

**PRODUCTION OF POLYHYDROXYALKANOATE (PHA)  
BIOPOLYMER FROM WASTE PRODUCTS OF FRUIT-BASED  
FOOD INDUSTRY, SRI LANKA**

Bastiangamage Sanali Upeksha Jayalath

Degree Master of Science in Environmental Management

Department of Civil Engineering

University of Moratuwa

Sri Lanka

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Bastianganage Sanali Upeksha Jayalath

Thesis/Dissertation submitted in partial fulfillment of the requirements for the  
degree Master of Science in Environmental Management

Department of Civil Engineering

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July 2022

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## ABSTRACT

Recently, conventional petrochemical-based plastic waste has been a global crisis, creating numerous environmental and economic challenges. Addressing this problem, the present study was aimed to assess the feasibility of bacterial polyhydroxyalkanoates (PHA) synthesis from locally available lignocellulosic fruit waste obtained from the fruit-based industry in Sri Lanka. The present study was focused on assessing the feasibility of bacterial PHA synthesis from locally available lignocellulosic fruit waste obtained from the fruit-based industry in Sri Lanka. This study investigated the viability of using papaya (*Carica papaya*), mango (*Mangifera indica*), watermelon (*Citrullus lanatus*), and pineapple (*Ananas comosus*) fruit wastes as substrates for PHA production. The pure strain of *Bacillus subtilis* bacterium was selected as the microorganism for 72 h fermentation process to produce PHAs under ambient temperature conditions. The bacterial strain cultured in modified LB agar media was stained with Sudan Black B (SBB) as a preliminary screen to confirm PHA production. Two pretreatments (Crude aqueous extract: CAE, and sulphuric acid treatment: SAT) were performed to analyze total reducing sugars in fruit waste. Comparatively the highest concentration of reducing sugar was obtained with SAT pretreatment method. Maximum average value of cellular dry weight (CDW) was obtained in papaya waste ( $2.15 \pm 0.15$ ) g/L at 60 h of fermentation and minimum value was obtained in watermelon ( $1.23 \pm 0.06$ ) g/L after 72 h of Fermentation. Fourier transform infrared spectra (FTIR) were performed for the obtained extracted samples, and PHA synthesis was confirmed by the corresponding peaks of PHA functional groups. The proof-of-concept stage was realized to promote PHA to be developed from fruit wastes.

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## List of Abbreviations

|            |   |
|------------|---|
| 3HB        | 3-hydroxybutyrate                       |
| 3HD        | 3-hydroxydecanoate                      |
| 3HDD       | 3-hydroxydodecanoate                    |
| 3HH        | 3-hydroxyheptanoate                     |
| 3HHx       | 3-hydroxyhexanoate                      |
| 3HV        | 3-hydroxyvalerate                       |
| 3HV        | 3-hydroxyvalerate                       |
| 4HB        | 4-hydroxybutyrate                       |
| 5HV        | 5-hydroxyvalerate                       |
| Acetyl CoA | acetyl coenzyme A                       |
| BioPE      | biopolyethylene                         |
| CAE        | crude aqueous extract                   |
| CDM        | cell dry mass                           |
| CFU        | colony forming units                    |
| CoA        | coenzyme A                              |
| DCW        | dry cell weight                         |
| DNS        | 3,5-Dinitrosalicylic acid               |
| FTIR       | Fourier transform infrared spectroscopy |

|          |  |
|----------|--|
| LB media | luria-bertani media                                |
| lcl-PHA  | long chain length PHA                              |
| mcl-PHA  | medium chain length PHA                            |
| OD       | optical density                                    |
| P(3HB)   | poly-3-hydroxybutyrate                             |
| P(3HP)   | poly-3-hydroxypropionate                           |
| P(3HV)   | poly-3-hydroxyvalerate                             |
| P(4HB)   | poly-4-hydroxybutyrate                             |
| PHA      | Polyhydroxyalkanoates                              |
| PHB      | poly(3-hydroxybutyrate)                            |
| PHBHHx   | copolymer of 3HB and (R)-3-hydroxyhexanoate(3HHx). |
| PHBV     | Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)       |
| PHHx     | poly(3-hydroxyhexanoate)                           |
| PHV      | poly(3-hydroxyvalerate)                            |
| PLA      | polylactic acid                                    |
| SAT      | sulphuric acid treatment                           |
| SBB      | sudan black B stain                                |
| scl-PHA  | short chain length PHA                             |
| TPS      | thermoplastic starch                               |