SCHOLARLY RESEARCH JOURNAL FOR INTERDISCIPLINARY STUDIES ISSN: 2319-4766

PHB PRODUCTION FROM BACTERIA UTILIZING WASTE ENGINE OIL AS CARBON SOURCE

Nausheen Sadaf & D. C. Kamthane
Research student, NSB College, Nanded.
Head and Research Guide, Dept. of Microbiology, SGB College, Purna.

Abstract

Used oil has been classified as hazardous wastes by the Ministry of Environment and Forests, Government of India which demands its proper management to avoid serious threat toenvironment and for economic gains. Four species of bacteria were isolated from soil collected from automobile workshop using Bushnell Hass medium having waste engine oil as sole carbon source. The isolated bacteria were subjected to morphological and biochemical characterization. Same were used for PHB production using waste engine oil as sole carbon source. The produced PHB was conformed with FTIR analysis.

Keywords: Automobile workshop, Bushnell Hass medium, Engine oil, FTIR analysis, PHB.

Introduction: In India, there are 36,165 industries, generating 62, 32,507 Metric Tonnes of Hazardous Waste (HW) every year. As per Schedule IV of the Hazardous Waste Rules, 2008, both used oil & waste oil have been categorized as 'hazardous waste' and also listed in Schedule I under the rules. Spent Oil (used & waste oil) has been classified as 'RED" category.(P.K Selvi et al). Motor oil picks up a variety of hazardous contaminants when used in engines and transmissions. These contaminants include lead, cadmium, chromium, arsenic, dioxins, benzene and polycyclic aromatics. If used motor oil and the contaminants it contains are disposed of inappropriately and released into the environment, they can harm humans, plants, animals, fish and shellfish. water, oil is a visible pollutant, floating as a scum on the surface. This oil scum can stop sunlight and oxygen from getting into the water, affecting fish and water plants. It can kill fish, frogs and other animals that breathe from the water's surface. Low temperature burning of used oil can create airborne pollutants which can get into people's lungs and have adverse health effects (Australian Government Department of Environment).

An important difference between new and used motor oil is the heavy metal content. This difference is extremely important because many of the metals are harmful to human health and living organisms. These metals originate from the fuel and from motor wear. Used oil contains high concentrations of lead, zinc, calcium, barium, and magnesium along with lower concentrations of iron, sodium, copper, aluminium, chromium, manganese, potassium, nickel, tin, silicon, boron, and molybdenum. Used oil that is leaked, spilled or improperly discarded may enter storm water runoff and eventually enter into and adversely affect the environmental health of receiving water bodies(Wasiu Olalekan Akintunde et al).Used oil dumped on the ground, sewers or sent to landfills is capable of seeping into ground and s urface water. Just one litre of used oil can render one million litres of water undrinkable (NUOMAC, 2004). It is also a serious threat to plant and animal life. Marine species can be adversely affected by oil concentrations as low as one ppm. The oil film on water blocks sunlight, making it harder f or plants to photosynthesize (Jhanani. S et al). 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 importan[illegible] polymers in the future in light of limiting natural resources. The properties of I[illegible]ilar to those of polyethylene (PE) and polypropylene (PP)(Reddy Vishnuvar[illegible]mala). The present paper concentrate on

isolation of potential bacteria which are present in the environment contaminated with used engine oil, and produce PHB which is considered as bioplastic.

Materials and methods- The soil samples were collected from automobile workshops of HONDA situated in Nanded region.

Enrichment of Bacteria: The samples were inoculated in sterile Bushnell Hass broth supplemented with 2% used engine oil which was previously filter sterilized. The flasks were kept on orbital rotatory shaker with 120rpm and 37°C temperature. After every 24hrs, the medium is sub cultured into fresh medium and this was repeated four times. The final flask medium was used as inoculums for isolation.

Isolation of Bacteria: The samples were serially diluted using sterile saline and 10^{-6} was used to inoculate Bushnell Hass agar plates supplemented with 2% used engine oil obtained from same workshop. The plates were incubated at 37°C for 48hrs.

Identification of PHB producers: Sudan black B staining was performed for isolates colonies and the colonies showing positive for accumulation of storage granule were taken forward for PHB production.

PHB Production: The selected strains were inoculated in sterile Bushnell Hass medium supplemented with 2% used engine oil. The flasks were kept on orbital rotatory shaker with 120rpm and 37°C temperature for 24hrs, 48hrs and 72hrs.

Extraction of PHB: The PHB was extracted by Rawte and Mavinkurve method. In this method the bacterial cell were hydrolyzed 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).

Confirmation of PHB: PHB 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 *et al*).

Results and Discussion: The bacteria were identified as *Pseudomonas, Bacillus Micrococcus* and *Proteus*. The medium used was Bushnell Hass medium which is specially designed to isolate hydrocarbon degraders. As far as PHB production is concerned, Pseudomonas showed maximum PHB accumulation (70%), followed by Bacillus (54%), Micrococcus (47%) and least by Proteus (35%). A huge impact of incubation period is observes on PHB accumulation. All bacteria showed maximum production at 48hrs. Present study is purely an innovative attempt in which waste engine oil is used as carbon source. As already mentioned in introduction about harmful effects of waste engine oil, if PHB, a bioplastic is being produced from this hazardous waste, it will be really promising and profitable attempt.

Observation Table

Bacteria	PHB produced (%)		
	24hrs	48hrs	72hrs
Pseudomonas	63	70	67
Bacillus	48	54	50
Micrococcus	41	47	43
Proteus	31	35	33

Bioscience Discovery, 10(1):10-15, Jan. - 2019

Print & Online, Open Access, Research Journal Available on http://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)
Research Article

PHB production with bacteria isolated from cardboard industry soil

Nausheen Sadaf[1] and D. C. Kamthane[2]

1 Research student, NSB College, Nanded.
2 Head and Research Guide, Dept. of Microbiology, SGB College, Purna.
E mail: daiwa.kamthane@rediffmail.com

Article Info

Received: 20-10-2018,
Revised: 29-11-2018,
Accepted: 10-12-2018

Keywords:
Plastic materials,
Bioplastic, Paper pulp,
PHB, FTIR

Abstract

Plastic materials that have been universally used in our daily lives but now they are causing serious environmental problems. To overcome this, the only alternative is use of bioplastic and for this, a decrease in its production cost is required. My work aims to use paper pulp as carbon source for PHB production. Three isolates have used here which are actually isolated from paper industry soil. They were biochemically characterized with Bergey's Mannual of Systematic Bacteriology. The isolates produced more PHB with paper pulp as compared with pure sugar as carbon source. Effect of different parameters like effect of pH, temperature and incubation time was also determined. The produced PHB was confirmed with FTIR analysis.

INTRODUCTION

Plastic materials that have been universally used in our daily lives but now they are causing serious environmental problems. Millions of tons of these nondegradable plastics accumulate in the environment per year. For efficient management of used-plastic materials, recycling is one solution. But as the speed of utilization is much higher than speed of recycling, it cannot be used as a practical method that could be used on commercial scale. Another solution to reduce plastic residue is the use of biodegradable plastics and among them polyhydroxybutyric acids (PHB) are drawing much attention. They are common intracellular compounds found in bacteria, archaea, and in few eukaryotes such as yeasts and fungi. PHAs are carbon and energy reserve polymers produced in some microorganisms when carbon source is in plentiful and other nutrients such as nitrogen, phosphorus, oxygen or sulfur are limited. PHB is found to accumulate in varieties of microorganisms as reserve food material. They are the most common biodegradable polymer that can be used as promising alternative to synthetic nondegradable plastics. These polymers are accumulated intracellular membrane enclosed inclusion up to 90% of the cell dry weight under conditions of nutrient stress and act as energy reserve material. It has similar mechanical properties as those of the oil-derived conventional plastics like polypropylene or polyethylene which can be molded, made into films, spun into monofilaments, and used to make heteropolymers with other synthetic polymers and many more applications in agriculture, packaging, and medical field being biodegradable and also immunologically compatible with human tissues (Anish Kumari *et al.*, 2013).

Cellulose is always been the most abundantly available waste in the world. It can be in the form of agricultural waste, food industry waste, sugar industry waste, or paper waste. The waste paper is generally recycled in paper or cardboard industry. The paper pulp of waste paper of this industry can be used as an efficient source of carbon for PHB production (Muthu Kumar A, 2017).

References

T. Sivaramasundari, Dr. N. Jevakumar,(2018) Isolation and screening of Diesel oil degradinf bacteria using redox indicator. IJSDR 3(5).

Ugare M. Shah N. and Jadhav M.(2015) Enrichment, Isolation and Identification of Hydrocarbon Degrading Bacteria. Int Jr. of current microbiology and applied sciences 4(6).

Himedia Technical Data.

P K Selvan,*, Mita Sharmaa, J S Kamyotra (2013). Spent oil management and its recycling potential in India inventory and issues. Elsevier Procedia Environmental Sciences 18 (2013) 742 – 755.

Wasiu Olalekan Akintunde,[1*] Ojo A. Olugbenga,[2] and Ogundipe O. Olufemi (2015) Some Adverse Effects of Used Engine Oil (Common Waste Pollutant) On Reproduction of Male Sprague Dawley Rats.

Jnanam, S.L., Kurian Joseph (2011) Used oil generation and management in the automotive industries. Int. Jr of Applied engineering research Dindigul 2(1).

Zagheer A, Dobhal S and Tomar V (2017). A Comparative Study of Oil Degradation with Used and Unused Engine Oil by Microbes Isolated From Water Sample of Mechanic Workshops. Journal of Agricultural research and technology. 10(2).

T. Rawte, Mavinkurve (2002). A rapid hypochloritc method for extraction of polyhydroxyalkanoates from bacterial cell. Ind. Jr. of experimental Biology. Vol 40.

N. Vishnuvardhan Reddy, and 2M. Thirumala (2012) Isolation of polyhydroxyalkanoates (PHA) producing bacteria fromcontaminated soils. Int. Jr. of Environmental Biology 2(3).

Sajida Munir and Nazia Jamil (2015) Characterization of Polyhydroxyalkanoates Produced by Contaminated Soil Bacteria using Wastewater and Glucose as Carbon Sources. Tropical Journal of Pharmaceutical Research September 2015; 14 (9): 1605-1611.

Bioscience Discovery, 10(1):10-15, Jan. - 2019

Print & Online, Open Access, Research Journal Available on http://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)
Research Article

PHB production with bacteria isolated from cardboard industry soil

Nausheen Sadaf[1] and D. C. Kamthane[2]

1 Research student, NSB College, Nanded.
2 Head and Research Guide, Dept. of Microbiology, SGB College, Puma.
E mail: daiwa.kamthane@rediffmail.com

Article Info

Received: 20-10-2018,
Revised: 29-11-2018,
Accepted: 10-12-2018

Keywords:
Plastic materials,
Bioplastic, Paper pulp,
PHB, FTIR

Abstract

Plastic materials that have been universally used in our daily lives but now they are causing serious environmental problems. To overcome this, the only alternative is use of bioplastic and for this, a decrease in its production cost is required. My work aims to use paper pulp as carbon source for PHB production. Three isolates have used here which are actually isolated from paper industry soil. They were biochemically characterized with Bergey's Mannual of Systematic Bacteriology. The isolates produced more PHB with paper pulp as compared with pure sugar as carbon source. Effect of different parameters like effect of pH, temperature and incubation time was also determined. The produced PHB was confirmed with FTIR analysis.

INTRODUCTION

Plastic materials that have been universally used in our daily lives but now they are causing serious environmental problems. Millions of tons of these nondegradable plastics accumulate in the environment per year. For efficient management of used-plastic materials, recycling is one solution. But as the speed of utilization is much higher than speed of recycling, it cannot be used as a practical method that could be used on commercial scale. Another solution to reduce plastic residue is the use of biodegradable plastics and among them polyhydroxybutyric acids (PHB) are drawing much attention. They are common intracellular compounds found in bacteria, archaea, and in few eukaryotes such as yeasts and fungi. PHAs are carbon and energy reserve polymers produced in some microorganisms when carbon source is in plentiful and other nutrients such as nitrogen, phosphorus, oxygen or sulfur are limited. PHB is found to accumulate in varieties of microorganisms as reserve food material. They are the most common biodegradable polymer that can be used as promising alternative to synthetic nondegradable plastics. These polymers are accumulated intracellular membrane enclosed inclusion up to 90% of the cell dry weight under conditions of nutrient stress and act as energy reserve material. It has similar mechanical properties as those of the oil-derived conventional plastics like polypropylene or polyethylene which can be molded, made into films, spun into monofilaments, and used to make heteropolymers with other synthetic polymers and many more applications in agriculture, packaging, and medical field being biodegradable and also immunologically compatible with human tissues (Anish Kumari *et al.*, 2013).

Cellulose is always been the most abundantly available waste in the world. It can be in the form of agricultural waste, food industry waste, sugar industry waste, or paper waste. The waste paper is generally recycled in paper or cardboard industry. The paper pulp of waste paper of this industry can be used as an efficient source of carbon for PHB production (Muthu Kumar A, 2017).

The present study attempts to use paper pulp as crude carbon source for PHB production and the efficiency will be compared with use of pure glucose sugar.

Isolation of Bacteria

1g of paper pulp sample was taken from cardboard industry located in Nanded region. The sample was serially diluted with sterile saline and inoculated on Sterile nutrient agar plates. The plates were incubated for 24hrs at 37°C. After incubation, selected colonies were grown on minimal media supplemented with 1% of sterile paper pulp as carbon source. The plates were incubated for 24hrs at 37°C. After incubation, the appeared colonies were subjected for morphological and biochemical analysis. The obtained results were compared with Bergey's manual of systematic Bacteriology for strain identification (Bhairavi *et al.*, 2011).

Screening of PHB producers

For isolating PHB producing bacteria, Sudan black B and Nile blue A staining were performed and only the colonies showing positive results were carried forward for production (Anish Kumari *et al.*, 2014)

PHB Production

Modified Mineral Salt medium without glucose was used as production medium supplemented with 1% paper pulp which is separately autoclaved and then mixed with the medium (Anteneh and Fantahun, 2016).

A comparative set with mineral salt medium with its proper composition was also set with same selected strains. The medium is incubated at 37°C for 24hrs. Effects of pH, temperature and incubation time on PHB production were also determined. (Obruca *et al.*, 2015)

Extraction and Confirmation of PHB

The extraction procedure was performed as described by Anteneh and Fantahun in 2016.

FTIR spectrophotometer analysis of PHB

About 1 mg extracted sample of PHB was dissolved in 5 ml chloroform. After pellet was formed by adding KBr, spectra were recorded at 4000–400 cm^{-1} range by Spectrum 65 FT-IR (Anteneh and Fantahun, 2016).

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, 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, which are showing similarity with the previous work.

Table-1: Morphological characters

Character	Isolate P1	Isolate P2	IsolateP3
Size	3mm	3mm	2mm
Shape	Rod	Rod	Rod
Colour	Cream	white	Cream
Margin	Irregular	entire	entire
Elevation	Concave	Concave	Convex
Consistancy	Smooth	Smooth	Mucoid
motility	motile	motile	motile
Opacity	Opaque	Opaque	Opaque
Gram's nature	Negative	positive	negative

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

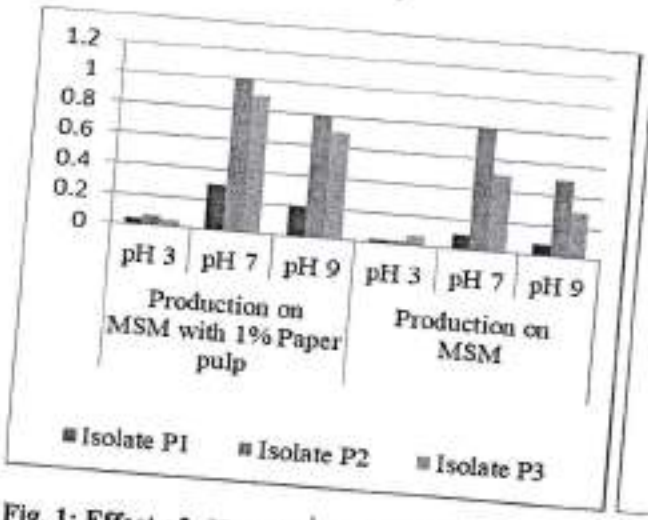

Fig. 1: Effect of pH on PHB production

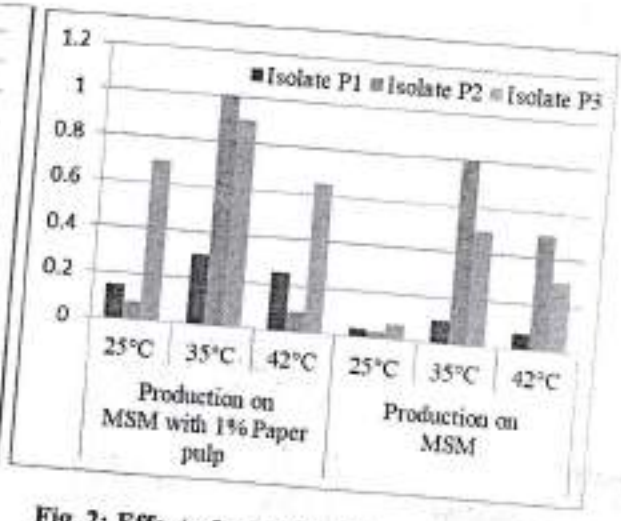

Fig. 2: Effect of temp on phb production

Co-ordinator
IQAC

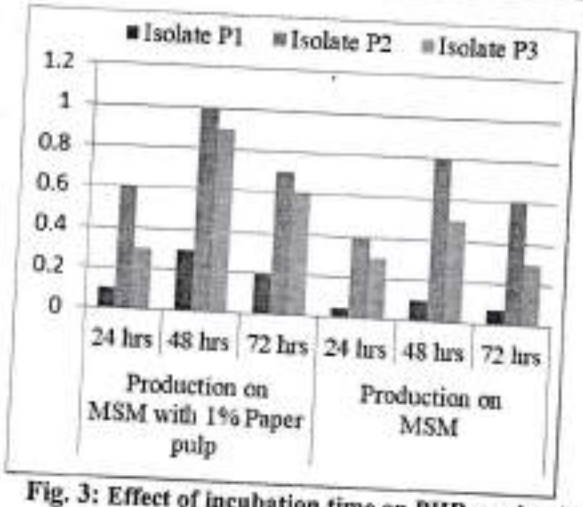

Fig. 3: Effect of incubation time on PHB production

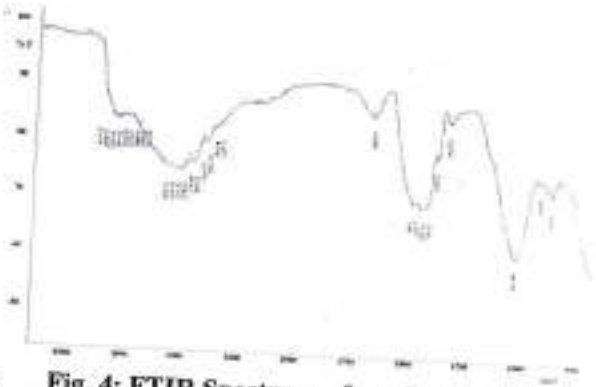

Fig. 4: FTIR Spectrum of produced PHB

REFERENCES

Anish Kumari Bhuwal, Gulab Singh, Neeraj Kumar Aggarwal, Varsha Goyal and Anita Yadav, 2014. Poly-β-hydroxybutyrate production and management of cardboard industry effluent by new *Bacillus sp.* NA10. *Bioresources and bioprocessing.* **1**(19):1-11.

Anish Kumari Bhuwal, Gulab Singh, Neeraj Kumar Aggarwal, Varsha Goyal and Anita Yadav, 2013. Isolation and Screening of Polyhydroxyalkanoates Producing bacteria from pulp, paper and cardboard industry. *International journal of Biomaterials*, 2:13.

Anteneh Getachew and Fantahun Woldesenbet, 2016. Production of biodegradable plastic by polyhydroxybutyrate (PHB) accumulating bacteria using low cost agricultural waste material. *BioMed Central.*, 9:509.

Bhairavi Ghate, Prashant Pandit, Chandrashekahar Kulkarni, Deepti D. Mungi and Twisha S. Patel, 2011. PHB production using novel agro industrial sources from different *Bacillus* Species, *Int. Journal of Pharma and biosciences*, **2**(3):242-249.

Muthu Kumar A, 2017. Wood waste - carbon source for Polyhydroxyalkanoates (PHAs) production, *International journal of foresty and wood science*, **4**(1):36-40.

S Oruca, P Benesova, L. Marsalek and I Maro, 2015. Use of Lignocellulosic materials for PHA production, *Chem. Biochem. Eng. Q.*, **29** (2) 135–144.

How to cite this article

Nausheen Sadaf and D. C. Kamthane, 2019. PHB production with bacteria isolated from cardboard industry soil. *Bioscience Discovery*, **10**(1):16-20.

Co-ordinator
IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn) Dist. Parbhani - 431511 (M.S.)

PRINCIPAL
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist.Parbhani