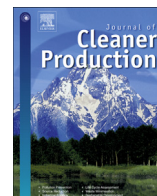




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Review

Plant oils as promising substrates for polyhydroxyalkanoates production

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ABSTRACT

The development of processes for the production of biopolymer materials is being stimulated by a combination of factors. These factors include the negative effects of petrochemical-derived plastics on the global environment, depletion of global fossil fuel supplies, and the growing demands of an ever-increasing population for the products deemed necessary for an affluent modern lifestyle. In particular, polyhydroxyalkanoates have attracted attention as environmentally friendly alternatives to the synthetic polymers that are commonly used. Polyhydroxyalkanoates are polyesters produced and accumulated in intracellular granules by many microorganisms. Because they are biodegradable and biocompatible and can be produced by fermentation of renewable feedstocks, they are considered attractive substitutes for petroleum-derived polymers. To create bacterial polyesters, crude and waste plant oils, which can be difficult to dispose of, can be recovered and used as feedstock. This paper gives an overview of the potential for the production of polyhydroxyalkanoates with useful physicochemical properties by bacteria grown on renewable resources such as plant oils.

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1. Introduction

Petroleum-based plastics have excellent physical properties that resulted in their wide application in almost all industries. The useful life time of articles made of plastic is usually very short, especially when they are used for packaging. After use they are usually discarded, thus the use of synthetic plastics has led to solid waste management problems. Indeed, plastics are especially troublesome to recycle, because different types of plastic need to be processed differently to be reformulated and re-used as raw material. Even in the most developed countries in Europe, plastic recycling does not exceed 50% (BIO Intelligence Service, 2013).

In response to plastic waste's problems and harmful effects on the environment, there has been considerable interest in the development of materials which could be more biodegradable than petrol-based plastics. As a result, polymers such as polylactides, aliphatic polyesters, polysaccharides and polyhydroxyalkanoates

have been taken under consideration as substitutes for conventional plastics. Polyhydroxyalkanoates (PHAs) are especially attractive because they have the proper physical properties and can be completely biodegraded under various conditions by a multiplicity of microorganisms within a period of one year (Anderson and Dawes, 1990).

Due to high PHAs synthesis costs, their production on a large scale is limited, therefore there is a need for the development of novel processes using inexpensive carbon sources. Such substrates could be plant oils. Especially, the usage of waste plant oils, generated by the food industry and food service, could be reasonable from the economic and environmental point of view. The conversion of these residues to new biomaterials is fully an eco-innovative approach.

The aim of this review is to give a background on the polyhydroxyalkanoates, their properties, applications and to systematize recent research focused on the potential use of plant oils in their production. This review highlights challenges in the PHAs technological approaches to make these environmentally friendly biopolymers as competitive as conventional plastics. In this paper, the authors consider the opportunities of the usage of plant oils as a

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promising utilizable carbon source towards valuable biomaterials production.

2. Methods

This review was designed to summarize recent progresses in the PHAs production field using plant oils. Papers from numerous scientific journals, commercial websites were selected and reviewed. The literature search was carried out using the most important and available databases, such as Scopus and PubMed. Firstly, specific keywords were selected: polyhydroxyalkanoates, waste plant oils, biopolymers, food waste, byproducts food industry, agricultural feedstock. Then, the abstracts were examined and selected based on the criteria that were chosen to determine the publications related to the objective of this paper. In particular, the authors identified studies: (1) dealing with PHAs production during bacterial cultivations on pure and spent form of plant oils; (2) providing fermentations strategies together with the description of crucial technological parameters; (3) focusing on material properties of PHAs from oils; (4) addressing future prospects of introducing biopolymers into global market.

3. PHAs structure and metabolism

PHAs are divided into two groups according to the number of carbon atoms in the monomer units: short-chain length PHAs (scl-PHAs) with 3–5 carbon atoms in each monomer unit, and medium-chain length PHAs (mcl-PHA) with 6–14 carbon atoms in each unit (Fig. 1). The most commonly produced monomer unit is poly-3-hydroxybutyrate [P(3HB)]. Scl-PHAs with poly(3-hydroxybutyrate-co-3-hydroxyvalerate copolymers [P(3HB-co-3HV)] have mechanical properties that are considered more desirable than P(3HB) homopolymers; mcl-PHAs are even more desirable, because of their superior thermo mechanical properties. The type of polymer produced is determined by the strain of bacteria used.

Although the composition of PHAs was determined by Lemoigne in 1927, interest in PHAs only started to increase in 1958. That was the year when Macrae and Wilkinson (1958) observed that *Bacillus megaterium* stored a homopolymer especially swiftly when the glucose-to-nitrogen source ratio of the medium was high. This homopolymer turned out to be poly-3-hydroxybutyrate [P(3HB)], nowadays the most commonly known and widely recognized polymer. Since 1958, knowledge about PHAs has greatly increased. These biopolymers are accumulated by both Gram positive and Gram negative bacteria, cyanobacteria and even Archaea (Chee et al., 2010b). Hundreds of bacterial species that can synthesize PHAs have been counted, and the number is still increasing.

Accumulating PHAs is a natural way for bacteria to store carbon and energy when environmental conditions are disturbed. In laboratory conditions these polyesters are accumulated when bacterial growth is limited by depletion of nitrogen, phosphorous or oxygen and an excess amount of a carbon source is still present (Anderson and Dawes, 1990; Ciesielski et al., 2010).

PHAs are accumulated in intracellular granules inside the cells. In *Cupriavidus necator* (formerly *Ralstonia eutropha*), 8–13 granules

per cell with sizes ranging from 0.2 to 0.5 μm were detected (Byrom, 1992). The surface of PHA granules is coated with phospholipids and proteins, which play a major role in PHA synthesis, degradation and even the whole process of PHA synthesis regulation. These proteins facilitate PHA metabolism, protect the granule from coalescence, and separate the hydrophobic polymer from the aqueous cytoplasm. By far the most abundant proteins on the granule are the phasins which has been shown to cover from 27 to 54% of the PHA granules' surface in *Cupriavidus necator* (Tian et al., 2005).

From a chemical point of view, PHAs are polyesters are composed of hydroxycarboxylate monomers. At least 150 different monomer constituents of PHA have been found, which possess straight, branched, saturated, unsaturated and aromatic structures (Steinbüchel, 2001). These monomers are all in the *R*-configuration because of the stereospecificity of the biosynthetic enzymes. Monomers with various functional groups on the chain, such as halogen, hydroxy-, epoxy-, cyano-, carboxyl- and esterified carboxyl groups, have been discovered in mcl-PHAs (Steinbüchel and Valentin, 1995).

The mechanical properties of individual PHAs depend on the composition of the monomer units. P(3PHB) is highly crystalline because it is a completely stereoregular polyester, with all asymmetric carbon atoms in the *R*-configuration. The high crystallinity (typically 55–80%) makes it relatively stiff and brittle. The glass transition temperature (T_g) of P(3HB) lies between 5 and 9 $^{\circ}\text{C}$, and the melting point (T_m) ranges from 173 to 180 $^{\circ}\text{C}$. P(3HB-co-3HV) is another well-studied PHA, composed of both 3HB and 3HV monomers. Its mechanical properties are considered more desirable than P(3HB) monomers because its melting point is much lower, and it is less crystalline, easier to mold and tougher than pure P(3HB) (Luzier, 1992). These thermo mechanical properties can be widely varied because the composition of P(3HB-co-3HV) can range from 0 to 30 mol% 3HV (Braunegg et al., 1998).

Mcl-PHAs have significantly lower melting point temperatures than scl-PHAs; they range from 39 to 61 $^{\circ}\text{C}$ and are strongly dependent on the thermal history of the material. Their glass transition temperature is usually below room temperature, ranging from -43 to -25 $^{\circ}\text{C}$, and they are about 25% crystalline. These characteristics make mcl-PHAs more flexible and elastic materials than scl-PHAs (Sudesh et al., 2000).

The type of PHA produced depends on the metabolic pathway of the particular bacteria being used. Scl-PHAs consisting of P(3HB) homopolymers are generally biosynthesized in a three-step process that is regulated by 3-ketothiolase (PhaA), acetoacetyl-CoA reductase (PhaB) and the SCL PHA synthase (PhaC). The most common metabolic pathway, found in a wide range of bacteria producing scl-PHAs, is probably the generation of P(3HB) from acetyl-CoAs. This pathway has been extensively studied in *Cupriavidus necator*. In this bacteria two acetyl-CoA moieties are condensed to yield acetoacetyl-CoA by 3-ketothiolase (PhaA). The product is subsequently reduced to (R)-3HB-CoA by an NADPH-dependent acetoacetyl-CoA reductase (PhaB). Only (R)-isomers are accepted as substrates by PHA synthase (PhaC), the polymerizing enzyme (Oeding and Schlegel, 1973).

There are three kinds of known pathways for mcl-PHA synthesis, found mostly in *Pseudomonas* species. Each pathway uses a different substrate to create 3HAs, which are then used to synthesize mcl-PHAs. All *Pseudomonas* spp. will only accept 3HAs of 6–14 carbon atoms. This is due to the specificity of PHA synthase, the enzyme responsible for polymerization of hydroxyalkanoic acid thioesters (Koller et al., 2010a).

In the first pathway that creates 3HAs for mcl-PHA synthesis, aliphatic carbon sources are degraded via β -oxidation to produce 3-hydroxyacyl-CoA. This β -oxidation intermediate is in the (*S*) form,

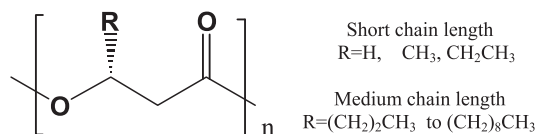


Fig. 1. Structure of PHAs.

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