Advances in the Production of Polyhydroxyalkanoates by the Use of the Surplus Product Whey and Acceleration of the Process by the Application of Renewable Nitrogen Sources

Cometitive and sustainable growth programme „WHEYPOL“

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Abstract

Polyhydroxyalkanoates (PHAs) are completely biodegradable polyesters produced by numerous bacterial strains in fermentation processes. They can be applicated in many fields as **sustainable** alternatives to end-of-pipe plastics deriving from petrol (*1,2*). Till today, high substrate prices avoid the displacement of common plastic materials by PHAs. Therefore we use the **surplus product whey** from diary industry containing high amounts of lactose as main carbon source for the production of PHAs in **bioreactor fermentations** in order to make the process economically more competitive (*3*). We tested several bacterial strains successfully for biomass- and PHA production from whey and its hydrolyzation products. The influence of the addition of **renewable complex nitrogen sources** with agricultural origin such as grass juice and silage juice to the fermentation process was investigated. These substances showed to effect the kinetics for biomass production using PHA – accumulating strains in a positive way thus leading to **higher volumetric productivities** in PHA production

Experiments and Methods:

The strains *Bacillus megaterium KM*, *Pseudomonas hydrogenovora* DSM 1749, *Nocardia opaca* DSM 6427 and others were tested in shaking flasks and discontinuous stirred tank bioreactors in order to test their ability to utilize whey (lactose as main carbon source) and hydrolyzed whey (glucose and galactose as main carbon sources) for PHA production. Kinetic data for bacterial growth and accumulation of PHA were determined. The hydrolysation of whey lactose, if necessary, was performed by the application of the commercially available enzyme *Maxilact 2000 at 37°C and pH = 6,5*. Substrate concentrations were monitored via HPLC, the concentration and composition of PHAs was determined gaschromatographically by acid catalyzed methanolysis (*4*). Fig. 3 shows a representative fermentation pattern for growth and PHA production by the strain *Pseudomonas hydrogenovora* DSM 1749 on hydrolyzed whey followed by the most significant kinetic data.

We further did a series of shaking flask and bioreactor fermentations using the well – known PHA-producing strain *Ralstonia eutropha* DSM 545 adding cheap complex nitrogen sources such as grass juice and silage juice (fermentation pattern and table with important kinetic data see fig. 5) to the fermentation medium and compared the effects with fermentations using already known additives such as corn step liquor, yeast extract, meat extract and casein hydrolysate. The aim of these experiments was the investigation of the influence of these additives on the kinetc of bacterial growth and the volumetric productivity of PHA production.

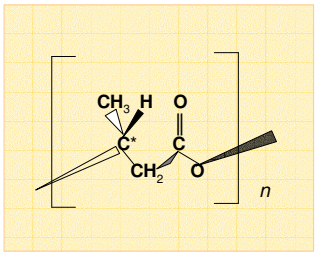

Fig.1: Structure of Poly-3-Hydroxybutyrate

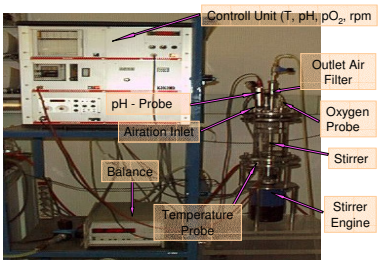

Fig.2: 2L Bioreactor (Discont. Stirred Tank)

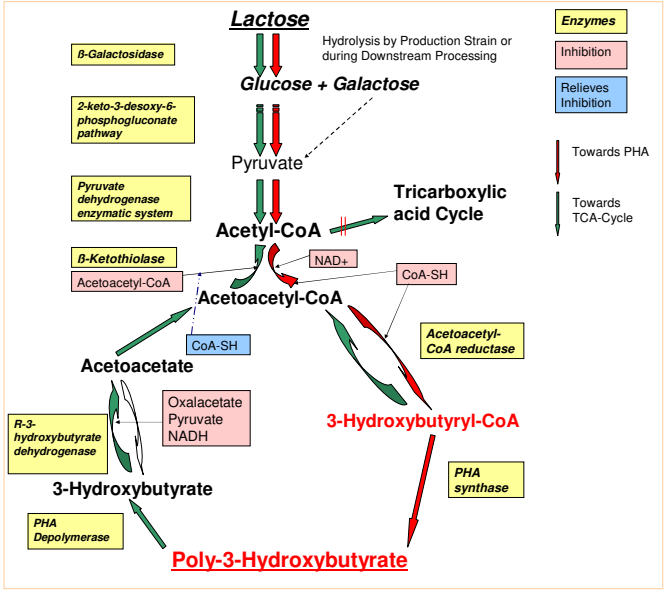

Fig. 3: Metabolic pathway for bacterial production of Poly-3-Hydroxybutyrate from whey lactose

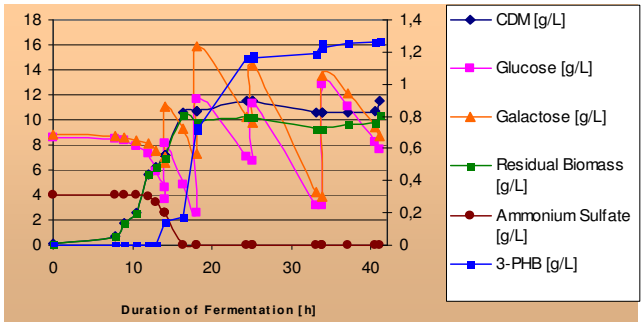

*Fig. 4: Fermentation with **Pseudomonas hydrogenovora** DSM 1749 on **hydrolyzed whey***

Max. 3-PHB / CDM [%]:	11,6
Max Conc. 3-PHB [g/L]:	1,27
μ max. [1/h] :	0,24
Y (3-PHB / Carbon source):	0,03
π max. [1/h]:	0,03
Vol. Productivity [g/Lh]:	0,03

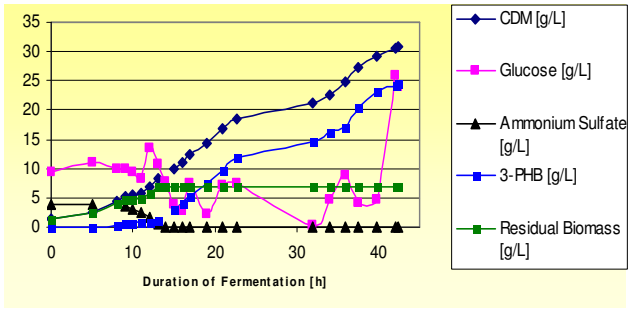

*Fig. 5: Fermentation with **Ralstonia eutropha** DSM 545 on glucose and **silage juice***

Max. 3-PHB / CDM [%]:	79,9
Max Conc. 3-PHB [g/L]:	26,40
μ max. [1/h] :	0,17
Y (3-PHB / Carbon source):	0,43
π max. [1/h]:	0,32
Vol. Productivity [g/Lh]:	0,58

Conclusion:

We tested and found several bacterial strains that are able to convert lactose, the main carbon compound of whey, towards polyhydroxyalkanoate formation. In future, our research could lead to an industrial – scale production of polyhydroxyalkanoates from the waste product whey in order to achieve an economical and ecological profitable process. The use of alternative complex nitrogen sources always showed an acceleration of bacterial growth kinetics as well as an higher yield for biomass formation. The volumetric productivity reached during the fermentation depicted in *fig. 4*, using 5 % v/v silage juice as additive to the fermentation medium, showed a value twice as high compared to a controll fermentation (not depicted here!) without addition of this compound.

References:

(1) Braunegg et al., J.Biotechnol. 65 (1998) 127-161
(2) Doi, Microbial polyesters (1990), VCH Publisher, Inc., New York
(3) Biopolyester – Ein Beitrag zur Nachhaltigen Industriellen Entwicklung Braunegg et al., Arbeitstagung ZERIA (2003)
(4) Braunegg et al., Eur. J. Appl. Microb. 6 (1978) 29-37