ISSN: 2348-1900
Plant Science Today
http://www.plantsciencetoday.online

OPEN ACCESS

Research Article

SPECIAL ISSUE on Current Trends in Plant Science Research

Production of poly hydroxy butyrate (PHB) from *Eichhornia crassipes* through microbial fermentation process

Varsha Upadhayay, Samakshi Verma & Arindam Kuila*

Department of Bioscience & Biotechnology, Banasthali Vidyapith, Rajasthan 304 022, India

Article history
Received: 27 November 2019
Accepted: 26 December 2019
Published: 31 December 2019

Publisher
Horizon e-Publishing Group

Correspondence
Arindam Kuila
✉ arindammcb@gmail.com

Abstract

Polyhydroxybutyrate (PHB) is one of the highly biodegradable and biologically acceptable thermoplastics synthesized by many microorganisms collectively called polyhydroxyalkanoates (PHAs). All available biopolymers are viewed as perfect answers for the resolution of natural contamination issue by supplanting ordinary plastic business. They are likewise utilized as osteosclerotic stimulants attributable to their piezoelectric properties, in bone plates, during operations as suture material and vein substitutions. Synthesis of PHB is found in a wide range of Gram's negative and gram's positive bacteria belonging to distinct genera. Optimum culture condition for the PHB producing microbes are provided, including restricted centralization of nitrogen, sulfur, phosphorus, or the trace elements and maximum convergence of carbon source Indeed, to market PHAs, significant exertion has been dedicated towards a decline in the production cost through the improvement of bacterial strains and enhancing effectiveness of recovery/fermentation procedure. This is being done considering the fact that substrate prices show the greatest impact on PHA's manufacturing cost. The price of the substrate used has the most significant influence on the production cost of PHA. In this research, a potential bacterial strain was isolated from the soil and tested for its PHB producing ability. The use of cheaper substrate for lowering the cost is prerequisite. For PHB production, water hyacinth was used as a carbon source. Bacterial growth was optimized for maximum PHB production. The optimum condition was found to be 30 °C, 8% substrate concentration and 72 h of incubation time.

Keywords: Bacteria, Bioplastic; Polyhydroxybutyrate; Optimum condition

Citation: Upadhayay V, Verma S, Kuila A. Production of poly hydroxy butyrate (PHB) from *Eichhornia crassipes* through microbial fermentation process. Plant Science Today 2019;6(sp1):541-550. https://doi.org/10.14719/pst.2019.6.sp1.673

Indexing: Plant Science Today is covered by Scopus, Web of Science, BIOSIS Previews, ESCI, CAS, AGRIS, UGC-CARE, CABI, Google Scholar, etc. Full list at http://www.plantsciencetoday.online

Introduction

Every discovery in the world is directly or indirectly affects the environment. Plastic was discovered more than a century ago (1). From the beginning era of plastic discovery is considered as a boon, but with the time passes this boon changed as a curse that is causing harm to the environment as well as the organisms inhabiting in terrestrial and marine ecosystems.

The exploitation of fossil fuels is not a new thing to mankind. In future, we will face the deficiency of non-renewable sources because of its continuous exploitation (2).

Many problems arise due to petrochemical plastic, to deal with the problems of petrochemical plastic the concept of bioplastic aroused. Bioplastic is easily degradable and contains many thermal and chemical properties that are nearly similar to conventional plastic. The biggest advantage of bioplastic is that it has fewer carbon footprints that doesn't tend to float on the water surface as it settles on the surface of the water and decomposes with the help of bacteria. The bioplastic production will be fruitful as well as economically good with the cheap substrate like agricultural waste and whey produced from dairy products. Bioplastic production is easy and cheap with the help of microbes but the problem lies in its extraction and purification. The methods for purification are generally solvent-based. The solvents require in these methods are in large amount which is costly and are not reusable. These solvents increase the cost of production. PHB is one of the bioplastics that has a variety of uses as it considers as one of the best type of bioplastic. PHB is biobased and biodegradable plastic (3). The present study focused on the isolation of PHB producing bacteria and optimization of PHB production utilizing water hyacinth as a substrate. There are some species that are capable of production of biopolymer. But for the industrial purpose there are selected species that can produce on large scale and have high yield. There are various parameter that are needed to select the microorganism *i.e.* polymer synthesis rate, growth rate, substrate utilization, polymer accumulation. Recovery efficiency is also one of most important parameter needs for the smooth economics of PHB. Here is a list of some bacteria that are PHB producing with their yield in Table 1.

Table 1. Biopolymer producing microbes

S.No	PHB producing microbes	PHB Yield (wt) %	References
1.	*Alcaligenes latus* DSM 1124	33-71	(20)
2.	*Bacillus megaterium*	~50	(21)
3.	*Burkholderia sp.* USM (JCM 15050)	22-70	(22)
4.	*Comamonas testosteroni*	79-88	(23)
5.	*Cupriavidus necator*	54	(24)
6.	*Cupriavidus necator* H16	65-90	(25)
7.	*Cupriavidus necator* DSM 545	50	(26)
8.	*Brevundimonas vesicularis*	64	(27)
9.	*Pseudomonas aeruginosa.*	65.51	(28)

Table 2. Parameter and code for Response Surface Methodology (RSM)

S. No	Parameters	Code 1	Code 2	Code 3
1.	Substrate concentration (%)	8	10	12
2.	Temperature (°C)	25	30	35
3.	pH	5	7	9
4.	Incubation time (days)	2	3	4

Table 3. Absorbance for standard curve of PHB

S. No	PHB (µg/ml)	OD (nm)
1.	10	0.360
2.	50	0.455
3.	75	0.572
4.	100	0.639
5.	150	0.786
6.	200	0.863

Table 4. Effect of pH on PHB production

S. No	pH	PHB (µg/ml)
1.	5	135
2.	6	165
3.	7	167.25
4.	8	129.25

Table 5. Effect of temperatures on PHB production

S. No	Temperature (°C)	PHB (µg/ml)
1.	25	117
2.	30	154.5
3.	35	234.5

Table 6. Effect of incubation time on PHB production

S. No	Incubation Time (days)	PHB (µg/ml)
1.	1 days	136
2.	2 days	124.75
3.	3 days	209.5
4.	4 days	176.5

Table 7. Effect of substrate concentration on PHB production

S. No	Substrate Concentration (%)	PHB (µg/ml)
1.	1%	111.5
2.	2%	84.5
3.	5%	90.75
4.	8%	125
5.	10%	132.25
6.	15%	98.75

Materials and Methods

Isolation of bacteria

Collection of soil samples

PHB producing bacterium isolated from the soil samples of nearby locality of Banasthali Vidyapith (Tonk) Rajasthan. The soil samples were collected from the premises of Department of Biotechnology. The soil samples were collected in a beaker and kept in lower temperature. The samples were dried then crushed with mortar pestle.

Isolation of bacterial strain from soil samples

The soil samples were subjected to serial dilution. 1 gm of soil was suspended in 9 ml water and serially diluted in different concentration. The diluted sample was used to plate on nutrient agar medium. These plates were incubated for 48 h at 30 °C. The colonies of different bacteria were formed in the plate. The different remarkable

colony was recuperated and re-streaked in order to obtain pure culture.

Screening and characterization of bacterial isolates

Screening of bacterial isolates was carried out by using lipophilic stain Sudan black B. Sudan black B is helpful for identifying the positive isolates of PHB it can be done by two methods under light microscope and by agar plate method (4).

Agar plate method

For detecting the PHB positive isolates, we used agar plate method. In this method we checked five isolates. All the five isolates were streak in a single plate. The plate was then incubated for 24 h at 30 °C. The plate was immersed in 3% ethanolic Sudan black stain for 30 min. The plate was then washed with 96% ethanol to destain un-adsorbed Sudan black. The colonies that retain black stain were selected as PHB positive isolates.

Light microscope method

The microscopic study of the isolates was done by smear formation. The smear of selected colony was formed on a glass slide. The smear was dried by air drying. The slide was then stained with 0.3% Sudan black stain (in 70% ethanol) for 10-15 min. The slide was washed with water then it was counterstained with 5% safranin (in distilled water). The slide was observed under light microscope. The black coloured strains were PHB positive isolates while red colour safranin stained strain were negative isolates.

PHB production

Collection of substratum

The substratum used for the experimental purpose was *Eichhornia crassipes* (water hyacinth). This is an aquatic weed. The collection of plant was done from a lake near Ganesh temple, village Jhagar Balakot, district Damoh (M.P.). The sample was washed and cleaned, then oven dried till it was devoid of moisture. The sample was grinded in grinder.

Growth condition and media

The culturing of bacteria requires certain parameter and nutrients. For this purpose we used Minimal Salt Media (MSM), the components of MSM were as follows:

Sodium nitrate ($NaNO_3$): 2.5 g/L, Potassium dihydrogen phosphate (KH_2PO_4): 1 g/L, Potassium chloride (KCl): 0.5g/L, Magnesium sulphate ($MgSO_4$): 0.5 g/L. To the above content we added 1% yeast extract (5). The 2% substratum (hyacinth powder), pH at 7, temperature at 28 °C and incubation time is 48 h which was maintained as standard. The media was inoculated with 1% of bacterial culture.

Quantification of PHB from selected isolates

The quantification of the PHB is very important step in order to determine the amount of PHB produced in the given amount of conditions. For quantifying PHB, we filtered the culture and centrifuged it at 10000 rpm for 10 minutes at 4 °C. The supernatant was discarded from the tube. The pellets were washed with acetone after that with ethanol, then washed pellets suspended in 4% sodium hypochlorite with of equal volume of pellets and incubate them at room temperature for 30 min. The mixture ions were then centrifuged at 10000 rpm for 10 min at 4 °C. The supernatant was discarded and the pellets were dissolved in hot chloroform. The mixture was filtered with the help of Whatman filter paper number 1 already treated with hot chloroform. To the filtrate add 10 ml of concentrated H_2SO_4. Concentrated H_2SO_4 convert the polymer into crotonic acid which imparts brown color to the solution (4, 6). The quantification was done with the help of standard curve prepared by the synthetic PHB. Then, 2 g of PHB was added in 10 ml of conc. H_2SO_4. Then, the mixture was heated for 10 min to be converted into crotonic acid.

Optimization of different cultural aspects for better growth

Optimization is one of the aspects to find out the best of all the parameter for the better yield of product and growth of microbes. The optimization is carried out by two approach *i.e.*

- OVAT: One Variable At a Time
- Statistical technique

OVAT: One Variable at a Time

In this approach, one factor varies while all other factors are kept constant.

Effect of pH on the PHB production

The pH plays important role in the growth and metabolism of bacteria. For the determination of optimum pH, we inoculated PHB positive isolate in 50 ml culture with 2% substrate concentration at 30 °C for 2 days. The pH considered was 5, 6, 7 and 8. The quantification of the product determined the best pH for PHB production.

Effect of temperature

The PHB positive isolate was inoculated in 50 mL culture with 2% substrate concentration at pH 7 for 2 days. The culture was provided with different temperature of 25, 30 and 35 °C. The quantification of PHB shows about the best temperature for production.

Effect of incubation period

The PHB positive isolate was inoculated in 50 ml of culture with 2% substrate concentration at pH 7 and 30 °C. The incubation time varies from 1 day to 4 days. The optimum temperature for production was determined by quantification of PHB.

Effect of substrate concentration

The PHB positive isolate was inoculated in 50 ml of culture at pH 7, 30 °C for 2 days. The substrate concentrations provided was 1, 2, 5, 8, 10 and 15%. The best concentration for the growth of culture was identified by the quantification of product.

Response surface methodology

The optimization of PHB production was carried out using central composite design (CCD) based on response surface methodology (RSM). The parameters of experiment were as follows: Water hyacinth concentration (8-12%, w/v), temperature (25 – 35 °C), pH (6-10) and incubation time (2-4 days) (Table 2). The Response Surface Regression (RSREG) was used to analyze experimental data to fit in the second order polynomial equations:

$$Y = \beta_{k0} + \sum_{i=1}^{5}\beta_{ki}x_i + \sum_{i=1}^{5}\beta_{kii}x_i^2 + \sum_{i=1}^{4}\sum_{j=i+1}^{5}\beta k_{ij}x_i x_j$$

Where Y is the response PHB production; β_{k0}, β_{ki}, β_{kii} and βk_{ij} were constant coefficients and x_i, xj were coded as independent variables, which influence the response variable Y.

Cell cultivation, harvesting and PHB extraction

For the large scale cell cultivation, 300 ml minimal salt media was prepared in 500 ml flask. The media was inoculated with the PHB positive strain. The flask was then incubated at pH 7 for 3 days at 30 °C with gentle shaking. After incubation, the harvesting of cells was done by filtration with double layer of muslin cloth and rotates the filtrate at 10000 rpm for 10 min at 4 °C. After centrifugation pellets were washed with sterile water. The process was repeated twice and dry biomass at 60 °C. The extraction of PHB was done with the help of solvent extraction method. The lipids from the bacterial cells were removed by using 40 volumes of methanol than to pellets. The above mixture was then incubated for 60 min at 95 °C. The mixture was removed from methanol by filtration and kept for drying at 65 °C. The chloroform were added in dried samples and kept for incubation at 95 °C for 10 min. The mixture was then cool down and subjected to mixing overnight. The overnight mixture was filtered. The filtered granules were precipitated with 70% methanol. The precipitate was dried and washed with acetone.

Characterization

Characterization was done with the help of instrument Fourier Transformed Infrared Spectroscopy (FT-IR), X-Ray Diffraction and Scanning Electron Microscopy (SEM). The characterization is important for the knowledge of structure, surface and crystalline properties. The characterization was performed for water hyacinth, extracted PHB and standard PHB.

Characterization of biomass

i) The parameters for FT-IR were mid infrared region for 32 scans at 400–4000 cm^{-1} at resolution 4 cm^{-1}. This parameter helps in the detection of functional groups.

ii) The X-Ray Diffraction parameter were at 30 kV/ kV, 10 mA; grade range between 12° and 45° with step size of 0.02° and the radiation was Cu (1.54A).

iii) The SEM was used to visualize surface structure of the plant. The sample was coated with platinum. The subject was visualized at 20 kV.

Characterization of PHB

The different parameter for the PHB characterization was same as for lignocellulosic biomass.

[1] The parameters for FT-IR were mid infrared region for 32 scans at 400–4000 cm^{-1} at resolution 4 cm^{-1}. This parameter helps in the detection of functional groups.

[2] The X-Ray Diffraction parameter was at 30 kV, 10 mA; grade range between 12° and 45° with step size of 0.02° and the radiation was Cu (1.54A).

[3] The SEM was used to visualize surface structure of the plant. The sample was coated with platinum. The subject was visualized at 20 kV.

Results and Discussion

Isolation and screening of PHB accumulating bacteria

Banasthali campus was the main source of sample collection and a total of five different soil samples were collected. All these soil samples were serially diluted and then the bacterial colonies were enumerated on nutrient agar plates. Depending upon the appearance, colour, size, shape and texture of bacterial colonies, a total of 40 representative bacterial colonies were picked, isolated and stored as a pure culture for screening with Sudan black dye. Among 40 bacterial colonies only five bacterial isolates were found to be PHB positive. The strain A1 retains most of the stain and was taken further for PHB production as shown in Fig. 1. The screening method was done by staining the bacterial isolates with Sudan Black dye on petri plates and also under the microscope. There are various scientists who utilize Sudan Black dye as a screening measure for PHB production (7-10). It was observed that among 40 bacterial colonies, only 5 isolates were found as a potential PHB producing strain which gives blue/black colour both on the petri plates and under the light microscope on staining with Sudan Black dye as shown in Fig. 2 and 3.

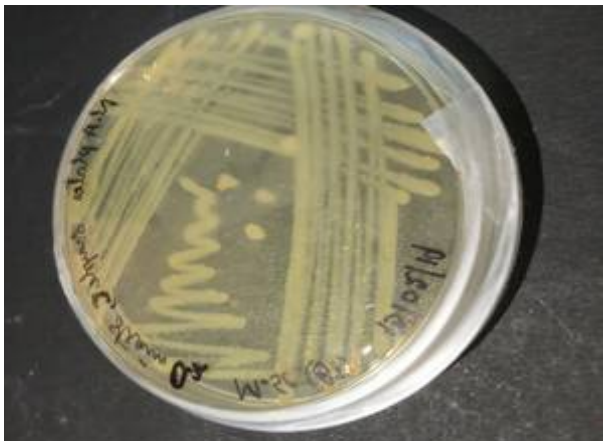

Fig. 1. Isolation of bacteria from sample A1

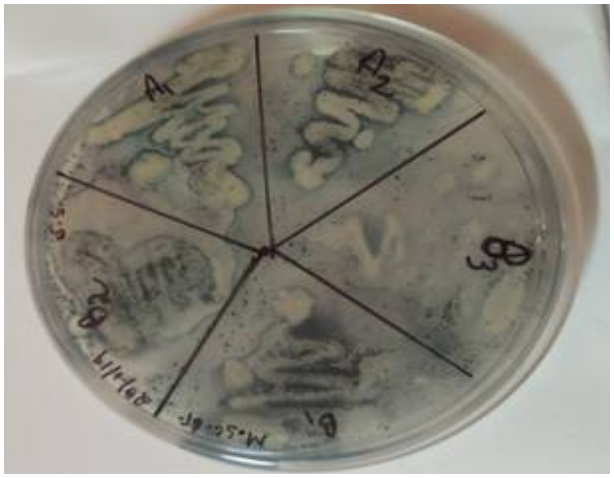

Fig. 2. Solid agar plate screening

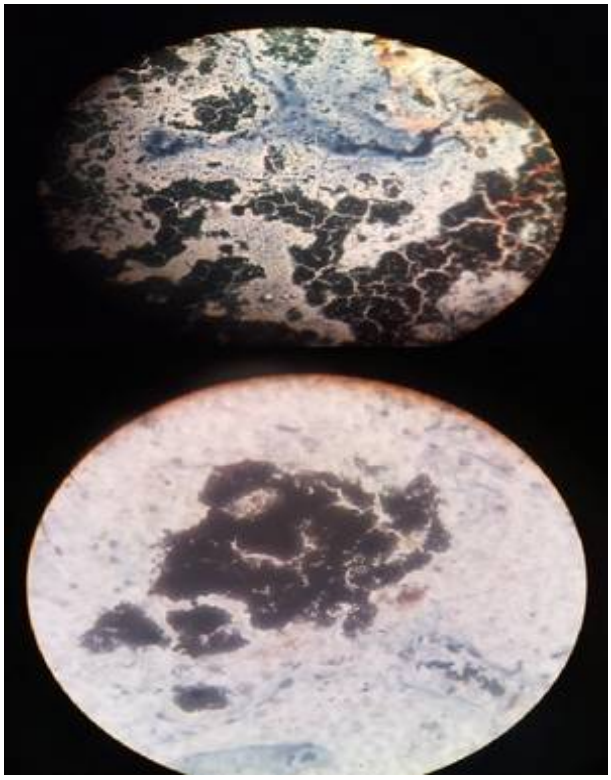

Fig. 3. Screening of strain A1 with Sudan Black dye-PHB producing strain shows blue/black colour under light microscope

Characterization of PHB positive isolates

All the five Sudan Black positive isolates were processed with microscopic and morphological characterization. Colour, shape, size and texture were the morphological characteristic that was studied for all the five isolates which gives high variability such as: the size varies from small to large; having an irregular or round shape; being off-white/ white/ yellowish-orange in colour; with a glossy/ smooth/ dry texture. Gram staining was performed (11) and isolates showed gram negative characteristics. On a preliminary basis, isolates were identified as member of these five genera *viz*., *Bacillus*, *Enterobacter*, *Escherichia*, *Pseudomonas* and *Staphylococcus*. For the molecular identification of strain, DNA isolation and its amplification have been done and the strain identification is under process.

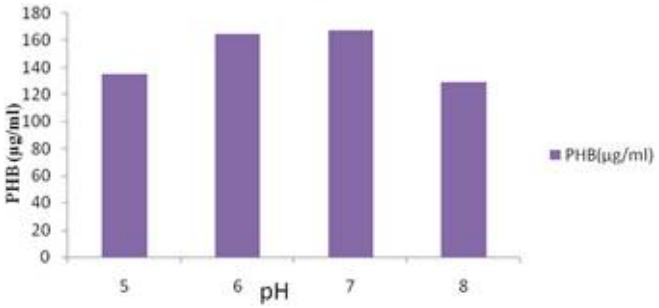

Fig. 4. Effect of pH on PHB production

Quantification of PHB production

Quantification of PHB and the standard graph was prepared (4). From the standard curve the calculation of PHB quantity was evaluated with the help of slope value (Table 3).

Optimization via different parameters

PHB was produced by using different parameter *viz*. pH, temperature, and substrate concentration and incubation time. The production of PHB was affected by different parameter. We will be discussing the effect of parameter in OVAT approach.

Optimization via OVAT approach

Effect of pH

pH was varied between 5 to 8 and best yield has been found at 7 pH, according to literature studies it is noted that basic pH is not appropriate for the metabolism of the organism at pH 8 lowest rate of PHB has been found (6, 12) (Table 4).

Effect of temperature

The effect of Temperature is important for the growth of PHB, the temperature was varied at 25, 30 and 35 °C (6, 12). The optimum temperature for good production of PHB was found at temperature 30 °C. The optimum temperature for PHB production was at 35 °C. The lowest amount of PHB was observed at temperature 25 °C that shows that the organism was not able to perform metabolism at lower temperature (Table 5). The data has been shown in Fig. 5.

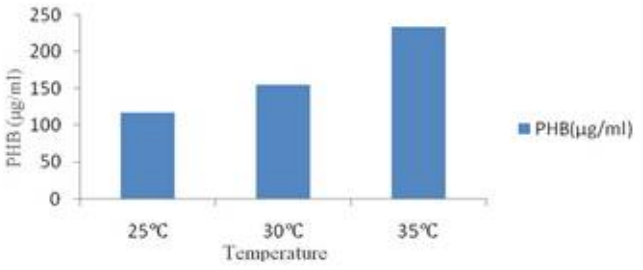

Fig. 5. Effect of temperatures on PHB production

Effect of incubation time

The incubation time was varied *viz*. 1, 2, 3, 4 days. The effect of incubation time is important for growth. The optimum incubation time for good

production of PHB was found after incubation time of 3 days. The lowest amount of PHB was observed after incubation time of 1 day that shows that the organism need to have certain high incubation time to reach log phase (Table 6). The data has been shown in Fig. 6.

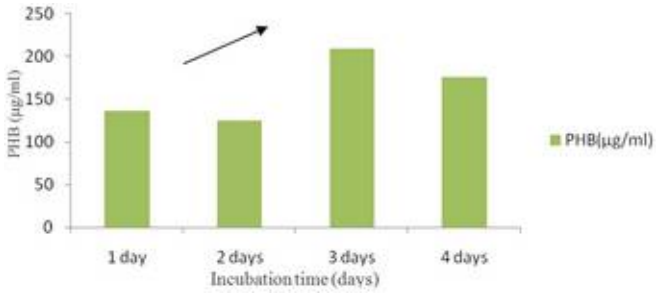

Fig. 6. Effect of incubation time on PHB production

Effect of substrate concentration

The substrate concentration was varied *viz*. 1, 2, 5, 8, 10 and 15%. The effect of substrate concentration is most important for growth and nutrition of organism. The optimum substrate concentration for good production of PHB was found at 10% substrate concentration (Table 7). The lowest amount of PHB was observed at substrate concentration 2% that shows that the organism need to have certain high substrate concentration of water hyacinth to reach at log phase (Fig. 7).

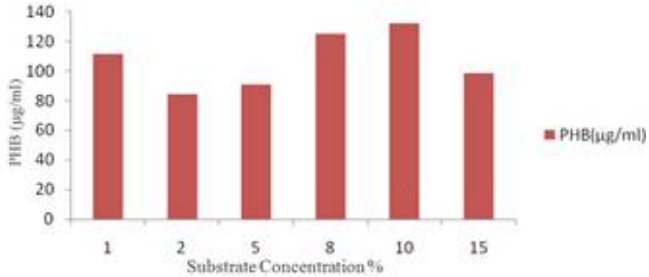

Fig. 7. Effect of substrate concentration on PHB production.

Optimization by Response Surface Methodology (RSM) model

The data obtained from experimental conditions and response was summarized in the Table 8. The experimental data from Table was used to calculate the coefficients of polynomial equations. The predicted PHB production (µg/ml) was obtained using polynomial equation. The experimental and predicted responses were very similar.

The analysis of variance (ANOVA) has been shown in Table 9, which demonstrated that the resultant quadratic polynomial models adequately presented the experimental data with the coefficient of multiple determinations (R^2) of 0.8103. *P* values generally consider for analyzing the importance of the entire regression coefficient. The smaller *P* values higher will be the significance of corresponding coefficient.

The mathematical expression of relationship of PHB production (µg/ml) with different variable (substrate concentration, temperature, pH, incubation time) is given in terms of non-coded factors:

PHB production (µg/ml) = 4796.25 – 17.43 x substrate concentration + 340.97 x temperature – 169.37 x pH + 318.59 x incubation time – 1.24 x substrate concentration x substrate concentration – 5.42 x temperature x temperature + 16.85 x pH x pH – 13.86 x incubation time x incubation time + 0.70 x substrate concentration x temperature + 1.45 x substrate concentration x pH + 0.85 substrate concentration x incubation time - 1.72 x temperature x pH - 2.60 x temperature x incubation time - 6.93 x pH x incubation time.

Table 9 shows the calculated F-value (5.19) was less than tabulated value at 4 and 14 degree of freedom. It implies the significance at linear and square effect at quadratic equation for PHB production. The observation can be evaluated by the fact that some parameters have effect on other for PHB production is evident from the parallel mesh plot.

From the RSM plot between pH and incubation time (Fig. 8), it was concluded the PHB production increase with the incubation time and PHB production increase with increase in pH until certain point.

The observation is simple as the incubation time increases, microbial growth will increases and increases PHB production. But after completion of log phase of microbial growth, microbial growth starts to decline that will lead the decrement of PHB production.

From RSM plot between temperature and incubation time (Fig. 9), it was concluded that the increase in temperature will also increase PHB production but after 30 °C the production starts declining while on the other hand increase in incubation time increases PHB production.

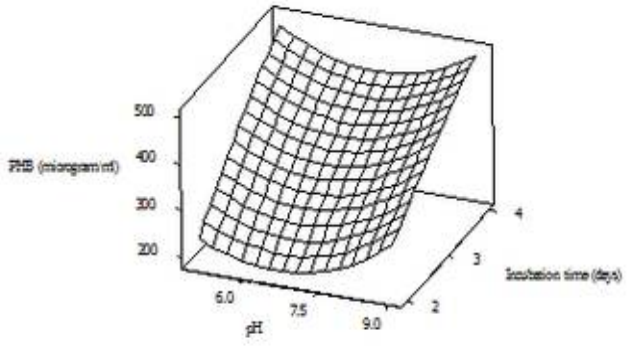

Fig. 8. RSM plot showing the effect of pH and Incubation time on PHB production

Table 8. Experimental design (conditions and responses) for PHB

Run order	Substrate concentration (%)	Temperature (°C)	pH	Incubation time (days)	PHB (µg/ml)	
					Experimental	Predicted
1	8	25	5	4	365.75	362
2	12	25	5	3	105.25	173.96
3	8	35	5	4	367.5	386.82
4	12	35	5	4	365.25	356.01
5	8	25	9	2	154.25	162.42
6	12	25	9	4	357.25	359.19
7	8	35	9	4	380.5	348.07
8	12	35	9	2	153.5	159.18
9	12	25	5	4	380.75	306.01
10	8	35	5	4	373	386.82
11	12	35	5	2	156.75	119.26
12	8	25	9	4	372.25	391.96
13	12	25	9	2	173.75	122.82
14	8	35	9	2	148	170.60
15	12	35	9	4	361.25	343.47
16	8	30	7	3	386	338.52
17	12	30	7	3	194	304.81
18	10	25	7	3	145	175.88
19	10	35	7	3	174	206.46
20	10	30	5	3	356.25	376.57
21	10	30	9	3	368.5	411.51
22	10	30	7	3	342	326.64
23	10	30	7	3	387.75	326.64
24	10	30	7	2	143.5	195.45
25	10	30	7	4	350	430.10
26	10	30	7	3	327.5	326.64
27	10	30	7	3	327.5	326.64
28	10	30	7	3	342	326.64
29	10	30	7	3	395.75	326.64
30	10	30	7	3	325.25	326.64
31	10	30	7	3	370	326.64
32	10	30	7	3	380.75	326.64

Table 9. ANOVA of RSM model for PHB production

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	14	252102	252102	18007.3	5.19	0.001
Linear	4	165607	55640	13910.0	4.01	0.018
Square	4	80094	76083	19020.8	5.48	0.005
Interaction	6	6401	6401	1066.9	0.31	0.924
Residual Errors	17	59008	59008	3471.1		
Lack of fit	8	52638	52638	6579.7	9.30	0.002
Pure error	9	6371	6371	707.8		
Total	31	311111				
R^2	81.03%	65.41%				

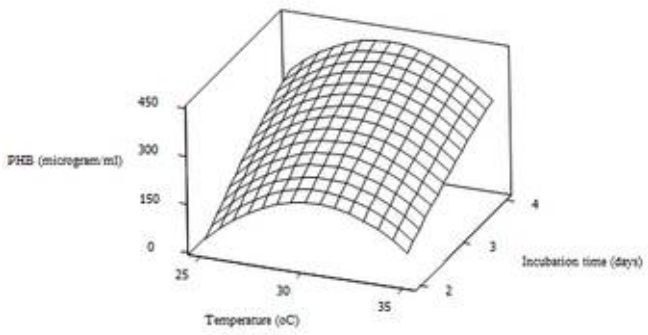

Fig. 9. RSM plot showing the effect of Temperature and Incubation time on PHB production

From the RSM plot between temperature and pH (Fig. 10), it was concluded that the temperature required for higher PHB production is 30 °C above or below this temperature the production starts declining. PHB production is highest at basic pH.

Characterization of PHB

The microbial cells producing PHB were collected from the culture. The cells were separated in the form of pellets. The pellets were dried and weighed. The dry weight of microbial cells was 0.8 grams. The PHB was then extracted from cells. The weight of PHB extracted was 0.5 gms.

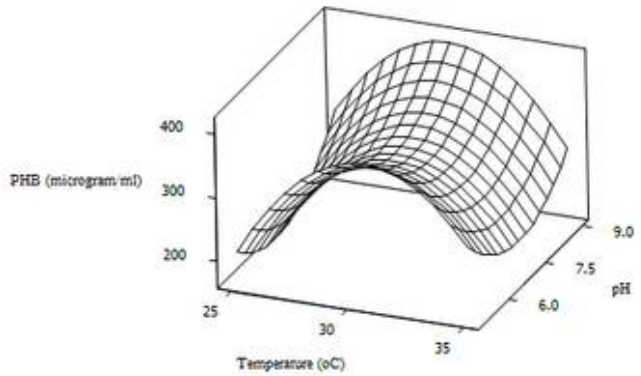

Fig. 10. RSM plot showing the effect of Temperature and pH on PHB production

X-ray diffraction

XRD was performed for the structural characterization of the matter for the analysis, at 30 mA and 40 kV with graphite filtered radiation, the 2 scales were set as 5◦ to 80◦. Fig. 11 shows the crystalline metallic nature of extracted PHB by main peaks of 2θ values. The crystalline index of standard PHB was 64.37%; biomass was 15.77%; extracted PHB was 3.45%. Degree of crystallinity reported for bioplastic on the base of XRD results reported as 52.23% (13) and 58% (14).

SEM study

The morphological analysis of PHB was done with the help of Scanning Electron Microscopy. The SEM image shows (Fig. 12) spherical particles for the PHB. The image was magnified till 2 nm. The voltage provided for the electron is 5.0 kV.

FT-IR study

FTIR characterization of PHB was carried out in the region range of 4000-400 cm^{-1}. FTIR spectral profile of standard and extracted PHB has been shown in Fig. 13. The organic compounds were from 3854.93 to 3067.41 cm^{-1}. The band at 3741cm^{-1} (O–H stretching in hydroxyl group), 2359 cm^{-1} (C–H stretching), 1646 cm^{-1} (conjugated C=O stretch), 1320 cm^{-1} (C-O stretching or OH deformation), 852.49 cm^{-1} (structural and nonstructural carbohydrate band). The observations imply that the PHB extracted from bacterial cells was same as that of standard PHB. Similar results have been studied in earlier literature by Alarfaj, Altaee, Brinda Devi and Pradhan (15-19).

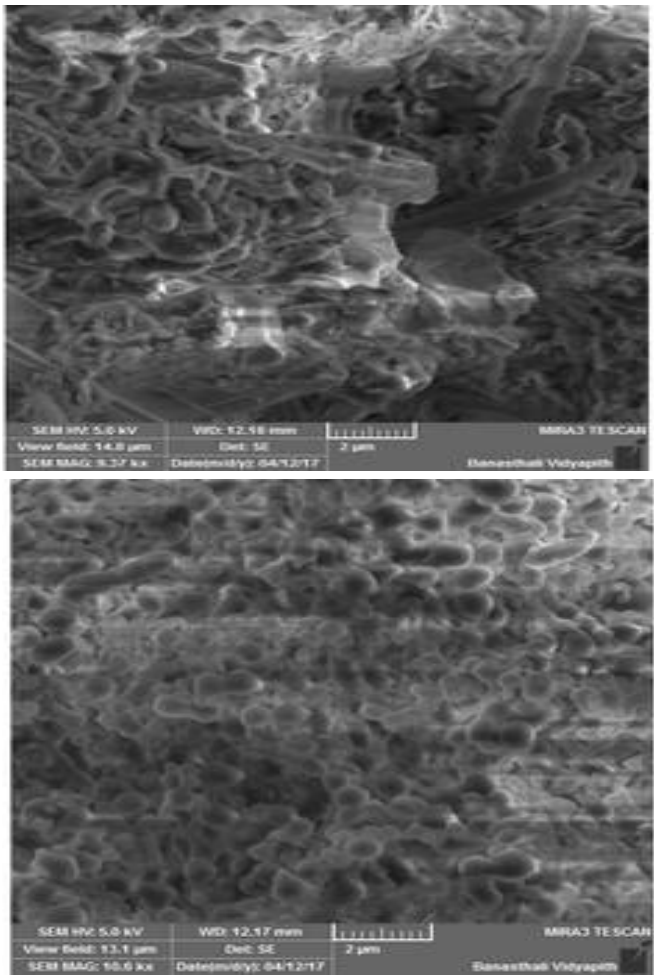

Fig. 12. SEM images of a) Extracted PHB b) Standard PHB

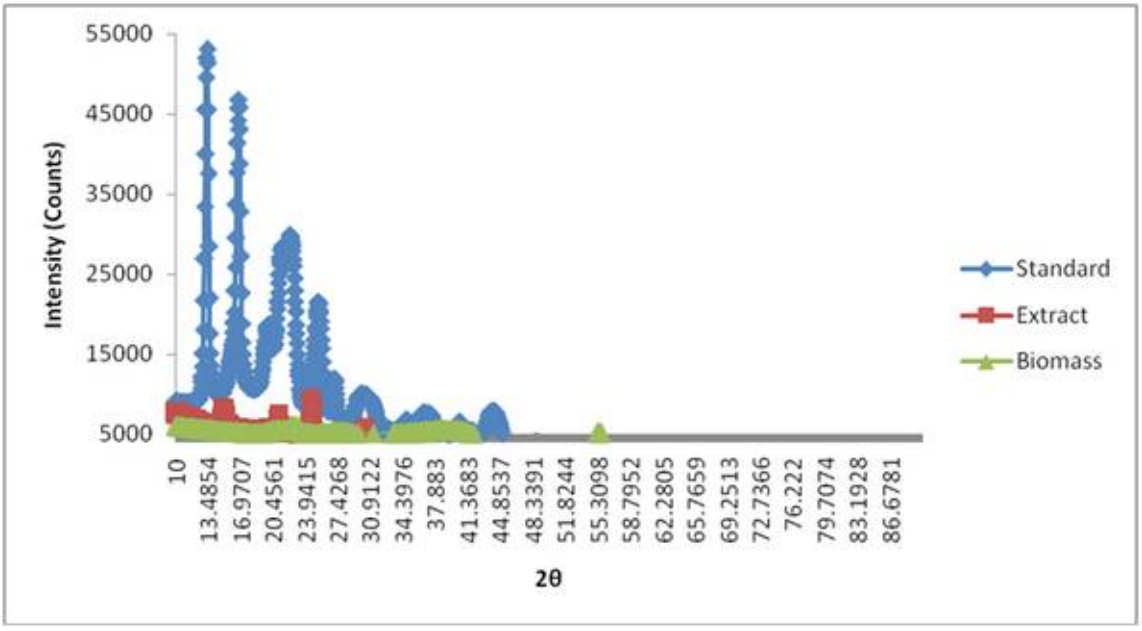

Fig. 11. X-Ray diffraction pattern for PHB

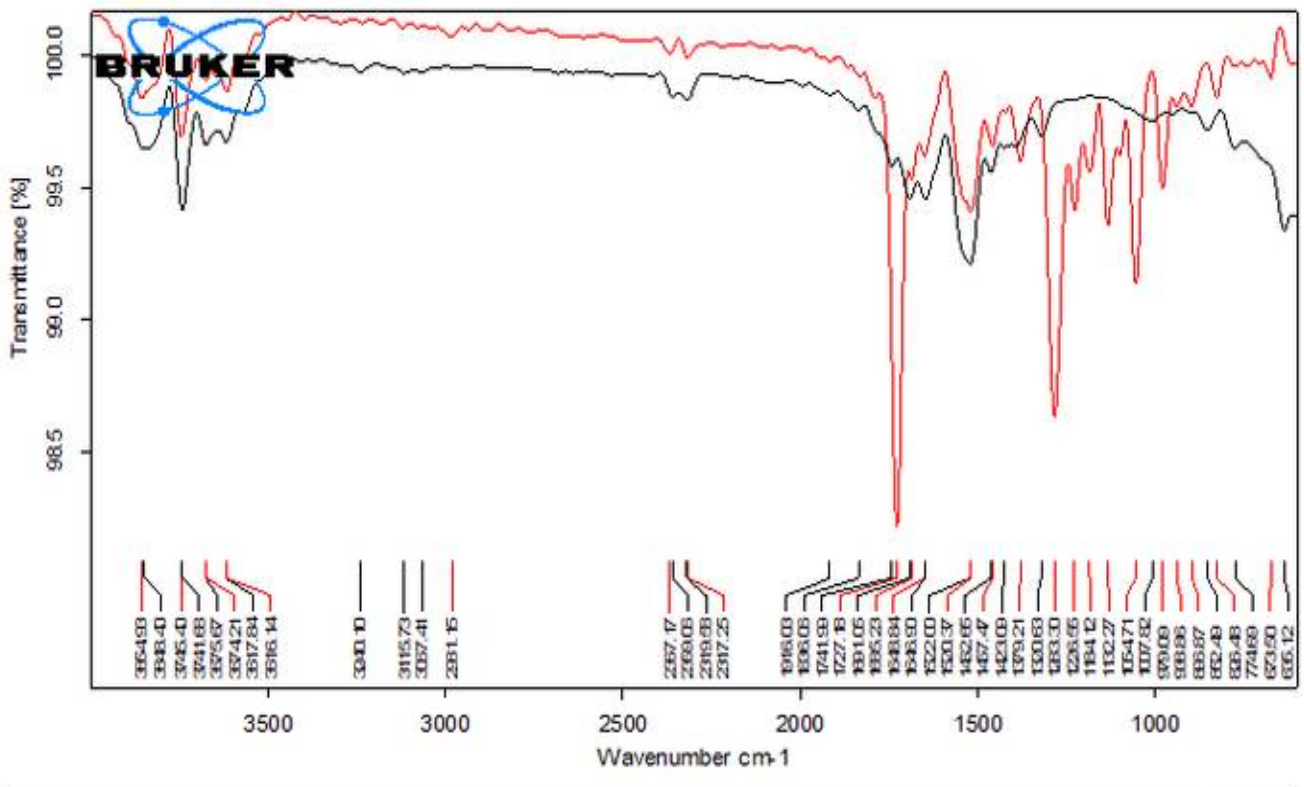

Fig. 13. FT-IR graph of PHB

Conclusion

The PHB extraction was performed with the help of isolated bacteria from soil. The substrate used for this purpose is an aquatic weed *Eichhornia crassipes* (Water Hyacinth), which grows in polluted and stagnant water and easily available which can reduce the production cost of PHB. Work is focused on to obtain the maximum yield of PHB. Optimization was performed by using various parameters responsible for the growth of bacteria and production of PHB. The parameters considered were incubation time, pH, substrate concentration and temperature, and results conclude a great potential in PHB production.

Acknowledgements

The authors are grateful to Prof. Aditya Shastri, Vice Chancellor, Banasthali Vidyapith for providing all necessary support. We acknowledge the Bioinformatics Center, Banasthali Vidyapith supported by DBT for providing computation support, and DST for providing networking and equipment support through the FIST and CURIE programs at the Department of Bioscience and Biotechnology. CESME, Banasthali Vidyapith, supported by MHRD, Government of India under the PMMMNMTT is acknowledged for organizing the symposium.

Conflict of interest

The authors declare that they don't have any conflict of interest in the publication.

Authors' contributions

First author performed and analysed all the experimental work. Second author wrote the manuscript. Corresponding author planned the work and revised the final manuscript.

References

1. Lackner M. Bioplastics. Kirk-Othmer Encyclopedia. Chem Technol. 2000;1-41.
2. Pathak S, Sneha CLR, Mathew BB. Bioplastics: its timeline based scenario & challenges. J Polym Biopolym Physics Chem. 2014;2:84-90. https://doi.org/10.12691/jpbpc-2-4-5
3. Chen YJ. Bioplastics and their role in achieving global sustainability. J Chem Pharm Res. 2014;6:226-31.
4. Nehra K, Jaglan A, Shaheen A, Yadav J, Lathwal P. Production of Poly-β-Hydroxybutyrate (PHB) by bacteria isolated from rhizospheric soils. Int J Microbial Resource Technol 2015; 2: 38-48.
5. Getachew A, Woldesenbet F. Production of biodegradable plastic by polyhydroxybutyrate (PHB) accumulating bacteria using low cost agricultural waste material. BMC Res Notes. 2016;12:509. https://doi.org/10.1186/s13104-016-2321-y
6. Law JH, Slepecky RA. Assay of poly-β-hydroxybutyric acid. J Bacteriol. 1961;82:33-36.
7. Poirier Y, Nawrath C, Somerville C. Production of polyhydroxyalkanoates, a family of biodegradable plastics and elastomers in bacteria and plants. Biotechnol. 1995;13:142-50. https://doi.org/10.1038/nbt0295-142
8. Kumar S, Mudaliar MSN, Reddy KMK, Chakrabarti J. Production of biodegradable plastics from activated sludge generated from a food processing industrial waste water. Bioresource Technol. 2004;95:327-30. https://doi.org/10.1016/j.biortech.2004.02.019
9. Singh G, Mittal A, Kumari A, Goel V, Aggarwal NK, Yadav A. Optimization of poly-B-hydroxybutyrate production from *Bacillus* species. Europ J Biol Sc. 2011;3:112-16.

10. Soam A, Singh AK, Singh R, Shahi SK. Optimization of culture conditions for bio-polymer producing *Bacillus mycoides* (WSS2) bacteria from sewage. Curr Discover. 2012;1:27-32.
11. Gregersen T. (1978). Rapid method for distinction of Gram-negative from Gram-positive bacteria. Eur J Appl Microbiol Biotechnol. 1978;5:123-27. https://doi.org/10.1007/BF00498806
12. Indira M, Karlapudi AP, Venkateswarulu TC, Babu DJ, Nath SB, Kodali VP. Isolation, screening and extraction of polyhydroxybutyrate (PHB) producing bacteria from sewage sample. Int J PharmTech Res. 2014;6:850-57.
13. Campos MI, Figueiredo TVB, Sousa LS, Druzian JI. The influence of crude glycerin and nitrogen concentrations on the production of PHA by *Cupriavidus necator* using a response surface methodology and its characterizations. Ind Crop Prod. 2014;52:338-46. https://doi.org/10.1016/j.indcrop.2013.11.008
14. Bengtsson S, Pisco AR, Johansson P, Lemos PC, Reis MAM. Molecular weight and thermal properties of polyhydroxyalkanoates produced from fermented sugar molasses by open mixed cultures. J Biotechnol. 2010;147:172-79. https://doi.org/10.1016/j.jbiotec.2010.03.022
15. Alarfaj AA, Arshad M, Sholkamy EN, Munusamy MA. Extraction and Characterization of Polyhydroxybutyrates (PHB) from *Bacillus thuringiensis* KSADL127 Isolated from Mangrove Environments of Saudi Arabia. Braz Arch Biol Technol. 2015;58:781-88. http://dx.doi.org/10.1590/S1516-891320150500003
16. Hu S, McDonald AG, Coats ER. Characterization of polyhydroxybutyrate biosynthesized from crude glycerol waste using mixed microbial consortia. J Appl Polym Sci. 2013;129:1314-21. https://doi.org/10.1002/app.38820
17. Devi AB, Nachiyar CV, Kaviyarasi T, Samrot AV. Characterization of polyhydroxybutyrate synthesized by *Bacillus cereus*. Int J Pharm Pharm Sci. 2015;7:140-44. https://innovareacademics.in/journals/index.php/ijpps/article/view/4378
18. Altaee N, Fahdil A, Yousif E, Sudesh K. Recovery and subsequent characterization of polyhydroxybutyrate from *Rhodococcus equi* cells grown on crude palm kernel oil. J Taibah Univ Sci. 2016;10:543-50. https://doi.org/10.1016/j.jtusci.2015.09.003
19. Pradhan S, Borah AJ, Poddar MK, Dikshit PK, Rohidas L, Moholkar VS. Microbial production, ultrasound-assisted extraction and characterization of biopolymer polyhydroxybutyrate (PHB) from terrestrial (P. hysterophorus) and aquatic (E. crassipes) invasive weeds. Bioresource Technol. 2017;242:304-10. https://doi.org/10.1016/j.biortech.2017.03.117
20. Peter HY, Chua H, Huang AL, Ho KP. Conversion of industrial food wastes by *Alcaligenes latus* into polyhydroxyalkanoates. In: Twentieth Symposium on Biotechnology for Fuels and Chemicals. Humana Press, Totowa, NJ. 1999;445-54.
21. Omar S, Rayes A, Eqaab A, Voß I, Steinbüchel A. Optimization of cell growth and poly (3-hydroxybutyrate) accumulation on date syrup by a *Bacillus megaterium* strain. Biotechnol lett. 2001;23:1119-23. doi: https://doi.org/10.1023/A:1010559800535
22. Chee JY, Tan Y, Samian MR, Sudesh K. Isolation and characterization of a *Burkholderia* sp. USM (JCM15050) capable of producing polyhydroxyalkanoate (PHA) from triglycerides, fatty acids and glycerols. J Polym Environ. 2010;18:584-92. doi: https://doi.org/10.1007/s10924-010-0204-1
23. Thakor N, Trivedi U, Patel KC. Biosynthesis of medium chain length poly (3-hydroxyalkanoates)(mcl-PHAs) by *Comamonas testosteroni* during cultivation on vegetable oils. Bioresource Technol. 2005;96:1843-50. doi: 10.1016/j.biortech.2005.01.030
24. Yu J, Stahl H. Microbial utilization and biopolyester synthesis of bagasse hydrolysates. Bioresource Technol. 2008;99:8042-48. https://doi.org/10.1016/j.biortech.2008.03.071
25. Lee WH, Loo CY, Nomura CT, Sudesh K. Biosynthesis of polyhydroxyalkanoate copolymers from mixtures of plant oils and 3-hydroxyvalerate precursors. Bioresource Technol. 2008;99:6844-51. https://doi.org/10.1016/j.biortech.2008.01.051
26. Cavalheiro JMBT, de-Almeida MCMD, Grandfils C, da-Fonseca MMR. Poly (3-hydroxybutyrate) production by *Cupriavidus necator* using waste glycerol. Proc Biochem. 2009;44:509-15. https://doi.org/10.1016/j.procbio.2009.01.008
27. Bhuwal AK, Singh G, Aggarwal NK, Goyal V, Yadav A. Isolation and screening of polyhydroxyalkanoates producing bacteria from pulp, paper, and cardboard industfigry wastes. Int J Biomat. 2013;1-10. http://dx.doi.org/10.1155/2013/752821
28. Preethi K, Umesh VM. *Water hyacinth*: a potential substrate for bioplastic (PHA) production using Pseudomonas aeruginosa. Int J Appl Res. 2015;1:349-54