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Development of Biopolyesters (PHA) as Part of the Swiss Priority Program in Biotechnology

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Abstract: The Swiss Priority Program in Biotechnology of the Swiss National Science Foundation that lasted between 1992 and 2001 had a boosting effect on many biotech disciplines and on the developments of polyhydroxyalkanoates (PHAs) in Switzerland in particular. The funding organization led by Prof. Oreste Ghisalba enabled a better understanding of the PHA biosynthesis and the development, as well as the implementation of novel bioprocesses (*e.g.* two-phase fermentations, multiple nutrient limited growth conditions, multi-stage chemostats, and product formation in different host organisms). However, production of PHA in Switzerland appeared to be impossible for cost reasons due to the strong competition from cheaper, petrol-based plastics. The recent reports on environmental issues with non-degradable plastics has triggered a general change in the perception of biodegradable plastics, giving them an added value and thus justifying a higher price. Ongoing research focuses on the sustainable production of PHAs using carbon waste streams, synthesis gas or even CO_2

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1. Introduction

In 1994, Oreste Ghisalba explained in a *CHIMIA* article[1] the key elements of the Swiss Priority Program in Biotechnology (SPP BioTech) that would become one of the biggest success stories of the Swiss National Science Foundation (SNSF). This governmental funding initiative was a logical consequence of a previous study (Früherkennungsstudie Biotechnologie) published by Ghisalba and Vogel[2] that compared in detail the international public funding programs in biotechnology and concluded that Switzerland significantly lagged behind Germany, France, The Netherlands, Great Britain, Scandinavia, USA, and Japan with respect to funding opportunities in research and development. The authors recommended that for a sustainable growth of the Swiss biotech industry a substantial and national program was urgently needed. As a good example Germany was mentioned, where DM 805 M (today's value of € 411.6 M) were invested into projects and infrastructure between 1985 and 1988. In Switzerland, Prof. Ralf Hütter at ETHZ strongly supported the requests by the Swiss biotechnology scene in the ETH research council to establish a similar funding opportunity like in Germany. It also became clear that only a long lasting and national program could have a sustainable, boosting effect on the development of biotechnology in Switzerland, as exemplified successfully by Japan.

Thanks to the strong promotion by Oreste Ghisalba (at that time at Ciba-Geigy AG) and Urs Christ from SNSF, the Swiss Federal Council was convinced to support a Swiss Priority Program in Biotechnology (1992–1994 with option for prolongation) with CHF 83 M (originally requested were CHF 150 M). However, the Swiss councils then reduced the funding by another CHF 33 M to the disappointment of Oreste Ghisalba. The SNSF was put in charge of the administration of the grants and calls, and Oreste Ghisalba was elected as program director. The SPP BioTech had six subprograms[1] called Modules and one of them, Module 2, was dedicated to 'Bioengineering und Stoffumwandlung' that significantly influenced the research on bioprocess engineering and on biopolyesters in Switzerland. Within Module 2, two newly elected professors at ETHZ, Jay Bailey and Bernard Witholt, were asked to establish new competences at the Institute of Biotechnology after the retirement of its founder Prof. Armin Fiechter. With Bernard Witholt, a new era of research on the biosynthesis of polyhydroxyalkanoates, a biodegradable polymer and candidate to replace petrol-based plastics, was initiated in Switzerland.[3]

1.1 *Current State of Plastics*

These days it appears difficult to imagine how our daily life would look like without plastics and in particular how one should replace well-established plastics without losing their unique and valuable properties. Packaging, *e.g.* for cosmetics and other high-value products, is frequently made of plastics because they give designers an enormous freedom to create new shapes with unique colors and a particular grip at a low cost. Sportswear is made from polyester fibers and is comfortable to wear because it is breathable, once wet it dries much faster than natural fibers and additionally keep the color for a longer period. Also, in industry, transportation, and construction more and more traditional materials (*e.g.* steel) have been replaced by high-performance plastics and composites.

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It is therefore not surprising that the need for plastics is increasing year by year. In 2018 the production of plastics reached 359 M t[4] and will rise further due to increasing population and ongoing industrialization in developing countries.

In the past 20 years, more and more negative aspects of plastics have become obvious.[5] Inappropriate disposal of waste has resulted in the accumulation of plastics in the environment. The non-degradability of plastics seen in earlier times as a clear advantage has become more and more an issue, particularly for packaging that usually has a very short lifetime. A critical stage was reached when it was noticed that the plastic trash accumulated in the sea.[6] Due to the low density of plastics, these materials do not sink but rather float in seawater. Five regions, so-called gyres, could be identified in the oceans that contain a significantly enhanced concentration of plastics. Due to the marine currents, the plastics were concentrated and are estimated to amount to a total of 9 –263'000 t.[7] At the same time, reports on harmful additives became public. The plasticizer bisphenol A was found to have endocrine disruptive activities and was banned for baby bottles in Europe in 2011 but is also in discussion to be prohibited completely.[8]

Further studies revealed that plastics undergo an ageing process and become more brittle over time because of polymer chain scission due to exposure to UV illumination, leaching of softening additives or recrystallization.[9] A few companies started to add catalysts that significantly enhanced this deterioration process under UV exposure and promoted this feature under the term oxo-degradable or even oxo-biodegradable materials. Because such modified plastics decay into very small pieces in the environment and concomitantly fulfilled the norm ASTM D6954-18, these companies claimed to have solved the plastic waste problem. The fact is that Spain, France, and Italy banned the use of oxo-degradable plastics in 2017 because of the lack of circular economy options[10] (recycling such materials is no longer possible). Nevertheless, in Switzerland they are still in use.

1.2 *Fate of Plastics in the Environment*

As previously mentioned, regular plastic materials are degraded mechanically and hydrolytically at a slow rate and therefore may persist in the environment for many centuries. Recent studies showed that the surface water had already become contaminated not only with easily detectable plastic products but also with microplastics (particles smaller than 5 mm) that sediment only slowly.[11] A Swiss study revealed that the river Rhine was found to be more heavily polluted by microplastics than lakes.[12] Recently, microplastics have also been found in Swiss soil.[13] Presumably its contamination occurred by intensified agriculture (*e.g.* mulching foils) and transport of degraded material *via* the atmosphere. Microplastics are currently under investigation whether they are able to accumulate toxic compounds and contribute to their mobilization. More recently, textile fibers have been found that do not sediment but also do not degrade.[14] These fibers originate from all kinds of synthetic textiles and are released during wear or washing processes into the environment. Microplastics have now been found in snow layers in very remote regions (*e.g.* in Arctic regions and in the Swiss Alps).[15] In December 2019, it has been reported that plastic particles are also transported *via* the atmosphere.[16] Thus, it was found that London has one of the highest air pollution by plastics worldwide. To date, these plastics are also considered to become a health issue because tiny particles can enter the lung and may lead to serious health problems.[17]

1.3 *Bioplastics: A Potential Solution?*

As a consequence of the increasing pollution problem, the call for bioplastics has increased. Unfortunately, the definition of bioplastics is quite ambiguous and is currently under discussion in the European Union. The Swiss Academy of Technical Sciences (SATW) recently published a factsheet on this topic[18] and defined the term bioplastic in a very simplistic way in agreement with the definition given by the European Bioplastics Organization:[19] "Bioplastic is a plastic material that is bio-based, degradable in the environment or both." This problematic reasoning resulted from the fact that plastics produced from natural resources (*viz.* non-petrol based) were also called bioplastics even though they are not degraded in the environment. On the other hand, there are also petrol-based bioplastics that do degrade in the environment (*e.g.,* polybutylene adipate terephthalate (PBAT) within 90 days) and thus fulfil the EN 13432. Fig. 1 helps to categorize the different examples of plastics according to their resources and degradability.

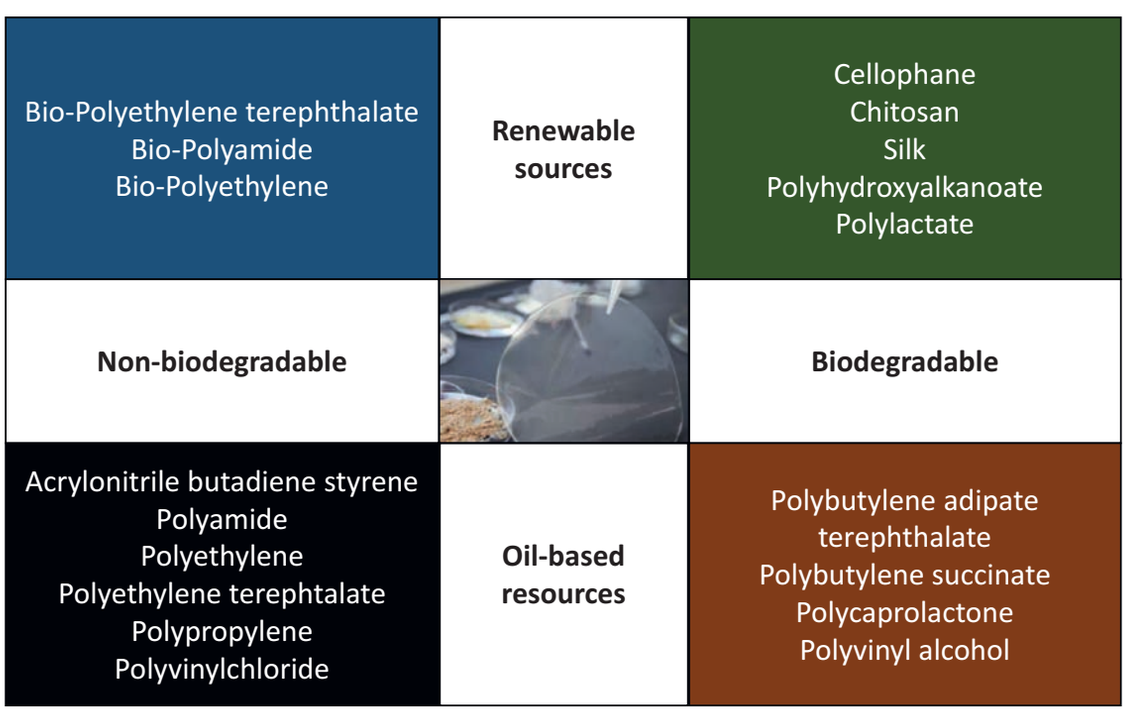

Fig. 1. Classification of plastics into four categories according to the source of carbon and their fate in the natural environment (adapted from ref. [18]).

Two particular polyesters, poly(L-lactic acid) (PLLA) and poly(3-hydroxyalkanoate) (PHA) are both biobased and biodegradable. PLLA has been successfully commercialized for packaging applications (*e.g.* drinking cups and bottles)[19] or as textiles used in medical applications.[21] Recently, PHA has been shown to degrade in seawater[22] and is therefore an interesting material for packaging.

1.4 *Polyhydroxyalkanoates: A Versatile Class of Biodegradable Biopolyesters*

The discovery of an intracellular carbon and energy storage compound consisting of poly(3-hydroxybutyrate) (PHB) in *Bacillus megaterium* by Lemoigne in 1926[23] was not recognized until the late 1950s, when its biosynthesis was elucidated and was considered to be a wide-spread storage compound among bacterial, archaeal, yeast, and fungal species.[24] These findings and the fascinating properties of the isolated polyester triggered the interest of industry. In the early 1960s, the W.R. Grace Company in Maryland, U.S.A., made the first attempt to produce PHB at a commercial level; however, with little success.[25] In late 1970s, Imperial Chemical Industries (ICI, UK) began with the production of PHB with a mutant strain *Cupriavidus necator* NCIB 11599 and reached a production scale of 300 t y^{-1}. The commercialized product was trade-named Biopol and was used by the German company Wella for a selection of shampoo bottles for several years. The involved patents were later on sold to Zeneca, then to Monsanto and to Metabolix, Inc. (USA),[25] who finally sold the PHA-related patents to an affiliate of the South Korean firm CJ CheilJedang Corp. at a price of US$ 10 M in 2016.[26]

The reason for the slow commercialization of Biopol after the initial success was certainly to some extent due to the stringent patent situation and the limited availability, but also due to the difficulty to compete economically with petrol-based polymers. The discovery of large oil fields in Mexico and the North Sea resulted in the oil price falling below the long-time average of US$ 100 per barrel thus ending the oil crisis[27] and consequently further slowed down the commercialization of PHA. Nevertheless, suddenly, petrol could also be considered as a cheap carbon source for biotechnological processes and biodegradable polymers in the early 1980s!

Bernard Witholt, while still at the University of Groningen, analyzed the metabolic activities of *Pseudomonas oleovorans* GPo1 (later on reclassified as *Pseudomonas putida* GPo1[16]) in two-phase liquid systems with n-octane as sole carbon source.[28] While studying electron micrographs of freeze-fractured cells, he discovered inclusion bodies that formed a mushroom-type structure rather than the well-known needle-type that was typical for PHB granules. In 1983, his group published the first paper on biopolymer production with a significantly different composition than the known Biopol PHA type.[29] It was soon found that these intracellular polyesters were copolymers and had thermoplastic properties with a high elasticity rendering them suitable for coating applications. This new class of medium-chain-length polyhydroxyalkanoates grew over the years to more than 130 different monomers including functionalized ones suitable for medical applications.[30]

In 1992, Bernard Witholt accepted the call to ETHZ and moved with his team to Zurich. The Swiss National Science Foundation supported his research with a significant number of projects leading to many PhD theses and postdoctoral projects[3c,31c,e,f] that were also carried out together with other research institutes in Switzerland.

The collaboration between Thomas Egli, the environmental microbiologist at Swiss Federal Institute of Aquatic Science and Technology in Dübendorf and Bernard Witholt, the microbial biotechnologist, was very fruitful. During the first of the three funding phases of SPP BioTech, cell physiology and PHA production under different nutrient limitations were addressed. One PhD thesis investigated the interaction of PHB with polyphosphate in mixed cultures in wastewater treatment plants,[32] a technique which was considered 10 years later as a cheap production method for PHA.[33] In two other PhD theses the multiple nutrient limited growth conditions were assessed in continuous cultures.[34] It was found that under dual (carbon and nitrogen) limited growth in chemostat, optimal growth and most efficient PHA production conditions could be established. Multiple nutrient limited growth was further followed up in the second round of SPP BioTech by two PhD students and resulted in highly sophisticated two-stage chemostat fermentations using two-phase systems[35,36] and the first PHA block copolymers produced with a lipase.[37] During the last four years of the SPP BioTech (1998–2001), the so-called phasing out phase, the collaboration with industry was a requirement in order to get funding. The goal was to foster the collaboration between academia and industry and thus identify partners for potential follow-up projects. The non-profit organization Biotectra (in 2001 renamed to Unitectra) was founded and was put in charge of assistance in patenting new findings originating from SPP BioTech. During this phasing out period, Thomas Egli and Bernard Witholt collaborated with Roland Wohlgemuth at Fluka and I had the pleasure to be a part of the SPP Biotechnology for the second time, this time as a postdoc. A chemostat production process was developed at Eawag using dual (C,N) limited growth conditions that enabled the production of random copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate with tailored monomeric unit content.[38] This production method was successfully transferred and implemented at Fluka in Switzerland and, last but not least, the products were introduced into the Fluka catalogue of chemicals and sold worldwide.

During the past five years, PHA-based materials have become attractive again due to the environmental problems caused by non-degradable plastics but also due to new processing methods established during large EU projects (*e.g.* PHBottle, P4SB, RES URBIS, and YPACK). In industry a change of attitude can be observed and new programs to become 'greener' in its product range partially also motivated by the tightening of governmental regulations. Moreover, the new materials offer much more than just the replacement of petrol-based plastics, they allow a complete redesign of plastic materials and the integration of new properties. Nevertheless, in order to profit from the interesting features of PHAs, production must become cheaper. Recently, interesting carbon sources and production conditions have been proposed, *e.g.* using synthesis gas or even CO_2 as a cheap carbon source,[39] seawater as a cheap and selective medium to avoid expensive sterilization procedures,[40] or even wastewater treatment plants as suitable production facilities providing attractive PHA yields.[41]

2. Conclusions

The Swiss Priority Program in Biotechnology initiated and led by Oreste Ghisalba had a sustainable impact on biotech activities in Switzerland. Thanks to this program, extensive know-how in biotechnology in general but also with respect to biosynthesis and tailored material properties of PHAs could be generated. Further and intensified developments of large-scale production of PHA are taking place in Asia, in North and South America, and in the European Union: Time for a new SPP Biotech in Switzerland!

In order to be more effective in the support of biotechnologies, I would also address the fact that boosting PHA and the development of new sustainable plastics are priorities not only because of environmental reasons but also because they represent an opportunity for innovating materials and discovering new functional plastics, not achievable through conventional chemistry. Biosynthesis is by far more effective in achieving such results, with respect to organic chemistry. Mimicking classical fossil-based chemistry (namely drop-in bioplastic products), is not profitable, since these

old processes are highly optimized. However, policies (unexpectedly) are pushing the transitions to new, more sustainable plastics and industries are aware of that. Scientists (not young scientists fortunately!) and consumers are somehow more reluctant to surf the change. These two categories must be informed and educated.

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