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Exploiting microorganism for bio-plastic: An overview

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ABSTRACT

Plastic is the general common term for a wide range of synthetic or semi synthetic organic amorphous solid. It refers to their malleability, or plasticity during manufacture, that allows them to be cast, pressed, or extruded into a variety of shapes-such as films, fibers, plates, tubes, bottles, boxes, and much more. They are made of petrochemicals, non-renewable, contain a variety of toxic additives and they last for hundreds of years, they damage to natural habitats and killing animals that mistake them for food. Bioplastics is made by using renewable biomass. Many polymers were proposed and tested for their possible industrial applications and their biodegradability, e.g., cellulose, starch, blends of those with synthetic polymers, polylactate, polyester-amide, and polyhydroxyalkanoates (PHAs). PHAs gained particular interest since they were shown to be biodegradable and biocompatible. Bioplastics are divided in to biodegradable bio-plastic, non-biodegradable bio-plastic and mixed bio-plastics. Bioplastics are natural biopolymers that are synthesised and catabolised by various micro organisms. PHB (poly-β-hydroxybutyrate) is one of the important storage reservoirs providing energy. It is the cellular inclusion bounded by lipid, non-unit membrane separated from cytoplasm. β-hydroxy butyrate is connected by easter linkage and form PHB. These materials do not cause toxic effects in the host and have certain advantages over petroleum derived plastic. Currently there are four biosynthetic approaches to produce PHA: *in vitro* via PHA-polymerase catalyzed polymerization, and *in vivo* with batch, fed-batch, and continuous (chemostat) cultures.

KEYWORDS

Bioplastics;
PHB (Poly-β-Hydroxybutyrate);
Polyhydroxyalkanoates (PHAs) Biodegradable;
Biosynthetic production.

INTRODUCTION

Plastic

Plastic is the general common term for a wide range of synthetic or semi synthetic organic amorphous solid. The word is derived from the Greek (plastikos) meaning fit for molding, and (plastos) meaning molded. It refers to their malleability, or plasticity during manufacture, that allows them to be cast, pressed, or extruded into a variety of shapes-such as films, fibers, plates, tubes, bottles, boxes, and much more. Growth in the human population has led to the accumulation of huge amounts of non-degradable waste materials across our planet.

The accumulation of plastic wastes has become a major concern in terms of the environment[6,44]. Within the last 50 years petrochemical plastics have become one of our most applied materials. Their versatility, outstanding technical properties and relatively low price (1kg of polypropylene costs about US$ 0.70) caused their success. Today's applications are nearly universal: components in automobiles, home appliances, computer equipment, construction, sport and leisure equipment, packages, and even medical applications are areas, where plastics clearly have become indispensable. However, we all know that these plastics are environmentally unfriendly, i.e., they are not biologically degraded[47]. Conventional plastics not only take many decades to be decomposed in nature, but also produce toxins during the process of degradation.

Disadvantages of plastic

Significance

Plastic bags are not renewable, which means they cannot be easily recycled like paper bags. They are made of petrochemicals, which is what makes them non-renewable and a risk to the health of the planet. They last for hundreds of years, all the while doing damage to natural habitats and killing animals that mistake them for food. The more plastic bags people use, the greater the chances of environmental damage.

Effects

If not carefully disposed of, plastic bags can be devastating to animal life. DEFRA (Department for Environment Food and Rural Affairs) reported that 1,678,900 tons of plastic packaging was in the UK waste stream in 2001. Because plastic bags do not decay quickly, they stay in environments longer, causing more build-up on the natural landscape than a more degradable material like paper would. The Marrickville Council reports that over 100,000 whales, turtles and birds die every year as a result of plastic in their environment.

Toxicity

Due to their insolubility in water and relative chemical inertness, pure plastics generally have low toxicity in their finished state, and will pass through the digestive system with no ill effect (other than mechanical damage or obstruction).

However, plastics often contain a variety of toxic additives. For example, plasticizers like adipates and phthalates are often added to brittle plastics like polyvinyl chloride (PVC) to make them pliable enough for use in food packaging, children's toys and teethes, tubing, shower curtains and other items. Traces of these chemicals can leach out of the plastic when it comes into contact with food. Out of these concerns, the European Union has banned the use of DEHP (di-2-ethylhexyl phthalate), the most widely used plasticizer in PVC. Some compounds leaching from polystyrene food containers have been found to interfere with hormone functions and are suspected human carcinogens.

Moreover, while the finished plastic may be non-toxic, the monomers used in its manufacture may be toxic; and small amounts of those chemical may remain trapped in the product. The World Health Organization's International Agency for Research on Cancer (IARC) has recognized the chemical used to make PVC, vinyl chloride, as a known human carcinogen. Some polymers may also decompose into the monomers or other toxic substances when heated.

The primary building block of polycarbonates, bisphenol A (BPA), is an estrogen-like endocrine disruptor that may leach into food. Research in Environmental Health Perspectives finds that BPA leached from the lining of tin cans, dental sealants and polycarbonate bottles can increase body weight of lab animal's offspring. A more recent animal study suggests that even low-level exposure to BPA results in insulin resistance, which can lead to inflammation and heart disease.

Bio-plastic

It is a plastic made using renewable biomass. Many polymers were proposed and tested for their possible industrial applications and their biodegradability, e.g., cellulose, starch, blends of those with synthetic polymers, polylactate, polyester-amide, and polyhydroxyalkanoates (PHAs). PHAs gained particular interest since they were shown to be biodegradable and biocompatible[5,18].

Both properties can best be achieved by production in bacteria, thus, guaranteeing complete stereospecifity [all chiral carbon atoms in the backbone are in the R (2) configuration], which is essential for their biodegradability and biocompatibility. The type of bacterium and growth conditions determine the chemical composition of PHAs and the molecular weight, which typically ranges from 2×10^5 to 3×10^6 Da[7].

Bioplastic can be broken down in the environment by microorganisms in a process called biodegradation. This process produces carbon dioxide (CO_2) and water (H_2O) under aerobic conditions. Methane (CH_4) under anaerobic conditions. Mixed bioplstic are usually biodegradable, but some are not and can be either reused or processed for energy recovery.

Comparatively bio plastics decompose and combine with the soil quite fast. It is said that bio plastics are 30-80% free from carbon compared to normal plastics made from petroleum. Studies say that when we use bio plastics the shelf life of food items increases. There is a 20-30% increase in the usage of bioplastics every year. Statistics show that last year 0.2 million ton bioplastics was manufactured.

Types of bio-plastic

Biodegradable bio-plastic

A plastic derived from renewable biomass that can be broken down in the environment by micro-organisms. They are made by,

Starch-based bio-plastics can be manufactured from either raw or modified starch (e.g. thermoplastic starch or TPS) or from the fermentation of starch-derived sugars (e.g. polylactic acid or PLA). Common starch sources include maize, wheat, potatoes and cassava.

Cellulose-based bio-plastics are typically chemically-modified plant cellulose materials such as cellulose acetate (CA). Common cellulose sources include wood pulp, hemp and cotton.

Lignin-based bio-plastics contain wood (or lignocellulosic plant material) produced as a byproduct of the paper milling industry.

Plant proteins such as maize 'zein' can also be used to manufacture bio-plastics.

Non-biodegradable bio-plastic

A plastic derived from renewable biomass that cannot be easily broken down in the environment by micro-organisms. They are made by,

Conventional plastic resins can be made from plant oils and are manufactured using compounds extracted from castor, soya bean or oilseed rape oil. Examples include polyurethane (PU) manufactured from soya bean oil and nylon (polyamides or PAs) made using castor bean oil.

Conventional polyethylene (PE) can be manufactured from bioethanol.

Mixed bio-plastics

Mixed bioplastics can be both biodegradable and non-biodegradable depending on the polymers used to manufacture them. For example, a mixed bioplastic containing starch and polycaprolactone (PCL) is biodegradable, whereas a plastic containing a 1:1 mix of biomass and oil-derived polypropylene (PP) is not.

Bio-plastic from micro organisms

Bioplastic are natural biopolymers that are synthesis and catabolised by various micro organisms[1,19,20]. These materials do not cause toxic effects in the host and have certain advantages over petroleum derived plastic[31,39,47]. Bioplastics are manufactured using biopolymers which alter renewable and sustainable alternative to oil based plastics (petro plastic).

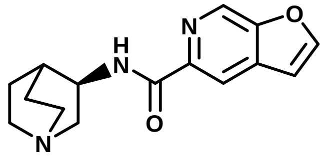
It is one of the important storage reservoirs providing energy. It is the cellular inclusion bounded by lipid non-unit membrane separated from cytoplasm. β-hydroxy butyrate is connected by easter linkage and form PHB[3]. It is now well recognized that this lipid inclusion is accumulated by many bacteria as they enter the stationary phase of growth to be used later as an internal reserve of carbon and energy.

Microorganism

Bradyrhizobium japonicum CB 1809, *Rhizobium sp.* CC 1192[19,20], *Aeromonas hydrophila*[37], *Salmonella enterica*[2], *Rhizobium meliloti*[26], *E.coli* and *Synechocytis sp.*[45], *Azotobacter vinelandii*[41], *Comamonas acidovorans* and *Alcaligenes eutrophus*[42], *Halomonas bolivensis*[14,41], *Saccharophagus degradans* ATCC 43961[10,12], *Streptomyces sp.*[46], *Bacillus megaterium*[11,22], *Azotobacter chroococcum* 6B[38], *Pseudomonas putida*[10], *Corynebacterium glutamicum*[16], *Wautersia eutropha*[6], *Alcanivorax borkumensis* SK2[40], *Pseudomonas oleovorans*[34,35], *Nocardia*[31], *Methylobacterium extorquens* (DSMZ 1340)[15], *Paracoccus, Micrococcus, Staphylococcus, Rhodococcus*[8], *Actinobacillus, Agrobacterium, Azotobacter, Rhodobacter* and *Sphaerotilius*[27], *Beneckea*[4], and *Vibrio*[32].

Microbial bioplastics

Poly 3-hydroxy butyrate (PHB)
PHB granules



Poly 3-hydroxy alkanoate (PHA)
Structure of PHA

Advantages of bioplastics

Biodegradable, Eco-friendly synthesis, High processibility, Derived from renewable resources, Good mechanical properties shortcomings of bioplastics, Poor interactions with fibers, Narrow processing window, Lack of reactive groups, Thermal degradation, Brittleness.

Production of PHA

Currently there are four biosynthetic approaches to produce PHA: *in vitro* via PHA-polymerase catalyzed polymerization, and *in vivo* with batch, fed-batch, and continuous (chemostat) cultures.

In vitro synthesis of PHA

Much effort has been put into research for the *in vitro* production recently because of its advantages over the in vivo synthesis, e.g., production can be controlled through the addition of PHA-precursors and cofactors[36]. Moreover, isolation of PHA is much easier since no extraction from cells is necessary. However, the recycling of cofactors appears to be problematic and expensive. Nevertheless, it was reported that purified polymerase from *R. eutropha* formed granules up to 3 mm in size when exposed to 3-hydroxybutyryl-CoA[13]. The molecular weight of 10 Da was surprisingly high. In vitro systems would also allow the synthesis of block-copolymers through the sequential supply of different substrates[21].

In vivo synthesis of PHA

The synthesis of PHA *in vivo* has been and is still investigated using batch cultures. Batch cultures are easy to handle and are suited for growth studies and screenings for potential PHA accumulating organisms. Generally, the medium is designed in such a way that one nutrient, mostly nitrogen, limits growth of biomass while other nutrients including the carbon source are in excess. Depending on the microorganism and the substrate the experiments are per formed within 1-2 days. During this time the cells go through a sequence of growth stages[33], such as lag, exponential growth, PHA production, stationary, and finally death phase. Concomitantly, the cells perceive a continuous change of their environment due to the ever-changing nutrient concentration caused by the cell metabolism. Since cells that become starved for carbon degrade PHA again, this method rarely gives an indication of the maximum capacity of the cells to accumulate PHA.

The fed-batch culture is basically a batch culture that is continuously supplemented with selected nutrients after it enters the late exponential phase[33]. Biopol, a copolymer that consists of 3-hydroxy butyrate and 3-hydroxyvalerate in various mixing ratios, was produced by Monsanto (St. Louis, USA) in such a fed-batch mode on a large scale until 1999 with a mutant of *Ralstonia eutropha* that could grow on glucose. The production process consisted of two main phases: In a first phase, the cells were cultured in a minimal medium which contained the essential growth nutrients, glucose, and low amounts of phosphate, supporting cell growth to a certain biomass concentration and only minor PHA accumulation[7].

In a second phase, after all the phosphorus was consumed by the cells, PHA accumulation took place. PHA accumulation was driven through the continuous addition of glucose and propionic acid to the culture at well defined rates. After 48 h of feed the PHA consisted of about 80% 3-hydroxybutyrate and 20% 3-hydroxyvalerate. The process was stopped when the PHA content of the cells had reached a desired level, generally between 70 and 80% of the cell dry weight. The fed-batch process was the culture method of choice since propionic acid is toxic to the cells and can inhibit cell growth at high concentrations.

The advantage of fed-batch cultures in general is the high cell densities[18,23-25] that can be obtained which reduce the costs of PHA production significantly[23-25]. A disadvantage of the process is that the cells grow at a decreasing growth rate when the feed rate and feed concentration are kept constant. The reason for this is that the added nutrients are consumed by an ever-increasing cell concentration during identical time units this can lead to unexpected losses in PHA production[43] and a shift in copolymer composition[28].

The fourth method to produce PHA biotechnologically, the chemostat, is the most controlled cultivation method. In such a system the culture broth is continuously exchanged with sterile growth medium[33]. According to the theory of Monod[29,30] the specific growth rate of the culture can be set by the ratio of feed rate to volume of the culture broth. This allows the determination of the influence of a well defined growth condition (e.g., nutrient limitation at a specific growth rate) on PHA accumulation[9,34,35,47]. However, to date this method is not yet applied to PHA production on a large scale; although a high PHA productivity can be obtained when appropriate growth conditions are selected. The potential of chemostat production can be increased further when two chemostats are connected in sequence. Jung reported that the mclPHA productivity in *P. oleovorans* could be increased to a volumetric productivity of 1.06 g l^{-1} h^{-1} and that the PHA content attained 63% (w/w) of the cell dry weight[17].

CONCLUSION

Bioplastics are natural biopolymers that are synthesised and catabolised by various micro organisms. These materials do not cause toxic effects in the host and have certain advantages over petroleum derived plastic. Currently there are four biosynthetic approaches to produce PHA and PHB by microorganisms. In future, we carried out to produce and use bioplastics in lot and to eradicate the chemical plastics.

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