



Production, Optimization and Characterization of Polyhydroxyalkanoates (PHA) Bioplastics Using Sweet Potato Peels as Renewable Carbon Source

Benjamin P. C¹, D. A. Ajiya¹, A. A. Mahmoud¹, U.F Hassan¹, D.S John², B.C Okoli³

¹ Department of Chemistry, Faculty of Sciences, Abubakar Tafawa Balewa University Bauchi, P.M.B 0248, Bauchi State, Nigeria.

² Department of Chemical Sciences, Faculty of Sciences, Federal University of Kashere, P.M.B 0182, Gombe State, Nigeria.

³ Department of Mechanical Engineering, Faculty of Engineering, Abubakar Tafawa Balewa University Bauchi, P.M.B 0248, Bauchi State, Nigeria.

E-mail: beverlypresh223@gmail.com

ABSTRACT:

Persistent petroleum-based plastics and the high cost of microbial polyhydroxyalkanoate (PHA) production have increased interest in low-cost renewable feedstocks. This study investigated the production, optimization and characterization of PHA from sweet potato (*Ipomoea batatas* L.) peel hydrolysate using *Bacillus subtilis*. Peels were dried, milled and hydrolysed with 1% (v/v) H₂SO₄ at 80 °C for 90 min, and the neutralized hydrolysate was used as the main carbon source in a medium supplemented with peptone and MgSO₄. A 13-run Box–Behnken design was used to evaluate the effects of peel concentration (60–100 g/L), incubation time (24–72 h) and temperature (30–37 °C) on PHA yield, expressed as a percentage of dry cell weight. Yields ranged from 10.9% to 45.0%, the highest being obtained at 100 g/L, 48 h and 37 °C. The full quadratic model was not significant ($p = 0.264$), whereas a reduced model was ($p = 0.002$; $R^2 = 0.79$, adjusted $R^2 = 0.72$). Peel concentration was the most influential variable ($p = 0.0008$), the effect of incubation time was not significant, and no significant interactions were detected. Because the design had a single centre point and no replicates, pure error and lack of fit could not be assessed. PHA recovered by chloroform extraction and ethanol precipitation was a brown, brittle solid. FTIR showed a strong ester carbonyl band at 1728 cm⁻¹ together with C–O–C, CH₂ and CH₃ bands typical of PHA. SEM revealed a heterogeneous, granular and aggregated morphology, and DSC gave melting temperatures of 176.5 °C and 173.6 °C and a crystallization temperature of 82.1 °C, consistent with a PHB-rich polyester. The polymer was insoluble in water, ethanol and n-hexane. These findings indicate that sweet potato peel is a promising substrate for PHA production by *B. subtilis*.

These findings demonstrated the potential of sweet potato peel biomass as an inexpensive and renewable substrate for microbial PHA production while highlighting the importance of optimizing fermentation conditions to enhance polymer yield. Characterization confirmed the successful production of biodegradable polyhydroxyalkanoates with physicochemical properties suitable for biodegradable plastic applications. The study concludes that the utilization of sweet potato peel biomass provides an environmentally sustainable and economically viable approach for PHA production while contributing to agricultural waste valorization and the development of eco-friendly bioplastics.

1.0 Introduction

The increasing dependence on plastics has contributed substantially to modern economic and technological development because of their versatility, durability, light weight, chemical resistance, and relatively low production cost. Conventional petroleum-derived plastics are widely used in packaging, agriculture, construction, healthcare, transportation, electronics, and household products. However, their extensive production and disposal have created serious environmental concerns because most conventional plastics are resistant to natural degradation. Consequently, plastic wastes can persist in the environment for prolonged periods, contributing to pollution of terrestrial and aquatic ecosystems and increasing concerns about microplastic contamination. The environmental challenges associated with conventional plastics have consequently intensified the search for sustainable and biodegradable alternatives (Abd El-malek *et al.*, 2020).

Bioplastics have emerged as promising alternatives to conventional petroleum-based plastics because they can be produced from renewable biological resources and, depending on their chemical composition, may undergo biodegradation under suitable environmental conditions. Among the different classes of biodegradable bioplastics, polyhydroxyalkanoates (PHAs) have attracted considerable scientific attention. PHAs are naturally occurring polyesters synthesized by numerous microorganisms and accumulated intracellularly as carbon and energy reserves. Their synthesis is commonly promoted when an adequate carbon source is available while another essential nutrient, such as nitrogen, phosphorus, oxygen, or another growth-limiting nutrient, becomes restricted. The accumulated polymer can subsequently be utilized by the microorganism as an internal source of carbon and energy (Meereboer *et al.*, 2020).

PHAs possess several properties that make them attractive alternatives to conventional plastics, including biodegradability, biocompatibility, thermoplasticity, and the potential for use in diverse applications. Different PHA polymers, including polyhydroxybutyrate (PHB) and related copolymers, have been investigated for applications in packaging, agriculture, biomedical materials, tissue engineering, drug delivery, and other value-added products. Their ability to biodegrade in various environments gives PHAs an important advantage over many conventional petroleum-derived plastics, particularly in applications where end-of-life environmental impact is a major consideration (Adak *et al.*, 2024).

Despite these advantages, the relatively high cost of PHA production remains one of the major limitations to its widespread commercial application. The cost is influenced by several factors, including the carbon substrate, culture medium, fermentation conditions, microbial productivity, polymer recovery, purification, and downstream processing. Among these factors, the choice and cost of the carbon source are particularly important because carbon substrates provide the primary source of carbon required for microbial growth and polymer biosynthesis. Consequently, the use of inexpensive renewable substrates and waste materials has become an important strategy for reducing the cost and improving the sustainability of PHA production (Wang *et al.*, 2021).

The utilization of agricultural and food-processing residues as alternative carbon sources provides an opportunity to address both the economic challenges of PHA production and the environmental problems associated with organic waste disposal. Agro-industrial wastes contain carbohydrates, proteins, lipids, cellulose, hemicellulose, starch, and other organic compounds that can potentially be converted into value-added products through microbial processes. Recent reviews have highlighted the potential of agricultural residues and other organic waste streams as inexpensive substrates for PHA production, while emphasizing the importance of appropriate pretreatment and fermentation optimization for efficient conversion (Saratale *et al.*, 2021).

Sweet potato (*Ipomoea batatas* L.) is an important food crop cultivated and consumed in many parts of the world. Processing and preparation of sweet potato generate considerable quantities of peel residues. Rather than being regarded solely as waste, sweet potato peel is increasingly being considered a valuable biomass resource because it contains carbohydrates and other potentially useful compounds that can be recovered or converted into value-added products. A recent comprehensive review reported that sweet potato peels contain nutritional components, bioactive compounds, polysaccharides, and other substances that provide opportunities for industrial and biological valorization (Jiang *et al.*, 2024).

The carbohydrate-rich nature of sweet potato peel makes it particularly interesting as a potential renewable carbon source for microbial fermentation. Previous research has demonstrated that sweet potato peel can serve as a substrate for microbial bioconversion processes. For example, sweet potato peel starch hydrolysate has been investigated as a sustainable substrate for microbial production of valuable fermentation products, demonstrating the potential of the material to provide fermentable carbon following appropriate pretreatment and hydrolysis. The conversion of complex biomass into simpler fermentable sugars is particularly relevant to PHA production because microorganisms generally utilize accessible carbon compounds more efficiently than untreated complex biomass.

The conversion of sweet potato peel into PHA therefore represents a potential waste to value approach. Instead of allowing the peel to accumulate as discarded organic material, its carbohydrate components can be made available to microorganisms for the production of a biodegradable polymer. Such an approach supports the principles of waste valorization and the circular bioeconomy, in which wastes and residues are converted into useful products rather than being treated merely as materials for disposal (Chavez *et al.*, 2022).

Microbial selection is another important consideration in PHA production. Several bacterial genera, including *Cupriavidus*, *Pseudomonas*, *Bacillus*, and *Halomonas*, have been investigated for their ability to synthesize PHAs. *Bacillus subtilis* is particularly attractive for biotechnological applications because of its established industrial relevance, physiological versatility, and ability to utilize different substrates. The organism has also been investigated for PHB production, demonstrating that it can accumulate PHA under suitable cultivation conditions (Su *et al.*, 2020).

Recent studies further demonstrate the importance of *Bacillus* species in the conversion of low-cost substrates into biodegradable polymers. For example, bacterial PHA production using waste carbon sources has been investigated with *Bacillus subtilis* alongside other bacterial producers, highlighting the potential of waste-derived substrates for reducing dependence on expensive refined carbon sources (Naseem *et al.*, 2024).

The efficiency of PHA production is influenced by several nutritional and environmental parameters. Factors such as carbon-source concentration, temperature, pH, incubation period, inoculum concentration, nitrogen availability, aeration, and carbon-to-nitrogen ratio can affect microbial growth, substrate utilization, intracellular polymer accumulation, and final PHA yield. Because these factors may interact with one another, changing one parameter independently may not adequately reveal the conditions required for maximum polymer production. Consequently, systematic optimization of fermentation conditions is important for improving PHA productivity and reducing production costs (Diankristanti *et al.*, 2024).

Response Surface Methodology (RSM) provides a useful statistical approach for optimizing processes in which several independent variables influence a measurable response. Rather than evaluating each factor independently, RSM allows the individual and interactive effects of selected process variables to be evaluated and used to establish optimum operating conditions. Its application in fermentation and bioprocess development can therefore facilitate the identification of conditions that maximize polymer production while reducing the number of experimental runs required.

Following microbial production and recovery, characterization of the synthesized polymer is essential to establish its chemical, structural, morphological, and thermal properties. Fourier Transform Infrared Spectroscopy (FTIR) provides information about the functional groups present in the polymer and assist in identifying characteristic chemical bonds associated with PHA. Scanning Electron Microscopy (SEM) provides information on the surface morphology of the recovered material, while Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) provide information concerning thermal transitions and thermal stability. While, X-ray diffraction (XRD), provide information about the crystalline structure of the polymer.

Comprehensive characterization is then necessary to determine whether the recovered material possesses characteristics consistent with a biodegradable PHA and to assess its potential usefulness as a bioplastic (Adak *et al.*, 2024).

Abubakar *et al.* (2022) applied response surface methodology with a central composite design to optimize biodiesel production from *Jatropha curcas* seed oil. They reported an optimum yield of 98.32% at a catalyst mass of 0.30 g, an ethanol-to-oil molar ratio of 12:1, a temperature of 65 °C and a reaction time of 2 h. Their study illustrates the application of RSM to modelling process responses and identifying favourable operating conditions. This provides methodological support for applying RSM in the present study to evaluate the effects of sweet potato peel concentration, incubation time and temperature on PHA yield.

Therefore, the present study focuses on the production, optimization, and characterization of biodegradable polyhydroxyalkanoates using sweet potato peel as a renewable carbon source. The study utilized sweet potato peel as an inexpensive substrate for microbial polymer production, optimized important fermentation conditions for improved PHA yield, and characterized the recovered polymer using appropriate analytical techniques. The study contributes to the valorization of sweet potato peel, development of low-cost bioplastic production processes, reduction of dependence on petroleum-derived plastics, and advancement of sustainable waste-to-value technologies.

2.0 Materials and Methods

2.1 Materials

Yellow sweet potatoes peels (*Ipomea Batatas*) were collected from a processing farm in Kagadama, Bauchi. Distilled water, diluted H₂SO₄, NaOH, MgSO₄ all of analytical grade were obtained from the school store.

2.1.1 Hydrolysis of Sweet potato

Fresh sweet potato peels were collected, sorted, washed, oven-dried at 60 °C, milled and sieved to obtain a uniform powder. The powder was subjected to dilute acid hydrolysis using 1% (v/v) H₂SO₄ at 80 °C for 90 minutes to convert complex carbohydrates into fermentable sugars. The hydrolysate was cooled, filtered using Whatman No. 1 filter paper and neutralized with NaOH to pH 7.0. The hydrolysate was then used as the primary carbon source for fermentation and supplemented with 5 g/L peptone and 0.2 g/L MgSO₄, with the pH maintained at 7.0.

2.1.2 Fermentation Medium Preparation and *Bacillus* inoculation

The fermentation medium was sterilized, cooled and inoculated with 15 mL of *Bacillus subtilis* culture into 100 mL of medium. PHA production was carried out by submerged fermentation in an orbital shaker at 150 rpm using the Box–Behnken experimental design. The three variables investigated were sweet potato peel concentration (30–50 g/L), incubation time (24–72 h) and temperature (30–37°C), with PHA yield as the response. A total of 13 experimental runs were conducted and analysed using Response Surface Methodology (RSM) and ANOVA to determine the optimum conditions.

2.1.3 Chloroform Extraction and Ethanol Purification

After fermentation, the culture broth was centrifuged at 10,000 rpm for 15 minutes to recover the bacterial cells containing intracellular PHA. Chloroform was added to the cell pellet and gently heated to extract the polymer. The PHA was precipitated by adding 100 mL chilled absolute ethanol, allowed to stand for 30–60 minutes, filtered, washed with ethanol and distilled water, and dried at 40–50 °C to constant weight. The dried polymer was weighed to determine PHA yield and stored for further analysis.

2.1.4 Optimization and Characterization

The extracted PHA was subjected to solubility testing using selected solvents and characterized using FTIR to identify functional groups, SEM to examine surface morphology, and DSC to determine thermal properties. The experimental data were analysed statistically using RSM to evaluate the individual and interactive effects of substrate concentration, incubation time and temperature on PHA production and to determine the optimum fermentation conditions.

In simple flow form:

Sweet potato peel → Washing → Drying → Milling/Sieving → Acid hydrolysis → Filtration → Neutralization → Fermentation medium preparation → *Bacillus subtilis* inoculation → Fermentation → Centrifugation → Chloroform extraction → Ethanol precipitation → Filtration/Washing → Drying → PHA yield determination → Solubility test → FTIR/SEM/DSC characterization → RSM optimization.

3.0 RESULT AND DISCUSSION

3.1 Physical Characteristics of Sweet Potato Peel Hydrolysate

Table 3 describes the physical changes observed in the sweet potato peel after acid hydrolysis. It shows the materials and conditions used for hydrolysis and the resulting physical appearance of the hydrolysate.

Table 3.1 Physical Characteristics of Sweet Potato Peel Hydrolysate

Parameter	Observation
Feed Stock	Sweet Potato Peels
Hydrolysis Reagent	1% (v/v) H ₂ SO ₄
Hydrolysis Temperature	80 °C
Hydrolysis Time	90 Minutes
Colour After Hydrolysis	Brownish
Appearance	Softened and partially degrade biomass
Filtrate colour	Brownish-yellow

The sweet potato peel was hydrolysed using 1% (v/v) sulphuric acid at 80°C for 90 minutes, followed by filtration and neutralization to pH 7.0. Hydrolysis caused the peel to soften and partially disintegrate, with a colour change from cream-orange to brownish and the filtrate appearing brownish-yellow, indicating structural breakdown and release of soluble compounds. The hydrolysate was subsequently used for fermentation. After chloroform extraction and ethanol precipitation, the recovered PHA appeared as a brown, dry and brittle flake-like solid. The colour was attributed to residual pigments and other biomass components. The successful recovery of polymer from all experimental runs demonstrated that *Bacillus subtilis* utilized the sweet potato peel hydrolysate as a carbon source for PHA production.

3.2 Percentage Yield

Table 3.2 presents the experimental results for the production of polyhydroxyalkanoates (PHA) using sweet potato peel hydrolysate as a carbon source and *Bacillus subtilis* as the producing microorganism. The experiment evaluated the effects of sample mass, fermentation time, and temperature on PHA production across 13 experimental runs. The dried bacterial cell weight and extracted PHA weight were determined for each run, from which the PHA yield was calculated.

Table 3.2 The experimental design matrix and the corresponding responses are presented

Run	A: Peel (g/L)	B: Time (h)	C: Temp (°C)	W ₁ (g)	W ₂ (g)	DCW (g)	PHA (g)	Yield (%)	Predicted (%)
1	100	48	37	10.350	10.890	0.540	0.243	45.0	38.4
2	80	24	37	10.520	10.745	0.225	0.077	34.2	25.8
3	80	24	30	10.535	10.712	0.177	0.036	20.3	25.8
4	80	72	37	11.225	11.710	0.485	0.158	32.6	32.2
5	100	24	33.5	12.350	12.562	0.212	0.049	23.1	27.2
6	60	24	33.5	10.425	10.581	0.156	0.017	10.9	8.4
7	100	72	33.5	11.318	11.896	0.578	0.176	30.4	33.5
8	60	48	37	11.820	12.203	0.383	0.056	14.6	19.6
9	80	48	33.5	10.915	11.340	0.425	0.083	19.5	21.0
10	60	48	30	11.352	11.612	0.260	0.042	16.2	19.6
11	60	72	33.5	10.430	10.745	0.315	0.066	21.0	14.8

Run	A: Peel (g/L)	B: Time (h)	C: Temp (°C)	W ₁ (g)	W ₂ (g)	DCW (g)	PHA (g)	Yield (%)	Predicted (%)
12	80	72	30	11.145	11.617	0.472	0.141	29.9	32.2
13	100	48	30	11.625	12.127	0.502	0.197	39.2	38.4

W₁ = weight of empty crucible; W₂ = crucible + dried cells; DCW = W₂ – W₁; Yield = (PHA/DCW) × 100

PHA yield ranged from 10.9% (Run 6: 60 g/L, 24 h, 33.5 °C) to 45.0% (Run 1: 100 g/L, 48 h, 37 °C). Dry cell weight ranged from 0.156 to 0.578 g, and the mass of extracted PHA from 0.017 to 0.243 g. The highest yield corresponded to 0.243 g of PHA from 0.540 g of dry cells, while the lowest corresponded to 0.017 g from 0.156 g. A clear grouping by peel concentration was evident. All runs at 60 g/L gave yields of 21.0% or less (10.9 to 21.0%), whereas runs at 100 g/L gave 23.1 to 45.0%. The highest biomass (0.578 g, Run 7: 100 g/L, 72 h, 33.5 °C) did not correspond to the highest PHA yield (30.4%), which indicates that biomass formation and intracellular PHA accumulation did not follow the same trend.

3.3 Main Effect Plots of Variables on PHA Yield

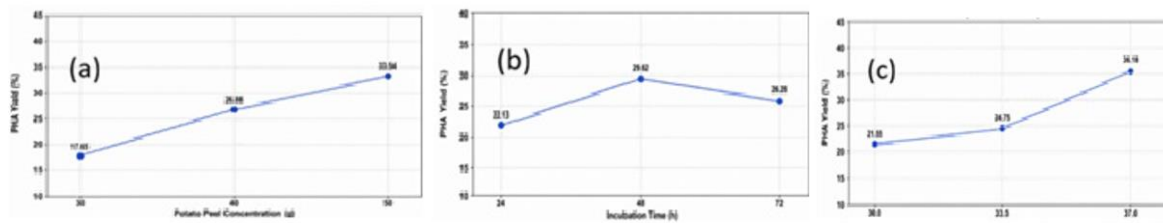


Fig 3: (a) Main Effect plot: Potato peel concentration; (b) Incubation Time on PHA Yield; (c) Temperature on PHA yield

The results showed that sample concentration, incubation time, and fermentation temperature influenced PHA production by *Bacillus subtilis*. Increasing the sweet potato peel concentration generally improved PHA yield, with higher substrate availability providing more fermentable carbohydrates for microbial growth and PHA biosynthesis. However, excessive substrate concentration may adversely affect microbial activity through factors such as increased viscosity and reduced oxygen transfer. Similarly, increasing the incubation period from 24 to 48 hours enhanced PHA accumulation, with 48 hours producing favourable results, whereas prolonged incubation to 72 hours did not consistently improve yield, possibly due to nutrient depletion and subsequent utilization of stored PHA. Fermentation temperature also affected PHA production, with an increase from 30 °C to 37 °C, generally enhancing polymer accumulation due to favourable microbial growth and enzymatic activity. Overall, the findings indicate that appropriate control of substrate concentration, incubation time, and temperature is essential for efficient PHA production from sweet potato peel hydrolysate, supporting its potential as a low-cost renewable carbon source for sustainable bioplastic production.

3.4 Interaction Effect of Variables on PHA Yield

3.4.1 Interaction Effect of Potato Peel Concentration and Incubation Time on PHA Yield

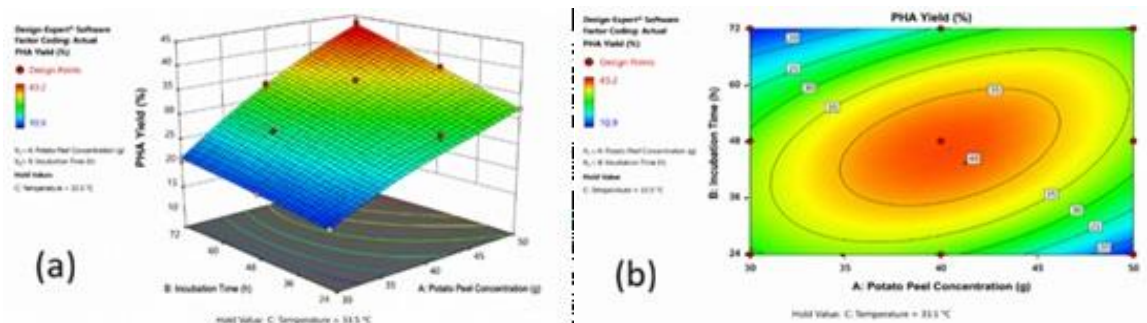


Fig 3.4.1: (a) Three-Dimensional Response Surface Plot showing the interaction between Potato Peel Concentration and incubation Time on PHA Yield; (b) Contour-Plot showing the interaction between Potato Peel Concentration and incubation Time on PHA Yield

The response surface analysis showed a positive interaction between potato peel concentration and incubation time. Increasing the concentration from 30 g to 50 g alongside increasing incubation time from 24 to 48 hours substantially improved PHA yield. However, extending incubation beyond 48 hours did not further increase yield, possibly due to nutrient depletion and utilization of stored PHA by *Bacillus subtilis*. The elliptical contour pattern indicated

a significant interaction between the two factors. Overall, a high potato peel concentration combined with an intermediate incubation period of about 48 hours provided the most favorable conditions for PHA production.

3.4.2 Interaction effect of Potato Peel Concentration and Temperature on PHA Yield

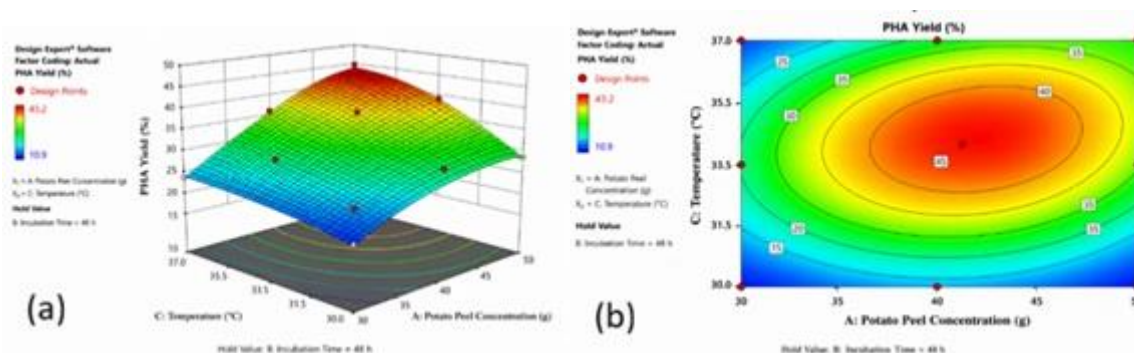


Fig 3.4.2: (a) Three-Dimensional Response Surface Plot showing the interaction between Potato Peel Concentration and Temperature on PHA Yield; (b) Contour Plot showing the interaction between Potato Peel Concentration and Temperature on PHA Yield

The response surface and contour plots showed that potato peel concentration and fermentation temperature interacted positively to influence PHA production. Increasing the substrate concentration alongside temperature up to 37 °C improved PHA yield, likely due to increased carbon availability and enhanced microbial and enzymatic activity. The elliptical contour pattern indicated a significant interaction between the two factors. Overall, combining a high potato peel concentration with an optimum temperature of about 37 °C provided favorable conditions for maximizing PHA production by *Bacillus subtilis*.

3.4.3 Interaction Effect of Incubation Time and Temperature on PHA Yield

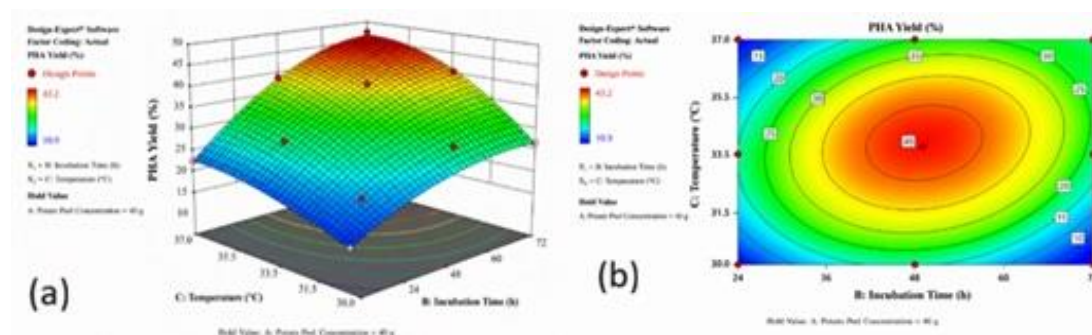


Fig 3.4.3: (a) Three-Dimensional Response Surface Plot showing the interaction between Incubation Time and Temperature on PHA Yield; (b) Contour Plot showing the interaction between Incubation and Temperature on PHA Yield

The response surface analysis showed that incubation time and fermentation temperature jointly influenced PHA production. Increasing incubation time from 24 to about 48 hours and temperature from 30 °C to 37 °C improved PHA yield, with the optimum region occurring around 48 hours and 37°C. Beyond this period, PHA production slightly decreased, possibly due to nutrient depletion and utilization of stored PHA by *Bacillus subtilis*. The elliptical contour pattern confirmed a significant interaction between the two factors. Overall, 48 hours incubation at 37 °C provided favourable conditions for maximizing PHA production.

3.5 FTIR Result

3.5.1 FTIR Result of Sweet Potato Peel

The FTIR spectrum of the raw sweet potato peel showed a broad O–H band at 3316 cm⁻¹, indicating the presence of hydroxyl-rich compounds such as starch, cellulose, hemicellulose and pectin. The 2920 cm⁻¹ band was attributed to aliphatic C–H stretching from carbohydrate structures and possible lipids or waxes. The band at 1648 cm⁻¹ was associated with O–H deformation and possible contributions from other components of the complex biomass. The 1392 cm⁻¹ band indicated C–H bending/O–H deformation, while the strong 1054 cm⁻¹ band represented C–O and C–O–C stretching associated with starch and cellulose. The 888 cm⁻¹ band was related to glucose-ring and anomeric-carbon vibrations. The FTIR spectrum confirmed that raw sweet potato peel is mainly composed of hydroxyl-rich carbohydrate materials, with no prominent ester carbonyl band observed.

The FTIR spectrum of the uninoculated sweet potato peel hydrolysate showed major peaks at 3283.8, 2924.1, 1636.3, 1418.3, 1338.1, 1148.0, 1075.3, 993.3 and 928.1 cm^{-1} . Overall, the spectrum confirmed that the hydrolysate was predominantly composed of hydroxyl-rich carbohydrate compounds produced from the hydrolysis of starch and other polysaccharides.

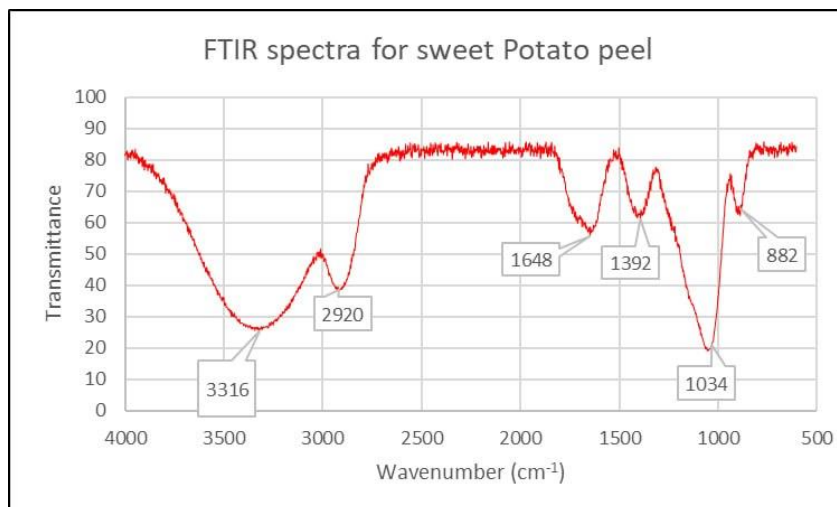


Fig 3.5.1 FTIR result for ground sweet potatoes peel

The 3283.8 cm^{-1} peak indicated O–H stretching, while 2924.1 cm^{-1} represented aliphatic C–H stretching. The 1636.3, 1418.3 and 1338.1 cm^{-1} bands were associated with O–H deformation, C–H bending and other vibrations from carbohydrate and biomass components. The fingerprint region, particularly 1148.0 and 1075.3 cm^{-1} , indicated C–O–C and C–O stretching associated with glycosidic bonds and carbohydrate structures. The 993.3 and 928.1 cm^{-1} peaks were related to glucose-ring, C–O–C and anomeric vibrations.

The FTIR result indicates that acid hydrolysis successfully produced a carbohydrate-rich hydrolysate containing accessible sugars and partially hydrolysed carbohydrate fragments, making it suitable as a carbon source for *Bacillus subtilis* fermentation and PHA production.

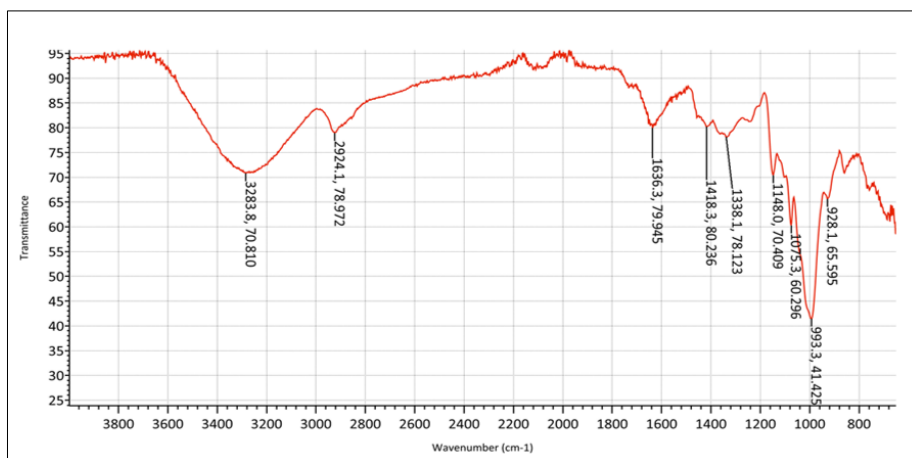


Fig 3.5.2 FTIR result for uninoculated sweet potato hydrolysate

The FTIR spectrum of the extracted PHA showed characteristic peaks at 984, 1040, 1102, 1108, 1234, 1278, 1378, 1456 and 1728 cm^{-1} , with broader bands around 3400 and 2900 cm^{-1} . The broad 3400 cm^{-1} band was attributed to O–H stretching, while the 2900 cm^{-1} region represented aliphatic C–H stretching.

The most important peak was the strong 1728 cm^{-1} absorption, corresponding to the ester carbonyl (C=O) group, which provides strong evidence for the presence of a PHA-type polyester. The peaks at 1456 and 1378 cm^{-1} were associated with CH_2 and CH_3 deformation vibrations. The bands between 1278 and 1040 cm^{-1} represented C–O and C–O–C stretching of ester linkages and formed an important PHA fingerprint region. The 984 cm^{-1} peak was associated with skeletal and CH_3 -related vibrations.

Overall, the FTIR spectrum showed the characteristic ester carbonyl, C–H, C–O and C–O–C vibrations expected in PHA, supporting the successful recovery of a PHA-type polymer from the fermentation process.

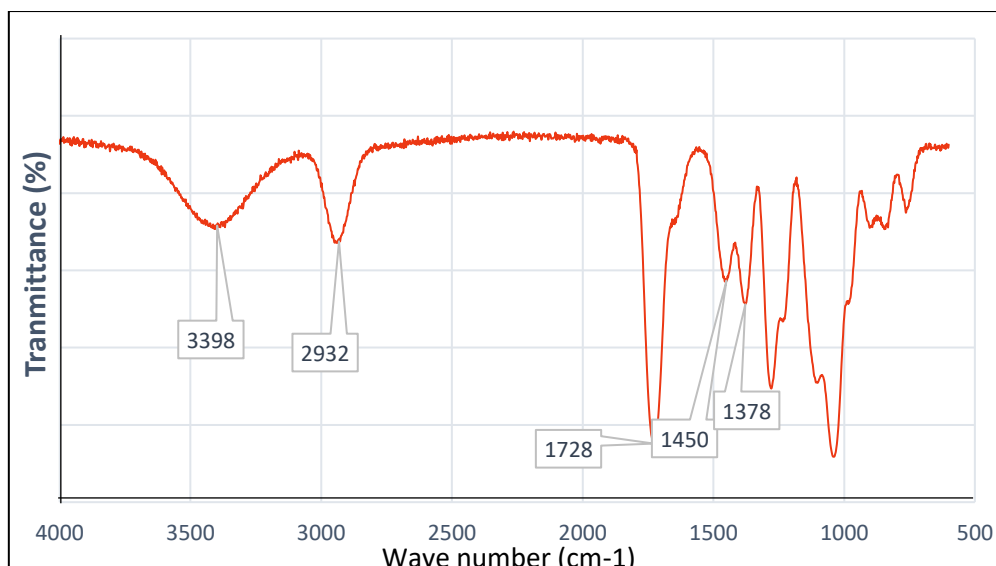


Fig 3.5.3 FTIR Result of Extracted PHA

3.6 SEM Result

The SEM micrograph obtained at 5,000× magnification showed a heterogeneous, granular and irregular morphology rather than a smooth, continuous surface. The sample contained particles of different shapes and sizes, including rounded, oval and irregular structures, with smaller particles surrounding larger micrometer-sized particles. Extensive particle agglomeration and partial fusion were also observed.

The morphology of the extracted PHA was characterized by rough, irregular and clustered particles, which is consistent with previously reported morphologies of recovered or extracted PHA/PHB (Nguyen *et al.*, 2020). The observed irregularity may be associated with the extraction, precipitation and drying processes and does not necessarily indicate impurities. The SEM result therefore supports the formation and recovery of granular PHA material.

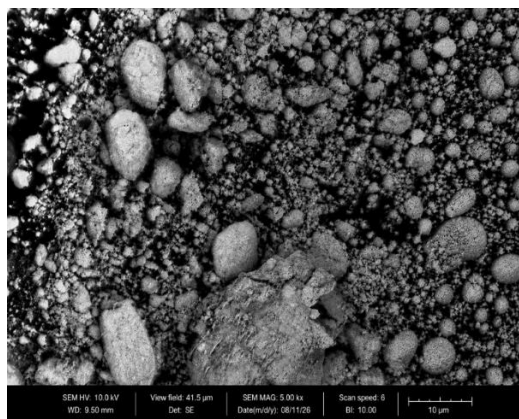


Fig 3.6 SEM image of Extracted PHA

3.7 DSC Result

The DSC thermogram of the extracted PHA showed three major thermal transitions: a **first melting temperature (T_m) of 176.5 °C**, a **crystallization temperature (T_c) of 82.1 °C**, and a **second melting temperature (T_m) of 173.6 °C**. The melting peaks indicate the presence of a substantial crystalline phase, while the crystallization peak confirms that the polymer can reorganize into a crystalline structure after melting.

The melting temperatures obtained are comparable to reported values for PHB (Guzmán-Lagunes *et al.*, 2022), suggesting that the extracted polymer is likely PHB-rich PHA. The small difference of 2.9 °C between the first and second melting temperatures indicates relatively consistent thermal behaviour despite differences in thermal history. However, the exact PHA composition cannot be confirmed from DSC alone. Overall, the DSC results demonstrate that the extracted polymer possesses semicrystalline characteristics and good thermal stability, supporting its potential as a biodegradable polymer material.

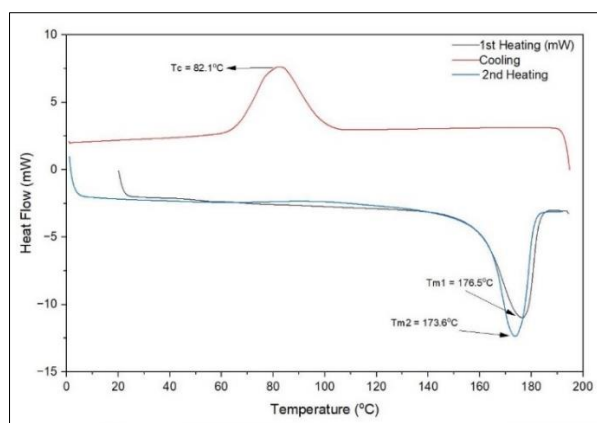


Fig 3.7 DSC image of Extracted PHA

3.8 Solubility Test Result

The extracted PHA showed **different levels of solubility in the tested solvents** at 25 °C. Partial solubility was observed in **acetone, methanol and ethyl acetate**, while **toluene showed the strongest interaction**, characterized by swelling and partial dissolution. In contrast, **ethanol, n-hexane and water showed no observable dissolution**.

The insolubility in water supports the **hydrophobic nature of PHA**, while the varying responses to the organic solvents indicate that solubility depends on factors such as solvent–polymer compatibility, molecular structure, crystallinity and molecular weight. The results demonstrate that the extracted PHA had **limited solubility and predominantly hydrophobic characteristics**, consistent with the expected behaviour of PHA-based biodegradable polyesters (Bartels *et al.* (2020)).

Table 4.4: Solubility behaviour of the extracted PHA in selected solvents

Solvent	PHA Mass (g)	Polarity	Solvent Vol. (ml)	Temp. (°C)	Solubility	Observation
Acetone	0.2	Polar	2ml	25	+	Some dissolves, substantial residue of PHA remains
Ethanol	0.2	Polar	2ml	25	-	PHA remains undissolved and settles at the bottom
Methanol	0.2	Polar	2ml	25	+	Some dissolves, substantial residue of PHA remains
n-Hexane	0.2	non-polar	2ml	25	-	PHA remains largely undissolved
Toluene	0.2	Non -polar	2ml	25	++	Some swelling / partial dissolution
Ethyl Acetate	0.2	Moderately polar	2ml	25	+	Slightly/ partially soluble
Water	0.2	Polar	2ml	25	-	PHA remains undissolved and settles at the bottom

4.0 Conclusion

Based on the findings of this study, sweet potato peel biomass demonstrated potential as a renewable substrate for the microbial production of polyhydroxyalkanoates using *Bacillus subtilis*. Acid hydrolysis improved the availability of carbohydrate components in the biomass, thereby providing a suitable carbon source for microbial fermentation.

The fermentation experiments showed that PHA production increased significantly with sweet potato peel concentration within the range of 60 to 100 g/L, whereas the effect of incubation time was not statistically significant and the effect of temperature remained uncertain. The best condition observed was 100 g/L, 48 h and 37°C. Because this condition lies at the upper boundary of the design, the true optimum may lie at a higher peel concentration. This demonstrates the importance of controlling fermentation conditions when converting agricultural waste into biodegradable polymers.

The characterization results further supported the successful recovery of a PHA-type polymer. FTIR provided strong evidence of ester linkages through the characteristic carbonyl absorption at 1728 cm⁻¹ and associated C–O/C–O–C bands. SEM revealed a granular, irregular and aggregated morphology typical of recovered microbial polymer materials, while DSC demonstrated distinct melting and crystallization behavior with melting temperatures of 176.5°C and 173.6°C. The solubility behavior, particularly complete dissolution in chloroform and limited solubility in water and several polar solvents, was also consistent with the physicochemical behavior of the recovered polymer.

In particular, the results strongly support the presence of a PHA-type polyester and suggest a PHB-rich material, but definitive identification of the precise PHA composition would require additional confirmatory techniques such as NMR or more specific compositional analysis.

Therefore, the study concludes that sweet potato peel represents a promising agricultural waste feedstock to produce biodegradable PHA by *Bacillus subtilis*. The approach simultaneously provides an opportunity for agricultural waste valorization and the development of an alternative material to persistent petroleum-derived plastics.

5.0 Recommendations

Based on the findings of this study, the following recommendations are made:

- i. Further optimization studies should investigate additional fermentation parameters such as inoculum concentration, pH, nitrogen source, carbon-to-nitrogen ratio, agitation rate and mineral composition to further improve PHA production.
- ii. Further purification of the recovered polymer should be undertaken to reduce the brown coloration and possible residual cellular or substrate-derived materials observed in the crude extract.
- iii. Alternative and less hazardous PHA recovery methods should be investigated because chloroform-based extraction, although effective for polymer recovery, presents environmental and safety concerns.

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