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Review

Dual production of biopolymers from bacteria

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ABSTRACT

Rapid depletion of natural resources with continued demands of an increasing population and high consumption rates of today's world will cause serious problems in the future. This, along with environmental concerns, has directed research towards finding alternatives in variety of sectors including sustainable and environmentally friendly consumer goods. Biopolymers of bacterial origin, with their vast range of applications, biodegradability and eco-friendly manufacturing processes, are one of the alternatives for a more sustainable future. However, the cost of their production is a drawback. Simultaneous production processes have always been an option for researchers in order to reduce cost, but the variable requirements of microorganisms to produce both different and valuable products are a hindering factor. This review will look at some examples and identify ideas towards developing a successful strategy for simultaneous production of bio-products.

Contents

1. Introduction

Globalisation of commodities accompanied with increasing consumption rates will result in depletion of natural resources in the coming decades. In the United States alone, consumption of goods grew more than six fold between 1960 and 2008 yet the U.S. population only grew by a factor of 2.2, meaning consumption itself tripled within the period (Eco-cycle, 2010).

A major unsustainable resource is mineral oil (crude oil); a significant percentage of which is used to manufacture plastic products; for example in 2005, 18 million barrels of crude oil-equivalent were used to manufacture 2 million polyethylene terephthalate (PET) bottles (Eco-cycle, 2010). Due to excessive fossil fuel consumption, the atmospheric concentrations of CO_2 and CH_4 have exceeded natural levels. The rapid depletion of crude oil and the mounting adverse effects on the environment, accompanied by their increasing rates of consumption are driving forces for alternatives to petroleum mineral-based products and processes. Bio-refinery concept, where biomass is converted into fuel, heat, energy and value-added chemical products through an integrated process, has been introduced as a consequence of these new challenges (Kaparaju, Serrano, Thomsen, Kongjan, & Angelidaki, 2009). Biopolymers with their biodegradability, eco-friendly manufacturing processes and vast range of applications are important alternatives to non-sustainable products and can be produced through bio-refineries as part of integrated bioprocesses.

Biopolymers in nature are produced by a range of microorganisms and plants (Marjadi & Dharaiya, 2011). Microbial biopolymers are produced either directly via fermentation or by chemical polymerisation of monomers, which are in turn produced through fermentation. Biopolymers produced by microorganisms require specific nutrients and controlled environmental conditions (Marjadi & Dharaiya, 2011). Many biopolymers are biocompatible; they have no adverse effects on biological systems. It is

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believed that biopolymers of bacterial origin are produced either as a result of their defence mechanism or as storage material. In both cases, the produced biopolymers can be decomposed by bacteria. Some petroleum-based polymers and some biopolymers of plant origin are classified as biopolymers and are biodegradable (Matsuura, Ye, & He, 2008). Having such desirable functionalities and properties, biopolymers find many applications in various sectors from medical field to consumable goods (Philip, Keshavarz, & Roy, 2007).

An interesting group of biopolymers produced by a vast range of microorganisms are Exopolysaccharides (EPS). EPSs generally consist of monosaccharides and some non-carbohydrate substituents (such as acetate, pyruvate, succinate, and phosphate). Due to their vast diversity in composition, EPSs have found various applications, particularly in food and pharmaceutical industries. Moreover, considerable progress has been made in discovering and developing new microbial EPS that possess novel industrial significance as biomaterials or as rheology modifiers of aqueous systems. The limitation of the applications bacterial EPSs has been mostly due to cost of production relative to their commercial value (Kumar & Mody, 2009).

While some biopolymers are already produced at industrial scale (e.g. polylactic acid, cellulose), many other classes are yet to be produced at commercial scale. For example, the majority of polyhaydroxyalkanoates (PHAs), with their great potential in a variety of bio-sectors, are still at the research and development or early commercialisation stage. A significant problem to overcome for their industrial production is the high cost of raw material and relatively low conversion rates (Castilho, Mitchell, & Freire, 2009; Valappil, Rai, Bucke, & Roy, 2008). One approach to minimize the cost of raw materials is expanding the variety of valuable products obtained from a single batch.

Simultaneous production of two or more microbial products through the same process has always been desirable. This is due to the potential reduction in cost and simplicity of operation. This bio-refinery approach, however, faces problems due to the diversity of microorganisms' demands to produce products. Whilst simultaneous or sequential production of biopolymers with another biopolymer or bio-product has been reported, in many cases this has been based on unplanned observation rather than prior deliberate design. In this context, there is great opportunity for investigation, leading to potential economic advantages for bio-industries. Simultaneous production of biopolymers is an attractive approach for a high profit bio-refinery. Therefore, this short review will focus on the simultaneous and sequential production of biopolymers of bacterial origin, concomitant with various products. It will discuss some examples to aid the identification of approaches towards the development of a successful strategy for simultaneous bio-product production.

2. Examples of dual production

2.1. Dual production with PHAs

PHAs are a family of linear polyesters of 3, 4, 5 & 6-hydroxyacids. They are water insoluble and have thermoplastic and elastomeric properties. They are synthesized and stored intracellular by a wide variety of bacteria including, *Bacillus* sp., *Pseudomonas* sp., *Azobacterium* sp. and many recombinant strains. This is through the fermentation of sugars, lipids, alkanes, alkenes and alkaloid acids in the presence of excess carbon, while another essential nutrient, such as nitrogen or phosphorus, is limiting (Albuquerque, Eiroa, Torres, Nunes, & Reis, 2007; Keshavarz & Roy, 2010).

Due to their vast range of applications and intracellular synthesis, they have attracted the attention of researchers for dual production. Intracellular production of at least one product provides the advantage in the seperation of two products, the second being extracellular. Several researchers have investigated the potential of PHAs production along with an extracellular product to exploit this advantage (Table 1).

The most common and abundant class of extracellular products in many bacteria is Exopolysaccarides (EPS). Lama et al. (1995) studied the effects of growth conditions on PHAs and EPS production. However, what makes their study different is the production of two different intracellular biopolymers along with an extracellular EPS. The occurrence and characterization of polyhydroxy butyric acid (PHB) were investigated in *Anabaena cylindrical* strain cultivated under different growth conditions in batch culture. It was reported that using appropriate precursors, the strain could accumulate PHB and co-polyester, which is a blend of poly hydroxyvalerate (PHV) and PHB as hetero-polymer (PHB + PHV). EPS production was also observed during this process. EPS was partially characterized and the importance of growth conditions was investigated for all products. A production of 0.012 g/L PHB + PHV and 0.325 g/L EPS was reported using the standard medium for cyanobacteria (BG-11 medium), supplemented with acetate, in a single-stage batch fermentation. Addition of acetate or glucose to the BG-11 growth medium enhanced PHB biosynthesis, while the addition of citrate caused a strong decrease in PHB content of the cells. For production of copolymer (PHB + PHV), the medium was supported by propionate or valerate as carbon sources to initiate the hetero-polymer production. Addition of these substrates caused a large decrease in cell-yield. The presence of $NaNO_3$ resulted in a sevenfold increase compared to the control, but addition of fatty acids caused a sharp decrease in the production of EPS.

The physico-chemical factors influencing the production of PHB and EPS by a yellow-pigmented *Azotobacter beijerinckii* was investigated (Pal, Manna, & Paul, 1999). Under nitrogen-free conditions, PHB accumulation started after 20 h and reached its maximum at the late exponential phase (around the 30th hour) followed by a sharp decline, whereas EPS production was associated with cell growth. According to the report 2.73 g/L PHB and 1.5 g/L EPS were produced from a single-stage batch fermentation. It was indicated that the PHB production was carbon-source dependent while organic nitrogen sources enhanced both PHB and EPS production. Inorganic nitrogen sources however had a negative effect on production of both classes of biopolymers. In this strain, oxygen limited conditions favoured production of both PHB and EPS, independent of carbon sources.

Quagliano and Miyazaki (1999) investigated production of PHB and EPS with *Azotobacter chroococcum* strain isolated from soil samples. The effects of various carbon sources with and without the addition of ammonium were investigated. The addition of ammonium resulted in a reduction in both PHB and/or EPS production in media containing glucose, fructose or sucrose. Also PHB was accumulated only in cultures grown on glucose and sucrose. However, EPS was secreted copiously in the presence of fructose and glucose. When a complex carbon source, molasses, was used PHB accumulated in the cells while EPS was produced from the start for 24 h. After 48 h of fermentation, PHB started to decay but EPS continued to be produced. When sugar cane molasses was used, 2.75 g/L PHB and 1.5 g/L EPS was obtained from single-stage batch fermentation.

Wang and Yu (2007) investigated different concentrations of glucose and $(NH_4)_2SO_4$ in batch cultures of *Ralstonia eutrophia*. Production of EPS occurred along with cell growth, whereas PHB was produced only under nitrogen limited and cell growth limited conditions. Specific PHB production-rate had an exponential correlation with both specific cell growth-rate and EPS production-rate. Under statistically optimised conditions, using central composite design, the final production level reported was 12 g/L PHB and 0.13 g/L EPS.