Utilization of Hydrocarbon Wastes for Bioplastics (Polyhydroxyalkanoates) Production by Marine Bacteria

F.No: MRP-6777/16(SERO/UGC)

Final Report

Submitted

To

UNIVERSTY GRANTS COMMISSION (UGC)
SOUTH EASTERN REGIONAL OFFICE (SERO)
HYDERABAD

Submitted

by

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Annexure - VI

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002
CONSOLIDATED REPORT

Final report of the work done on the Minor Research Project

1.	Project report No.	**Final Report**
2.	UGC Reference No.	**No.F MRP-6777/16(SERO/UGC). dt 30.06.2017**
3.	Period of report	**From 01-01-2017 to 29-06-2019**
4.	Title of the Research Project	**Utilization of Hydrocarbon wastes for Bio plastics (Polyhydroxyalkanoates) production by Marine bacteria**
5.	(a) Name of the Principal Investigator (b) Department (c) College where the work has progressed	**Dr.M.Karthikeyan** **Zoology and Microbiology** **Thiagarajar College(Autonomous)** **#139-140,Kamarajar salai** **Teppakulam** **Madurai-625009**
6.	Effective date of starting of the Project	**01-01-2017**

7. Grant approved and expenditure incurred during the period of the report:

a. Total amount Sanctioned **Rs. 40000.00**
b. Total expenditure **Rs. 32,888.00**
c. Report of work done : Refer Attachment I

i. Brief objective of the project

- Site selection and sampling - Physico-chemical analysis of the samples collected from hydrocarbon contaminated coastal regions of Ennore oil spill area and RameshwaramGulf of Mannar

- Isolation and screening of hydrocarbon degrading and PHA accumulating ability of the isolates

- Determination of surfactants, cellsurface hydrophobicity,emulsification activity of the Isolates

- Utilization of hydrocarbon wastes for the production of PHA
- Extraction, Purification and structural characterization of PHA
- Optimization of media composition for PHAs production using Response surface methodology.

ii. **Work done so far and results achieved and publications, if any, resulting from the work**

Hydrocarbon contaminated marine water samples was collected along the coastal regions of Ennore oil spill area and Gulf of Mannar, Rameshwaram. Different bacterial strains were isolated and screened for simultaneous hydrocarbon degradation and PHB production. PHB accumulation was ascertained by Sudan Black staining. Hydrocarbon degrading ability of the isolates was confirmed by 2,6 dichlorophenol indophenol assay. Biosurfactant production by the isolates was assessed by emulsification index analysis and MATH assay. Selected isolates were further screened for their ability to produce bioplastics (PHB) using different hydrocarbons like diesel, kerosene, benzene, toluene and crude oil. Further, production of PHB was carried out using filter sterilized hydrocarbon contaminated waste water by the isolates. PHB produced from hydrocarbon contaminated waste water was isolated by sodium hypochlorite method and purified. The purified PHB was spectrally characterized by FTIR and NMR analysis. Medium optimization studies was carried out using Response surface methodology. **A research paper entitled 'Bioconversion of oily bilge waste to polyhydroxybutyrate (PHB) by marine *Ochrobactrum intermedium*' was published in Scopus indexed journal Bioresource Technology Reports. Findings of the project was delivered as invited talk in DST SERB funded "7th National conference on Emerging Trends and New Challenges in Biotechnology-Advances in Bioplastics organized by PG and Research centre in Biotechnology, MGR college, Hosur. January 30st – January 31, 2019.**

iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons

Yes

iv. Please enclose a summary of the findings of the study. One bound copy of the final report of work done may also be sent to the concerned Regional Office of the UGC.

Copy enclosed

PRINCIPAL INVESTIGATOR

PRINCIPAL

PRINCIPAL
Thiagarajar College, Madurai.

ATTACHMENT –I

Project Title: Utilization of Hydrocarbon Wastes for Bioplastic (Polyhydroxyalkanoates) production by Marine Bacteria

INTRODUCTION:

Plastics are utilized in almost every manufacturing industry ranging from automobiles to medicine. Most of the synthetic polymers are produced from petrochemicals, which are derived from fossil fuels. The focus has been diverted from synthetic polymers towards biopolymers, because of two major reasons. Firstly they cause environmental pollution and secondly they are derived from fossil fuels. The reservoir of fossil fuels is now reaching its bottom line, which will lead to an increase in the production cost of synthetic polymers. It is a well-acknowledged fact that commercial use of biodegradable polymers will greatly reduce the increasing dependence on synthetic polymers and its environmental impacts. Polyhydroxyalkanoate (PHA) is an intracellular microbial thermoplastic that is widely produced by bacteria present in soil (Anderson and Dawes, 1990). They possess material properties similar to the common petrochemical-based synthetic thermoplastics and elastomers currently in use. They can be completely degraded to carbon dioxide and water (and methane under anaerobic conditions) by microorganisms in the environment (Du *et al*., 2001). The properties of pure PHB, including thermoplastic processability, hydrophobicity, complete biodegradability and biocompatibility with optical purity suggest that PHB could be an attractive alternative to the common plastics (Sharma and Mallick, 2005).

PHAs can be produced in bacteria, recombinant bacteria and genetically engineered plants. The preferences of using a microbial source for large-scale production of biodegradable polymers can be advocated because of the advantages of rapid mass production and malleability of bacterial genetic makeup. Also, bacteria have been found to integrate novel monomers in the polymer, which increases the diversity of PHAs produced. There is still ongoing interest to find novel PHAs with novel constituents or a new combination of already known constituents. Owing to their biocompatibility and biodegradability, PHAs are receiving an increasing attention for their commercial application as substitutes for synthetic plastics. The applications of PHAs are not limited to packaging consumables but extend to quality applications in medicine and pharmacy and nanocomposites in biomedical devices (Zinn *et al*., 2001, Pandey *at al*., 2005).

The presence of stress in form of nutrient limitation or presence of xenobiotics leads to physiological response in the form of unbalanced growth leading to an increase in the PHA content. It is thought of as a strategy to increase survival and stress tolerance in changing environments (Ayub *et al*., 2004). PHA producing bacteria have been repeatedly isolated from oil-contaminated soils (Haba *et al*., 2007). Oil-contaminated soils contain about 84% carbon, 14% hydrogen, 1-3% sulfur and less than 1% of nitrogen and other compounds (Atlas, 1995). Excess carbon with less than 1% nitrogen make these sites a potential source for isolating PHA producers since the synthesis of PHA is favored by environmental stresses (Anderson and Dawes, 1990). Several studies have reported the isolation of PHA producing bacteria from oil contaminated sites (He *et al*, 1998; Haba *et al*., 2007). However, our understanding of diversity

of PHA producers at oil contaminated sites remains limited. Further, present literature on PHA producing bacterial strains is limited to a relatively small number of well characterized marine bacteria. Due to this, from oil contaminated sites, producers belonging to the *Pseudomonas* genera have been rediscovered in different studies. Hydrocarbon-degrading bacteria that can produce PHA have an extended advantage of degrading recalcitrant pollutants and production of a commercially important polymer from them. In spite of the advantages of PHAs compared with petroleum-derived plastics, their use is currently limited due to their high production costs (Kellerhals *et al*., 2000). A significant portion of the cost associated with PHA production is the cost of the substrate used for growth and polymer accumulation.

One approach to reduce the cost of PHA is to use inexpensive carbon source. Alkanoates such as octanoate are the best carbon sources for all MCL-PHA synthesizing *Pseudomonas*. However, using pure alkanoates for MCL-PHA synthesis increases the production costs of these polymers. Considering that pure fatty acids are expensive, vegetable oils and their fatty acids have greater suitability for production of MCL- PHA economically (Lee *et al*., 2000). Though a variety of industrial wastes are used for PHA production, no reports are available on the utilization of polyaromatic hydrocarbons (PAHs) (Thakor, 2003). Microbial production of PHA utilizing PAH will not only be cost effective but also reduce the oil spill reduction costs. In this study, it is intended to use native bacteria isolated from oil contaminated marine environment for hydrocarbon degradation and PHB production. Therefore the present work is designed to isolate PHA accumulating bacteria from hydrocarbon contaminated coastal regions of Ennore oil spill area and rameshwaram Gulf of Mannar in order to investigate the production of polyhydroxyalkonates using hydrocarbon wastes.

OBJECTIVES:

- Site selection and sampling - Physico-chemical analysis of the samples collected from hydrocarbon contaminated coastal regions of Ennore oil spill area and RameshwaramGulf of Mannar
- Isolation and screening of hydrocarbon degrading and PHA accumulating ability of the isolates
- Determination of surfactants, cellsurface hydrophobicity and emulsification activity of the
  Isolate
- Utilization of hydrocarbon wastes for the production of PHA
- Extraction, Purification and structural characterization of PHA
- Optimization of media composition for PHAs production using Response surface methodology.

METHODOLOGY:

Determination of elemental composition of the sample

Elemental composition of hydrocarbon contaminated sea water was collected from Ennore oil spill area and hydrocarbon contaminated coastal regions of Gulf of mannar. The chemical composition of oil polluted water samples was assessed by standard protocols ascertained by Manivasagam et al (1987).

Screening for PHA accumulation

PHA accumulation in the oil degrading bacteria was determined by Sudan Black staining and viable colony staining method (Spiekermann *et al.*, 1999). For viable colony staining, 0.25mg Nile red (Hi Media, India) per ml of dimethyl sulfoxide (DMSO) was be added to the sterilized BH agar medium (pH 8.0) to a final concentration of 0.5µg dye per ml. Filter sterilized sodium gluconate (1%) was added as a carbon source. The isolate was streaked on agar plates and incubated for 72 hrs. PHA accumulation by the isolate was detected by fluorescence emission on exposure to UV light. Further, the cells were subjected to microscopic analysis (Olympus CKX 41, Japan excitation, 546/10 nm; barrier filter, 590 nm; dichroic mirror, 575 nm) for fluorescence imaging.

Identification of the isolate

Oil degrading bacteria that exhibit fluorescence was be subjected to morphological and physiological analyses according to Bergy's manual of determinative bacteriology (Holt 1994)**.** 16S rRNA gene sequencing was carried out for species identification.

Influence of different hydrocarbon on growth of the isolate

Tolerance and growth of the isolate in different hydrocarbons was be determined following the procedure described by Martino *et al.* (2012). Tolerance of the isolates to different hydrocarbons were confirmed by its growth on Zobell marine broth after exposure to the respective hydrocarbons for 12 hr. For the growth determination, the isolate was inoculated on BH medium supplemented with different hydrocarbons and incubated for 7 days at 30°C with shaking (120 rpm). Hydrocarbon utilization and growth of the isolate were confirmed by the appearance of turbidity. In addition

Biosurfactant production, Emulsification Index and Cell surface hydrophobicity assay

Biosurfactant production by the isolate was be confirmed by oil spread method and drop collapse test (Bodour and Miller-Maier 1998).Emulsification index will be calculated following the method of Satpute *et al.* (2008). Hydrophobicity of bacterial cells to different hydrocarbons was determined following ''microbial adhesion to hydrocarbon'' (MATH) assay (Rahman *et al.*, 2008). The isolate were grown in BH medium supplemented with 1% sodium gluconate for 24 hours. Cells were harvested by centrifugation (10,000g for 15 min.) washed and resuspended in 50 mM K_2HPO_4 (pH 8.0) to an absorbance of about 0.6 at 660 nm. Bacterial suspension (5ml) was vortexed with 1 ml of different hydrocarbons and incubated at room temperature for an hour. Difference in the absorbance of suspension due to bacterial adhesion to hydrocarbons was be recorded at 660 nm and expressed as surface hydrophobicity percentage (SHb %)

$$SHb\ \% = [(A_0-A) / A_0] \times 100$$

where A_0 and A are the absorbance before and after the addition of hydrocarbons respectively.

Extraction and quantification of PHA

One ml of mid log phase bacterial culture was inoculated in BH medium supplemented with different hydrocarbons (1% v/v) and incubated at 30ºC on a rotary shaker (120rpm) for a week. After incubation the culture broth was centrifuged (10,000g for 15 min.), resultant cell pellet was washed with dichloromethane to remove the residual hydrocarbons and subjected to PHA quantification and dry cell weight (DCW) determination as per the procedure described by Taran (2011) with slight modifications.

The cell pellet (1g) will be digested with 10 ml of sodium hypochlorite at 40°C for 1 hour, washed with 10 ml of acetone followed by 10 ml of ethanol. The residue was dissolved in 10 ml chloroform and evaporated to dryness at 40°C. Polymer accumulation were qualitatively determined by crotonic acid assay (Salgaonkar and Braganca et al. 2015). 100µg of the polymer was acid hydrolyzed with conc. H_2SO_4 at 100°C for 10 min in a boiling water bath and subjected to UV-Visible spectroscopy (Biospectrophotometer, Eppendorf). PHA will be quantified as the percentage composition in the DCW (dry cell weight). The residual mass was calculated as the difference between DCW and PHA (Kumar *et al.*, 2006). After quantification the resultant polymer was purified and subjected to spectral analyses.

Fourier transformed infrared spectroscopy (FTIR):

The purified polymer will be pelleted with potassium bromide (KBr) and subjected to FTIR spectroscopy. Spectra were recorded over a range of 4,000 to 400 cm^{-1} and the results were averaged over 100 scans.

Optimization and Simulation studies

Influence of hydrocarbon wastes on biopolymer production by the bacterial isolates was be analysed. Factor treatments and experimental design was employed by Central Composite Experimental Design (CCD) using Response Surface Methodology (Design expert software Statease Inc., Minneapolis, (USA).On the basis of experimental design formulation of nutrient sources was adopted for optimization studies.

RESULTS:

Elemental characteristics of hydrocarbon contaminated samples collected from two different locations Ennore oil spill area (Sample A), and Gulf of Mannar namely Rameshwaram (Sample B) was presented in Table 1.No wide variation in pH, temperature, total dissolved solids and hardness were observed between the samples. Hydrogen ion concentration (pH) in the three sampling sites A and B was found to be 5.3 and 6.2 respectively. The total dissolved solids content in sample A was estimated to be 21589 mg/L, 24321 mg/L in sample B. Total petroleum hydrocarbons in the sample A and B was found to be 24136 mg /kg and 16705 mg /kg respectively. Oil contamination may be the reason for the foul odor in the samples.Concentration of inorganic salts namely calcium, magnesium, chlorides, phosphate and nitrate in the samples are presented in Table 1. A conspicuous difference was observed with the concentration of the inorganic salts in the three different locations. Total dissolved solid of water sample collected from Ennore Oil spill area (sample A) was greater when compared to other two. Nitrite and nitrate level was found to be minimum in all the samples analyzed. The amount of phosphate

was found to be 12 µg/L in sample A. The concentration of chloride was found to be 11300 µg/L in sample A, it was found to be 11901 µg/L in sample B respectively.

PHA producing ability of the isolates was confirmed by a series of tests using lipophilic dyes such as Sudan black and nile red. To detect the colonies that produce PHA, 72 hr bacterial colonies onBH medium were flooded with Sudan black and nile red separately. Appearance of blue-black inclusions on addition of Sudan black indicates the presence of PHA within the cells

Hydrocarbon degrading potential of the isolates was determined by 2, 6 DCPIP redox indicator assay using different hydrocarbons as a sole carbon source. The isolates were able to accumulate PHAs when grown in BH medium supplemented with different hydrocarbons. BH medium is used in the present study because its composition is similar to the chemical constituents of marine environment and used by earlier workers for the isolation of hydrocarbon-degrading bacteria. 2,6 DCPIP assay is an easy and reliable method for determining hydrocarbon degrading potential of the microorganisms. Synthesis of surface-active compounds is considered as one of the prerequisite for hydrocarbon degradation by the bacteria. In aqueous solution, hydrocarbons remain partitioned as a separate non-aqueous liquid phase, thus their availability to microbial cells for degradation is limited. Biosurfactant production differs with the isolates and hydrocarbons. Two isolates found to produce bio surfactants with complex hydrocarbons like diesel, crude oil and kerosene including aliphatic and aromatic hydrocarbons.

In this study, biosurfactant produced by the isolate emulsify all the hydrocarbons evaluated. Emulsification activity was found to differ with hydrocarbons tested. MATH assay carried out in this study sheds light on the adhesion of the microbial cells to different hydrophobic compounds. Adhesion of the microbial cells to hydrocarbons found to vary with the isolates and the different hydrocarbons analyzed. Diesel exhibited maximum emulsifiying activity (61%) followed by crudeoil (56%).The emulsifying activity with toluene was found to be 12%. MATH assay was performed to elucidate the adhesion property of the isolate towards hydrocarbons. Results obtained in MATH assay were found to be similar with that of the emulsification index. Influence of different hydrocarbons on the tolerance and growth of *Bacillus species* was presented in Figure 3. The isolate fails to kerosene. Tolerance and growth of the isolate was observed when the media was supplemented with benzene (6%), Xylene (6%), Toluene (4%). Surfactant production was considered to be a desirable characteristic for oil degrading bacteria. In the present study, formation of flattened drops in drop collapse test confirms the production of biosurfactant by the isolate. In this study, biosurfactant produced by the isolate emulsify all the hydrocarbons evaluated. Emulsification activity was found to differ with hydrocarbons tested. Benzene exhibited maximum emulsifiying activity (78) followed by xylene (70%).The emulsifying activity with toluene was found to be 45%. MATH assay was performed to elucidate the adhesion property of the isolate towards hydrocarbons. Results obtained in MATH assay were found to be similar with that of the emulsification index.

In this study of the six hydrocarbons tested only three supported the cell growth and biosynthesis of PHB in *Pseudomonas species*. Therefore, it is evident that the ability of the isolate to grow and accumulate PHB appears to be substrate specific. Of the three hydrocarbons evaluated maximum growth (2.14 mg $L^{-1)}$ and PHB accumulation (45.1% DCW) was observed with diesel. In the presence kerosene and crude oil, the growth and PHB accumulation was found to be 1.97g $L^{-1,}$ 1.56 97g $L^{-1,}$ and .38.5% , 37.2 % DCW respectively Surfactant production was considered to be a desirable characteristic for oil degrading bacteria. In the present study, formation of flattened drops in drop collapse test confirms the production of biosurfactant by the isolate. Emulsification activity is considered as one of the important criteria in selecting the potential biosurfactant producers. In case of *Bacillus species* of the three hydrocarbons evaluated maximum growth (53.23 mg $L^{-1)}$ and PHB accumulation (4.6% DCW) was observed with xylene. In presence of toluene and benzene growth and PHB accumulation was found 49.98 g L^{-1} and 39.67 g L^{-1} and 4.23% and 3.52% DCW respectively.

FTIR spectrum of the PHA extracted from *Pseudomonas species* reflects the presence of highly saturated biodegradable polymer. The spectrum is characterized by the sharp high intense peak at 1602 cm^{-1} due to the amorphous stretching vibration of carbonyl groups (C=O). A band at 1226 cm^{-1} may be due to valence vibration of carboxyl group. The band at 2954 cm^{-1} is attributed to the bending vibrations of aliphatic C–H. The pronounced vibration at about 1382 cm^{-1} is typically associated with deformation of C-H bond in CH_3 group.Infrared analysis of the polymer produced by *B.megaterium* showed an intense absorption band at 1060 cm^{-1} and 1226cm^{-1} corresponding to C=O stretching. A band at 2891 cm^{-1} corresponds to the vibration of aliphatic C-H. The band at 1371 cm^{-1} corresponds to the asymmetrical deformation of C-H bond in CH_3 group. A band at 1031 cm^{-1} corresponds to the stretching of the C–O bond of the ester group.

Investigations were carried out to optimize the media components (BH medium) to enhance biomass and PHB production in the bacterial isolates. The 3-D response surface plots graphically represent the regression equation. By using the response surface plot, the interaction between two variables and their optimum levels can be easily understood. Figure 6 and d represent the influence of interaction between oily bilge water and NH_4NO_3, oily bilge water and K_2HPO_4, $NH_4NO_3$and K_2HPO_4 on PHB production respectively. It is evident from the Figure 6 that the interactive effect of hydrocarbon waste and NH_4NO_3 had a maximum effect on PHB production in pseudomonas species. However in case of Bacillus interactive effect of K_2 HPO_4. Gradual replacement of synthetic plastics with biodegradable polymers is an environmental and social issue of great significance. However, attempt made and the results obtained in this study illustrate a remarkable achievement in biopolymer production strategies. From the ecological point of view, utilization of hydrocarbon waste for PHA production would certainly reduce the toxic effects of the pollutant in marine environment. Therefore, investigation of this kind of microbe mediated transformation of harmful substances into value added end products has represented a more recent research thrust which not only enhances the production but also reduces the production cost. Further, this approach was found to be beneficial from the both environmental and economic point of view.

Table 1

Elemental compositional analyses of the Sampling Sites

Parameters	Sample A	Sample B
Appearance	Blackish	Slightly brown
Colour	Blackish	Slightly brown
Odour	Foul smell	Foul smell
Total Petroleum Hydrocarbons	24136	16705
Total diss.solids mg/L	21589	24321
pH	5.30	6.20
Total alk .as caco3	200	340
Total hardness as caco3	6200	5700
Calcium	1513	1391
Magnesium	580	534
Iron	13	0
Manganese	0	0
Zinc	23	41
Chromium	0.09	0.01
Lead	0.002	00.5
Copper	21	12
Free ammonia as NH_3	40	23
Nitrate as NO_2	16	13
Nitrate as NO_3	54	31
Chloride	11300	10901
Sulphate as so4	548	411
Phosphate as PO4	12	5

Table 2.Morphological and Biochemical Characterization of the isolates

Test	*Pseudomonas species*	*Bacillus species*
Morphology	White colony	Creamy colony
Gram staining	G –ve	G+ve
Cell size (µm)	1-1.5 x 1.2	1.2x1.2
Growth temperature (^{o}C)	21-33	27-35
Growth in NaCl		
0% - 4%	+	+
8.5%	-	+
15%	-	-
Motility	+	-
Oxidase	+	-
Catalase	+	-
Salt tolerance	2-4 %	2-4%
Arginine dihydrolase	-	+
Ornithine decarboxylase	+	-
Lysine decarboxylase	+	+
Luminescence	+	+
Urease	-	_
Citrate Utilization	-	+
MR test	+	+
VP test	-	+
Mannitol	+	+
Gelatin hydrolysis	-	

Table 3. Emulsification activity (%, E_{24}), cell surface hydrophobicity (%, MATH) of *Pseudomonas species* with different hydrocarbons

Hydrocarbon source	EI %	Hydrophobicity %
Diesel	61	68
Crude oil	56	64
Kerosene	39	41
Toluene	12	33
Xylene	15	10

Table 4. Emulsification activity (%, E_{24}), cell surface hydrophobicity (%, MATH) of *Bacillus species* with different hydrocarbons

Hydrocarbon source	**EI %**	**Hydrophobicity %**
Diesel	13	15
Crude oil	7	9
Kerosene	8	10
Toluene	45	41
Xylene	70	60
Benzene	78	66

Figure 1. 2,6dichlorophenol indophenols assay

Pseudomonas species ***Bacillus species***

Figure 2.Influence of different carbon hydrocarbons on growth and PHB accumulation of Pseudomonas species

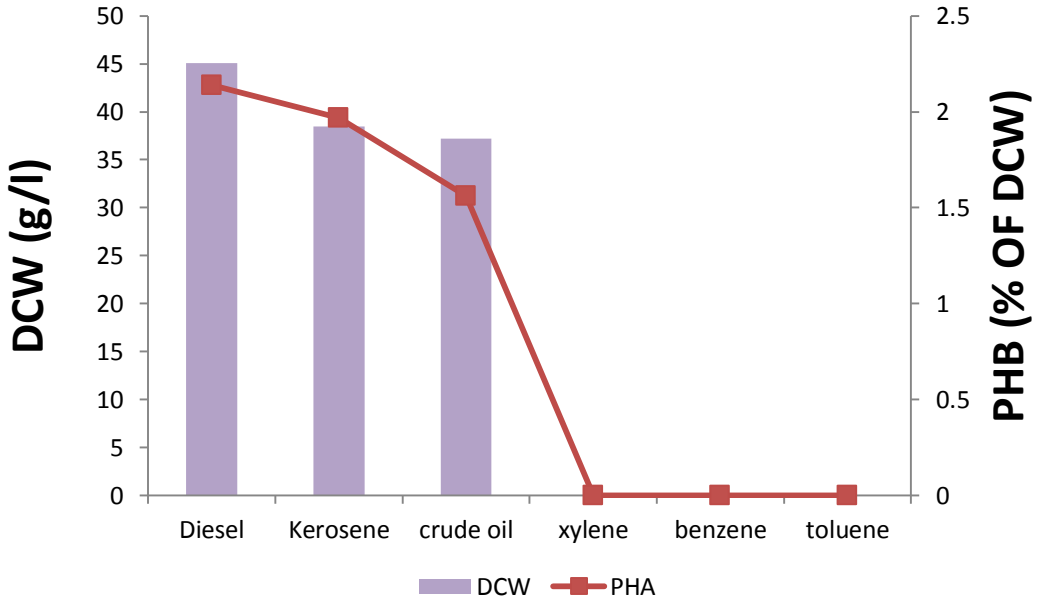


Figure 3.Influence of different carbon hydrocarbons on growth and PHB accumulation of Bacillus species

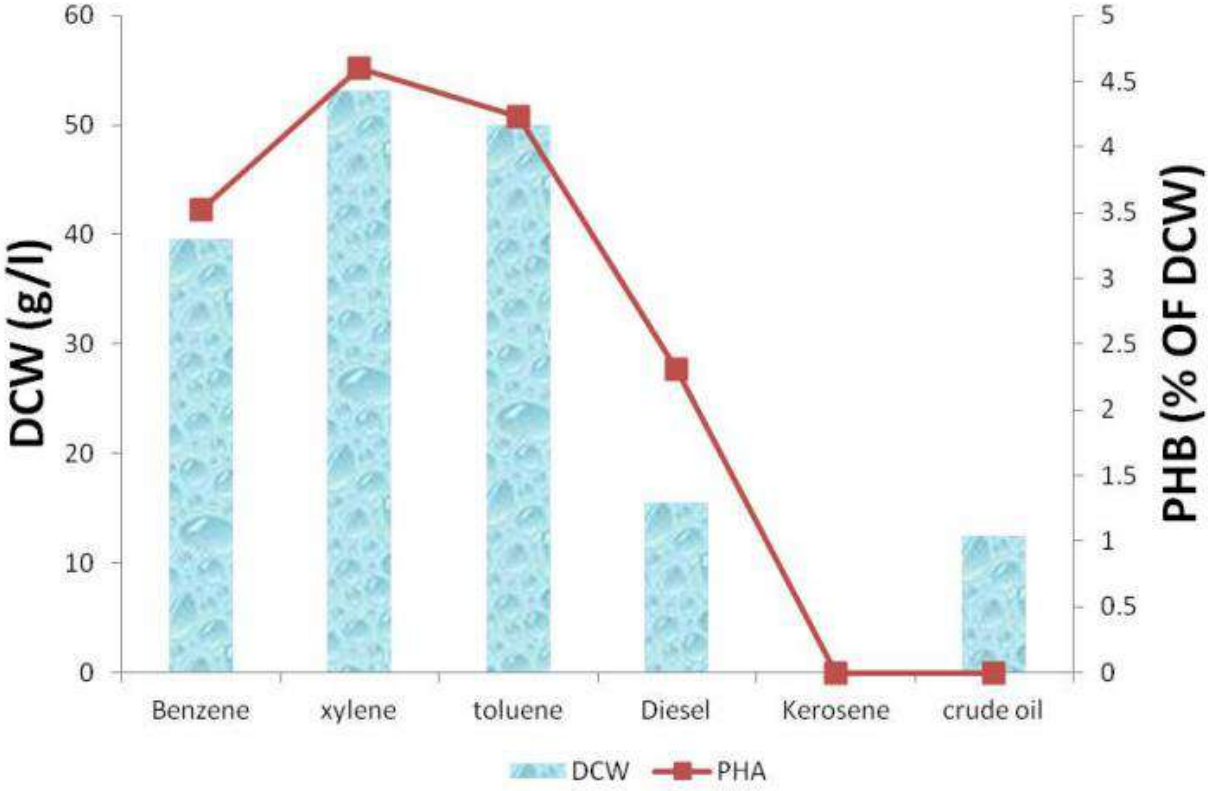


Figure 4.Infrared spectrum of PHB produced by *Pseudomonas species*

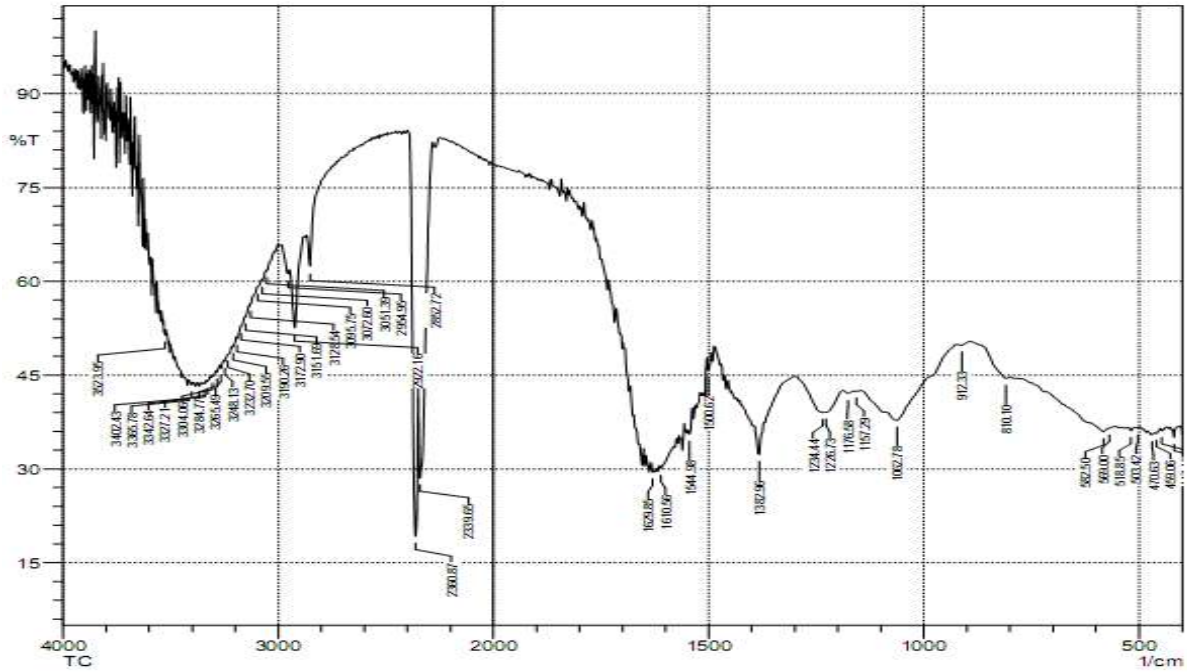


Figure 5. Infrared spectrum of PHB produced by *Bacillus species*

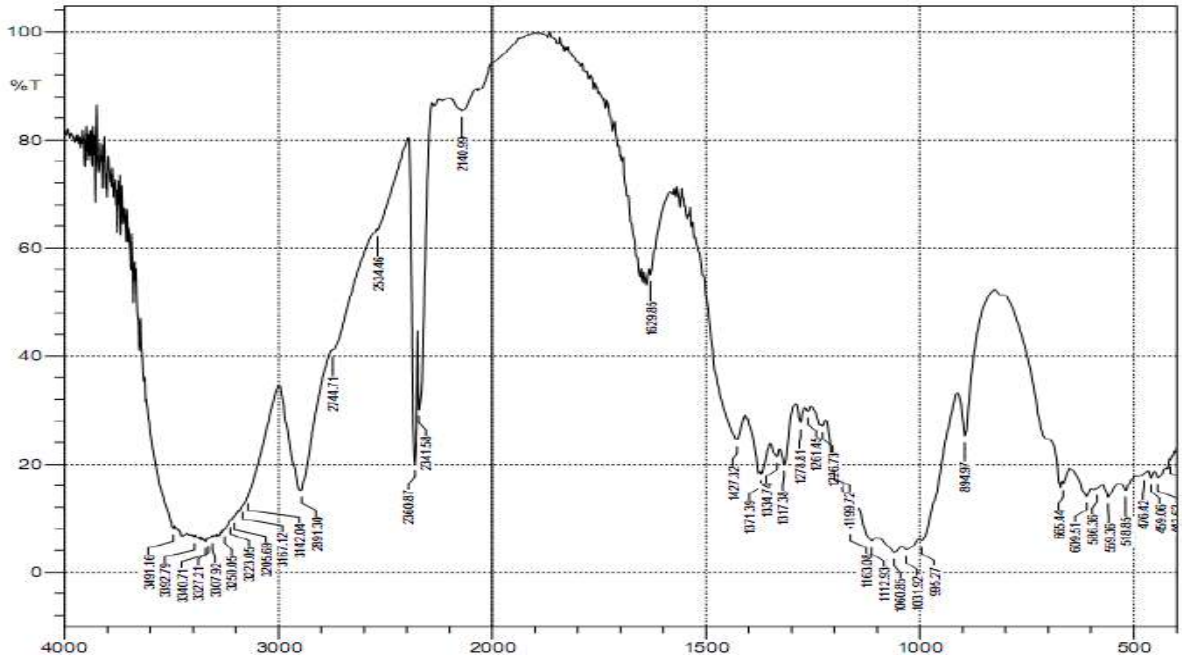


Figure 6. Medium Optimization Studies in *Pseudomonas species*

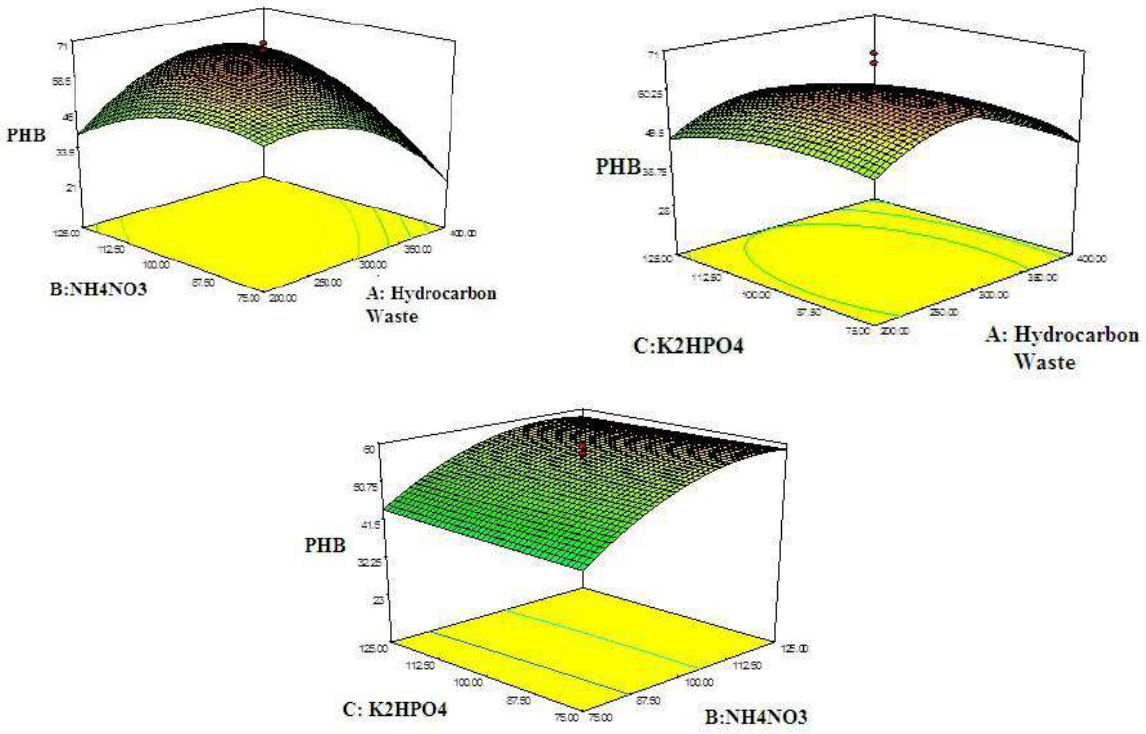
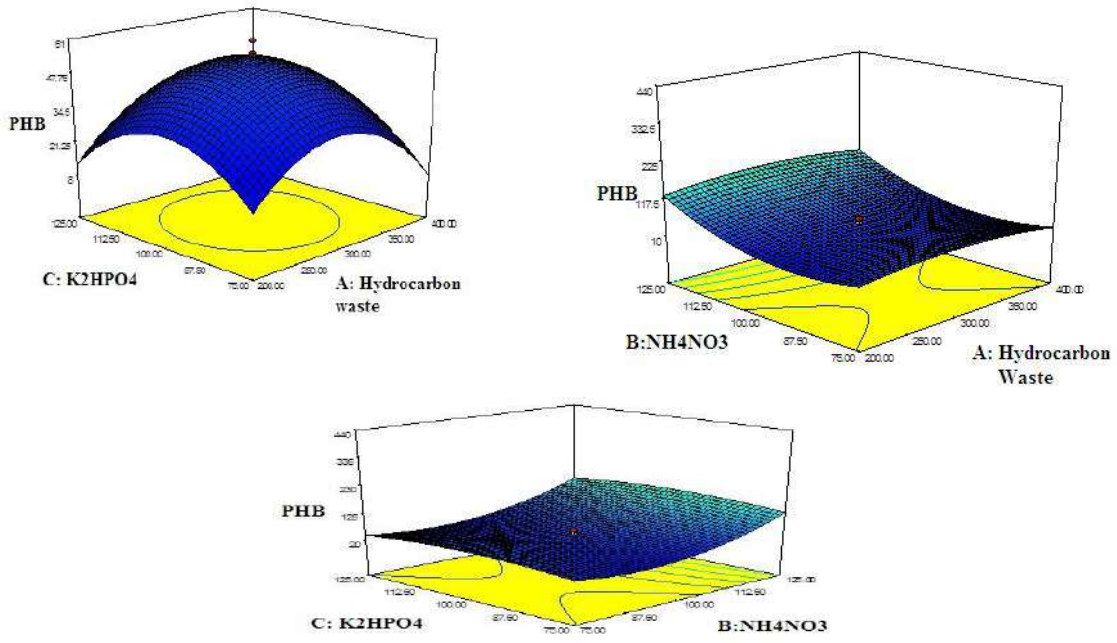


Figure 6. Medium Optimization Studies in *Bacillus species*



Annexure - VII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002
CONSOLIDATED REPORT
PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1.	**TITLE OF THE PROJECT**	**Utilization of Hydrocarbon Wastes for Bioplastic (Polyhydroxyalkanoates) production by Marine Bacteria**
2.	**NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR**	**Dr.M.Karthikeyan** **Department of Zoology and Microbiology** **Thiagarajar College, Madurai**
3.	**NAME AND ADDRESS OF THE INSTITUTION**	**Thiagarajar College** **#139-140, Kamarajar salai** **Teppakulam, Madurai-625009**
4.	**UGC APPROVEL LETTER NO. AND DATE**	**No.F MRP-6777/16(SERO/UGC). dt 30.06.2017**
5.	**DATE OF IMPLEMENTATION**	**July 01, 2017**
6.	**TENURE OF THE PROJECT**	**2 years**
7.	**TOTAL GRANT ALLOCATED**	**Rs.75000.00**
8.	**TOTAL GRANT RECIEVED**	**Rs. 40000.00**
9.	**FINAL EXPENDITURE**	**Rs.32,888.00**
10.	**TITLE OF THE PROJECT**	**Utilization of Hydrocarbon Wastes for Bioplastic (Polyhydroxyalkanoates) production by Marine Bacteria**
11.	**WHEATHER OBJECTIVES WERE ACHIVED**	Yes
12.	**ACHIVEMENTS FROM THE PROJECT** Green approach for the bioconversion a pollutant to biodegradable plastics was attempted. Potent bacterial strains with maximum PHB accumulation were isolated from the marine environment will be helpful for the treatment of oil spills in future.	
13.	**SUMMARY OF THE FINDINGS** This study reveals the occurrence and distribution of bio plastics (polyhydroxyalkanoates) producing bacteria in hydrocarbon contaminated coastal regions of Ennore Oil spill area and Rameshwaram, Gulf of Mannar. Furthermore, it unravels the ability of the bacteria to convert a wide range of hydrocarbons to biodegradable polymers. The bacterial strains was subjected to biochemical and 16s rRNA partial gene sequencing. Tolerance and growth of the isolates with different hydrocarbons were analysed. Hydrocarbon degrading ability of isolates were determined by 2,6 dichlorophenol indophenol assay, emulsification index andcell surface hydrophobicity analysis. Based on the results of the study it is evident that hydrocarbon degrading efficiency of the bacterial strains are unique in nature.	

	PHB accumulation by the isolates was confirmed by Sudan black staining. FTIR and ^{1}H and studies confirm the polymer produced by the isolates to be of poly3-hydroxybutyrate type. Optimization of medium constituents was carried out by response surface methodology (RSM). The large scale industrial production of PHB is impeded by the lack of efficient and cost- effective processes. Cleaner production approaches is not well established in the field of PHB production. Utilization of microorganisms in biotransformation of toxic anthropogenic compounds render bioremediation technologies environmentally benign and ecologically viable. Understanding the physiology of hydrocarbon degrading bacteria with respect to PHB production help to overcome the economic obstacles in commercialization of biopolymers. To the best of our knowledge, this study is one of the few reports on illustrating the potential of marine hydrocarbon degrading bacteria in the utilization and bioconversion of hydrocarbon waste to value added biopolymer.	
14.	**CONTRIBUTION TO THE SOCIETY** Different bacterial strains capable of producing bio plastics using hydrocarbon waste were isolated. The isolated strains will be useful for the treatment of oil spills in marine environment and further the isolates can be useful for the bioconversion of oily industrial wastes to bio plastics.	
15.	**WHEATHER ANY PH.D ENROLLED / PRODUCED OUT OF THE PROJECT**	No
16.	**NO PUBLICATIONS OUT OF THE PROJECT**	One

SIGNATURE OF THE
PRINCIPAL INVESTIGATOR

PRINCIPAL

PRINCIPAL
Thiagarajar College, Madurai.