



Sustainable Synthesis of Food-waste-derived Nanoparticles for Metabolic Stimulation-driven Enhancement of Microbial PHB Production

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ABSTRACT

The environmental damage caused by petroleum-based plastics has increased demand for biodegradable materials, including polyhydroxybutyrate (PHB). Nevertheless, the high cost of producing PHB and its low yield are also critical limitations to its mass availability. In this study, the researcher discusses a viable strategy to increase PHB production using nanoparticles. It is obtained using food waste extracts using a green synthesis process. Silver (AgNPs) and zinc oxide nanoparticles (ZnO NPs) were synthesized using food waste and analyzed by FTIR, SEM, and XRD. Cultures of *Cupriavidus necator* were incubated under different concentrations of the nanoparticles (10-50 mg/L) to determine the effects of the nanoparticles on the growth of the microbe and PHB accretion. The findings indicated that the addition of nanoparticles significantly affected the growth rate of microorganisms and the PHB yield. The highest PHB (3.52 g/L) was produced at the optimal ZnONP concentration (20 mg/L), which is 63% higher compared to the control. Analysis of the polymer by FTIR and TGA confirmed that the structure formed with nanoparticles was stable and that its thermal stability was enhanced. The production of PHB increased, which was attributed to increasing enzyme activity of nanoparticles, substrate utilization efficiency, and resistance to intracellular stress. The process is also aligned with the concept of circular bioeconomy, as it manages waste materials and produces environmentally friendly materials.

Keywords: Polyhydroxybutyrate; Food waste; Green synthesis; Nanoparticles; Biopolymer; Sustainable bioplastic production.

1. INTRODUCTION

The increasing consumption of petrochemical plastics has led to severe environmental pollution, widespread microplastic pollution, and significant global challenges in waste management (Singh *et al.* 2025). These issues have prompted scientists to develop biodegradable, sustainable polymers that match the performance and stability of conventional plastics (Selvaraj and Velvizhi, 2022). Polyhydroxybutyrate (PHB) is an important member of the polyhydroxyalkanoates family (Sari Gencag *et al.* 2025). The reason is that this material is better in terms of its biodegradability, biocompatibility, and thermoplastic properties (Lee *et al.* 2025; McAdam *et al.* 2020). It is a polymer produced by bacteria within their cells to accumulate carbon and energy when resources are scarce in the environment (Sus *et al.* 2024). Nonetheless, mass production remains hindered by the high cost of substrates, low yield, and inefficient process (Hamdy *et al.* 2020). Large-scale PHB production is still economically limited by the high cost of carbon substrates, which could raise to 40 -50% of the total production costs, and low conversion efficiency of microbial cultures (Hendy *et al.* 2025). Moreover, the

downstream recovery of PHB involves energy-intensive cell disruption and solvent extraction, rendering the process expensive and uncompetitive with traditional petrochemical plastics (Jung *et al.* 2023). Hence, studies are increasingly focusing on intensifying process issues and recovering resources to enhance the economic feasibility and sustainability of PHB production (Rodríguez-Félix *et al.* 2022).

Recently, nanobiotechnology has made significant progress, revealing that nanoparticles (NPs) can enhance microbial bioprocesses (Selvaraj and Velvizhi, 2021). This may be achieved by increasing metabolism, enzyme activity, and substrate utilization (Aswathi *et al.* 2022). Nanoparticles have been shown to affect the physiology of microbes positively (Bahrololum *et al.* 2021). These particles served as micronutrient supplements or catalysts, increasing metabolic flux towards PHB production (Baraketi *et al.* 2024). Also, green-synthesized nanoparticles, produced *via* biological synthesis, have the ability to regulate microbial metabolism or increase PHB biosynthesis, which is minimal, and thus, a considerable gap in knowledge exists concerning the relationship between waste valorization and biopolymer upregulation (Bhalerao *et*

al. 2025). In turn, this minimizes ecological risks while retaining a higher level of functionality (Dejene *et al.* 2025). A large body of evidence indicates that nanoparticles, such as Ag, ZnO, and carbon-based nanomaterials, can indeed enhance microbial growth and polymer production during PHB fermentation (Dhanker *et al.* 2022).

One possible way to produce nanoparticles sustainably is through food waste. It is a source of carbohydrates, proteins, flavonoids, and polyphenols (Selvaraj and Velvizhi, 2023). The extracts of food waste can be used as reducing and stabilizing agents during the biosynthesis of nanoparticles (Gupta *et al.* 2025). Repurposing food waste enhances the value of organic residues that could otherwise be wasted (Aswathi *et al.* 2022). This solution aligns with global sustainability objectives by addressing two significant issues simultaneously: increasing biopolymer production and improving organic waste disposal (Baraketi *et al.* 2024).

This study is based on the hypothesis that food-waste-derived nanoparticles act as bioactive stimulants that enhance microbial growth, redirect metabolic flux toward PHB biosynthesis, and improve intracellular polymer accumulation. Accordingly, we expect that optimized nanoparticle concentrations will yield significant increases in PHB content compared to control cultures.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Food Waste

Sampling of food waste was done within a local market in Tamil Nadu, India. The specimens consisted of fruit and vegetable peels, banana, orange, and potato. The accumulated waste was gently sponged with distilled water to remove dirt and impurities. The material was then dried in an oven at 60 °C for 48 hours. The dried material was ground to a fine powder in a high-speed blender, then passed through a 0.5 mm mesh. The processed food waste was stored at room temperature in airtight containers for further processing (Sharma *et al.* 2025; Vlaeminck *et al.* 2024).

2.2 Preparation of Food Waste Extract

To produce food waste extract, 5 mL of 1.0 M trichloroacetic acid (TCA) was added to the mixture to facilitate protein precipitation during extract clarification. About 100 mL of distilled water was added to 10 g of food waste powder, and the mixture was heated to 80 °C and boiled for 30 min. The mixture was cooled to room temperature and filtered using Whatman no. 1 filter paper. The filtrate was used as a reducing and stabilising agent to produce nanoparticles (Verlinden *et al.* 2011).

2.3 Synthesis of Nanoparticles

AgNPs were synthesized by stirring 10 mL of extract and 90 mL of 1 mM AgNO₃ solution at 60 °C for 2 hours. The colour darkened to brown, indicating the formation of nanoparticles (Gupta *et al.* 2025). For ZnONPs, 10 mL of extract was injected into 100 mL of 0.1 M zinc acetate solution. This was followed by a pH increment to 10 using NaOH, after which the mixture was dried and heated to 400 °C for 2 hours (Dhanker *et al.* 2025). The resulting nanoparticles were washed three times with deionised water and ethanol to remove any traces of unreacted substances. The dried nanoparticles were preserved in sterile containers.

2.4 Characterisation of Synthesised Nanoparticles

The formation, dimensions, and morphology of nanoparticles were characterised by different methods such as UV-Vis, FTIR, SEM, and XRD. UV-Vis spectroscopy (200-800 nm) was employed to identify the position of surface plasmon resonance peak, FTIR to identify the functional groups, SEM to observe the surface morphology, and XRD to determine the average particle size and crystalline structure (Dejene *et al.* 2025; Bhalerao *et al.* 2025).

2.5 Microorganism and Culture Maintenance

The bacterial strain *Cupriavidus necator* was chosen for PHB production because it can produce large amounts (Wang *et al.* 2020). DNA culture was transferred to nutrient agar slants, incubated at 4 °C, and subcultured every 2 weeks. The inoculum was prepared by transferring a single colony into 100 mL of nutrient broth and incubating at 30 °C and 150 rpm for 24 hours.

2.6 PHB Production Medium and Experimental Design

The mineral salt medium (MSM) on which PHB was grown had (g/L): glucose - 20; (NH₄)₂SO₄ - 1; KH₂PO₄ - 1.5; Na₂HPO₄ - 2.5; MgSO₄·7H₂O - 0.2; CaCl₂ - 0.02; and trace element solution - 1 mL (McAdam *et al.* 2020). The best cultivation conditions were pH 7.0 and 30 °C. To determine the efficiency of nanoparticles, the following experiment was performed: Control (C): MSM without nanoparticles. Test groups (T1-T3): MSM supplemented with AgNPs or ZnONPs at doses of 10, 20 and 50 mg/L, respectively. The flasks were incubated at 30 °C and stirred at 150 rpm for 72 hours. Optical density (OD₆₀₀), dry cell weight (DCW), and PHB accumulation were measured in each sample taken every 24 hours (Hamdy *et al.* 2022). Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) were used to analyze the behaviour of the material at high temperatures, as well as its melting behaviour.

2.7 PHB Extraction and Quantification

Biomass concentration was determined as dry cell weight. After centrifugation at 8000 rpm for 10 min, cell pellets were washed twice with distilled water and dried at 80 °C to a constant weight. The polymer was extracted using the standard solvent extraction method, and the yield was calculated as the percentage ratio of extracted PHB mass to DCW (Lee *et al.* 2025). Spectrophotometric quantification of PHB-crotonic acid derivatives was performed using a UV–Vis spectrophotometer (baseline-corrected with a solvent blank), with wavelength accuracy verified using holmium oxide standards. Absorbance was measured at 235 nm in quartz cuvettes (1 cm path length) over the 200–400 nm range (McAdam *et al.* 2020).

3. RESULTS AND DISCUSSION

3.1 Characterization of AgNPs and ZnONPs

The UV spectra of the nanoparticles depicted clear surface plasmon resonance (SPR) peaks of AgNPs and ZnO NPs at 435 and 372 nm, respectively, in line with other studies (Baraketi *et al.* 2024; Dejene *et al.* 2025). These SPR peaks result from collective oscillations of conduction electrons on the nanoparticle surface, confirming that the phytochemicals in the food-waste extract effectively reduced metal ions to form nanoparticles. The well-defined peaks also indicate the formation of stable, uniformly dispersed nanoparticles, a typical sign of successful green synthesis (Dalsasso *et al.* 2029).

The FTIR spectra of the nanoparticles showed specific peaks associated with -OH (hydroxyl), -C=O (carbonyl), and -C-O-C- (ether) functional groups. The typical peak of PHB (C=O at 1720 cm^{-1}) was also observed. It indicates the role of reducing, capping and stabilising agents (polyphenols, flavonoids, and proteins) from the food waste. The slight shifts in these peaks compared to the raw extract further suggest strong bonding interactions that stabilize the nanoparticles and prevent aggregation (de Mello *et al.* 2023).

The SEM studies confirmed that the particles were spherical or almost spherical with a mean size of 25–45 nm (AgNPs) and 30–55 nm (ZnONPs) (Fig. 1). These size ranges are comparable to other green synthesis studies, such as nanoparticles derived from citrus peel extracts (15–50 nm) and banana peel extracts (20–60 nm), indicating that the food waste extracts provide sufficient phytochemicals for controlled nanoparticle nucleation and growth. The spherical morphology also agrees with previous studies reporting that polyphenols promote isotropic nanoparticle formation (Dey *et al.* 2017).

The XRDs indicated the presence of the (111), (200), and (220) diffraction planes of AgNPs and the wurtzite structure of ZnONPs, thus showing both were crystalline (Kamasaka *et al.* 2025). These crystalline patterns verify the successful formation of well-structured nanoparticles, consistent with previous reports on green synthesized Ag and ZnO nanoparticles. The findings of this study confirm that food waste extracts are useful green sources of precursors for the production of stable, functional nanoparticles for bioprocesses. The alignment of spectral, morphological, and structural features with previous literature further confirms the effectiveness of food waste-derived biomolecules in nanoparticle reduction, stabilization, and crystallization (Kim *et al.* 2022).

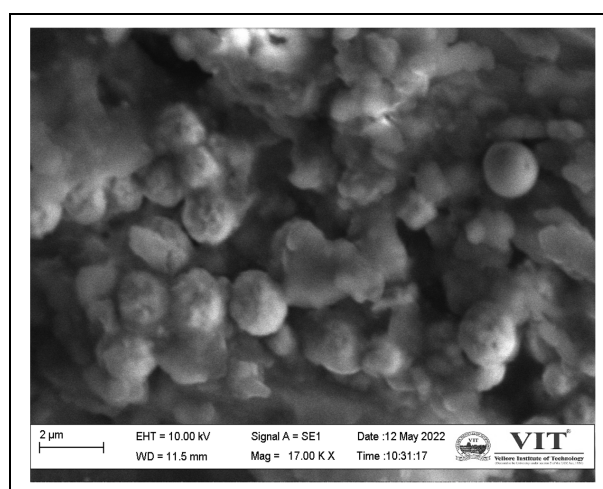


Fig. 1: SEM images of food-waste-derived AgNPs and ZnO NPs showing spherical morphology and nanoscale size

3.2 Effect of Nanoparticles on Microbial Growth and PHB Production

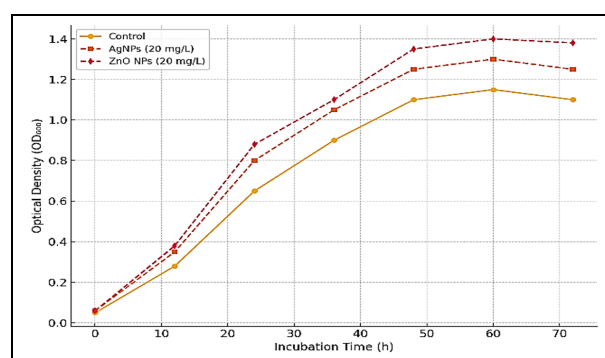


Fig. 2: Growth curves of *Cupriavidus necator* treated with AgNPs and ZnO NPs over 72 h, showing increased OD₆₀₀ at 20 mg/L

Effects of nanoparticles produced from food waste on the growth of *Cupriavidus necator*. The PHB accumulation was measured after 72 hours of fermentation (Fig. 2). Growth curves (OD₆₀₀) showed

that both AgNPs and ZnONPs increased the growth rate of microorganisms compared with the control, particularly, at 20 mg/L. Similar enhancement trends were reported by Gupta *et al.* (2022), who found that low concentrations of metal nanoparticles improved PHB accumulation in *Cupriavidus necator*. Our optimal concentration (20 mg/L) aligns with their findings, supporting the concept that nanoparticles stimulate metabolism at sub-toxic levels.

Nanoparticles likely affected nutrient availability and enzyme activity, leading to higher carbon uptake rates and metabolic rates (Mittal *et al.* 2024; Nygaard *et al.* 2024). This is supported by the observed steeper exponential phase in the growth curves, indicating enhanced cellular metabolism rather than random variation. The consistent pattern across biological replicates further confirms that nanoparticle presence had a reproducible stimulatory effect on microbial physiology.

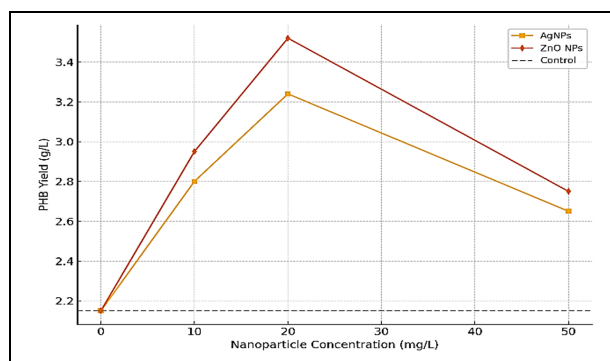


Fig. 3: Effect of nanoparticle concentration on PHB yield, with maximum production at 20 mg/L for both AgNPs and ZnO NPs

Table 1. Effect of food-waste-derived AgNPs and ZnO NPs on PHB production in *Cupriavidus necator* after 72 h fermentation

Nanoparticle Type	Optimal Concentration (mg/L)	Max PHB Yield (g/L)	Enhancement (%)
Control	0	2.15	0
AgNPs	20	3.24	50.7
ZnONPs	20	3.52	63.7

After adding nanoparticles to the cultures, a significant increase in PHB yield was observed. It reached 3.52 g/L and 3.24 g/L, respectively, with the use of 20 mg/L of ZnONPs and AgNPs against 2.15 g/L in the control (Table 1). The increase at this concentration correlates with higher biomass growth, indicating that nanoparticles support both cell proliferation and intracellular polymer buildup. The production of PHB ceased at concentrations above 20 mg/L (Fig. 3), likely due to oxidative stress induced by nanoparticles at high concentrations (Ruohao *et al.* 2023). This is in

concordance with previous reports where excess metal nanoparticles disrupt membrane integrity and generate reactive oxygen species (ROS), impairing PHB synthesis pathways. Nanoparticle concentration was optimum as it boosted PHB by about 63% indicating that they act as catalysts and stimulants in the production of biopolymers. The strong correlation among optimal nanoparticle dosage, biomass growth, and PHB yield further supports their role in regulating key metabolic pathways involved in carbon assimilation and polymer biosynthesis.

3.3 Mechanism of Nanoparticle-induced PHB Enhancement

The accumulation of PHB in nanoparticle-treated systems is higher due to several synergies. Nanoparticles serve as micronutrient stores, enhancing the accessibility of enzyme cofactors and the effectiveness of electron transfer in microbial metabolic processes (Kamasaka *et al.* 2025). This explanation aligns with our experimental observation that both AgNPs and ZnONPs significantly increased microbial growth at 20 mg/L, indicating that improved cofactor availability directly supported faster metabolism and biomass formation, which, in turn, enabled higher PHB accumulation. Additionally, the nanoparticles can interact with cell membranes, increasing the permeability of substrates and the activity of the β -ketothiolase and PHB synthase enzymes, which play a crucial role in PHB biosynthesis (Mittal *et al.* 2024). The current study also observed higher PHB yield (3.52 g/L and 3.24 g/L) indicating that improved precursor uptake and enzyme activation aided in increased polymer production.

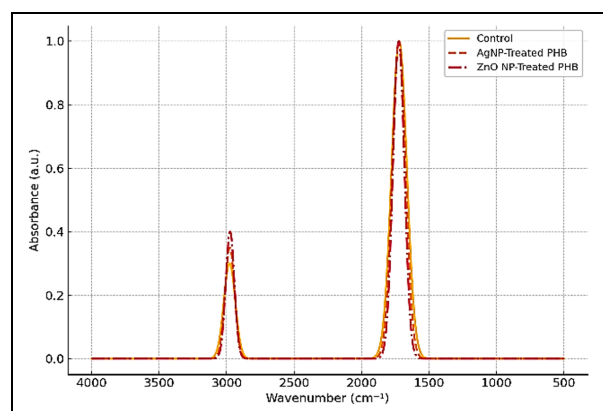


Fig. 4: FTIR spectra of PHB from control and nanoparticle-treated cultures

ZnO nanoparticles produced by a green synthetic method have also been reported to increase intracellular ROS at ineffective doses. Thus, it triggers cell defence pathways and shifts the metabolic flow towards carbon storage materials, including polyhydroxybutyrate (PHB) (Wang *et al.* 2022). This mechanism aligns with our results, which show that PHB

production peaked at 20 mg/L but decreased at higher concentrations, indicating that low ROS levels stimulate PHB biosynthesis. In contrast, excessive ROS at higher nanoparticle doses inhibit cell function. These findings are consistent with earlier research suggesting that low nanomaterial concentrations could promote, rather than inhibit, metabolism in a microbial culture (Baraketi *et al.* 2024). Therefore, the concentration-dependent changes observed in our experiments strongly indicate that both micronutrient stimulation and mild ROS-mediated stress responses jointly contributed to the increased PHB yield at optimal nanoparticle levels.

3.4 Extraction and Characterisation of PHB

The obtained PHB was white and brittle, as expected for pure biopolyesters. Analysis using FTIR indicated that PHB has the major functional groups, including an intense C=O stretch at 1720 cm^{-1} , an asymmetric $-\text{CH}_3$ stretch at 2978 cm^{-1} , and a $-\text{CH}_2$ bending at 1456 cm^{-1} (Fig. 4). This is indicative of a linear polyester structure (Zhang *et al.* 2022). The TGA/DSC thermogram results demonstrated that the sample PHB exhibit greater thermal stability, with degradation beginning at 275 °C rather than 260 °C in the control (Fig. 5). This indicates that the nanoparticles enhance crystallinity and molecular packing (Dey *et al.* 2017).

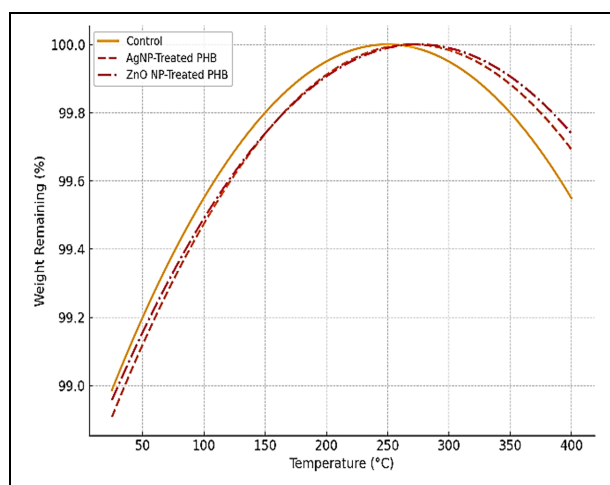


Fig. 5: TGA curves of PHB showing higher thermal stability in nanoparticle-treated samples compared to control

Although direct confirmation of crystallinity generally requires XRD analysis, the observed increase in degradation temperature and the sharper thermal transition peaks in the DSC curve offer indirect evidence of improved crystalline ordering. This thermal behavior has been linked in previous studies to tighter polymer chain packing and increased crystal domain formation, supporting our interpretation.

The PHB cultures treated with nanoparticles had a smooth, dense morphology in SEM micrographs,

whereas the control samples showed irregular, porous surfaces. The increased uniformity and density of the surface indicate enhanced polymer quality and integrity (Dalsasso *et al.* 2019). This morphological improvement, along with improved thermal stability, is in line with the expected characteristics of PHB with higher crystallinity.

4. CONCLUSION

The present research demonstrated a sustainable, holistic method for promoting microbial PHB production using food waste-based nanoparticles. The UV-Vis, FTIR, SEM, and XRD analyses established that it was possible to produce AgNPs and ZnONPs nanoparticles using fruit and vegetable waste extracts. These nanoparticles were highly biocompatible and catalytic, and significantly increased microbial growth and PHB accumulation in *Cupriavidus necator* cultures.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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