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TAILOR-MADE SYNTHESIS OF POLYHYDROXYALKANOATE

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The French scientist Lemoigne discovered in *Bacillus megaterium* inclusion bodies consisting of poly([R]-3-hydroxyalkanoate) (PHB) in 1926 (9). Laboratory studies showed that PHB and other polyhydroxyalkanoates (PHAs) in general are found in combination to a shortage in a non-carboneus nutrient, e.g. nitrogen. When the shortage is relieved, PHA is recycled and used as a carbon and energy source. Since the discovery of PHB more than 90 genera of archae and eubacteria (gram$^+$ and gram$^-$) have been detected in aerobic and anaerobic habitats able to produce PHA. Only in 1983 PHAs with longer side chains have been discovered (1). To date, PHAs have been separated into 3 classes of PHAs: short chain length PHAs (sclPHA, monomers ranging from C3 to C5), medium chain length PHAs (mclPHA, C6 to C14) and long chain length PHAs (lclPHAs, >C14) (8). Research over the past 20 years focused on the substrate specificity of PHA polymerases. It was found that the supply of bacteria with a particular fatty acid is often reflected in the polymer composition. Thus, PHA monomers with straight, branched, saturated, unsaturated, and also aromatic monomers have been described in literature. Nevertheless only little is known about the chemical and mechanical properties of the polymer because only small amounts of PHA have been produced. Of special interests are functionalized groups in the side chain that allow further chemical modification, e.g., halogens, carboxyl, hydroxyl, epoxy, phenoxy, cyanophenoxy, nitrophenoxy, thiophenoxy, and methylester groups (see also (6, 7). The length of the side chain and its functional group considerably influence the properties of PHA, e.g. melting point, glass transition temperature, and crystallinity (flexibility/ stiffness). The average molecular weight of all PHAs ranges between 10^5 and $5*10^6$ g mol^{-1} and is clearly dependent on the microorganism and its growth condition.

Continuous culture studies of microbial growth revealed that PHA-accumulating bacteria are able grow simultaneously limited by carbon (C) and nitrogen (N) substrates (2-5). It was found that dual (C,N) limited growth is an appropriate method to produce PHA from a toxic carbon source. Since substrate toxicity is dependent on the concentration of the toxic substance, usually growth is not disturbed when the substrate is fully metabolized and, thus, keeping its concentration below a threshold level of toxicity, This beneficial effect of dual (C,N) limited growth of could be shown with growth of the bacterium *Pseudomonas oleovorans* on n-octane (5).

Recently, it has been proposed that this dual (C,N) limited growth regime offers a new approach to tailor PHA composition during biosynthesis since all carbon substrates were metabolized completely (10). A first test with *Ralstonia eutropha* (DSM 428) confirmed the concept. Growth limiting concentrations of ammonium, butyric, and valeric acid were fed to a chemostat culture (D = 0.1 h^{-1}, C/N = 17 mol/mol). It was found that the composition of the isolated poly(3-hydroxbutyrate-*co*-3-hydroxyvalerate) (PHB/HV) was a function of the carbon substrate mixture. Thus, the content of 3-hydroxyvalerate in PHB/HV could be controlled reproducibly between 0 and 62 mol% (see Figure 1). Interestingly, the polymer composition had a significant influence on the melting temperature and flexibility of the material. In another approach dual (C,N) limited growth (D = 0.1 h^{-1}, C/N =16 mol/mol) was used to tailor functionalized medium-chain-length PHA in *Pseudomonas putida* GPo1 using 10-undecenoic acid and octanoic acid as carbon substrates. Ten mol% of 10-undecenoic in the carbon feed resulted in the formation of 10 mol% of unsaturated PHA monomers, which allowed subsequent chemical modification.

Concluding, dual (C,N) limited growth offers a new way to produce PHA with tailor-made properties. Current research is focused on the scale-up of the production method.

Figure 1: Tailor-made PHB/HV can be produced in dual (C/N) limited chemostat cultures of Ralstonia eutropha with HV contents between 0 and 62 mol%. The straight line depicts the values for HV in PHB/HV when all valeric acid would be incorporated perfectly proportional to the ratio of butyrate and valerate supplied in the feed. The melting temperature is significantly influenced by the co-polymer composition.

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