

Available online at [GSC Online Press Directory](#)

GSC Advanced Research and Reviews

e-ISSN: 2582-4597, CODEN (USA): GARRC2

Journal homepage: <https://www.gsonlinepress.com/journals/gscarr>

(RESEARCH ARTICLE)



Characterization and blending of polyhydroxyalkanoate (PHA) produced by *Bacillus safensis* FO-366(T) on cassava peels

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Publication history: Received on 02 October 2020; revised on 17 November 2020; accepted on 20 November 2020

Article DOI: <https://doi.org/10.30574/gscarr.2020.5.2.0084>

Abstract

Plastic industry has contributed significantly to the current state of environmental damage because the polymeric materials used so far in various applications are not biodegradable. Thus, the need for viable and sustainable solutions to reduce the environmental impact and have better performance than the options currently available. In this regard, biodegradable polymers emerged as one of the solutions to cushion impact of plastics waste disposal on the environment. Cassava peels were dried and made into powder, which was used for this study with *Bacillus safensis* FO-366(T). After 48 hrs of incubation the pellets were extracted and purified with chloroform. The pellets were dried and characterised. The FTIR and GCMS revealed the presence of 3HD methyl ester as the prominent monomers of PHA. The blends showed better mechanical properties than PLA and PE. This study revealed the potential of cassava peels as a carbon source for production of biodegradable polymer.

Keywords: *Bacillus Safensis Fo-366(T)*; Cassava Peel Flour; PHA; Blending

1. Introduction

Plastics was introduced in the 1950s and used in daily life for numerous application [1], the continuous usage of plastics has become a major cause of environmental pollution, and this is because of the inability of these plastics to degrade easily. It decomposing and incineration process became limited because of the toxic gases that are involved [2, 3, 4]. Thus, there have been scientific concerns and research in regards to the development of bio-degradable plastics as alternatives for synthetic plastics which are non-biodegradable which belong to the class of polyesters, known as Poly hydroxyalkanoate (PHA) [4, 5, 6]. PHA are characterized with excellent mechanical properties similar to synthetic plastics and are biodegradable by microorganism such as *Bacillus megaterium* and *Rhizobium spp* into water and carbon dioxide [7-14]. The production of Polyhydroxyalkanoate was limited industrially because of the expensive carbon source used for the production which depends mainly on the cost of substrate and known to account for not less than 40% of the total production costs [15].

One of the requirements for decreasing the production costs is the development of better PHA-producing bacterial strains [16] which has led to the isolation of bacterial strains capable of growing and producing PHA from inexpensive raw materials and using different bacterial such as *Alcaligene eutrophus*, *Azotobacter beijerinckia*, *Pseudomonas Oleovorans*, *Rhizobium sp.* and *Bacillus sp. Schlegelella sp* [17, 18]. Poly (lactic acid) (PLA) is gotten from renewable resources [19]. It can contribute to the control of green-house gas (CO₂) emission as a result of carbon capture during plant growth and eventually complete biodegradability of the PLA matrix. Wang et al [20] blended PLA and PBAT by solvent-casting methods, obtaining homogeneous films with increased flexibility; this occurs without loss of tensile

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properties, transparency, or water vapor barrier. Pan and coworkers prepared the biodegradable PLA/PBAT blends by melt compounding using MDI as chain extender [19]. It was found that the impact strength of biodegradable PLA and PBAT blends was significantly enhanced successfully by using MDI as chain extender through reactive melt processing. Upon increasing the content of the MDI, the blends showed increased yield tensile strength, modulus, and elongation at break. Jiang and coworkers prepared PLA/PBAT blends by melt blending [21]. It was found that the PBAT component accelerated the crystallization rate of PLA but had little effect on its final degree of crystallinity. With the increase in PBAT content, the blend showed decreased tensile strength and modulus; however, elongation and toughness were dramatically increased. In addition, with the addition of PBAT, the failure mode changed from brittle fracture of the neat PLA to ductile fracture of the blend. Debonding between the PLA and PBAT domains induced large plastic deformation in PLA matrix ligaments.

There are plenty of low-cost, renewable, carbon-containing raw materials available around the world, cassava peels is one of such renewable agricultural commodity. This study deals with the isolation of *Bacillus safensis* FO-366(T) and use of cassava peels for the production of poly- β -hydroxybutyrate and blending the polymer with polyethylene PE and polylactic acid PLA and compare the properties of the blends.

2. Material and methods

2.1. Material collection

Fresh peels of *Manihot esculenta* (cassava) peels were washed with distilled water, sun dried for 8 days and made to powder by grounding and sieving with 32 mesh size sieve while glucose was used a control in the process of PHA production and to compare yield to the subtract studied. Pure strain of *Bacillus safensis* FO-366(T) was collected from Microbiology Research Laboratory of Federal University of Technology, Akure, Nigeria.

2.2. Media preparation and Sub-culturing of *Bacillus safensis* FO-366(T)

The pure culture of *bacillus safensis* FO-366(T) was introduced with sterile loop to already prepared homogenized media (2g of nutrient agar and 100mL of distilled water and 5mL in bottle and autoclave at 110°C) followed by 37°C incubation for 24 hrs. Preservation of colonies obtained were done on the nutrient agar at 4°C.

2.3. PHA Production and Quantification

Kannan and Rehacek medium was modified to make a PHA production medium stated as (g/L), KCl 2.5, (NH₄)SO₄ 3.0, 2H₂O 2.0, yeast extract 2.0, glucose 15.0, cassava peels subtract (10 g/L) was added as the sole carbon source in with pH adjustment of 6.8 using 1 N HCL and 1 M NaOH [20, 21, 22]. *Bacillus safensis* FO-366(T) was inoculated at level of 2% on 100 mL of production medium, incubated on a rotary shaker (120rpm) at 35°C for 48 hrs. During the 48 hrs of production process, yield of production of PHA were monitored every 8 hours by centrifuging 50 mL of the aliquots at 4000rpm. Pellets were extracted at the end of the fermentation hours. The slurry were poured into 50mL centrifuging tube and centrifuge at 4000rpm for 20min, the tubes were stand for 10min then decanted, 10mL sodium hypochlorite was introduced, after 2 hrs of incubation, the slurry was centrifuged again at 4000rpm for 15min then washed with distilled water follow by addition of 10mL acetone and 10mL ice cold methanol. PHA were obtained at the second layer and purified with chloroform [23, 24].

2.4. Cell Dry Weight (CDW)

The pellets harvested was weighed before drying to attained constant weight at 50°C.

2.5. Characterization of the Polymer

Exactly 2 mg of the dried sample of the polymer produced (PHA) was dissolved in chloroform and added to KBr pellets and solvent evaporated. Elmer RX1 Fourier transform infrared (FTIR) spectrophotometer was used for the analysis within spectral range 4400-350 cm⁻¹ for chemical structure [25]. Identification of polymer produced was done using GC-MS analysis of the methanolysed polymer. The polymer was prepared and dissolved in chloroform as described by [26]. The sample in chloroform (1 μ l) was injected with helium (1ml min⁻¹) as the carrier gas into the GC-MS (Agilent 1909IS-933H-1MS), the injector temperature was 290°C.

2.6. Preparation of 3HD with PE and PLA blends

Blending of PHB with PE and PLA were prepared by manual mixing 3HD with PE and PLA at different proportion. The sample compounding in chloroform was performed using heating mantle at 95°C. The ratio of 3HD with the PE and PLA

blends were shown in the table below, after 20 min complete met in the solvent, the samples were cooled in square mould at room temperature to form the sheets needed for characterization.

Table 1 Composition of the blends

3HD(g)	PE(g)	PLA (g)
10	50	50
20	40	40
30	30	30

2.7. Mechanical properties of the blends

Properties of the blends were measured on an Instron-5369 tensile tester 50KN capacity. The specimens were prepared into dumbbell type with dimensions of 50 mm (total length) × 10 mm (width) × 4 mm (narrow portion width) × 2.0 mm (thickness) then sent to central laboratory, Obafemi Awolowo University Ile-Ife, Nigeria.

3. Results and discussion

3.1. PHA production

Figure 1 Showed PHA yield with respect to 8 hours' time interval in the cassava peels and glucose respectively. PHA yield and time was inversely proportional to the pH of the media. The result clearly shows that the higher the time the more yield was obtained which is proportional to increase in optical density. The pH of the glucose sample became more acidic than the cassava peels sample with increase in time which may be due to other basic component in the peels affecting media pH. The dry cell weight (Table 1) shows that the percentage yield of PHA from glucose was higher than cassava peels, although the readily availability of the cassava peels can substitute for the yield percentage at industrial level of production.

Table 2 Dry cell weight and Polyhydroxyalkanoate accumulation of substrates

Substrate	Dry cell weight (g/L)	Polyhydroxyalkanoate (%)
Cassava peels	4.2	42
Glucose (Control)	6	60

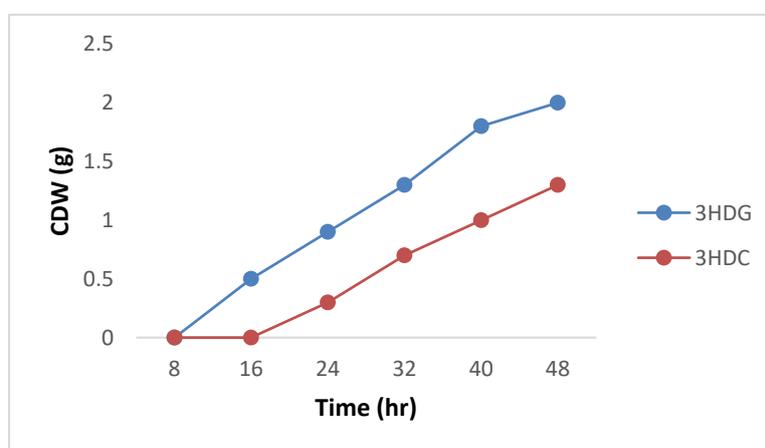


Figure 1 Substrate production of polymer during fermentation

3.2. Characterization of the polymer

Table 2 The FT-IR results shows polymers absorption in Figure 2 and Figure 3

Peaks cm^{-1}	Functional
3359 - 3405	O-H
2944 - 2949	C-H
1656 - 1643	C=O
1421 - 1409	-CH ₃
1027 - 1022	Sugar derivatives

[27, 28, 29]

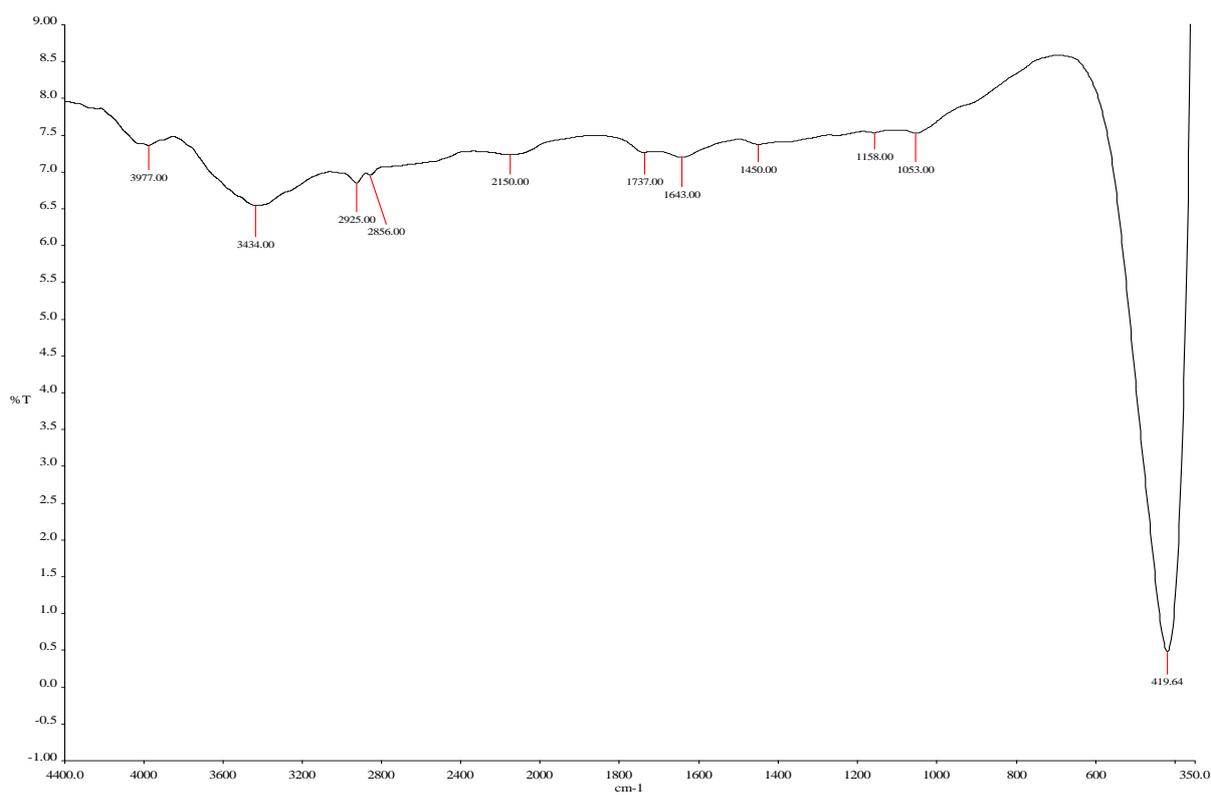


Figure 2 FTIR curve of the polymer extracted from glucose substrate

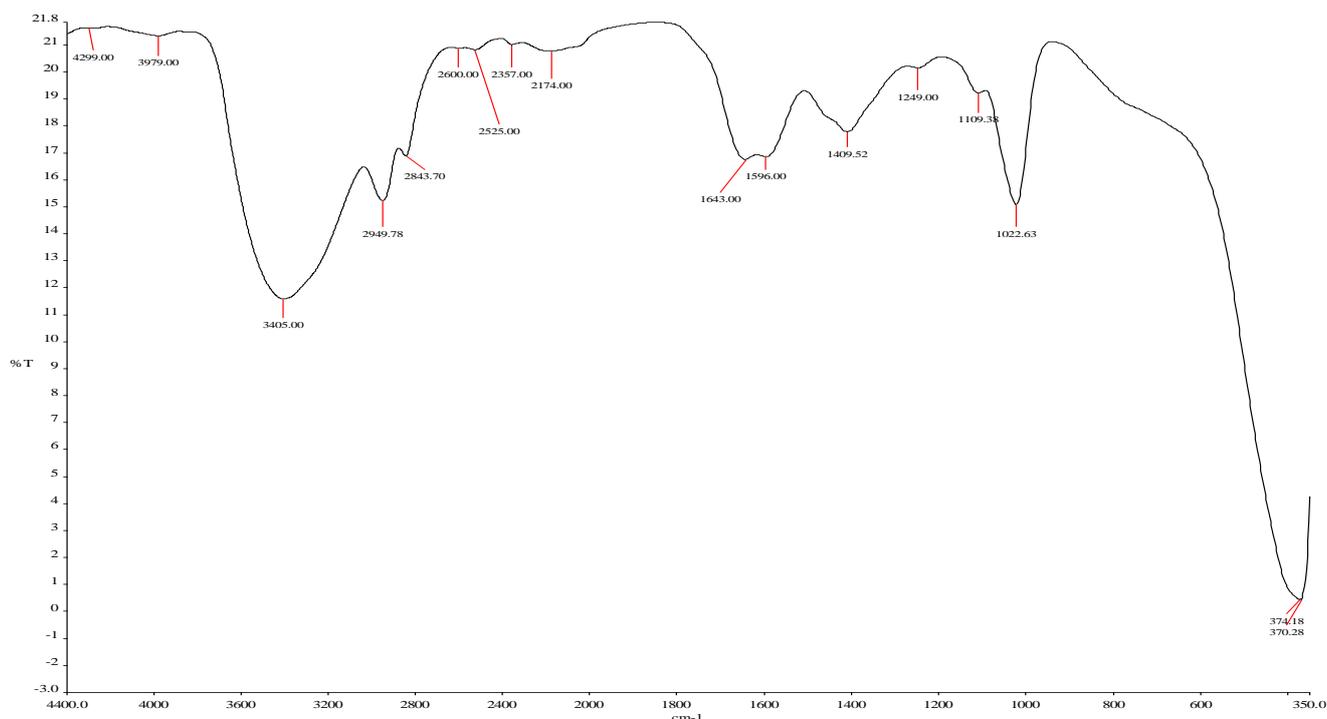


Figure 3 FTIR curve of polymer extracted from cassava peels substrate

3.3. GC-MS Analysis of polymer extracted using cassava peel as the substrate

The mass spectrum of the peak at a retention time (Rt) of 7.9 min was identical to methyl ester of 3-hydroxydecanoic acid in the MS library, confirming that the polymer produced was the polymer of 3-hydroxydecanoate. 3HD was the main monomer unit. 2-ethyl-2-hydroxybutyric acid was used as an internal standard and was represented by the peak at a retention time of 5.55 min

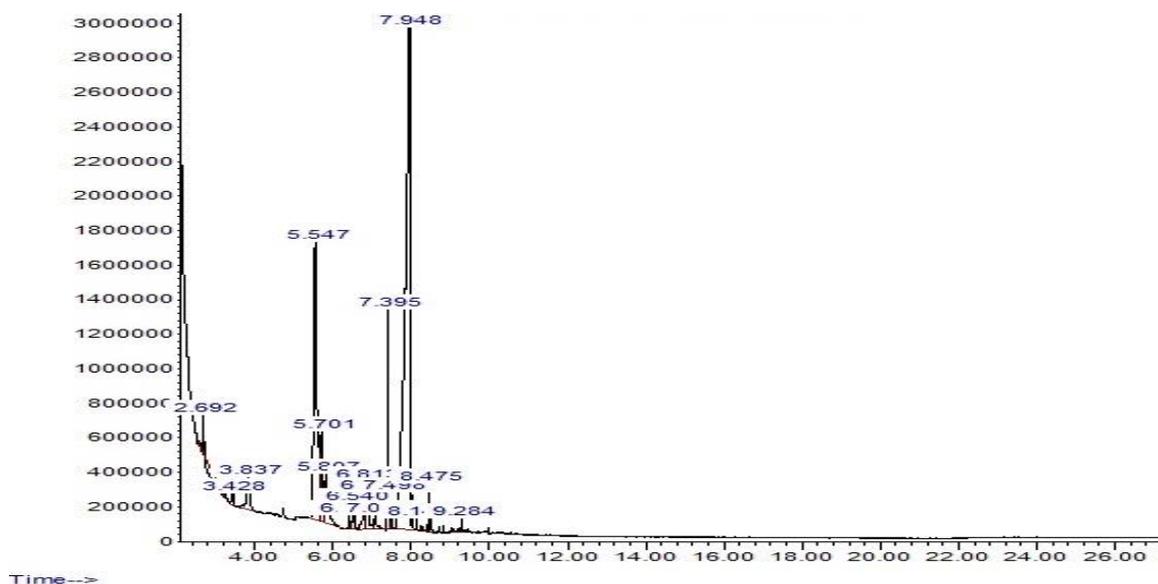


Figure 4 GC-MS analysis of polymer extracted from cassava peels substrate

3.4. Mechanical properties of the blends

Table 3 showed the mechanical properties of 3HD, PE and PLA blends, the PLA content in 3HD/PLA matrix increases, an increase in Young's modulus was noted. Comparing the results obtained for 3HD/PLA and PE matrix with higher and lower PLA and PE contents, respectively, corroborate previous reports [30] Elongation at break were 3HD/PLA/PE 40/20 (51.368 ± 0.98 and 55.021 ± 0.37). These results are expected since both polymers are rigid in their pure state, 3HD/PLA/PE blends comparative relationships can be established regarding this mixing ratio. Therefore, the observed

value of the modulus, strain and strength shown by both blends with respect to their pure form, an approximate increase of 264.56% is obvious, whereas if compared with 3HD/PLA/PE Young modulus, an approximate increase was observed. Regarding the final strength, behaviour marked by a pattern does not occur as in the case of the Young modulus. It was observed that the values of the properties vary independently of the content of PLA or 3HD; however, it can be noted that 3HD/PLA blends exhibit higher values than 3HD/PE matrix.

Table 3 Tensile strength of PHB blends with Polyethylene and PLA

Samples	Elongation at Break (%)	Maximum Tensile strength (mPA)	Tensile strain (Standard) (mm)	Young Modulus (E)
3HD/PE10	27.433± 0.58	0.275± 0.01	0.567± 0.03	0.497± 0.04
3HD/PE20	51.368± 0.98	0.959± 0.17	1.267± 0.03	0.599± 0.06
3HD/PE30	44.552± 0.23	1.421± 0.02	3.534± 0.05	0.830± 0.01
3HD/PLA10	28.001±1.20	0.756±031	0.431±5.30	0.521±270
3HD/PLA20	55.021±0.37	1.542±810	0.926±300	2.180±001
3HD/PLA30	43.530±185	1.032±002	0.351±001	0.941±110

4. Conclusion

The substrate (Cassava peels) was used for PHA production using the *Bacillus safensis* FO-366(T). The fermentation lasted for 48 hours, the yield increases with time and optical density was inversely proportional to the pH of the media. Dry weight shows that the pure glucose yielded more than the cassava peels. The FT-IR and GC-MS confirmed hydroxydecanoate (3HD) as most prominent class of polyhydroxylakanoate. The blends showed better mechanical properties and conformity in the composites formed. The study shown that cassava peels could be used as a good source of carbon for the production of 3HD.

Compliance with ethical standards

Authors' contributions

This study was collectively done by all the authors. Author BRB conceived the idea, carried out the experimental work and interpreted the data. Author OVC reviewed literatures and edited the manuscript. Author BOA performed preliminary data analysis. All authors read and approved the manuscript.

Acknowledgments

We acknowledged the contribution of Dr. Agbabiaka Sola (USA) and Mr. Aransiola Sesan Abiodun.

Disclosure of conflict of interest

No conflict of interest.

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