Enzymatic degradation of poly(3-hydroxybutyrate): qualitative analysis and kinetical representation

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Background

In order to make the future of polymer industry sustainable, we have to eliminate the need for fossil resources, as these resources are inevitably depleting, on the one hand, and the carbon footprint of polymers produced from fossil material sources is enormous, on the other. A possible solution to these drawbacks is offered by the results of research projects conducted in the field of biopolymers: these materials can now be used as potential substituents of conventional polymers, e.g. polyolefins. Moreover, these materials can also biodegrade, which makes their recycling fast, easy and cost efficient. These advantageous features are originating from the fact that the depolymerization of the vast majority of these polymers can be catalyzed by enzyme molecules.

Aim of the work

The goal of the research is to investigate the degradation of poly(3-hydroxybutyrate) (PHB) initiated and catalyzed by enzyme molecules natively produced by the strain *Bacillus Megaterium*, and also to describe the reaction with a modified Michaelis-Menten model quantitatively. Enzyme molecules are expressed in the Department of Applied Biotechnology and Food Science, while the experiments aiming to the characterization of depolymerization are executed in the Laboratory of Plastics and Rubber Technology.

Methods

A) Enzyme expression with a recombinant *Escherichia coli* strain

Recombinant bacteria:

Strain:	Origami DE3 (Novagen)
Plasmid	pGS1865 (pET22b+, bearing the *Bacillus megaterium* intracellular PHB depolymerase gene)

Enzyme separation and purification:

Method	affinity chromatography
Column	Ni-NTA Agarose

B) Amorphous PHB film preparation

Method	Compression molding
Temperature	220 °C
Pressure	120 kN

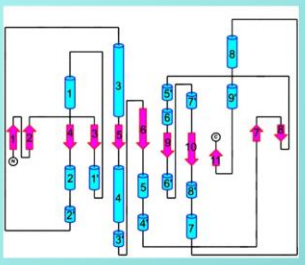
C) Enzymatic degradation monitored by UV-VIS spectrophotometry

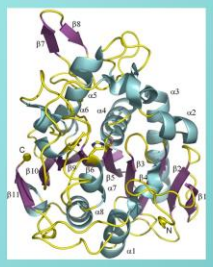
Instrument	Unicam UV-500 PharmaTest PTWS 600
Resolution	1 nm
Wavelength range	190-400 nm

D) Enzymatic degradation monitored by liquid chromatography

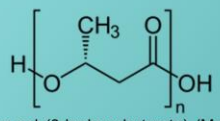
Chromatograph	Merck-Hitachi LaChrom Elite
Eluent:	pH 3.0 H_3PO_4/KH_2PO_4 buffer
Flow:	1 ml/min
Column:	LiChroChart 250-4
Stationary phase:	LiChrospher 100 RP-18 5 µm
Detector	DAD UV-VIS
Wavelength range	190-400 nm

Materials

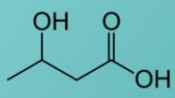




Secondary and tertiary structure of a PHB depolymerase enzyme



Polymer: poly(3-hydroxybutyrate) (Metabolix)



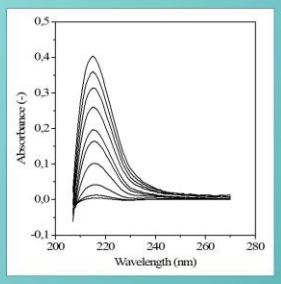
Degradation product: 3-hydroxybutyric acid

Results

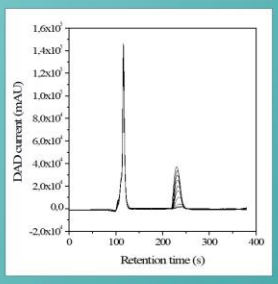
1) Increasing absorbance at 215 nm

Both the UV-VIS and HPLC-DAD measurements show an increasing absorbance at 215 nm, which can be attributed to the formation of metabolite molecules.

The monomer, (3-hydroxybutyric acid) proved to be the sole degradation product by the recursively recorded HPLC chromatographs.



Time dependent UV spectra of the aqueous media

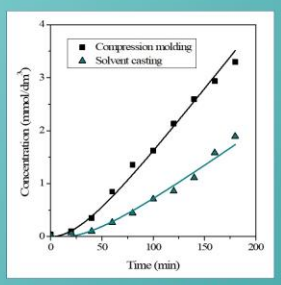


Time dependent chromatograms of the aqueous media

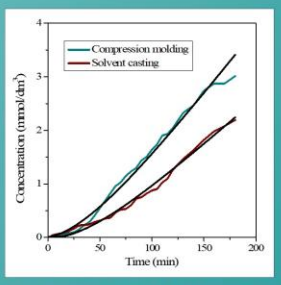
2) Time dependent monomer concentration

The monomer concentration vs. time function has is nonlinear. The enzymatic reaction shows a slightly accelerating characteristic at the beginning, which is later followed by a stationary phase.

The explanation of the nonlinear reaction characteristics shown by the graphs presented here requires the application of a kinetic model.



Absorbance values at the UV-VIS spectrum peak maximums



Absorbance at 215 nm (measured with an online UV-VIS spectrophotometer)

3) Kinetic characterization based on the modified Michaelis-Menten model

The modification takes into consideration the heterogeneous nature of the enzymatic PHB degradation.

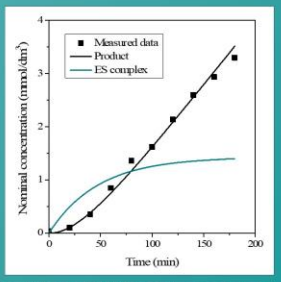
$$\frac{d[ES](t)}{dt} = +k_1 \cdot E_0 \cdot S_0 - k_{-1} \cdot [ES](t) - k_{cat} \cdot [ES](t)$$

$$\frac{d[P](t)}{dt} = +k_{cat} \cdot [ES](t)$$

An analytical solution of the differential equation system provides the [P](t) function looked for

$$[P](t) = C \cdot \frac{-k_{cat}}{(k_{-1} + k_{cat})} \cdot e^{-(k_{-1}+k_{cat})\cdot t} + k_{cat} \cdot \frac{k_1 \cdot E_0 \cdot S_0}{k_{-1} + k_{cat}} \cdot t + C'$$

An adequate fitting of the model onto the measured data provides the reaction rate coefficients, which can be applied to predict the time dependence of the product concentration.



[ES](t) and [P](t) functions predicted by the kinetic model

Conclusions

1. The intracellular PHB depolymerase of the strain *Bacillus megaterium* is an excellent catalyst of enzymatic degradation.
2. HPLC measurements prove that the sole product of the reaction is the monomer (3-hydroxybutyric acid).
3. Monomer formation can be described with, or even predicted by a modified Michaelis-Menten model developed in the project.