Sustainable Environment and the built Space: Integrating Ecology, Planning and Human Well-being
Proceedings of the 6th International Conference (FESCON 2026) at Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Uli, Nigeria
18 - 19 June 2026

PRODUCTION OF BIOPLASTIC (POLYHYDROXYBUTYRATE, PHB) USING BACTERIA ISOLATED FROM SLAUGHTERHOUSES IN OGWASHI-UKWU TOWN, DELTA STATE

Uba, B. O[1]*., Dokubo, C. U.[2] and Akaun, I. P.[3]
[1]*Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, P.M.B. 02 Uli, Anambra State, Nigeria*
[2]*Department of Science and Laboratory Technology, Delta State Polytechnic Ogwashi – Uku, Delta State Nigeria*
[3]*Department of General Studies, Delta State Polytechnic, Ogwashi-Uku, Delta State, Nigeria*

Corresponding Author*: [1]bo.uba@coou.edu.ng

Abstract

This study was designed to produce bioplastic (Polyhydroxybutyrate, PHB) using bacteria isolated from slaughterhouses in Ogwashi-Ukwu town, Delta State. The collected slaughter house samples were cultured on nutrient agar, incubated at 370C for 48 hrs and later sub cultured on Luria Bertani agar plates. The bioplastic producing bacteria were screened, confirmed using Sudan B black and Nile blue staining techniques, respectively. Classical morphological and biochemical tests were used to identify the five higher reactive bacterial strains as Bacillus spp. These bacterial strains were subjected to different analysis including growth rate, dry weight and residual biomass. The results revealed that most strains (8 out of 10) showed positive results (++ or +) for Sudan Black B staining, indicating the presence of bioplastic. Also, 5 out of 10 strains showed positive results (+ or ++) for Nile Blue staining, indicating the presence of specific types of bioplastics. The consortium of BPO1 and BPO7 showed higher growth (OD600 nm = 1.05) and bioplastic production (PHB = 2.50 g/L) compared to individual strains. The consortium also showed a higher PHB content (62.50% w/w) compared to individual strains. Based on the biochemical characteristics, all isolates were identified as Bacillus sp. The results suggest that the Bacillus sp. isolates may have potential applications in various fields, such as biotechnology or industry.

Keywords: Bioplastics, Ogwashi-Ukwu, Slaughterhouses

1.0 INTRODUCTION

Petroleum based polymers are undisputable from the world as it offered excellent support in various technology and applications for the benefits of human kind. However, the origin of this polymer is from petroleum, hence, it is non-biodegradable, persists in the environment for a prolonged period and causes severe ecological reduction by unbalancing the environmental conditions. Besides that, during fabrication and incineration of the petroleum - based polymers, these create serious air, water, and soil pollutions. These lead to cause health issues to humans, animals and entirely collapse the biological systems and cycles.

Bioplastics otherwise known as biopolymers, polyhydoxyalkanoates (PHAs) are plastic materials produced from renewable biomas sources, such as vegetable fats and oils, corn starch, straw, woodchips, sawdust, recycled food wastes as well as other biodegradable agricultural wastes (Siracusa & Blanco, 2020).

Increased demand and drive towards search for an attractive, alternative and sustainable bio-based materials to replace fossil fuel-based plastics in different industrial applications has led to the substantial interest in Polyhydroxyalkanoates (PHAs) (Koller & Rodriguez, 2018, Shamsuddin et al., 2020).

PHA is insoluble in water and exhibit an amorphous mobile liquid state in vivo but in laboratory settings, it displays variety of mechanical and material properties, from rigid and brittle crystals to elastomers and molds (Danial et al., 2021). Biochemically, PHA biosynthetic pathway is initiated by condensation of two molecules of Acetyl Co Enzyme A (Acetyl-CoA) by $\beta - ketothiolase$ to Acetyl-CoA which is then reduced to 3-hydroxybutyryl-CoA by Acetyle-CoA reductase and then be polymerized into PHA-by-PHA synthase (Llimos et al., 2020). PHA degrades quickly into carbondioxide, water, methane and biomass due to the enzymatic activities of microbes (Rhatika et al., 2019). PHA properties and qualities are dependent on monomer composition. They are stratified into three classes (Scl-PHA, Mcl-PHA and Icl-PHA) on the basis of their monomeric unit. Scl-PHA have less than 5 carbon atoms, mcl-PHA have six to fourteen carbon atoms while Icl-PHA have more than fourteen carbon atoms but are rare and understudied (Johri et al., 2021). PHA is stored intracellularly as granules and the size of the granules varies depending on the microorganism (D' Anna et al., 2019).

The environmental and health concern brought poly-β −hydroxybutyrate (PHB) as sustainable and the best alternative to synthetic plastic and an ideal material for making biodegradable plastics, although it is completely biodegradable in a natural environment after disposal (Tripathi & Srivastava, 2019). Although PHB has been found as an eco-friendly biopolymer, the cost associated with carbon substrate used in the fermentation, has a major limiting factor in the commercialization of biodegradable polyesters (Gabr, 2019, Hong et al., 2019).

The vast application possibilities of chemical polymers are inevitable substances in the world due to its excellent benefits like being less weight, chemically inert, comfortable, durable, flexible to make desire shape and size (Bose et al., 2020). There has been increasing demand to fabricate and use biodegradable polymeric materials from biological (microbes, plants, etc) and biomolecule sources such as polysaccharides, proteins and lipids which have been used as an expensive and renewable alternate for traditional polymers (Rathika et al., 2019). Fortunately, nature offers an alternative for petroleum-based polymers from natural biological resources (El-kadi et al., 2021). Among the various biopolymers, the polyhydroxybutyrate (PHB) is a preferable one due to high thermosplastic like traditional polymers and possesses most suitable physical, mechanical, immunological properties, which promotes this PHB as suitable alternate for traditional polymers (Corchado et al., 2021). Among the microbes like bacteria, fungi, yeast, algae e.t.c., the bacteria-based biopolymers fabrication gain more attention due to its easy control, maximum biomass, simple downstream processing and more gene modification possibilities (Johro et al., 2021). Several abounds on the production of polyhydroxybutyrate (PHB) by bacterial strains from different sources. There is paucity of information on the bioplastic production from biogas slurry and therefore necessitated this study. The present study is designed to produce bioplastic (Polyhydroxybutyrate, PHB) using bacteria isolated from slaughterhouses in Ogwashi-Ukwu town, Delta State.

2.0 MATERIALS AND METHODS

2.1 Description of Sampling Site

The site of collection for this study was around the slaughter house within, Ogwashi – Ukwu modern market with coordinates of 6°10'59.06" N latitude and 6°31'27.72"E longitude located west of the state capital Asaba, which is the headquarters for the Local Government Area, Aniocha South Delta State with a population of about 50,234 people. Slaughter house of Ogwashi Ukwu modern market shall be chosen for the sample collection site due to enormous anthropological activities that are taking place there.

2.2 Sample Collection and Processing

The water samples were aseptically collected randomly from five different sampling points using a sterile labelled 2 L container and mixed to have a representative sample. The samples were placed in ice packed coolers and sent to the Laboratory. The water samples will be filtered through Whatman No. 1 filter paper, the residue was discarded and the filtrate was stored in the refrigerator (40C) for subsequent processing (Uba et al. 2019).

2.3 Serial Dilution Technique

The slurry shall be suspended in sterilized saline solution (0.85% w/v) and shall be streaked on nutrient agar medium containing 5.0g/L peptone, 3.0g/L beef extract, 5.0g/L sodium chloride and 15g/L agar. pH shall be adjusted at 7.2 ± 0.2.

2.4 Isolation of Predominant Bacteria

Standard serial dilution practices shall be performed in sterile water, 0.1ml of 10-6 serial dilution shall be taking and inoculated on sterile Luria Bertani (LB) agar plate by using a spread plate method and using sterile cotton swab according to Bhuwal et al., (2020). Triplicate plates shall be inoculated and incubated for 48hrs at 370C with proper labeling with sterile control. Major diverse colonies shall be selected and sub cultured by the streak plate method on the LB agar plate to be used for further study. The different calories shall be picked and subjected to series of steps for obtaining purified strain.

2.5 Screening of PHBs Producing Bacteria

The primary screening of PHBs producing isolates shall be achieved by following the method and techniques described by Khardenavis et al., (2019). Predominant bacterial isolates shall be chosen for qualitative analysis of PHBs producing bacterial isolates by viable colony method using Sudan Black B stain as described by Juan, Gonzalez and Walker, (2021). The test isolates shall be inoculated on the LB agar medium (enriched with 1% of sterile glucose) by spread plate inoculation. The inoculated plates shall be incubated for 24hrs at 370C. After the incubation, 0.3% of Sudan Black B stain shall be gently spread over the colonies and shall be retained uninterrupted for 30 mins. The excess stain from the plate shall be washed with ethanol. The development of dark blue colored colonies shall indicate the PHBs producer. The positive isolates shall be further subjected to Nile Red staining for confirmation of PHB accumulation.

2.6 Confirmation of PHB Producing Bacteria Using Nile Red Staining

The screening method described by Spiekermann et al. (2019) shall be undertaking and shall be conducted for detecting the ability of the bacterial isolates to accumulate PHBs. The strains to be isolated shall be grown on Mineral Salt Medium (MSM) and shall be supplemented with 20g/L glucose as carbon source. 1.2mg/L Fe (III) NH4 and citrate 0.1ml/L. The glucose shall be autoclaved separately and shall be allowed to cool down to 55 0C. In addition, MSM shall also be supplemented with a solution of 0.25mg Nile Red and bacterial strain shall be incubated at 37 0C for 5 days. The agar plates shall be exposed to ultraviolet (UV) light (312nm) to confirm the accumulation of PHBs in positive isolates.

2.7 Characterization of PHBs producing bacterial isolates

The primary and secondary screening shall confirm a single isolate that possesses PHB producing potential. This PHBs producing isolate shall be characterized by standard microscopic (staining), macroscopic (colony morphology, appearance, shape etc.), biochemical tests and growth on selective media (EMB agar media).

2.8.1 Biochemical tests for PHB detection

The following biochemical tests shall be carried out in order to identify and characterize PHB producers. The biochemical tests shall be Oxidase, Catalase, Nitrate reduction, Starch hydrolysis, Methyl red, Vogues Proskauer, Citrate utilization, Urea hydrolysis, Casein hydrolysis, Triple sugar iron, Gelatin hydrolysis, Hydrogen sulphide production and dehydrogenase (Sambrook & Russell, 2021).

2.8.2 Sugar Utilization Test

The following sugars shall be used for sugar utilization test in order to confirm whether PHB to be produced can make use of any of these sugars for its carbon source: Arabinose, Fructose, Mannitol, Maltose, Sucrose, Dextrose, Galactose and Glucose (as described by Sambrook & Russell, 2021).

2.9 Media Composition for PHB production

HM-2 media shall be used with the following composition (percentage, w/v) MgSO4 . 7H20 (0.037), KH2PO4 (0.02), CaCl2, 2H2O (0.012), NaCl (4.5), KCl (0.074), Peptone (0.025), NaBr (0.02), Yeast extract (0.1). Furthermore, the substrate shall be supplied with glucose and trace element solution which shall be inoculated with 5% v/v inocular of selected bacterial strains which shall be incubated at 370C for 72hrs.

2.10 Extraction assay for PHBs

After three days of incubation, the bacterium from all the setup shall be separated from media following the method of Aljuraifani et al., (2021) with slight modifications. The fermented media containing the bacterium shall be centrifuged at 1000 rpm for 10 minutes and the obtained pellets shall be suspended with the identical quantity of sodium hypochloride (NaClO) and shall be incubated at 450C for 3hrs. the extract shall be centrifuged at 7000rpm for 15minutes and the pellets containing polymers shall be collected after centrifugation. Then the pellets shall be suspended in an equal volume of 4% sodium hypochlorite and shall be incubated at 370C for 24hrs. the pellet shall be rinsed with a 2:1 ratio of ethanol and acetone. Undissolved debris in the pellet shall be removed by dissolving in 5ml boiling chloroform and shall be centrifuged at 7000rpm for 10minutes. The obtained final pellet shall be allowed to air dry by keeping open for chloroform evaporation and yield of PHBs shall be determined as mg/L. PHB granules shall be observed more colourful and clearer. Positive PHB isolates shall appear as being black while bacterial cytoplasm shall be appeared red.

2.10.1 Determination of the Cell Dry Weight

After incubation, bacterial culture shall be centrifuge (10,000 rpm) for 15 mins. The suspended pellet shall be suspended in 1ml distilled water and shall be transferred to pre weighed boats and dried to constant weight at 600C. Cell pellets shall be dried to determine dry cell weight (DCW) as a g/L unit.

2.11 Percentage PHB Quantification

2.11.1 Analysis method

The liquid phase containing PHB shall be made to form pellets. The pellets shall be washed twice with distilled water and sterilized water and shall be dried in an oven at 1000C for 10 minutes. Also, 10 ml sulphuric acid (H2SO4) shall be added to the biomass in the tube for the conversion of PHB to crotonic acid and the samples shall be placed in water bath at 1000C for 1hr. The volume of remaining sample in the water bath shall be kept and stored at -40C. However, 0.2ml of the residual sample shall be picked up and placed in Spectrophotometer to determine the amount of polymer produced on the basis of observed absorption in the presence of produced crotonic acid. The maximum absorbance of each sample at 235nm which is the maximum absorbance for crotonic acid and also PHB shall be noted in that crotonic acid absorbs in the range 100 – 1000 μg/ ml. UV irradiation at 235nm which shall appear as a sharp peak in the spectrum. The extent of UV irradiation absorbed by crotonic acid shall be directly correspond to the amount of PHB present in the sample since PHB is converted to Crotonic acid.

The following equation shall be used to determine the amount of produced PHB in samples (as described by Gnanasekhar, 2012).

$$y = 0.0226\,x - 0.0945$$

Where y is the absorbance, and x is the amount of crotonic acid corresponding to PHB content in μg.

$$\text{PHB}\ \% = \frac{X}{CDW} \times 100$$

CDW is the cell dry weight obtained by weighing method.

2.12 Data Analysis

The data will be presented in tables and charts and the means statistically analyzed using GraphPad Prism version 9.0.0 by adopting Analysis of Variance (ANOVA). Values less 0.05 are considered significant.

3.0 RESULTS

Table 1: Bioplastic profile of the selected bacterial strains

Strain	Sudan B black	Nile blue
BPO1	+	++
BPO2	++	-
BPO3	++	_
BPO4	+	_
BPO5	++	+
BPO6	++	+
BPO7	++	++
BPO8	++	-`
BPO9	++	+
BPO10	+	-

Key: ++ = Strongly reactive; + = Weakly reactive; - = Non - reactive

Table 2: Biochemical characteristics of the isolated and selected bacterial strains

Isolate/ Parameter	BPO1	BPO5	BPO6	BPO7	PHB9
Gram reaction	Positive	Positive	Positive	Positive	Positive
Cell shape	Rod	Rod	Rod	Rod	Rod
Oxidase	Positive	Positive	Positive	Positive	Negative
Hydrogen sulphide	Positive	Negative	Positive	Positive	Positive
Glucose	Positive	Positive	Positive	Positive	Positive
Sucrose	Negative	Positive	Negative	Positive	Negative
Lactose	Negative	Positive	Negative	Positive	Negative
Citrate	Positive	Negative	Positive	Positive	Positive
Indole	Negative	Negative	Negative	Negative	Negative
Nitrate reduction	Positive	Positive	Positive	Positive	Positive
Voges Proskauer	Positive	Positive	Positive	Positive	Positive
Xylose	Positive	Positive	Positive	Positive	Positive
Inositol	Positive	Positive	Positive	Positive	Positive
Arabinose	Positive	Positive	Positive	Positive	Positive
Galactose	Positive	Positive	Positive	Positive	Positive
Methyl red	Negative	Negative	Negative	Negative	Negative
Catalase	Positive	Positive	Positive	Positive	Positive
Identity	Bacillus sp.	Bacillus sp.	Bacillus sp.	Bacillus sp.	Bacillus sp.

Key + = Reactive; - = Non - reactive

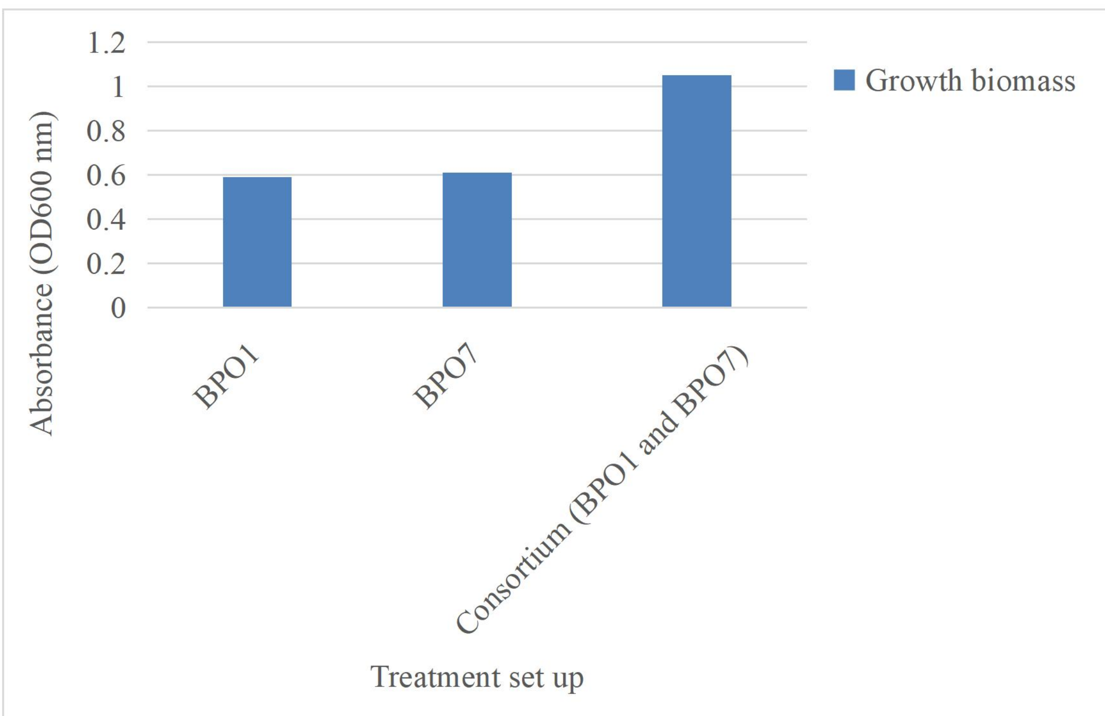

Figure 1: Growth biomass of bioplastic of the selected bacterial strains

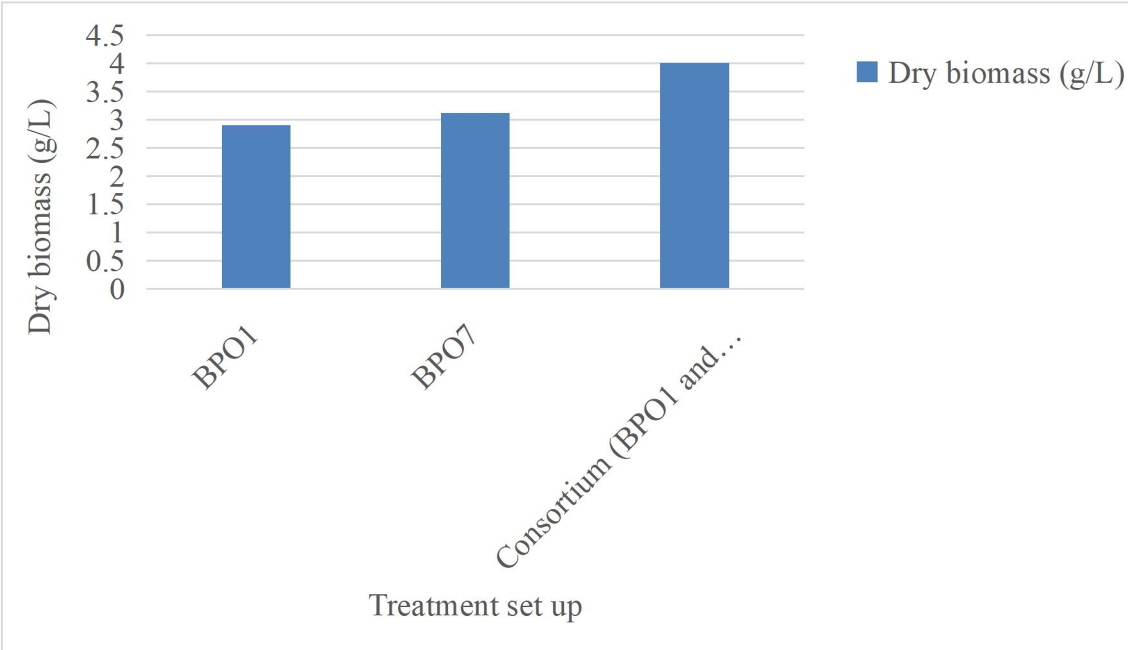

Figure 2: Dry biomass of bioplastic of the selected bacterial strains

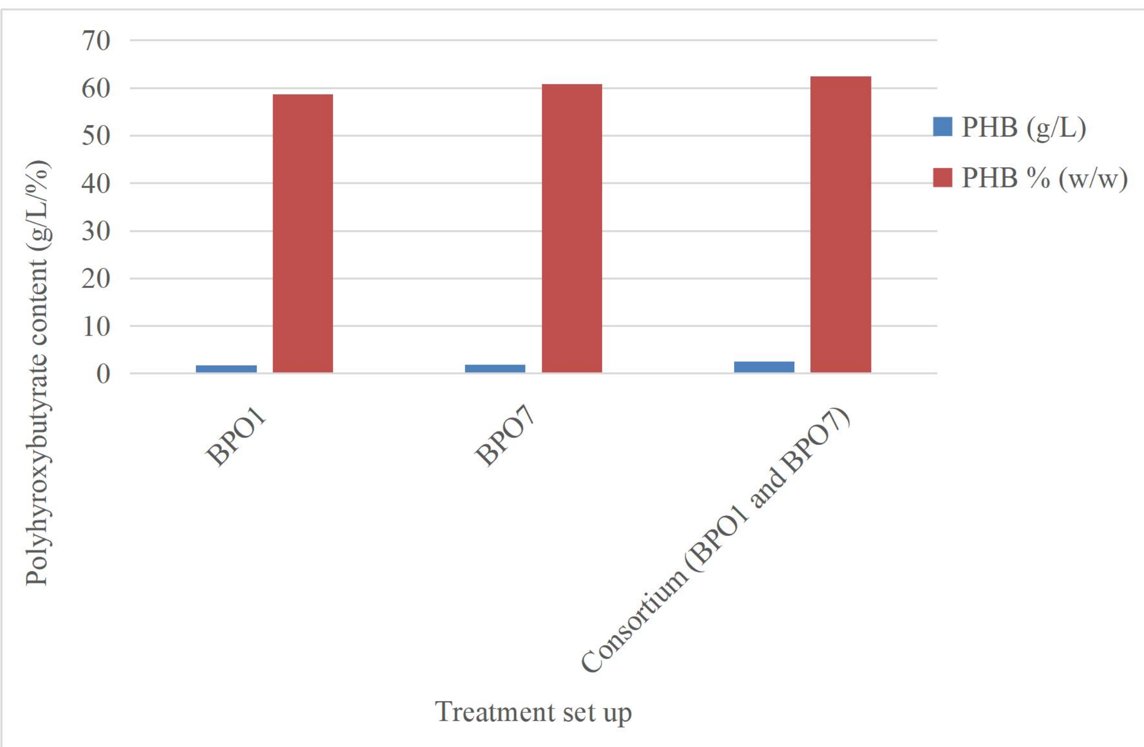

Figure 3: Polyhydroxybutyrate content of the selected bacterial strains

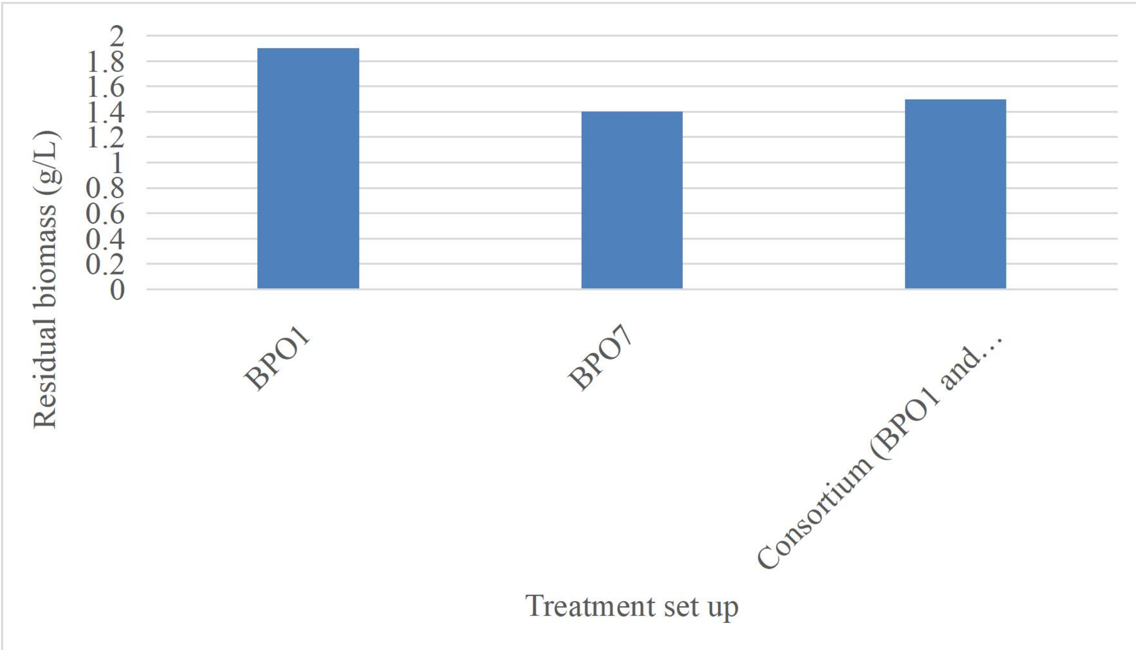

Figure 4: Residual biomass of the selected bacterial strains

Table 4: Bioplastic production profile of the selected bacterial strains

Treatment	Growth (OD600 nm)	Dry biomass (g/L)	PHB (g/L)	Residual biomass (g/L)	PHB % (w/w)
BPO1	0.590	2.90	1.70	1.90	58.62
BPO7	0.610	3.12	1.90	1.40	60.90
Consortium (BPO1 and BPO7)	1.05	4.00	2.50	1.50	62.50

4.0 DISCUSSION

The Table 1 presents the bioplastic profile of 10 bacterial strains, assessed using Sudan Black B and Nile Blue staining. The bacterial strains showed varying levels of bioplastic production, as indicated by the staining results. Most strains (8 out of 10) showed positive results (++ or +) for Sudan Black B staining, indicating the presence of bioplastic. Also, 5 out of 10 strains showed positive results (+ or ++) for Nile Blue staining, indicating the presence of specific types of bioplastics. The results suggest that the selected bacterial strains have the potential to produce bioplastics, which could be explored for biotechnological applications. These observations were in accordance with reports presented by Mathiyazhaghan et al. (2020), Sayyed et al. (2021) and Mohammed et al. (2019) that most PHB bacterial strains are highly or strongly reactive to Sudan B black stain.

The Table 2 presents the biochemical characteristics of five bacterial strains (BPO1, BPO5, BPO6, BPO7 and BPO9) isolated and selected for further study. All isolates showed similar characteristics, such as Gram-positive reaction and Rod-shaped cells. Positive reactions for oxidase, glucose, citrate, nitrate reduction, Voges-Proskauer, and catalase tests. Some isolates showed variations in hydrogen sulphide production (BPO5 showed negative result), sucrose and lactose utilization (variable results among isolates) and oxidase test (BPO9 showed negative result). Based on the biochemical characteristics, all isolates were identified as Bacillus sp. The results suggest that the Bacillus sp. isolates may have potential applications in various fields, such as biotechnology or industry.

The Figures 1, 2, 3, 4 and Table 3 presented the bioplastic production profile of two bacterial strains (BPO1 and BPO7) and their consortium. The consortium of BPO1 and BPO7 showed higher growth (OD600 nm = 1.05) and bioplastic production (PHB = 2.50 g/L) compared to individual strains. The consortium also showed a higher PHB content (62.50 % w/w) compared to individual strains. The results suggest a synergistic effect between the two strains in the consortium, leading to enhanced bioplastic production. The results suggest that using a consortium of bacterial strains could be a promising approach for enhancing bioplastic production.

CONCLUSION

The study showed the Slaughter house in Ogwashi – Ukwu town harboured a lot of bioplastic producers. The variability in bioplastic production among the strains highlights the importance of screening and selecting strains with desired bioplastic production capabilities. Further characterization, such as molecular identification and analysis of specific properties, is necessary to fully understand the potential of these isolates. The high PHB content and production rates achieved by the consortium make it a potential candidate for industrial-scale bioplastic production.

REFERENCES

Abol, F.D., (2020). Bacterial nanocellulose from agro industrial wastes: low cost and enhanced production by Komagataeibacter saccharivorans MDI. *Sci Rep* 10:3491.

Ahmed, O.B., Dablool, A.S., (2017). Quality improvement of the DNA extracted by boiling method in gram-negative bacteria. *International Journal of Bioassays. 6*(4): 5347-5349.

Alabi, O.A., Ologbonjaye, K.I., Awosolu, O., Alalade, O.E., (2019). Public and environment health effects of plastic wastes disposal. A review. *J. toxical risk assess 5* (21):1-13.

Al-Battashi, H. Annamalal, N., Al-kindi, S., Nair, A.S., Al-Bahry, S., Verma, J.P., Sivakumar, N., (2019). Production of bioplastic (poly-3-hydroxy butyrate) using waste paper as a feedstock: Optimization of enzymatic hydrolysis and fermentation employing Burkholderia sacchari. *Journal of cleaner production*. 1:4-5.

Alfano, S., Lorini, L., Majone, M., Sciubba, F., Valentinno F., Valentino, F., Martinelli, A., (2021). Ethylic esters as green solvents for the extraction of intracellular polyhydroxyalanoates produced by mixed microbial culture. *Polymers* 13 (16): 2782-2789.

Aljuraifani, A.A., Berekaa, M.M., Ghazwani, A.A, (2019). Bacterial biopolymers (polyhydroxyalkanoates) production from low-cost sustainable sources. *Microbiology open 8* (6): 755-785.

Aljuraifani, A.A., Berekaa, M.M., Ghazwani, A.A., (2019). Bacterial polymer (polyhydroalkanoate) production from low cost sustainable sources. *Microbiology open. 10* (3):1002.

Alves, M.I., (2019). Optimization of Ralstonia solanacearum cell growth using a central composite rotational design for the P (3HB) production: effects of agitation and aeration. *PLoS One.* 14:1-13.

Aly, M.M., Alharbi, S., Bokhari, F., (2019). Synthesis of biodegrdable polymer (PHB) by Bacillus subtilis isolated from rhizosphere soil sample and optimization of growth conditions. *IOSR Journal of pharmacy and biological sciences. 14* (6): 34-44.

Amadu, A.A., (2021). A review of biopolymer poly-B-hydroxybutyrate synthesis in microbes cultivated on wastewater. *Sci. Total. Environ.* 756: 143729.

Amadu, A.A., Qiu, S., Ge, S., Addico, G.N.D, Ameka , G.K., Yu, Z., (2021). A review of biopolymer (polyhydroxybutyrate) synthesis in microbes cultivated on wastewater. *Science of the total environment 7* (56): 143729 – 143737.

Ansari, S., Fatma, T., (2016). Cyanobacterial polyhydroxybutyrate (PHB); screening, optimization and characterization. *PLOS ONE. 11* (6): 158-168.

Atiwesh, G., Michael, A., Parish, C.C., Banoub, J., (2021). Environmental impact of bioplastic use: A review. *Heliyon* 7 (9): 7918 – 7926.

Barekaa, M., Salama, K., Alkaharsah, K., (2019). Antibiotics sensitivity and heavy metals resistance in PHB-producing bacilli isolated from Eastern province, Saudi Arabia. *International journal of agriculture and biology. 18* (6): 1032-1036.

Baria, D.M., Dodiya, K.R., Shaikh, J.A., Patel, J.M., Rana, J.R., (2023). Isolation, screening and quantifying of polyhydroxyalkanoates (PHA) using FTIR analysis. *Research square. 29*(6): 503-507.

Baria, D.M., Patel, N.Y., Yagnik, S.M., Panchal, R.R., Rajput, K.N., Raval, V.H., (2022). Exopolysaccharide from marine microbes with prowess for environment clean up. E*nviron sci. pollut. Res. 29* (51): 76611 – 76625.

Bejagam, K.K., Gupta, N.S., Le, K.S., Iverson, C.N Marrone, B.L, Pilania, G., (2022). Predicting the mechanical response of polyhydroxyalkanoate biopolymers using molecular dynamics simulations. *Polymers*. 14:345.

Bhuwal, A.K., Singh, G., Aggarwal, N.K., Goyal, V., Yadav, A., (2020). Isolation and screening of polyhydroxyalkanoates producing bacteria from pulp, paper and cardboard industry wastes. *Int. J. Biomaterial*. 20 (13): 1-10.

Bose, S.A, Raja, S., Jeyaram, K., Arockiasam, S., Velmuruga, S., (2020). Investigation of fermentation condition for production enhancement of polyhydroxyalnoate from cheese whey by Pseudomonas sp. *Journal of microbiology, biotechnology and food science. 9*(5): 890-989.

Britto, J.S.J., Priyakumar, A., Nair, N.R., (2023). Production and optimization of bioplastic from Bacillus isolated from environment sample. *International journal of advanced research. 11*(5): 1674-1686.

Chandani, N., Mazumder, P.B, Bhattacharjel, A. (2020). Potential of dumpsite bacterial isolate in producing polyhydroxybutyrate under stress prone environment. *Indian journal of experimental biology*.58:643-650.

Choi, T.R, Park, Y.L., Song, H.S. Lee, S.M., Park, S.L, Lee, H.S (2021). Fructose based production of short-chain-length and medium-chain-length polyhydroxyalkanoate copolymer by Arctic Pseudomonas sp. *Polymers*. 13 (1398): 1-14.

Choi, T.R., Park, Y.L., Song, H.S., Lee, S.M., Park, S.L., Lee, H.S., Yang, Y.H., (2021). Fructose based production of polyhydroxybutyrate by Pseudomonas sp. *Polymers*. 13 (11): 345-351.

Corchado, L.C., Martinez, A.O., Marti, E., Lim'os, J., Busquets, A.M., Kucera, D., Obruca, S., Lienas, L., Ponsa, S., (2021). Brewer's spent grain as a no cost substrate for polyhydroxyalkanoates production. Assessment of pretreatment strategies and different bacterial strains. *New biotechnology* 62:60-67.

D'Anna, A., Arrigo, R., Frache, A., (2019). PLA/PHB blends: Biocompatibilizer effects. *Polymers*. 11:1416.

Denial, A.W., Hamdy, S.M, Alrumman, S.A., Gad, S.M.F Shoreit, A.A.M, Hesham, A.E.L, (2021). Bioplastic production by Bacillus wiedmannii AS-02 OK576278 using different agricultural wastes. *Microorganisms* 9 (2395):1-19.

Dienye, B.N., Agwa, O.K., Abu, G.O., (2022). Molecular characterization, optimization and production of PHA by indigenous bacteria using alternative nutrient sources as substrate. *Microbiology research journal international 32* (12): 12-26.

El-Abd, M.A., El-Sheikh, H.H., Desouky, S., Shehab, A., (2021). Identification, biodegradation and bio-evaluation of biopolymer produced from Bacillus thuringenesis. J*ournal of applied pharmaceutical science. 7* (4): 103-110.

El-kadi, S.M., Elbagory, M., El-Zawaivy, H.A.H., El-Shaer, H.F.A., Shoukry, A.A., El-Nahrawy, S., Omara, A.E.D., Ali, D.F.T (2021). Biosynthesis of polyhydroxybutyrate (PHB) from different bacterial strains grown on alternative cheap carbon sources. *Polymers*. 13 (3801):1-20.

Folino, A., Karageorgu, A., Calabro, P.S, Komilis, D., (2020). Biodegradation of wasted bioplastic in natural and industrial environments: *A review. Sustainability 12* (15): 6030-6037.

Gabr, G.A., (2019). Isolation and identification of bacterial strains able to biopolymer polyhydroxyl butyrate (PHB) Production from soil of Al-kharj probes, Saudi Arabia. *J. pharm. Res. Inter.* 21 (76): 254-261.

Gnanasekhar, J.D., (2012). Production and analysis of Polyhydroxylbutyrate from Halomonas boliviensis. *Sweden KTH Royal Institute of Technology.* 12:14683-86.

Hamdy, S.M., Danial, A.W., Sanaa, M.F., El Ra, G., Shoreit, A.A.M., Hesham, A.E.L, (2022). Production and optimization of bioplastic (polyhydroxybutyrate) from Bacillus cereus strain using response surface methodology. *BMC microbiology.* 22:183.

Hassan, M.A., Amara, A., Abuelhamd, A., Haroun, B., (2010). Leucocytes show improvement growth on PHA polymer surface. *Pak. J. Pharm. Sci.* 23:332-336.

Hong, J.W., Song, H.S., Moon, Y.M., Hong, Y.G., Bhatia, S.K., Jung, H.R., Choi, T.R., (2019). Polyhdroxybutyrate production in halophilic marine bacteria Vibrio proteolyticus isolated from the Korean peninsula. *Bioproc. Biosyst. Eng.* 42: 603-610.

Idris, S., Rahim, A.R., Amiru, A.A., (2022). Bioprospecting and molecular identification of used transformer oil-degrading bacteria for bioplastics production. *Microorganisms*. 10:583-588.

Jain, R., Twari, A., (2019). Biosynthesis of planet friendly bioplastics using renewable carbon source. J*ournal of environmental health, science and engineering. 13* (11): 2-5.

Johri, M., Rajaguru, B., Pillai, G., (2021). Optimization of cultural conditions for polyhdroxybutyrate (PHB) production by Alcaligens sp from mangrove soil. *Journal of advanced scientific research* 19:19-25.

Juan, M.L., Gonzalez, W.G.C. and Walker, A., (2021). Novel screening method for isolating exopolysaccharide deficient mutants. *Appl. Environ. Microbiol*. 64:4600-4602.

Kalia, V.C., Ray, S., Patel, S.K., Singh, G.P., (2019). *Biotechnological applications of polyhdroxy alkanoates. Applications of polyhydroxyalkanoates and their metabolites as drug carriers*: Springer, Singapore.

Karan, H., Funk, C., Grabert, M., Hankamer, B., (2019). Green bioplastics as part of a circular bioeconomy. *Trends plant sci. 24* (3): 237-249.

Kate, S.A., Hire, D.R., Pawar, N.M., (2019). Production and optimization of polyhydroxybutyrate and its application in bioplastic production. *International Journal of Scientific Research in Biological Sciences. 6* (2): 50-58.

Khardenavis, A., Sureshkumar, J., Chakrabarti, T., (2019). Biodegradable plastics production from industrial waste water by activated sludge. In *44th Annual Conference of Association of Microbiologists of India.* 107: 356-362.

Koller, M., Rodriguez, C.A., (2019). Techiques for tracing PHA producing organisms and for qualitative and quantitative analysis of intra and extracellular PHA. *Engineering in life sciences*. 15:558-581.

Kurian, N.S., Das, B., (2021). Comparative analysis of various extraction processes based on economy, ecofriendly, purity and recovery of Polyhydroxyalkanoate: A review. I*nt. J. Biol. Macromol*. 183:1881-1890.

Kurian, N.S., Das, B., (2021). Comparative analysis of various extraction processes based on economy, ecofriendly, purity and recovery of polyhydroxyalkanoate. A review. *Int. J. Biol. Macromol*. 183:1881-1890.

Lee, S.Y., Choi., J., Wong, H., (2019). Recent advances in Polyhydroxyalkanoate production by bacterial fermentation: mini-review. *International Journal of Biological Macromolecules*, 25:31-36.

Liu, F., Li, W., Ridgway, D., Gu, T., (2020). Production of Polyhydroxybutyrate on molasses by recombinant Escherichia coli. *Biotechnology letter*. 20: 345-348.

Llimo's, J., Martinez, A.O., Mart, E. Corchado, L.C., Lienas, L., Geg,T., (2020). Brewer's spent grain biotransformation to produce lignocellulolyte enzymes and polyhydroxyalkanoates in a two-stage volarization scheme. *Biomas conversion and biorefinery*. 12:3921-3932.

Lopez, J.A., Naranjo, J.M., Higuita, J.C., Cubitto, M.A., Cardona, C.A., Villar, M.A., (2021). Biosynthesis of PHB from a new isolated Bacillus megaterium strain: outlook on future developments with endospore forming bacteria. *The Korean society for biotechnology and bioengineering 17* (10): 250-258.

Mahato, R.P., Kumar, S., Singh, P., (2021). Optimization of growth conditions to produce sustainable. Polyhydroxyalkanoate bioplastic by Pseudomonas aeruginosa EQI. *Frontier in microbiology*. 12 (711588):1-9.

Mahitha, S., Sasi, P.J.M., Abiya, C.D., (2023). Optimization of culture conditions for polyhydroxybutyrate (PHB) production in the positive bacterial isolates of the garden soil. *Biological forum: an international journal. 15* (3): 192-198.

Mahmoud, M.B & Al lssa, A.M., (2020). Enhanced production of polyhydroxybutyrate (PHB) from agro-industrial wastes: Fed batch cultivation and statistical. *Journal of microbiology, biotechnology and food science (JMBFS) 5* (6): 606-611.

Mannima, G., Presti, D., Montiel, J.G., Suarez, O.M.E., (2019). Bioplastic recovery from wastewater. A new protocol for polyhydroxyalkanoates (PHA) extraction from mixed microbial cultures. Bioresour. *Technol*. 282:361-369.

Margino, S., Martani, E., Prameswara, A., (2020). Poly-B-hydroxybutyrate (PHB) production by Amylolytic Micrococcus sp. PGI isolated from soil polluted arrowroot starch waste. *Indonesian journal of biotechnology. 19* (1): 56-63.

Mascarenhas, J., Aruna, K., (2019). Production and characterization of polyhydroxyalknoates (PHA) by Bacillus megaterium strain jha using inexpensive agro-industrial wastes. *International journal of recent scientific research. 10* (7): 33359-33374.

Mathiyazhagan, N., Kumarasamy, S., Ranganathan, M., Kandasamy, S., Kandasamy, G., Gnanavel, K., Mamtha, K., (2020). Production and characterization of polyhydroxyalkanoates synthesised by E. coli isolated from sludge soil. *Materials today proceedings* 5:725.

Mathiyazhaghan, N., Sabariswaran, K., Suresh, K., Keerthama, G., Muthsamy, R., Gajendiran, K., (2020). Screening of polyhydroxybutyrate producing indigenous bacteria from polluted lake soil. *Heliyon journal.* 6:5381-5386.

McAdam, B., Fournet, M.B., McDonald, P., Mojicevic, M., (2020). Production of polyhydroxybutyrate (PHB) and factors impacting its chemical and mechanical characteristics. *Polymers*. 12:2-20.

Mitra, R., Xu, T., Xiang, H., Ha, J., (2020). Current developments on polyhdroxyalkanoates synthesis by using halophiles as a promising cell factor. *Microbial cell factory. 19*(86):1-30.

Moayedi, S., Farzi, A., Dehnad, A., (2022). Optimization of production of polyhydroxybutyrate biopolymer using Streptomyces native bacteria. *Research square* 10: 212-217.

Mohamed, A.H., Elsayed, K.B., Salah, G.A., Hussien, R.H., (2019). Production and characterization of polyhydroxybutyrate (PHB) produced by Bacillus sp. isolated from Egypt. *Journal of applied pharmaceutical science. 6* (4): 46-51.

Mohammad, A., Naushin, B., Abu, B., Qamar, Z., Saeed, B., Mohd, R.Z., Mohammad, S., Sarfaraz, N., Mohd, F., Khan, Z.R., Azaz, A., Azad, R., (2021). Isolation and optimization of extracellular PHB depolymerise produce Aeromonas caviae Kukl-(34) for sustainable solid waste management of biodegradable polymers. *PLOS ONE.* 21:27980.

Moreno, S.A.S., Montoya, M.A.M., Martinez, M.L.A., Perez, Y.S.M., (2020). Identification of polyhydroalkanoate producing bacteria in soils contaminated with fique wastes. Rev. *Colomb. Biotechnol. 14* (2): 89-100.

Mostafa A., Otaif, A.S., (2020). Production and characterization of bioplastic by polyhydroxy butyrate accumulating Erythrobacter aquimaris isolated from mangrove rhizosphere. *Molecules* 25:179.

Mostafa, Y.S. (2020). Bioplastic (poly-3-hydroxybutyrate) production by the marine bacterium Pseudodonghicola xiamenensis through date syrup volarization and structural assessment of the biopolymer. *Sci. Rep*. 10:8815.

Mozar, B.K., Miloloza, M., Martinjak, V., Cvetnic, M., Bulatovic, O.V., Mandic, V., Bafti, A., Ukic, S., Dajana, K.G., Bolanca, T., (2023). Bacteria and yeasts isolated from the environment in biodegradation of PS and PVC microplastics: screening and treatment optimization. *Environments. 10* (12): 207-211.

Naik, P., Ranade, V., (2020). Bioanalytical evaluation of PHA Co-polymer produced by Massila spp. Isolated from rhizosphere soil. *Int. J. Pharm. Sci., 11*(1): 106-113.

Palmieri, S., Tittarelli, F., Sabbatini, S., Cespi, M., Bonacucina, G., Eusebi, A.L., Stipa, P., (2021). Effects of different pretreatments on the properties of polyhydroxyalkanoates extracted from sidestreams of a municipal wastewater treatment plant. *Sci. Total environ.* 801:149633.

Rathika, R., Janaki, V., Shanthi, K., Kamah, K.S., (2019). Bioconversion of agro-industrial effluents for polyhydroxyalanoates production using Bacillus subtilis RSI. *International journal of environmental science and technology. 16* (10): 5725-5734.

Ratnaningrum, D., Saraswaty, V., Priatni, S., Lisdiyanti, P., Purnomo, A., Pudjirarharti, S., (2019). Screening of polyhydroxyalkanoates (PHA) producing bacteria from soil bacteria strains. *Earth and environmental science 277* (1): 120-123.

Rocha, C.J.L., Alvareez, C.E., Yanzee, M.R.E., Bengoechea, C., Guerrero, A., Ledesma, M.T.O., (2020). Development of bioplastics from a microalgae consortium from waste water. *Journal of environmental management. 263* (110353): 1-8.

Rogala, M.M., Gawor, J., Gromadka, R., Kowalczyk, M., Grzesiak, J., (2020). Biodiversity and habitats of polar region polyhydroxy-alanoic acid producing bacteria: bioprospection by popular screening methods. *Genes. 11* (8): 873-878.

Sadaf, N., Kamthane, D.C., (2019). PHB production from bacteria isolated from oil contaminated soil. *Bioscience discovery.10* (2): 103-107.

Sambrook, J. & Russell, D.W., (2021). *Molecular cloning: A Laboratory Manual* (Vol. 2).New York, Cold Spring Harbor Laboratory Press.

Samrot, A.V., Samanvitha, K.S., Shobana, N., Renitta, R.E., Senthilkumar, P., Kumar, S.S., (2021). The synthesis, characterization and applications of polyhydroxyalkanoates (PHA) and PHA-based nano particles. Polymers 13 (3302):1-29.

Sanchueza, C., (2020). Influence of the carbon source on the properties of poly(3) hydroxybutyrate produced by Paraburholderia xenovorans LB 400 and its electrospun fibers. *Int. J. Biol. Macromol*. 152:11-20.

Saratele, R.G., Choi, S.K., Saratele, G.D., Ghodake, G.S, Bharagava, R.N., Kim, D.S, Nair, S., Shin, H.S., (2021). Efficient bioconversion of sugarcane baggase into polyhydroxybutyrate (PHB) by Lysinibacillus sp and its characterization. *Bioresource technology* 324:124673.

Satchasatapom, K, Duangsri, C. Charunchaipitat, W. Laloknam, S., Burut, A.S. Powtongsock, S., Akrimajirachoote, N., Raksajit, W., (2022) Enhanced production of poly-3-hydroxybutyrate and carotenoids by Arthrospira platensis under combined glycerol and phosphorus supplementation. *Science Asia*. 48:1-9.

Sayyed, R.Z, Shaikh, S.S, Wani, S.J, Rehman, M.T., Al Ajmi, M.F., Haque, S., El Enshasy, H.A., (2021). Production of biodegradable polymer from agro wastes in Alcaligenes sp. And Pseudomonas sp. *Molecules* 26:2443.

Sayyed, R.Z., Shaikh, S.S., Wani, S.J., Rehman, M.T., Al Ajmi, M.F., Haque, S., El Enshasy, H.A., (2021) production of biodegradable polymer from agro-wastes in Alcaligenese sp. And Pseudomonas sp. *Molecules 26* (9): 2443.

Sayyed, R.Z., Wani, S.J., Alyousef, A.A., Alqasim, A., Syed, A., El-enshasy, H.A., (2019). Purification and kinetics of the PHB depolymerase of Microbacterium paraoxydans RZ56 isolated from a dumping yard. *PLoS ONE* 14: 212-234.

Senthilkumar, B. & Prabhakaran, G., (2019). Production of PHB (bioplastics) using bio effluents as substrates by Alcaligens eutrophas. *Indian Journal of Biotechnology*. 5:76-79.

Shamsuddin, I.M, Jafar, A.J, Abubakar, S.A.S, Yusuf, S. Lateefah, M. Aminu, I, (2020). Boiplastics as better alternative to petroplastics ad their role in national sustainability: A review. *Advances in bioscience and bioengineering. 5*(4):63-70

Singh, A., Srivastava, V., (2022). Polyhydroxybutyrate (PHB) production by Halomonas boliviensis isolated from waste water. *International journal of health sciences.6* (2): 14934-14943.

Singh, M.K., Rai, P.K., Rai, A., Singh, S.I., Singh, J.S., (2019). Poly-B-hydroxybutyrate production by the Cyanobacterium scytonema and Bhatadwaja geitleri under varying environmental conditions. *Biomolecules. 9*(2):198.

Siracusa, V., Blanco, I. (2020). Bio-polyethylene (Bio-PE), Bio-polypropylene (Bio-PP) and Bio-poly (ethylene terephthalate) (Bio – PET): Recent developments in Bio-based polymers analogs to petrol- derived ones for packaging and engineering applications. *Polymers* 12:1641.

Spiekermann, P., Rehm, B.H.A., Rainer, K., Dirk, B., Steinbuchel, A.A., (2019). A sensitive, viable colony staining method using Nile Red for direct screening of bacteria that accumulate Polyhydroxyalkanoic acid and other lipid storage compounds. *Archives of microbiology 171* (7): 73-80.

Srinvivasan, R., Karaoz, U., Volegova, M., Macichen, J., KatoMaeda, M., Miller, S., Nadarajan, R., Brodie, E.l., Lynch, S.V., (2015). Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PLOS ONE 10* (2):117617-19.

Stanley, A., Murthy, P.K, Viyayendra, S.V.N., (2020). Characterization of polyhydroxyalkanoate produced by Halomonas venusta. *J. Polym Environ. 28* (6): 973-983.

Tan, W.A, Wijaya, I. Purwadaria, T., (2019). Bioprospecting of polyhydroxyalkanoates producing bacteria from Indonesian marine environment. *Biodiversitas journal of biological diversity. 20* (5): 13057-13063.

Thapa, C., Shakya, P., Shrestha, R., Pal, S., Manandhar, P., (2019). Isolation of polyhydroxybutyrate (PHB) producing bacteria, optimization of culture conditions for PHB production, extraction and characterization of PHB. *Nepal J. Biotechnol.* 6:62-68.

Thiruchelvi, R., Das, A., Sikdar, E., (2021). Bioplastics as better alternative to petroplastic. *Materials today*. 37:1634-1639.

Trakunjae, C., Boondaeng, A., Apiwatanapiwat, W., Kosugi, A., Arai, T., Sudesh, K., Vaithanomsat., P., (2021). Enhanced polyhydroxybutyrate (PHB) production by newly isolated rare actinomycetes Rhodococcus sp. strain BSTT1-i using response surface methodology. *Scientific reports*. 11:1896-1901.

Tripathi, A.D., Srivastava, S.K., (2019). Kinetic study of biopolymer (PHB) synthesis in Alcaligenes sp. In submerged fermentation process using TEM: *J.Polym. Environ.* 19:732-738.

Zaki, N.H., (2019). Biodegradable plastic production by Bacillus sp. Isolated from agricultural wastes and genetic determination of PHA synthesis. *Al Mustansiriyah journal of science 29* (1); 115 – 118.