King Midas approach - Turning waste plastic into valuable bioplastics

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What are bioplastics?

Due to our **decreasing reserves of fossil fuels** we need to find **alternatives** to **petro-chemically** made **plastics**. Polyhydroxyalkanoates (PHAs) could be an alternative **bioplastic** [1].

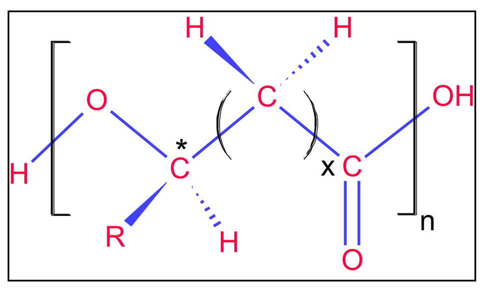
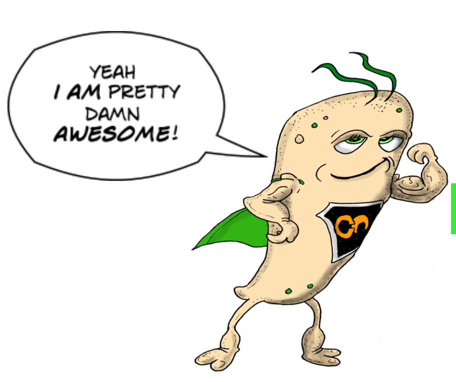


Figure 1: PHA structure

PHAs are a group of **biocompatible, non toxic, recyclable** bioplastics that can be produced by certain types of bacteria. One such bacterium is ***Cupriavidus necator***, selected for this study because it is very **robust**, able to produce **high yields** of PHAs and it **grows well at relatively low temperatures**, making it economically viable [2].



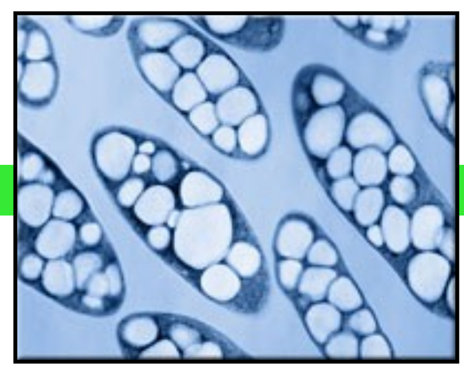


***Figure 2: C. necator bacterial cells with PHA granules inside* [3]**

PHAs can be adapted for a **wide range** of applications:

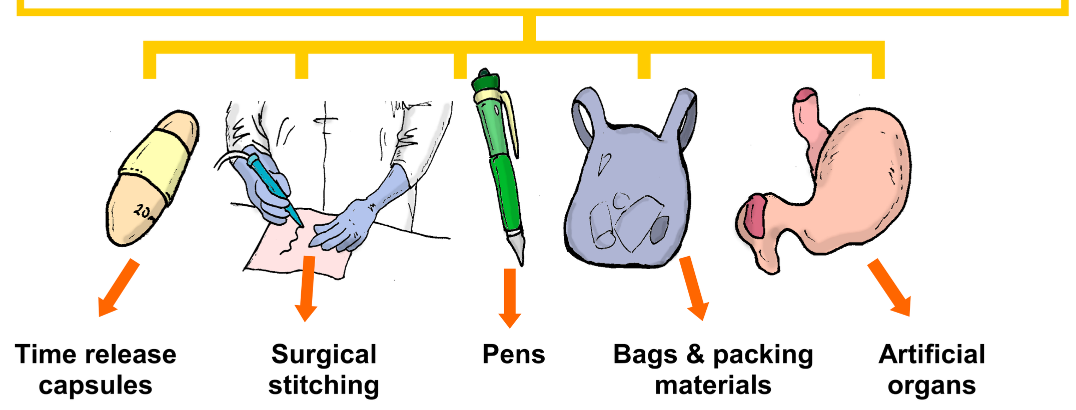


Figure 3: Applications of PHAs [1- 4]

The factors **limiting** mass usage of PHAs today are **high costs** of the carbon sources needed and the expensive processing requirements to extract and develop stable PHA structures in comparison to petrochemical plastics used currently [4]. **This research** takes a novel approach using waste shredded plastics to make use of oxidised polyethylene wax (O-PEW) as a food source for bacteria with a hope to increase their ability to make PHAs at **lower cost**, using more **environmentally friendly** processes [5-6]. The overall process will not only use a recycled carbon source but also use green chemicals for the polymer extraction from the cell biomass.

Project aims

- To use *C. necator* and a modified strain to produce PHAs with **recycled plastic waxes**.
- To successfully detect and analyse the waxes and PHAs using a range of analytical techniques to confirm project practical and economical validity.

Materials

- *Cupriavidus necator* formally *Ralsonia eutropha* H16 (NCIMB 10442, ATCC 17699) obtained from the University of Wolverhampton culture collection.
- O-PEW at varying acid number (**AN**– *an indication of the amount of oxygen exposure time the wax had*) produced by The Department of Chemical Organic Technology and Petrochemistry, Silesian University of Technology, Gliwice, Poland.
- All chemicals in this study were provided by Sigma Aldrich.

Methodology

Upstream: To prevent any impurities that could have a negative affect on the bacteria in the BSM/TSB growth media, no initiators or catalysts were used in the production of the waxes (***orange boxes 1 & 2***). This has the added advantage of making the process more **green**.

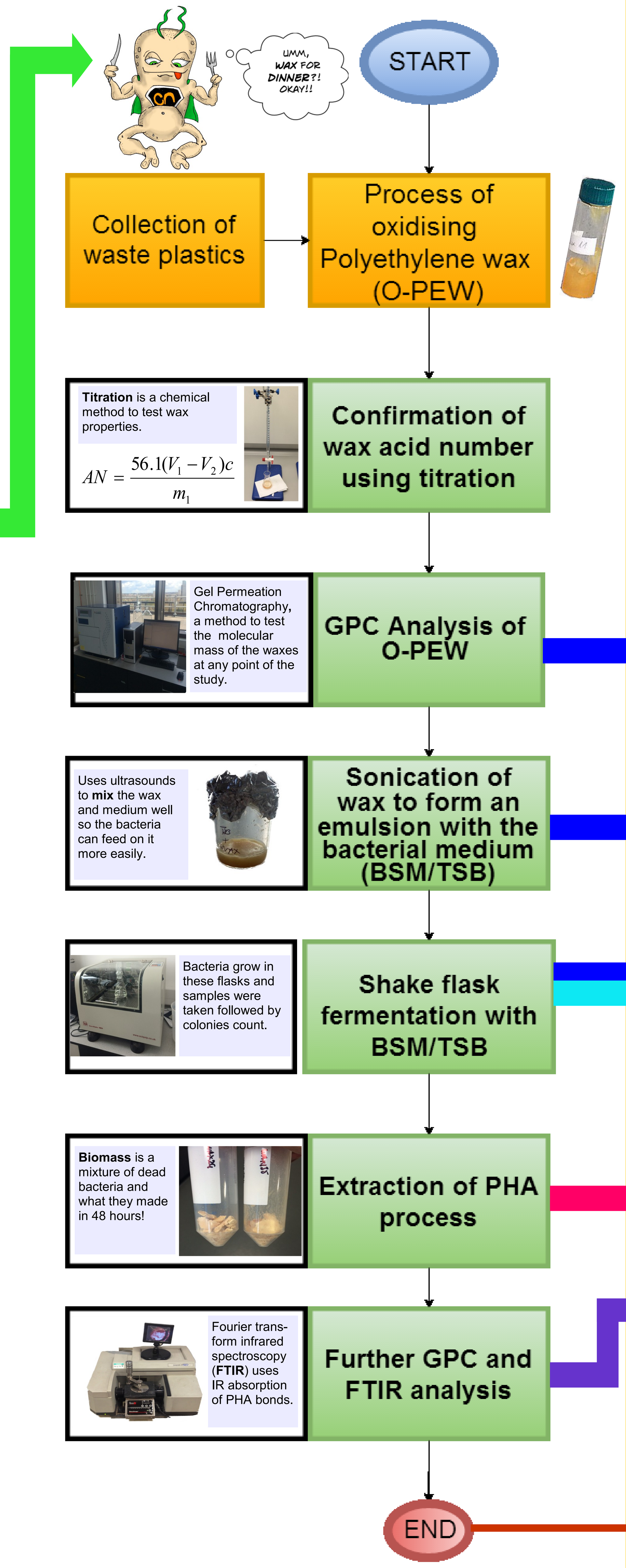
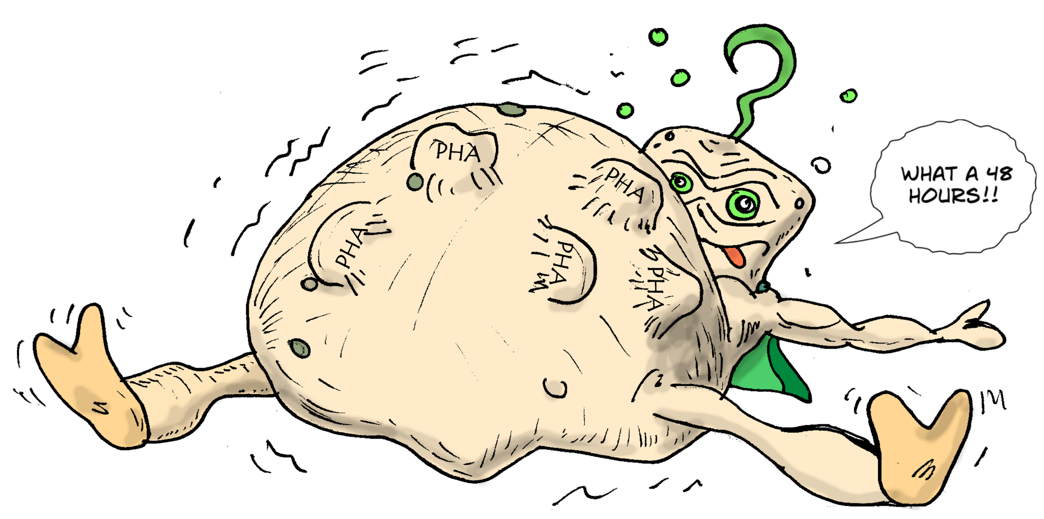


Figure 4: Summary of PHA production and analysis. (green boxes are processes investigated)

Downstream: PHA **extraction** was done after 48 hours of bacterial growth. The biomass obtained was frozen overnight at -20 °C and then dried in a vacuum. Chloroform and heating was then used to separate the PHA from any debris. The PHA produced could then be further processed to make biodegradable items.



Results

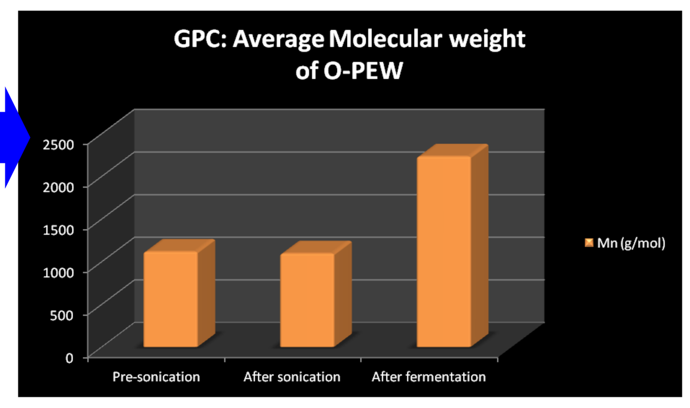


Figure 5:

GPC results of wax AN195 throughout the research process.

Here the average molecular mass (Mn) of the wax decreases slightly after sonication but post fermentation it increases from 1100 g/mol to 2200 g/mol, indicating **smaller wax molecules were most likely being used up by the bacteria as a food source.**

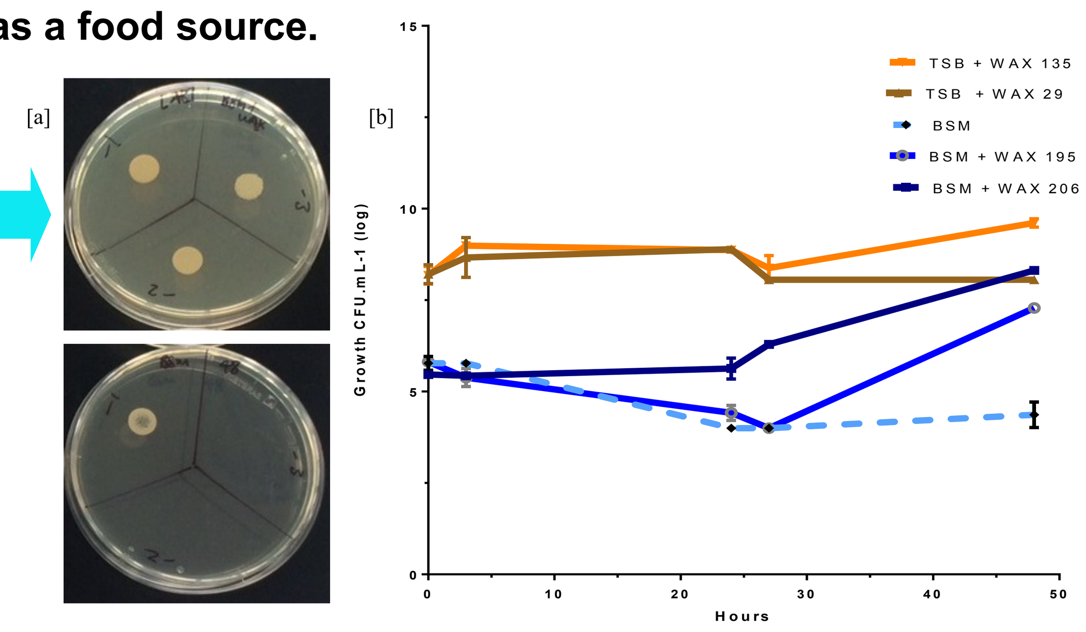


Figure 6: (a) Growth of C. necator on TSA plates after serial dilutions of 10^{-1}, 10^{-2} and 10^{-3} from a BSM medium. (b) Growth of bacteria in BSM/TSB over 48 hours.

(a) Top plate - shows bacteria grown from a **basic medium** (BSM) with wax (on TSA); the bottom plate shows bacteria from a BSM only medium. **Better growth is seen with wax, showing it is a viable carbon source**.

(b) Shake flask results of TSB (a nutrient rich medium) and BSM with differing AN waxes.

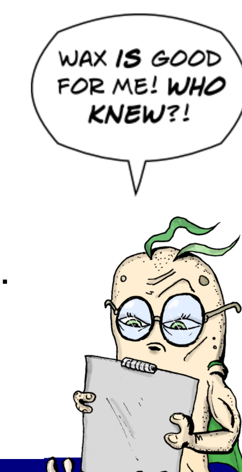
PHA production in TSB/BSM

Media	Average CDW (g/L)	PHA Average (g/L)	PHA (%w/w)
TSB only	0.98	0.20	20.0
TSB + wax 200*	3.66	1.24	33.8
TSB + wax 197	1.76	0.52	30.0
TSB + wax 135	2.46	1.00	41.0
TSB + wax 29	2.26	0.94	41.6
BSM only	0.00	0.00	0.00
BSM + wax 195	0.64	0.04	6.25

* From a joint study conducted at the University of Wolverhampton [6]

Figure 7:

Bacterial PHA production in BSM/TSB media.



Without waxes there are less PHAs produced.

PHA structure was confirmed using FTIR analysis (data not shown).

Conclusions

- PHAs have been produced from O-PEW and waxes can increase biomass.
- **Lower AN waxes** require more time and cost more to process for bacterial use.
- The increase in AN and changes in wax average mass after fermentation indicates wax was **oxidised** and broken down by either *C. necator* or processing, so further analysis into exactly how is required.

Future work

- PHAs will be analysed thermally to identify physical properties and detailed structural analysis to be conducted.
- Investigation into green methods of PHA extraction.

References

[1] Verlinden R. A. J., Hill D. J., Kenward M. A., Williams C. D. and Radecka I. (2007) Bacterial synthesis of biodegradable polyhydroxyalkanoates. *Journal of Applied Microbiology* 102(6), pp.1437-1449.
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[5] Zawadiak, J.; Orlinska, B.; Marek, A.A. (2013) Catalytic oxidation of polyethylene with oxygen in aqueous dispersion. *Journal of Applied Polymer Science*, 127, 976-981, doi: 10.1002/app.37515.
[6] Radecka I., Irorere V., Jiang G., Hill D., Williams C., Adamus G., Kwiecień M., Marek A.A., Zawadiak J., **Johnston B.** and Kowalczuk M. (2016) Oxidized Polyethylene Wax as a Potential Carbon Source for PHA Production. *Materials*, *9* (5), 367; doi:10.3390/ma9050367.