BIOCONVERSION OF WASTE INTO VALUE ADDED BIOMATERIAL

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Background

- **Polyhydroxyalkanoates (PHAs)**; a non-toxic, **biodegradable and eco-friendly bioplastic synthesized intracellularly by certain microbes,** could be an **alternative to petroleum-based plastics** .
- PHAs are considered a more attractive biomaterial in research with high future impact due to its desirable properties and extensive applications especially for packaging materials and in medicine for the production of biodegradable implants, drug carriers and biocontrol agents.

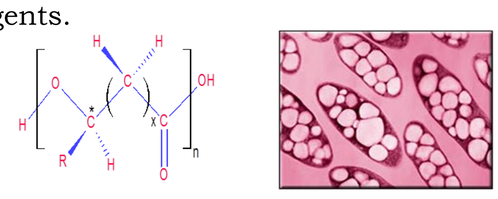
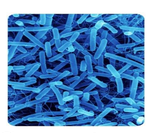
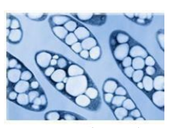


Figure 1: PHA structure (left) PHA granules within microbes (right)

- However, factors currently limiting the advanced application of PHAs are:
  (i) **High cost** of nutrient sources for its biosynthesis
  (ii) Hydrophobic nature with fewer functional groups
  (iii) Poor mechanical properties
- To reduce the production cost of PHAs and make it more affordable than petroleum-based plastics, various cheap waste materials are currently being researched upon as a sustainable carbon source for PHA production.
- **Tetra Pak (TP)** is a type of packaging (made up of aluminum foil, cardboard paper and low density polyethylene) widely used for aseptically packaging billions of litres of liquid food items, such as milk and juice.
- To reduce the environmental impact of waste tetra pak and brewery waste, this study proposes a **novel recycling technique of using brewery waste and the LDPE component in tetra pak waste as a source of feed for the production of bioplastics (polyhydroxyalkanoates - PHA) by *Cupriavidus necator*;** a bacterial strain.

Tetra Pak +  ***C.necator*** =  ***PHA granules in C.necator*** →  ***Packaging materials from PHA***

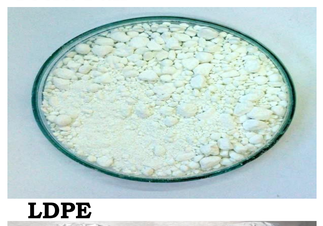
Objectives

- To isolate PHA from brewery waste (BW)
- To use LDPE from waste tetra pak (TP)as a carbon source for PHA production by ***C.necator***
- To characterize the PHA obtained using NMR and ESI/MS

Methodology

Tetra Pak

Tetra Pak was pre-treated to obtain LDPE using solvent separation techniques

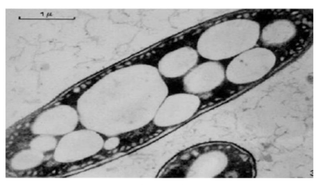


LDPE

0.50 g of LDPE was added into the fermentation flask

Fermentation

fermentation flasks containing *C.necator* and LDPE in either Tryptone soy broth (TSB) or Basal salt medium (BSM) was incubated for 48 h at 30°C

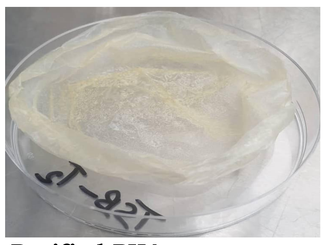


PHA granules in *C. necator*

PHA in *C.necator* was extracted using chloroform for 48 h

Extraction and purification of PHA

Extracted PHA was purified using rotatory evaporation and hexane precipitation



Purified PHA bioplastic

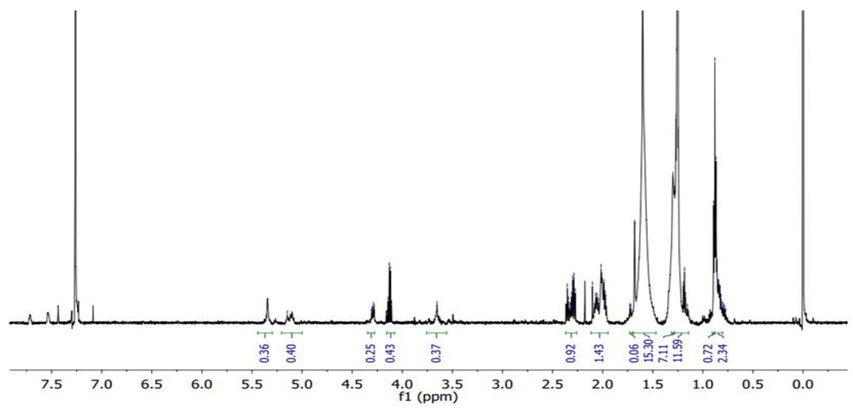
PHA yield was determined and purified PHA was characterized using NMR and ESI-MS

Results

Fermentation Conditions	Average Cell dry Weight (g)	Extracted PHA (g)	PHA (% w/w)
Brewery Waste	2.14	0.033	1.5%
TSB only	0.865	0.013	1.5%
TSB with Tetra Pak	0.820	0.330	40%
BSM only	0.073	ND	ND
BSM with Tetra Pak	0.087	ND	ND

Table 1: PHA yield obtained from brewery waste and tetra pak waste

(a)



(b)

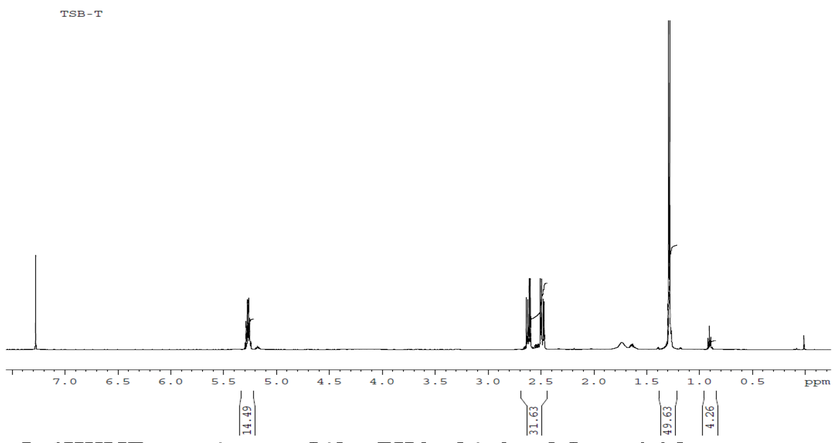


Figure 2: ^{1}HNMR spectrum of the PHA obtained from (a) brewery waste and (b) tetra pak waste

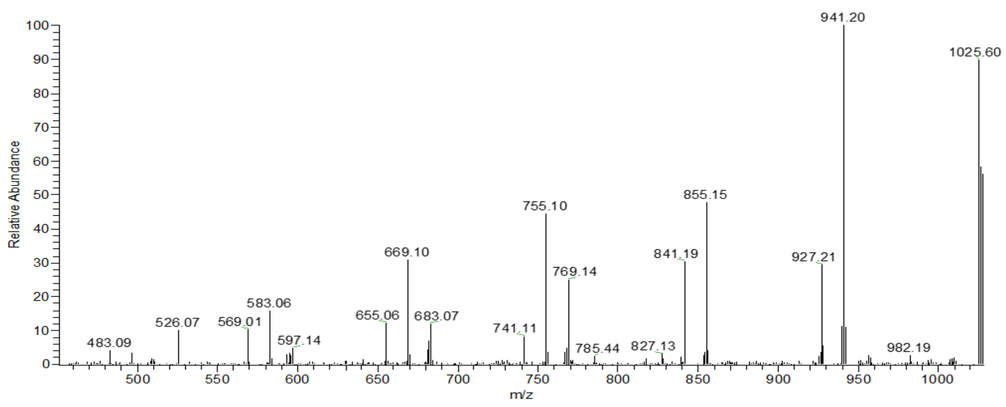


Figure 3: The ESI-MS/MS spectrum (positive-ion mode) of the PHA oligomers parent ion at *m/z* 1025, obtained *via* partial thermal degradation of the biopolyester produced by *C.necator* H16 in TSB using TP as an additional carbon source.

Discussion

- The Highest PHA yield (40%) was observed when TSB was supplemented with TP showing that the addition of TP stimulated the production of PHA in *C. necator* when compared to TSB only (1.5%) and brewery waste (1.5%)
- NMR spectrum of brewery waste confirmed the presence of 3-hydroxybutyrate units (a monomer type of PHA) at 1.25 ppm (CH_3), 2.40 ppm (CH_2) and 5.25 ppm (CH).
- NMR spectrum of tera pak waste confirmed the presence of 3HB and 3-hydroxyvalerate units (a copolymer type of PHA) at 1.28 ppm (CH_3), 2.64 ppm (CH_2) , 5.32 ppm (CH) and 0.9 ppm (CH_3), 2.49 ppm (CH_2), 5.19 ppm (CH) respectively.
- ESI-MS/MS spectrum of PHA obtained from TSB supplemented with tetra pak waste further confirmed the presence of 3-HB and 3-HV units.

Conclusion

- This study shows that Tetra Pak waste containing low density polyethylene is a suitable and cheap carbon source for the production of bioplastic (polyhydroxyalkanoate) by *Cupriavidus necator* than brewery waste.

Acknowledgements

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