Enzymatic degradation of bioplastics

Basic information about bioplastics

Biogas and biodiesel have been well known to the general public for several years. But what are bioplastics and how are they formed?

For many million years, bacteria have been using carbon compounds as storage substances. Among other things, microorganisms require nitrogen and phosphorus for growth and cell division. If one of these two elements is missing, cell division stops. At the same time, the cells switch their internal program to «storage» by taking up carbon, mainly from sugars, fatty acids and other compounds. This carbon is then stored inside the cells in the form of granula[1] that are made up of poly(3-hydroxyalkanoate) (PHA), a group of polyesters of which poly(3-hydroxybutyric acid) (PHB) is particularly widespread (Fig. 1). As soon as the bacteria have everything they require for cell division again, the stored carbon is consumed.

PHA compounds (Fig. 2) have material properties that very closely resemble those of conventional plastics such as PET. Additionally, they are biodegradable (Fig. 3) and can be obtained from self-regenerating raw materials. Other types of bioplastics have been developed as well, for example on the basis of corn starch or polylactic acid (PLA). Up to now, PHA products have only been used for test purposes: PHA isolation and processing is still much more expensive than the manufacturing of plastics from crude.

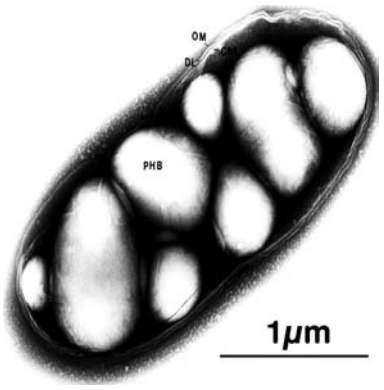

Fig. 1: Electron micrograph of Ralstonia eutropha H16 cleary showing stored PHB granula (from [2]).

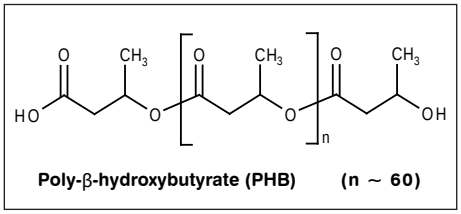

Fig. 2: Structural formula of polyhydroxybutyrate (PHB).

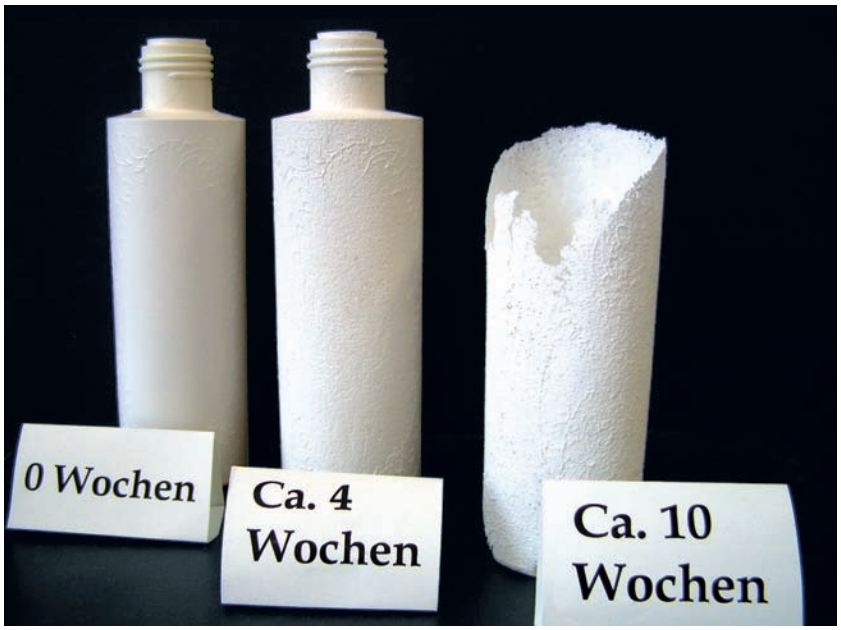

Fig. 3: PHB bottle after incubation in a sewage plant for 0, approx. 4 and approx. 10 weeks.

Depolymerization studies at the University of Stuttgart

In Prof. Dr. Jendrossek's workgroup in the Institute for Microbiology at the University of Stuttgart, the focus of interest is the degradation of the polymer in the cell itself (depolymerization [1]). It is chiefly the enzymes (so-called PHA depolymerases) that are responsible for this and they are therefore being studied in detail in order to learn more about their exact role within the cell system. Part of this enzyme is located on the granula surface and, if needed, splits the long polyester molecules into shorter ones that serve as building blocks for cell metabolism. The enzyme activity can be measured by the acid that is released by the hydrolysis of each ester bond.

[1] Granula are grain-shaped deposits in biological cells that usually contain storage or secreted substances. They can be easily recognized under a microscope.

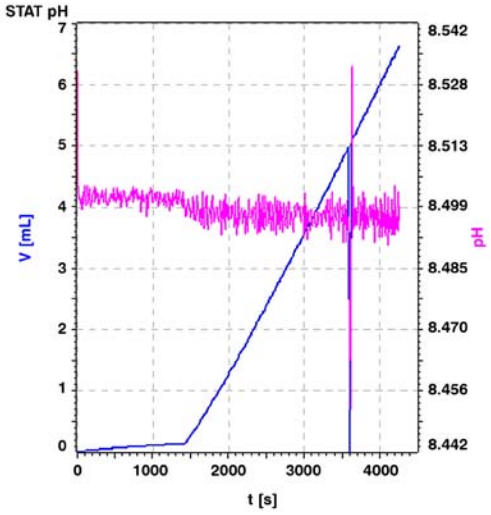

Fig. 4: Measurement of the hydrolysis rate of native PHB granula by PHB depolymerase.

Incubation in tris buffer for 6 hours. pH set to 8.5 and kept constant by automatic addition of c(NaOH) = 10 mMol/L. pH variation shown as pink curve with y-axis at right; volume of added NaOH solution shown as blue curve with y-axis at left.

After 1400 seconds the acid release rate and therefore the NaOH consumption increased greatly due to the addition of concentrated depolymerase enzyme to the solution.

Activity test procedure based on titration

For the activity test, the granula are isolated from the bacteria and placed in the reaction vessel of the 842 Titrando together with several milliliters of a weak buffer (Fig. 4). Using this pH-stat titrator allows to detect the weak acid release at the small PHB spheres. A very precise and robust pH micro-electrode transmits any variation from the set pH value via the ***tiamo***™ software (Version 1.1) to the Dosing Unit of the 800 Dosino. To again obtain the set pH, NaOH (e.g. c(NaOH) = 0.01 mol/L) is added by the Dosino with an extremely variable dosing rate ranging theoretically from 0.01 µL/min up to 60 mL/min. By continuously recording and plotting the amount of base consumed versus time, the acid release rate can easily be monitored in real time on the computer display (Fig. 4). This procedure is ideally suitable for studying the influence of various substances on the hydrolysis rate of the PHB granula. A thermostatted reaction vessel equipped with a temperature sensor also allows the effects of temperature changes on the release rate of acid to be investigated. As two pH-stat titrations can be carried out in parallel with ***tiamo***™, the acid release can be recorded simultaneously at two different temperatures. Two measuring cells and two electrodes (Fig. 5) connected to the 842 Titrando are required to do this.

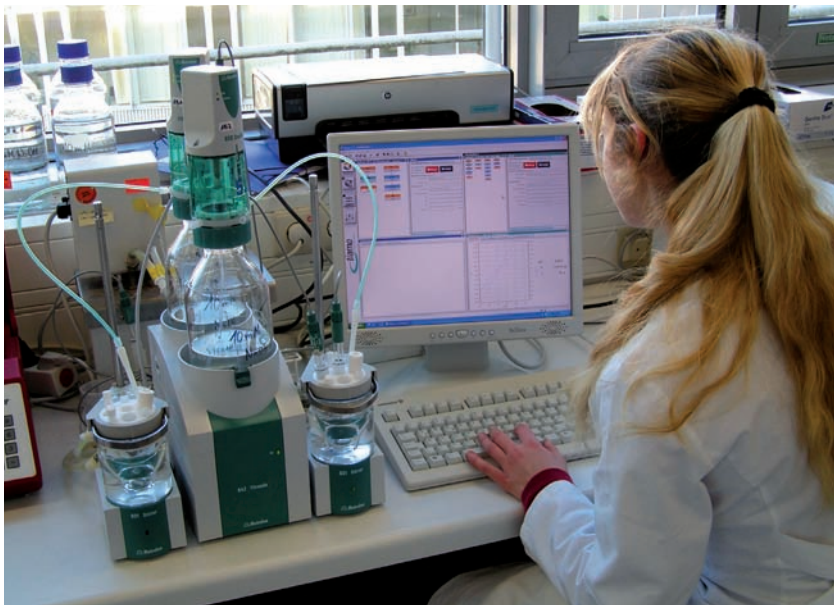

Fig. 5: Operation of the 842 Titrando with ***tiamo***™ control and data acquisition software.

Evaluation of the results

At the end of the measurement, the data and the graphs can be entered in a prestructured report data sheet and printed out; they can also be exported into other programs (Excel, PowerPoint, etc.) for further processing. With ***tiamo***™ the graphs can be compared, reports optimized, comments inserted, etc. There are virtually no limits to result presentation.

We would like to thank Dr. Gebauer from the University of Stuttgart for this summary. Further information can be found in the literature or on the Internet [1].

Literatur

[1] Birgit Gebauer and Dieter Jendrossek
Assay of Poly(3-Hydroxybutyrate) Depolymerase Activity and Product Determination
Applied and Environmental Microbiology, 72/9 (2006) 6094-6100
http://aem.asm.org/cgi/content/abstract/72/9/6094

[2] Jendrossek, D. and Handrick, R.
Microbiodegradation of polyhydroxyalkanoates
Annu. Rev. Microbiol. 56 (2002) 403-432