, while the melting temperature of the DTA peak is 256°C. hOne of an affordable substrate that yields more PHB is Palmyra haustorium

I. Introduction

Plastic has been a part of almost every area of our life and was once considered a wonder of contemporary ingenuity. Its uses are endless, with applications in the fields of packaging, building, healthcare, electronics, and automobiles, among others. Because it is affordable, strong, and lightweight, plastic has emerged as the main material in our consumer-driven culture. But plastic's pervasiveness comes at an incredible cost to the ecosystem (Geyer et. al. 2017).

Globally, the abundance of single-use plastics—from bottles to bags—has overflowed landfills and clogged rivers. Convenience-driven products like these have come to symbolize our disposable society, which is catastrophic for biodiversity and ecosystems. Carelessly dumped plastic debris clogs rivers, floods streets, and builds up in enormous gyres that span the seas, upsetting marine environments and endangering numerous species. Even in the most distant regions of the world, micro plastics—the sneaky leftovers of bigger plastic objects or micro beads in personal care products—are present in food chains and pose unanticipated health concerns to people (Jambeck, 2015).

The environmental impacts of plastic are many and diverse. In addition to ruining the oceans and landscapes, plastic waste threatens the fragile ecological balance that supports all life. When marine life mistakenly swallows hazardous compounds from plastic waste, it may cause hunger, asphyxia, and even reproductive failure. Coral reefs, essential marine environments rich in biodiversity, are under risk of suffocation and illness due to entanglement and smothering by plastic. When plastic garbage is left on land, it contaminates food and water sources, erodes groundwater tables, and poses a health risk to people (Barnes 2019).

Plastic pollution is a sneaky problem that goes beyond its obvious effects. The manufacture and disposal of plastics increase emissions of greenhouse gases, which exacerbate climatic variability and jeopardize the stability of the earth (Lebreton 2018). Plastic pollution has an equally startling financial impact that affects governments, businesses, and communities equally. Municipal finances are strained by clean-up operations, and medical costs are rising as a result of diseases and accidents brought on by plastic. Many economies rely heavily on tourism, but when plastic pollution taints once-pristine sites and discourages travellers, tourism suffers (Agenda 2016).

The battle against plastic pollution is more than just an environmental necessity; it is a moral commitment to protect the earth for future generations. As we face the legacy of our plastic addiction, let us learn from the past and chart a course toward a more sustainable products or ways to protect our environment (Rochman 2015). There has been a lot of attention lately in in this study arena for the evolution of CRU using bio-derived polymers as coating ingredients such as starch (Versino et al., 2020). Bio-based polyurethane includes lignin (Elhassani, 2019), cellulose (Pang et al., 2019), and biodegradable polyurethane (Feng et al., 2019). Lemoigne discovered polyhydroxybutyrate (PHB) *in Bacillus megaterium* in 1926. Poly (3-hydroxybutyrate) (P3HB) is a well-known PHA that has physical properties similar to polypropylene. Bacteria synthesize and store polyhydroxybutyrates as granules in their cytoplasm. According to Aman et al., (2018) in Sri Lanka there are 10 million palms on 25 000 hectares (two-thirds in Jaffna area), and in India 60 million palms (two- thirds in Tamil Nadu). Central Burma (Myanmar) has 2.5 million palms on 25 000 acres, while central Cambodia has 1.8 million palms. Palmyrah palm, beautify the parched landscape of the semi-arid areas of Tamil Nadu, Andhra Pradesh, Odisha, West Bengal, Bihar, Karnataka and Maharashtra. India has over 102 million palms and half of them are in Tamil Nadu. Distribution Of Palmyra In Tamil Nadu (Veilmuthu et. al. 2020). India has the biggest amount of Palmyra (85.9 million) in the globe and out of which 60% are in Tamil Nadu (51 million). According to Kanthimathi (2015), Tamil Nadu has the main share with 51.9 million trees with the district of Thoothukudi ranking top. Hence its widely cosmopolitan in distribution it is producing.

The fruit of the Palmyra palm is enormous and fibrous which comprises of nut-like sections which consist of seed in it. The fruits are three-sided when the fruit ripens, It's a profound brown to black. Every segment of the palmyra tree retains its distinct nutritional characteristics. Palmyra roots are abundant in fibres It is a reliable source of Vitamin E and carbs also. Palmyra haustorium is the palatable, sweet in flavor food contained within the endocarp of Palmyra nut which is abundant in Carbohydrates.

The discovery and use of renewable feedstock for the synthesis of multifunctional chemicals has attracted a lot of attention from the food and pharmaceutical industries. The production of ecologically friendly products requires the effective utilization of resources. PHB production is efficiently created when bacteria are given the acid hydrolysate of the aquatic weed water hyacinth as a nutrition source. (Radhika and Murugesan 2012). Furthermore to reducing the cost of manufacturing for Azotobacter chroococcum's PHB biosynthesis, groundnut shell hydrolysate is an effective agricultural waste management strategy. The process increases the production of PHB, reducing our dependence on fossil fuel-derived plastics (Nagajothi and Murugesan 2023).

The species known technically as *Borassus flabellifer*, or Palmyra palm, is found in tropical climates, especially in Southeast Asia and the Indian subcontinent. Its haustorium, a unique structure essential to its life and development, is one of its most intriguing characteristics. (Muralidharan 2011). The Palmyra palm has an underutilised carbohydrate containing food stuff called the haustorium, which allows the plant to take up water and nutrients from surrounding host plants. It grows horizontally underground from the base of the palm stem and is basically a modified root structure. By connecting with other plants' roots, this system enables the Palmyra palm to access their resources. because to its structure that connects to their roots.

While the connection between the Palmyra palm and its host plants is not always destructive, the haustorium functions as a parasitic organ. Rather, hemiparasitism, a kind of symbiosis, would be a more realistic description. The Palmyra palm gains nutrients and water from the host plant in this kind of interaction, but the host plant may sometimes gain as well, for example, via better soil conditions or erosion protection.

Numerous environmental elements, including as soil quality, moisture content, and the presence of compatible host plants, affect how the haustorium develops. Once developed, the haustorium may reach great lengths, allowing the Palmyra palm to have access to resources love a large region.

Apart from aiding in the intake of nutrients and water, the haustorium also functions to stabilize the Palmyra palm within its natural environment. It aids in anchoring the palm in place by forming linkages with other plants, especially in regions vulnerable to floods or soil erosion.An interesting possibility for sustainable material development might be the haustorium of the Palmyra palm, which can be used to produce bioplastic. As environmentally beneficial substitutes for conventional plastics made from fossil fuels, bioplastics are becoming more and more popular. Making use of natural resources such as the haustorium of the Palmyra palm may provide a sustainable and biodegradable means of producing bioplastics.

The creation of bioplastics may be facilitated by the haustoria's abundance of organic substances, including as cellulose, lignin, and polysaccharides. In order to effectively extract these substances from the haustoria, extraction techniques would have to be created.

One interesting approach to sustainable material development might be to produce bio plastics from the haustorium of the Palmyra palm. Natural plastics made from fossil fuels are being replaced with bio plastics, which are becoming more and more popular. A sustainable and biodegradable supply of bio plastic might be provided by using natural resources such as the haustorium of the Palmyra palm. To produce polyhydroxybutyrate from *Bacillus megaterium* MTCC 6544, Palmyra haustorium was used as a substrate. A physicochemical study was conducted on the selected Palmyra haustorium used in order to produce PHB.

II. Material and Methods

2.1 Collection of PALM HAUSTORIUM

Substrates of Palmyra haustorium were gathered and delivered to the laboratory from the nearby market. After being thoroughly cleaned and cut into small pieces, the The raw material was dehydrated for 48 hours at 70°C in a hot air oven. It was then ground into a fine powder using a standard mixer grinder and stored in an airtight plastic container for later use. (Radhika and Murugesan., 2012).

2.2 Pretreatment of raw material

The substrate for Palmyra haustorium was pre-treated with 5% H_2SO_4 and autoclaved for 30 minutes at 121°C. Following a fast cooling process to ambient temperature, the suspensions were filtered through muslin fabric to get rid of the insoluble particles. After adding 20M NaOH to the filtrate, the suspension was once again filtered using Whatman No. 1 filter paper. Subsequently, the filtrate was used directly as the fermentation's carbon source. (Radhika and .Murugesan, 2012).

2.3 Preparation of Seed Culture

A 250 ml flask holding sterile culture medium (100 ml) containing the following nutrients contents was injected with the *Bacillus megaterium* MTCC 6544 bacteria cultured in nutrient agar medium.: peptone (10 gL^{-1}), yeast extracts (5 gL^{-1}), NaCl (10 gL^{-1}), glucose (10 gL^{-1}) incubated at 27°C for 96 h at a rate of agitation of 100 rpm.

2.4 PHB Production Medium

Glucose peptone broth media (20 gL^{-1} of Peptone, 10 gL^{-1} of dextrose, 5 gL^{-1} of NaCl) was used to produce a seed culture of *Bacillus megaterium*. The media that had been infected were treated at the agitation speed in a revolving shaker of 100rpm for 24 - 48 h. Then 5% of the respective turbid cultures were inoculated into the standard minimal medium (Radhika and Murugesan 2012). *B. megaterium* was cultivated under the following nutrients existed mineral media; 0.79 g of $MgSO_4$. $7H_2O$, 2.98 g of Na_2HPO_4,1.5g of KH_2PO_4, 15 mg of $FeSO_4.7H_2O$.

2.5 Shake Flask Fermentation

PHB was produced in a laboratory setting. The hydrolysate was made based on the quantity of reducing sugar released. Carbon resource hydrolysate, minimum mineral supply, and trace elements were sterilized separately in a laboratory size autoclave at 121°C for 30 minutes at 15 lbs of pressure for the creation of fermentative medium. After steam sterilization of the raw material hydrolysate, the solutions of minimal mineral salts and tiny elements were thoroughly mixed in, and 3% inoculum was applied. It was then grown at ambient temperature in a laboratory-sized shaking incubator with a spinning speed of 100 rpm.

2.6 Extraction of PHB

The production of PHB by bacteria was collected using the dispersion method. The culture mixtures were centrifuged at 5000 rpm for 15 minutes after incubation, and the culture pellets were cleaned with distilled water and acetone. The washed cell pellets were then treated with an equal amount of Six percent sodium hypochlorite and chloroform and incubated at 37°C for 1 hour. The previously described solution mixture was centrifuged for 20 minutes at 5000 rpm after incubation. After centrifugation, three distinct layers (upper-degraded cell materials, middle-non-degraded cell materials, and the bottom layer of chloroform that contained PHB) were produced. The process of non-solvent precipitation was used to create the chloroform layer containing PHB molecules (methanol and water in a 7:3 ratio) and held at 4°C for subsequent analysis.

2.7 Estimation of Physio-chemical properties

The chemical composition of Palmyra haustorium determines their physio-chemical qualities. PHB is created by bacteria using carbon sources as an energy source, while bacterial cell biomass is produced by bacteria using nitrogen. (Stefana et al., 2019). Palmyra haustorium extract contains cellulose, hemicellulose, phosphorus, carbohydrate, protein, lignin and nitrogen. These were estimated through different techniques that have been mention below and data was collected.

S.No	Content	Method of Estimation	Reference
1.	Moisture	Oven Drying	Jeandson et al., 2018
2.	Ash	Combustion	DI Sánchez et al.,2004
3.	Phosphorus	Spectrophotometric Determination	Y Zhai 2013
4.	Nitrogen	Kjeldahl-digestion Method	WH Baker et al, 1992
5.	Cellulose	Medium-throughput Method	Manoj Kumar & Simon 2015
6.	Hemicellulose	ADF (Acid detergent fiber) Method	Goering, H D and Vansoest, P J (1975)
7.	Lignin	ADF (Acid detergent fiber) Method	Goering, H D and Vansoest, P J (1975)
8.	Carbohydrate	Anthrone Method	Sumanta 2021

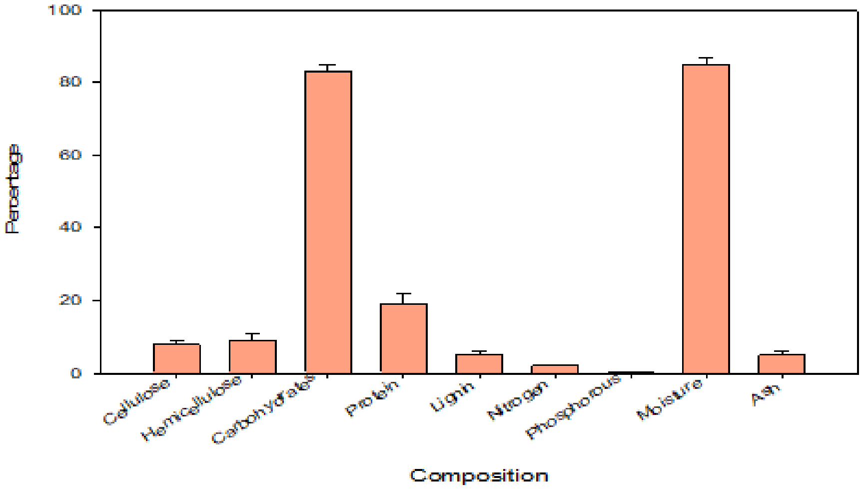
2.8. Optimization of Culture Medium Conditions for PHB Production

To maximize the carbon supply, several carbon sources including maltose, glucose, sucrose, fructose starch, and Palmyra haustorium hydrolysate were put into the medium for fermentation. at different 1% concentrations. (Saba et al., 2021). Different nitrogen sources were added to the medium individually at 1% concentrations in order to optimize the nitrogen source (ammonium nitrate, ammonium sulfate, ammonium chloride, peptone and Palmyra haustorium). Physical parameter of growth medium is also important as it is affecting growth and development of bacteria. pH and temperature were evaluated for favorable growth condition. To analyze the impact of pH, various fermentation media were prepared at different pH values (5, 6, 7, 8, and 9). To analyze the impact of temperature, incubation temperatures were maintained at 25°C, 30°C, 35°C and 45°C.

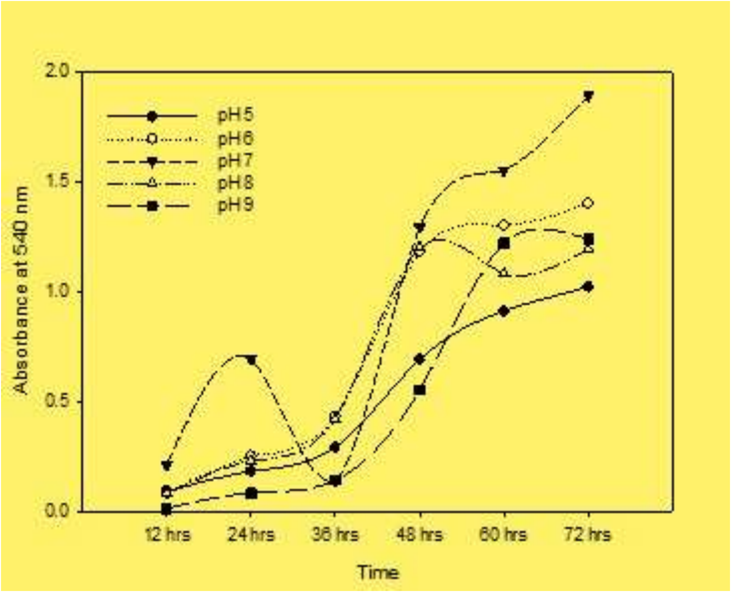
3. Results

3.1. Physio chemical composition of raw material (Figure 1)

The raw material consists of lignin, cellulose and hemicellulose polymers in a predominant proportion compared to other mineral compounds. The chemical parameters of the raw material Palmyra haustorium are presented. In the composition of the raw material, carbohydrates are the maximum, which is 83%, and phosphorus is the minimum, which is 0. 02%. Of these elements, cellulose and hemicellulose are the most important because they contain strong carbon building blocks needed in bioplastics study.



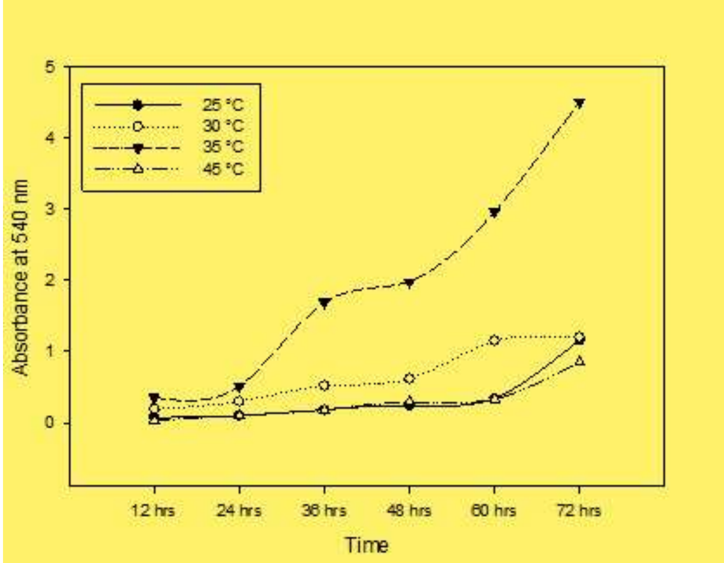
3.2. Growth of *Bacillus megaterium* MTCC 6544 at different pH (5, 6, 7, 8 and 9) at various time of intervals (Figure 2)



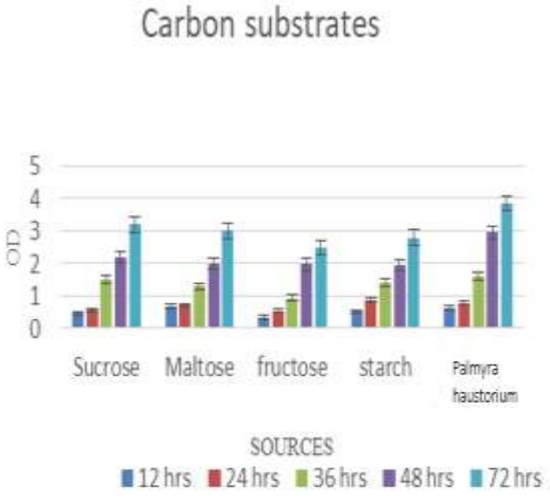
The effect of pH on the growth of *B.megaterium* poses impact on growth of bacteria the optical density of the cultures was analyzed at 540 nm at various intervals as well as affect the PHB production rate. Adequate pH ranges play vital role for PHB production. Even slight variation on pH, metabolic processes of PHB could be impacted adversely. As per the result that was taken from different pH ranges at pH parameters (5 - 9), the minimum growth rate was achieved after 24 hours of incubation and the maximum growth rate was achieved after 72 hours of incubation. Compared to all pH parameters at pH 7, the growth rate was maximum. Also, minimal growth was observed at pH 9. These results conclude that the suitable pH for *B.megaterium* MTCC 6544 is pH 7.

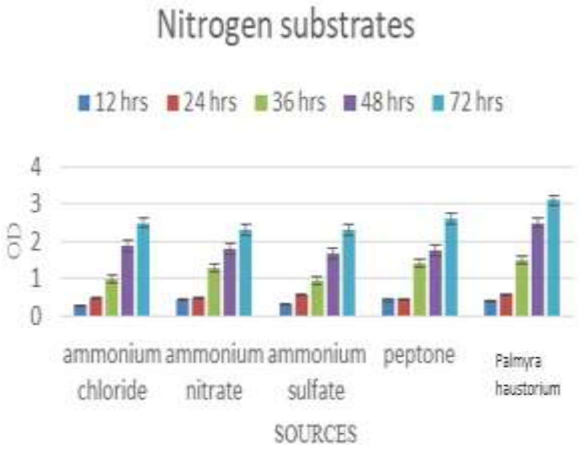
3.3. Growth of Bacillus *megaterium* MTCC 6544 in different temperature at various time intervals (Figure 3)

The effect of temperature on the growth of *B. megaterium* MTCC 6544. This effect was analysed by estimating the optical density of the cultures at 540 nm at different time intervals. Temperature is an important factor involved in bacterial growth. Minimal growth was observed at a temperature of 45 °C, and similarly maximum growth was observed at a temperature of 35°C. The optimal temperature for growth of PHB-producing bacteria is 35°C.



3.4. Growth of Bacillus *megaterium* MTCC 6544 in different Carbon and Nitrogen sources at various time intervals (Figure 4, Figure 5)





The impacts of carbon and nitrogen sources on development of *Bacillus megaterium* are shown in Figure 4. This study showed that Palmyra haustorium is the leading source of carbon for the most extreme development rate. The least number of developments had a place to the fructose as carbon source. Figure 5 appeared that Palmyra haustorium is the foremost reasonable nitrogen source among diverse nitrogen sources with development concentration. The most reduced level of development was observed within the medium with ammonium sulfate as nitrogen source.

Figure 6. PHB sheet produced from using Palmyra haustorium *Bacillus megaterium* MTCC 6544 culture

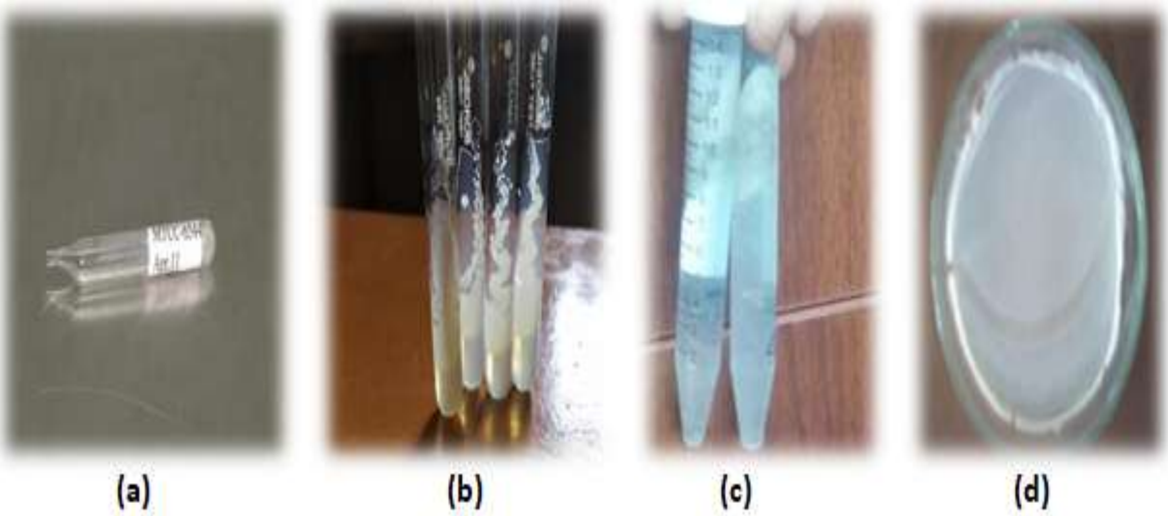
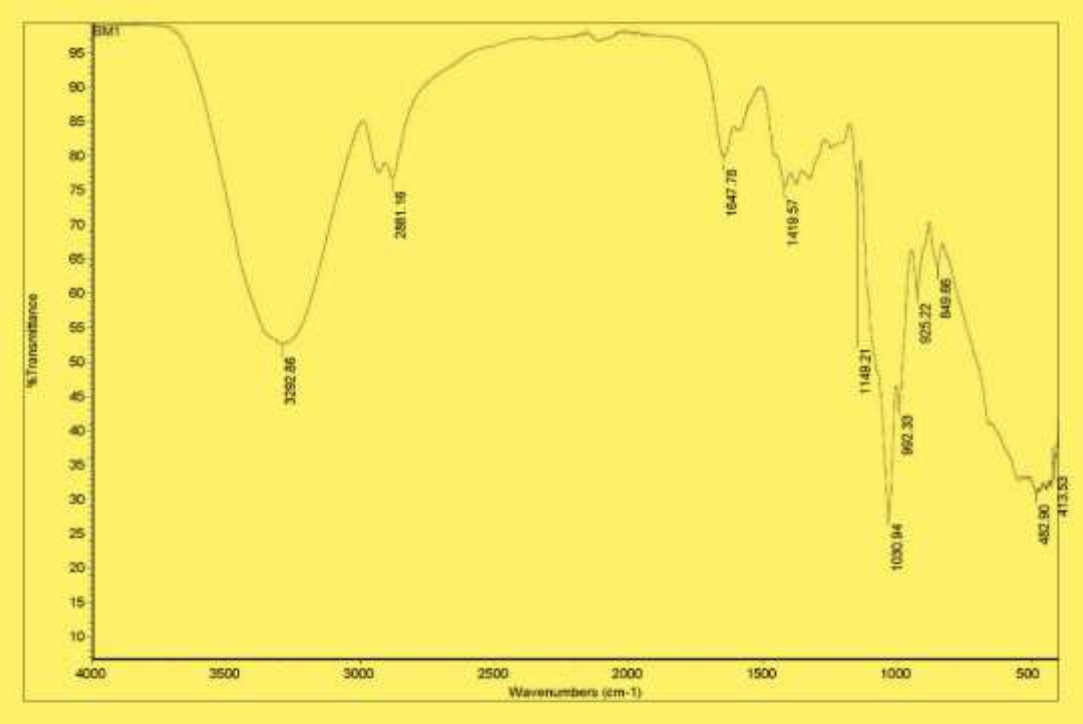
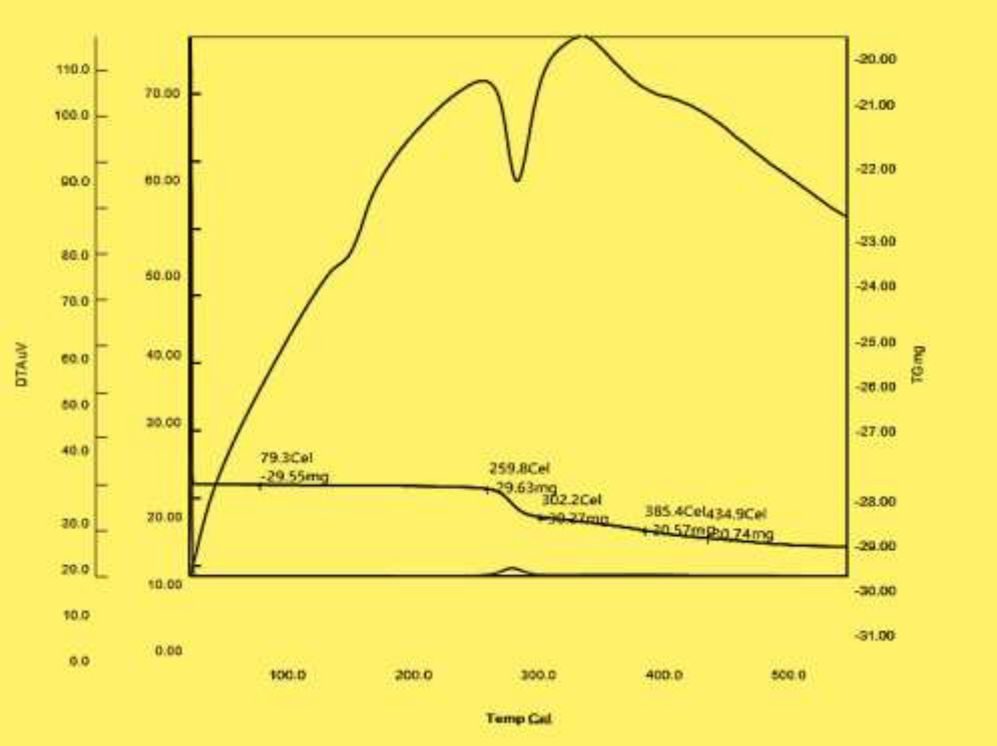


Figure: 6 (a) *Bacillus megaterium* MTCC 6544 culture was bought from MTCC, (b) Sub-culture of *Bacillus megaterium* MTCC 6544, (c) Centrifuged Extraction of PHB from *Bacillus megaterium* MTCC 6544 culture, (d) PHB sheet produced from *Bacillus megaterium* MTCC 6544 using Palmyra haustorium

3.5. FTIR SPECTRUM OF PHB SHEET



3.6. THERMAO GRAVITOMETRIC ANALYSIS



4. DISCUSSION

The utilization of Polyhydroxy butyrate as a substrate for non-biodegradable petroleum-based plastics taken a toll considerably more than its fossil fuel-based partners and offer a number of execution advantage other than biodegradability. To play down the taken a toll of Polyhydroxy Butyrate generation, the Palmyra haustorium was utilized as the source for the generation of Polyhydroxy Butyrate. In this consider PHB was delivered utilizing microscopic organism's *B. megaterium* MTCC 6544. Youhong et al, 2013 detailed that PHB is delivered by *B. megaterium* R11, was disconnected in Singapore and might amass PHB up to 51.3% of its cell dry weight (CDW) from both glucose and xylose. PHB substance and generation come to 58.5% and 9.32 g/L.

Investigation of low-cost cellulose has been inquire about center for biomass change over the past few decades. On-site chemical generation could be more cost-effective and cruder chemical can be straightforwardly utilized for corrosive hydrolysis without the requirements of protein concentration and conservation. In spite of the fact that li3egnocellulosic materials are potential substrates for low-cost PHB generation, so distant as it were a couple of such ponders have been detailed. The most elevated PHB substance, 73.9% (w/w), was gotten by Yu and Stahl (2008). Optimization is an efficient technique for standardize the supplement components and required physical conditions to the superior development and formative of mechanically appropriate microorganisms since the enzymatic reactions and other formative instruments of microorganisms may be superbly carry out with the assistance of different components of developing environment. Medium containing carbohydrates as the excellent carbon source with PHB production appeared impressive metabolic capacity. A basic sugar like glucose can be rapidly utilized by bacteria effectively and this process increments PHB yield (Issazadeh et al., 2015).

On the restricting, a complex particle like starch can't is effectively utilized by bacteria. In this way, an increment in starch complexity leads to diminish in PHB yield (Nehra et al., 2015). Palmyra haustorium consist of cellulose, carbohydrate and nitrogen contribute to most elevated development rate. In this manner, the sort of nitrogen source and utilized microorganisms are key components for effective PHB generation. In addition, precise pH control is of great importance because small changes in pH can affect the metabolism of an organism. Research on the influence of pH shows that pH 7 is the optimal pH for PHB production. Furthermore, considering the low production efficiency of pH 5 and 9, it can be concluded that pH values above and below the optimal limit can affect the enzymes involved in PHB synthesis and destroy them (Saba et al., 2021). Bharathi et al., (2016) also reported that pH 7 was optimal for PHB production by *Bacillus cereus* BB613-A. Among the five different temperature conditions, the maximum PHB production was observed at 35°C and the minimum at 45°C. The decrease in PHB efficiency at high temperatures may be due to low activity of the PHB polymerase enzyme (Saba et al., 2021). Hamieh et al., 2013 introduced 37°C as the optimal temperature for PHB production *by Bacillus thuringiensis* and *Bacillus subtilis*.

The results obtained in this study and other reported results confirm the findings of Thirumala et al. 2010 stated that PHB can be produced by *Bacillus* species at temperatures of 30–38 °C. The pH and temperature were standardized. The group societies of *Bacillus megaterium* MTCC 6544 explored and optimized for the generation of the microbial PHB (Silva et al., 2007 and Lee, 1998.)

In FT-IR analysis, the band observed at 1030.94 was attributed to the C–O bond. These band results are corroborated by the reports of Sushobhan Pradhan 2021. Pure PHB has a C–O ester bond represented by a wavenumber of 1149.21 cm-1. It shows a closely related group of PHBs declared by Mathiyazhagan et al., (2020). The band found at 1419.57 cm-1 corresponds to the asymmetric deformation of the C–H bond in the CH2 group. Likewise, this was confirmed by comparing the results with Jimmy et al. (2012), who obtained PHB from *B.megarium*. Furthermore, the bands at 1647.78 cm-1 indicate the occurrence of O-H bonds of aliphatic compounds. This is related to the findings of Mathiyazhagan et al., (2020) who obtained PHB from *Bacillus cereus* and recorded the frequency band at 1633.71 cm -1. Bands were recorded at 3292.86 cm-1 and 2881.16 cm-1, corresponding to the C=O and C-H stretches of aliphatic carbonyl esters of most biopolymers (Mathiyazhagan et al., 2020). The band and peak profiles of PHB change due to microbial metabolism. The fingerprint region from 1000 to 400 is distinguished by the large number of infrared bands there. These can be many different vibrations, including stretching of the C-O, C-C, and C-N single bonds, C-H bending vibrations, and some bands due to benzene rings that can be found in this region. The TGA analysis of PHB synthesized from Palmyra haustorium as raw material shown in fig. 3.6. TGA curves show two types of weight loss. The initial stage weight loss corresponds to evaporation of physically absorbed solvents of the polymer. The degradation of the polymer commences with melting in temperature range of 200°c to 300°c.

5. CONCLUSION

According to this present study, the optimal culture conditions for maximum PHB production by bacteria isolated from Palmyra haustorium as carbon source and nitrogen source, were at pH 7 and at a temperature of 35°C. The local *Bacillus megaterium* strain can be used as a suitable candidate for industrial PHB production. In addition, Palmyra haustorium is a suitable substrate for isolating bacteria that produce this biopolymer. To improve the capacity of microbial biodegradable polymer production and reduce production costs, it is proposed to use inexpensive Palmyra haustorium as a carbon source substrate.

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