# Strategical Approaches for Quantification and Optimization for Polyhydroxybutyrate Production from Bacterial Strains of Salty Water Body Origin from South west of Lagos State, Nigeria.

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**Abstract**
The current research aimed at analyzing quantification and optimization of polyhydroxyalkanoate (PHA) in bioplastic production for controlling environmental pollutions course by petroleum derived plastics. The water and soil sample were used in order to isolate the PHB producing organisms, the medium used is modified mineral salt broth for the culturing and production of PHB produce organisms. Some amount of salt (NaCl) in different concentration is added to the medium during preparation since PHB producing organisms grow only in the present of salt. Bacterial growth and bar chart graphs were also plotted. The isolated organisms from these samples are gram positive and gram negative bacilli. The quantification analysis of these bacterial strains at different carbon sources showed that the highest PHB yield percentage is occurred in the medium containing lactose as source of carbon. The best temperature for the growing of these bacterial strains is 37$^{o}$C. PHB recovery and extraction of these bacterial strains was carried out using chloroform extraction method. Fourier transform infrared (FTIR) and thin layer chromatography (TLC) analysis of the extracted PHB was also performed. Biochemical analysis was done for the tentative identification of these bacterial strains. Bio-plastics are bio-based, biodegradable plastics with nearly similar properties to synthetic plastics. So bio-plastics are used in assisting the world to reduce the escalating harms of litter mostly in the rivers, sand and seas.
**Keywords:** Environment, Pollution, Petroleum, Bio-plastic, Biodegradability.

## INTRODUCTION

Utilization of synthetic rubber within the environment causes a lot of negative effects to the entire world. To have pleasant substitute of synthetic plastics, scientists discovered polymers from bacterial origin. Numerous microbial organisms are able decompose these polymers [1]. These biopolymers have advantage against synthetic polymers they are not artificial and they can be decompose easily within the environment. The biopolymer in microorganism was discovered in the year 1920s, by Lemoigne and he announced the presence PHB within bacteria [2,3]. Plastics generated from PHB can be applied in every aspect. More than hundred PHAs were recognized from diverse group of microorganisms [4]. Until 1980s, scientists do not have chance to investigate better solution for synthetic plastics in order to reduce its effects. Early 80s, Anthony Sinskey together with his mates discovered enzyme 'thiolase' this enzyme play major important role in the production of biopolymers. Rights appliances of biopolymers was prepared in 1987 and lastly get acknowledged in1993 [4].

PHAs are members of biopolymers isolated from diversity of Gram-positive and Gram-negative bacteria. Over 300 species, mostly bacteria are reported to generate these polymers [5]. These bacteria can gather biopolymers in their inner cell (cytoplasm) as an intracellular carbon and energy reserve. Bacteria that are used for the manufacturing of PHAs can be devided into two groups based on the stress conditions required for PHA synthesis. The first division of bacteria

requires the restriction of an important nutrient such as nitrogen, phosphorous, magnesium or sulphur with carbon source in abundance. The bacteria falling in this class are *A. eutrophus*, *Protomonas extorquens* and *Protomonas oleovorans*. The second division of this bacteria, are *Alcaligenes latus*, a mutant strain of *Azotobacter vinelandii* and recombinant *E. coli* do not need nutrient restriction for the synthesis of PHAs.

Biopolymers are essential substitute to petroleum derived polymers because of their ability to degrade easily, and have simple production procedure they can also be used in a variety of areas such as health and agriculture. And also produce via bio-refineries as a division of biotechnology. Bioplastics of microbial origin are formed from diverse group of bacteria. Majority of bioplastics have the ability to decay in the environment with no serious health problem. All agreed with bioplastic from microbial source have the ability for storage of objects [6]. In any problems, bacteria decayed biopolymers [7]. Biopolymers are degradable tools that come from reusable source to decrease difficulty in disposing of plastic which overpowering the universe by polluting our environment. Biopolymers or nutural plastics are form of plastics from biomass origins, like corn, starch or microbiota, they are pleasant than synthetic plastics which are derived from petroleum. Biodegradation is a procedure by which bacteria within environment modify substance into natural form like water and $CO_2$ etc. Genera of bacteria like *Beneckea* and *Vibrio* is reported as the number one organism to produce PHB extracted in the sediments of marine [8]. Numerous types of bacteria, like, *Pseudomonas* spp. *megaterium, Ralstonia eutropha* score lots of attraction by scientists and these group of bacteria is extensively used because of their ability to produce PHB. Production of PHB by *Bacillus megaterium* is 84% under anaerobic conditions [9]. However various group of bacteria such as *Actinobacillus, Azotobacter, Agrobacterium, Rhodobacter* and *Sphaerotilius* gained more attraction due to thire ability of changing organic waste such as wheat straw and rice husks to PHA. For industrial production of PHB, many haloarchaeal species such as *Haloterrigena, Halococcus, Haloquadratum, Haloferax, Haloarcula, Halobacterium, Halorubrum, Natronococcus Natronobacterium,* and *Natrialba* are considered as PHB production.

## METHODOLOGY

### SAMPLE COLLECTION

Water and soil sample were collected from saline Atlantic ocean in South west of Lagos State, Nigeria. The sample was stored in sterile bottles and polythene bag respectively. The sample was transported to laboratory in air tight bags for further isolation and analysis.

### ISOLATION PROCEDURE

Isolation was carried out on the method used by Okwuobi and Ogunjobi 2013 [10]. The sample was serially diluted with sterile saline water. Following serial dilution, nutrient agar was prepared at different salt concentration (NaCl) of 1 molar, 2 molar, 3 molar and 4 molar respectively. Spread plate method was used for isolation of halophiles. Growth of the bacterial colony was observed after 24 hours of incubation period.

### MORPHOLOGICAL CHARACTERIZATION

Morphology was identification for growing colonies from pure culture plates by using simple and gram staining technique.

### SCREENING OF PHB PRODUCING ISOLATES BY SUDAN BLACK B STAINING:

Thin smear was prepared on microscope glass slide and heat fixed it. Staining with Sudan black B solution of 0.3% was utilized and was applied on the slide. Further, slide was allowed for twenty minutes, followed by rinsing with sterile water then counter stain with safranin for 10 seconds. Again rinsing with distilled water and air dried the slide at room temperature. The slides were viewed at 100X magnification for PHB granules analysis [11, 12]

**PHB OPTIMIZATION FOR CARBON SOURCE**

The screened isolated bacteria was grown in 100 ml conical flasks containing 50 ml modified mineral salt medium of different concentrations with 0.5g of glucose in each flask. These flasks were inoculated with bacterial strains isolated from soil and water sample and incubated at different temperature of 25$^{o}$C, 30$^{o}$C and 37$^{o}$C. Further, spectrophotometer analysis was done for observing the growth curve analysis for the determination of stationary phase for maximum biomass production after every 12 hours interval for 7 days. After taking reading by spectrophotometer, bacterial growth curve graphs were plotted against time. Best grown temperature for PHB was chosen among the above mentioned temperature and high biomass growth was noticed for PHB production.

**PHB PRODUCTION**

Production of PHB was studied by using modified mineral salt medium (nutrient broth) with appropriate carbon sources i.e glucose, lactose followed with sucrose. Optimization at best carbon source was done by performing the experiment with different carbon sources. Three flasks were inoculated with colonies isolated from the soil sample and the remaining three flasks also inoculated with water sample colonies and incubated at 37$^{o}$C in orbital shaker for 48 hours.

**CELL DRY WEIGHT MEASUREMENT AND PHB PRODUCTION ANALYSIS**

After 48 hours incubation period, culture was transferred into six centrifuge test tube and then centrifuged at 3000 rpm for 30 minutes. At the end of centrifugation, the supernatant was discarded and the pellet was dried at 80$^{o}$C in the hot air oven. The total cell weight concentrations were determined by weighing the total cell dry weight which were dried at 80$^{o}$C in the hot air oven to a constant mass and then cooled down at room temperature. The drying process was continued tilled constant mass is obtained.

**PHB RECOVERY AND EXTRACTION**

The content of PHB from each dried cell was determined by adding 5ml of 4% sodium hypochloride solution in each tube and incubated the tubes at 37$^{o}$C for sixty minutes followed by centrifugation at 12,000 rpm for ten minutes in order to break the cell wall of the bacteria and also to obtain supernatant. The supernatant were transferred into six different beakers for the extraction of PHB by adding 5 ml of 96% ethanol and 5 ml of 96% acetone in each beaker. 10 ml of chloroform was also added to the mixture and the beakers and then placed inside the water bath at 60$^{o}$C. The mass of the dried chloroform extract was the one to be determined. The extracted PHB was taken for Fourier transform infrared (FTIR) analysis.

$$\text{PHB Yield \%} = \frac{\text{PHB g/l}}{\text{Cell dry weight g/l}} \times 100$$

**FOURIER TRANSFORM INFRARED (FTIR)**

The extracted polyhydroxybutyrate was analyzed by FTIR in order to know their chemical morphology and different functional groups. Extracted PHB powder was dissolved in methanol and pellets of PHB was prepared by mixing it with bromide and then taken to another laboratory for FTIR analysis.

**THIN LAYER CHROMATOGRAPHY (TLC).**

TLC is carried out for the extracted PHB polymer and purified PHB procured from Sigma Aldrich in order to know the individual chemical substance. Both extracted PHB and commercially PHB served as control were dissolved in chloroform, while the solvent was the combination of chloroform and methanol in the ratio of 1:1. Retention factor was calculated by this formula below.

Rf = distance move by substance
distance move by solvent

## RESULT AND DISCUSSION

Even though some previous research work have shown that many Gram positive and Gram negative bacteria synthesized Polyhydroxybutyrate, a lot of researchers put more emphasis on the recognition what strains have the ability to generate enough quantity of PHB by using low-priced minerals resources. The work focused on the soil and water collected from the ocean of south west region of Nigeria for isolating PHB using modified mineral salt (nutrient agar) medium with different carbon of sources. Sudan Black B screening for PHB production revealed that isolated colonies are PHB positive.

## MORPHOLOGY CHARACTERIZATION AND SUDAN BLACK SCREENING OF PHB

The structure of the organisms isolated from water sample appeared as dark blue colored cell wall, indicated that they were gram positive bacteria while that from soil sample plates appeared to be pink in colour, which indicated they were gram negative. All the organisms from both the sources were rod shape bacilli. Following sudan black staining the organism showed dark blue colored granular structures inside the membrane were positive for PHB production.

**TABLE 1: Morphology and Sudan Black B Screening of isolates**

| S/N | Samples | Molarity | Shape | Gram stain | Sudan stain |
|---|---|---|---|---|---|
| 1 | H1 water | 1 molar | Rod | Gram positive | Blue violet |
| 2 | H2 water | 2 molar | Rod | Gram positive | Blue violet |
| 3 | H1 soil | 1 molar | Rod | Gram negative | Blue violet |
| 4 | H2 soil | 2 molar | Rod | Gram negative | Blue violet |
| 5 | H3 soil | 3 molar | Rod | Gram negative | Blue violet |

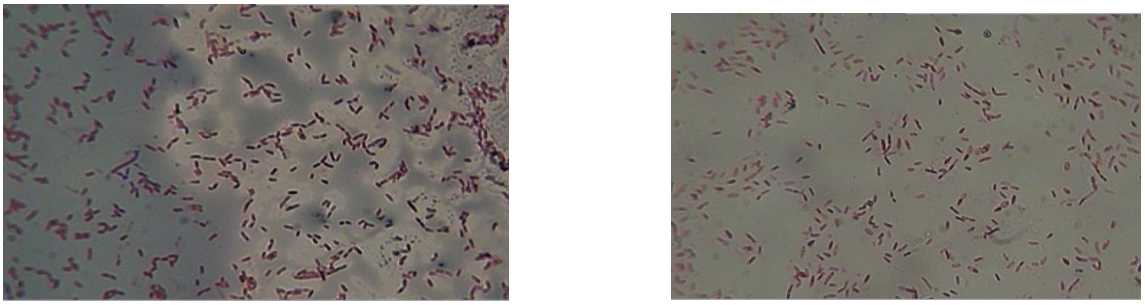


**Figure 1: Positive strains for PHB production by Sudan Black B**

**PRODUCTION OF PHB**

Production of PHB was studied by using modified mineral salt medium (nutrient broth) with appropriate carbon source such as glucose, lactose and sucrose viz. Reading of PHB optimization were taken with spectrophotometer at 600 nm for seven days at an interval of twelve hours.

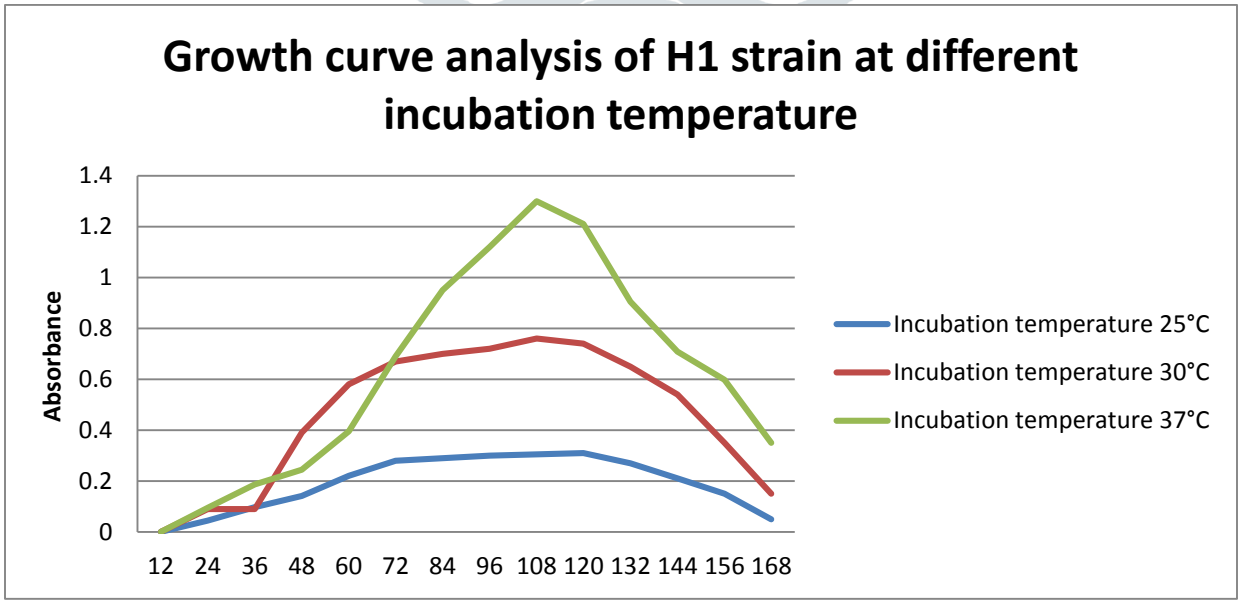
**OPTIMIZATION PARAMETERS AT DIFFERENT TEMPERATURE**

Optimization process was carried out at different temperatures. The different temperature used to grow the bacteria was 25$^{o}$C, 30$^{o}$C and 37$^{o}$C respectively. Best growth temperature for PHB was chosen among the above mentioned temperature.

**TEMPERATURE OPTIMIZATION FOR BACTERIAL STRAINS H1 SOIL**

The H1 soil bacteria strain were grown at three different temperatures. H1 soil bacteria strain reached the stationary phase at the period of 120 hours with optimal density of 0.310 nm at a temperature of 25$^{o}$C. H1 soil bacteria strain reached the stationary phase at the period of 96 hours with optimal density of 0.740 nm at a temperature of 30$^{o}$C. At a temperature of 37$^{o}$C, the bacteria strain has the maximum growth rate at 108 hrs and optical density of 1.299 nm. The best temperature observed for the maximum cell mass density was at 37$^{o}$C, as for the successful PHB production, the first objective is to get maximum cell weight, because maximum growth leads to large amount of PHB extraction from bacterial cells. During this optimization the medium used was modified with 1 molar salt (NaCl2) concentration (MSM broth medium).

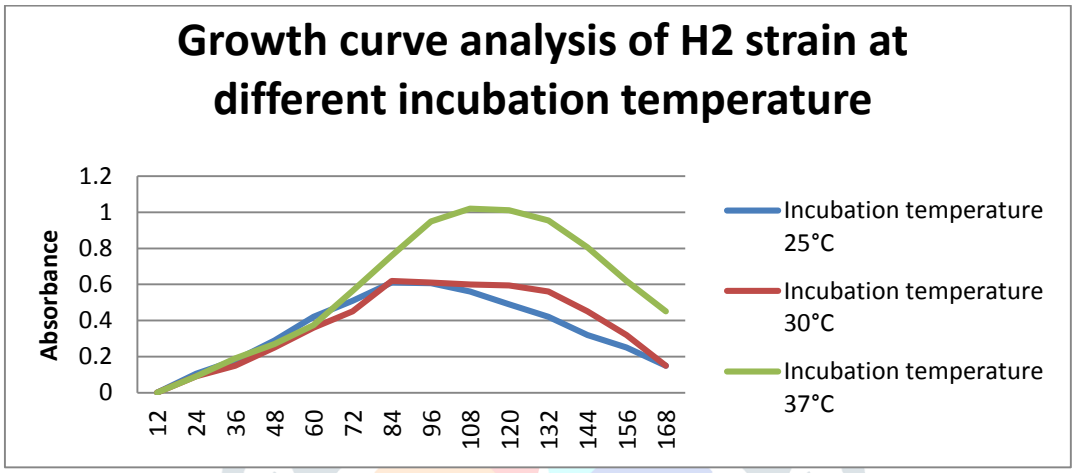
**Figure 2: H1 soil bacteria strain grown in MSM broth at 25$^{o}$C, 30$^{o}$C and 37$^{o}$C**



**TEMPERATURE OPTIMIZATION FOR BACTERIAL STRAINS H2 SOIL**

The bacterial strain H2 was grown at three different temperatures 25ºC, 30ºC and 37ºC. At temperature of 25ºC, H2 soil bacteria strain reached the stationary phase at the period of 84 hours with optimal density of 0.610 nm . At 30ºC , H2 soil bacteria strain reached the stationary phase at the period of 84 hours also with optimal density of 0.620 nm. At 37ºC , bacterial strains has the highest rate of growth at 108 hrs and optical density of 1.020 nm (Graph 6). The best temperature observed for the maximum cell mass density is 37ºC, as for the successful PHB production. During this optimization the medium used was modified with 2 molar salt ($NaCl_2$) concentrations (MSM broth medium).

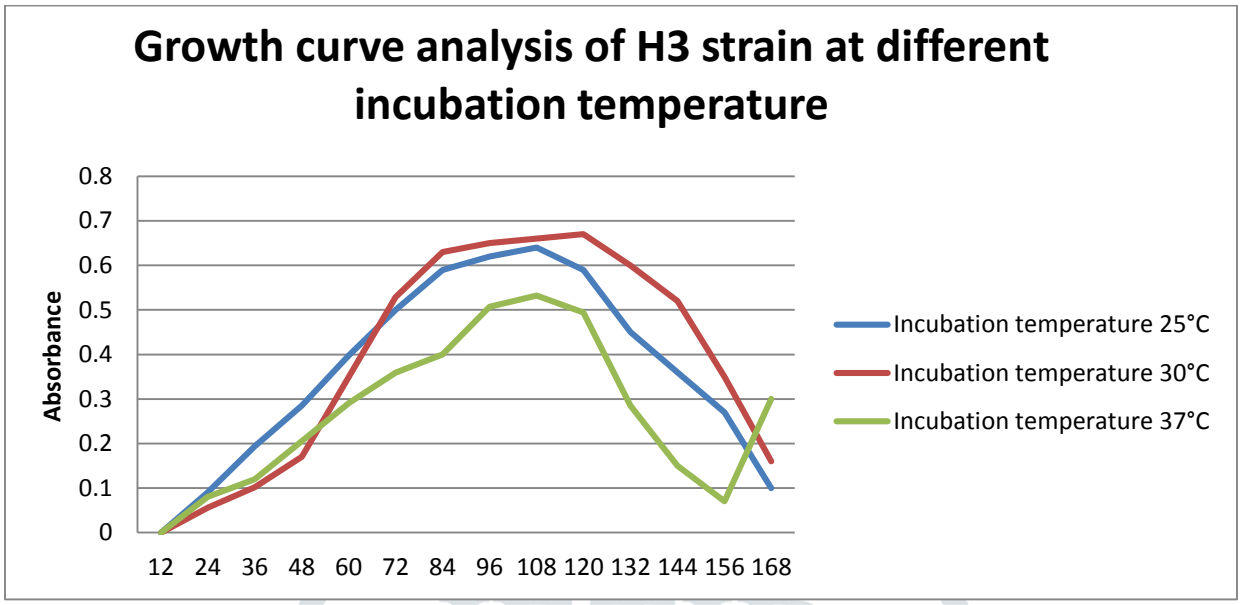
**Figure 3: H2 soil bacteria strain grown in MSM broth at 25ºC, 30ºC and 37ºC**




**TEMPERATURE OPTIMIZATION FOR BACTERIAL STRAINS H3 SOIL**

Nevertheless, this bacterium also grown at 25ºC 30ºC and 30ºC temperatures respectively. At 25ºC temperature, H3 soil bacteria strain reached the stationary phase at the period of 108 hours with optimal density of 0.640 nm. Another temperature was 30ºC , the highest rate of growth at the period of 120 hours with optimal density of 0.670 nm at 30ºC. At 37ºC temperature, H3 soil bacterial strain has the high rate of growth at 108 hrs and optical density of 0.532 nm. The best temperature observed for the maximum cell mass density was 37ºC, as for the successful PHB production. During this optimization the medium used was modified with 3 molar salt ($NaCl_2$) concentrations (MSM broth medium).

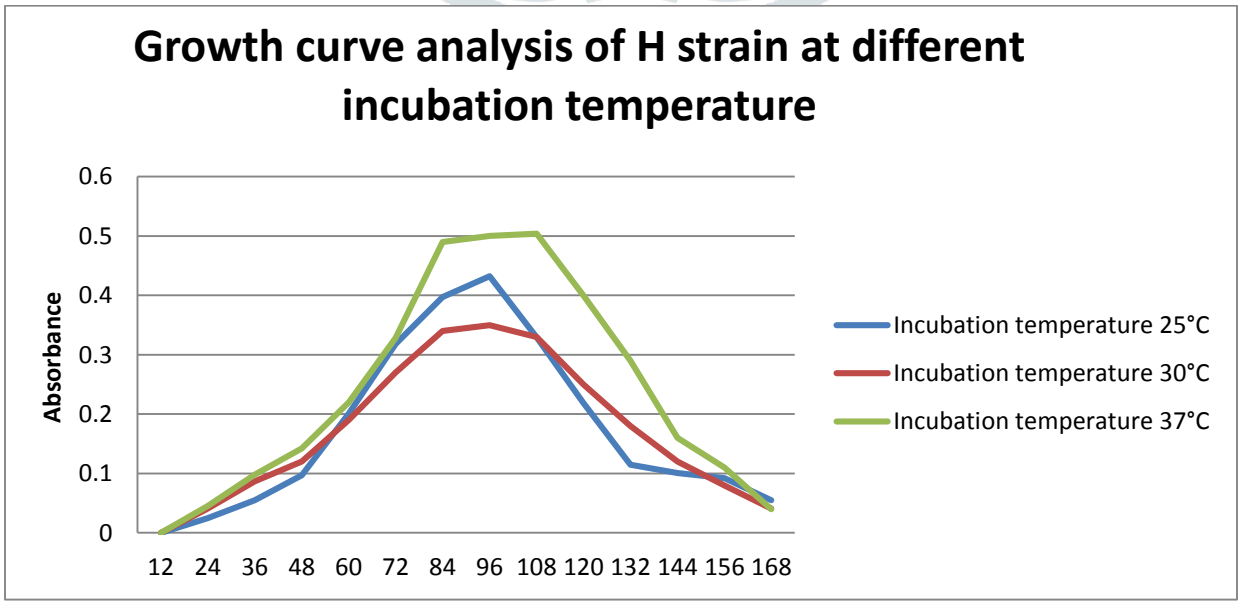
**Figure 3: H3 bacteria strain grown in MSM broth at 25ºC, 30ºC and 37ºC**




### 6.10. TEMPERATURE OPTIMIZATION FOR BACTERIAL STRAINS H WATER

The H water bacteria stain was also grown at three different temperatures. H water bacteria strain had reached the stationary phase at the period of 96 hours with optimal density of 0.432 nm at 25ºC. At 30ºC temperature, H water bacteria strain reached the stationary phase at the period of 96 hours also with optimal density of 0.350 nm. At 37ºC, the maximum growth rate at this temperature by H water bacterial strains was at 108 hrs and optical density of 0.504 nm. The best temperature observed for the maximum cell mass density is 37ºC, as for the successful PHB production. During this optimization the medium used was modified with 1 molar salt (NaCl2) concentration (MSM broth medium).

**Figure 4: H water bacteria strain grown in MSM broth at 25ºC, 30ºC and 37ºC**



## QUANTIFICATION ANALYSIS OF DIFFERENT BACTERIAL STRAINS AT DIFFERENT CARBON SOURCES

Optical density of bacterial strains was performed using different sources of carbon. Glucose, lactose and sucrose were used as carbon sources. Spectrophotometer was used for taking the reading at 600 nm. The optical density from H1 soil bacterial strains showed in different carbon sources i.e. glucose is 0.65 g/l for PHB weight and 0.6 g/l for cell dry weight (cdw) with PHB percentage yield of 30.76 % while that of lactose is 0.3 g/l for PHB weight and 0.35 g/l for cdw, with PHB percentage yield of 58.3 %. PHB weight from sucrose was observed as 0.2 g/l and 0.3 g/l for cell dry weight (cdw) and PHB percentage yield of 66.6 %. Lactose has the highest PHB percentage yield followed by sucrose and glucose with low percentage yield. The optical density of H2 soil bacterial strains, the strains grown in glucose has PHB weight analysed as 0.2 g/l and 0.3 g/l for cell dry weight and 66.6 % was the PHB yield, the reading from lactose is 0.3 g/l for PHB weight and 0.4 of cell weight with PHB percentage yield of 75%. For sucrose PHB weight was 0.2 g/l and cell weight was observed as 0.45 and PHB percentage yield of 44.4%. Lactose has the maximum percentage of PHB yield. The analysis showed that of H3 soil bacterial strains, the PHB and cell dry weight (cdw) (g/l) from glucose was observed as PHB weight was 0.15 g/l and cell dry weight (cdw) was 0.2 g/l and PHB yield percentage is 75%, while that from lactose PHB weight was 0.1 g/l and 0.2 g/l for cell weight with 50% PHB yield and that from sucrose was 0.1 g/l for PHB weight and 0.35 g/l for cell dry weight with 28% PHB yield respectively. But in the reading obtained from these bacterial strains, glucose has the highest yield percentage of PHB. The bacterial strain H water, showed that the PHB weight as 0.2 g/l and 0.7 g/l for cell dry weight with PHB yield of 29% from glucose as carbon source, while the optical density from lactose was 0.2 g/l for PHB weight and 0.5 for cell dry weight with PHB yield of 40%. The sucrose with PHB weight have been 0.1 g/l and 0.5 g/l for cell dry weight with PHB yield of 20The optical density analysis of these bacterial strains at different carbon sources showed that the highest PHB yield percentage is occurred in the medium containing lactose as source of carbon with only high PHB yield percentage in glucose from H3 soil bacterial strain. The temperature used for the growing of bacterial strains is 37$^{o}$C. Considering the highest growth of these bacterial strains in the medium containing lactose as carbon sources, it could be concluded that

lactose was analysed the best in PHB extraction and production. The PHB recovery and extraction of these bacterial strains was carried out using sodium hypochlorite extraction method followed by chloroform dried method.

Formula for calculating the PHB yield percentage (%) is as follow:

$$\text{PHB yield \%} = \frac{\text{weight of PHB}}{\text{Cell weight}} \times 100$$

**Table 2: Optimization at different carbon sources and quantification of PHB isolated from H1 soil bacterial strain**

| H1 soil bacteria strains | PHB weight g/l | Cell weight g/l | PHBWT yield % = (cdw g/l/PHB g/l)*100 |
|---|---|---|---|
| Glucose | 0.2 | 0.65 | 30.76% |
| Lactose | 0.3 | 0.35 | 58.3% |
| Sucrose | 0.2 | 0.3 | 66.6% |

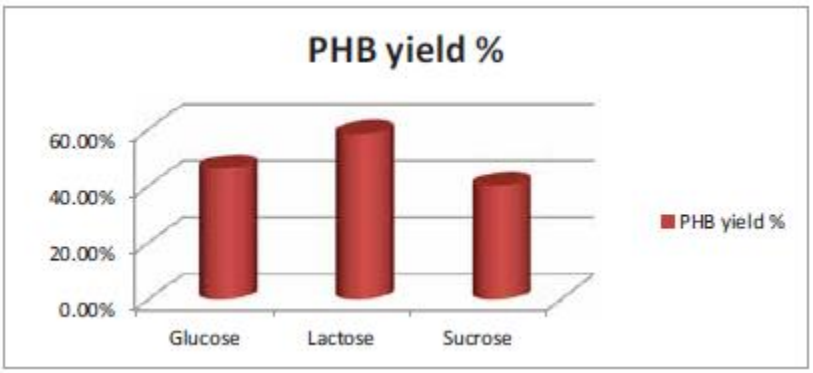



**Figure 5: PHB yield % extracted from H1 soil**

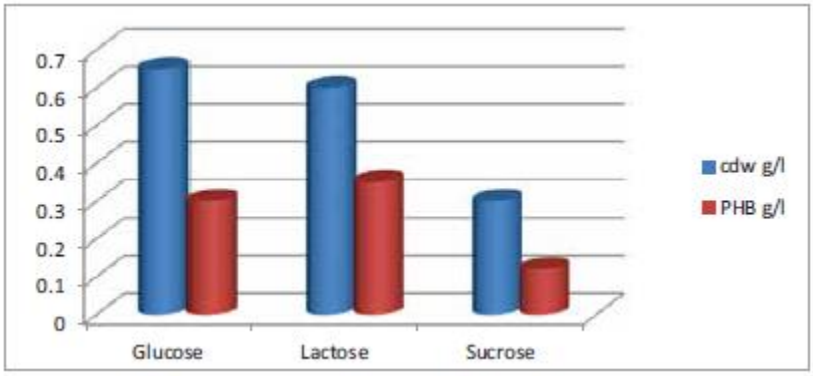



**Figure 6: Cell dry weight and PHB weight extracted from H1 soil**

**Table 3: Optimization at different carbon sources and quantification of PHB isolated from H3 soil bacterial strains**

| H3 soil bacterial stains | PHB weight g/l | Cell weight | PHB yield % = (PHBwt/cdrw) *100 |
|---|---|---|---|
| Glucose | 0.15 | 0.2 | 75% |
| Lactose | 0.1 | 0.2 | 50% |
| Sucrose | 0.1 | 0.35 | 28.6% |

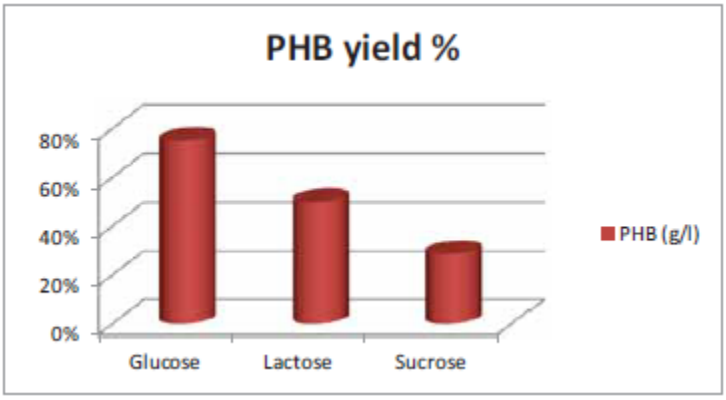



**Figure 7: PHB yield % extracted from H3 soil**

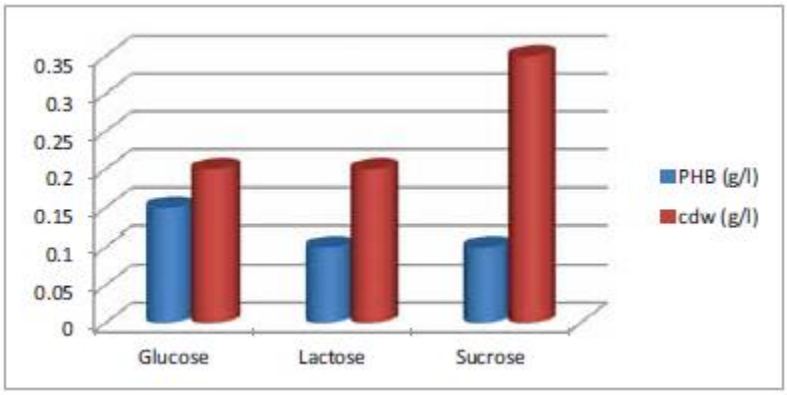



**Figure 8: Cell dry weight and PHB weight extracted from H3 soil**

**Table 4: Optimization at different carbon sources and quantification of PHB isolated from H water bacteria strain**

| H3 soil bacterial stains | PHB weight g/l | Cell weight | PHB yield % = (PHBwt/cdw)*100 |
|---|---|---|---|
| Glucose | 0.2 | 0.7 | 29% |
| Lactose | 0.2 | 0.5 | 40% |
| Sucrose | 0.1 | 0.5 | 20% |

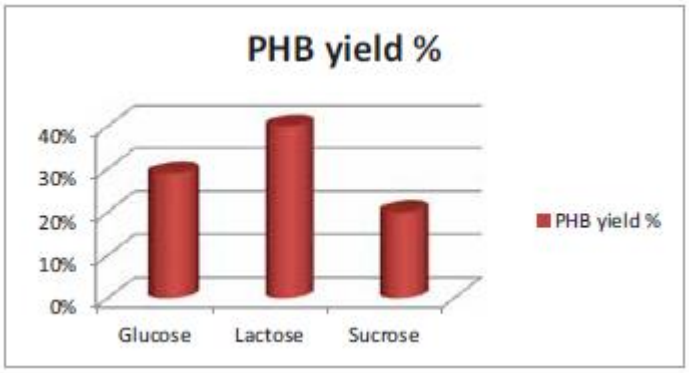



**Figure 9: PHB yield % extracted from H water**

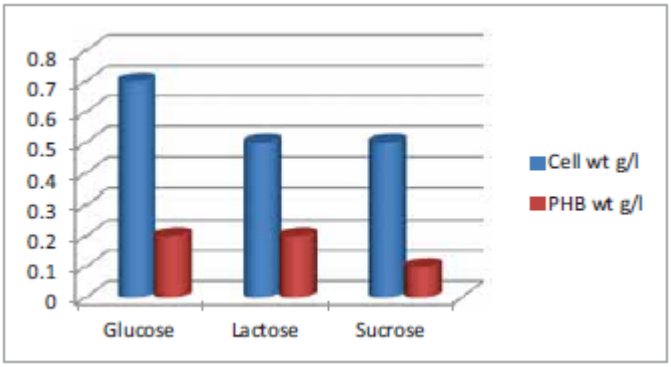



**Figure 10: Cell dry weight and PHB weight extracted from H water**

**EXTRACTED PHB**

The content of PHB for from each dried cells were extracted from the mass of the dried chloroform. The extracted PHB was put in eppendorf tube and further analysed for Fourier transform infrared (FTIR) analysis.

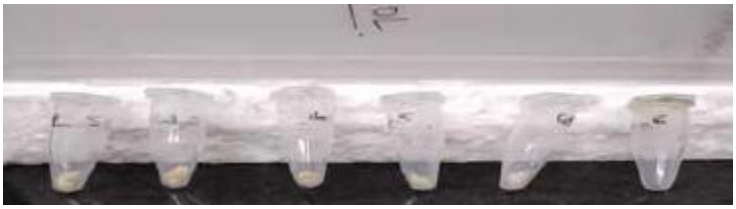


Figure 11: Extracted PHB in powder form in eppndoff

**FOURIER TRANSFORM INFRARED**

FTIR is chemical technique for studying structure and functional groups of extracted PHB. IT was utilized as a preliminary test for PHB confirmation (Naumann et al.1991). Adsorption peak of the study substance signifies the composition, since each peak plays a major role in the structure of the substance. The functional groups of the extracted PHB polymers showed the presence of expected
functional groups in the structure of PHB. Results obtained from that extracted polymers was closely related to PHB (Gomaa, 2014). FTIR carried out within the ranges of 4000- 500 $cm^{-1}$.

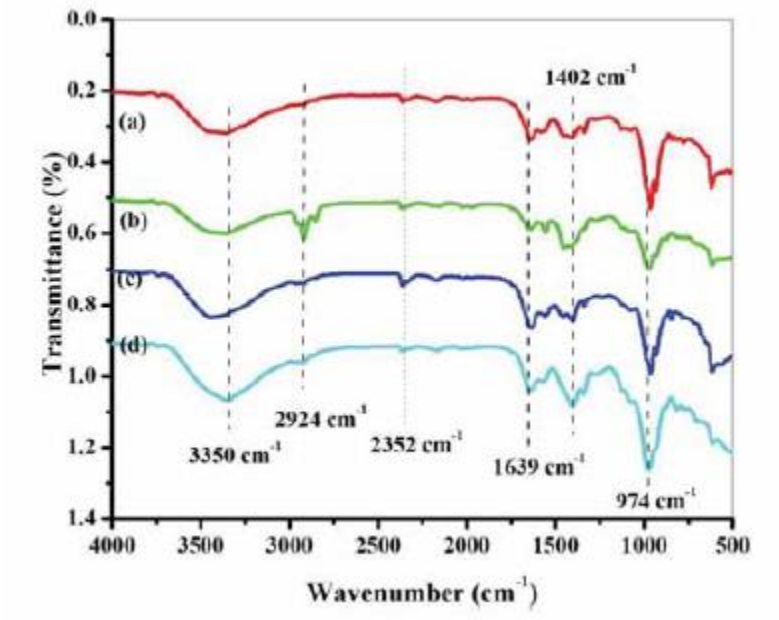



**Figure 12: FTIR graph from the extracted PHB**

**THIN LAYER CHROMATOGRAPHY (TLC).**

TLC was carried out between the extracted PHB polymer and commercially purified PHB polymer (Sigma Aldrich) in order to know the individual chemical substance between them. Commercially PHB served as control. The retention factors (Rf) of both polymers was studied. The extracted polymer from H1 soil bacteria strain has the Rf value of 0.5 while that of control PHB was 0.66 and the extracted PHB from H2 soil bacteria strain has the Rf value of 0.5 and for control PHB was analysed as 0.66. Likewise the H3 soil bacteria strain has the Rf value of 0.57 and control PHB has the value of 0.66 and H water bacteria strain has the Rf of 0.45 while that of control PHB was 0.66 respectively. H3 soil bacteria has the highest PHB content when compared with other bacterial strains but all the isolated bacterial strains produce PHB in 50 to 70 % when compared with the control PHB.

## CALCULATED TLC RESULT

**Table 5: Rf values observed by Thin layer Chromoatography**

| S/N | Extracted PHB $R_f$ value | Control PHB $R_f$ value |
|---|---|---|
| H1 Soil | 0.5 | 0.66 |
| H2 Soil | 0.5 | 0.66 |
| H3 Soil | 0.57 | 0.66 |
| H water | 0.54 | 0.66 |

## BIOCHEMICAL CHARCTERIZATION OF ISOLATED BACTERIAL STRAIN

Several biochemical tests were conducted for the tentative identification of bacteria using Bergey's manual of systemic bacteriology. H1 soil bacterial strain have features similarity with *Halomonas species*. This most likelihood identification was compared with Bergey's manual of systematic bacteriology as describedin the below table. The bacterial strains of H2 soil and H3 soil bacteria have the features similarities with *Pseudomonas species.* However, for the H water bacteria strain this strain of bacteria possess the similarities of *Bacillus species*

**Table 6: Biochemical analysis of H1 and H2 soil bacteria strain**

| S/N | TEST | OBSERVATION |
|---|---|---|
| 1 | Catalase | Positive |
| 2 | Citrate | Negative |
| 3 | MR | Positive |
| 4 | VP | Positive |
| 5 | Oxidase | Negative |
| 6 | Urease | positive |
| 7 | Indole | Negative |
| 8 | Motility | Motile |
| 9 | Hydrogen sulphide | Positive |
| 10 | Gram stain | Gram negative |
| 11 | Cell shape | Rod |

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|---|---|---|
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| 7 | Indole | Negative |
| 8 | Motility | Motile |
| 9 | Hydrogen sulphide | Negative |
| 10 | Gram stain | Gram negative |
| 11 | Cell shape | Rod |

**Table 7: Biochemical analysis of H3 and H bacteria strain**

| S/N | TEST | OBSERVATION |
|---|---|---|
| 1 | Catalase | Positive |
| 2 | Citrate | Positive |
| 3 | MR | Negative |
| 4 | VP | Negative |
| 5 | Oxidase | Positive |
| 6 | Urease | Negative |
| 7 | Indole | Negative |
| 8 | Motility | Motile |
| 9 | Hydrogen sulphide | Negative |
| 10 | Gram stain | Gram negative |
| 11 | Cell shape | Rod |

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| 5 | Oxidase | Negative |
| 6 | Urease | Negative |
| 7 | Indole | Negative |
| 8 | Motility | Motile |
| 9 | Hydrogen sulphide | Negative |
| 10 | Gram stain | Gram positive |
| 11 | Cell shape | Rod |

## Discussion

Water and soil samples were used in order to isolate the PHB producing organisms, the medium utilized for carrying out the analysis was modified mineral salt broth for production of PHB produce organisms. Since, PHB producing organisms grow only in the present of salt. The isolated bacterial strains were analysed for their morphology and further, Sudan Black staining was done for screening the positive strains for PHB production. The quantification analysis of these bacterial strains carried out at different carbon sources showed that the highest PHB yield percentage was occurred in the medium containing with only high PHB yield percentage in glucose from H3 soil bacteria strain. Temperature used for the growing of bacterial strains was 37$^{o}$C as optimized. Considering the highest growth of these bacterial strains in the medium containing lactose as carbon sources it could be concluded that lactose was analysed as the best source for PHB extraction. The PHB recovery and extraction of these bacterial strains was carried out using sodium hypochlorite, 96% ethanol, 96% acetone and chloroform extraction method. The content of PHB for from each dried cells were extracted from the mass of the dried chloroform. The extracted PHB was analysed for Fourier transform infrared (FTIR) analysis. FTIR analysis of the extracted PHB polymers showed that extracted PHB produced has the present stretching bonds in it structure by comparing with the study of (Gomaa 2014). TLC also proved that the extracted polymers from all extracted PHB have the chemical substances which are similar with commercially purified PHB. TLC was performed between the extracted PHB and commercially purified PHB. Biochemical test was also performed for the tentative identification of the isolated bacteria from the samples.

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## Conflict of Interest

There is no conflict of interest in the research paper.

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