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Research Article

PHB production from bacteria isolated from oil contaminated soil

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Abstract

PHB was prepared from the bacterial species isolated from oil contaminated soil. Five efficient producers were proceeded for PHB production. Two crude carbon sources, groundnut oil cake powder and molasses were tested versus pure sugar glucose as carbon source for production. The isolates were biochemically characterized by Bergey's Mannual of Systematic bacteriology 2nd edition 2001. The produced PHB was confirmed by Spectrophotometric method and FTIR. Better production was observed with groundnut oil cake powder as compared with pure glucose. Optimum pH, temperature and incubation time for PHB production was also determined.

INTRODUCTION

Biodegradable plastics are a group of biopolymers synthesized by many bacteria and archaea. Among the various biodegradable polymer materials, polyhydroxyalkanoates (PHA) provide a good alternative to petrochemical plastics because they are biodegradables well as biocompatible and eco friendly. Non petroleum based biological polyesters are considered to be one of the most important next-generation polymers in the future in light of limiting natural resources. The properties of PHA are also similar to those of polyethylene (PE) and polypropylene (PP) (Reddy Vishnuvardhan and M.Thirumala, 2012). Groundnut oil cake contains carbohydrates-22 to 30%, protein-45 to 60%, minerals-4 to 5%,. Molasses contains 25% carbohydrates.

For PHB production, various researches were performed using different habitat. In present study, groundnut oil mill soil was taken for isolation of PHB producing bacteria. As the soil was contaminated with groundnut oil, therefore, groundnut oil cake powder was taken as sole source of carbon in production media.

MATERIALS AND METHODS

Collection of samples

The soil samples were collected from Gruhaseva Tel Bhandar Nanded, Maharashtra, and stored in sterile containers.

Isolation of Bacteria

1g of soil sample diluted with 100ml sterile saline was considered as standard suspension. It was serially diluted and 10^{-9} dilution was used to spread on medium. The medium used here was Luria Bertani (LB agar) medium supplemented with 1% groundnut oil cake powder. The plates were incubated for 24 hours at 37°C. (Vishnuvardhan Reddy and M.Thirumala, 2012).

Screening of PHB producing bacteria

The isolates were induced to accumulate PHB by culturing on modified E2 mineral medium with 2% groundnut oil cake powder {(NH4)2 HPo4 .4H2O : 3.5g, K2HPO4.3H2O : 7.5g, KH2PO4 : 3.7g, MgSO4 : 0.17g, Yeast extract: 0.04g, Microelement solution:1ml(Micro element stock solution/1000ml-FeSO4.

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7H2O: 2.7 mg, MnCl4. 4H2O: 1.98 mg, CoSO4. 7H2O: 2.8 mg, CaCl2. 2H2O: 0.17 mg, ZnSO4. 7H2O: 0.29 mg), Agar: 1.8g, pH: 7±0.2}(Vishnuvardhan Reddy and M.Thirumala, 2012).

After incubation the colonies were subjected for sudan black B and Nile blue A staining. The colonies showing presence of accumulation of granules were uesd to proceed further. After Nile blue A staining, the PHB accumulating colonies showed bright orange fluorescence on irradiation with UV light and their fluorescence intensity increased with increase in PHB content of the cell. The finalized isolates were subjected for morphological and biochemical analysis as per Bergey's mannual of systemic bacteriology 2nd edition 2001. (Vishnuvardhan Reddy & M.Thirumala, 2012; K. Susithra, 2017).

PHB Production

The above mentioned same modified E2 medium was used with three variations. In one flask E2 medium with 2% groundnut oil cake powder, in second flask E2 medium with 2% molasses, and the third flask contain the orignal E2 medium having 2% glucose as carbon source. All three variants were subjected to different pH conditions (pH 3,7 and 9), different temperature conditions (25°C, 35°C and 42°C), and different incubation time (24hrs,48hrs and 72hrs) (Sushma Shenoy *et al.,* 2012).

PHB extraction and estimation

The PHB was extracted by Rawte and Mavinkurve,2002 method.In this method the bacterial cell was hydrolysed with sodium hypochlorite proceeded by wash with cold diethyl ether. From bacterial cell, the PHB was precipitated after repeated centrifugation. The extracted PHB was quantified by UV Spectrophotometer. In this method the PHB was acidified with concentrated sulphuric acid to form crotonic acid which was measured at 235nm (Rawte and Mavinkurve, 2002).

Confirmation of PHB by IR

PHA in the biomass was determined by examining the dried biomass on the ATR-FTIR. The scanning conditions were spectral range between 4000 cm^{-1} and 400 cm^{-1}, 24–32 scans, at a resolution of 4 cm^{-1}. The specific bands for PHA were observed. (Sajida Munir and Nazia Jamil, 2015).

RESULTS AND DISCUSSION

PHB was produced from five bacterial isolates that are isolated from oil contaminated soil. By performing various biochemical and morphological tests and comparing their results with Bergey's Mannual of Systematic Bacteriology 2nd edition 2001, the isolates were assumed to be isolate-1: *Pseudomonas*, isolate-2: *Klebsiella*, isolate-3: *Bacillus*, isolate-4: *Enterobacter*, isolate-5: *Staphylococcus*.

PHB production was performed by using two crude carbon sources, groundnut oil cake powder and molasses against pure sugar glucose. Effect of different parameters like pH, temperature and incubation time was also performed. From the tables and their respective graphs it is clear that for all isolates, the most optimum pH is pH 7, the most optimum temperature is 35°C and most optimum incubation time is 48hrs. The results also declared that the crude carbon sources gave better production against pure sugar.

Among the two crude sources, the results with groundnut oil cake powder were promising as compared with molasses. All isolates showed higher production on groundnut oil cake powder as compared with molasses. This was a purely innovative initiative taken by me, as in the references, this type of combination was not performed earlier.

Among the five isolates, the isolate-1 showed maximum production on groundnut oil cake powder, followed by glucose and least with molasses. The isolate-2 also showed maximum production on groundnut oil cake powder, followed by glucose and least on molasses. The isolate-3 showed maximum production on groundnut oil cake powder, followed by molasses and least with glucose. The isolate-4 showed maximum production on groundnut oil cake followed by glucose the least with molasses. The isolate-5 showed same production on groundnut oil cake powder and glucose and least with molasses.

All results were approximately similar to the earlier studies.

Conclusion

The study finally concludes that crude carbon sources are better as compared to pure sugars for PHB production. Also the conditions for PHB production were also optimized that were pH 7, temperature 35°C and incubation time 48hrs.

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Table-1: Morphological Characters

Character	Isolate O1	Isolate O2	IsolateO3	Isolate O4	Isolate O5
Size	3mm	3mm	2mm	4mm	3.5mm
Shape	Rod	Rod	Rod	rod	cocci
Colour	Green	Cream	white	white	white
Margin	irregular	entire	entire	entire	entire
Elevation	concave	Concave	Concave	Concave	Concave
Consistancy	smooth	Smooth	Smooth	Smooth	Smooth
motility	motile (unipolar)	non motile	motile	motile	non motile
Opacity	opaque	Opaque	Opaque	Opaque	Opaque
Gram's nature	negative	negative	positive	Negative	Positive

Table-2: Biochemical characters

Isolate	Sugar fermentation test				Indole	MR-VP Test	Citrate Test	Oxidase Test	catalase Test	Amylase Test	Urease Test	Other
	Glucose	Lactose	Sucrose	Arabinose								
Isolate O1	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	-ve	Siderophores Positive
Isolate O2	+ve	+ve	+ve	+ve	-ve	MR-ve VP+ve	+ve	-ve	+ve	-ve	+ve	Purple colony on Hi chrome Klebsiella agar
Isolate O3	+ve	+ve	+ve	+ve	-ve	MR-ve VP+ve	+ve	-ve	+ve	+ve	-ve	endospore formation
Isolate O4	+ve	-ve	+ve	+ve	-ve	MR-ve VP+ve	+ve	-ve	+ve	+ve	-ve	Positive for growth in KCN and EMB
Isolate O5	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	+ve	Haemolysis

Table-3: Effect of pH on PHB production

	Production on Groundnut oil cake powder 2%			Production on Molasses 2%			Production on Glucose 2%		
	pH 3	pH 7	pH 9	pH 3	pH 7	pH 9	pH 3	pH 7	pH 9
Isolate O1	0.3g	1g	0.5g	0.2g	0.8g	0.6g	0.2g	0.9g	0.7g
Isolate O2	0.22g	0.96g	0.4g	0.1g	0.92g	0.32g	0.11g	0.93g	0.34g
Isolate O3	0.42g	0.98g	0.7g	0.34g	0.97g	0.2g	0.4g	0.96g	0.33g
Isolate O4	0.2g	0.8g	0.3g	0.12g	0.7g	0.3g	0.2g	0.75g	0.32g
Isolate O5	0.12g	0.75g	0.45g	0.1g	0.73g	0.33g	0.1g	0.75g	0.22g

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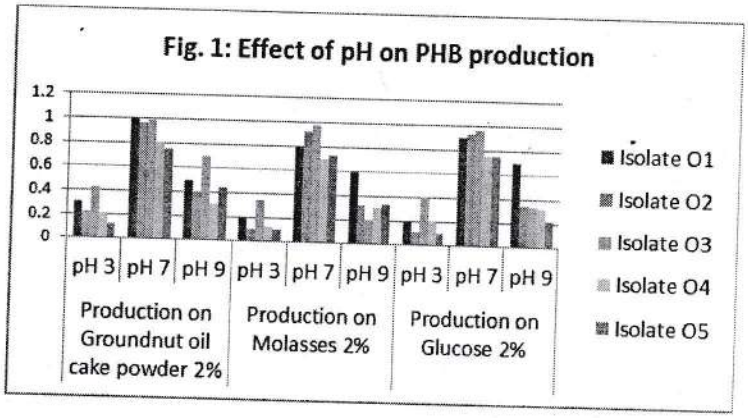
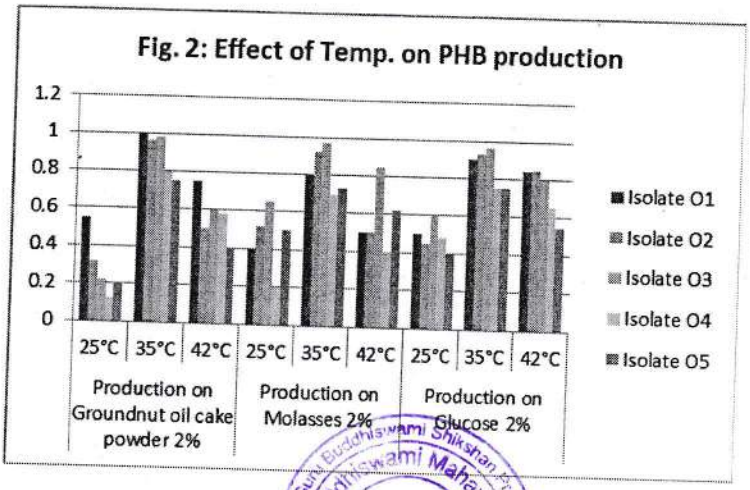
Table-4: Effect of Temp. on PHB production

	Production on Groundnut oil cake powder 2%			Production on Molasses 2%			Production on Glucose 2%		
	25°C	35°C	42°C	25°C	35°C	42°C	25°C	35°C	42°C
Isolate O1	0.55g	1g	0.75g	0.4g	0.8g	0.5g	0.5g	0.9g	0.84g
Isolate O2	0.32g	0.96g	0.5g	0.52g	0.92g	0.5g	0.45g	0.93g	0.85g
Isolate O3	0.22g	0.98g	0.6g	0.65g	0.97g	0.85g	0.6g	0.96g	0.8g
Isolate O4	0.12g	0.8g	0.58g	0.2g	0.7g	0.4g	0.48g	0.75g	0.65
Isolate O5	0.2g	0.75g	0.4g	0.5g	0.73g	0.62g	0.4g	0.75g	0.55g

Table-5: Effect of incubation time on PHB production

	Production on Groundnut oil cake powder 2%			Production on Molasses 2%			Production on Glucose 2%		
	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
Isolate O1	0.8g	1g	0.87g	0.7g	0.8g	0.75g	0.85g	0.9g	0.85g
Isolate O2	0.85g	0.96g	0.9g	0.85g	0.92g	0.89g	0.89g	0.93g	0.89g
Isolate O3	0.9g	0.98g	0.92g	0.88g	0.97g	0.9g	0.9g	0.96g	0.91g
Isolate O4	0.68g	0.8g	0.75g	0.65g	0.7g	0.68g	0.7g	0.75g	0.68g
Isolate O5	0.7g	0.75g	0.72g	0.68g	0.73g	0.7g	0.7g	0.75g	0.7g





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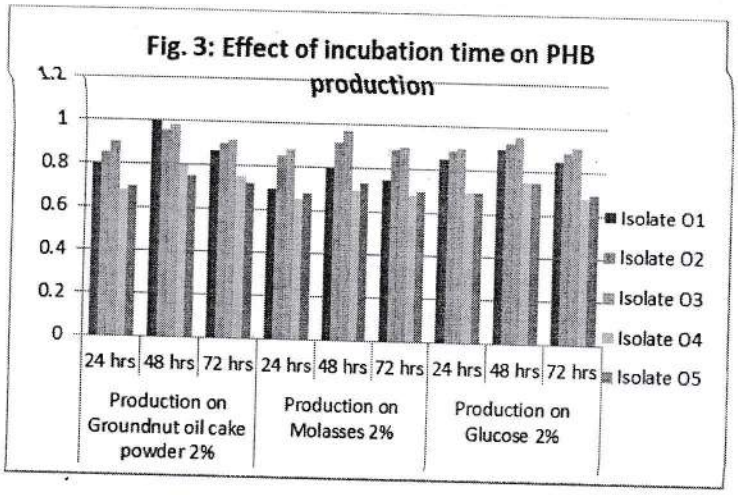

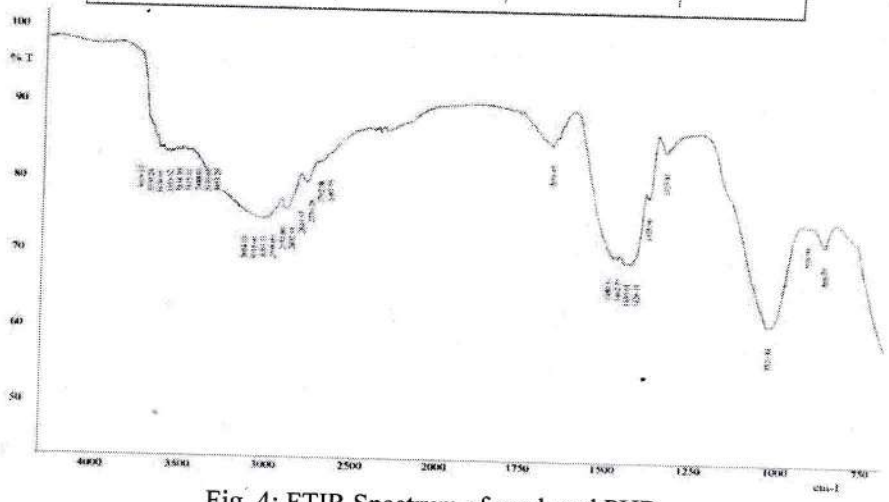

Fig. 4: FTIR Spectrum of produced PHB

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