



**EPOBIO: Realising the economic potential of sustainable resources - bioproducts from non-food crops**

**EPOBIO Workshop: Products from Plants – the Biorefinery Future**

Wageningen International Conference Centre, The Netherlands 22-24 May 2006

**Title of paper: Foundation Paper for the Biopolymers Flagship**

**Work Package: Biopolymers Flagship**

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## **Summary**

During this century renewables will gradually replace petrochemical-based industrial products, including polymers. This paper gives an overview of the current state of affairs in research, development and production of biopolymer-based products. In some cases, quality and price already allow bioplastics to successfully compete with petrochemical plastics. In other cases, a significant effort is required either on the side of the raw materials or on the processing to achieve production of useful and economic materials. The goal of the EPOBIO project, as detailed in this paper for biopolymers, is the identification of technical and scientific bottlenecks in the development and placement of existing or novel bioproducts, based on plant biopolymers. Genes may be altered to change the substrate range of polymerases, the precursors available for polymerization, or new genes may be introduced in plants to obtain polymers with different ionic charge, composition, chemical reactivity, stability, solubility, melting and other thermoplastic behaviour. Here, four specific groups of biopolymers and their applications were singled out for special attention. However, many other potential sources and products may be considered, based on new raw material streams, opportunities, and processing technologies.

## **Introduction**

It is taken for granted that plastics are made from mineral oil. One of the first plastics was made from cotton cellulose in the mid 19<sup>th</sup> century: collodion. The body of a 1941 Ford demonstration vehicle consisted of plant fibers, soy protein polymers, and rubber tires made from goldenrods. The low cost and reliable supply of fossil fuels put an end to that: petroleum-derived plastics took over. Now, the pendulum appears to swing back due to the rising cost of oil, regulation and mounting concern over climate change. Production of plastics from plant biopolymers offers the potential to replace non-renewable materials derived from petroleum with renewable resources, resulting in reliable (domestic) supplies, jobs in rural communities, sustainable production, lower greenhouse gas production, and competitive prices. It is the task of this Flagship project to identify

bottlenecks in research, development, production, and application of materials based on plant biopolymers.

#### *Alternative routes to bioplastics and other biomaterials*

Bioplastics and biomaterials compete with petrochemicals-based equivalents, the production of which has been optimized over the last decades. Optimization of these production methods according to green or sustainable chemistry principles may still significantly reduce costs, waste production, energy and raw materials use for petroleum-based plastics. However, many chemical processes are mature and have little room for optimization, while biotechnological processes are in their infancy; there is great potential for streamlining and improved process integration. In petrochemical refineries, the raw materials cost are critical as processing costs have gradually decreased, more products are developed, and less waste is produced.

White biotechnology provides new routes to biopolymers; biomass can be converted to glucose, fatty acids, or other small compounds, either as the main product or as a waste stream from other production processes. These small compounds serve to produce polymers by microbial fermentation or chemical polymerization (18). For example, poly- $\beta$ -hydroxyalkanoates, biocellulose, xanthan, silk, and polythioesters, can be produced by fermentation processes (34), while polylactic acid, poly-caprolactone, and other (partially renewable) polyesters such as Sorona (Dupont), and Bionolle (Showa) are produced using chemical polymerization of substrates that are at least in part produced by bacterial fermentation. It is likely that these processes will be part of future biorefineries, which are now in a very early stage of development, with the exception of starch and paper mills. This implies that in biorefineries, the processing costs still determine the economic viability of bio-products. As biorefineries mature, the focus will also shift to the cost of producing the raw materials.

The cheapest and easiest to handle biopolymer is starch. Due to its abundance and low price (the world production is 57'000'000 T/A, at around € 0.30 per kg depending on the source of the starch), it has found numerous applications in the non-food sector, which includes its use as a thermoplast in bioplastics. The current rise of the oil and natural gas prices is reflected in the plastics market, and is making renewable bioplastics more

competitive. However, it appears that prices of raw materials for the production of bioplastics are also strongly increasing. For example, sugar prices have increased from 270 US\$ per ton to more than 400 US\$ per ton in the course of 2005 due to the strong demand for bioethanol and food applications. Similarly, some vegetable oils (including waste products from the food industry) are cheaper than diesel (also because they are less heavily taxed). Here, it should be noted that the current world production capacity of vegetable oils is barely higher than the demand for food applications. In other words, the price of sugars and vegetable oils (and any energy-rich waste stream) will become tightly linked to the price of oil.

### *Eco-efficiency*

Bioplastics and other biomaterials have a marketing advantage due a perceived lower environmental impact. However, this idea must be supported by data. According to some reports, the production of petroleum-based plastics consumes less raw material and energy, and produce less CO<sub>2</sub>, compared to the production of bioplastics by fermentation, especially when energy and materials consumed for the production of fertilizers, pesticides, transport, and process energy are factored in.

Accordingly, life cycle, eco-efficiency, ecological footprint, carbon efficiency, and sustainability analyses are key to determine whether the production of (biodegradable) bioplastics truly makes sense. For example, recycling of classical plastics may be better than composting bioplastics. Furthermore, the energy content of bioplastics can be recovered by incineration or conversion to biogas, which may create a critical advantage. The possibility that it is more eco-efficient to use biomass for fuel and energy, while continuing the use of oil-based plastics (13) should also be considered, although evidence points in the other direction (9). In the end, the net greenhouse gas production may be crucial as evidence for climate change is mounting. On a timescale of 10-15 years it is practically certain that carbon-credits will exert an sizeable influence on the market, favoring (bio)plastics and materials with a good carbon-balance.

## Plant biopolymers

Four specific (classes of) biopolymers were singled out for special attention by EPOBIO: natural rubber and other polyisoprenes, starch, protein-based polymers, and poly- $\beta$ -hydroxyalkanoates (Table 1). Starch, natural rubber, and proteins such as zein, gluten, and soy-protein are natural plant products. Their productivity, quality, and harvesting may be optimized by plant breeding, biotechnology, and processing technology. Poly- $\beta$ -hydroxyalkanoates, proteins made of repeated blocks of amino acids e.g. silk and non-ribosomal proteins are considered for production in transgenic plants, but can also be produced in transgenic microorganisms. There is a clear need for a better understanding of plant genetics, metabolic pathways, storage, deleterious effects of biopolymer production can be made out.

Table 1. Production methods and volume of plant biopolymers

	Plant	Fermentation	Chemical
Polyisoprenoids	7'000'000 T/A		
Natural rubber (cis-PI)	7'000'000 T/A from Hevea	R&D	10'500'000 T/A (equivalent)
Balata (trans-PI)	100-1000 T/A		
Gutta Percha (trans-PI)	100-1000 T/A		
Starches (many different types, different properties)	57'000'000 T/A from maize, potato, rice, cassava, etc.	Not applicable	Many processes to produce derivates of starch, 3'600'000 T/A for non-food applications (EU15)
Only amylopectin (waxy)	GMOs and breeding		
High amylose	GMOs and breeding		
Protein polymers:	100 – 1000 T/A		
Cross-linked plant proteins	Commercial (1930-50)	Single cell protein (1950-80)	
Silk & other repeat-proteins	GMOs	GMOs	
Non-ribosomal proteins	GMOs	GMOs	Thermal polyaspartate
Poly- $\beta$ -hydroxyalkanoates	GMOs Monsanto stopped in 1998 Metabolix develops PHA production in switchgrass R&D in academia	GMOs Monsanto stopped in 1998 P&G stopped development of PHBH (Nodax) in 2006 Metabolix and ADM announced a 50'000 T/A plant	R&D Lab-scale for defined polymers

Cellulose, hemicellulose and lignin are major plant biopolymers, which are considered in the Cell Walls Flagship, and have important applications as bioplastics and materials. For example, the annual production of cellulose acetate is about 750'000 tons (29). Plants

produce many other biopolymers that presently have no applications in the non-food sector (Table 2). This could change if more material becomes available, for example as co-products of bio-fuels production. Other biopolymers that are now isolated from fungi or bacteria could also be produced in plants.

Table 2. Some other plant biopolymers and their applications in the non-food sector

	Chemical structure and source	Applications as material
Cellulose	Polysaccharide: 1,4-linked $\beta$ -D-glucose, most abundant component of terrestrial biomass. Can be derivatized to ethers and esters (with acetate, propionate, butyrate, etc.)	Nitrocellulose, cellophane, CMC, Tencel fiber, cellulose acetate (expensive but great potential)
Hemicellulose	Polysaccharides: xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan, present in almost all cell walls along with cellulose	Limited use as source of chemicals (great potential)
Lignin	Complex (irregular) polyphenolic macromolecule making up a quarter to a third of the dry mass of wood	Limited use as polymer and source of chemicals
Pectin	Various polysaccharides containing 1,4-linked $\alpha$ -D-galacturonic acid units, and L-rhamnopyranose units, linear and branched molecules.	Edible films
Inulin	Polysaccharide: Linear $\beta$ -(2 $\rightarrow$ 1)-linked fructose chains attached to a sucrose molecule. Belongs to fructan-group: alternative storage carbohydrate in the vacuole of ~ 15% of flowering plant species.	Mainly used to produce inulin syrup. Carboxymethyl inulin (CMI) is used as antiscalant.
Cutin	Polyester found on the surface of plants	None
Suberin	Complex (irregular) biopolymer consisting of $\omega$ -hydroxyalkanoates, dicarboxylic acids and aromatic compounds. It is a waste product available in large amounts (80'000 T/A from cork production alone)	None
Pullulan	$\alpha$ -(1 $\rightarrow$ 4)-linked glucose trimer, linked by $\alpha$ -(1 $\rightarrow$ 6) bonds, fungal polymer that could be produced in plants	Edible films, fibers
Hyaluronic acid	Repeating disaccharide unit consisting of an N-acetyl-hexosamine and a hexose or hexuronic acid, either or both of which may be sulfated	Surgery

### **Bio-based polymers produced by chemical methods or fermentation**

Many (partially) bio-based polymers are produced by fermentation or chemical polymerization. The building blocks are small molecules such as alcohols and acids, that are ultimately derived from plant polymers, sugars and vegetable oils. The production of bio-based polymers competes with chemicals and biofuels for the same resources. As such, they do not require specific measures related to plant biotechnology or agriculture in the context of EPOBIO. They are included for comparisons, but the production methods are not discussed here. Life-cycle analysis and related research may show that it is advantageous to produce bio-based polymers by fermentation, instead of using biopolymers produced in plants (either in wild-type or in transgenic plants). In addition,

many of the bio-based polymers shown in Table 3 are essential in making superior biomaterials consisting of blends with starch, cellulose, latex, or fibers.

Table 3. Other (potentially) bio-based polymers

Polymer group	Structure and production method	Company
Polyurethanes	Petrochemical aromatic or aliphatic diisocyanate reacted with a bio-based polyol (sorbitol, isosorbide, vegetable oil polyols)	Nawaro, SoyOil
Polyesters (diacids plus diols) 1 a) Polytrimethyleneterephthalate b) Polybutyleneterephthalate c) Polybutylene succinate	Often copolymers of more than one diacid and/or diol monomers Petrochemical terephthalic acid plus bio-based propanediol Petrochemical terephthalic acid plus bio-based butanediol Bio-based succinate and butanediol	Sorona (DuPont) PBT (BASF) Bionolle (Showa)
Polyesters (hydroxy acids) 1 a) Poly- $\beta$ -hydroxyalkanoates (PHA) b) Polycaprolactone (PCL) c) Polylactic acid (PLA) d) Polyglycolic acid	Produced by fermentation from sugars or vegetable oils Produced by fermentation from sugars, used mainly in blends Polymerized lactic acid from fermentation Polymerized glycolic acid from sugar cane syrup or chemical	Metabolix Cargill DuPont
Polyamides a) Nylon 6 b) Nylon 66 c) Nylon 69	Bio-based caprolactam produced by fermentation Bio-based adipic acid produced by fermentation Bio-based monomer obtained from oleic acid (chemical steps)	R&D stage

1. Most polyesters are not used in a pure form, but as copolyesters containing different monomers for better control over properties.

### Natural rubber and other polyisoprenes

Natural rubber (NR) consists of *cis*-polyisoprene, with many minor additional components that are key to the superior properties of this material compared to all synthetic rubbers. 80% of all NR is produced by only three countries (Malaysia, Indonesia, and Thailand), and from one biological source: the Brazilian rubber tree (*Hevea brasiliensis*). For many applications, synthetic rubber cannot replace NR (for example: heavy duty tires for trucks, busses, and airplanes, latex products for the medical profession). According to rubber industry research group ISRG, tire makers consume around 70% of global NR production, with average synthetic rubber substitution levels estimated at around 8%.

NR is considered a strategic commodity, difficult to access in case of war (e.g. during WWII). In 1934, South American leaf blight (SALB) wiped out the production of NR in Brazil, and it has not been possible to restart large-scale production due to the endemic leaf blight pathogen *Microcyclus ulei* (the present production in Brazil is only 96'000 T/A). The same could easily happen in Asia, as *Hevea brasiliensis* is genetically very

homogeneous: the millions of hectares of rubber plantations are all derived from a small sample of seeds collected in Brazil by Dr. Henry Wickam in 1876 (8). Thus, *Hevea* is studied to generate leaf-blight resistant varieties, increased yield, and altered properties in France and Brazil. Recently, efficient transformation of calli and regeneration of plants was shown to be possible (2). However, the narrow genetic base, prolonged breeding cycles and juvenile period, and highly heterozygous nature of *Hevea* make breeding complex, time-consuming and labor-intensive. In view of the critical importance of NR, these efforts appear extremely limited: it makes sense to increase these efforts, and investigate alternative production methods. Strongly rising demand and the general drive to renewables are further strong incentives that could enable a breakthrough on any of the following options.

Table 4. Alternative sources of polyisoprenes

Material	Source	Production in T/A (year)	Price	Current R&D related to NR	Reference
Natural rubber (poly- <i>cis</i> -isoprene)	Rubber tree 2 <i>Hevea brasiliensis</i>	7'000'000 (2005)	€ 1.80 / kg	Resistance to SALB Rubber polymerase	(2)
Natural rubber (poly- <i>cis</i> -isoprene)	Guayule shrub <i>Parthenium argentatum</i> Gray	10'000 (1910) R&D (Yulex)	n.a.	Processing technol. Rubber polymerase	(21)
Natural rubber (poly- <i>cis</i> -isoprene)	Goldenrod <i>Solidago virgaurea minuta</i>	Demonstration project (1931)	n.a.	Presently none related to NR	(28)
Natural rubber (poly- <i>cis</i> -isoprene)	Russian dandelion <i>Taraxacum kok-saghyz</i>	WWII emergency projects USSR / USA, 3'000?	n.a.	Domestication Processing technol.	(38)
Chicle 1 (poly-isoprene (cis and trans))	Sapodilla tree <i>Manilkara zapota</i>	Decreasing		No	(28)
Gutta percha 1 (poly- <i>trans</i> -isoprene)	Gutta Percha <i>Palaquium gutta</i>	Decreasing	\$ 100	No	(28)
Balata 1 (poly- <i>trans</i> -isoprene)	<i>Manilkara didentata</i>	Decreasing	\$ 40	No	(28)

1. Total export value of chicle, gutta percha, balata and guayule: US\$ 13 million (2002). Total production around 3000 T/A

2. Other tropical trees including several *Hevea* and *Ficus* species, and several tropical vines also produce latex.

### *Guayule as an alternative source of NR*

Only one other plant has been used in large-scale commercial production of NR: in 1910, 10'000 T/A of NR was obtained from the guayule shrub (30). As production from *Hevea* became more efficient, this production strategy was gradually abandoned. Guayule was studied intermittently for strategic reasons, and more recently also because many consumers are allergic to *Hevea* NR, but not to guayule-rubber. Over the years guayule breeding efforts have improved latex yield to 1000 kg per hectare (compared to 3000 kg for *Hevea*) (30). In Europe, guayule has not attracted much attention, except for limited cultivation studies in Spain and Greece. As the plant is quite vulnerable to cold winters, the initial priorities might include the development of more hardy strains that can be grown in Southern Europe, or the identification of more suitable regions for growing this crop (North Africa). General research areas requiring attention are breeding for higher yield, harvesting methods, processing, and co-products utilization (22).

### *Natural rubber from plants grown in temperate climates*

The last major research activity of Thomas Edison was the development of natural rubber production from Goldenrod. Extensive research proved Goldenrod, a common weed growing to an average height of 1 meter, produced 5% yield of latex. Through hybridization, Edison produced Goldenrod in excess of 3 meters, yielding 12% latex. However, even though Edison turned his research over to the U.S. government a year before his death in 1931, Goldenrod rubber never went beyond the experimental stage (<http://en.wikipedia.org/wiki/Goldenrod>), probably because the latex harvesting method was extremely labor-intensive. Another potential source of rubber is the Russian dandelion. The root is a source of high quality latex, used in making rubber during WWII, with yields between 150 and 500 kilos per hectare, and 45 kg of rubber per ton of roots (28, 38). Several other plants have been considered, but all projects were abandoned because of agronomic, yield, or harvesting considerations (28). However, it is likely that the application of modern breeding, harvesting, and downstream processing methods, and co-products utilization would enable fast progress.

### *Other natural polyisoprenes*

Chicle is a mixture of *cis*- and *trans*-polyisoprene, produced mainly for use in chewing gums. It has been almost completely replaced by petrochemical plastics. Gutta percha and balata consist of *trans*-polyisoprenes. Balata is hard, inelastic, tough and leathery but contains some resin, which makes it (unlike gutta-percha) useless for electrical insulation. It is used for machine belts and for covering very high-quality golf balls. Gutta percha is a brownish-grey rubbery substance that has lost most of its applications (33).

### **Starch**

Starch is the second major agricultural commodity after cellulose, is the least expensive food commodity, and has numerous industrial applications. Currently, about 25'000 T/A are converted to biodegradable polymers by a range of small and large companies. Most of this bioplastic is marketed as biodegradable, and is used for packaging films and foams, and for disposables (e.g. cups and plates, plant pots, bags). The growth potential of this market is high with many studies referring to future market sizes in the range of 1'000'000 T/A (7): about one third of current non-food uses of starch in the EU15 (3'600'000 T/A), or about 13% of total EU15 starch market (35). Figure 1 shows the general uses of starch in food and non-food applications (from (26)).

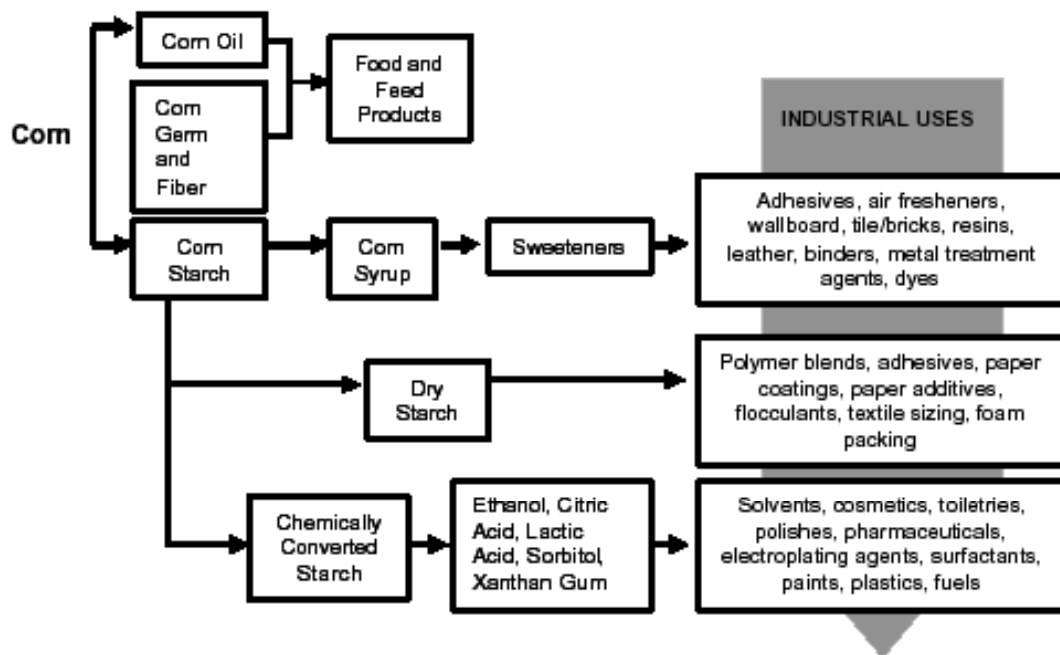


Figure 1. Food and non-food uses of corn

Due to the importance of this commodity, starch has been studied in great depth. The so-called starch-enhancement technology has increased the amount of starch relative to the other components in potato, yielding more starch per hectare and lower processing costs (reviewed in (36)). Efforts to change the properties of starch *in planta* have focused on the ratio between amylose and amylopectin, the branching pattern of amylopectin, synthesis of phosphate-substituted starches, and starches from new or alternative crops (3, 4, 14). Most work was carried out with food applications in mind, but also aid the production of thermoplasts from starch (TPS). For example, high-amylose TPS were reported to have better properties than “normal”-starch TPS: they are less sensitive to water, and less subject to cracking and shrinking (37). It is likely that these starches modified *in planta* will find additional non-food applications. Further properties that could be engineered into starch by plant genetic engineering, and methods to accomplish these targets will need to be identified.

One of the main barriers to applications of TPS is its high moisture sensitivity and difficulties in processing. Such problems can be remedied by chemical derivatization, e.g. by introducing ester and ether-groups, which is not possible in plants. Blending TPS with

polycaprolactone or other biodegradable hydrophobic polymers, or by coating TPS films with a waterbarrier (1) is also extensively used. Both types of research have been carried out for many decades and are covered by numerous patents. New opportunities arise out of the increased availability and reduced price of bio-based polymers for blending. Other novel research fields involve using clay for nanocomposites, making graft copolymers with latex (for coated paper) or with polyacrylate (via acrylonitril, for superadsorbents) (29).

### **Protein-based bioplastics or biopolymers**

Three groups of protein-based plastics and biomaterials can be distinguished: 1) Protein co-products of starch or vegetable oil production, 2) Proteins with potential uses in engineering, like spider silk, mussel adhesive protein, collagen elastin, and 3) Non-ribosomally produced proteins such as polyaspartate and polylysine.

The first - very heterogeneous group - consists of the materials that can be derived from natural proteins, usually co-products of carbohydrate and vegetable oil production.

Examples are plastics and resins based on zein (corn protein) (16), soy protein (19), and gluten from wheat (25). These materials are produced by cross-linking proteins with glutaraldehyde, formaldehyde, or other chemicals, in combination with starch, polyphosphate or other fillers. Zein is the major protein in corn. In 1950 about 2700 T/A of zein plastics (glossy, scuff-proof, grease-proof coatings) and 2'200 T/A of Vicara fiber were produced. If produced on the same scale as in the 1950's and as a by-product from ethanol production, zein would cost about € 2.50 /kg (16), the actual cost now being 10 times higher. Soy protein was already used by Henry T. Ford as a source of bioplastics to construct car parts. However, after a brief bloom in the 1930's and 40's soy protein-based plastics were replaced by petroleum-based plastics, in part because of microbial degradation and water permeability issues (19). Gluten-based bioplastics are being studied, but are also too expensive for large scale use.

The increased demand for biofuels may put huge amounts of waste protein on the market that cannot be absorbed by feed production, enabling the development of a protein-based bioplastics industry (Table 5). There is potential to alter the structural properties of zein

and other plant proteins by genetic engineering. However, these changes must be justified by a specific increase in added-value.

Table 5. Protein co-products potentially available for the production of bioplastics or biopolymers

Protein	Total crop harvest T/A	Protein content %	Uses of protein as material	Price and volume
Zein (maize)	692'000'000	4%	Films, bioplastic, fibers	€ 10-20 per kg, < 1000 T/A
Soy protein (soybean)	209'500'000	38-45%	Films, extruded foams, injection molded products	Price is slightly higher than conventional plastics (6)
Gluten (wheat)	626'000'000	9-15%	Films, coatings, bioplastics, resins	Not available

The second group of protein biopolymers that are useful as materials contains natural protein fibers or adhesives that typically consist of short blocks of repeated amino acids. Examples are silk proteins, elastin, and adhesin from mollusks. These are potentially very attractive materials, but expensive and labor-intensive to produce. Heterologous expression in plants would enable production on a much larger scale, and open up new markets. In addition, genetic engineering can be used to produce completely new materials such as block-copolymers, combinations of different proteins like silk and elastin, completely synthetic sequences with even better properties, and sequences optimized for production in specific plants. Presently production of these proteins in plants suffers from low yield (31) and difficult processing (spinning of heterologously produced silk needs to be addressed). Some of these proteins are thermostable and can be isolated by simple heat-treatments (32). For these proteins, microorganisms (bacteria, yeast or fungi) may be more favorable hosts, as much higher product concentrations can be attained in these organisms, genetic engineering is much easier (especially in view of the multitude of different proteins in this class), as is downstream processing. In addition, if these proteins are to be used as high-end engineering materials, used on a relatively small scale, the fermentation costs are less relevant than the material properties. Only if used as a commodity production in plants can be envisaged.

The third group of protein biopolymers consists of non-ribosomally produced polypeptides such as cyanophycin (a protein-like copolymer composed of a polyaspartate backbone and arginine side-groups), produced by cyanobacteria and a few non-

photosynthetic bacteria; polylysine, an antimicrobial polymer used as food additive; and polyglutamate, also used in food. Polyaspartate can be used as superadsorbant or antiscalant. Recombinant *E. coli* can produce cyanophycin up to 29% of CDW on protamylase, a waste product of starch production from potato (10). Transgenic plants have been created that contain up to 1.1 % cyanophycin of dry weight (23). Due to the low-price applications of these compounds, the critical question is whether production levels in plants can be high enough for cheap production.

### **Poly- $\beta$ -hydroxyalkanoates**

Poly- $\beta$ -hydroxyalkanoates (PHAs) are a class of polymers produced by microorganisms primarily as carbon and energy storage material. The polymer properties depend strongly on the nature of the monomer, which can range from linear C4-C16  $\beta$ -hydroxy fatty acids to  $\beta$ -hydroxy acids substituted with aromatic rings, other functional-groups, or containing double-bonds. The simplest PHA, poly- $\beta$ -hydroxybutyrate is a relatively hard and brittle material with a melting point slightly below the thermal decomposition temperature (17). Inclusion of C5-monomers (PHBV: co-valerate) gives slightly better properties (Monsanto, Metabolix). Adding small amounts of longer monomers (C6 and longer) has resulted in materials that are much easier to process (Nodax: Proctor & Gamble, Kaneka) (24). PHAs consisting of higher molecular weight monomers (C6-C16) typically are rubber-like materials with an amorphous soft-sticky consistence. These polymers still have to find applications apart from the production of enantiopure *R*-3-hydroxy-carboxylic acids (39).

As a group PHAs are very attractive polymers for consumer products such as bottles, films and fibers, due to their water and air impermeability. As a potential large scale commodity polymer plant production of PHA has to be considered (20). In *A. thaliana* PHB levels of 4% w/w (40% based on dry weight for some plant parts) were obtained, but plant growth was severely affected (5). Isolation of PHA from plant tissues is likely to be always be more difficult than isolation from bacteria where there is no need to break up tissue, and much higher concentrations can be reached without affecting the viability of the host organisms (at least 50% for mcl-PHAs, up to 85% for PHB, PHBV and PHBH). The timing of production is also much easier as the typical substrates for

bacterial growth (sugars and oil-containing wastes, or purified compounds) can be stored, and production can take place throughout the year. Previously, PHAs produced by microbial fermentation were considered too expensive and lacking environmental benefits (12). Present efforts to develop cellulosic ethanol (11), and the rapid development of biogas technology to convert waste biomass into process heat and electricity, should make PHA fermentation much more energy and CO<sub>2</sub> efficient (15). The same methods, however, would also facilitate co-production of PHAs from biomass. According to some sources, PHAs can now be produced by fermentation for as little as € 1.80 per kg. The critical question to ask is: can PHA production in plants compete with fermentation methods using agricultural products that are already efficiently produced on an enormous scale, or using waste streams from agribusiness or food industry? If the answer is yes, many technical hurdles need to be identified that are generally related to production levels in plant tissue, deleterious effects of PHA production, and processing technology.

### **Technical issues in the production of polymers in plants that could be considered in future EU research**

Based on the above analysis of four biopolymer areas, the responses from industry and academia, a number of general and specific targets for further research can be identified. Several of these targets are very specific, while others are much more general and likely to overlap with the targets of other projects.

1. Modifications of biopolymer properties *in planta* (chain length, branching, monomer composition, physical characteristics, substituents, macromolecular properties).
2. Increased amounts of the target biopolymer (relative to other components).
3. Decreased amounts of other compounds in the plant (lignin, protein, pectin, hemicellulose, amylose, amylopectin).

4. Production of polymers in transgenic plants (natural rubber in sunflower or lettuce, silk-elastin in Arabidopsis or tobacco, cyanophycin in tobacco or potato).
5. Increased stability of biopolymers in the plant between harvest and extraction (are PHAs and protein polymers stable in harvested material treated as common crops).
6. Better understanding of plant genetics, metabolic pathways, and deleterious effects to plant growth of biopolymer production.
7. New crops for the production of raw materials for bioplastics or biomaterials (root chicory and Jerusalem artichoke for inulin, guayule or Russian dandelion for natural rubber).
8. Resistance of *Hevea brasiliensis* to *Microcyclus ulei* and other pathogens by genetic engineering or classical breeding.
9. Improved extraction and processing methods (technologies developed for biofuels production, expression of cellulases or other hydrolases in plants to release target material, co-production of fibers, oils, protein, plant-meal, energy, or energy carriers, ionic liquids as solvents for biopolymers (27)).
10. Technical aspects of the application of plant biotechnology for biopolymer production (crop identity preservation, confining genes in crops, limited flexibility of production in plants, long time to market for transgenic plants, GMO methods vs. fast-track breeding, use of non-food plants to avoid controversy).
11. Consequences of increased competition for use of biomass for food, fuel, energy, chemicals, and materials (leading to higher prices for food).
12. Identification of new product or waste streams available due to the use of renewables for the production of chemicals and fuel.
13. Effects of economies of scale (much larger production volumes are required if petroleum-based plastics are to be replaced on a significant scale, which will undoubtedly reduce prices).
14. Life cycle, eco-efficiency, ecological footprint, carbon efficiency and sustainability analyses (carbon as precious commodity after peak oil, green better than white biotechnology; minimized greenhouse gas emissions).

15. New technology (role of technology development time gap; acceptance: yield enhancement for non-food crops (36) may be easier to accept in Europe than changes in food crops; costs and time frame of developing, registering, and patenting transgenic crops; low marginal costs for established technologies).

Important key questions that can be asked are:

1. Can these research targets be addressed with current or near future technology? Is it possible to gain sufficient control over the properties of biopolymers “*in planta*”, compared to the relative ease of control in chemical polymerization and in fermentations?
2. Is the research justified by the economic value of the product? Is the significant investment of creating transgenic plant or plant breeding for bioplastics production justified, in view of the current main use of bioplastics in low-cost applications such as packaging and in biocomposites?
3. Is the research justified by the strategic value of the product?
4. Which of the bioplastics are clearly superior to petrochemical plastics in regard to all or many of the factors mentioned above (price, performance, energy and CO<sub>2</sub>-balance, availability of raw materials)?

## References

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