Biodegradable plastics are those that can be completely degraded in landfills, composters or sewage treatment plants by the action of naturally occurring micro-organisms. Truly biodegradable plastics leave no toxic, visible or distinguishable residues following degradation. Their biodegradability contrasts sharply with most petroleum-based plastics, which are essentially indestructible in a biological context. Because of the ubiquitous use of petroleum-based plastics, their persistence in the environment and their fossil-fuel derivation, alternatives to these traditional plastics are being explored. Issues surrounding waste management of traditional and biodegradable polymers are discussed in the context of reducing environmental pressures and carbon footprints. The main thrust of the present review addresses the development of plant-based biodegradable polymers. Plants naturally produce numerous polymers, including rubber, starch, cellulose and storage proteins, all of which have been exploited for biodegradable plastic production. Bacterial bioreactors fed with renewable resources from plants – so-called ‘white biotechnology’ – have also been successful in producing biodegradable polymers. In addition to these methods of exploiting plant materials for biodegradable polymer production, the present review also addresses the advances in synthesizing novel polymers within transgenic plants, especially those in the polyhydroxyalkanoate class. Although there is a stigma associated with transgenic plants, especially food crops, plant-based biodegradable polymers, produced as value-added co-products, or, from marginal land (non-food), crops such as switchgrass (Panicum virgatum L.), have the potential to become viable alternatives to petroleum-based plastics and an environmentally benign and carbon-neutral source of polymers.

Key words: biodegradable plastic, natural polymer, poly-hydroxyalkanoate, transgenic plant.

INTRODUCTION

The term ‘plastic’ is defined as any of numerous organic synthetic or processed materials that are mostly thermoplastic or thermosetting polymers of high molecular mass and that can be made into objects, films or filaments (Merriam–Webster Dictionary definition). The majority of plastics are synthetic, using petroleum both as feedstock and as energy during manufacture. With the price of oil rising and easily accessible reserves dwindling, alternative sources of fuel and of oil-based commodities such as plastics are being explored across the world. The environmental concerns of the oil-based economy are also being widely voiced as companies and individuals attempt to reduce their carbon footprints.

Plastics are truly a huge industry. Data for first-quarter 2008 U.S. production show that total output of all petroleum-based plastics exceeded 11.34 billion kg (25 billion lb; American Chemistry Council press release April 2008). With this volume of production, the economies of scale become evident [for example, polypropylene or polyethylene cost approx. 40 U.S. cents/lb or $1 (or approx. €1.5/€1.3)/kg]. With a market value of $100 billion (or approx. £25 billion), plastics are also one-third of plastic use in the United States, followed by construction and consumer products [1]. The simple ‘paper or plastic’ decision one makes in the supermarket eventually has consequences for the environment. These consequences have been recognized in the form of bans on plastic bags in many of the EU (European Union) countries, China, Australia, the City of San Francisco and an attempted ban in the State of California.

The search for alternatives to traditional petroleum-based plastics is progressing to the point that not just the source, but also the downstream consequences, are being addressed in the form of biodegradable plastics. Although photodegradable plastics continue to be explored [2], these alternatives must be constantly exposed to sunlight and so are not suitable for landfill disposal. Biodegradability in composters or municipal landfills is the goal. Even large chemical companies, like BASF with its Ecoflex® product, are touting their biodegradable-plastics efforts to address the downstream consequences on the environment of conventional plastics.

In this context, production of biodegradable plastics in plants is an enviable goal. Plants naturally produce many polymers, such as starch or cellulose, and these have been exploited for plastics production. Additionally, novel plastics, like the PHAs (polyhydroxyalkanoates), are also being synthesized in plants. Plants can be considered as solar-driven biofactories with the potential for being renewable, sustainable, scaleable and relatively environmentally benign sources of edible vaccines or ‘plantibodies’ [3,4], novel oils and fatty acids (reviewed in [5] and [6]) and biodegradable plastic. The present review will detail the efforts to produce biodegradable plastics in plants and will be extended to include the broader topics of biodegradable polymers derived from plant materials, including starch and cellulose.

Abbreviations used: ADM, Archer Daniels Midland; ASTM, American Society for Testing and Materials; CaMV, cauliflower mosaic virus; CAP, cellulose acetate phthalate; CEN, European Committee for Standardization; ER, endoplasmic reticulum; EU, European Union; GMO, genetically modified organism; HB, hydroxybutyrate; HV, hydroxvvalerate; IPP, isopentenyl diphosphate; PA, protective antigen; PCLH, polycaprolactone (polyhexanolactone)/hexamethylene di-isocyanate; PDC, pyruvate dehydrogenase complex; PLA, poly(lactic acid); PHA, polyhydroxyalkanoate; PHB, polyhydroxybutyrate; PHBV, PHB-co-polyhydroxyvalerate; SPI, soy protein isolate; UTR, untranslated region.

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Studies on the ecological footprint of humanity have suggested that sustainability of human pressures on the world’s ecological resources shifted from 70% of global regenerative capacity in 1961 to 120% in 1999 [7]. This translates to a clear and simple fact: that we consume more than can be regenerated by the biosphere. A clear example is crude oil, which obviously cannot be regenerated on a human timescale. Petroleum-based plastics add to this ecological imbalance, both from a source point of view and from a waste management standpoint. Traditional plastics have been engineered to be stable in many types of environments and to persist for many years. This central ‘purpose’ of plastics and their cost-effective production (i.e. that they are cheap) has led to their ubiquitous use and is also the main reason that their disposal presents such problems. The ubiquitous use of plastics has led to their comprising about 12% of the 227 tonnes/metric tons (250 million short tons) of municipal waste produced annually in the United States. Recycling recovered about 30% of this plastic from the wastestream, but that still allows plastics to accumulate in the environment at a rate in excess of 18.2 metric tons (20 million short tons) per year (source: http://www.epa.gov/epaoswer/non-hw/muncpl/msw99.htm). Similar values for plastic waste apply in the EU, where the typical generation of solid waste in 2001 was 520 kg/year per person, of which approx. 10–15% was plastics. Most of the EU’s municipal waste is sent to landfills (45%), but almost 40% is recycled or composted, and a further 18% is used for energy production by incineration (source: http://www.eea.europa.eu/themes/waste/about-waste-and-material-resources). Most of the common types of petroleum-based plastic (e.g. polyethylene, PVC, polypropylene and polystyrene) are non-degradable, although incorporating starch into polyethylene allows breakdown of the polymer state (more correctly called ‘disintegration’ rather than true ‘biodegradation’) in composting environments [8]. Additionally, municipal solid waste (including plastics) is being tested for power generation due to a relatively new EU landfills directive [9]. In Asia, plastic waste is also being explored as a source of fuels for power generation; for example, in Korea, polyethylene has been tested as an additive to coal-burning blast furnaces [10].

Although efforts have been explored in source reduction (i.e. replacing plastics at the manufacturing/packaging stage), in most cases recycling and disposal are the main ways in which plastics are dealt with post-consumer. A clear example of one of the more recent increases in use of plastics is bottled-water sales. Annual U.S. sales of bottled water increased almost threefold between 1997 and 2006, growing from a $4 billion to a $10.8 billion industry. In the space of 20 years (1987–2006), U.S. per capita consumption of bottled water increased from 21.6 to 104.5 litres (5.7 to 27.6 U.S. gallons) [11]. Unfortunately, many water and soda bottles are used ‘on the go’ and so are more likely to end up in ordinary rubbish collections rather than separated for recycling. Although there have been some ingenious strategies for separation of different types of plastics using electrostatics [12] and hydraulics [13], consumer separation of classes of plastics and recycling them in bulk is typically the best course of action. However, even with recycling, traditional plastics have a finite re-usability and eventually are only suitable for bulk applications such as engineered lumber, WPC (wood plastic composite) [14] or additives to concrete [15].

Clearly a source of biodegradable plastics is an obvious alternative. A number of countries have implemented certification standards for biodegradability, with concomitant labelling based on their degradability and with an emphasis on composting environments (Figure 1). North America, Europe, and some countries in Asia have enforced standard product testing to achieve certification for labelling based on the ASTM/CEN (American Society for Testing and Materials/European Committee for Standardization) system of classification. The ASTM 6400-99 designation covers ‘standard specification for compostable plastics’ and EN 13432 covers ‘proof of compostability of plastic products’. Two pertinent definitions from the ASTM standard are as follows:

- **biodegradable plastic**: a degradable plastic in which the degradation results from the action of naturally occurring micro-organisms such as bacteria, fungi and algae
- **compostable plastic**: a plastic that undergoes degradation by biological processes during composting to yield CO₂, water, inorganic compounds, and biomass at a rate consistent with other compostable materials and leaves no visible, distinguishable, or toxic residue [16]

The ‘residue’ clause in the second definition is an important distinction that favours truly biodegradable plastics rather than blended materials that disintegrate or partially biodegrade.

**PLANT-BASED BIODEGRADABLE PLASTICS**

Although plant-based biodegradable plastics are not new, the current interest in green technologies has lead to a renewed interest in using plants for a number of applications. Many of these...
applications are traditionally based on crop plants, be it for ethanol production, biomass production for power generation and sources of novel compounds such as pharmaceuticals. Additionally, with pressures on land use for farming, the concomitant use of fertilizers and pesticides, and various (perceived?) threats of GMOs (genetically modified organisms) to the human food chain, non-food crops are also being explored as biofactories.

For plastics production, plant materials can be harvested and used directly (natural rubber is a clear example), or plant polymers can be derivatized to produce plastics. Plants can be used indirectly as a nutrient source in bioreactors for biodegradable plastic production. Plants can also be genetically modified to directly synthesize novel polymers.

**NATURAL PLANT POLYMERS**

Plants naturally produce a number of structural and carbon-reserve polymers. Polysaccharides are estimated to make up approx. 70% of all organic matter. Cellulose accounts for about 40% of total organic matter and is the most abundant macromolecule on earth. Lignin comprises 15–25% of a typical woody plant. Starch is also a major component of global biomass, and the ability to digest starchy foods is thought to have played a role in human evolution and its success over other species [17]. A number of natural polymers have been exploited for commodity manufacturing, and most of these products retain the inherent biodegradability of their carbohydrate building blocks.

**Rubber**

Rubber, a polymer of isoprene (cis-1,4-polyisoprene; Figure 2A), is the most widely used natural plant polymer. All commercial production of rubber comes from the Brazilian rubber tree (*Hevea brasiliensis*). Despite the plant’s common name, more than 90% of the world’s natural rubber supply now actually comes from South-East Asia. Although natural rubber production only accounts for 40% of demand (the remainder is synthetic), natural rubber is superior to synthetic, owing to its molecular structure and high-molecular mass (>1 MDA), which confers resistance to abrasion and impact, elasticity, efficient heat dispersal and resilience and malleability at low temperatures. These properties have not been duplicated in synthetic rubber because of the unique, somewhat undefined, secondary compounds (proteins, lipids, carbohydrates and minerals) in natural rubber.

So although synthetic-rubber production has reached a plateau, natural-rubber production continues to increase, particularly in China and Vietnam [18]. The main reason production has switched to Asia from South America is the South American leaf blight fungal infection caused by *Microcyclus ulei*, which originates in the Amazon region. All attempts at commercial-scale rubber cultivation in South and Central America have been thwarted by this, as yet, uncontrolled fungus. More recently, molecular-breeding approaches have been explored to attempt to confer resistance traits, and, with strict quarantine, plantations in Asia have been unaffected by the blight. Rubber plantations are composed of clonal trees; in fact, the commercial rubber tree is one of the most genetically restricted crops grown, making the *Hevea* rubber tree particularly susceptible to pathogen attack [19].

Natural rubber’s isoprene monomers are derived almost exclusively from IPP (isopentenyl diphosphate). IPP is derived from cytosolic acetyl-CoA through the mevalonate pathway. Polymerization of the isoprenyl units is catalysed by ‘rubber elongation factor’ [20] or ‘particle-bound rubber transferase’. This enzyme is a cis-prenyltransferase, which adds isoprenyl units from IPP to form the polymer. Two transferase cDNAs were cloned from *H. brasiliensis*, then expressed in, and purified from, *Escherichia coli*. Although low-molecular-mass rubber (<10 kDa) was made in vitro using the purified enzymes, the addition of a fraction from latex containing washed rubber particles permitted the formation of a high-molecular-mass product [21]. Clearly, then, other factors present in the natural-rubber particles are required for high-molecular-mass polymer production, but these components are ill-defined [22], although magnesium cations seem to regulate the activity of the prenyltransferase [23].

Other natural sources of rubber are being explored owing to *H. brasiliensis*’ disease susceptibility and also because of the prevalence of allergy to latex. Type I latex allergy is based on the reaction to natural-rubber latex proteins, and type IV is based on chemical additives, in gloves, for example [24]. Latex allergies occur at a rate of about 1% of the general population, but at a 10–20-fold higher rate in healthcare workers [25]. Although a number of plant species (about 2500) synthesize rubber, many of these are not suitable for commercialization because they do not produce the high-molecular-mass form of the polymer with its concomitant useful properties.

Two promising alternatives to *Hevea* that do produce high-molecular-mass rubber are guayule (*Parthenium argentatum* Gray) and Russian dandelion (*Taraxacum kok-saghyz*) [26]. Guayule is a non-tropical shrub native to Mexico and parts of the U.S. South-West. Guayule rubber is considered hypoallergenic compared with that from *Hevea* because it is less proteinaceous and the proteins that are present do not cross-react with *Hevea* immunoglobulins [27]. However, unlike *Hevea*, the guayule shrub cannot be ‘tapped’ for latex extraction, and the kilogram yield per acre of rubber is about one- to two- thirds that of *Hevea*. This obviously increases the cost of production, and without significant improvements in cultivation, harvesting and processing, guayule rubber will likely remain as a specialized-use product (reviewed in [26]). The Yulex Corporation in Arizona has built a pilot plant
for its Yulex Natural Rubber product based on guayule latex and they plan to couple latex production with cellulosic biomass for biofuels and power generation.

Russian dandelion produces a higher-molecular-mass polymer than either Hevea or guayule (about 2 MDa), which accumulates in lactifiers in the roots. It was discovered in Kazakhstan in the 1930s and was used as a source of motor-tyre rubber during World War II by a number of countries, including the United States and the U.K. Although production yields are poor and large-scale cultivation is hampered by cross-breeding with weedy species and labour-intensive crop maintenance, it can be useful as a model species for rubber biosynthesis because, in part, of its relatively short life cycle (altered rubber phenotypes can be screened within 6 months) compared with guayule and Hevea. A further advantage is the double-crossing potential of Russian dandelion, both as a source of rubber and its fructose-based storage sugar, inulin, which accumulates at 25–40% of root dry weight and could be used in bioethanol production [26].

Proteins

Proteins can be considered polymers of amino acids that are combined in various combinations that confer function on the basis of their side-chain structure and on the arrangement of the amino acid monomers within a protein for tertiary/quaternary structure. Although plants are being used to synthesize novel proteins (see the subsequent section), a number of naturally occurring proteins have been exploited as plastics. For example, proteins from wheat (Triticum aestivum), maize (Zea mays) and soybean (Glycine max), particularly zein and gluten, have been used as the basis for biodegradable polymers.

Gluten is a composite of the proteins glutenin and gliadin (with other globulin and albumin components). Two-dimensional gel-electrophoretic analysis shows multiple spots corresponding to multiple isoforms of glutenin [28]. The gluten fraction comprises about 80% (w/w) of the wheat seed protein and about 8–15% of seed dry weight. Gluten is easily harvested from seed by washing away soluble compounds (mainly starch) to produce an essentially pure protein isolate [28]. Gluten is used as a protein source in a number of food products, for example in preparing fibrous meat analogues composed of gluten, soy protein and starch [29]. Gluten confers elastic properties on dough, owing to the presence of disulfide-linked glutenin chains [28], gliadin intercalating with the glutenin chains (Figure 2B). This property has led to research in using gluten and zein/gluten composites as plastics. Gluten coated with zein has been used to produce a plastic that is biodegradable and yet has a compressive strength similar to that of polypropylene. Production of this polymer is very simple; gluten and zein are mixed in ethanol and then formed in moulds. Essentially the zein forms a ‘glue’, which binds the matrix (gluten) and causes aggregation; pressure moulding then removes the ethanol and allows the polymer to form [30]. Maize gluten has been used in the manufacture of wood composites. In this process, wood fibres are mixed with maize gluten, plasticized using glycerol, water and ethanol, and then extrusion-moulded into pots that are reasonably water-resistant and biodegradable [31]. Gluten-based polymers are very efficiently degraded in soil and in liquid environments, being degraded completely after 50 days in soil and about ten times more quickly in liquid [32]. Medical uses for gluten-based polymers also exist. When gliadin is purified from wheat gluten using ethanol extraction, it can be spun into fibres that allow adhesion and growth of muscle cells. Using plant proteins for this purpose (rather than collagen) is postulated to be superior, owing to the mechanical properties of the gliadin fibre and because it obviates the risk of disease transmission [33].

Zein is an alcohol-soluble protein extracted from maize gluten meal. Zein accounts for approx. 65% of the protein content of the meal. Zein is a member of the prolamin family, which are proteins characterized by a high percentage of hydrophobic amino acid residues, including proline [34]. Its purification scheme and its use as a plastic resulted in the award of a number of patents in the late 19th and early 20th Centuries. Commercial production of zein started in the 1930s and was used in the manufacture of buttons, fibres, adhesives etc., with production peaking in the 1950s at around 6.8 million kg (15 million lb)/year (reviewed in [35]). More recently, production has fallen to about 0.45 million kg (1 million lb)/year, and it is produced by two companies, one in the United States (Freeman Industries) and a second in Japan (Showa Sangyo Corp.). Its current price ranges from $10 to $40/kg, depending on the purity and so is considered a value-added by-product of maize [36]. Zein fibres were used for clothing and furniture stuffing, and were marketed under the brand name Vicara™ in the 1950s (reviewed in [36]). Zein has also been used for film production, for example for use as a grease-resistant coating for paper that is resistant to microbial attack [37,38]. Addition of food-grade antimicrobial agents, such as lysozyme and the peptide nisin, enhanced the application for food-packaging films [39]. Pure zein polymers tend to be brittle and hygroscopic [40], but the addition of various plasticizers has allowed production of useful plastics. PCLH [polycaprolactone (polyhexanolactone)/hexamethylene diisocyanate] was used to coat a zein matrix by the addition of 10% zein to about 50% PCLH. Through interactions with glutamate, glutamine, tyrosine and histidine side chains, a polymer with superior mechanical properties (specifically flexibility) was produced [41]. Porous zein three-dimensional scaffolds with the potential for use in bone-tissue engineering have been produced by mixing zein with NaCl crystals. Once this polymer is pressure-moulded, the salt is removed in hot water. Following freeze-drying, the zein forms a porous polymer with interconnecting pores; this interconnection allows blood-vessel proliferation during bone growth, but the mechanical properties (i.e. brittleness) of the polymer would limit it to non-weight-bearing applications [42]. An improved polymer was subsequently achieved by coating the zein with hydroxyapatite [43]. Multiple uses of zein microspheres for sustained or targeted drug delivery have also been reported [44–46]. These reports state that simply mixing the drug and purified zein in aqueous ethanol solutions results in the production of microspheres that have an average diameter of about 1 μm and allow gradual release of the drug.

An early use of plasticized soy protein was by the Ford Motor Company. In 1940, Henry Ford applied for patent protection on his invention for automobile body construction, which stated that “the object of my invention is to provide a body construction in which plastic panels are employed, not only for the doors and side panels, but also for the roof, hood and all other exposed panels on the body.” The panels were made from soy meal (about 50% protein) that was cross-linked with formaldehyde with the addition of phenol or urea to increase strength and resistance to moisture. The panels were layered over a unique (at the time) tubular steel cage that provided the structural rigidity for the car, which was actually the focus of the patent application. The patent was issued on 13 January 1942 (Automobile body construction, U.S. Patent 2269451). Henry Ford was photographed demonstrating the strength of the panels by swinging an axe at the car (although the photograph fails to show the impact or any damage associated with it). The prototype was never put into production,
partly because the plastic was susceptible to microbial degradation (it was biodegradable!) and was not adequately moisture resistant, and apparently the car smelled strongly of formaldehyde [47].

SPI (soy protein isolate) is a minimum of 90% protein (by dry weight) and is purified from defatted soy flour. The glycinin and conglycinin seed storage proteins are the bulk of the protein content. Although SPI has been widely used as a food ingredient, it also has been used as a basis for plastic production. Heat-induced cross-linking of the proteins creates a thermoplastic polymer based on three-dimensional networks of disulfide bonds, as well as hydrophobic interactions and hydrogen bonding. Addition of plasticizers, such as glycerol, improves the structural properties of the film. Combining 5% (w/v) SPI and 3% (w/v) glycerol in water and then adjusting the pH outside the pI of the major storage proteins to prevent aggregation/coagulation (< pH 3 or > pH 6), followed by heating to 80°C, allowed casting of films on a levelling table. Alkaline-cast films are more flexible than acid-cast ones. Although these films are poor moisture barriers, they are good oxygen barriers and so can be used as a layer in a multilayer sheet to prevent oxidation of packaged food [48].

Using γ-irradiation for protein cross-linking in film production has the added benefit of being a common sterilization technique. γ-Irradiation causes the production of free radicals in the protein solution, and cross-linking through biphenoI compounds is achieved. These films have superior puncture strength and deformation properties than have the thermoplastic SPI films, and this could be further improved by the inclusion of carboxymethyl-cellulose or poly(vinyl alcohol) [49]. More recently, composites of SPI and chitin or cellulose microfibres (‘whiskers’) have been produced that have superior properties compared with pure SPI-based plastics [50,51]. Cellulose whiskers with average dimensions of 1.2 μm long × 90 nm diameter were prepared from cotton by hydrolysis in sulfuric acid. Mixing SPI, whiskers and glycerol in water was followed by heating and pressure moulding to produce the polymer. The SPI–cellulose composites have a superior moisture resistance, tensile strength, thermal stability and flexibility to SPI-based polymers, but retain biodegradability [51]. Similar improvements in the quality of SPI-based plastics have been achieved by using polylactide in the composite [52].

**Cellulose**

Although much research using starch and cellulose reserves is based on their use for biofuels (reviewed in [53]), they have also been used for plastic production through derivatization.

Cellulose is a linear polymer of β-1,4-linked d-glucose (Figure 3). Although cellulose cannot be thermally processed into plastics, owing to decomposition of its hydrogen-bonded structure, derivatized cellulose has been employed for plastics production. Parkesine™ (named after its inventor, Alexander Parkes) was an early pressure-mouldable form of nitrocellulose (Figure 3) used as a replacement for ivory in the late 19th Century. The cellulose was derived from cotton fibres solubilized with nitric acid and ethanol. This solution, called ‘collodion’, could be cast in sheets or pressure-moulded. Parkesine was eventually updated and replaced by celluloid, which used camphor, a tough gummy volatile aromatic crystalline compound (C_{10}H_{16}O), as a plasticizer. The addition of the camphor plasticizer made celluloid more flexible and mouldable and less likely to fracture compared with Parkesine. Celluloid was also used as a substitute for ivory, specifically for billiard balls and, towards the end of the 19th Century, was used as a photographic film for still photographs and movie films and even as windshields [47].

Cellulose acetate (Figure 3), another derivative of cellulose, is prepared by dissolving cellulose in acetic acid and acetic anhydride in the presence of sulfuric acid. Sheets are then cast, or

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**Figure 3 Natural polymers: cellulose and starch**

Cellulose and starch can be modified to produce biodegradable polymers with various R groups in place of hydrogen. Nitrocellulose is cast as films/paper, and cellulose acetate and CAP have been used for both films and nanospheres. Derivatization is achieved by chemical means from the isolated plant material. Starch has been used directly for polymer production and also through derivatization with caprolactone, cellulose and various vinyl alcohols. The degradation properties of starch polymers are dependent on the relative proportion of pure starch to secondary compounds. The degradation of the Mater-Bi™ starch polymers is shown in the bottom left-hand panel.
fibres spun, typically from an acetone solution. Cellulose acetate was widely used as film stock in the early 20th Century.

There are a number of medical uses reported for a further derivative of cellulose acetate, namely CAP (cellulose acetate phthalate) (Figure 3). CAP is widely used as a coating for pills, but more specialized uses also exist. For example, CAP microspheres have been used to improve the retention of anti-diabetic drugs in the gut. The microspheres float in the stomach and so are not evacuated as quickly and therefore aid drug release [54]. Controlled release of drugs at sites of bone regeneration is also an application of CAP [55]. Additionally, there are reports of the use of CAP as an anti-HIV1 agent [56,57]. Cellophane is restructured cellulose prepared from wood pulp using NaOH to break down the crystalline structure, followed by gelling with acid [47]. Despite being mostly replaced by synthetics, it is still used in some food-packaging applications, where, for example, the cellophane is impregnated with an antibacterial peptide, nisin, and used to wrap fresh meat [58], and some specialized medical uses, for example by preventive adhesion of intestine and abdominal wall tissues to allow proper healing [59]. Additionally, cellophane is still widely used for packaging.

**Starch**

Starch is one of the cheapest and most abundant agricultural products and is completely degradable in a number of environments [60]. These properties have lead to exploring starch as a polymer for various applications. Thermoplastic starch is prepared by temperature and pressure extrusion and/or moulding. This pure-starch polymer degrades very quickly in a composting environment (the process lasting about a month), but it tends to age poorly and is not moisture-resistant [61]. Production of various thermoplastic starch composites is based on mixing starch with vinyl alcohols, and these types of polymers tend to be more stable. However, the biodegradability of these composites is inversely proportional to their starch content [62]. An Italian company, Novamont, has commercialized four starch-based biodegradable plastic composites that vary in their additives and are trademarked Mater-Bi™ (Figure 3). Their output is about 36 300 metric tons (40000 short tons) of biodegradable plastic pellets/year, which are supplied to a number of packaging companies. Their V-class polymer is about 85% thermoplastic starch, degrades very quickly in compost and is used as a polystyrene replacement. Their Z-class polymer is composed of starch and polycaprolactone (a synthetic polyester), is biodegradable in composting environments (20–45 days) and is mainly used for bags and films. The Y-class polymer can be injection-moulded, is composed of starch and modified cellulose and lasts about 4 months in composting environments. Finally the A-class polymer, which is a mixture of starch and ethylene vinyl alcohol, is their most stable form of polymer, which is not compostable, but does biodegrade (in about 2 years) in a simulated sewage- sludge treatment plant. The A-class polymer can be used for rigid and expanded items [63]. The Mater-Bi™-based products include garden-waste (leaves, twigs etc.) and rubbish bags, nappies (diapers) and organic food packaging used by the U.K. supermarket chain Sainsbury’s.

Medical uses for starch-based polymers are also being explored, for example as scaffolds for bone-tissue engineering because of their biocompatibility, biodegradability and porous nature, which allows blood-vessel proliferation during bone growth. The SEVA- C type polymer used is a 1:1 (w/w) blend of cornstarch and ethyl vinyl alcohol, which is mouldable, non-toxic and does not inhibit cell growth [64]. Additionally, implantation of the SEVA- C polymer in rats did not produce a severe inflammatory response, demonstrating it as a good candidate for implants for bone regeneration [65].

**BIOCONVERSION OF PLANT POLYMERS: FERMENTATION**

Currently, bioethanol production is a major focus for the use of plant starch and sugar reserves. Attempts at increasing the amounts of reserves accumulated have been addressed through altering the activity of the ADP-glucose pyrophosphorylase enzyme, altering source-sink allocation of carbon or altering carbon partitioning within storage organs in favour of starch synthesis. These and other approaches were reviewed recently by Smith [66]. Use of plant carbon reserves for plastics has also been addressed through fermentation using various strains of bacteria for different types of polymer production, such as PLA [poly(lactic acid)] and polyhydroxyalkanoates.

**PLA [Poly(lactic acid)]**

PLA (also known as polylactide) is a polymer of lactic acid (2-hydroxypropionic acid) that was first developed by Dow Chemicals in the 1950s. However, because of the high cost of production, its use was limited to specialized medical devices such as sutures, soft-tissue implants etc. Figure 4(A) shows the structure of the PLA polymer. More recent advances in fermentation have resulted in a dramatic cost reduction to the point that PLA is widely used in packaging. PLA is produced by naturally occurring lactic acid bacteria of the genus *Lactobacillus*, which ferment hexose sugars. Lactate dehydrogenase converts pyruvate into lactate, with concomitant oxidation of NADH (reviewed in [67]). Production of PLA is classed as heterofermentative or homofermentative. The former...
method produces less than 1.8 mol of lactic acid/mol of hexose, along with a number of other metabolites, such as acetic acid, ethanol, glycerol, mannitol and CO₂. The latter homfermentative method produces an average of 1.8 mol of lactic acid/mol of glucose and only minor amounts of other metabolites, which allows about a 90% conversion rate of glucose into lactic acid (reviewed in [68]). Polymerization of lactic acid to PLA by chemical synthesis produces a large amount of racemic D,L-lactic acid, but is very expensive. The method employed by Dow–Cargill to produce their NatureWorks® PLA product involves condensation of lactic acid to lactide (circularized lactic acid; two monomers both D-lactic acid, both L-lactic acid or a mixture of the two enantiomers), followed by ring-opening polymerization [69] to produce high-molecular-mass (> 100 kDa) PLA. NatureWorks LLC uses dextrose maize sugar (D-glucose), from Cargill, as a carbon source for fermentation. They produce over 136 million kg (300 million lb)/year in their plant in Blair, Nebraska, U.S.A. Over 70 companies are using their PLA for various film and moulded applications, because of its high gloss, high transparency and physical properties similar to those of poly(ethylene terephthalate) (source: http://www.natureworksllc.com). PLA is compostable and degrades in a two-stage process that involves reduction in molecular mass by hydrolysis, followed by biodegradation by microorganisms [68]. For example, a number of filamentous fungi naturally present in soil can biodegrade hydrolysed PLA [70]. In the United States, NatureWorks LLC also runs a ‘buy-back’ program for PLA-based products as part of their commitment to producing an environmentally friendly plastic. A recent advance in PLA production has been made by utilizing a ‘microbial factory’ instead of ring-opening polymerization for generation of high-molecular-mass PLA. A Japanese group (including researchers for the Toyota Motor Corporation) has modified a polyhydroxyalkanoate-synthesizing enzyme to facilitate polymerization of lactic acid, thereby allowing polymer synthesis within the microbe without the need for chemical polymerization [71].

**PHAs**

PHAs are hydroxyalkanoate polyesters of various chain lengths. PHAs are carbon and energy reserves stored as insoluble inclusion bodies in most bacteria under nutrient-limiting and carbon-excess conditions [72]. The inclusion bodies were first observed in the late 19th Century, and the composition of PHA was first examined in the 1920s by Maurice Lemoigne at the Pasteur Institute in Paris (reviewed in [73]). PHB (polyhydroxybutyrate; Figure 4B) has properties that resemble those of polypropylene and is amenable to injection moulding, extrusion blow moulding and fibre spray-gun moulding. p(3HB-co-3HV) or simply PHBV, the co-polymer of hydroxybutyrate and hydroxyvalerate (Figure 4C), is similar to the PHB homopolymer, although more flexible [72]. PHAs have been used for the manufacture of films, coated paper and board, compost bags and disposable flatware and can also be moulded for the production of bottles and razors and are completely biodegradable (to CO₂ and water). These properties make PHAs an attractive source of non-polluting renewable plastics and elastics [74]. A considerable body of research has gone into production of various PHAs in transgenic plants (see the subsequent section), but the use of plant products as carbon sources for bacterial bioreactors has also been explored.

Fermentative production of PHAs using the bacteria *Ralstonia eutropha* and *Alcaligenes latus* has been established for almost 40 years. The *R. eutropha* PHA synthase operon encodes a thiolase (phaA), a reductase (phaB) and a synthase (or polymerase, phaC) [75]. Although wild-type *R. eutropha* can only use fructose as a carbon source, a glucose-utilizing mutant of *R. eutropha* was engineered and used in the production of Biopol®. Biopol® was originally developed by ICI (Imperial Chemical Industries), which began worldwide commercialization of a family of PHBV polymers in the 1980s. In 1990, the agricultural and pharmaceutical divisions of ICI were spun off as Zeneca Ltd, and then Biopol® was bought by Monsanto in 1996. However, soon thereafter, Monsanto closed its bioplastics division as it refocused on agricultural applications of biotechnology and moved away from industrial applications. The patent rights were then bought by a Cambridge, (MA, U.S.A.) company called Metabolix (source: press release 16 May 2001, Metabolix.com).

In addition to the numerous naturally occurring PHA-producing prokaryotes, other bacteria that do not normally produce PHAs can be modified to do so. For example, cloning the PHA operon into *E. coli* has also allowed various types of PHAs to be produced. Specifically, the co-polymer PHBV is produced by adding glucose and propionic acid to the culture medium (reviewed in [76]). Figure 4(C) shows the structure of the co-polymer. Growing modified *E. coli* strains under specific growth conditions can be used to alter the structure of the co-polymer, specifically the relative abundances of the HB (hydroxybutyrate) and HV (hydroxyvalerate) monomers. Adding valine to the glucose-based culture medium results in 2% HV co-polymer, and including threonine along with valine results in 4% HV [77]. Typically PHAs are produced using pure cultures of defined bacterial isolates and supplying highly purified carbon sources like glucose. This type of production yields an average cost of $14/kg ($9/kg; £7.9), considerably more than petroleum-based plastics (e.g. polyethylene). However, production of PHA from mixed cultures using various wastestreams has been proposed as an effective way to reduce these costs [78]. Yu et al. [79] described using industrial food wastewater as a carbon source for production of PHA by *A. latus*. They investigated the use of soy wastes from a milk dairy and malt wastes from a brewery as carbon sources. The malt-waste-fed bacteria produced 70% polymer (on a cell weight basis), whereas the soy-waste-fed and sucrose-fed cultures produced a little over 30% polymer. A recombinant strain of *E. coli* was used to produce PHB with whey and maize steep liquor as the main carbon and nitrogen sources respectively. The whey liquor is a by-product of the cheese industry and maize steep liquor is a by-product of wet-milled maize. *E. coli* was transformed with a plasmid containing the phaABC genes expressed from the phage T5 promoter and two lac operator sequences. PHB polymer accumulated to over 70% of the cell dry weight, although this amount tended to decrease as the cultures aged, a phenomenon attributable to plasmid loss during growth. The PHB obtained was very similar to the naturally produced PHB, with a high molecular mass and a similar crystalline structure [80]. Interestingly, Metabolix and ADM (Archer Daniels Midland) have co-operated to produce a bacterially produced PHA with the trade name Mirel®. They are in the process of constructing a commercial-scale production plant adjacent to ADM’s wet maize mill in Clinton, Iowa, and plan to produce polymer in the second quarter of 2009 with a production capacity of 49.8 million kg (110 million pounds)/year (source: http://www.mireplastics.com/).

Plant oils have also been successfully used to produce the typical thermoplastic PHAs. PHB and the PHBV co-polymer can be produced by wild-type *A. eutropha* and a modified strain containing the synthase gene from *Aeromonas caviae* respectively. Wild-type *A. eutropha* accumulates 80% of its cell weight as PHB when fed olive, maize or palm oils, and similar amounts of PHBV are produced by the recombinant strain using the same plant-oil carbon sources. Using these cheap oils as a
renewable carbon source could increase the economic viability of fermentative PHA production [81].

Despite these advances in using relatively cheap plant oils and carbohydrates or industrial effluents as carbon sources, the limitations of bioreactor production still exist. PHA or PLA production in bioreactors requires careful monitoring of the conditions within the bioreactor, including the cell density, nutrient reserves, accumulation of waste products etc. Bioreactors also have certain size and production capacity limits [82]. Additionally, pure culture production of PHA is thought to consume more fossil-fuel resources than petroleum-based plastics [83,84]. Although mixed-culture PHA fermentative production is proposed to have significantly less environmental impact than traditional petroleum-based plastics such as high-density polyethylene [85], using plants for de novo synthesis of polymers could theoretically prove an economically viable option, either as a value-added co-product of food crop plants or perhaps as a novel use for some marginal land species such as switchgrass (Panicum virgatum L.).

DE NOVO SYNTHESIS OF POLYMERS WITHIN PLANTS

Traditional plant breeding to produce valuable traits has been employed since prehistory, when selection of visual phenotypes led to the domestication of the first crop plants. Wheat, for example, is thought to have been domesticated about 10 000 years ago in the Near East [86]. However, it was not until the late 20th Century that molecular manipulation to produce the first transgenic plants was conducted. Altering plant metabolism to produce value-added products by transgenic methods has slowly begun to yield some promising results for plastics production.

Proteins

A number of hurdles need to be tackled before commercial-scale production of value-added heterologous proteins (and other polymers) can be achieved in plants. The DNA encoding the foreign protein must be stable across a number of generations, and synthesis of the protein from mRNA, through processing, translation and, addition of post-translation modifications, must be considered. Additionally, the type of crop used and the site of accumulation must be carefully considered to allow high levels of expression and ease of purification (reviewed in [87]).

Among the number of novel proteins being expressed in transgenic plants, many of these confer traits on the plant itself, for example the Bacillus thuringiensis cry toxin for insect resistance (see [88]). However, in the context of polymer production, proteins with an inherent commercial value following purification from the plant are also being produced. Typically, these are high-value proteins with specialized uses, for example as vaccines. Production of heterologous proteins within plants has numerous advantages over production in animal or bacterial systems. Plant-based therapeutic proteins, for example, are theoretically safer from a disease point of view than those purified from animal sources (whether native or recombinant). Also, incorporation of post-translational modifications such as glycosylation can be achieved by in planta production, in contrast with bacterial systems. Although eukaryotic culture systems such as insect and mammalian cell cultures can be used in many cases to produce viable proteins, an additional advantage of plants is that they are renewable and scaleable and require less energy and nutrient input than bioreactors, which should make plants cost-effective for bulk applications (reviewed in [89]).

Heterologous protein accumulation within plants can be achieved through standard transgenic approaches (i.e. nuclear transformation), through plastid transformation and also through viral vectors. The latter approach typically leads to transient expression most commonly targeted to the leaves [90,91] and, thus, cannot be considered a sustainable approach. Nuclear transformation, typically mediated by Agrobacterium infection, allows incorporation of the heterologous genes into the nuclear genome, (often) followed by stable expression and heritability of the trait. A number of crop plants, including maize, soybean, rice and cabbage (Brassica) species, have been successfully transformed using this technique. In terms of heterologous protein expression for purification purposes, the goal, obviously, is to achieve high levels of expression. Avidin was produced in transgenic maize using a constitutive ubiquitin promoter to levels exceeding 2% of aqueous-soluble protein from seed, where more than half of the protein accumulated within the embryo. The extracted avidin could be highly purified using affinity chromatography and was identical with the native egg-white protein. The study reported that 150–300 mg of purified avidin could be extracted/kg of seed, and one high-expressing line contained 20 g of avidin in 100 kg of maize seed, which was the equivalent of 900 kg of eggs. The authors postulated that with the levels of accumulation, the stability and viability of the protein, and the heritability of the trait, that commercial-scale production was quite feasible [92]. Nandi et al. [93] reported production in rice grain of human lactoferrin, a protein which is involved in iron absorption and has properties ranging from antimicrobial to immune-system modulation and promotion of cell growth. It was demonstrated that stable expression over nine generations could be achieved with production amounts ranging from 4.5 to 5.5 g/kg (about 5% of the rice flour dry weight). The recombinant protein was very similar to the native protein from human milk, although the glycosylation patterns were not identical. Process simulations suggested that 1 g of lactoferrin would cost $6 (€4.64, £4.06) to produce, assuming a 5% (dry weight) level of expression and using a single cation-exchange purification step [93]. There are potential problems with nuclear transformation, however; for example, in the case of avidin production in maize, the plants exhibited male sterility [92]. Additionally, silencing of transgenes is also a frequent problem in plants. Gene silencing can occur from position effects – the influence of the local environment on the chromosome around the site of transgene insertion, which can affect, for example, the rates of transcription. Additionally, when the transgene and native genes are similar in sequence, homology-dependent gene silencing of the transgene and/or co-suppression of transgene and native gene can occur. Achieving high levels of heterologous protein expression that is stable over a number of generations can be severely hampered by these events, although there are strategies to mitigate silencing of nuclear-transformed plants (reviewed in [94]).

Production of spider silk proteins in plants genetically modified by Agrobacterium-mediated nuclear transformation has been explored. Spider silk proteins used in web construction tend to be elastic Lycra®-like fibres, whereas the dragline (lifeline) silks are Kevlar®-like and are stronger than steel on a per-weight basis. Silk proteins are composed of GPGXX and GGX (one-letter amino acid code) motifs, along with [GA], repeat sequences that confer elasticity and high tensile strength respectively. Transgenic tobacco (Nicotiana tabacum), potato (Solanum tuberosum) and thale cress (Arabidopsis thaliana) have been used for silk production (reviewed in [95]). Yang et al. [96] reported production of DP1B, a synthetic analogue of spider dragline silk protein, in A. thaliana. Expression was driven from the CaMV (cauliflower mosaic virus) 35 S promoter introduced by the standard floral-dip method for nuclear transformation by Agrobacterium. When protein accumulation was targeted to the ER (endoplasmic...
was produced. This level could be increased to 8.5 % in the leaf apoplast and as high as 18 % in seed-targeted expression. In tobacco and potato, the CaMV 35 S promoter was used to drive expression in nuclear-transformed plants and expression was targeted to the ER using the KDEL (one-letter amino acid code) ER retention signal [97]. These workers concentrated on a silk–elastin fusion protein and achieved laboratory-scale production of 80 mg of pure protein/kg of tobacco leaves using a simple buffer-extraction procedure followed by heating to 95 °C for 1 h to cause aggregation of most proteins, leaving the silk protein in the supernatant. Purification of the protein, however, does not confer the structural properties of the silks, as this is accomplished by the correct assembly of the proteins during the process of spinning in specialized organs in the spider (i.e. spinnerets), and this natural process has not been replicated for the recombinant proteins [98].

Generating transgenic plants through plastid genome (plastome) transformation has a number of advantages. High levels of transgene expression are typically achieved due to the plastome copy number and the presence of multiple chloroplasts per cell [99]. For example, choler toxin B was expressed at very low levels by nuclear transformation. However, 400–3000-fold increases were observed when chloroplasts were used as the site of transformation/expression [100]. Another advantage of the transplastomic approach is that multiple genes can be inserted in a single event [101] without problems of gene silencing, since genes are targeted to a specific locus on the plastid genome by homologous recombination. Plastome transformation has the added advantage of maternal inheritance, so that transmission of transgenes through pollen is eliminated, resulting in improved containment of the transgene in the environment [102]. Tobacco has been widely used for expression of proteins from the plastome as have soybean, cotton (Gossypium hirsutum) and carrot (Daucus carota) [103]. Lutz et al. [104] produced a guide to aid selection of vectors for plastid transformation that include marker genes to allow selection of transformants and also to follow insertion and expression of the transgene of interest.

Human growth hormone, or somatotropin, is one of the many proteins produced in tobacco through plastid transformation. Chloroplasts were shown to accumulate soluble active and disulfide-bonded somatotropin. Additionally, use of a fusion-protein approach (N-terminal ubiquitin) facilitated production of a functional protein lacking the N-terminal methionine residue; the native protein starts with a phenylalanine residue following signal sequence removal in the pituitary gland. High levels of somatotropin protein accumulation within tobacco chloroplasts was achieved by driving expression of the gene from the plastid psbA promoter and other domains in the UTRs (untranslated regions) [105]. The psbA gene encodes the D1 protein, part of the reaction centre of Photosystem II. Using the psbA promoter and other sequences in the gene’s UTRs was previously demonstrated to confer high expression of foreign genes in light-exposed leaves [106]. The amount of somatotropin produced was >7 % of total soluble protein, which corresponded to a 300-fold increase relative to levels achieved by nuclear transformation [105].

Attempts at producing an anthrax vaccine have also been made by plastid transformation of tobacco leaves. The Bacillus anthracis PA (protective antigen) was expressed in chloroplasts, also under the control of the psbA promoter. The widely used anthrax vaccine derived from B. anthracis itself has other minor components in addition to PA that cause side effects when administered. Using tobacco leaves as a source for a recombinant PA should obviate these reactions. During normal day/night light regimes PA was produced at between 1.7 and 2.7 % of total soluble protein from mature leaves. However, using a continuous light regime up to a ten-fold increase in expression was observed. About 170 mg was harvested from a single plant, equivalent to 2.5 mg/g fresh weight. The authors extrapolated this expression level to a supply of about 990 million doses of vaccine/hectare (400 million doses/acre) using their experimental cultivar and possibly more than 18-fold higher using a commercial cultivar, which would be the equivalent of a world supply of vaccine from just a few acres of transgenic tobacco [107].

Although extensive research has gone into using plants for heterologous-protein expression, apart from those that impart a specific valuable trait on the crop plant itself, few have made the leap to commercial/agricultural-scale production. In fact, this can be said of transgenic plants as a whole [108].

**PHAs**

The chemical properties of various types of PHAs impart multiple potential applications. Additionally, these polymers are completely biodegradable. As a result, PHAs have been proposed as alternatives to conventional petroleum-based plastics. The bacterial genes responsible for polymer synthesis have been studied in great detail, and numerous recombinant bacterial strains have been generated to facilitate PHA production. However, production of PHAs in transgenic plants has been proposed as an economically viable alternative to bioreactor-based or chemical synthesis.

Production of the PHB homopolymer in transgenic plants was first achieved by Chris Somerville’s group (then at Michigan State University in East Lansing, U.S.A.). These workers generated A. thaliana plants that were transformed with the *R. eutropha* genes encoding the acetoacetyl-CoA reductase and PHB synthase. Polymer synthesis was initiated by a cytosolic β-oxoohiolase naturally present in *A. thaliana* along with the introduced bacterial enzymes (under the control of the CaMV 35 S promoter) that facilitated production of high-molecular-mass PHB polymer in the cytosol, nucleus or vacuoles. However, the amount of PHB produced was quite low (about 0.1 % dry weight) [109], and this level of accumulation was not improved by cytosolic production in other transgenic plants such as tobacco [110]. Additionally, the transgenic plants showed serious side effects of PHB accumulation, namely a notable decrease in growth and seed production. The precursor for PHB production is acetyl-CoA (Figure 5), and diversion of much of this toward PHB production and away from natural pathways, such as those for flavanoid and phytosterol production and fatty acid elongation, has been postulated as the likely cause of the deleterious side effects [95].

Plastids were considered as a good site for polymer production for a number of reasons. Plastids contain large quantities of acetyl-CoA destined for fatty acid and amino acid synthesis, so the metabolic flux should be sufficient for PHA production. Plastids also naturally accumulate bulky starch grains; therefore they should tolerate accumulation of PHAs with less deleterious effects. However, plastids do not contain the β-oxoohiolase, so this must be included in the generation of transgenic plants. As discussed above, nuclear-transformation-mediated heterologous-protein production has been demonstrated to be far inferior to plastome transformation in terms of accumulation levels. Lössl et al. [111] introduced the *R. eutropha* PHA-synthesis operon into the plastome under the control of the psbA promoter by biolistic (‘gene gun’) transformation. The average amount of PHB synthesized by their transplastomic tobacco lines was 715 p.p.m. on a dry-weight basis (<0.01 %), although one line accumulated 1.7 % (by dry weight) polymer. Unfortunately, there was a strong correlation between PHB levels and reduced plant...
growth. Additionally, the transformed plants were male-sterile, and maintenance of the transgenes could only be achieved through fertilization with wild-type pollen [111]. Unlike the numerous success stories for protein production by transplastomic plants, PHA production was poor when driven from the plastid. The plastidial synthase operon comprising three enzymes (blue italics) allows accumulation of the polymer precursors of the polymer – acetyl-CoA and propionyl-CoA. Introduction of the bacterial PHA deaminase did raise the levels of propionyl-CoA relative to plants without it, the amounts were still 4–6-fold less than acetyl-CoA.

Figure 5 Scheme for production of PHBV in plants

Accumulation of PHBV within plastids uses the endogenous PDC for generation of the two precursors of the polymer – acetyl-CoA and propionyl-CoA. Introduction of the bacterial PHA synthase operon comprising three enzymes (blue italics) allows accumulation of the polymer within the plastid. The plastidial \( \alpha \)-oxobutyrate (\( \alpha \)-ketobutyrate) is produced from threonine by an introduced threonine deaminase (not shown).

Production of PHBV is relatively limited in plants, with most success reported in transgenic plants. Despite the potential of PHBV as a biodegradable polymer, commercial production has been challenging due to low levels of accumulation and maintenance of transgenes.

Most recently, switchgrass has been employed as a source of the PHB polymer. Switchgrass is a warm-season grass and one of the dominant species of the central North American tall-grass prairie. Switchgrass is seen as a valuable biomass crop with the potential for use in bioethanol production with concomitant reduction in greenhouse-gas emissions compared with gasoline/petrol [120]; it was recently targeted for funding as one of the useful bioenergy crops by the U.S. Department of Energy [121]. In addition to their bacterially produced polymer, researchers at Metabolix are also exploring plant-based PHAs. They used both constitutive and light-inducible promoters to drive expression of PHB synthesis genes appended with N-terminal chloroplast transit peptides. Greenhouse-grown switchgrass was found to accumulate almost 4% (by dry weight) PHB in the leaves and 1.2% (by dry weight) in whole tillers. Even their highest producing lines [3.72% (by dry weight) PHB in leaves] developed normally and accumulated biomass at levels similar to those of the wild-type. Additionally, stable high-level PHB accumulation was maintained in the subsequent generation. Although the amounts of PHB produced were below commercially viable levels, this was the first successful expression of a functional multigene pathway in switchgrass and bodes well for future engineering of the plants for value-added-product formation [122]. The hope eventually would be to produce a ‘double-crop’ for both PHAs and cellulose ethanol production.

Polymers consisting of PHB tend to be crystalline and brittle, whereas PHBV, the co-polymer of HB and HV, is considerably more flexible. PHBV precursors are acetyl-CoA and propionyl-CoA, and, although acetyl-CoA is present in the cytosol and plastids, propionyl-CoA is restricted to the mitochondria. An alternative route for production of propionyl-CoA is introduction of a threonine deaminase, which converts threonine into \( \alpha \)-oxobutyrate. The \( \alpha \)-oxobutyrate can then be utilized by the endogenous plastid PDC (pyruvate dehydrogenase complex) to produce propionyl-CoA (Figure 5). Slater et al. [123] employed this route to propionyl-CoA synthesis in plastids of \( B. \) napus and \( A. \) thaliana. This required introduction of the three bacterial PHA synthesis genes, along with the threonine deaminase – the pylA gene from \( E. \) coli. Although the presence of the threonine deaminase did raise the levels of propionyl-CoA relative to plants without it, the amounts were still 4–6-fold less than acetyl-CoA. Additionally, the poor efficiency of the plastid PDC for conversion of \( \alpha \)-oxobutyrate into propionyl-CoA resulted in accumulation of \( <3 \)% (by dry weight) polymer in rapeseed and about 1.6% in \( A. \) thaliana leaves. The relative amounts of the HV monomer in the PHA produced also decreased with increasing levels of accumulation, again pointing to inefficiency in generating HV. An encouraging point, however, is that no deleterious phenotypes were linked to the presence of the transgenes or PHBV content, although the authors concede that this might be due to the low levels of accumulation [123].

It is clear from the research to date that plastids are the most useful and widely used site for PHA accumulation in a number of crop plants, including alfalfa (\( Medicago \) sativa), cotton, potato, canola, tobacco, sugar crops [beet (\( Beta \) vulgaris) and cane (\( Saccharum \) officinarum)] and fibre crops such as flax (\( Linum \) usitatissimum) [95]. A goal of 7.5% (by dry weight)
The present review discusses three main themes for synthesis of biodegradable polymers. There are a number of naturally occurring plant polymers that have been exploited by direct harvesting for biodegradable plastics – rubber, starch, proteins and cellulose – sometimes followed by derivatization. Industrial or ‘white’ biotechnology using plant extracts as carbon sources for bioreactor fermentative production of PLA and PHA polymers is an alternative. Through engineering of bacteria, a number of valuable polymers can be produced with various monomers based on the nutrient input. Finally, using transgenic technologies, plants can be engineered to produce novel polymers, including value-added proteins and PHAs, and therefore function as solar-driven biofactories. In all cases, the polymers produced are biodegradable, so the carbon cycle is maintained – the transgenic route to plastics is considered the closest to a carbon-neutral method for polymer production.

The polymer has been set by Metabolix as a commercially viable level of accumulation [122], and some of the research into the slightly less desirable PHB polymer have clearly shown accumulation in excess of this (e.g. 40%; [114]). Unfortunately accumulation is only part of the process; extraction of the polymer is probably an even greater component for commercialization in a cost-effective and environmentally friendly manner to allow plant-based biodegradable polymers to replace petroleum-based plastics. Bacterial culture systems for PHA can accumulate 50–85% (by dry weight) polymer, with 80 g/litre and 2 g/h per litre capacities relatively easily met in fed-batch cultures [125]. Laboratory-scale purification of PHAs from bacterial cells can be achieved by simple mechanical (20 h constant stirring) organic-solvent extraction using chloroform and methanol, which exploits the solubility of PHA in chloroform and insolubility in methanol, allowing fractionation of the PHA [126]. An alternative approach is to use a ‘cocktail’ of enzymes and detergents to remove proteins, nucleic acids and cell walls, leaving the PHA intact [127]. However, a low-cost, highly efficient and environmentally friendly commercial-scale PHA extraction process has yet to be implemented. Two cost-effective and more environmentally friendly approaches have been developed recently, namely supercritical CO2 extraction and cell-mass dissolution by protons in aqueous solution (sulfuric acid extraction at high temperature). Recovery efficiencies of 90–95% with very high purity were achieved with these methods respectively (reviewed in [82]). Whether these methods can be used for PHA extraction from plants has not been tested. There are a number of patent filings for PHA extraction from plants based on non-halogenated solvent extraction (U.S. Patent 6043063) at high-temperature (U.S. Patent 6087471) and those that allow simultaneous extraction of PHAs and oils from oilseed crops using differential solvent extraction (U.S. Patent 6709848). None of these methods seem particularly environmentally friendly, and none have been published to date for commercial-scale extraction from plants.

CONCLUDING REMARKS

As discussed in the present review, the production of plant-based polymers can be approached in a number of ways (Figure 6). Some progress has been made in commercialization of plant-based materials, for example, the starch-based Mater-Bi™ range from the Italian company Novamont and U.S. NatureWorks LLC PLA polymer. However, commercialization of transgenic plants specifically designed to synthesize polymers has not been achieved. Parameters affecting commercialization of transgenic plants for bioplastics include the time and costs associated with their development (often 10–12 years and tens of millions of U.S. dollars in total) [128], the typically poor accumulation levels of novel polymers (compared with bacterial systems), the lack of proven processing and extraction methods, the price per unit of polymer from plants, and the stigma associated with GMOs in many parts of the world.

Many of these problems can be addressed by more research. Building on current seed stocks of plants that have already been developed for PHB production, such as those from Metabolix, for example, through cross-pollination or further genetic manipulation, could conceivability reduce the timeline for development and might, in part, reduce the costs associated with regulatory testing if the original plants already have a GRAS (‘generally recognized as safe’) designation. As more knowledge...
is gained on carbon flux in plants, perhaps, through emerging metabolomics methods for example, this will give us a better understanding of how to increase polymer accumulation. Judging from the numerous patent awards, processing technologies are being developed for transgenic plant polymers. In terms of price per unit polymer, fermentative PHA production could produce S2 (€1.55, £1.35)/kg prices, which is still about twice the price of polyethylene [129], but using crop plants in a ‘double-cropping’ value-added manner could allow exploitation of agronomic plants for polymer production. Additionally the scalability and renewable nature of plants should address price issues. Finally the acceptance of GMOs is still a point of contention. The StarLink maize incident in 2000 gained national and worldwide attention for the problems of segregation of food crops [130]. Utilizing non-food crops, like switchgrass, might ameliorate the stigma associated with transgenic crops, although food-chain contamination is only one of the issues that are used to argue against GMOs. Having said all this, the pressures on fossil fuels, seen in rising oil prices and environmental concerns, could soon precipitate a revolution against traditional plastics.

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