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Production of polyhydroxyalkanoates (PHA) by pseudomonas from waste products

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***Abstract*---**Polyhydroxyalkanoate commercial manufacture is hampered by high production costs (PHAs). Up to 50% of the total process expenses might be attributed to the medium used for cultivation. With this research, the researchers hoped to determine if acidogenic fermentation of food waste may yield volatile fatty acids as low-cost carbon sources for bacterial growth essential to make polyhydroxyalkanoates (PHAs). The bacterium Pseudomonas pseudoflava was used to examine the generation of polyhydroxyalkanoates (PHA) from waste food at varied carbon concentrations (4-55g/l). Bacteria produced the most PHA at a concentration of 19 g/l (52.5 percent) and removed the most carbon at a concentration of 7 g/l (67.6 percent). Various analytical methods were used to examine the synthesized PHA's structure, molecular weight, and thermal characteristics, among other things. According to the results of this study, organic carbon persisting in wastewaters may be degraded and converted into PHA by using P. pseudoflava.

***Keywords*---**total organic carbon, biopolymers, waste product, polyhydroxyalkanoates.

Introduction

Many plastics are created from nonrenewable materials and will stay in the environment for a long time due to their sluggish degradation rate. Researchers have been investigating bioplastic alternatives to try to resolve this problem. Polyhydroxyalkanoates (PHA) are biopolymers that bacteria create in their cells. PHA polyesters have gotten a lot of interest lately from the scientific community

and industry alike due to their biodegradability and physical similarities to polypropylene Sheu and colleagues (2009). Poly-3-hydroxybutyrate (P3HB) is a polyhydroxy acid produced by several microorganisms (PHA). According to Kim et al. 2012, for non-biodegradable plastics, agricultural methods for the long-term release of fertilizers, agrochemicals, and medicine PHA may be used (Rehm et al., 2001; Sudesh et al., 2000). PHA isn't a feasible alternative to more typical polymers because of its high price. It's based on (Fradinho et al., 2014). Low-cost fatty acid sources, such as molasses and plant oils, have been used to minimize the cost of generating PHAs. Please refer to the mentioned sources for further information (Ntaikou and colleagues 2009; Koller and colleagues 2005).

PHA synthase, an essential enzyme in PHA manufacture, feeds on R-3-hydroxy acyl-coenzyme A (CoA). A cloning and analysis of 45 different bacteria have yielded approximately 60 PHA synthase genes. There are three kinds of PHA synthases based on their substrate specificity and the number of subunits. *Allochromatium vinosum* class III PHA synthase enzymes have two 40 kDa subunits. *Pseudomonas aeruginosa* is the most regular source of class II PHA synthases, which have a single subunit like class I enzymes (Rehm and Steinbüchel., 1999). Class II PHA synthases vary most from the other two among the three PHA synthases. Class II PHA synthase incorporates medium-chain fatty acids (C6 to C14) into PHA to make latex.

PHA is mostly composed of 3H2MB and 3H2MV, as well as PHB and PHV (3H2MV). A variety of crystalline, stiff, and brittle properties characterize the PHB and PHV, respectively. The carbon source for 3HB was acetate, according to Satoh and colleagues (1996), whereas the carbon source for 3HV was propionate or succinate, they also claim. Lemos et al. (1998) found that less PHB could be synthesised when propionate comprised 72% of the carbon supply. Matsuo et al. (1992) synthesized PHA using a carbon source of propionate, 3 percent PHB, 43 percent 3HV, and 44 percent 3H2MV (Akiyama et al., 1992; Song et al., 1993). Carbon supplies with even-numbered carbon (acetate and butyrate) may raise PHB content, whereas carbon sources with odd-numbered carbon (propionate and valerate) may improve 3HB-co-3HV content (Doi et.al., 1988, Steinbuchel and Pierper, 1992; Song et al., 1993),

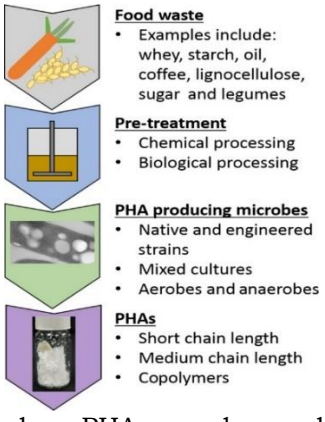

Figure 1. Microbes that produce PHAs may be used to convert food waste into PHAs

Every day, Korea produces 48,000 metric tonnes of municipal solid waste, collected using 42 metric tonnes of plastic bags. The bulk of plastic bags is made of polyethene. Non-biodegradable plastic makes it difficult for water to enter or exit the bag. To improve water flow and biodegradability, plastic bags in the United States use a 30% starch-polyethylene mix. A total of 12,700 metric tonnes of food waste makes up around 26.5% of the daily municipal rubbish production. 75 to 85 percent of food waste is water, and 60 to 70 percent of it is biodegradable. Because of the strong leachates and unpleasant odors that landfill sites release, municipalities prefer to collect food waste for repurposing rather than incineration or landfilling it. Fermented food waste was investigated to determine whether it might be utilized to make PHA, an important component of biodegradable polymers. Plastic bags may be replaced with this in the future, but it's not a certainty.

PHAs from food waste using pure cultures

Because of the different waste streams created during the manufacture, processing, and consumption of food, many ways have been suggested to transform food waste into PHAs. Because of its complexity, a variety of pretreatments, bacteria strains, growing conditions, and post-processing techniques are required for each food source. It is not possible for PHA-producing microbes to directly use organic waste that contains complex chemicals (Anderson and Dawes, 1990). Complex compounds contained in food waste must be pre-treated or processed before they may be used to produce PHA precursors. Other nutrients may be derived from sugars and fatty acids like acetic or propionic acid. Chemical precursors may be hydrolyzed from simpler food wastes and then given to a clean microbial culture. Whey, starch, oils, lignocellulosic materials, legume waste, and sugar waste may all be used to make polyhydroxyalkanoate (PHA).

Materials and Methods

Culture Collection

The NBRC Biological Resource Center (*Pseudomonas pseudoflava*) in Japan collected the bacteria (NBRC-102513). Bacteria belonging to the Comamonadaceae family include *P. pseudoflava*, which belongs to the *P. pseudoflava* genus. This bacterium has been generally reported to be very effective at making PHA from carbs.

Media Preparation

Media utilized to cultivate *P. pseudoflava* included nutritious broth and food scraps. Venkatewastear Reddy et al. (2016) discuss the chemical composition of the waste stream. Waste comprises acetate and propionate in its carbon source make. In waste, carbon concentrations ranged from 5 to 60 g/l. Before adding the medium to the flasks, the pH was adjusted to 7 and autoclaved.

Growth Curve Studies

At 30 degrees Celsius and 180 rotations per minute, a shaking incubator was used to keep the *P. pseudoflava* strain alive for 24 hours in a dark environment for the initial injection. The overnight-formed colony was inoculated into 100 ml of waste with carbon contents ranging from 5 to 60 g/l in different shaking flasks. The research took a total of 192 hours to complete. Samples were taken at various intervals, and absorbance measurements at 600 nm were made using a UV spectrometer.

Cell dry Weight Measurement

10 minutes at 10,000 g removed the bacteria from the sample (Megafuge 8, Thermo Fisher Scientific Gmb H, Dreieich, Germany). TERMAKS (Bergen, Norway) centrifuges were used to dry the samples to a consistent weight in an oven at 70°C. The grams of cells per millilitre of medium and grams of cells per gram of carbon source ingested may be used to compute CDW (CCR).

PHA Production

Centrifugation at various carbon concentrations in waste was used to differentiate cultures after 120 hours. After being soaked in waste, the pellets formed were collected. To stress PHA granule production in waste, low nitrogen and phosphorus concentrations (0.1 g/l) were injected. Tests using just acetate (at a concentration of 20 g/l) as a carbon source in waste were also performed to identify the changes in PHA composition. Every aspect of life remained unchanged throughout the growth era. PHA was extracted from the culture at 72 hours and analyzed.

PHA Characterization by *Fourier-Transform Infrared Spectroscopy (FTIR)*

The functional groups in the sample structure were studied using FTIR. Nicolet OMNIC 4.1 software was used to scan the sample 64 times across a spectrum spanning 400 to 4000 cm1. Essential FTIR was used to examine the resulting spectra (eFTIR, Madison, WI, USA).

TOC and VFA analysis

Shimadzu TOC automated analyzers were employed to keep tabs on the waste's carbon content removal. The non-purgeable organic carbon (NPOC) approach was used to assess total organic carbon. By sparging the sample with acid, the IC is converted to CO_2. After the carbon dioxide has been removed, the treated sample's total organic carbon (TOC) is calculated. The experiments were performed in triplets.

Analysis and Presentation of Results

There were no exceptions for the DSC tests, run in triplicate for each sample. Tables and graphs show statistical averages and standard deviations.

Results and Discussion

Growth Curve Detection

Various carbon sources (6–59 g/l) have grown *P. pseudoflava* in waste at 30°C. Carbon content has a major impact on bacterial growth, as shown by the growth curve study. OD 120 hours, 2.239, was shown to be the optimal concentration for bacteria. At 80 hours, the OD was 1.53. There is a difference in bacterial growth rates across carbon concentrations because bacteria have varying maximum life spans at various carbon concentrations. It takes 80 hours to get a 5 grams per liter substrate concentration. It takes 120 hours to reach 10 grams per liter of substrate concentration. The availability of acetate and propionate was considered while looking at growth curve data. There were 5g/l concentrations after 48 hours, but only a little quantity remained after 80 hours. At 10g/l concentrations, propionate and acetate were accessible for 48 and 80 hours, respectively. Even after 120 hours, acetate and propionate were still inaccessible at substrate concentrations of 5-15 g/l. After 48 and 80 hours at 20 g/l, acetate and propionate were available at 11.9 g/l as well as 0.42 g/l, respectively (propionate).

It is now possible to acquire acetate (0.38 percent) and propionate (0.38 percent) (0.12 percent). Acetate and propionate concentrations of 14.9 g/l and 0.87 g/l were obtained 48 hours after the 29 g/l. 80 and 120 hours of incubation produced acetate and propionate concentrations of 10.9 g/l and 6.9 g/l, respectively. After 48 hours, acetate availability decreased from 25.4 g/l to 8.13 g/l at 40 g/l (120 h). From 1.05 g/l to 0.07 g/l, the accessibility of propionate was reduced (120 h). In both 50 and 60 g/l substrate solutions, the same pattern could be detected for substrate concentrations. In 48 hours, acetate availability reduced from 27.3 g/l to 12 g/l at an amount of 49 g/l of acetate (120 h). Within 48 hours, the propionate availability dropped from 1.29 to 0.199 g/l (120 h). Acetate content went from 42.5 grams per liter to 16 grams per liter in 48 hours at 60 grams per liter. After 48 hours, it went from 1.23 g/l to 0.49 g/l (150 h). Two hours into the log phase, the log phase started between 5 and 39 g/l and 50 to 100 g/l. Higher dosages of VFA take longer to show their full potential due to the toxicity of VFA. Bacterial growth is considerably slowed when VFAs are present in high concentrations. In the previous investigation, *P. palleronii* and identical amounts of VFA were used to analyze growth curve data (Venkatewar Reddy et al., 2016). At larger dosages, *P. palleronii* grew faster than *P. pseudoflava*. When given VFA concentrations of 29-62 g/l, *P. palleronii* grew 1.2-1.05 times faster than *P. pseudoflava*. When VFA concentrations were lower, *P. palleronii* grew more slowly than *P. pseudoflava*. Compared to *P. pseudoflava*, *P. palleronii* grew 1.04 and 1.2 times slower, respectively, at concentrations of 5 and 10 g/l. When interacting with their environment, two strains of bacteria from the same genus are unique species, each with its own set of features and skills. Despite feeding both strains the identical substrate, the growth rates of the two bacteria were vastly different. Because of their diverse metabolic activities, bacteria may be to blame for this. Currently, we don't have a solution and will need to do more research.

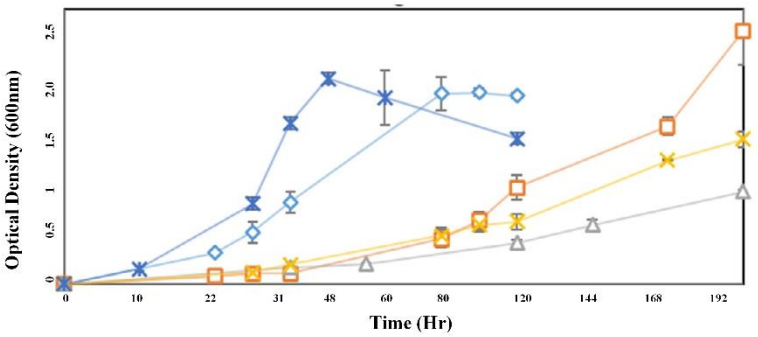

Figure 2. The absorbance of carbon source during cultivation

Carbon removal

However much carbon was in the analysed waste samples, the treatment method worked as shown by the reduction in TOC. 3.7 g/l of TOC was removed from the 5 g/l substrate concentration in 120 hours. Removal rates for total organic carbon (TOC) ranged from 4.9 g/l (at 80 hours) to 7.9 g/l (at a 120-hour time point) (at 48 h). At a substrate concentration of 15 g/l, TOC removal by *P. pseudoflava* was at its highest after 120 hours (10.2g/l). Using 18 g/l substrates, the greatest TOC removal rate was 12.5, then 7.8, then 4.44 g/l after 120 hours, while the lowest removal rate of TOC was after 48 hours at 4.4 g/l. *P. pseudoflava* eliminated the most TOC in 120 hours, followed by 9 grams/liter for 80 hours, and 5.9 grams/liter for 48 hours. After 120 hours of incubation, the TOC removal rates for bacteria cultured at substrate concentrations of 40 and 50 g/l were determined to be 16.9 g/l and 24.9 g/l, correspondingly. Higher TOC removal rates were recorded after 120 and 80 hours at 59 g/l raw material concentration.

Preliminary results show that when the concentrations are lower, *P. palleronii* is superior to *P. pseudoflava* in terms of total organic carbon removal (TOC) from the substrate. Each of the 120-hour substrate concentrations was subjected to a mass balance. Starting at 5 g/l of a substrate, further experiments indicated that 3 g/l was an acceptable concentration. There were just 1.3 grams of carbon left in the solution after the substrate was used up, very little carbon. The 3.7-gram-per-liter substrate included 1.6 grams of biomass and PHA, while the rest of the carbon was made up of unknown organic molecules. Only 7.0 g/l of the substrate was utilized in this experiment, resulting in 2.5 g/l of cell concentration and PHA going to waste. There was an 8.4-gram carbon source remaining after eating 11.6 grams of the substrate from a total solution concentration of 20 grams per liter. There was a total of 3.9 g/l biomass and PHA in the 11.6 g/l substrates. Acetate with a concentration of 0.38 g/l was detected. The remaining carbon in the solution was found to be 16.8 g/l or 30 percent. An unknown chemical molecule and acetate and propionate were also found to be left behind as a consequence of the 12.9 g/l substrates. When it came to the 17.5 g/l substrates, just .10 g/l was kept as propionate, while 95% was conserved as PHAs and biomass rich in PHAs. 25.5 g/l of the substrate was produced at a 50 g/l substrate concentration. Moreover, two-thirds (65 percent) of the 25.5-gram-per-litre substrate included 13 grams of acetate, 0.02 grams of propionate, and 4.8 grams of biomass and polyhydroxyalkanoate. Using 60 g/l of substrate left 34.9 g/l of carbon behind. All

of the carbon in the 24.6 gram-per-liter (g/l) raw material was maintained as carbon compounds, except for the unidentifiable organic materials. Glycerol and amino acids may be formed due to the breakdown of unidentified organic material (Jin et al., 2013).

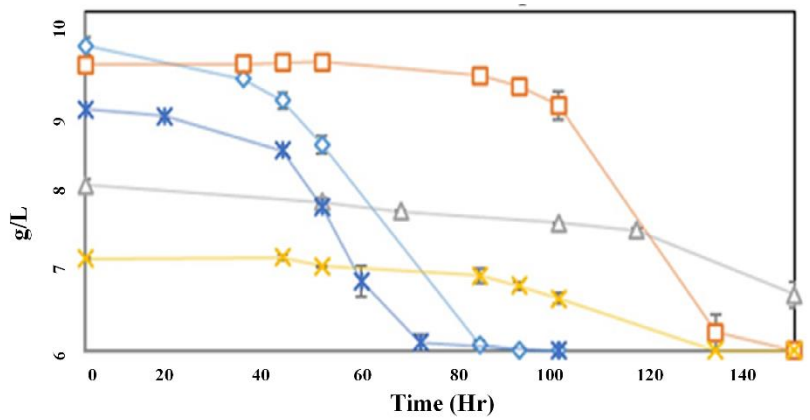

Figure 3. Concentration profile of carbon source during fermentation

Cell dry weight (CDW) and PHA accumulation

Figure 3 shows the dry weight of the cells and the accumulation of PHAs during the development of *P. pseudoflava* in the medium described in Section 3.2. After 72 hours, cells created 1.70 g/L (0.23 kg dry weight per gram carbon source) and 0.16 g/L (0.09 kg CDW) of PHA was built up in all of the circumstances evaluated. The findings were the same across all media. *P. pseudoflava* accumulated 8–9% of its PHAs when fed glucose or other volatile fatty acids as its major carbon source.

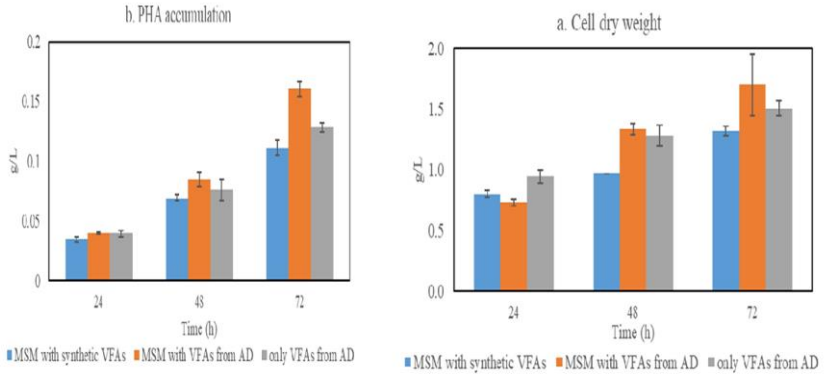

Figure 4. Concentrations of PHAs accumulation and cell dry weight mass

VFA removal

HPLC analysis determined the waste's VFA composition at various concentrations (ranging from 5 to 60 g/l) and time intervals (Table 1). There was no acetic or propionic acid in the water after 120 hours of growth of bacteria at 5, 10, and 15

g/l. Bacteria cultivated at a 20 g/l concentration extracted 99.8% pure acetic and propionic acid. Using 50 and 60 g/l concentrations of bacteria, respectively, acetic and propionic acids were eliminated by 73.4 and 58.5 percent. However, despite several descriptions, little is known regarding this bacteria's capacity to break down VFA.

Table 1
Removal of acetate and propionate from waste using *P. pseudoflava* at different intervals

Initial substrate concentration (g/l)	Acetate removal (%)	Propionate removal (%)
5	100±6	100±6
10	100±4	100±4
15	100±5	100±5
20	98±5	100±5
30	73±6	94±5
40	76±6	88±5
50	73±4	89±5
60	58±5	65±5

Conclusion and Future Research Perspectives

Pure bacterial strains are used in commercial PHB production, which is mostly based on refined carbohydrates. Plastic-like polyhydroxyalkanoates (PHAs), despite their six- to tenfold higher cost than regular plastics (72–74), are available from many companies at costs ranging from 2–10 US$/kg. PHB production methods that are take into consideration the expense of the fermentation medium and the need for sterile conditions for the use of pure cultures. Using waste VFA as food, the bacterium *P. pseudoflava* was able to produce a variety of valuable compounds. The generated PHA was examined for its thermal and structural characteristics. PHA synthase enzyme activity is greater in *P. palleronii* than in *P. pseudoflava* in this species. These results suggest that *P. pseudoflava* might be fed VFA-rich fermented wastes to manufacture PHA. Regeneration of VFAs from PHA-based waste products is made possible by the biodegradability of PHAs, which can be easily recycled by acidogenic fermentation. PHAs may be made and recycled in a closed-loop manner for the first time, reducing the environmental contamination.

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