Cost reduction and study of PHA bioplastic production from *Pseudomonas putida* ATCC 49128 utilizing fruit and vegetable peel waste as carbon substrate.

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Abstract : PHA(Polyhydroxyalkanoate) bioplastic holds the potential to replace the petroleum plastic by virtue of its high elasticity, stress resistance and low brittleness. However, for its large-scale production, cost of production is a major bottleneck of which the carbon substrate alone accounts for 28-50% of the production cost. So, studies are carried out to test the PHA bioplastic production by microbial culture on crude carbon substrates such as whey, starch, oil, lignocellulosic material and sugar wastes. In this study, one such attempt was made to produce PHA bioplastic from *Pseudomonas putida* ATCC 49128 grown on waste carbon substrate such as fruit and vegetable peel. The effect of nutrient deficiency and excess on PHA yield was also studied, wherein it was found that on imparting nutrient deficiency to grown culture medium PHA yield increased and presence of easily utilizable nitrogen source like peptone inhibited the PHA production. By GC-MS analysis, the PHA produced was found to be mcl-PHA (medium chain length-PHA) consisting of Hexadecanoate C-16 monomer and Octadecanoate C-18 monomer constituent which directly corresponded to the waste fruit and vegetable peel substrate fatty acid content. PHA bioplastic exhibit wide range of application also its newly recognized potential to be recycled into novel biofuels is entering a new development phase.

Index Terms **– Polyhydroxyalkanoate, Crude waste substrate, Bioplastic.**

I. INTRODUCTION

Most prevalent environmental problem now is Solid Waste Management and Global Warming. Solid Waste Management of plastic waste is very serious problem as the petroleum based non-biodegradable plastic keep on accumulating in the environment day by day (1). In India plastic waste accounts for 1 to 4% of the total 80,000 metric tons of Municipal Solid Waste generated daily of which only 10% of disposed waste is recycled rest seeps into drainage or is deposited on land affecting both marine and soil environment (2). The recently laid ban on plastic as per section 5 of EPA (Environment Protection Act) further emphasis on the need for generation of biodegradable and environmentally friendly plastic which includes PHA, PLA, aliphatic polyester and other polymer of polysaccharide. Among them PHA is considered to be potent substitute for petroleum plastics in terms of tensile strength, stiffness, brittleness and break limit (3) Polyhydroxyalkanoate (PHA) are polymer of hydroxy fatty acids that are naturally produced by many different bacteria as intracellular carbon and energy reserve material (1). PHA are structurally simple macromolecules synthesized by many gram positive and gram negative bacteria. PHA are accumulated as discrete granules to a level as high as 90% of the cell dry weight. When nutrient supplies are imbalanced, it is advantageous for bacteria to store excess nutrients intracellularly especially as their general fitness is not affected because by polymerizing soluble intermediate into insoluble molecules, the cell does not undergo alteration of its osmotic state and leakage of these valuable compounds out of the cell is prevented(4) Majority of the PHA identified are linear head to tail polyester composed of 3-hydroxy fatty acid monomer in which carboxy group of one monomer forms an ester bond with hydroxyl group of neighbouring group. At C-3 or β position an alkyl group is present which varies in length from methyl to tridecyl (2). 150 monomers with different alkyl chain have been reported with saturated, unsaturated and halogenated side chain(5). According to monomer constituent carbon chain length, PHAs are classified as scl-PHA (short chain length) and mcl-PHA (medium chain length). *Alcaligenes eutrophus*, *Rhodospirillum rubrum* and *Pseudomonas pseudoflava* are known to accumulate co-polyester composed of scl monomer units only, while *Pseudomonas oleovarans, Pseudomonas putida* and other few other *Pseudomonas* strains biosynthesize co-polyester principally composed of mcl monomer units (6). The first PHA to be identified was Poly 3-hydroxybutyrate (PHB) that has been extensively studied but due to brittleness its potential application is limited. PHA however are elastic that increases its ability to replace non-degradable petroleum based plastics (4) While the properties of PHA seems suitable as potential petrochemical plastic replacements, there are still bottlenecks for scaling up microbial production systems. One of the major bottlenecks is the cost of carbon substrates, which have been estimated to be 28–50% of the total production process. There are a number of complex waste streams that can potentially act as carbon substrates for microbial PHA manufacture, such as waste streams from biodiesel production, municipal wastewater, agricultural waste, syngas production and others. Food waste is a prime candidate for an inexpensive carbon source, due to its wide spread availability and the potential to solve significant wastage problems when used to produce PHAs. Food wastage is a global problem and occurs at different stages in food production systems, starting from the harvesting of food to storage, packaging and end of life (7). There are many different systems proposed to convert food waste into PHAs. Use of crude carbon waste substrate would reduce the cost of production considerably. Previous studies were carried out to produce PHA using crude waste substrate. S Y Lee and H Wang used whey for PHA accumulation in recombinant Escherichia coli (1998) (8). Likewise M C Santimano (2009) used Agro Industrial waste substrate to produce PHA from *Bacillus spp* CO1/A6 and was able to show accumulation of PHA upto 60% of Dry Cell Weight (DCW) (9). Palmeri Rosa (2012) demonstrated use of crude glycerol as carbon substrate for PHA production from *Pseudomonas medditerranea* and *Pseudomonas corrugate*. Both

recombinant strain and natural isolates have been found to show active accumulation of PHA. Johnson Katja (2009) was able to demonstrate PHA accumulation up to 90% of DCW in a mixed consortia of cultures using Sequence Batch Reactor (10). The aim of this study was to check whether crude fruit and vegetable peel could be use as carbon substrate by *Pseudomonas putida* ATCC 49128 to produce PHA, as a step towards cost reduction of PHA production.

II. MATERIALS AND METHODOLOGY

Materials

Culture of *Pseudomonas putida* ATCC 49128 was kindly provided by Bhavan's Research Centre, Andheri. 3% Sudan black made in 70% ethanol and counter stain 0.5% Safranin was obtained from Microbiology Department, Bhavans College. Carbon source of Fruit waste and Vegetable waste (peels of mixed fruit and mixed vegetables dried and shredded to obtain fine powder) were taken from household waste. Chemicals used were Crotonic acid, Glycerol, Sodium hypochlorite, Chloroform and Sulphuric acid and minerals for media Preparation. UV –Vis Spectrophotometer (model number = Bio-Era BE/CL/SP/S/03) along with 10mm Quartz Cuvette with cap (Cat No- Bio-Era BI/CL/ACC/09) was used. GC-MS analysis for PHA monomer determination was carried out at SAIF (Sophisticated analytical Instrument Facility) at IIT-Bombay Powai using Model = JEOL AccuTOF GCV with mass range of 10-2000 amu and mass resolution of 6000.

Methodology

2.1 Determination of PHA granule production by the *Pseudomonas putida* ATCC 49128

This was done by inoculating loopful of *Pseudomonas putida* ATCC 49128 colony into sterile Nutrient Broth (NB) containing 1% glycerol incubating flask on shaker condition at 120rpm at Room Temperature (RT) for 24hrs. After incubation period, smear of the spent medium was stained using Burdon's method of lipid granule staining and presence of lipid granules was observed microscopically. On determination of the selected strain to be positive for PHA (lipid granule) production, inoculum preparation was performed in which a loopful of colony was inoculated in 200ml of sterile NB in a 500ml flask and incubated on shaker at 120rpm at RT for 24hrs. After 24 hrs, culture was harvested by centrifugation and the cells were suspended in distilled water, washed two times and then the cells were suspended in saline and this was used as inoculum for further processing.

2.2 Characterization of crude waste substrate for use as nutrient medium for growth of selected culture

Medium used was mineral medium containing Ammonium chloride (0.5g), Sodium dihydrogen orthophosphate (3.3g), Potassium dihydrogen phosphate (2.8g), Magnesium sulphate (0.25g) and 1ml of mineral medium solution in 100ml of medium to this 1% pulverized fruit and vegetable waste was added and then autoclaved at 121ºC at 15 psi for 20 min. In order to check if the culture was able to utilize crude carbon waste substrate, 100ml medium of fruit and vegetable waste each were inoculated with 0.1 ml of cell suspension followed by incubation at RT for 48hrs under shaker condition. After incubation period of 48 hr, 2-3 loopful were used to make a smear on clean grease free slide, the smears were heat fixed and Lipid Granule staining by Burdon's method was performed to detect presence of both cells and PHA granules to check growth on waste substrate medium and lipid accumulation respectively. Separation of cells from coarse media particulate for extraction of PHA from cells by centrifugation was difficult in case of liquid medium so in order to check PHA production from solid medium, solid medium was prepared. For this first infusion medium was prepared by adding 1% pulverized waste carbon substrate into mineral medium followed by vigorous shaking on shaker for 30 min, for infusion of the nutrients from the finely pulverized waste substrate into the mineral medium which was then filtered to remove coarse particle, to this infusion medium was then added 2, 2.5 and 3% agar as solidifying agent. The waste carbon substrate containing solid medium thus prepared were autoclaved at 121ºC at 15 psi for 20 min. The molten mediums were poured onto sterile petri-plate and allowed to solidify, inoculated with culture suspension using sterile swab and incubated at RT for 24 hrs. Post incubation period the growth obtained on solid media was scrapped out and dried in oven to get dry cell mass.

2.3 Study of effect of Nutrient Stress on PHA production

For this study first 400ml liquid mineral medium containing 1% Fruit/Vegetable waste was prepared as described in 2.2(Flask 2) and inoculated with 0.4 ml of cell suspension, incubated for 48hrs at RT under shaker condition, after which 200ml aliquot was removed from flask 2 and added into a sterile empty 250ml flask (Flask 1). To the remaining 200ml medium in Flask 2, 200ml of St DW was added under sterile condition and this diluted medium in Flask 2 was then further incubated for 48hrs under similar incubation condition as the medium is diluted 1:2 times it is expected to impart nutrient stress. Flask 2 containing diluted medium is referred to as 2nd aliquot and Flask 1 containing undiluted medium is referred to as 1st aliquot. Grown culture medium is diluted, rather than using separated diluted and undiluted medium and inoculating them separately in order to suspend the same cells grown in nutrient rich condition into subsequent nutrient deficient condition. 200ml of both 1st and 2nd aliquots were centrifuged to obtain pellet from each 1st and 2nd aliquots containing both cell biomass as well as coarse medium particle and were dried in oven for 24hrs.

2.3.1 Extraction of PHA using Sodium Hypochlorite

The oven dried pellet from 1st aliquot(from undiluted medium imparting nutrient sufficient condition)and 2nd aliquot(from diluted medium imparting nutrient deficient condition) each were treated with Sodium Hypochlorite such that the concentration of pellet was 4% in the Sodium Hypochlorite Solvent in a 100ml clean dried flask. The flask was incubated at RT under shaker condition for 60min for the extraction process and then the content was spin down and the pellet was washed with DW and then ethanol, the residue remaining after last washing with ethanol was collected in watch glass and dried in oven for 24hrs, after which the dried residue was added into Hot Chloroform for dissolution of PHA into chloroform. The chloroform extracts were filtered using filter paper to removed non-dissolved impurities.

2.3.2 Crotonic Acid Assay for Quantification of PHA

20µl of chloroform extract from cells grown on both fruit and vegetable medium under Nutrient Sufficient (1st aliquot) and Nutrient Stress (2nd aliquot) were taken in a dry S-line to which 4.5ml of concentrated Sulfuric Acid was added and the content in the tubes were kept under BWB for 10 min. The content of tubes was then cooled and their absorbance were recorded at 230nm. For Standard Curve 100µg/ml of Crotonic Acid was used as stock and diluted with DW to make standard concentration range of 10,20,30,40 µg/ml. Absorbance of these standard concentrations was recorded at 230nm using UV Vis Spectrophotometer to obtain standard plot which was used for extrapolating the values of test.

2.3.3 Protein Assay of Cell biomass

Before the process of extraction 10ml of grown culture medium were removed from both 1st and 2nd aliquot for determination of the biomass protein content as in this case cell dry weight could not be taken into consideration due to presence of insoluble coarse medium constituent in the pellet. The samples were centrifuged at 1000rpm for 5min to obtain pellet, the pellet contained both cells and medium coarse matter, to the pellet was added 2ml DW to obtain a cell suspension and this pellet was subjected to Robinson and Hodgson method.(To 2ml cell suspension, 1ml of NaOH was added mixed and kept under BWB for 5 min and cooled, to these tubes was then added 1ml of 2.5% $CuSO_4$, incubated 5min and centrifuged at 700rpm for 5 min) as the aliquot taken for cell biomass protein content estimation also contained coarse medium particle so controls of each diluted (1st aliquot) and undiluted(2nd aliquot) medium were made similar to the preparation of medium and this un-inoculated mediums were used as Control. By subtraction of absorbance of control from the test, the color development due to presence of coarse medium constituents could be eliminated as both control and test are expected to contains similar waste substrate composition and concentration. 5mg/ml Bovine Serum Albumin (BSA) was used as Stock to make standard Concentration of 1,2,3,4 and 5mg/ml of BSA using DW as diluent to obtain standard plot that was used for extrapolating values of test samples.

PHA Yield was calculated as

$$\text{PHA Yield (\%)} = \frac{\text{PHA content as per Crotonic acid assay(mg/ml)}}{\text{Protein Content of cell Biomass as per Robinson and Hodgen method(mg/ml)}}$$

2.4 Study of Effect of Nutrient Excess on PHA Production

400 ml medium containing 3% Fruit and 3% Vegetable waste respectively were prepared and inoculated with 1ml of cell suspension and incubated at RT for 48hrs under shaker condition. After PHA extraction as per 2.3.1, the PHA content by Crotonic acid assay and the protein content of cell biomass were determined as per 2.3.2 and 2.3.3 respectively to get the PHA yield under 1% and 3% carbon substrate concentration in the medium.

2.5 Determination of monomer composition of PHA produced by *Pseudomonas putida* ATCC 49128 on Fruit and Vegetable Waste Substrate

1600ml of both solid and liquid mediums containing different medium composition were prepared as shown in **Table 1** and inoculated with 0.1% cell suspension and incubated at RT for 48 hrs. Liquid medium were kept under shaker condition. NB + 1% Glycerol was kept as a nutrient rich control. Fruit/vegetable waste with 1% glycerol and 1% peptone were set up to check the effect of readily utilizable organic nitrogen source on PHA production (On addition of peptone the medium was solidifying at 2.5% agar concentration hence solid substrate fermentation was carried out). Fruit/ Vegetable waste with 1% glycerol were set up in order to check if glycerol imparted any changes in the PHA monomer composition. After incubation period, medium was centrifuged and pellet was collected and subjected to Sodium Hypochlorite Extraction process as described in 2.3.1 and the chloroform extract was solvent casted on petri-dish and 100µl of it was used for GC-MS analysis to determine the monomer composition obtained under different medium composition. For analysis using GC-MS it is important to first convert PHA into its volatile methyl ester derivative. This derivatization was done by subjecting 1ml of chloroform extract with 0.5ml methanol and 1ml H_2SO_4 and heating the mixture in a screw capped bottle for 1 hr in a boiling water bath.

Sr No.	Fermentation Mode	Medium composition
1.	Solid Substrate Fermentation	NA+ 1% Glycerol
		Fruit waste infusion+ 1%Glycerol +1% Peptone
		Vegetables Waste Infusion +1%Glycerol +1%Peptone
2.	Liquid Submerged Fermentation	Fruit Waste
		Vegetable waste
		Fruit Waste + 1% Glycerol
		Vegetable Waste + 1% Glycerol

Table1. Medium composition of different medium used for mass production

III. RESULTS AND DISCUSSION

3.1 Determination of PHA granule production by *Pseudomonas putida* ATCC 49128

Culture grown on NB supplemented with 1% glycerol (lipid rich medium) was stained using Burdon's method. Microscopic observation for a possible accumulation of intracellular PHA by *Pseudomonas putida* ATCC 49128 was confirmed by staining as done by Gumel *etal* 2012(11), it was found that when culture was grown in nutrient broth supplemented with 1% glycerol the culture showed accumulation of intracellular granules visible as dark purple to black colored granules with light pink colored cytoplasm. In this study, standard culture has been used however, isolation of PHA producer from different lipid rich environmental samples is beneficial as few environmental samples happen to show robust PHA producers for example *Pseudomonas putida* Bet001 isolate from palm mill effluent went used by Gumel *etal* (11) it was found to accumulate 49.7 to 68.9 % PHA per DCW(dry cell weight).

3.2 Characterization of crude waste substrate for use as nutrient medium for growth of selected culture

To assess the ability of the culture to grow on these crude substrates and exhibit any PHA granule accumulation, 0.1ml of culture suspension was inoculated in 100ml of these mediums and incubated under shaker condition at RT for 24hrs after which Sudan Black Staining was performed to check growth and PHA granule production. Confluent growth was observed with intracellular accumulation of PHA granules visible under microscope as dark purplish black granules with light pink cytoplasm. In order to extract this PHA from cells, the cells had to be separated from the medium which in case of liquid medium containing fruit and vegetable waste coarse particle was difficult. Similar problem was reported by S Y Lee and HH Wang (8) when working on PHA production using whey substrate and by M C Santimano while using low cost Agro-Industrial waste as substrate (9) So an attempt was made to use Solid Substrate by adding solidifying agent (agar) into an infusion form of the medium. Different concentration of agar was added in the infused medium to determine the least concentration of agar needed for solidification of the infused mineral mediums, it was found that 2% and 2.5% agar was not sufficient for proper solidification of the nutrient infused mineral medium, only 3% agar was able to solidify the mineral mediums. 3% agar addition is also not acceptable keeping the primary aim of Cost Reduction in mind. Also the growth on Solid Substrate was not confluent, even after an incubation period of 48 hrs transparent, scanty growth was observed giving a very low output of dry cell mass. So the rest studies were carried out in liquid medium. When *Bacillus spp* CO1/A6 was used for PHA accumulation on similar Agro-Industrial Waste like citrus pulp and cane molasses it was able to accumulate 47% to 60% PHA per DCW (9). Different other substrate like whey and crude glycerol have been tested for potential use as waste carbon substrate for PHA production and have found to be successful in accumulation of 50 to 60 % PHA per DCW(8), whichever medium is used it is first important to assess if the producing culture is actually able to thrive on such crude waste substrate and accumulate PHA to a considerable level.

3.3 Study of Effect of Nutrient Stress on PHA production

To Study the effect of nutrient stress on PHA production the PHA yield was found out under Nutrient suffice and Nutrient stress condition and the values obtained were compared to check any effect of nutrient stress on PHA production. The results obtained are shown in **Table 2**.

Substrate	Aliquot Number(diluted/undiluted medium)	Absorbance at 230nm via Crotonic acid assay	PHA (µg/ml)	PHA (mg/ml) (A)	Biomass Protein content (mg/ml) (B)	PHA yield (%) (A/B*100)
Fruit waste	1st Aliquot (Undiluted medium)	0.234	292	0.292	2.91	10.03
	2nd Aliquot (1:2 Diluted medium) (Nutrient Stress)	0.606	756	0.756	1.52	49.73
Vegetable waste	1st Aliquot (Undiluted medium)	0.271	338	0.338	3.56	9.49
	2nd Aliquot (1:2 Diluted medium) (Nutrient Stress)	0.604	754	0.754	1.67	45.14

Table 2. Study of Effect of Nutrient stress on PHA production

The PHA yield by *Pseudomonas putid*a ATCC 49128 on supplementation of 1% carbon substrate came out to be 10% which is normally shown by *Pseudomonas putida* as seen in Huisman *etal* 1989 (13). It was seen that the PHA production was increased considerably on imparting Nutrient Stress from roughly 10% to 50% thus goes well along with the generalization that PHA accumulates intracellularly under condition of Nutrient Stress (2,5). It is also observed that among the two waste substrates the growth was more confluent in case of Vegetable substrate medium but the PHA yield per biomass protein content was higher in case of fruit waste substrate medium.

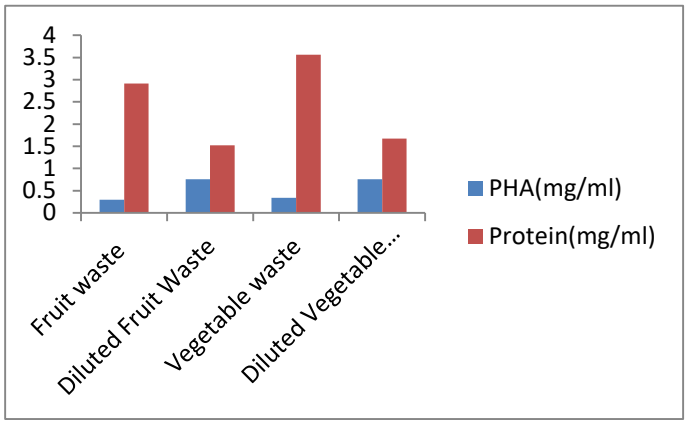
3.4 Study of Effect of Nutrient Excess on PHA Production

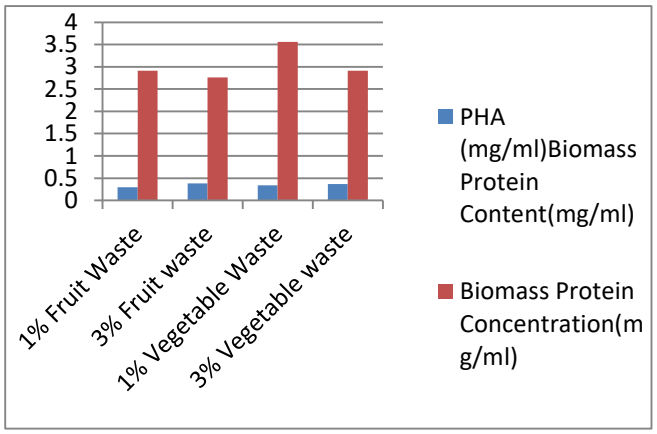
To study the effect of nutrient excess on PHA production, the PHA yield was found out under Nutrient suffice and Nutrient Excess condition and the values obtained were compared to check any effect of nutrient Excess on PHA production. The results obtained are shown in **Table 3**

Substrate	Substrate Concentration	Absorbance at 230nm via Crotonic acid assay	PHA(ug/ml)	PHA(mg/ml) (A)	Biomass Protein Content (mg/ml) (B)	PHA yield (%) (A/B*100)
Fruit Waste	1%	0.234	292	0.292	2.91	10.03
	3%	0.308	384	0.384	2.76	13.91
Vegetable Waste	1%	0.271	338	0.338	3.56	9.49
	3%	0.297	370	0.370	2.91	12.71

Table 3. Study of Nutrient Excess on PHA production

It was seen that increase in the concentration of carbon substrate increased the PHA yield, although the increase was not that significant as seen in case of Nutrient Deficiency, similar to the results obtained by Shrivastava (14) and Rodrigo Yoji (15). According to the PHA yield obtained under Nutrient Stress and Nutrient Excess condition as shown in **Table 1 and Table 2**, a





plot comparing these results is shown in **Fig 1.**

Figure 1. Comparison of PHA yield with biomass protein content in both nutrient excess and deficient Carbon Substrate

3.5 Determination of monomer composition of PHA produced by *Pseudomonas putida* ATCC 49128 on different medium composition and biopolymer extracted on mass production

The chloroform extract was subjected to derivatization followed by subsequence GC-MS analysis, results of the same are shown in **Table 4.** The fraction casted on petri-dish are shown in **Fig 2.** In case of peptone supplementation no PHA polymer was obtained after casting as can be seen in **Fig 2.**

Sr No	Supplements in the mineral medium	Relevant Peaks	Retention Time	Synonyms (methyl- ester of)
1.	NA+Glycerol	Hexadecanoic acid methylester	24.01	Palmitic acid
		Octadecanoic acid methylester	26.97	Stearic acid
		Docosanoic acid methylester	31.28	Behenic acid
2	Fruit Waste	Hexadecanoic acid methyl ester	23.77	Palmitic acid
		9-Octadecenoic acid methyl ester	26.56	Oleic acid
		Octadecanoic acid methyl ester	26.91	Stearic acid
3.	Vegetable Waste	Hexadecanoic acid methyl ester	25.93	Palmitic acid
		9-Octadecenoic acid methyl ester	29.78	Oleic acid
		Octadecanoic acid methyl ester	30.28	Stearic acid
4.	Fruit waste+Glycerol	Hexadecanoic acid methyl ester	25.92	Palmitic acid
		9-Octadecanoic acid methyl ester	29.88	Oleic acid
5.	Vegetable waste+Glycerol	Hexadecanoic acid methyl ester	26.04	Palmitic acid

Table 4. Monomer constituent of PHA extracted from cells grown on different medium composition.

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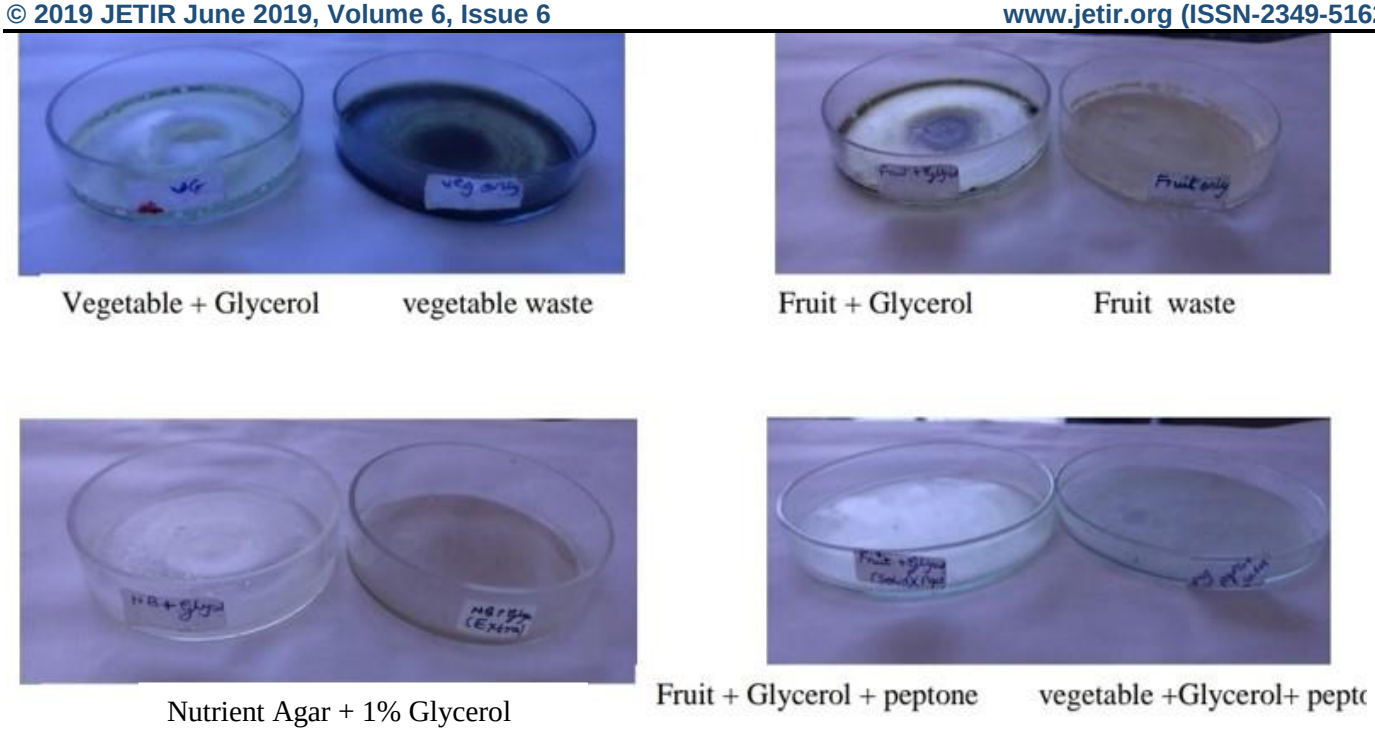

Figure 2. PHA polymer obtained after solvent casting chloroform extract on petri-dish.

By the composition of the mcl-PHA produced by *Pseudomonas putida* ATCC 49128 it is found that *Pseudomonas putida* exhibits presence of group two PHA synthase enzyme. As the mixture of different fruit peel and different vegetable peel wastes were used as carbon substrate, exact relationship between substrates fatty acid composition and PHA monomer composition cannot be drawn. However, the monomer composition in both fruit and vegetable waste as per GC-MS analysis were found to be hexadecanoic acid methyl ester (derivative of Palmitic acid) octadecanoic acid methyl ester (derivative of Oleic acid) which corresponds to the prominent fatty acid constituent present in fruit and vegetable waste-Palmitic acid and Oleic acid (16). Thus establishing a direct relationship between chemical composition of growth medium and monomer composition of mcl-PHA as described in various reviews (4)

IV. CONCLUSION

Plastic Waste Management and Pollution related to non-biodegradable petroleum based plastic is a threat to our environment. Both utilization of fossil fuel for petroleum plastic production and potential harm of these petroleum plastic to environment by large scale deposition in nature has driven the need to replace these non-biodegradable petroleum-based plastics with bio-degradable plastics that could be prepared from renewable resources. So hereby an attempt was made to assess PHA bioplastic production from *Pseudomonas putida* ATCC 49128 using fruit and vegetable waste as crude carbon substrate in production medium. The use of agrochemical waste for PHA production has been assessed but not by using this strain of *Pseudomonas putida* before. In this study, a standard culture was used keeping in mind the primary aim of cost reduction rather than isolation of PHA producing organism. However, it is found that isolation PHA producer from environmental sample is beneficial as they are known to exhibit robust production of PHA. Food waste was used as carbon substrate as high amount of food waste is generated in food industry starting from the initial step of harvesting to last step of packaging, thus proving food waste to be a superior option as compared to whey or crude glycerol or any waste substrate which has some or the other application yet to its credit. Also, other waste materials are not produced in such high amount as like food waste and the presence of readily utilizable carbohydrate constituent makes fruit and vegetable waste substrate readily utilizable by a number of different bacteria. The growth condition prevailing during the PHA production process in the bioreactor are extremely important as these conditions have been manipulated to obtain PHA production as high as 90% of the cell dry weight in mixed culture production. In this study, it was found that subjecting culture to nutrient stress was able to induce PHA production increasing it from 10% to 45% while on other hand presence of high amount of organic nitrogen component in production medium inhibited PHA production. The repeated monomer units in PHA are important to be characterized as these monomer constituents determine the physical attribute of the bioplastic produced. In this study, repeating units of biopolymer reflected the fatty acid profile of substrate provided for biosynthesis. Thus a study on PHA production by *Pseudomonas putida* ATCC 49128 using crude fruit and vegetable waste substrate was performed, further experimentation to study the effect of various growth condition such as pH, temperature, dissolved oxygen, etc and by using same fruit and vegetable waste substrate or any other waste substrate can be performed in order to determine the best suited PHA production setup, along with determination of more cost efficient extraction method must be performed in order to make production of this PHA bioplastic production on industrial scale feasible.

V. ACKNOWLEDGMENT

We would like to acknowledge all the members of Department of Microbiology Bharatiya Vidya Bhavan's College for their co-operation.

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