3.6 Using Cane Molasses As A Carbon Source

Culture was inoculated in varying concentrations of Cane Molasses such as 2.5%, 5%, 10%, 15%, 20%, 25%, 30% and after successive incubation concentrations were measured colorimetrically.

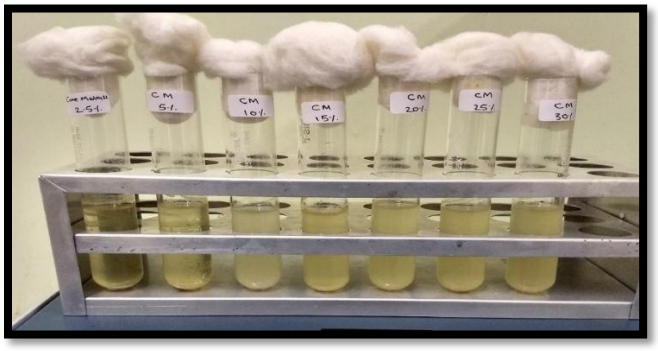


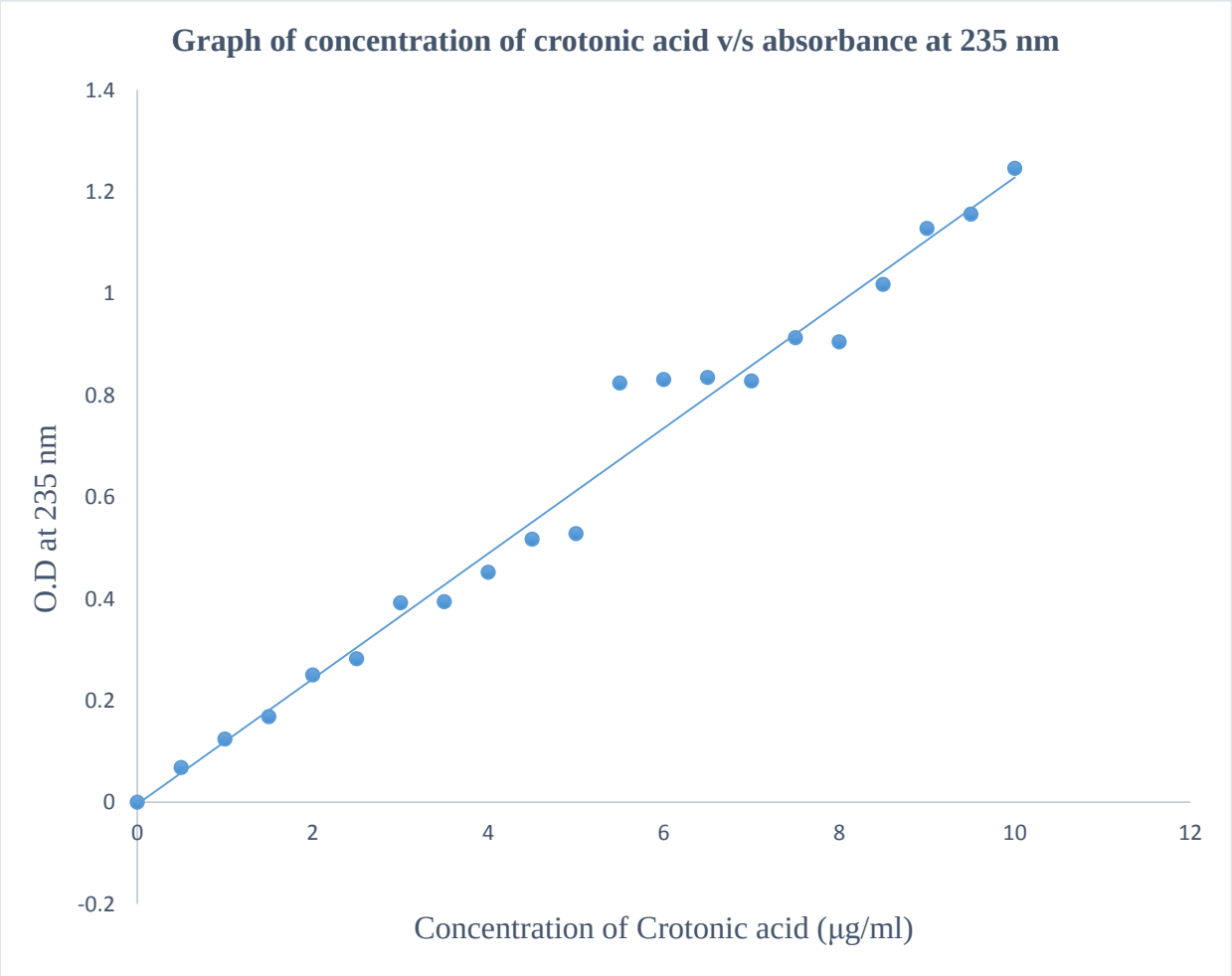
Figure 10: Growth in different Cane molasses concentration

Table 4: Effect of different concentrations of Cane molasses on PHB production by the selected bacterial isolate.

ISOLATE	DIFFERENT CONCENTRATIONS OF CANE MOLASSES (%)	PHB YIELD (mg/100 ml)
Kalina soil	2.5	2.8
	5	4.42
	10	7.86
	15	6.25
	20	3.63
	25	3.0
	30	2.3

3.7 Uv-Vis Analysis Of PHB Accumulation

Graph 1: Standard curve of concentration of Crotonic acid (mg/100 ml) v/s absorbance at 235 nm



Here, different concentrations of crotonic acid was measured against H_2SO4 blank using UV-Vis spectrophotometer at 235 nm. The absorbance of the sample was found to be at 0.60 nm and after plotting a graph of concentration of crotonic acid v/s absorbance at 235 nm, the concentration was found to be 4.9 µg/ml. As the sample was 1:2 diluted, the final concentration was found to be 9.8 µg/ml.

3.8 Preparation Of Bioplastic

Bioplastic was produced by the isolate from Kalina Campus using best carbon source (cane molasses) at 10% concentration and best nitrogen source (peptone) at 2.5% concentration and evaporating the filtrate at 4^{o}C.

Figure 11: Bioplastic

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3.9 PHB Degradation Study

Bioplastic which was produced was degraded by the same organism by performing spot inoculation method. It might be due to the fact that due to starvation conditions the organism might be breaking down lipids to obtain energy.

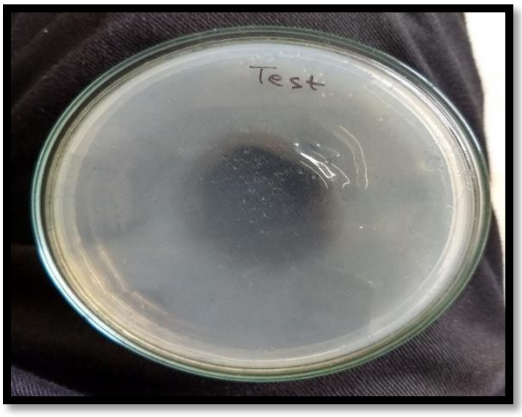

Figure 12: PHB degradation by *Bacillis subtilis*

4. CONCLUSION

The results of this study confirmed that cheaply available agro-residues can be used for the production of PHB serving triple purposes of reducing the cost of biodegradable plastics, reducing environmental pollution problems caused by conventional plastics and solving disposal problem of the agricultural wastes. The organism was able to grow well using cane molasses as a carbon source instead of glucose. It produced bioplastic in the presence of best carbon and nitrogen source at 4°C after pouring the filtrate into the plate.

Poly-β-hydroxybutyrate (PHB) is a natural, biodegradable polymer accumulated in the form of intracellular granules by a large variety of bacteria. In this project, PHB producing bacteria were isolated from different soil samples. The cultural parameters were optimized for the selected isolates. With a view to reduce the cost of PHB production, a cheaper substrate; molasses was evaluated for the production of PHB. Nowadays plastics and synthetic polymers are mainly produced using petrochemical materials that cannot be decomposed, thus resulting in environmental pollution. They are stored, burnt or recycled. During combustion, water and carbon dioxide are released into the atmosphere, i.e., an increase in the carbon dioxide concentration in the atmosphere occurs. Incineration is very difficult, dangerous and expensive and recycling is a long process and not very efficient. Some plastics still cannot be recycled or incinerated due to pigments, coatings and other additives added to the plastics when they are made. If plastics were made biodegradable, plastics would no longer accumulate as they do and recycling and incineration troubles would no longer be a problem.

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5. DISCUSSION

Microorganisms play a significant role in biological decomposition of materials, including synthetic polymers in natural environments. High-density and low-density polyethylenes are the most commonly used synthetic plastics and they are slow in degradability in natural environments, causing serious environmental problems. In this regard, there is a growing interest in non-degradable synthetic polymer biodegradation using effective microorganisms.

Plastics have become an important part of modern life and are used in different sectors of operations like packaging, building materials, consumer products and many more. Each year, about 100 million tonnes of plastics are produced worldwide. Most of the plastics and synthetic polymers are produced from petrochemicals. Because of their persistence in the environment, several communities are more sensitive to the impact of discarded plastics on the environment including deleterious effects on wild life and on the aesthetic qualities of cities and forests. Plastic bags or sheets do not allow water and air to percolate into earth causing reduction in fertility status of soil, depletion of underground water sources and damage to animal life. In seas too, plastic wastes choke and entangle the marine mammals. In cities, they choke drains leading to submergence of roads especially during rainy season. The increased cost of solid waste disposal as well as potential hazards from incineration of wastes such as dioxin emission from PVC makes synthetic plastic waste management a problem (Ojumun et al., 2004). Consequently

hence, for the past two decades, there have been a growing public and scientific interest in the development and use of biodegradable polymers as an ecologically useful alternative to plastics. Biodegradable plastics are made from renewable resources and do not lead 82 to depletion of finite resources. Polyhydroxyalkanoates (PHA) synthesized by at least 75 different genera of microorganisms are attracted as biodegradable plastics. They are accumulated intracellularly, as high as 90 per cent of cell dry weight under conditions of nutrient stress and act as a source of carbon and energy (Madison and Huisman, 1999). Hence, in this project, PHB accumulating bacteria were isolated from diverse sources to select the efficient strains. The process parameters for maximum PHB production were also optimized.

In the present study potential PHB accumulating bacteria were isolated from diverse sources and potential strains were selected for further studies. Most of the potential isolates were Bacilli. Bacillus sp are reported to be ideal PHB producers. The optimum growth and the maximum PHB accumulation by isolate happened at 48 hrs. This shows biomass and PHB production were concomitant with growth conditions and PHB production of a particular strain is related to its biomass. As biomass increases the bacteria starts accumulating PHB to the maximum level and the accumulated PHB decreases after the peak biomass production. This might be due to nutrient depletion, which forces the bacteria to use the accumulated PHB as energy source. The highest yield of PHB was obtained with glucose (15%) after 48 h incubation. Glucose is an easily assimilable carbon source that encourages bacteria to produce more PHB. Pre-treated sugarcane bagasse (15%) was the best cheap carbon source followed by corn cob. Similar results were reported by Yu et al. who obtained 54% PHB using bagasse hydrolytes from *Cupriavidus necator*. Paramjeet et al. obtained 60% PHB from sugarcane bagasse by *Pseudomonas aeruginosa.*

The optimum temperature for growth and accumulation of PHB by isolate was 37°C. The PHB and biomass yields increased till 37°C and sharply declined at temperature extremes. Bellard et al. also reported maximum cell density and PHB accumulation at 37°C after 48 h. The alteration in the PHB content by temperature variance can be due to the fact that extreme temperatures slow down the metabolic activity (enzyme activity) of microorganisms that ultimately reduces their ability to produce PHB. The maximum PHB production percentage per dry cell weight was achieved with peptone as a nitrogen source. UV–Vis scanning of the extracted polymers showed peaks between 235 nm readings. This peak range indicates the occurrence of PHB. The plastic nature and biodegradability of the extracted polymer was confirmed by preparing sample plastic film and the clear zone formed by soil born bacteria. PHBs are degraded by the action of microbial enzyme, PHB depolymerase, into water-soluble forms.

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