When biopolymers are manufactured from genetically modified crops by direct fermentation, they polymerize during the fermentation process. Due to natural biosynthesis, no additional synthesizing step is required for polymerization. By contrast, the fermentative generation of monomers, such as PLA from lactic acid, requires man-made polymerization.

Within the biopolymer group generated by direct biosynthesis, the best known and by far most important examples are the so-called polyhydroxy fatty acids and polyhydroxyalkanoates (PHA). Polyhydroxyalkanoates are polyesters that are intracellularly deposited by bacteria as energy storage or reserves. These polymers are formed mainly from saturated and unsaturated hydroxyalkanoic acids; thus the term polyhydroxyalkanoates. Their monomer building blocks can be branched or unbranched 3-hydroxyalkanoic acids or those with substituted side chains as well as 4- or 5-hydroxyalkanoic acids. PHAs are homo-, co- and terpolymers built from these various monomers. The variety of monomers, constitutional isomerism, wide range of molecular weights, as well as additional possibilities for manufacturing blends or chemically and/or physically modifying their microstructure create a potentially wide variety of biopolymers with different property profiles within this polymer family. In spite of the large number of theoretically possible PHAs, we can assume there will be a maximum of 10 industrially interesting different PHAs in the future [2, 3, 4].

From a chemist's point of view, these PHAs are optically active, aliphatic polyesters with a structure illustrated in Fig. 1.

For R = CH₃, the result is so-called polyhydroxybutyrate, also called polyhydroxybutyric acid (PHB). For R = C₂H₅, the result is polyhydroxyvalerate (PHV), for R = C₃H₇, polyhydroxyhexanoate (PHH), and for R = C₄H₉, polyhydroxyoctanoate (PHO), etc. We also distinguish between homo- and copolymers in polyhydroxyalkanoates, see Fig. 2.

The most prominent and best investigated representative of this biopolymer family is the homopolymer polyhydroxy butyrate. As a homopolymer, PHB from polyhydroxybutyric acid exhibits an absolutely linear isotactic structure and is highly crystalline (60–70%). Therefore, PHB is too brittle for many applications. If process parameters vary too widely, PHB’s relatively small difference between melting and decomposition temperature may also pose a problem. The small difference between these two temperatures can be attributed to the high melt temperature due to strong intermolecular interaction. Unfavorable conditions during PHB processing, e.g., humidity too high, temperature too high, or dwell time in the machine too long, can cause polymer degradation in the final products, such as films, coatings, or fibers. Another problem for PHB is the progressive decrease of its mechanical properties, such as tensile strength, because of secondary crystallization and gradual loss of plasticizers over time.

In analogy with conventional polymers, these problems with pure PHBs can generally be eliminated by polymerization with comonomers. The longer the side chain of the polymerized functional group is, the less crystalline and more ductile is the material, and the lower is its melting temperature because of the reduction in intermolecular
interaction caused by side chains.

The first PHA used for, among other things, a shampoo bottle from Wella, was ICI’s PHB/PHV copolymer with the brand name Biopol (Fig. 3), which is no longer available. ICI has transferred the corresponding rights to Zeneca. From Zeneca, they passed first to Monsanto and now belong to Metabolix.

PHAs can generally be processed well by injection molding, are insoluble in water, yet biologically degradable and biocompatible. Moreover, they exhibit good barrier properties against oxygen and, compared to other biopolymers, a slightly higher barrier effect against water vapor. Therefore, these PHAs are a promising group of materials for future development. Their molecular structure is variable, with the resulting range of property profiles, and there is a wide range of feedstock available for the production of these biopolymers. Beyond that, PHAs also represent an interesting source for smaller molecules or chemicals such as hydroxy acids or hydroxy alkanoles.

Manufacturing Process

In principle, three different approaches for the biotechnological production of PHA are known:

- Bacterial fermentation
- Synthesis in genetically modified plants
- Enzymatic catalysis in cell-free systems

Because the last two methods are (still) industrially irrelevant, they will be described only briefly in the following. With the aid of genetic engineering, PHA synthesis genes can be transmitted into useful crops. Transgenic crops yield PHA contents up to 10% of plant dry weight. However, to ensure economically viable and competitive PHA production, these PHA contents would have to be doubled and plant growth and yields would have to be significantly increased. Also, the plant preparation processes for PHA production and the monomer composition have to be further optimized [3].

In-vitro PHA synthesis can also be performed in cell-free systems by isolating the key enzymes. This method has the advantage that no by-products of cellular metabolism need to be removed. Pure polymers can be obtained, and monomers can be specifically polymerized that are not metabolized naturally. On the other hand, the disadvantages include limited stability, relatively high enzyme costs, as well as the use of relatively expensive substrates. Thus this approach is typically used for research purposes.

On an industrially scale the much more important method to produce PHA is bacterial fermentation, which is discussed in more detail in the following.

Various microorganisms can be used to produce PHAs [a comprehensive table of microorganisms can be found in the book (1)]. Over all, more than 300 different microorganisms are known that generate PHAs as natural energy reserves [2, 5, 6].
A lack of carbon or energy will cause the degradation of the PHA storage polymers. The choice of microorganisms for industrial applications depends on the microorganism’s stability and biological safety, its PHA production rates, PHA extractability, the molecular weight of the agglomerated PHA, as well as the spectrum of useable carbon sources. The maximum known production rate lies in the range of 5 g per liter fermenter volume and hour.

In general, two different types of microorganism can be used to generate PHB. One type produces PHB continuously, the other type only when basic growth supporting substances are depleted while there is still an oversupply from a carbon source available, i.e., discontinuously. The following process steps can be distinguished in bacteria fermentation:

a) Continuous synthesis (e.g., alcaligenes latus):
   1) Inoculation, i.e., multiplication and growth of the production organism and parallel PHA synthesis by continuously synthesizing microorganisms
   2) Isolation/production of the biopolymer, i.e., separation from biomass and purification
   3) Compounding and granulation

b) Discontinuous synthesis (e.g., alcaligenes eutrophus):
   1) Inoculation, i.e., multiplication and growth of the production organism
   2) PHA synthesis under altered fermentation conditions
   3) Isolation/production of the biopolymer, i.e., separation from biomass and purification
   4) Compounding and granulation

For PHAs, much as with PLA, inoculation is the first step of the bacterial fermentation process. Here, the bacteria required for the subsequent metabolization process multiply and grow in an aqueous medium enriched with a balanced nutrition supply (C, N, P, S, O, Mg, Fe) and air under optimum physical conditions. In the next step, the actual PHA synthesis begins under conditions not conducive to growth and multiplication (e.g., phosphate limitation) and a relative oversupply of C. The PHAs are usually stored in intracellular inclusion bodies and can account for up to 90% of dry cellular weight. Their molecular weight generally ranges from 100,000−500,000 g/mol. However, molecular weights of considerably more than 1,000,000 g/mol are obtained under special conditions (ultra-high molecular weight PHAs). The complete fermentation process typically takes approx. two days [7, 8].

Glucose and sugar-containing substrates, e.g., molasses, lactose, cellulose, starch, and whey hydrolysates, serve as nutrient sources for intracellular PHA generation. Other sources such as alcohols (e.g., methanol or glycerol), alkanes (hexane or dodecane), vegetable oils, or organic acids are also suitable nutrient sources.

The enzymes involved in the fermentation process are quite unspecific. Thus, a tailored substrate supply allows for the production of a wide variety of short (4−5 × C) or medium chain-length monomers [6−16 × C]. PHA copolymers or, in the future, PHA terpolymers, can also be

References
generated. For example, hydroxyvaleric acid can be incorporated by breeding the cells on glucose with additions of, e.g., propionic, methylpropionic, or valeric acid. A variety of copolymers can be generated by varying the fermentation conditions and the substrate supply. Other than with chemical (or man-made) synthesis, biosynthesis does not require catalysts or other auxiliary substances for polymerization. Thus, the microbial polyesters present in the cells are characterized by extremely high purity.

Often there is no spatial separation between the two processing steps of bacterial growth/multiplication and actual PHA generation. Different fermenters are not required because the transition from bacterial growth to PHA generation is initiated by a change in nutrient supply and fermentation conditions in a single fermenter.

PHAs are usually manufactured in batch or fed-batch processes because optimum conditions for the individual process steps in the growth and production phases can be achieved most easily in batch processes. They provide higher intracellular PHA contents than continuous processes. On the other hand, the potential variation in product quality is a disadvantage of batch-wise manufacture. In the next step, the polymer-containing microorganisms are isolated from the fermentation broth and the intracellular agglomerated PHAs are purified.

Classical mechanical separation techniques, such as centrifugation and filtration, are used in a first sub-step to separate the cells from the culture medium. In the second sub-step, the cells are destroyed and the raw polymer is isolated. PHA extraction can be carried out by various solvent extraction methods, but also by solvent-free, so called LF-methods. The solvents used are returned to the process in a closed circuit. Separation and lysis of bacterial cells and the subsequent separation of raw PHA essentially determine cost and quality of the final product and the ecology of the production method.

Although solvent-free methods are fundamentally more ecological than methods using solvents, they do not achieve similarly high product purity. Here, a new development using genetically modified bacteria represents progress: after fermentation has taken place at 28°C, the cell membranes are lysed by a virus incorporated into the bacteria genome and activated only above 42°C.

Subsequent to isolation, the PHAs are usually further purified and dried in vacuum processes. Further research is required to determine beneficial uses for the cell residue and/or biomass accruing during PHA production. Some potential options include conversion to biogas, production of animal feed, using it as substrate for further PHA production, or catalytical enzyme production from the biomass protein content.

In a final step, PHA powder is extrusion-granulated for further processing to plastics on injection molding machines. Simultaneously, additives such plasticizers and nucleation agents can be incorporated for targeted improvement of processing properties.

Compared to other biopolymers, the price of PHAs [...] is relatively high. [...] Initial manufacturers of various PHAs on a small scale include Biomer, Mitsubishi Gas Chemical Company, PHB Industrial Brazil S.A., Tianan Biologic Material Co., Ltd., and Kaneka Corporation. Meredian Inc. is also working on the development of a PHA material (Nodax). The US biotech company Metabolix bought all the rights to ICI materials patents from Monsanto. Metabolix (or Telles, a joint venture formed by Metabolix and ADM) [...] started a production plant of 50,000 tonnes/year in Clinton, Iowa, USA in 2010 - MT). Another approach of Metabolix Inc. is the utilization of genetically engineered tobacco to produce polyhydroxyalkanoates. A number of companies in the Brazilian bioethanol industry (besides PHB Industrial SA) are interested in expanding their product range. Fermentative, sugarcane-based PHA generation offers a product with higher added value and synergy effects. Not only is sugar obtained as substrate, but incidental manufacturing by-product, bagasse or cane-trash, can be used to provide processing energy for PHA production.