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REVALUATION OF AGRO-INDUSTRIAL WASTE THROUGH BIOTECHNOLOGY: PAPAYA PEEL WASTE AS FEEDSTOCK FOR PHB PRODUCTION BY NATIVE BACILLUS STRAINS

MDPI

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BACKGROUND

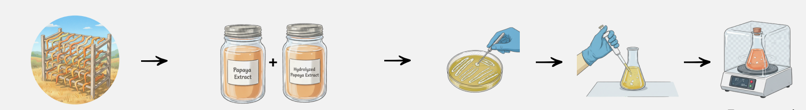
Conventional plastics pose a severe environmental crisis. Polyhydroxyalkanoates (PHAs) are fully biodegradable biopolyesters, offering a sustainable alternative.
However, high production costs, driven by expensive pure carbon substrates (e.g., glucose), currently limit their widespread use.
Agro-industrial by-products are a low-cost, sustainable solution. Papaya peel is a major, nutrient-rich waste stream (rich in maltose and minerals) but remains an unexploited resource for PHA production. This study aims to valorize this waste using *Bacillus thuringiensis* (C01).

AIM

To optimize the production of Poly(3-hydroxybutyrate) [P(3HB)] from papaya peel extract as the sole carbon source using Bacillus thuringiensis (C01).

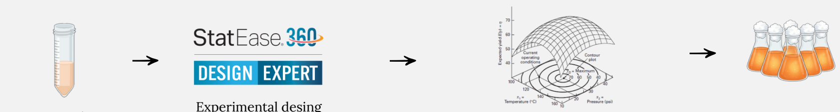
METODOLOGY

STAGE 1: SUBSTRATUM PREPARATION AND PREELMINAR FERMENTATION



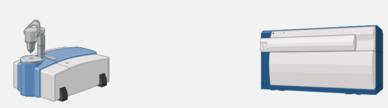
Drying: 7 days at room temperature → Sugar extraction: Aqueous (90-100°C - 30min) Hydrolysis (HCl 1, 2, 3% v/v) → Streak plate method *B.Thuringiensis* → Mc Farland Bacterial adjustment $3x10^8$ UFC/mL → Fermentation 72 hours 30°C 120 rpm

STAGE 2-3: OPTIMIZATION AND VALIDATION



Extraction 700 uL $CHCl_3$ 300 uL NaClO (5%) → Experimental desing Temperature (A) Sugar conc (B) pH (C) Inoculum concentration (D) → Model analysis and optimization for volumetric productivity → Model validation by confidence Interval (95%) Q_P

STAGE 4: CHARACTERIZATION



Fourier transform infrared spectroscopy

MALDI-TOF/TOF-MS

RESULTS

STAGE 1: SUBSTRATUM PREPARATION AND PREELMINAR FERMENTATION

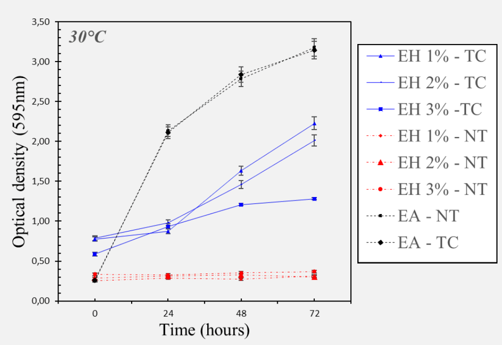
Treatment	Reducing sugars (g/L)	Soluble proteins (g/L)	Phenolic compounds (ppm)
EA	10.98 ± 0.16^a	0.759 ± 0.019^a	37.52 ± 1.44^d
EH 1%	11.07 ± 0.11^b	0.683 ± 0.087^{ab}	501.24 ± 4.5^b
EH 2%	11.55 ± 0.16^c	0.626 ± 0.100^{bc}	520.07 ± 7.45^c
EH 3%	12.08 ± 0.24^c	0.530 ± 0.15^c	549.99 ± 5.56^a

Note: AE: Aqueous Extract; HE 1%: Hydrolyzed Extract HCl 1% (v/v); HE 2%: Hydrolyzed Extract HCl 1% (v/v); HE 3%: Hydrolyzed Extract HCl 1% (v/v). Different capital letters in the same column indicate statistically significant differences (p < 0.05).

Parameter	Result
Ash	8%
Calcium	34035.63 mg/Kg
Magnesium	5720.15 mg/Kg
Sodium	1756.59 mg/Kg
Potassium	40081.57 mg/Kg

Note: Physicochemical analysis of papaya peel

Extract	Dry biomass (g/L) (<x>±IC 95%)	PHA accumulation % p/p	PHA (g/L) (<x>±IC 95%)
EH 1% -TC	2.594±0.055	54.6%	1.416±0.095
EH 2% -TC	2.292±0.157	42.0%	0.969±0.102
EH 3% -TC	1.347±0.142	34.3%	0.462±0.083
EH 1% - NT	0	0	0
EH 2% - NT	0	0	0
EH 3% - NT	0	0	0
EA – NT	3.694±0.040	62.0%	2.290±0.171
EA – TC	3.503±0.138	61.0%	2.231±0.014



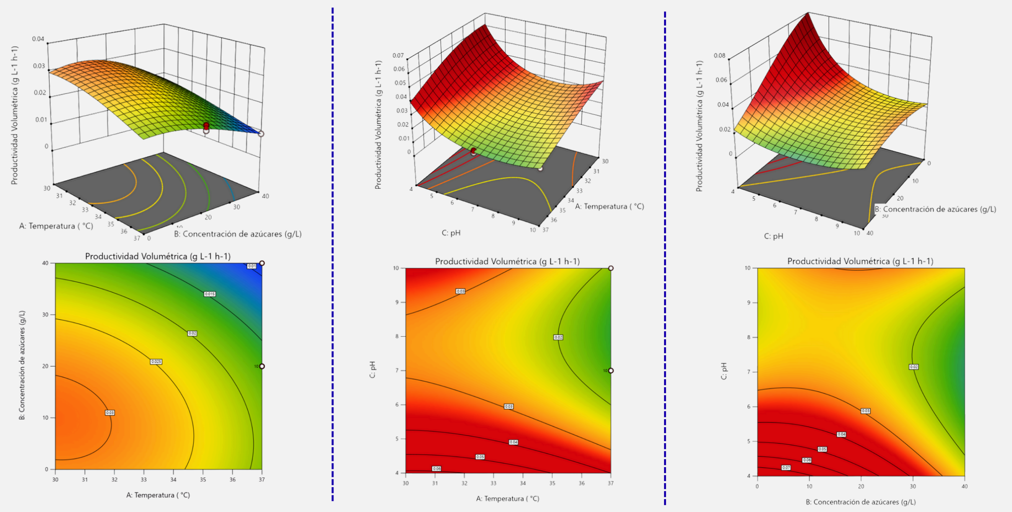
Note: AE: Aqueous Extract; HE 1%: Hydrolyzed Extract with 1% (v/v) sulfuric acid; HE 2%: Hydrolyzed Extract with 2% (v/v) sulfuric acid; HE 3%: Hydrolyzed Extract with 3% (v/v) sulfuric acid. TC: Treated with Activated Carbon; NT: Not Treated.

Aqueous extraction (AE) was selected as the ideal, non-inhibitory feedstock.

- Acid Hydrolysis (AH) generated severe microbial inhibitors, including >500 ppm of phenolic compounds and potential furfurals, which completely inhibited B. *thuringiensis* growth.
- Detoxification of AH with activated carbon was insufficient and failed to match the performance of simple AE.
- AE provided a nutrient-rich medium (sugars, minerals, proteins) that promoted robust bacterial growth.

STAGE 2-3: OPTIMIZATION AND VALIDATION

$$PHA\ (Y) = \{-1.60 - (0.1230\ x\ \boldsymbol{A}) - (0.1334\ x\ \boldsymbol{B}) - (0.1322\ x\ \boldsymbol{C}) - (0.0190\ x\ \boldsymbol{D})$$
$$- (0.0285\ x\ \boldsymbol{AB}) - (0.0289\ x\ \boldsymbol{AC}) + (0.0129\ x\ \boldsymbol{AD}) + (0.1278\ x\ \boldsymbol{BC})$$
$$- (0.0455\ x\ \boldsymbol{BD}) + (0.0077\ x\ \boldsymbol{CD}) - (0.0516\ x\ \boldsymbol{A^2}) - (0.1058\ x\ \boldsymbol{B^2})$$
$$+ (0.2179\ x\ \boldsymbol{C^2}) - (0.3708\ x\ \boldsymbol{D^2})\}$$

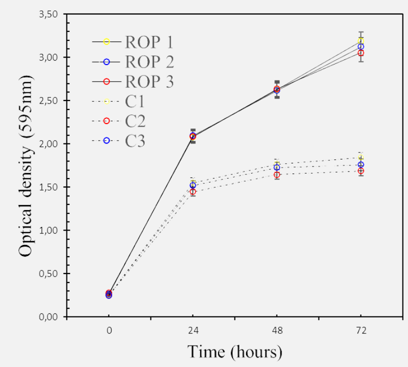


Note: Interaction between factors A-B; A-C; B-C

A Central Composite Design (CCD) successfully modeled the effects of Temperature (A), Substrate Concentration (B), pH (C), and Inoculum (D) on Volumetric Productivity (Q_p).

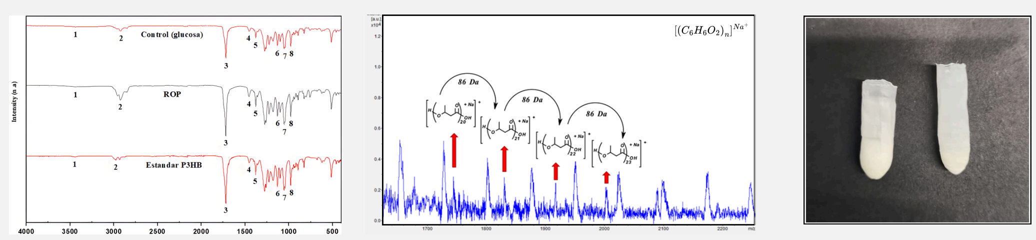
- The model was highly significant ($p < 0.0001$) with a strong coefficient of determination (R^2-adjusted = 0.9958) (R^2-predicted = 0.9902) .
- Temperature, Substrate Concentration, and pH were identified as the most critical factors influencing Q_p.
- Optimal conditions were validated at 31.4°C, 10.1 g/L substrate, pH 5.6 and 4% v/v ($1x10^7$ UFC/mL) inoculum conc.

Essay	Dry biomass(g/L)	PHA accumulation (% p/p)	PHA (g/L)	Q_p $g.L^{-1}h^{-1}$
ROP 1	3.38	81.2	2.75	0.038
ROP 2	3.31	80.7	2.67	0.037
ROP 3	3.23	80.54	2.61	0.036
C 1	1.66	25.33	0.42	0.005
C 2	1.46	26.33	0.39	0.005
C 3	1.54	25.66	0.4	0.005



Note: ROP: Aqueous extract test under optimal fermentation conditions; C: Control (glucose as carbon source) under optimal fermentation conditions.

STAGE 4: CHARACTERIZATION



The optimized process (ROP) was validated against a pure glucose control (C), and the resulting polymer was characterized.

- Superior Performance: The papaya peel substrate (ROP) was 6.6 times more productive (Q_p = 0.0371 g/L·h) than the glucose control (Q_p = 0.0056 g/L·h).
- High Accumulation: B. thuringiensis achieved a final PHA concentration of 2.67 g/L with an exceptionally high intracellular accumulation of 80.8% (p/p).
- FTIR Identity: FTIR analysis confirmed the polymer's identity as P(3HB) via the characteristic ester carbonyl peak at 1720 cm^{-1}.
- MALDI-TOF-MS Confirmation: MALDI confirmed the polymer structure, showing a clear repeating monomer series of 86 Da ($C_4H_6O_2$).
- High Quality Polymer: The polymer was found to be nearly monodisperse, with an extremely low Polydispersity Index (PDI) of 1.0057, indicating highly uniform chains desirable for industrial applications.

CONCLUSIONS

1. **Aqueous extraction (AE) of papaya peel is a simple, effective, and non-inhibitory method for creating a nutrient-rich feedstock for B. thuringiensis.**
2. **Response Surface Methodology (RSM) was a powerful tool to optimize PHA production, maximizing volumetric productivity (Q_p).**
3. **Papaya peel extract proved to be a vastly superior substrate to pure glucose, yielding 6.6x higher productivity.**
4. **B. *thuringiensis* (C01) successfully synthesized a high-quality, nearly monodisperse P(3HB) (PDI = 1.0057) from this agricultural waste, confirming the viability of papaya peel as a low-cost feedstock for a circular bioeconomy.**

References