

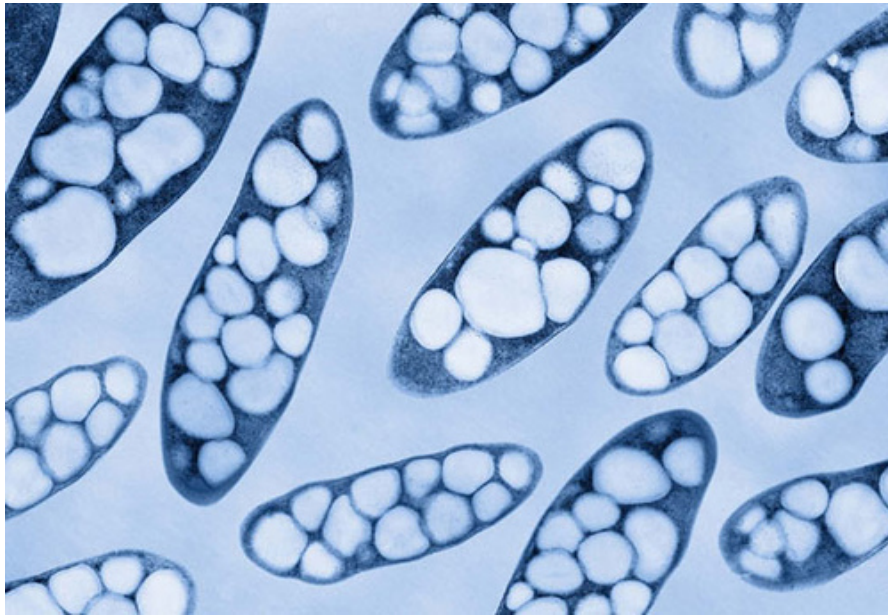
# PRODUCTION OF POLY-HYDROXYALKANOATES IN A TWO-STAGE BIOREACTOR

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Poly- $\beta$ -hydroxyalkanoates (PHA) are polyesters of 3-hydroxyalkanoic acids that are synthesized by several bacterial species. PHA can accumulate as intracellular granules when there are low concentrations of essential growth components such as nitrogen, phosphate, or oxygen in the presence of an excess carbon substrate. These polymers can allow for the sustenance of microorganisms during periods of starvation, encystment and sporulation. PHA have attracted increasing attention primarily due to their potential to replace petroleum-based plastics. In this study, however, PHA were explored as a source material for a second-generation biofuel. Since the economic viability of this approach requires a ready source of PHA, we developed a two-stage bioreactor for their production.

To minimize production costs, our approach was to use non-sterile feedstocks, anaerobic breakdown of lignocellulosics to volatile fatty acids (VFA; stage I) and subsequent microaerophilic production of PHA (stage II) using the VFA. The bioreactors were operated in semi-continuous batch mode, with the 1<sup>st</sup> stage being charged with 10 g (dry wt) of hay pretreated by heating in pH 11.0 water (final pH 10.4). 1L bioreactors were fed non-sterile tap water amended with mineral salts and adjusted to pH 10.0 with KOH at a flowrate of 0.7 mL min<sup>-1</sup>. The 1<sup>st</sup> stage was initially inoculated with 1 g of fresh cow

manure; subsequent batches were inoculated with 5 mL of digested hay. The 2<sup>nd</sup> stage was initially inoculated with 1 mL of sludge obtained from an aerobic secondary sewage reactor, aerated and fed water exiting the 1<sup>st</sup> stage.

Microbial biomass and community structure were determined using phospholipid phosphate (PLP) and phospholipid fatty acid (PLFA) analysis, respectively. PHA concentration was determined using gas chromatography, and VFA production was monitored by changes in pH. Results obtained indicated that this strategy is capable of producing PHB, and with further optimization, this approach has great potential to serve as an economically feasible source of PHB. Further advantages of this strategy include better management of waste materials obtain from wastewater treatment plants and agricultural processes through their conversion into useful products.