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Biosynthesis and Optimization of PolyHydroxyalkanoate (PHA) Production by Acinetobacter lwoffii Isolate

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Abstract

Polyhydroxyalkanoate (PHA) is an eco-friendly polymer that has various important biomedical uses, as well as biodegradability, drug delivery, and medical implants. It is a kind of polyester synthesized by various microorganisms as energy reserve material under inappropriate conditions. This study focused on testing waste cooking oil as the source of carbon to optimize PHA production. The tests included the most important environmental components within the culture medium affecting the growth of the bacterial isolate under the experiment and its ability to produce a polymer. Results revealed that the cultivation of *Acinetobacter lwoffii*, pre-isolated from hydrocarbon contaminated soil, under optimum conditions showed the highest productivity after 72 hours. Corn oil waste as the carbon and urea as the source of nitrogen were discovered to be the best nutritive sources for concentrated PHA production, with, 2% and 0.5 g/L as the best carbon and nitrogen sources concentrations, respectively. Through the results, it was found that there is an important role for the change, both qualitatively and quantitatively, in the components of the nutrient medium and the surrounding condition in increasing the efficiency of isolation, as it had a significant role in increasing the efficiency of the isolate. There was a significant increase in the PHA content ranging from 10 to 75% and the production of biomass to 3.6 g/L. respectively. Finally, this study concluded that the use of bacterial isolate in the production of PHA can contribute to solving the critical problem of environmental pollution caused by the use of industrial plastic and replacing it with environmentally friendly and low-cost materials.

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1. Introduction

One of the main pollutants of the earth is plastic, which needs proper management to get rid of its waste, which is continuously thrown into the environment because it is involved in many aspects of our daily life (Hussein *et al*., 2020). Plastic is used to manufacture daily products such as furniture and containers with its multiple uses (Hussein *et al*., 2018). Nowadays, most products are either made of plastic or manufactured in plastic-containing equipment (Noor, 2018). The plastic industries include many products, both durable and non-durable, such as

appliances, furniture, and cans used in packaging many materials such as shampoo, soft drinks, utensils, and, garbage bags. Many chemical pollutants are also produced as a result of the multiple and different manufacturing processes of plastic materials (Hussein, 2015). A major shift has occurred recently as a result of environmental awareness that has led to a shift in public opinion regarding the necessity of consciously dealing with the problem of plastic pollution. In addition to its accumulation in the environment, plastic pollution causes the creation of economic burdens due to the depletion of natural plastics as a used material for fuel (energy) and other natural resources (Varsha and Savitha,2011). Studies have tended to find biodegradable alternatives that are environmentally compatible and have the least impact on public health (Kazem and Amal, 2021). Bioplastics are one of the solutions to this issue, which is materials identical to plastic materials in terms of specifications produced by biomass, such as corn starch, fats, and oils (Anderson and Dawes 1990). "Bio-Based "is a term that represents the process of consuming raw materials resulting from renewable materials, especially polymers, which are materials that can be renewed through natural processes parallel to or superior to the consumption process so as not to affect environmental resources and their sustainability. (Ravenstijn *et al.*, 2010). The method of biodegradation of bioplastic materials depends on the ability of microorganisms to grow aerobically or anaerobically. To decrease pollution bioplastic developed instead of plastic (Aljanabi, 2018). Living organisms and their microbial products (such as enzymes) are working to achieve sustainable development in reality, through the production of fuels and polymers necessary for the various industries, so that sustainable production of these products can be achieved, which is called "white biotechnology" (Titz *et al*., 2012). Among the most important bioplastics are algae and microorganisms, which represent mostly degraded organic waste to form bioplastics. Therefore, the current plastic products can be replaced with the biological product Eco-friendly, degradable, and renewable (Matsusaki *et al*. 2000). Compared to traditional plastic, bioplastics are the least likely to emit hazardous and environmentally harmful gaseous emissions, such as carbon dioxide. Attention to the need to maintain projects for recycling vital products, in particular bioplastics, has become one of the main concerns of those concerned with the environment and industry. Because it is insoluble in the bacterial cytoplasm. The PHAs act as carbon and energy storage compounds. Bacteria that producing-PHA naturally include *A. latus, B. megaterium, C. necator and P. oleovorans* are characterized by being able to consume environmental, domestic and industrial waste as sources of carbon for the manufacture of PHA. (Anderson *et al*.,1990). These organisms can transform the compound by breaking it down to carbon dioxide and water after a period of time not exceeding one year. (Eman, 2017). The bacteria used in the production of PHA can be categorized into two groups depending on the available growth conditions. The first group includes species of bacteria that need few vital nutrients such as nitrogen and oxygen in addition to providing an extra carbon source in order to be able to efficiently produce PHA. While the second group is characterized by not requiring the limited nutrients in the culture medium for the synthesis of PHA. The most preferred sources for the production of PHA are vegetable oils such as soybean oil, palm oil and corn oil, which is lower in cost than most sugars. Also, glycerin, which is a side-product of palm oil refining, can be used as a carbon source for the production of polymers using bacteria. It is used in various applications such as oral hygiene products, cosmetics, food and beverages (Yu and Wang, 2001). Glycerol is used in oral hygiene products, cosmetics, food, and drinks. Microbes have been certified to be the effective product of PHA due to their great ability to adapt to various harsh environmental conditions (Poirier *et al.*, 1995). Out of these, *Bacillus* spp., *Pseudomonas* spp. and *Vibrio* spp. are found to be more effective for PHA production due to its ability to rapidly reproduce under environmental pressure and its high stability. This is to contribute to reducing the cost of producing these compounds that are used as biodegradable plastics and alternatives to industrial material. Aim of this study The current project aimed to: 1-investigate the PHA production by *A.lwoffii* bacterial isolate. 2-optimization of PHA production with different conditions, carbon and nitrogen sources and concentrations to determine the carbon source that will give the highest yield of the PHA.

2. Experimental Procedure

2.1. Test of Bacterial Isolate and Experimental Conditions

A.lwoffii Obtained from a previous study by isolating it from an area contaminated with hydrocarbon residues and identification with VITEK 2(Raghad and Nehia, 2021), and it was cultivated in Mineral Salt Medium Table1 at 30℃, pH 7.5, with 150 rpm of agitation. As a carbon source of 1% corn oil waste was added (Kim *et al.* 2000).

Table 1: Mineral salt medium composition (per litre distilled water).

Component	Weight (g/L)
Na_2HPO_4	4
KH_2PO_4	1
$MgSO_4.7H_2O$	0.2
$CaCl_2.2H_2O$	0.05
NH_4CL	3
NaCl	0.2
Glucose	1
$FeSO_4.7H_2O$	5.56
$MnCl_2.4H_2O$	3.96
$CoSO_4.7H_2O$	5.62
$CuCl_2.2H_2O$	0.34
$ZnSO4.7H_2O$	0.58
H_3BO_3	0.60
$NiCl.6H_2O$	0.04
$Na_2MoO_4.2H_2O$	0.06

2.2. Bacterial Isolates Staining by Sudan Black B

The microbial broth was fixed by exposure to direct heat after spreading it on a glass slide, then stained for 10 minutes with a 3 percent Sudan Black B (w/v in 70% ethanol, Sigma) solution. The slide was subsequently entirely decolonized by immersing it in xylene. Cells of the sample were immersed for 10 seconds in safranin dye as counter dye (5 percent w/v in distilled water), then rinsed and dried with distilled water. Optical microscopy was used to study the cells after applying few drops of immersion oil straight to the fully dry slide. (Kemavongse *et al*., 2008 and Kumari and Dhingra, 2013).

2.3. Detection of the Presence of Intracellular PHA Using Nile blue A

For the detection of bacterial isolate producing PHA, Nile Blue A melted in dimethyl sulfoxide (DMSO; 0.25 mg/mL) was added to the sterile production medium which contain the following components: (Table 2):

Table 2: PHA production medium composition.

Component	Weight (g/L)
NaCl	30
$MgSO_4.7H_2O$	0.25
$CaCl_2$	0.09
KCl	0.5
NaBr	0.06
Glucose	1
Peptone	5
Yeast extract	10
Agar	20

Depending on (Quillaguaman *et al*., 2004), pH was adjusted to 7and after culturing; the isolated strain was incubated at 35 ℃ for 48 hours. The accumulation of PHA in the cultivated colonies was detected after examining the cells under a fluorescent microscope by exposing the cultured agar plates to UV light (312 nm).

2.4. Measurement of Bacterial Growth

Bacteria were grown in a nutrient medium containing oil residues as a carbon source at a concentration of 1%, and the bacterial growth was estimated by calculating the dry weight after incubation by fixing all conditions and adopting one variable for the study. The dry weight of the cells was determined at different phases of incubation.

After centrifuging 25 mL of culture broth for 15 minutes at 4℃ at 10,000 rpm, the supernatant was collected. Before being weighed, cell pellets were put in a pre-weighed aluminum foil cup and baked for 2 hours at 80℃. The number of dry cells per litre of soup was determined. (Yuksekdag *et al*., 2004).

2.5. PHA Extraction and Quantification

The quantity of PHA generated was estimated after extracting the polyhydroxyalkanoate polymer Figure 1. The growth of the isolate containing the polymer was pelleted at 10,000 rpm at 40 ℃ for 10 minutes after it was grown in nutritional broth. To eliminate undesirable elements, the pellet was washed in acetone and ethanol, then resuspended in an equivalent amount of 4 percent sodium hypochlorite and incubated at room temperature for 30 minutes. To sediment the lipid granules, the mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant was discarded, and the cell pellet was washed in acetone and ethanol in the order listed. Polymer granules were pelleted and dissolved in hot chloroform before being filtered using Whatman No. 1 filter paper (previously treated with hot chloroform). 10 mL of hot concentrated H_2SO_4 was added to the filter, converting the polymer to crotonic acid and making the solution brown. The solution was cooled, and the absorbance was measured on a UV-VIS spectrophotometer at 200-600 nm against a concentrated H_2SO_4 blank. The peak at 235 nm was used to confirm the presence of PHA (Nehra *et al*., 2015).

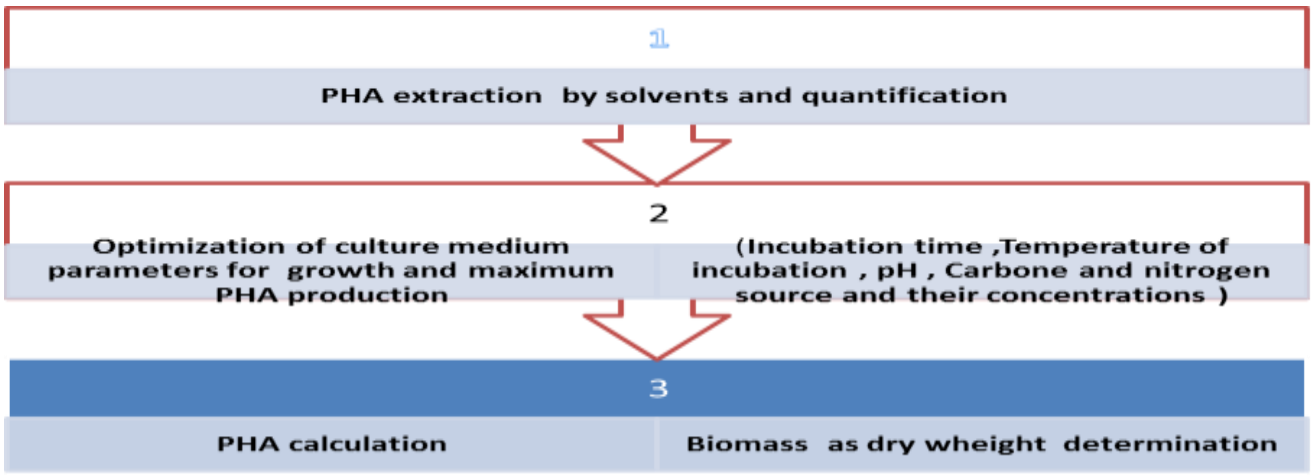


Figure 1: Schematic diagram **of** PHA extraction, quantification, and improvement of growth conditions

2.6. Optimization of Culture Medium Parameters for Growth and Maximum PHA Production

Acinetobacter lwoffii isolates were cultured in nutrient broth and incubated for 24 h. To be a 1% bacterial inoculum and the minimal medium was injected and cultured under different growing conditions. Different factors *viz*., carbon and nitrogen source and their concentrations, incubation time, pH, and incubation temperature play an important role in PHA production rate. Therefore, in order to determine the optimum conditions for maximum PHA production, the effect of all these parameters on PHA production by the PHA positive *Acinetobacter lwoffii* isolate was studied by varying all the conditions within a defined range**.**

2.6.1. Effect of Incubation Time on PHA Production

The effect of different incubation temperatures on PHA production was determined by inoculating the culture in MSM supplemented with the C and N source and then incubating at different time viz., 24, 48, 72, 96, and 120 hrs at 30℃, PHA yield was quantified; based on the yields the optimum incubation time for maximum PHA production was determined.

2.6.2. Effect of Temperature on PHA production

The effect of different incubation temperatures on PHA production was determined by inoculating the cultures in MSM supplemented with the C and N source and then incubating at different temperatures viz., 25 30, 35, 37 and 40℃. After 48 h of incubation at respective temperatures, PHA yield was quantified; based on the yields the optimum temperature for maximum PHA production was determined.

2.6.3. Effect of pH on PHA Production

For pH optimization, culture of the bacterial isolate was raised in MSM supplemented with the C and N source having different pH, viz., 6.0,6.5, 7.0,7.5 and 8.0. Culture was incubated at 37℃ on a rotary shaker (150 rpm) for 48 hrs. After incubation, biomass and PHA yield was quantified, and the pH exhibiting maximum yield was determined.

2.6.4. Optimization of Carbon to Nitrogen Concentration

As PHA accumulation has been found to be enhanced if the bacterial cells are cultivated in the presence of an excess carbon and limited nitrogen sources (Reddy et al., 2009), therefore, in addition to the determination of the best C and N sources, the effect of different C and N concentration on PHA production was also determined. For this, culture was inoculated in MSM supplemented with different concentrations of the C and N source (C concentration (0.5, 1, 2, 2.5, 3 and 5 g\l nd N concentration as 0.1, 0.25, 0.3, 0.5 and 1.0 g\l). The culture was incubated at 37℃ on a rotary shaker (150 rpm) for 48 h. After incubation, PHA yield and biomass were quantified after extraction and based on the yields the best C and N concentrations were determined**.**

2.6.5. Optimization of Different Carbon Sources

The effect of different carbon sources on PHA production was determined by raising the culture of the isolate in 100 ml of minimal salt medium (MSM) (Suresh Kumar *et al*., 2004) supplemented with different carbon sources (including glucose, molasses, waste corn oil, olive oil, glycerin) at 2% concentration. The culture was incubated at 37℃ on a rotary shaker (150 rpm) for 48 hrs. After incubation, growth and PHA produced by the isolate was quantified.

2.6.6 Nitrogen Source Optimization

The PHA positive isolate was inoculated in 100 ml of MSM broth containing the best carbon source and different nitrogen sources (Ammonium chloride (NH4Cl), peptone, tryptone, urea, and yeast extract) at 1% concentration. After 48 h of incubation at 37℃, PHA yield was determined for the isolate, and the best nitrogen source was selected on the basis of their yield.
(Marjadi and Dharaiya, 2012).

3. Results and Discussion

PHA granules appeared in the bacterial isolate in the form of blue-black droplets when stained with Sudan black dye, while the cytoplasmic part of the bacteria appears in pink color under the objective lens immersed in oil, as shown in Figure 2 A. Gerhardt *et al*. (1981) demonstrated that the lipid contents and lipid inclusions in bacteria can be monitored using Sudan Black B staining. PHA-stained granules appear as blue-black drops within the pink cytoplasm of cells after 24-48 hours of growth. The increase in the biomass of living organisms can refer to the increase in the rate of granule synthesis, which can be detected using the Sudan Black B staining method, which appears in the form of black-blue colonies, depending on (Adwitiya pal *et al., 2009*). After Nile blue treatment, the isolate fluoresced bright orange when exposed to UV light. Staining indicate that PHA was accumulating Figure 2 B. With the increase in the content of PHA in the bacterial cells, the density and intensity of the color increases.

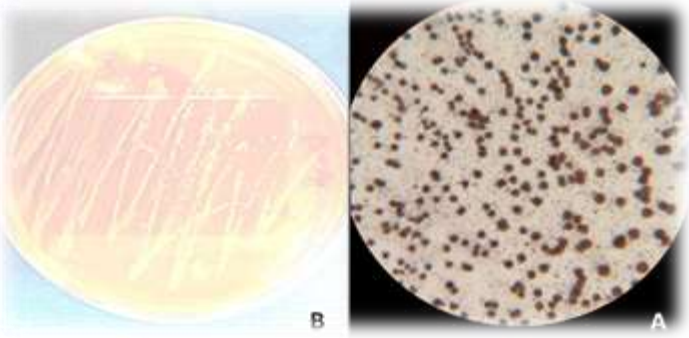


Figure 2: A- PHA granules (black section) on *A.lwoffii G3* were noticed. Under a 100X oil immersion objective with Sudan black B stain. B-Fluorescent PHA under UV light after staining bacteria with Nile blue.

By searching for new, inexpensive substrates, we can reduce the cost of biosynthesis of the product, as well as by adopting fermentation methods, methods of recovery and extraction, and new fermentation strategies, or by using highly produced organisms. Research experiments have shown that the process of accumulation of PHA by bacteria is affected by nutrition, temperature, growth and factors Physical, such as the carbon, nitrogen sources of the culture medium, pH and temperature Therefore, all these factors were optimized for higher PHA production by the isolate. There is an agreement between the increase in the weight of the bacterial cell and the amount of material produced in the form of PHA granules (Reddy *et al*., 2009). The culture medium and growth parameters for the isolate were optimized for maximum PHA production. Where it is noted that there is a gradual growth and an increase in production after 24 hours of growth, and the best growth was recorded by an amount of 2.8 g/liter after 48 hours of growth with an increase in the proportion of polymer production exceeding 30%. Accordingly, the best incubation period of 48 hours was fixed for subsequent experiment. Yuksekdag *et al*. (2004) mentioned that some types of bacilli, after growing them in the nutrient broth medium and under certain conditions for periods ranging from 6 to 48 hours, were able to produce different amounts of PHA. The result presented in Figure 3 indicates that maximum PHA production was observed at 48 h. Decreasing of biomass and PHA percentage after 48h may be due to the consumption of nutrients and using PHA as a carbon source.

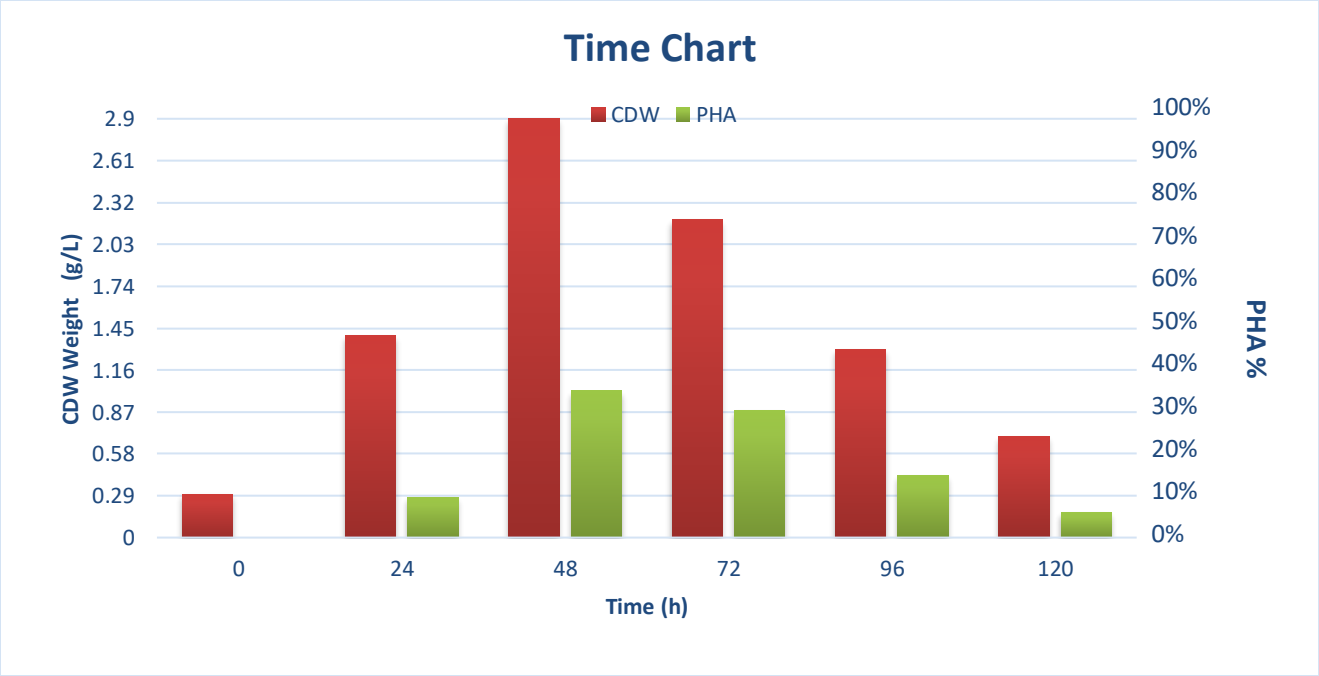


Figure 3: Effect of incubation time on growth rate and PHA production by *A. lwoffii* G3.

3.1. Effect of Culture Temperature

All parameters including temperature and pH impact the levels of dissolved oxygen and mass transfer efficiency, and thus these environmental elements greatly influence cellular growth and bio-product production (Bilal and Entesar, 2021). The effects of temperature on CDW and PHA production were evaluated by *A. lwoffii* G3 because these environmental factors are represented by temperature., acidity and other factors have a direct effect on dissolved oxygen and mass transfer efficiency, and thus they are factors that greatly affect cellular growth and production of biological products. In this work, PHA production by *A. lwoffii* G3 was examined at 25, 30, 35, 37and 40 °C (Dina and Entesar, 2021). Figures 4 show the temperature at 37^0C were discovered to be optimum conditions for obtaining higher yield of PHA 54 and 53 with 3.5 and 3.2 g\l of biomass respectively. Similar results have been obtained by Grothe *et al.* (1999), wherein they have concluded that temperature between 30-37^0C is optimum for PHA synthesis under fermentation conditions.

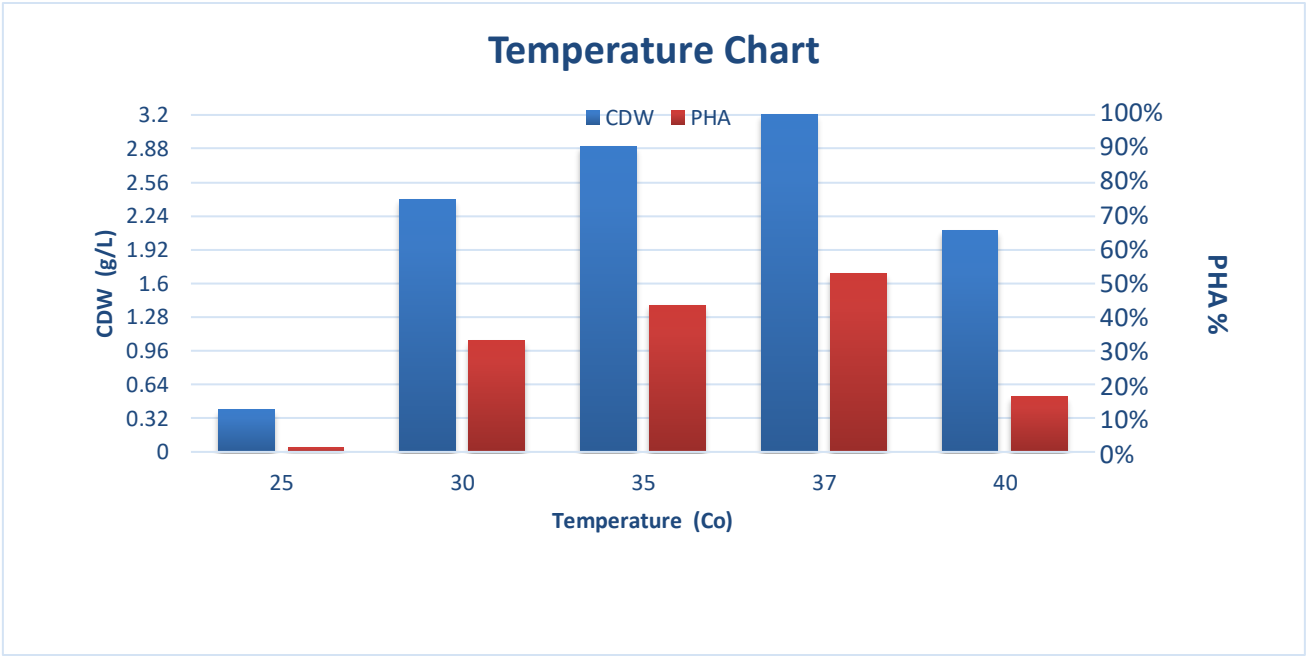


Figure 4: Effect of different incubation temperatures on biomass and PHA yield with *A. lwoffii G3*.

3.2. Effect of pH

For the purpose of improving productivity and reducing costs, a number of development methods for microorganisms have been followed, such as fed batches and continuous cultivation and proper pH control is critical because metabolic processes are highly sensitive to even small changes in pH. Figure 5 shows the Effect of pH on cell dry weight (CDW) and PHA production. The initial pH was controlled and attuned by the addition of concentrated hydrochloric acid or sodium hydroxide to MS medium (Du and Yu, 2002b). Figure 5 shows the optimum pH of the medium at 7.0. These results are in accordance with the results obtained by Grothe *et al*. (1999) who have reported that pH values ranging from 6.0 to 7.5 are optimum for PHA production.

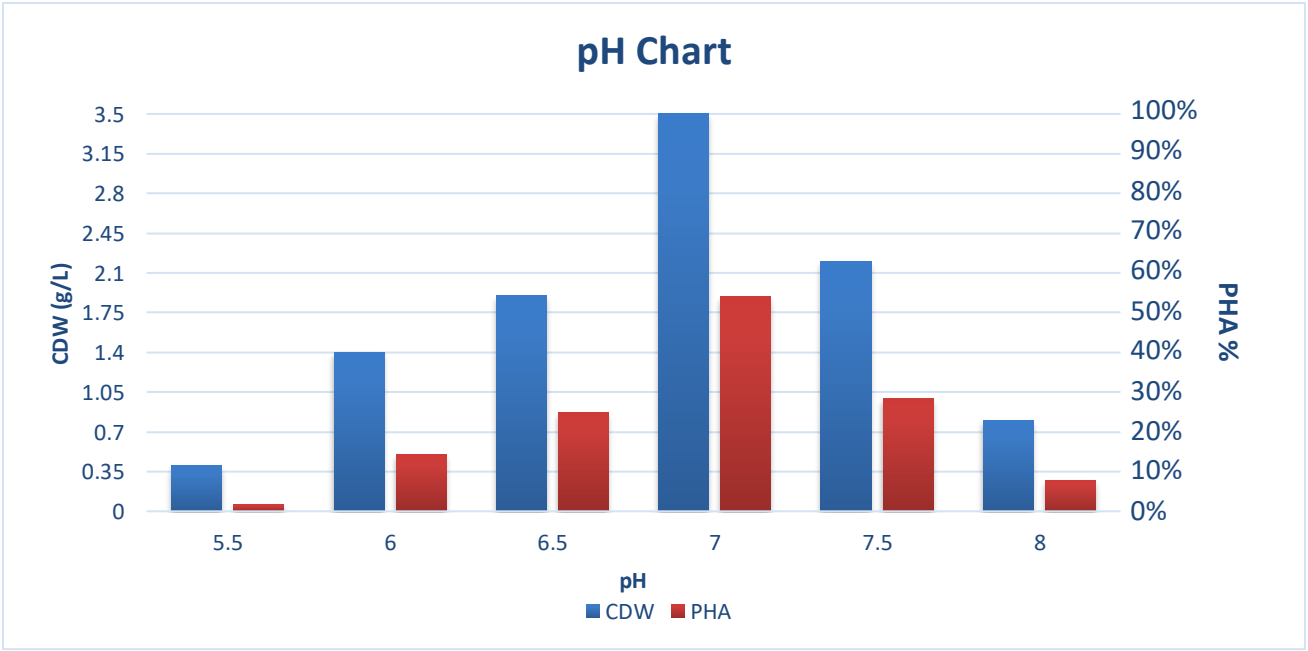
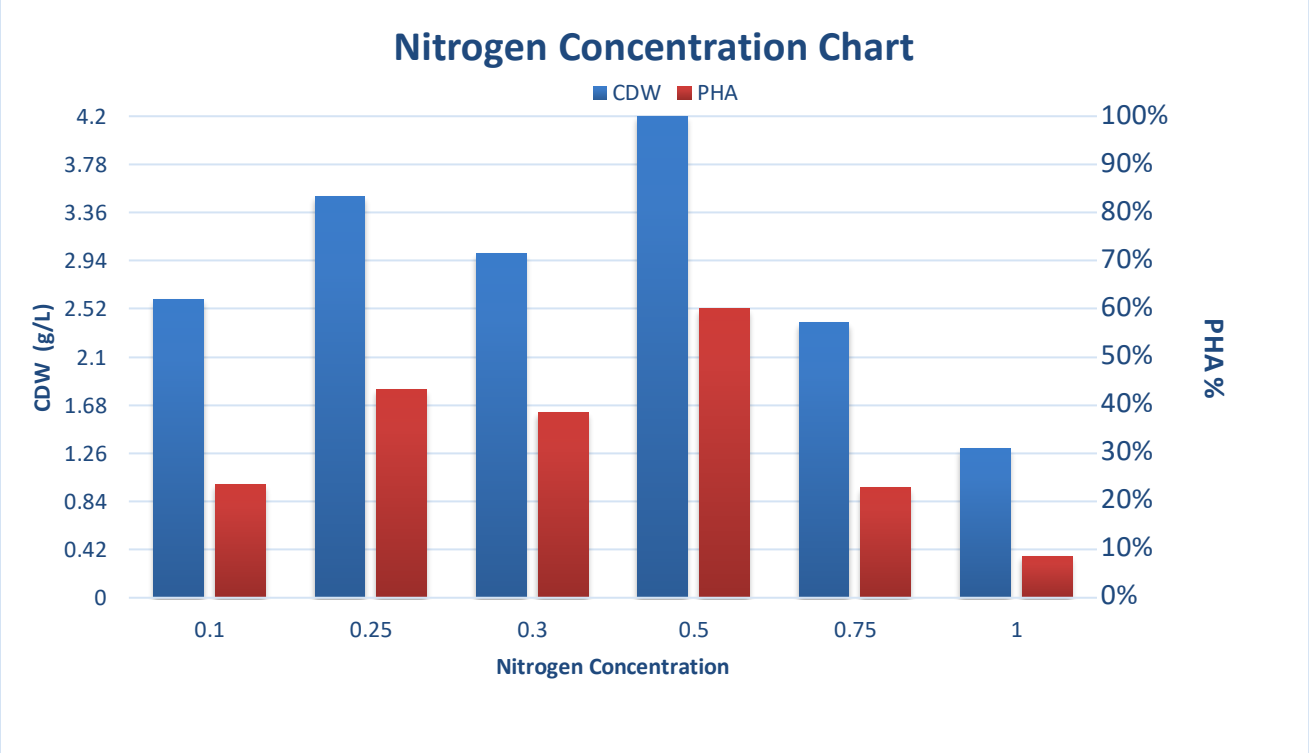


Figure 5: Effects of different pH values on growth rate and PHA production with *A. lwoffii G3*.

Figure 5 clearly displays that an initial pH of 7.0 achieved the uppermost 54% PHA content and 3.5 g/L biomass. These results are consistent with the findings in a study in which the two researchers were able to reach a recommendation because the best pH range from 6.0 to 7.5 for microbial growth and production of PHA (Kessler and Palleroni, 2000).

3.3. Effect of relative Concentrations of Carbon and Nitrogen Sources on PHA Production

With the supplementation of different concentration of nitrogen source in oil waste containing minimal medium, the cell biomass varied from 1.3 to 4.2 gm/L and maximum cell biomass was obtained with addition of ammonium chloride at 0.5% figure 6. After adding different concentrations of the nitrogen source to the culture medium containing the oil used as a carbon source, then growing the bacteria after inoculating the medium with bacterial isolate, and incubating it at a temperature of 30℃, the cell biomass varied from 1.3 to 3.5 g L-1. Maximum cell biomass was obtained with the addition of ammonium chloride as nitrogen source. The highest amount of PHA ranging from 28 to 57% was obtained in minimal broth containing 2.0% oil (v/v) as shown in Figure 7 and supplemented with ammonium chloride Under constant conditions. (Yuksekdag *et al.* 2004) reported that nitrogen sources of different complexity had a great effect on PHA yield, while (Dhawal et *al*, 2010) noted that the best yield of PHA was obtained by *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria using ammonium sulfate and ammonium phosphate as nitrogen sources than



if the source was used yeast extract. In addition to carbon, the nitrogen source must be controlled due to its importance as a precursor to vitamins, amino acids and growth factors (Saranya and Shenbagarathai 2010).

Figure 6: Effect of different nitrogen source concentrations on bacterial growth and PHA production.

Choosing the active bacteria producing PHA and improving the conditions to be the most suitable for the production of PHA is certainly one of the most important reasons for the success of the bioplastic production process. To one of these conclusions, this work indicates that *A lwoffii* G3 obtained from hydrocarbon-amended soils can be used in the industrial production of PHA (Kulkarni and Dalai, 2006).

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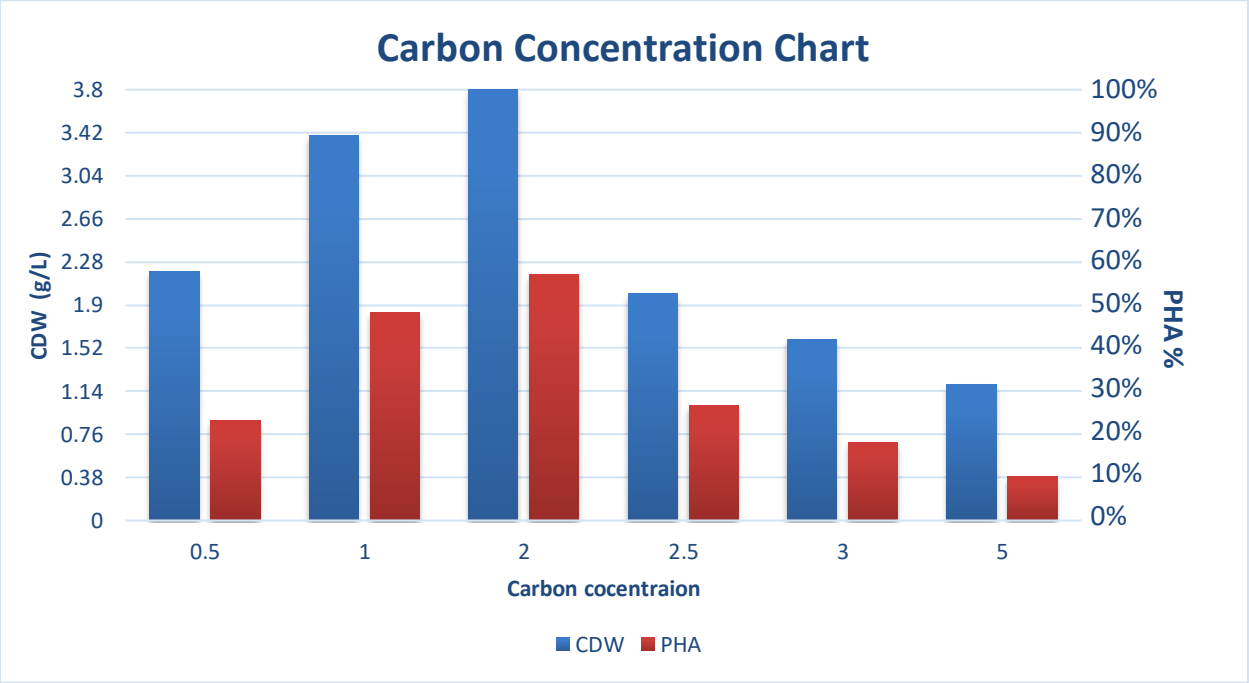

Figure 7: Effect of different carbon source concentrations on bacterial growth and PHA production.

3.4 Effect of the types of Carbon and Nitrogen on PHA Production by *A lwoffii G3*

The effect of different types of carbon and nitrogen sources on growth density and PHA production was estimated using different carbon sources (including glucose, molasses, waste corn oil, olive oil, glycerin and several nitrogen sources)., e.g., Ammonium chloride (NH_4Cl), peptone, tryptone, urea, and yeast extract. Separately sterilized carbohydrates were added to MS medium at a final concentration of 2% (w/v) after filtering. At quantities of 2 g/L and 0.5 g/L, several carbon and nitrogen sources were used. In MS medium, different hydrocarbon sources were used as a substrate to estimate their effects on bacterial growth and PHA accumulation. Cultivation was carried out at 37°C and a stirring rate of 150 rpm under aerobic conditions. The cost of carbon source alone accounts to about 50% of the production costs of the PHA (Halami, 2007) and therefore, use of inexpensive carbon substrates viz. agroindustrial waste or sewage sludge can contribute to as much as 40-50% reduction in the overall production cost (Ramadas *et al.*, 2009). It has been reported earlier (Khanna and Srivastava, 2005) that monosaccharides such as glucose and fructose are readily utilized by bacteria and, hence support growth and subsequently PHB production. On the other hand, complex molecules like starch and lactose are not easily utilized for effective PHB production. As the complexity of the carbon sources increases, PHB yield has been found to decrease (Joshi and Jayaswal, 2010). After *A. lwoffii* G3 was tested for the production of PHA in different carbon and nitrogen sources, it was found that all carbon sources were able to enhance and increase the synthesis of PHA. Although all nitrogen sources absolutely affected PHA synthesis, corn oil and urea had the greatest effect, the amount of dry weight exceeded 3.58g per liter, while the production of the polymer reached 56%. This agreed with several studies which stated that vegetable oils can be good carbon substrate for PHA production (Fukui and Doi, 1998; Budde *et al.*, 2010). Yuksekdag *et al.* (2004) reported that complex nitrogen sources increased the yield of PHA whereas Joshi and Jaysawal (2010) observed that better yield of PHA was obtained by different bacterial genus like ,*Bacillus*, *Staphylococcus* and *Pseudomonas* using ammonium sulphate and ammonium phosphate as nitrogen sources than that of yeast extract. Hence, Figures 8 and 9 shows that the medium containing waste corn oil and urea as a source of carbon and nitrogen, respectively, seemingly had a positive effect on PHA production. According to (Halami, 2007), the cost of carbon source only is about 50% of the costs of PHA production and therefore, the use of low-cost carbon substrates such as active sludge and agro-industrial residues can give a share in reducing

the total production costs by 40-50% (Khardenavis *et al*., 2005; Ramadas *et al*., 2009). Therefore, it can be concluded that the best final combination of MS medium contains corn oil residues as carbon sources and Yoruba as nitrogen sources at a concentration of 2% and 0.5 g/L, respectively. In order to reduce the cost of production and obtain high productivity for bioplastics, attention has been directed recently to exploring the best carbon substrates that are distinguished by their inexpensive and renewable (Poirier *et al*., 1995). The UV-transformed crotonic acid spectrophotometer method was used to detect the cellular content of PHA granules. The solution of dissolved polymer was cooled and the absorbance was measured at different wavelengths between 200-600 nm against a concentrated sulphuric acid as blank in the spectrophotometer.

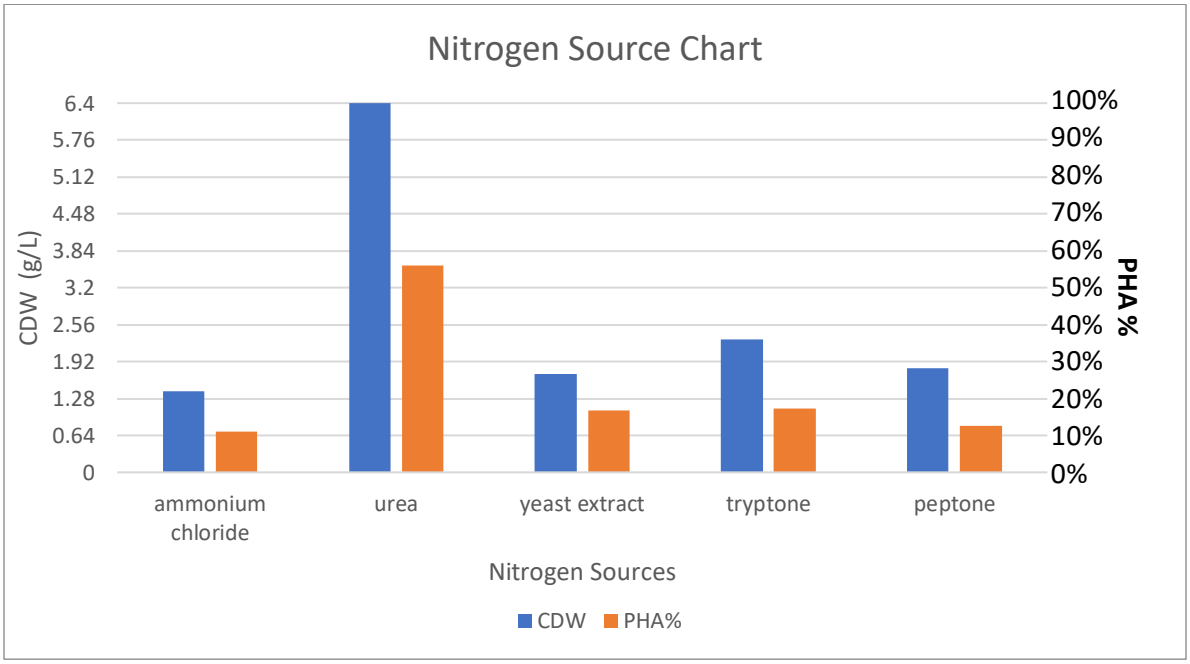

Figure 8: Effect of various nitrogen sources on bacterial growth and PHA production.

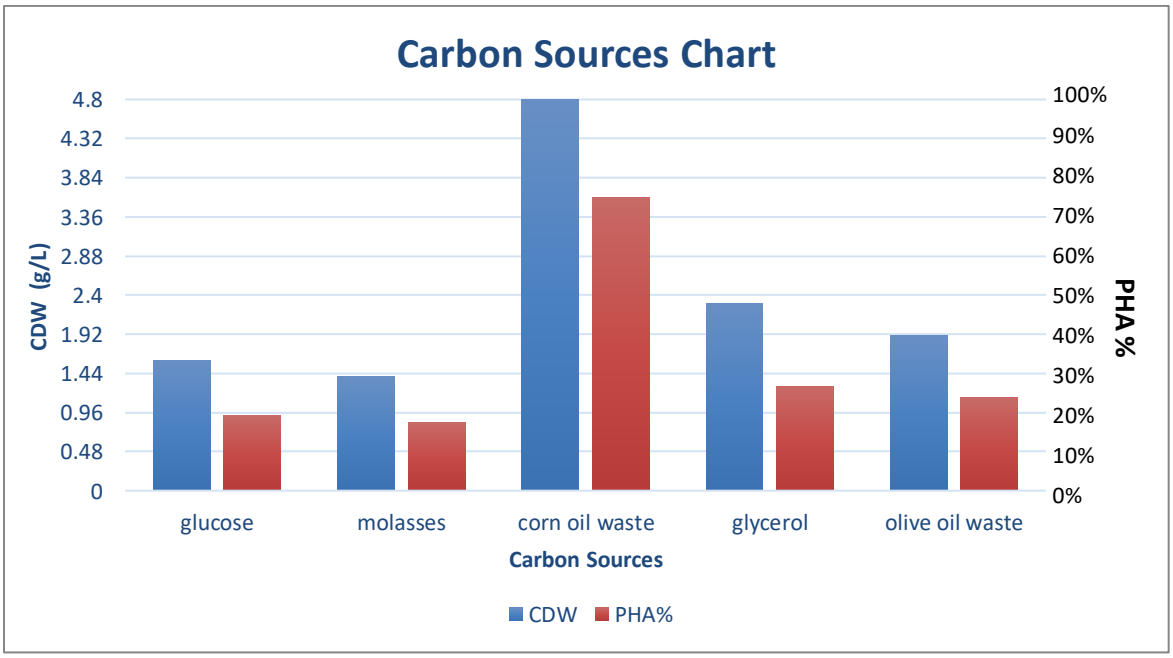

Figure 9: Effect of various carbon sources on bacterial growth and PHA production.

Figure 10 shows the best wavelength for detecting the polymer is 235, where the highest reading was recorded at this wavelength, which confirms that the polymer is a type of polymer (Valappil *et al.*, 2007). This analytical method used to determine PHA accumulation in bacteria which is based on the degradation of PHA with sulfuric acid to crotonic acid. The crotonic acid thus formed can be estimated by measuring the absorbance at 235 nm and therefore used for quantifying the polymer.

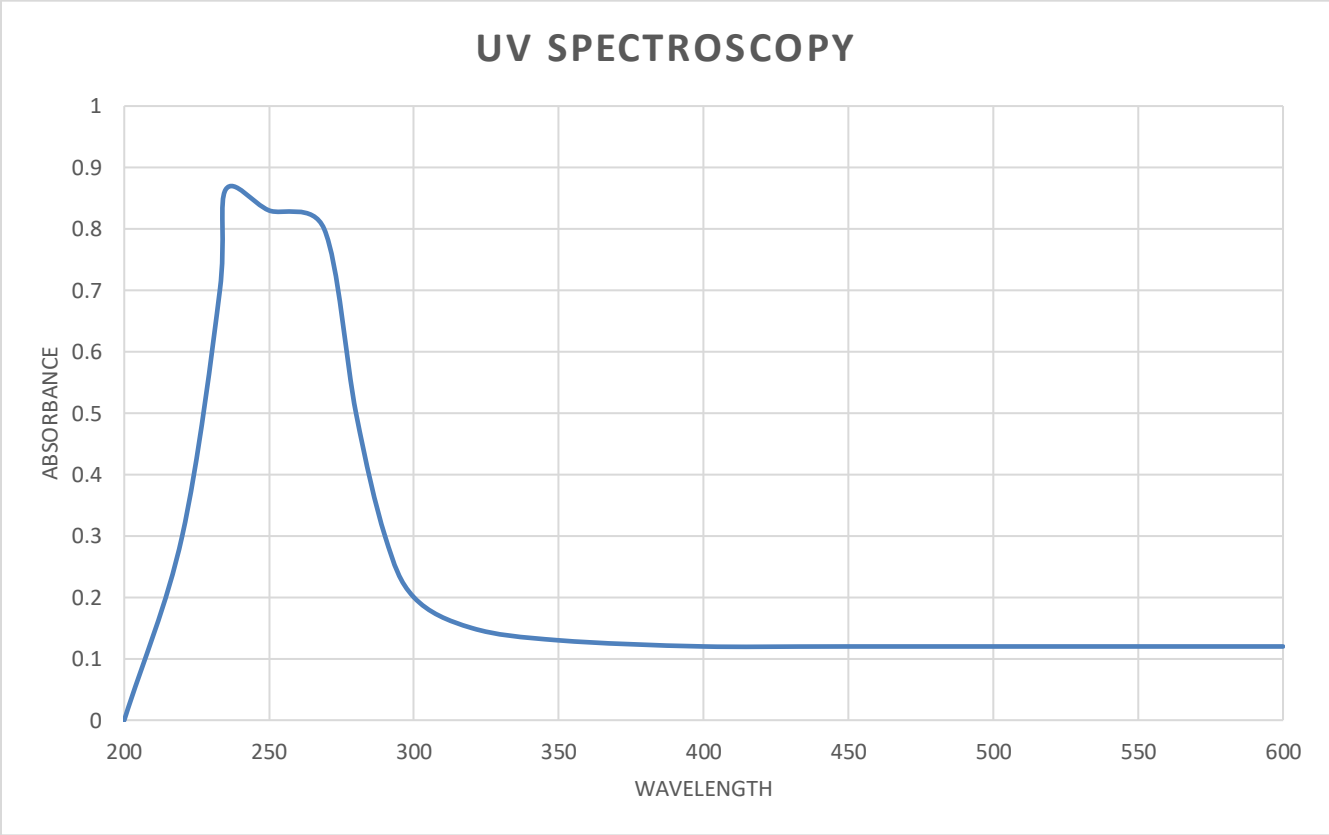

Figure 10: UV absorbance spectrum of PHA extracted from *A. lwoffii.*

4. Conclusions

The major objective of the present study was to optimize polyhydroxybutyrate producing isolate culture conditions so as to obtain the maximum PHA yield by native isolate of *A lwoffii*. The study of the appropriate time period for the growth and production of PHA for this isolate in the salt medium showed that the production of the polymer is related to the stationary phase of growth. Using spectrophotometry, the results showed that the absorbance peak of the polymer was recorded at 235 nm. It was found that *A. lwoffii* accumulated at a high level of PHA under optimal culture conditions after multiple tests of various conditions and food components; thus, the possibility of its exploitation in the production of PHA appears on an industrial scale. The high cost of biodegradable PHA production is due to the cost of the carbon source, fermentation process and other feedstock components and extraction. Therefore, the use of cheap agricultural industrial waste such as molasses, whey and oil residues to produce PHA can reduce 40-50% of the total production cost The present study provided useful data about the optimal conditions for the production of PHA that can be used for the industrial production of the polymer, which is the best and safest alternative to non-biodegradable plastics using vegetable oils exactly so waste cooking oil are a good economical source of carbon for production of PHA.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] A. Hussein, M. Alzuhairi; and N. Aljanabi. Degradation and Depolymerization of Plastics Waste by Local Bacterial Isolates and Bubble Column Reactor. Technologies and Materials for Renewable Energy, Environment and Sustainability TMREE18 Int'l Conf, pp.435-454, 2018.

[2] A. H. Amal Al-Obaidi, "Biodegradation of low-density polyethylene (LDPE) by local bacterial isolates from soil and plastic waste". A Thesis submitted to the council of the collage of science as a partial fulfillment of the requirement for the degree of doctorate of philosophy in biology university of Baghdad, 2015.

[3] Y. M. Varsha and R.Savitha. "Overview on polyhydroxyalkanoates: A promising biopol", *J. of Microbial and Biochem. Technol.*, 3, 99–105, 2011.

[4] A.J. Anderson and E.A. Dawes., "Occcurence, Metabolism, Metabolic Role and Industrial uses of Bacterial Polyhydroxyalkanoates", *Microbiol. Rev*., 54, pp. 450-472, 1990.

[5] J. Ravenstijn, "Bioplastics in consumer electronics", *Ind. Biotechnol*. 6, pp. 252-263, 2010.

[6] N. H. B Aljanabi "Chemical and Biological Treatment of Plastic Waste by Acinetobacter baumannii isolated from soil samples", A Thesis submitted to the council of the collage of science as a partial fulfillment of the requirement for the degree of MS in applied science, University of technology department of applied science, 2018.

[7] H. B. Noor, "Chemical and Biological Treatment of Plastic Waste by *Acinetobacter baumannii* Isolated from Soil Samples" A thesis Submitted to the School of Applied Science, University of Technology in a Partial fulfillment of the Requirements for the Degree of M.Sc. in Biotechnology, 2018.

[8] M. Titz, K.H. Kettl, K.Shahzad, *et al.*, Process optimization for efficient biomediated PHA production from animal-based waste streams. *Clean Technol. Environ. Policy.;* 14, pp. 495–503, 2012.

[9] H. Matsusaki., H. Abe, and Y Doi., "Biosynthesis and properties of poly (3-hydroxybutyrate-co-3-hydroxyalkanoates) by recombinant strains of Pseudomonas sp 61-3", *Biomacromolecules* 1, pp. 17–22, 2000.

[10] H. G. Eman, "Biosynthesis and Characterization of bioplastic (Poly-B-hydroxybutyrate) by Pseudomonas aeruginosa local isolate using waste oil. A thesis submitted to the council of the collage of science as partial fulfillment of the requirements for the degree of doctorate of philosophy in biotechnology university of Baghdad, 2017.

[11] J. Yu and J. Wang., "Metabolic flux modelling of detoxification of acetic acid by *Ralstonia eutrophus* at slightly alkaline pH levels", *Biotechnol. Bioeng*. 73, pp. 458-464, 2001.

[12] Y. Poirier, C Nawrath, C. Somerville., "Production of polyhydroxyalkanoates, a family of Biodegradable plastics and elastomers, in bacterial and plant". *Biotechnol*. 13: pp. 142-150, 1995.

[13] D.Y. KimY.B, Kim and Y.H. Rhee., "Evaluation of various carbon substrates for the biosynthesis of polyhydroxyalkanoates bearing functional group by Pseudomonas putida", *Int J Biol Macromol* 28, pp. 23–29, 2000.

[14] K. Kemavongse, P. Prasertsan, A Upaichit *et al*., Poly-β-hydroxylkanoate production by halotolerant *Rhodobacter sphaeroides* U7. *World J Microbiol Biotechnol*. 24, pp. 2073-2085, 2008.

[15] P. Kumari, and K. Dhingra Harish., "Isolation and Characterization of PHB Producing Microorganisms isolated from Root Nodules of Leguminous Plants". *The Bioscan*, vol. 1, no. 88, pp. 109 – 111, 2013.

[16] J. R. Quillaguaman, B. Hatti Kaul, M. T Mattiasson, et *a*l, "*Halomonas boliviensis* sp. nov., an alkalitolerant, moderate halophile bacterium isolated from soil around a Bolivian hypersaline lake", *Int. Journal of System. Evolution. Microbiol.*, 54, pp. 721 – 725, 2004.

[17] Z.N. Yuksekdag, B. Aslım, Y. Beyatli *et al*. "Effect of carbon and nitrogen sources and incubationtimes on poly-β-hydroxybutyrate (PHB) synthesis by Bacillus subtilis 25 and *Bacillus megaterium* 12". Afr. J. Biotechnol., 3, pp. 63-66, 2004.

[18] K. Nehra, N Dahiya, P. Lathwal *et al*. "Influence of nutritional and environmental parameters on PHB production by bacteria isolated from contaminated soils", *Int. J. Pharma Biosc.*, vol 1, no. 6B, pp. 676–691, 2015.

[19] D. Marjadi and N. Dharaiya., "Bioprospecting and Characterization of poly-β- hydroxyalkanoate (PHAs) producing Pseudomonas Spp. isolated from edible oil contaminated soil", *Res. Biotechnol*. 3:5, 2012.

[20] P. Adwitiya, P. Ashwini and A. K. Avinash, *et al.*, "Optimisation of process parameters for maximum poly (-β-) hydroxyl butyrate (PHB) production by *Bacillus thuringiensis* IAM 12077", *Polish J. of Microbiol*, vol.58, no. 2, pp. 149-154, 2009.

[21] C.S.K. Reddy, R. Ghai Rashmi and V.C. Kalia., "Polyhydroxyalkanoates: an overview". *Biores Technol* 87, pp. 137– 146, 2003.

[22] B. Kessler1 and N J. Palleroni2., "Taxonomic implications of synthesis of poly-beta-hydroxybutyrate and other poly-beta-hydroxyalkanoates by aerobic pseudomonads", *Int. J. of System and Evolution*. Microbiol, 50, pp. 711–713, 2000.

[23] P. T. Dhawal B. K. Mayur. *et al.*, "Exploring the ability of *Sphingobacterium* sp. ATM to degrade textile dye Direct Blue GLL, mixture of dyes and textile effluent and production of polyhydroxyhexadecanoic acid using waste biomass generated after dye degradation" *J. of Hazard.Mat.*, vol. 182, no. 1–3, pp. 169-176, 2010.

[24] V. Saranya and R. Shenbagarathai., "Effect of nitrogen and calcium sources on growth and production of PHA of *Pseudomonas* sp. LDC-5 and it's mutant", *Curr Res J Biol Sci*., 23, pp. 164-167, 2010.

[25] M. G. Kulkarni and A. K. Dalai, "Waste cooking oils an economical source for biodiesel", A rev. *J of Indust.and Engin .Chem.* Res. 45, pp. 2901 – 2913, 2006.

[26] P.M Halami., "Production of polyhydroxyalkanoate from starch by the native isolate Bacillus cereus CFR06", *World J. Microbiol. Biotechnol.*, 24,pp. 805-812, 2008.

[27] A. P.K Khardenavis,. M.S Guha, S.N Kumar, *et al*., "Activated sludge is a potential source for production of biodegradable plastics from wastewater", *Environ. Technol*. 26, pp. 545-552, 2005.

[28] N.V. Ramadas; S.K. Singh, C.R. Soccol et *al*., "Polyhydroxybutyrate production using agro-industrial residue as substrate by *Bacillus sphaericus* NCIM 5149". Braz. Arch. Biol. Technol., 52, pp. 17-23, 2009.

[29] Y. Poirier; C. Nawrath & C. Somerville"Production of Polyhydroxyalkanoates, a Family of Biodegradable Plastics and Elastomers, in Bacteria and Plants "*Bio/Technol.* vol. 13, pp. 142–150, 1995.

[30] M. Kazem, Kazem and A. A. Hussein. Biological treatment *of PAHs using genetically modified local bacterial isolates. IOP* Conf. Series: Earth and Environmental Science 779 012105, 2021.

[31] A. Hussein, M. Alzuhairi and M. T. Abeas. Depolymerization of polyethylene terephthalate (water bottles waste) using Nano-fluid catalyst. *Indian J Ecology*., vol. 9, no. 47, 2020.

[32] H. Jasim, Bilal and Entesar H. Ali. Enhanced Production of Fibrinolytic Enzyme from Pseudomonas aeruginosa by Optimization Media Components. *Journal of Applied Sciences and Nanotechnology*, vol. 1, no. 2, pp. 58-65, 2021.

[33] A. Jabbar, Raghad and Nehia N. Hussein. Evaluation The Antibacterial Activity of Biosynthesis Silver Nanoparticles by Lactobacillus Gasseri Bacteria. *Journal of Applied Sciences and Nanotechnology*, vol. 1, no. 3, pp. 86-95, 2021.

[34] H. Abdul Hameed, Dina and Entesar H. Ali. Obtaining L - Glutamate Oxidase Enzyme from Streptomyces and Estimation Its Toxicity on Blood Components. *Journal of Applied Sciences and Nanotechnology*, vol. 1, no. 4, pp:61-68, 2021.

[35] S.P. Valappil, A.R.Boccaccini, Bucke, C.; Roy, I. Polyhydroxyalkanoates in Gram-positive bacteria: Insights from the genera Bacillus and Streptomyces. *Antonie Van Leeuwenhoek*., 91, pp. 1–17, 2007.

[36] N.V Ramadas, S.K. Singh, C.R. Soccol and A. Pandey. Polyhydroxybutyrate production using agro-industrial residue as substrate by *Bacillus sphaericus* NCIM 5149. *Braz. Arch. Biol. Technol.*, 52, pp. 17-23, 2009.

[37] P. M. Halami, Production of polyhydroxyalkanoate from starch by the native isolate *Bacillus cereus* CFR06. *World J. Microbiol. Biotechnol*., 24: pp. 805-812, 2008.

[38] P.A. Joshi, and S.R. Jaysawal. Isolation and characterization of poly-β-hydroxyalkanoate producing bacteria from sewage sample. *J. Cell Tissue Res*., 10: pp. 2165-2168, 2010.

[39] S. Khanna, and A.K. Srivastava, Statistical media optimization studies for growth and PHB production by *Ralstonia eutropha*. *Process Biochem*., 4: pp. 2173-2183, 2005.

[40] E. Grothe, M. Moo-Young, and Y. Chisti. Fermentation optimization for the production of poly-(β -hydroxybutyric acid) microbial thermoplastic. *Enzym. Microbial. Tech*., 25: pp. 132-141, 1999.

[41] C. Budde, S. Riedel, F. Hübner, S. Risch, Popović, M., Rha, C., Sinskey, A. Growth and polyhydroxybutyrate production by *Ralstonia eutropha* in emulsified plant oil medium. *Applied Microbiology and Biotechnology*, vol. 5, no. 855, pp. 1611-1619, 2010.

[42] T. Fukui, and Y. Doi, Efficient production of polyhydroxyalkanoates from plant oils by *Alcaligenes eutrophus* and its recombinant strain. *Applied Microbiology and Biotechnology*, 49, pp. 333-336, 1998.

[43] S.V. Reddy, M. Thirumala, and S.K. Mahmood. Production of PHB and P(3HB-co-3HV) biopolymers by *Bacillus megaterium* strain OU303A isolated from municipal sewage sludge. *World J. of Microbiol. Biotechnol.*, vol. 3, no. 25, pp. 391-397, 2009.