Production of Poly-3(hydroxybutyrate-*co*-hydroxyvalerate) from Agricultural Surplus Materials

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Abstract

Polyhydroxyalkanoates (PHAs) are biotechnologically produced polyesters which are accumulated by numerous microbial strains. In contrast to common plastics deriving from petrol, they possess the outstanding property of biodegradablity. [1,2] In order to be also economically competitive, their production costs have to be minimized. Biotechnological PHA production occurs in aerobic cultivations, therefore only about 50% of the main carbon sources are metabolized towards the desired products. Complex nitrogen sources needed for microbial growth constitute another significant cost factor in PHA-production processes. For both types of substrates, cheap alternatives are available from agricultural waste and surplus materials. In this study, surplus whey from dairy industry and glycerol liquid phase (GLP) from the biodiesel production were applicated successfully as sole carbon sources; hydrolysis of meat and bone meal (MBM) led to a cheap nitrogen- and phosphorus source in laboratory scale fermentations, using an osmophilic microorganism. Kinetic data from the processes and product qualities were analyzed and compared. Depending on the applicated waste substrate, high-quality poly-3(hydroxybutyrate-*co*-hydroxyvalerate) copolyesters possessing made-to-order properties were produced. [3]

Objectives of the study

Whey:

40,420.800 tons of whey is produced per year as a by-product of cheese industry in the EU. From it, an annual amount of 13,462.000 tons, containing about 619.250 tons of sugar is nowadays not utilized for further production of lactose and therefore constitutes a surplus product.[4] On the other hand, studies show that the price of lactose from whey can be estimated to only $ 116.7/ton compared to $ 493/ton pure glucose which is a common substrate for biotechnological purposes.[5] The direct utilization of whey permeate as sole carbon source for high-scale PHA production is only investigated by recombinant *E.coli*.[6,7] In this study an osmophilic wild-type strain that is still under detailed characterization was used. The crude whey was separated from proteins (retentate fraction) and concentrated (permeate fraction) directly at a dairy company, and was obtained by the authors with a lactose concentration of 20% (w/v) that constitutes the upper limit of lactose solubility in water. This permeate was used as carbon source for the investigation of PHA accumulation.

Glycerol Liquid Phase:

The same organism was used for PHA-production on glycerol liquid phase (GLP), a by-product of the **biodiesel production** from plant oils and tallow, containing about 70 % (w/w) glycerol. In all Europe, a total production of biodiesel can be estimated for 2005 with 1,925.000 metric tons, for 2008 already with 2,649.000 metric tons, corresponding to 192.500 and 264.900 metric tons of glycerol, respectivly.[8] GLP nowadays constitutes a surplus material. The utilization of this product leads to an enormous cost advantage compared with commercially available pure glycerol, possessing a market value of 900 € per metric ton (year 2002).[9]

Meat and Bone Meal:

Severe problems have arisen during the last decennium in the EU from the topic **BSE (*bovine spongiphorme encephalophathie*)**. On its summit, the disease had infected 3500 head of cattle weekly in the U.K.[10] This encouraged several scientists at Graz University of Technology in 2001 to find methods for safe utilization of meat and bone meal.[11] In order to produce alternative cheap nitrogen sources, meat and bone meal (MBM) that was proven to be free of prions was chemically hydrolyzed and successfully used as complex substrate for the organism.

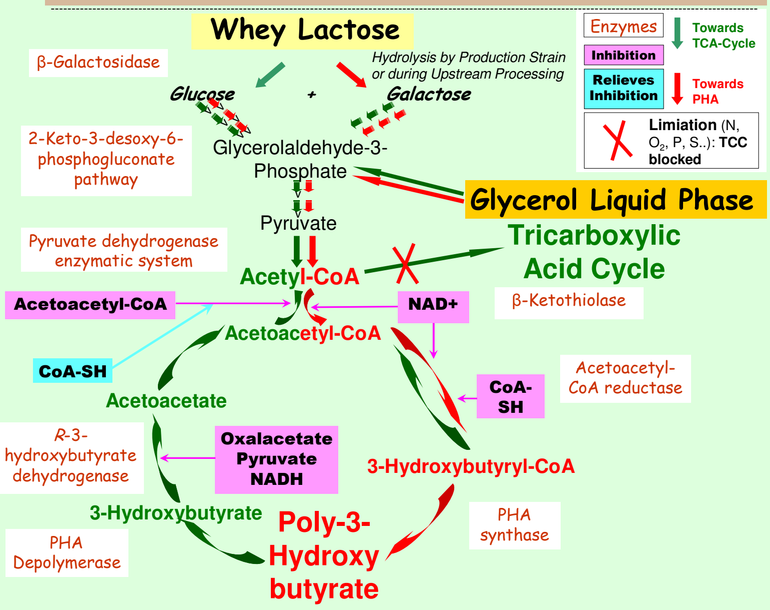

Fig. 1: Cyclic nature of the metabolic pathways for microbial production of Poly-3-Hydroxybutyrate from Whey lactose and glycerol from Glyerol Liquid Phase

Results

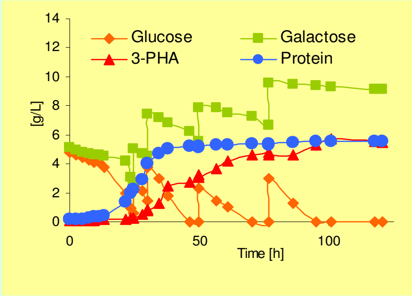

Fig. 2 A: Fermentation pattern: Production of Poly-3(hydroxybutyrate-co-hydroxyvalerate) from hydrolyzed whey

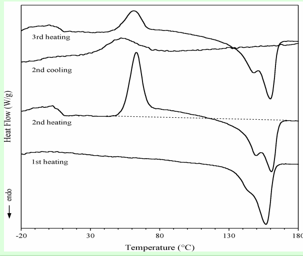

Fig. 2 B: **D**ifferential **S**canning **C**alorimetry traces of Poly-3(hydroxybutyrate-co-hydroxyvalerate) from hydrolyzed whey

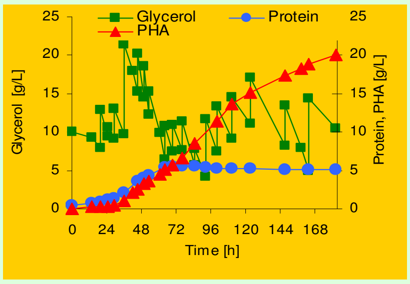

Fig. 3 A: Fermentation pattern: Production of Poly-3(hydroxybutyrate-co-hydroxyvalerate) from Glycerol Liquid Phase

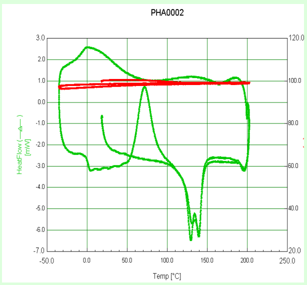

Fig. 3 B: **D**ifferential **S**canning **C**alorimetry traces of Poly-3(hydroxybutyrate-co-hydroxyvalerate) from hydrolyzed whey

Table 1: Kinetic data for Poly-3(hydroxybutyrate-co-hydroxyvalerate) from Whey and Glycerol Liquid Phase

	Whey	GLP
max. μ [1/h]	**0.11**	**0.06**
π_{max} [1/h]	**0.077**	**0.081**
Y (PHA/Whey sugars) [g/g]	**0.33**	**-**
Y (PHA/GLP) [g/g]	**-**	**0.23**
max. ***Protein*** concentration [g/l]	**5.5**	**5.5**
max. ***PHA*** concentration [g/l]	**5.5**	**16.2**
PHA/CDM [%] (end)	**49.6**	**76.0**
Vol. Productivity [g/lh] (PHA; total duration)	**0.05**	**0.14**

Table 2: Polymer data for Poly-3(hydroxybutyrate-co-hydroxyvalerate) from Whey and Glycerol Liquid Phase

	Whey	GLP
1st. Melting endotherm (T_g1), [°C]	**149.7**	**128.7**
2nd. Melting endotherm (T_g2), [°C]	**160.7**	**138.8**
Cold Crystallization Peak [°C]	**63.4**	**64.5**
Glass Transition Temperature [°C]	**7.0**	**7.0**
3-HV/PHA [% w/w]	**8-10**	**8-10**
Mw [kD]	**696**	**253**
Polydispersity Index (M*w*/M*n*)	**2.2**	**2.7**

Film of Poly-3(hydroxybutyrate-*co*-hydroxyvalerate) from hydrolyzed whey

Film of Poly-3(hydroxybutyrate-*co*-hydroxyvalerate) from Glycerol Liquid Phase

Conclusion:

According to the results, the biotechnological utilization of both agricultural surplus products, whey and GLP, as cheap carbon sources for production of the high price product PHA is possible. Together with the application of hydrolyzed meat and bone meal that has been proved by the authors to be a suitable complex nitrogen- and phosphorus source, production costs can be minimized considerably. Because of totally different molecular masses depending on the substrate (high Mw on whey hexoses, low Mw on GLP), a broad spectrum of biopolyesters with different properties are available starting from surplus products as raw materials. The production of 3-HV in a constant amount from both cheap substrates makes the process economically even more interesting and improves the product qualities.

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