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Making Green Polymers Even Greener: Towards Sustainable Production of Polyhydroxyalkanoates from Agroindustrial By-Products

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1. Introduction

This review addresses recent achievements on the development of energy-saving and environmentally-friendly bioprocesses for the synthesis of polyhydroxyalkanoates (PHAs), a kind of non-petrochemical bioplastics. Different cutting-edge strategies developed in order to achieve bioprocesses with enhanced sustainability will be described. These are mainly based on the use of cheap substrates concomitantly lowering energy consumption levels, thus diminishing the environmental impact of PHAs production. We will also cover studies that shed light on the physiology of PHA-producing microorganisms by means of metabolic flux analysis, and also those that analyzed polymer modifications aimed at modifying physico-chemical properties. An overview of the applications of PHAs, including novel functionalized varieties, will conclude this review.

1.1 Environmental preservation

Microbial fermentations and many other industrial processes mostly rely on two fossil fuels (petroleum and gas) as sources of energy. This biased strategy has contributed to global climate change by emitting large amounts of carbon dioxide to the atmosphere and, as a collateral consequence, has favored the generation of an extended range of difficult-to-dispose-of goods by petrochemical industries. Accumulation of microscopic plastic debris at sea is particularly alarming, as well as the exponentially increasing need of landfill for municipal solid waste disposal. This particular situation has renewed the interest in strategies based on energy-saving bioprocesses. These strategies propose the replacement of petroleum by renewable resources and the manufacture of non-petrochemical goods, such as bioplastics, in order to reduce the pollution phenomena.

1.2 Bioplastics

The name biopolymers is currently used for polymers that are either synthesized by living organisms or produced from substrates obtained from living organisms. Examples of the first kind of biopolymers are naturally occurring polymers such as cellulose, starch, and PHAs. Among the second kind, there are poly(lactic acid), that can be synthesized from biologically-obtained lactic acid, or even polyethylene, when it is produced from ethylene obtained from bio-ethanol. Bioplastics are biopolymers with plastic properties. Bioplastics synthesized by living organisms are generally biodegradable; and chemically synthesized polymers, especially those derived from petroleum, are generally non-biodegradable, while those that are "bio-based" (*i.e.*, obtained using a biologically produced substrate such as bio-ethanol), have several degrees of biodegradability. Polyethylene and polypropylene, whether bio-based or not, are considered non-biodegradable, even when there have been claims of slow degradation of these polymers in nature (Corti et al., 2010). There are exceptions to the relationship between biological origin and biodegradability, as not all biopolymers are biodegradable, and not all biodegradable polymers are biopolymers. There are some plastics obtained from non-biological processes that can also be biodegraded, such as poly(ϵ -caprolactone) and the petroleum derived polymer poly(butadiene adipate-co-terephthalate) (Queiroz & Collares-Queiroz, 2009); and there are also polymers synthesized by microorganisms that are not biodegradable, such as polythioesters, obtained by the polymerization of mercaptoalkanoic acids by PHA synthases (Steinbüchel, 2005).

Currently, there are many different biodegradable bioplastics. Among these, we found blends containing natural polymers, such as starch and cellulose; and polymers synthesized chemically from different substrates, such as poly(lactic acid), poly(ϵ -caprolactone), and others (Rehm, 2010). Starch can be blended with other compounds to obtain polymers which could be used for several applications, but this material is quickly damaged in contact with water. Poly(lactic acid) is not normally degraded by microorganisms, but it is easily hydrolyzed and can be composted. PHAs are natural bioplastics produced by many bacteria from different substrates. In sharp contrast to the other bioplastics mentioned above, these polymers are totally biodegradable, as all microorganisms that naturally accumulate PHAs can degrade them. Moreover, PHAs can also be degraded by many other microorganisms, both bacteria and fungi, under either aerobic or anaerobic conditions.

These polymers are synthesized naturally by a wide variety of bacterial species as a reserve for carbon and energy. Nowadays, PHAs continue to attract increasing industrial interest as renewable, biodegradable, biocompatible, and extremely versatile thermoplastics (Steinbüchel & Lütke-Eversloh, 2003; Suriyamongkol et al., 2007). PHAs are the only waterproof thermoplastic materials available that are fully biodegraded both in aerobic and anaerobic environments. Two classes of PHAs are distinguished according to their monomer composition: short-chain length (SCL) PHAs and medium-chain length (MCL) PHAs. SCL-PHAs are polymers of 3-hydroxyacid monomers with a chain length of three to five carbon atoms, such as poly(3-hydroxybutyrate) (PHB, the most common PHA); whereas MCL-PHAs contain 3-hydroxyacid monomers with six to sixteen carbon atoms. All of them are optically active *R*-(−) compounds. This versatility is partly due to the wide substrate range of the PHA-synthesizing enzymes, and gives PHAs an extended spectrum of associated properties which is a clear advantage *vis-à-vis* to other bioplastics. Around 200 different monomer constituents were found in the polymers analyzed so far (Steinbüchel & Lütke-Eversloh, 2003).

1.3 Environmental Issues

Current high-yield bioprocesses for the synthesis of PHAs require fully aerobic conditions, which means that they are high energy-consuming processes. The environmental impact of replacing oil-derived plastics with biopolymers has been the subject of several studies, among them, those regarding bacterial PHB production in bioreactors (Gerngross, 1999). A complete life cycle assessment for PHB production from the cradle to the factory gate has been published by Harding et al. (2007). Those studies pointed out that, in spite of the fact that PHB is more environmentally friendly than oil-derived polymers, the great amount of energy required for its production must be taken into account when assessing its environmental impact. Similar results were obtained from research applied to the manufacturing of polymers obtained from transgenic plants (Zhong et al., 2009), or from agricultural substrates such as corn (Kim & Dale, 2005).

From these researches and other studies it was concluded that when the amount of energy used for sterilization, aeration, and agitation (both in the bioreactor and downstream processing), as well as the energy needed for the production of the agricultural feed-stocks to be used as carbon sources is considered, the environmental performance of PHAs equals that of petrochemical polymers. Different initiatives to overcome this problem are described below.

2. Towards an enhanced sustainable production

In the following sections, we will discuss several cutting-edge strategies intended to enhance the sustainability of PHA production processes (as summarized in Fig. 1). Even when they will be discussed in a sequential fashion, beginning with the choice of suitable substrates up to the rational functionalization of polymer properties, it is important to mention that bioprocesses designed for PHA synthesis from agroindustrial by-products are subjected to continuous improvement.

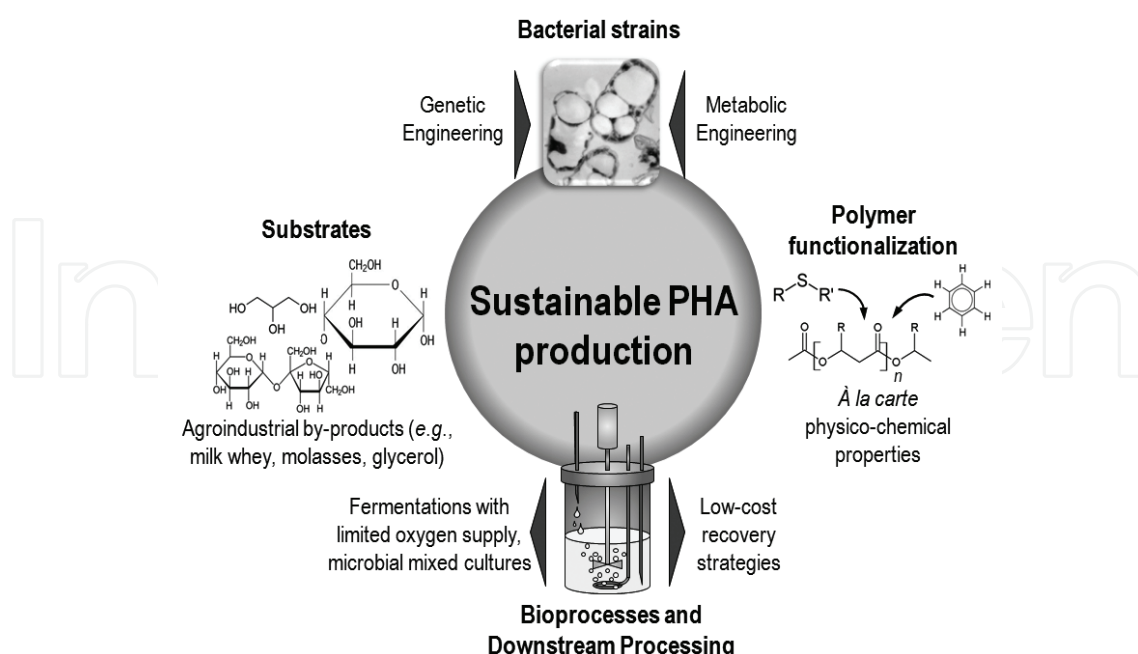


Fig. 1. Strategies used to enhance the sustainability of PHA production processes. Note that modifications to improve different steps in the process as a whole can be implemented in a cyclic, iterative fashion.

2.1 Substrates

It is widely accepted that the prize of the carbon source is one of the main factors affecting the cost of PHAs, influencing the sustainability of production processes. However, the choice of a suitable carbon source is not a clearcut issue. The use of industrial and agricultural by-products could require extensive purification, equalling or even surpassing the energy demand of cost-intensive agricultural feed-stocks.

Some early approaches to overcome this situation have integrated PHA and sugar production in a substrate- and energy-closed system (Nonato et al., 2001). Milk whey, a substrate which does not require extensive purification, was the most appropriate option for many other initiatives. Recently, glycerol has received attention as a potential carbon substrate due to its accumulation as a by-product of biodiesel synthesis.

2.1.1 Milk whey

About 80-90% of the processed milk volume is converted to whey during cheese and casein production by the dairy industry (Wong & Lee, 1998). Whey is rich in lactose, proteins, lipids, and lactic acid (Yang et al., 1994). After casein precipitation from raw milk, skimmed whey is produced, which is then concentrated and ultra-filtrated producing whey permeate (rich in lactose) and whey retentate (rich in proteins and containing a considerable amount of residual lactose).

Some components of whey retentate are useful in the pharmaceutical industry. Whey permeate contains *ca.* 81% of the original lactose in milk and is appropriate for biotechnological processes (Nath et al., 2008).

Young et al. (1994) evaluated for the first time the production of PHB from lactose by *Burkholderia cepacia*. Since then, many other isolated bacteria were evaluated for the production of PHB from lactose or milk whey (Nath et al., 2008). However, the cultures were always performed at low-cell-densities, thus hindering an appropriate evaluation of their economical relevance. The production of the copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(HB-co-HV)] from milk whey was also demonstrated in cultures either supplemented or not supplemented with valeric (pentanoic) acid (Koller et al., 2005, 2008). Production of PHAs with different monomer compositions using milk whey as the main carbon source will allow their use in different applications.

*Ralstonia eutropha*¹ has been extensively established as the platform for PHA production *par excellence* (Reinecke & Steinbüchel, 2009). However, it is unable to hydrolyse lactose, and galactose (its hydrolysis product) is not metabolized. Koller et al. (2007) proposed an alternative process consisting of two steps for PHB production from milk whey: lactose was first converted to lactic acid by lactobacilli, and the resulting lactic acid was then used as carbon source by *R. eutropha* for PHA production. Marangoni et al. (2002) hydrolyzed milk whey in order to make glucose available for *R. eutropha*, but it should be considered that galactose would remain unused in the culture medium since it is not metabolized by *R. eutropha*, as mentioned. After the expression of genes encoding β -galactosidase and

¹*R. eutropha* is currently known as *Cupriavidus necator*. In this review we will adopt the name *R. eutropha* which is most frequently used in the literature, including the announcement of its complete genome sequence (Pohlmann et al., 2006).

galactokinase in *R. eutropha* it became able to use lactose, albeit at a very slow rate (Pries et al., 1990). Recently, the *lacZ* (encoding β -galactosidase) and *lacI* (encoding the *lac* operon repressor protein) genes from *Escherichia coli* were introduced in the genome of *R. eutropha* interrupting *phaZ1* (encoding an intracellular PHB depolymerase). Cell concentration reached values higher than $8 \text{ g} \cdot \text{L}^{-1}$ and the PHB content was about 20-25% (wt/wt), demonstrating the capability of this recombinant *R. eutropha* strain to use lactose (Povolo et al., 2010).

Lee and co-workers studied high-cell density cultures of recombinant *E. coli* for the production of PHB from milk whey (Ahn et al., 2000; Wong & Lee, 1998). The best results were reached when using a highly concentrated milk whey solution associated to an external membrane module to retain the cell mass inside the bioreactor. Under these working conditions, and after 36.5 h of cultivation, PHB volumetric productivities as high as $4.6 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ were reached (i.e., cell and PHB concentrations of 194 and $168 \text{ g} \cdot \text{L}^{-1}$, respectively). These processes were scaled up (30 and 300 L), but the productivities attained were low (Park et al., 2002).

Kim (2000) also studied the production of PHB from milk whey by recombinant *E. coli* strains harboring the PHB biosynthetic genes from *R. eutropha*. After 35 h of cultivation, cell and PHB concentrations reached 55 and $32 \text{ g} \cdot \text{L}^{-1}$, respectively; corresponding to a PHB volumetric productivity of $0.9 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$. In a 24-h fed-batch process using milk whey and corn steep liquor as the main carbon and nitrogen sources, a recombinant *E. coli* strain (harboring the PHB biosynthetic genes from *Azotobacter* sp. strain FA8) reached cell and PHB concentrations of 70.1 and $51.1 \text{ g} \cdot \text{L}^{-1}$, respectively, corresponding to a PHB volumetric productivity of $2.13 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ (Nikel et al., 2006a).

PHB production processes from milk whey, based on fed-batch cultivation of recombinant *E. coli* strains and reaching high cell densities and PHB volumetric productivities, were established (Ahn et al., 2000; Nikel et al., 2006a; Wong & Lee, 1998), and are economically sound. However, further studies will be needed to scale up these processes keeping high productivities, cell concentration, and PHB content. Milk whey would satisfy the materials demands in processes for the production of other PHAs. However, taking into account the relevance of energy demands in these processes (Gerngross, 1999; Nonato et al., 2001), a renewable source must be considered to fulfill energy requirements and make the bioprocess truly sustainable.

2.1.2 Sugarcane molasses

Molasses is the residual syrup generated in sugar-refining mills after repeated sugar extraction by applying crystallization to sugarcane or sugar beet juice. At this point, sugar extraction is no longer economically viable, despite still having relatively high sucrose content. Low-grade molasses, inappropriate to be used in food or feed, has been suggested as substrate to produce PHAs (Solaiman et al., 2006).

A number of processes have been developed using molasses to produce PHA by bacteria. Most of the data provided on polymer content seem not to be competitive or sustainable at the moment. Beet and soy molasses have also been tested as alternative substrates (Solaiman et al., 2006). PHA production from molasses has been reported using Gram negative bacteria, such as recombinant *E. coli* and *Klebsiella* strains (Zhang et al., 1994), *R. eutropha*

(Oliveira et al., 2004), *Pseudomonas cepacia* (Çelik et al., 2005), or Gram positive bacteria such as *Bacillus* strains (Kulpreecha et al., 2009). In some cases, molasses was only used as additive [0.3 to 2.5% (wt/wt)] to grow *R. eutropha* in liquid or solid-state cultures along with other main substrates, reaching a maximum PHA content ranging from 26 to 39% (wt/wt) (Beaulieu et al., 1995; Oliveira et al., 2004). *P. cepacia* G13 accumulated PHA up to 70% (wt/wt) in culture media supplemented with 3% (wt/vol) beet molasses (Çelik et al., 2005).

Considering the use of sugarcane molasses as the main carbon source, Kulpreecha et al. (2009) tested *Bacillus megaterium* on sugarcane molasses and achieved a cell dry mass concentration of $72.7 \text{ g} \cdot \text{L}^{-1}$ in 24 h, with a PHB content of 42% (wt/wt); a good process that can still be improved since dissolved oxygen was a limiting factor. Brazil is currently one of the world leaders on sugarcane production (569 million tons in 2008-2009; Sawaya Jank, 2011). Initial attempts to use sugarcane molasses or high-test-molasses to produce PHAs were partially limited by its high nitrogen content (unpublished data).

Sugarcane molasses is no longer a waste material in Brazil but a by-product showing a good market value, and since 1970 it has been increasingly used on bio-ethanol production. The bagasse excess and sugarcane leaves are promising substrates to produce second-generation bio-ethanol. However, further developments are needed to solve the inability of yeasts to use the xylose fraction released from bagasse hydrolysis. Therefore, xylose and arabinose may be the new target by-products to be used in order to produce PHA in the integrated model of a sugar mill bio-refinery.

2.1.3 Glycerol

In the last years, a very important increase in the production of biodiesel has caused a sharp fall in the cost of glycerol, the main by-product of the biodiesel synthesis (da Silva et al., 2009; Solaiman et al., 2006). As a result, glycerol has become a very attractive substrate for bacterial growth. Additionally, because carbon atoms in glycerol are more reduced than in glucose or lactose, cells using glycerol are in a more reduced physiological state, favoring polymer synthesis. The use of glycerol for microbial PHA synthesis has been analyzed in natural PHA producers, such as *Methylobacterium rhodesianum* and *R. eutropha* (Borman & Roth, 1999), several *Pseudomonas* strains (Solaiman et al., 2006), the recently described *Zobellella denitrificans* (Ibrahim & Steinbüchel, 2009), and *Bacillus* sp. (Reddy et al., 2009), among others. Glycerol has also been investigated as a substrate for PHB synthesis in recombinant *E. coli* carrying the PHB biosynthetic genes from *Streptomyces aureofaciens* (Mahishi et al., 2003), and *Azotobacter* sp. strain FA8 (Nikel et al., 2008b).

PHAs obtained from glycerol were reported to have a significantly lower molecular weight (M_r) than polymers synthesized from other substrates, typically less than 1 MDa. In *Methylobacterium extorquens* and *R. eutropha*, PHB obtained from glycerol, ethanol, or methanol had a lower M_r than that obtained from other substrates (such as succinate, glucose, and fructose), and the M_r of the polymer was shown to decrease with increasing glycerol concentrations (Taidi et al., 1994). This effect was further analyzed and attributed to chain termination caused by glycerol (Madden et al., 1999). In studies performed using different *Pseudomonas* strains, the M_r of the polymers obtained, such as PHB produced by *P. oleovorans* and MCL-PHA synthesized by *P. corrugata*, was also observed to decrease with increasing glycerol concentrations [from 1% to 5% (wt/vol)] (Ashby et al., 2005). A recent study performed using *R. eutropha* describes PHB obtained from commercial glycerol and

from waste glycerol with a M_r of 957 and 786 kDa, respectively, less than half of that of PHB obtained from glucose (Cavalheiro et al., 2009). In contrast, in a recent report describing P(HB-co-HV) accumulation in a *Bacillus* strain, similar M_r s, lower than 700 kDa, were observed for the polymer obtained from the two carbon sources (Reddy et al., 2009). A low M_r is undesirable for industrial processing of the polymer, so the results available in the literature pointed to a drawback in the use of glycerol as a substrate for the microbial production of PHAs. However, based on recent results obtained with recombinant *E. coli*, it has been proposed that it is possible to obtain PHB from glycerol with M_r s similar to those of the polymer obtained from glucose or lactose by using adequate bacterial strains and culture conditions (de Almeida et al., 2010).

2.2 Strains

As stated before, industrial synthesis of PHAs must improve sustainability in order to reach an appropriate production cost and diminish environment damage. The manipulation of natural PHA producers and recombinant strains to achieve high PHA production has been the subject of many studies (reviewed in Aldor & Keasling, 2003; Jung et al., 2010; Keshavarz & Roy, 2010; Madison & Huisman, 1999; Steinbüchel, 2001), and will not be considered in this review. In spite of the fact that several bacterial species are currently being used in biotechnological processes, *E. coli* remains as the "workhorse" of industrial developments. This species has been the selected host for genetic techniques devised to introduce the PHA biosynthetic genes, improve their expression, provide suitable quality and concentration of substrates to the PHA synthase, as well as to modify the host strains to improve their performance in the bioreactor (Li et al., 2007).

In this section, we will focus on recent studies using different *E. coli* mutant strains and metabolic flux analysis with the objective of increasing sustainability in PHAs synthesis processes.

2.2.1 Modification of host strains

When they are grown in bioreactors, all microorganisms, including PHB-producing recombinant *E. coli* strains, are subjected to extreme (and often oscillating) conditions, such as shear forces, extreme aeration (either low or high), pH, and growth temperatures chosen to obtain maximum product yield. These extreme conditions often lead to membrane debilitation, cell filamentation, or protein precipitation. A strategy used to avoid filamentation was to over-express the gene encoding FtsZ (involved in cell division) in *E. coli* harboring the *pha* genes from *R. eutropha*, thus improving the polymer productivity from $2.08 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ in the wild-type strain up to $3.4 \text{ g} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ in the filamentation-suppressed derivative strain and consequently enhancing the process sustainability (Wang & Lee, 1997).

Current processes for the synthesis of PHAs require fully aerobic conditions, which mean that they are high energy-consuming processes. Most of the energy requirement is needed to fulfill aeration and agitation inside the culture vessel of the bioreactor. Therefore, strategies aimed towards improving the respiratory capacity of the host strain under micro-aerobic growth conditions were developed to reduce aeration needs. *Vitreoscilla* haemoglobin is supposed to facilitate intracellular oxygen transfer and assimilation, and the gene encoding this protein was introduced in PHB-producing *E. coli* improving the growth and polymer

yield, simultaneously avoiding the need of pure oxygen supplementation to achieve high-cell-density cultures (Hornig et al., 2010, 2011).

Another approach was based on the control of the redox state. In *E. coli*, the two-component signal transduction system ArcAB modulates, the expression of many operons according to the redox state of the environment (Lynch & Lin, 1996). The main targets for repression by the phosphorylated regulator are the genes that encode the enzymes involved in aerobic respiration and oxidative bioreactions, such as those of the tricarboxylic acid cycle. *E. coli arc* mutants are unregulated for aerobic respiration, and the genes encoding components of the tricarboxylic acid cycle are fully expressed under micro-aerobic growth conditions.

As a consequence, the pool of reducing equivalents is elevated and could be funneled into reduced bioproducts such as PHB. This approach enabled the increase of PHB content up to 35% (wt/wt) in an *arcA* mutant strain grown in a semi-synthetic medium with gentle (75 rpm) agitation, conditions in which no PHB was accumulated by the wild-type strain (Nikel et al., 2006b). Another global regulatory system manipulated to increase PHB synthesis is CreBC, a two-component signal transduction pair, where CreB is the regulator and CreC the sensor kinase. The *cre* regulon includes different genes, and some of them are involved in carbon metabolism (Avison et al., 2001). *E. coli* strain CT1061, an *arcA* and *creC* constitutive mutant, has enhanced carbon source consumption as well as a reducing intracellular environment (characterized by a high NADH/NAD⁺ ratio, *ca.* 1 mol · mol⁻¹), making it adequate as a candidate host for reduced biochemical synthesis (Nikel et al., 2008a). Introduction of the PHB biosynthetic genes from *Azotobacter* sp. strain FA8 in *E. coli* CT1061 resulted in increased PHB yield from glucose- or glycerol-supplemented semi-synthetic media, associated to the highly reduced redox state in this strain (Nikel et al., 2006b).

Another approach, based on the same rationale, was the use of anaerobic promoters to achieve PHB production under micro- or anaerobic conditions. Among the promoters tested, the one for *E. coli* alcohol dehydrogenase was the most effective in promoting micro-aerobic synthesis of PHB (Wei et al., 2009).

2.2.2 Metabolic engineering of PHA biosynthesis

Industrial microorganisms have been traditionally developed *via* multiple rounds of random mutagenesis followed by selection of desired phenotypes. However, these techniques do not take into account important features of the bioprocess itself, *inter alia*, increased sustainability. Approaches for microbial synthesis of valuable bioproducts have increasingly evolved towards more systematic strategies. Metabolic Engineering is a multidisciplinary field defined as the directed improvement of product(s) synthesis or cellular properties through the rationale modification of specific biochemical reaction(s), or the introduction of new one(s), as well as manipulating regulatory cellular processes (Stephanopoulos, 1999). In connection with this concept, Synthetic Biology is a newly coined term which defines a group of methodologies aimed at creating novel functional parts, modules, systems, and, ultimately, novel (micro)organisms through the integrated use of biological techniques and mathematical methods traditionally employed in Engineering designs (Lee et al., 2010). Metabolic Engineering and Systems Biology are different from other cellular engineering strategies since their systematic approaches focus on understanding the whole metabolic network in the cell. As a consequence, they can be used as powerful tools to increase bioprocess sustainability by taking into account different cellular and process features at the

same time. Metabolic Engineering is characterized by a cyclic process involving evaluation of metabolic performance of cells, establishment of appropriate target(s) for genetic engineering, and implementation of genetic modification(s) (Nielsen, 2001). The use of analytical tools and metabolic models to study the performance of cells and to identify the appropriate target for genetic modification allows distinguishing Metabolic Engineering from classical genetic engineering and characterize it as a system approach (Nielsen & Jewett, 2008).

PHAs synthesis is an interesting target for Metabolic Engineering manipulation as both polymer assembly and accumulation take place *in vivo*, offering the chance to optimize different metabolic and cellular processes at the same time (Jung et al., 2010; Tyo et al., 2010). The simplest Metabolic Engineering strategy for PHA synthesis manipulation would be to choose the appropriate carbon source(s) supplied to the bacterial host to control and direct carbon flux through relevant precursors and polymer biosynthesis enzymes. This strategy has traditionally been exploited to modulate polymer composition by varying the feed ratio of different substrate precursors (Lütke-Eversloh et al., 2001, Marangoni et al., 2002). Additionally, knowledge of the metabolic network operation under PHA-producing conditions would enable the rational streamlining of catabolic pathways to harness the greatest possible amount of carbon source for polymer synthesis. Knowing the distribution of fluxes is an important way to improve PHA production process towards efficient (and sustainable) polymer accumulation. Intracellular fluxes are quantitative descriptors which can be used to choose appropriate targets for modification of the metabolic network activity, increasing the formation of a desired product (e.g., PHAs).

In silico genome scale analysis of metabolic models were also implemented to identify potential targets for manipulation and strain improvement of efficient PHA producers. Using this approach, Lim et al. (2002) identified *zwf* and *gnd* (encoding glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, respectively) as relevant targets for manipulation in recombinant *E. coli* to redirect catabolic fluxes towards the pentose phosphate pathway, resulting in a high NADPH/NADP⁺ ratio that favored PHA accumulation [up to 41% (wt/wt)]. Another study dealing with *in silico* metabolic analysis of PHB-accumulating *E. coli* strains showed that the Entner-Doudoroff pathway represents an important contribution to PHB synthesis (Hong et al., 2003), a fact also evidenced in proteomic analysis (Han et al., 2001). These studies clearly pointed towards the fact that choosing the adequate mutant background through systematic analysis of metabolic networks allowed the enhancement of PHB production processes.

A breakthrough in Metabolic Engineering is related to the emergence of ¹³C-labeling methodologies to study the efficiency of complex metabolic networks. As the labeled substrate proceeds through the metabolic network, the pools of downstream metabolites become labeled and, at steady state, the fraction of labeled substrate in a given pool can be used to calculate the flux through that pathway. ¹³C-based metabolic flux analysis uses the labeling information in proteinogenic amino acids to infer the labeling patterns of the respective precursor metabolites from central carbon metabolism (Sauer, 2006). The labeling information can be determined either by gas chromatography-mass spectrometry or nuclear magnetic resonance spectroscopy. The resulting labeling information is used as additional constraints for metabolic network models that utilize the biochemical stoichiometry, the substrate uptake, product secretion, and biomass formation rates to compute the intracellular flux distribution. Two alternative labeling information interpretation methods

are used: comprehensive isotopomer modeling (Wiechert, 2001), and net-flux calculation utilizing results from metabolic flux ratio analysis (Fischer et al., 2004).

Metabolic networks are not the only targets for rational design of sustainable PHA production processes. In fact, regulatory circuits within the cell can be manipulated in order to obtain a desirable phenotype. Signal transduction pathways are involved in intercellular interactions and communication of extracellular conditions to the interior of the cell. The final outcome of such a signaling pathway is often the activation of specific transcription factor(s) that, in turn, control(s) gene expression. As stated before, in *E. coli* aerobic and anaerobic respiration, as well as fermentation pathways, are switched on and off by the ArcAB system, enabling bacterial cells to optimize energy generation according to the oxygen levels in the surrounding medium, and CreBC is responsive to the carbon source used and oxygen availability. Metabolic flux analysis based on ^{13}C -labelling showed that both ArcAB and CreBC systems have a deep impact on central metabolic pathways of *E. coli* under micro-aerobic growth conditions (Nikel et al., 2009), offering valuable information for rationale modification of regulatory networks aimed at polymer (and other bioproducts) synthesis. These results highlighted the idea that manipulation of the genes encoding global regulators could provide a relevant tool for the modulation of central metabolism and reducing power availability for biotechnological purposes, rather than manipulating the genes directly involved in the metabolic pathway of interest.

2.3 Bioprocesses and downstream processing

During the bioprocess conducting to PHAs production, energy is needed for the generation of steam used for sterilization, aeration and agitation in the reactor, and downstream processing. Several strategies which aimed to enhance both the polymer yield and the process sustainability by means of diminishing energy consumption were developed. Bacterial growth in the reactor was the target of these attempts, which were specially centered on two key aspects: (i) the growth of recombinant *E. coli* (facultative aerobe) under conditions not fully aerobic, thus decreasing aeration and agitation needs, and (ii) the development of mixed cultures, which circumvents sterilization.

Carlson et al. (2005) observed that recombinant *E. coli* DH5 α carrying the *pha* genes from *R. eutropha* can support PHB accumulation in anaerobiosis when grown in rich media. The authors also developed a theoretical model of the biochemical network to interpret the experimental results and to study the metabolic capabilities of *E. coli* under anaerobic conditions. One of the few reports in the scientific literature on fed-batch cultivation in micro-aerobiosis describes a process for the synthesis of PHB developed under these conditions using glycerol as substrate and the concomitant synthesis of a valuable by-product, bio-ethanol, during micro-aerobic PHB accumulation. Micro-aerobic fed-batch cultures allowed a 2.57-fold increase in volumetric productivity when compared with batch cultures, attaining a PHB content of 51% (wt/wt) (Nikel et al., 2008b). In this work, the authors introduced the *pha* genes from *Azotobacter* sp. strain FA8 into an *arcA creC* mutant of *E. coli*, unregulated for redox control and carbon catabolism. In a fed-batch aerobic cultivation of a recombinant *E. coli* it was also reported that a PHB content of 80% (wt/wt) was obtained with oxygen limitation and a small increase in agitation using milk whey as the main carbon source (Kim, 2000).

An alternative to fed-batch processes to produce PHA from waste materials is the use of open microbial mixed cultures (MMCs). MMCs are microbial populations, often with unknown composition, selected by the operational conditions imposed on the biological system (currently referred to as "feast and famine", or aerobic dynamic feeding) resulting on polymer accumulation not induced by nutrient limitation. This system reduces bioreactor and operation costs, including sterilization, and is suitable for the use of agroindustrial wastes with unknown or variable composition (Serafim et al., 2008). Studies using sugarcane molasses in MMCs showed that by controlling the concentration of the influent substrate in the bioreactor, 88% of the working microorganisms accumulated PHA up to 74.5% (wt/wt) (Albuquerque et al., 2010), corresponding to a PHA concentration of *ca.* 5.1 g · L⁻¹. MMC have been extensively studied, including the implementation of different strategies to manipulate the polymer monomer composition (Albuquerque et al., 2011). MMCs allow the use of already existing wastewater treatment plants to produce PHA but require long operation periods, on the opposite of some existing processes. The choice of one or another operational mode (*i.e.*, fed-batch or MMC) as a sustainable process depends on the scenario of each region.

As stated before, PHB and related copolymers are produced in Brazil in a bioprocess facility integrated into a sugarcane mill. The energy necessary for the production process is provided by waste biomass. Carbon dioxide emissions to the environment are photosynthetically assimilated by the sugarcane crop and liquid wastes are recycled to the cane fields (Nonato et al., 2001).

Considering downstream processing, the recovery of PHAs usually demand a considerable energy input for centrifugation and cell disruption (Harding et al., 2007). Several strategies have been used to diminish the downstream processing costs and the toxic effects of organic solvents traditionally used for polymer solubilization (Berger et al., 1989). The methods based on non-PHA cell mass dissolution are considered a smart alternative (Kapritchkoff et al., 2006; Martínez et al., 2011). These methods, extensively reviewed by Jacquél et al. (2008), utilize alkali, enzymes, slightly acid solutions, and different pre-treatments. Among the recent achievements in this area, there is a new method based on dissolution of non-PHA cell mass by protons in aqueous solution and the crystallization of PHAs (Yu & Chen, 2006). By applying these conditions, high purity (97.9%) and high recovery yield (98.7%) were obtained.

An eventual breakthrough in polymer recovery could be the generation of a suitable mutant of *Alcanivorax borkumensis* characterized by the extracellular deposition of MCL-PHA when grown on alkanes, allowing the recovery of the polymer from the culture medium (Sabirova et al., 2006).

2.4 Tailor made polymers

Microbiologists have the skills to engineer bacteria for the production of tailored polymeric reserve materials (Hunter, 2010). Since the discovery that some bacteria can incorporate 3-hydroxyalkanoates bearing functional groups from related substrates (Lenz et al., 1992), research has led to structural diversification of PHAs by modulated processes during biosynthesis and chemical modifications (Hazer & Steinbüchel, 2007). Holmes et al. (1984) described the controlled synthesis of P(HB-co-HV) in *R. eutropha*, in which the 3-hydroxyvalerate fraction in the polymer could be controlled by the concentration of

propionate in the growth medium. After the discovery of poly(3-hydroxyoctanoate-co-3-hydroxyhexanoate) in octane-grown *Pseudomonas oleovorans* (de Smet et al., 1983), the range of different constituents of PHAs expanded rapidly, and *ca.* 200 different PHA monomers have been identified (Steinbüchel & Lütke-Eversloh, 2003). However, the most commonly applied route for tailoring PHAs is their *in situ* functionalization by biosynthetically producing side chains with terminal double bonds followed by chemistry (revised in Scholz, 2010). PHAs with terminal double bonds were first described by Lageveen et al. (1988) and received a lot of follow-up research (Fritzsche et al., 1990; Hartmann et al., 2006; Park et al., 1998). In *Pseudomonads*, PHAs that are formed from glycerol, gluconate, or related sugars have a different composition with respect to PHAs obtained from fatty acids. Whereas the latter PHAs have 3-hydroxyoctanoate as the main constituent, sugar-grown cells accumulate PHAs in which 3-hydroxydecanoate is the main constituent, along with small amounts of unsaturated monomers (Huijberts et al., 1992).

The resulting tailor-made structural and material properties have positioned PHAs well to contribute to the manufacturing of second and third generation biomaterials for medical applications, which require a variety of tailor-made chemical architectures, physical properties, and surface characteristics (Chen, 2009; Escapa et al., 2011). Bacterial copolyesters with vinyl groups have attracted attention because the unsaturated terminal group is highly reactive when compared to other terminal groups. The evaluation of different plant oils as carbon source for PHA production by *Pseudomonas* spp. revealed the possibility of tailored synthesis of these polymers containing variable molar fractions of unsaturated monomers in a sustainable way (Silva-Queiroz et al., 2009). Some studies described the biosynthesis of alkyl esters substituted MCL-PHA (Scholz et al., 1994), as well as PHAs containing sulphur-groups in the side chains, comprising either thiophenoxy functional groups (Takagi et al., 1999), or thioester groups (Ewering et al., 2002). Moreover, biopolymers with thioester linkages in the polymer backbone, containing 3-mercaptopropionate or 3-mercaptoputyrate in addition to 3-hydroxybutyrate as the monomer constituents, were isolated from *R. eutropha* (Lütke-Eversloh et al., 2002). Molecular biology strategies designed to increase the production of MCL-PHA in *Pseudomonas* was firstly described in *P. putida* U (García et al., 1999). The existence in the genome of this strain of several sets of iso-enzymes encoding genes similar to those belonging to the *fad* regulon from *E. coli* from the β -oxidation of fatty acids have been described (Olivera et al., 2001a, 2001b). Engineered strains carrying mutations in the *fadA-fadB* genes had a strong intracellular accumulation of biopolyesters. Furthermore, the application of this strategy resulted in an over-accumulation of functionalized MCL-PHAs bearing aromatic side groups (Olivera et al., 2001b).

Similarly, the existence of several sets of *fad* genes in the model microorganism *P. putida* KT2440 has been mentioned in the literature, which is in agreement with the huge metabolic versatility of this strain (Nelson et al., 2002). When the *fadA* and *fadB* genes were knocked-out in its derived strain *P. putida* KT2442, PHAs with a higher fraction of long chain length monomers than the wild type, or even containing monomers with thioester-groups were produced (Escapa et al., 2011; Ouyang et al., 2007). Interestingly, terminal oxo- or thio-ester groups could undergo trans-esterifications reactions (Escapa et al., 2011).

3. Applications

The versatile copolymer P(HB-co-HV) was initially manufactured as shampoo bottles and other cosmetic containers (Hocking & Marchessault, 1994). Later on, pens, cups, and

packaging elements (*e.g.*, films) made with PHAs also appeared in the market. PHAs are biocompatible and for this reason they have also attracted attention as raw material to be used in medical devices (Wu et al., 2009). Being composed by *R*-(–) monomers, PHAs are a source of chiral compounds with a high demand from the pharmaceutical industries (Chen & Wu, 2005). However, the manufacture of PHAs is carried out at small facilities and, as a consequence, it lacks the economic benefit of a large scale production (Chanprateep, 2010). A complete description of the goods produced as prototypes or already traded is presented by Philip et al. (2007).

4. Future research

- The technical potential substitution of plastic applications (thermoplastic and thermosets) and man-made fibers (*e.g.*, staple fibers and filaments) by bio-based plastics have been estimated based on their typical physical properties. The potential of biobased plastics for replacement of petrochemical plastics is 90%, corresponding to 240 million tons per year. PHA would respond for *ca.* 30 million tons (Akaraonye et al., 2010). Realizing this potential represents a great challenge, especially in a sustainable way.
- Bacterial growth in bioreactors needs an *ad fundum* understanding of microbial physiology and regulatory processes in order to select cultivation conditions aimed at an enhanced energy-saving process. All the attempts to grow PHA microbial producers under low oxygen supply provide an interesting starting point for these processes, but polymer yields are lower than those obtained under aerobic conditions. Additional process development and optimization are needed to achieve high PHA volumetric productivities and polymer content.
- The use of industrial and agricultural by-products is certainly needed for sustainability. However, high amounts of energy are still needed for production, extraction, and purification of PHAs. Hence, the definition of renewable energy sources will be also quite important.
- Metabolic-Engineering driven approaches should be a relevant tool to establish processes allowing to reach PHA yields close to the theoretical maximum from a given carbon source. Considering the relevance of carbon source on PHAs production cost, it will be important to explore the full metabolic potential of microbial cells.
- The great diversity of monomers detected as PHAs constituents is certainly the feature determining their great potential for technical replacement of petrochemical thermoplastics. Therefore, directed evolution of enzymes involved in PHA biosynthesis and Metabolic Engineering approaches of bacterial hosts will be the driving force to establish bioprocesses for the controlled production of PHAs with monomer composition *à la carte* and hence suitable for a number of applications. The potential of technical replacement could even be increased as the outcome of intensive scientific and technological work to explore the diversity of PHAs composition.
- Systems-level analysis of metabolic, signaling, and regulatory networks makes it possible to comprehensively understand global physiological processes taking place in PHA-accumulating *E. coli* strains. New targets and strategies for the improvement of PHA production will certainly be developed in the next future, including tailor-made PHAs with desired monomer compositions and *M_r*s. Ideally, and in order to design a completely sustainable PHA production process, strains developed using these system-

based approaches should be further metabolically engineered to produce PHAs up to a sufficiently high polymer content with high productivity from the most inexpensive carbon source through fine-controlled fermentation schemes.

Despite these great challenges, the current scenario is highly promising for the development of sustainable PHA production bioprocesses which could fulfill our needs for biopolymers applications.

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6. References

- Ahn, W.S.; Park, S.J. & Lee, S.Y. (2000). Production of Poly(3-Hydroxybutyrate) by Fed-Batch Culture of Recombinant *Escherichia coli* with a Highly Concentrated Whey Solution. *Applied and Environmental Microbiology*, Vol. 66, No. 8, (August 2000), pp. 3624-3627, ISSN 0099-2240
- Akaraonye, E.; Keshavarz, T. & Roy, I. (2010). Production of Polyhydroxyalkanoates: The Future Green Materials of Choice. *Journal of Chemical Technology and Biotechnology*, Vol. 85, No. 6, (June 2010), pp. 732-743, ISSN 1097-4660
- Albuquerque, M.G.E.; Concas, S., Bengtsson, S. & Reis, M.A.M. (2010). Mixed Culture Polyhydroxyalkanoates Production from Sugar Molasses: The Use of a 2-Stage CSTR System for Culture Selection. *Bioresource Technology*, Vol. 101, No. 18, (September 2010), pp. 7112-7122, ISSN 0960-8524
- Albuquerque, M.G.E.; Martino, V., Pollet, E., Avérous, L. & Reis, M.A.M. (2011). Mixed Culture Polyhydroxyalkanoate (PHA) Production from Volatile Fatty Acid (VFA)-Rich Streams: Effect of Substrate Composition and Feeding Regime on PHA Productivity, Composition and Properties. *Journal of Biotechnology*, Vol. 151, No. 1, (January 2011), pp. 66-76, ISSN 0168-1656
- Aldor, I.S. & Keasling, J.S. (2003). Process Design for Microbial Plastic Factories: Metabolic Engineering of Polyhydroxyalkanoates. *Current Opinion in Biotechnology*, Vol. 14, No. 5, (October 2003), pp. 475-483, ISSN 0958-1669
- Ashby, R.D.; Solaiman, D.K.Y. & Foglia, T. (2005). Synthesis of Short-/Medium-Chain-Length Poly(Hydroxyalkanoate) Blends by Mixed Culture Fermentation of Glycerol. *Biomacromolecules*, Vol. 6, No. 4, (July 2005), pp. 2106-2112, ISSN 1525-7797
- Avison, M.B.; Horton, R.E., Walsh, T.R. & Bennett, P.M. (2001). *Escherichia coli* CreBC Is a Global Regulator of Gene Expression That Responds to Growth in Minimal Media. *Journal of Biological Chemistry*, Vol. 276, No. 29, (July 2001), pp. 26955-26961, ISSN 0021-9258
- Beaulieu, M.; Beaulieu, Y., Mélinard, J., Pandian, S. & Goulet, J. (1995). Influence of Ammonium Salts and Cane Molasses on Growth of *Alcaligenes eutrophus* and Production of Polyhydroxybutyrate. *Applied and Environmental Microbiology*, Vol. 61, No. 1, (January 1995), pp. 165-169, ISSN 0099-2240
- Berger, E.; Ramsay, B.A., Ramsay, J.A., Chavarie, C. & Braunegg, G. (1989). PHB Recovery by Hypochlorite Digestion of Non-PHB Biomass. *Biotechnology Techniques*, Vol. 3, No. 4, (April 1989), pp. 227-232, ISSN 0951-208X

- Borman, E.J. & Roth, M. (1999). The Production of Polyhydroxybutyrate by *Methylobacterium rhodesianum* and *Ralstonia eutropha* in Media Containing Glycerol and Casein Hydrolysates. *Biotechnology Letters*, Vol. 21, No. 12, (December 1999), pp. 1059-1063, ISSN 0141-5492
- Carlson, R.; Wlaschin, A. & Srienc, F. (2005). Kinetic Studies and Biochemical Pathway Analysis of Anaerobic Poly-(R)-3-Hydroxybutyric Acid Synthesis in *Escherichia coli*. *Applied and Environmental Microbiology*, Vol. 71, No. 2, (February 2005), pp. 713-720, ISSN 0099-2240
- Cavalheiro, J.M.B.T.; de Almeida, M.C.M.D., Grandfils, C. & da Fonseca, M.M.R. (2009). Poly(3-Hydroxybutyrate) Production by *Cupriavidus necator* Using Waste Glycerol. *Process Biochemistry*, Vol. 44, No. 5, (May 2009), pp. 509-515, ISSN 1359-5113
- Çelik, G.Y.; Beyatli, Y. & Aslim, B. (2005). Determination of Poly-β-Hydroxybutyrate (PHB) in Sugarbeet Molasses by *Pseudomonas cepacia* G13 Strain. *Zuckerindustrie*, Vol. 130, No. 3, (March 2005), pp. 201-203, ISSN 0344-8657
- Chanprateep, S. (2010). Current Trends in Biodegradable Polyhydroxyalkanoates. *Journal of Bioscience and Bioengineering*, Vol. 110, No. 6, (December 2010), pp. 621-632, ISSN 1389-1723
- Chen, G.G.Q. & Wu, Q. (2005). Microbial Production and Applications of Chiral Hydroxyalkanoates. *Applied Microbiology and Biotechnology*, Vol. 67, No. 5, (June 2005), pp. 592-599, ISSN 0175-7598
- Chen, G.G.Q. (2009). A Microbial Polyhydroxyalkanoates (PHA) Based Bio- and Materials Industry. *Chemical Society Reviews*, Vol. 38, No. 8, (August 2009), pp. 2434-2446, ISSN 0306-0012
- Corti, A.; Muniyasamy, S., Vitali, M., Imam, S.H. & Chiellini, E. (2010). Oxidation and Biodegradation of Polyethylene Films Containing Pro-Oxidant Additives: Synergistic Effects of Sunlight Exposure, Thermal Aging and Fungal Biodegradation. *Polymer Degradation and Stability*, Vol. 95, No. 6, (June 2010), pp. 1106-1114, ISSN 0141-3910
- da Silva, G.P.; Mack, M. & Contiero, J. (2009). Glycerol: A Promising and Abundant Carbon Source for Industrial Microbiology. *Biotechnology Advances*, Vol. 27, No. 1, (January-February 2009), pp. 30-39, ISSN 0734-9750
- de Almeida, A.; Giordano, A.M., Nikel, P.I. & Pettinari, M.J. (2010). Effects of Aeration on the Synthesis of Poly(3-Hydroxybutyrate) from Glycerol and Glucose in Recombinant *Escherichia coli*. *Applied and Environmental Microbiology*, Vol. 76, No. 6, (March 2010), pp. 2036-2040, ISSN 0099-2240
- de Smet, M.J.; Eggink, G., Witholt, B., Kingma, J. & Wynberg, H. (1983). Characterization of Intracellular Inclusions Formed by *Pseudomonas oleovorans* During Growth on Octane. *Journal of Bacteriology*, Vol. 154, No. 2, (May 1983), pp. 870-878, ISSN 0021-9193
- Escapa, I.F.; Morales, V., Martino, V.P., Pollet, E., Avérous, L., García, J.L. & Prieto, M.A. (2011). Disruption of β-Oxidation Pathway in *Pseudomonas putida* KT2442 to Produce New Functionalized PHAs with Thioester Groups. *Applied Microbiology and Biotechnology*, Vol. 89, No. 5, (March 2011), pp. 1583-1598, ISSN 0175-7598
- Ewering, C.; Lütke-Eversloh, T., Luftmann, H. & Steinbüchel, A. (2002). Identification of Novel Sulfur-Containing Bacterial Polyesters: Biosynthesis of Poly(3-Hydroxy-S-Propyl-ω-Thioalkanoates) Containing Thioether Linkages in the Side Chains. *Microbiology*, Vol. 148, No. 5, (May 2002), pp. 1397-1406, ISSN 1350-0872

- Fischer, E.; Zamboni, N. & Sauer, U. (2004). High-Throughput Metabolic Flux Analysis Based on Gas Chromatography-Mass Spectrometry Derived ^{13}C Constraints. *Analytical Biochemistry*, Vol. 325, No. 2, (February 2004), pp. 308-316, ISSN 0003-2697
- Fritzsche, K.; Lenz, R.W. & Fuller, R.C. (1990). Production of Unsaturated Polyesters by *Pseudomonas oleovorans*. *International Journal of Biological Macromolecules*, Vol. 12, No. 2, (April 1990), pp. 85-91, ISSN 0141-8130
- García, B.; Olivera, E.R., Miñambres, B., Fernández-Valverde, M., Cañedo, L.M., Prieto, M.A., García, J.L., Martínez, M. & Luengo, J.M. (1999). Novel Biodegradable Aromatic Plastics from a Bacterial Source. Genetic and Biochemical Studies on a Route of the Phenylacetyl-CoA Catabolon. *Journal of Biological Chemistry*, Vol. 274, No. 41, (October 1999), pp. 29228-29241, ISSN 0021-9258
- Gerngross, T.U. (1999). Can Biotechnology Move Us toward a Sustainable Society? *Nature Biotechnology*, Vol. 17, No. 6, (June 1999), pp. 541-544, ISSN 1087-0156
- Han, M.J.; Yoon, S.S. & Lee, S.Y. (2001). Proteome Analysis of Metabolically Engineered *Escherichia coli* Producing Poly(3-Hydroxybutyrate). *Journal of Bacteriology*, Vol. 183, No. 1, (January 2001), pp. 301-308, ISSN 0021-9193
- Harding, K.G.; Dennis, J.S., von Blottnitz, H. & Harrison, S.T.L. (2007). Environmental Analysis of Plastic Production Processes: Comparing Petroleum-Based Polypropylene and Polyethylene with Biologically Based Poly- β -Hydroxybutyric Acid Using Life Cycle Analysis. *Journal of Biotechnology*, Vol. 130, No. 1, (May 2007), pp. 57-66, ISSN 0168-1656
- Hartmann, R.; Hany, R., Pletscher, E., Ritter, A., Witholt, B. & Zinn, M. (2006). Tailor-Made Olefinic Medium-Chain-Length Poly[(R)-3-Hydroxyalkanoates] by *Pseudomonas putida* GPo1: Batch versus Chemostat Production. *Biotechnology and Bioengineering*, Vol. 93, No. 4, (March 2006), pp. 737-746, ISSN 1097-0290
- Hazer, B. & Steinbüchel, A. (2007). Increased Diversification of Polyhydroxyalkanoates by Modification Reactions for Industrial and Medical Applications. *Applied Microbiology and Biotechnology*, Vol. 74, No. 1, (February 2007), pp. 1-12, ISSN 0175-7598
- Hocking, P.J. & Marchessault, R.H. (1994). Biopolyesters, In: *Chemistry and Technology of Biodegradable Polymers*, G.J.L. Griffin, (Ed.), pp. 48-96, Blackie Academic & Professional, ISBN 0-7514-0003-3, Glasgow, United Kingdom
- Holmes, P.A.; Collins, S.H. & Wright, L.F. (1984). 3-Hydroxybutyrate Polymers. *U.S. Patent 4,477,654*, (October 1984)
- Hong, S.H.; Park, S.J., Moon, S.Y., Park, J.P. & Lee, S.Y. (2003). *In Silico* Prediction and Validation of the Importance of the Entner-Doudoroff Pathway in Poly(3-Hydroxybutyrate) Production by Metabolically Engineered *Escherichia coli*. *Biotechnology and Bioengineering*, Vol. 83, No. 7, (September 2003), pp. 854-863, ISSN 1097-0290
- Horng, Y.T.; Chang, K.C., Chien, C.C., Wei, Y.H., Sun, Y.M. & Soo, P.C. (2010). Enhanced Polyhydroxybutyrate (PHB) Production via the Coexpressed *phaCAB* and *vgb* Genes Controlled by Arabinose P_{BAD} Promoter in *Escherichia coli*. *Letters in Applied Microbiology*, Vol. 50, No. 2, (February 2010), pp. 158-167, ISSN 1472-765X
- Horng, Y.T.; Chien, C.C., Wei, Y.H., Chen, S.Y., Lan, J.C., Sun, Y.M. & Soo, P.C. (2011). Functional *cis*-Expression of *phaCAB* Genes for Poly(3-Hydroxybutyrate) Production by *Escherichia coli*. *Letters in Applied Microbiology*, Vol. 52, No. 5, (May 2011), pp. 475-483, ISSN 1472-765X

- Huijberts, G.N.; Eggink, G., de Waard, P., Huisman, G.W. & Witholt, B. (1992). *Pseudomonas putida* KT2442 Cultivated on Glucose Accumulates Poly(3-Hydroxyalkanoates) Consisting of Saturated and Unsaturated Monomers. *Applied and Environmental Microbiology*, Vol. 58, No. 2, (February 1992), pp. 536-544, ISSN 0099-2240
- Hunter, P. (2010). Can Bacteria Save the Planet? *EMBO Reports*, Vol. 11, No. 4, (April 2010), pp. 266-269, ISSN 1469-221X
- Ibrahim, M.H.A. & Steinbüchel, A. (2009). Poly(3-Hydroxybutyrate) Production from Glycerol by *Zobellella denitrificans* MW1 via High-Cell-Density Fed-Batch Fermentation and Simplified Solvent Extraction. *Applied and Environmental Microbiology*, Vol. 75, No. 19, (October 2009), pp. 6222-6231, ISSN 0099-2240
- Jacquel, N.; Lo, C.W., Wei, Y.H., Wu, H.S. & Wang, S.S. (2008). Isolation and Purification of Bacterial Poly(3-Hydroxyalkanoates). *Biochemical Engineering Journal*, Vol. 39, No. 1, (April 2008), pp. 15-27, ISSN 1369-703X
- Jung, Y.K.; Lee, S.Y. & Tam, T.T. (2010). Towards Systems Metabolic Engineering of PHA Producers, In: *Plastics from Bacteria: Natural Functions and Applications*, G.G.Q. Chen, (Ed.), pp. 63-84, Springer-Verlag, ISBN 978-3-642-03286-8, Berlin, Germany
- Kapritchkoff, F.M.; Viotti, A.P., Alli, R.C.P., Zuccolo, M., Pradella, J.G.C., Maiorano, A.E., Miranda, E.A. & Bonomi, A. (2006). Enzymatic Recovery and Purification of Polyhydroxybutyrate Produced by *Ralstonia eutropha*. *Journal of Biotechnology*, Vol. 122, No. 4, (April 2006), pp. 453-462, ISSN 0168-1656
- Keshavarz, T. & Roy, I. (2010). Polyhydroxyalkanoates: Bioplastics with a Green Agenda. *Current Opinion in Microbiology*, Vol. 13, No. 3, (June 2010), pp. 321-326, ISSN 1369-5274
- Kim, B.S. (2000). Production of Poly(3-Hydroxybutyrate) from Inexpensive Substrates. *Enzyme and Microbial Technology*, Vol. 27, No. 10, (December 2000), pp. 774-777, ISSN 0141-0229
- Kim, S. & Dale, B.E. (2005). Lifecycle Assessment Study of Biopolymer (Polyhydroxyalkanoates) - Derived from No-Tilled Corn. *The International Journal of Life Cycle Assessment*, Vol. 10, No. 3, (May 2005), pp. 200-210, ISSN 0948-3349
- Koller, M.; Bona, R., Braunegg, G., Hermann, C., Horvat, P., Kroutil, M., Martinz, J., Neto, J., Pereira, L. & Varila, P. (2005). Production of Polyhydroxyalkanoates from Agricultural Waste and Surplus Materials. *Biomacromolecules*, Vol. 6, No. 2, (March 2005), pp. 561-565, ISSN 1525-7797
- Koller, M.; Hesse, P., Bona, R., Kutschera, C., Atlić, A. & Braunegg, G. (2007). Potential of Various Archae- and Eubacterial Strains as Industrial Polyhydroxyalkanoate Producers from Whey. *Macromolecular Bioscience*, Vol. 7, No. 2, (February 2007), pp. 218-226, ISSN 1616-5195
- Koller, M.; Bona, R., Chiellini, E., Grillo-Fernandes, E., Horvat, P., Kutschera, C., Hesse, P. & Braunegg, G. (2008). Polyhydroxyalkanoate Production from Whey by *Pseudomonas hydrogenovora*. *Bioresource Technology*, Vol. 99, No. 11, (July 2008), pp. 4854-4863, ISSN 0960-8524
- Kulpreecha, S.; Boonruangthavorn, A., Meksiriporn, B. & Thongchul, N. (2009). Inexpensive Fed-Batch Cultivation for High Poly(3-Hydroxybutyrate) Production by a New Isolate of *Bacillus megaterium*. *Journal of Bioscience and Bioengineering*, Vol. 107, No. 3, (March 2009), pp. 240-245, ISSN 1389-1723
- Lageveen, R.G.; Huisman, G.W., Preusting, H., Ketelaar, P., Eggink, G. & Witholt, B. (1988). Formation of Polyesters by *Pseudomonas oleovorans*: Effect of Substrates on Formation and Composition of Poly-(R)-3-Hydroxyalkanoates and Poly-(R)-3-

- Hydroxyalkenoates. *Applied and Environmental Microbiology*, Vol. 54, No. 12, (December 1988), pp. 2924-2932, ISSN 0099-2240
- Lee, S.Y.; Kim, H.U., Yun, H., Sohn, S.B., Kim, J.S., Palsson, B.Ø., Herrgård, M.J. & Portnoy, V.A. (2010). Systems Biology, Genome-Scale Models, and Metabolic Engineering, In: *The Metabolic Pathway Engineering Handbook - Tools and Applications*, C.D. Smolke, (Ed.), pp. 15.1-15.11, CRC Press, ISBN 978-1-4200-7765-0, Boca Raton, Florida, United States of America
- Lenz, R.W.; Kim, Y.B. & Fuller, R.C. (1992). Production of Unusual Bacterial Polyesters by *Pseudomonas oleovorans* through Cometabolism. *FEMS Microbiology Letters*, Vol. 103, No. 2-4, (December 1992), pp. 207-214, ISSN 1574-6968
- Li, R.; Zhang, H. & Qi, Q. (2007). The Production of Polyhydroxyalkanoates in Recombinant *Escherichia coli*. *Bioresource Technology*, Vol. 98, No. 12, (September 2007), pp. 2313-2320, ISSN 0960-8524
- Lim, S.J.; Jung, Y.M., Shin, H.D. & Lee, Y.H. (2002). Amplification of the NADPH-Related Genes *zwf* and *gnd* for the Oddball Biosynthesis of PHB in an *E. coli* Transformant Harboring a Cloned *phbCAB* Operon. *Journal of Bioscience and Bioengineering*, Vol. 93, No. 6, (October 2002), pp. 543-549, ISSN 1389-1723
- Lütke-Eversloh, T.; Bergander, K., Luftmann, H. & Steinbüchel, A. (2001). Biosynthesis of Poly(3-Hydroxybutyrate-co-3-Mercaptobutyrate) as a Sulfur Analogue to Poly(3-Hydroxybutyrate) (PHB). *Biomacromolecules*, Vol. 2, No. 3, (August 2001), pp. 1061-1065, ISSN 1525-7797
- Lütke-Eversloh, T.; Fischer, A., Remminghorst, U., Kawada, J., Marchessault, R.H., Bögershausen, A., Kalwei, M., Eckert, H., Reichelt, R., Liu, S.J. & Steinbüchel, A. (2002). Biosynthesis of Novel Thermoplastic Polythioesters by Engineered *Escherichia coli*. *Nature Materials*, Vol. 1, No. 4, (December 2002), pp. 236-240, ISSN 1476-1122
- Lynch, A.S. & Lin, E.C.C. (1996). Responses to Molecular Oxygen, In: *Escherichia coli and Salmonella: Cellular and Molecular Biology*, F.C. Neidhardt, R. Curtiss III, J.L. Ingraham, E.C.C. Lin, K.B. Low, B. Magasanik, W.S. Reznikoff, M. Riley, M. Schaechter, H.E. Umbarger, (Eds.), pp. 1526-1538, ASM Press, ISBN 1-5558-1084-5, Washington, D.C., United States of America
- Madden, L.A.; Anderson, A.J., Shah, D.T. & Asrar, J. (1999). Chain Termination in Polyhydroxyalkanoate Synthesis: Involvement of Exogenous Hydroxy-Compounds as Chain Transfer Agents. *International Journal of Biological Macromolecules*, Vol. 25, No. 1-3, (June 1999), pp. 43-53, ISSN 0141-8130
- Madison, L.L. & Huisman, G.W. (1999). Metabolic Engineering of Poly(3-Hydroxyalkanoates): From DNA to Plastic. *Microbiology and Molecular Biology Reviews*, Vol. 63, No. 1, (March 1999), pp. 21-53, ISSN 1092-2172
- Mahishi, L.H.; Tripathi, G. & Rawal, S.K. (2003). Poly(3-Hydroxybutyrate) (PHB) Synthesis by Recombinant *Escherichia coli* Harboring *Streptomyces aureofaciens* PHB Biosynthesis Genes: Effect of Various Carbon and Nitrogen Sources. *Microbiological Research*, Vol. 158, No. 1, (January 2003), pp. 19-27, ISSN 0944-5013
- Marangoni, C.; Furigo Jr., A. & de Aragão, G.M.F. (2002). Production of Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate) by *Ralstonia eutropha* in Whey and Inverted Sugar with Propionic Acid Feeding. *Process Biochemistry*, Vol. 38, No. 2, (October 2002), pp. 137-141, ISSN 1359-5113

- Martínez, V.; García, P., García, J.L. & Prieto, M.A. (2011). Controlled Autolysis Facilitates the Polyhydroxyalkanoate Recovery in *Pseudomonas putida* KT2440. *Microbial Biotechnology*, Vol. 4, No. 4, (July 2011), pp. 533-547, ISSN 1751-7915
- Nath, A.; Dixit, M., Bandiya, A., Chavda, S. & Desai, A.J. (2008). Enhanced PHB Production and Scale up Studies Using Cheese Whey in Fed Batch Culture of *Methylobacterium* sp. ZP24. *Bioresource Technology*, Vol. 99, No. 13, (September 2008), pp. 5749-5755, ISSN 0960-8524
- Nelson, K.E.; Weinl, C., Paulsen, I.T., Dodson, R.J., Hilbert, H., Martins dos Santos, V.A.P., Fouts, D.E., Gill, S.R., Pop, M., Holmes, M., Brinkac, L., Beanan, M., DeBoy, R.T., Daugherty, S., Kolonay, J., Madupu, R., Nelson, W., White, O., Peterson, J., Khouri, H., Hance, I., Chris Lee, P., Holtzapple, E., Scanlan, D., Tran, K., Moazzez, A., Utterback, T., Rizzo, M., Lee, K., Kosack, D., Moestl, D., Wedler, H., Lauber, J., Stjepandic, D., Hoheisel, J., Straetz, M., Heim, S., Kiewitz, C., Eisen, J.A., Timmis, K.N., Düsterhöft, A., Tümmeler, B. & Fraser, C.M. (2002). Complete Genome Sequence and Comparative Analysis of the Metabolically Versatile *Pseudomonas putida* KT2440. *Environmental Microbiology*, Vol. 4, No. 12, (December 2002), pp. 799-808, ISSN 1462-2920
- Nielsen, J. (2001). Metabolic Engineering. *Applied Microbiology and Biotechnology*, Vol. 55, No. 3, (April 2001), pp. 263-283, ISSN 0175-7598
- Nielsen, J. & Jewett, M.C. (2008). Impact of Systems Biology on Metabolic Engineering of *Saccharomyces cerevisiae*. *FEMS Yeast Research*, Vol. 8, No. 1, (February 2008), pp. 122-131, ISSN 1567-1364
- Nikel, P.I.; de Almeida, A., Melillo, E.C., Galvagno, M.A. & Pettinari, M.J. (2006a). New Recombinant *Escherichia coli* Strain Tailored for the Production of Poly(3-Hydroxybutyrate) from Agroindustrial By-Products. *Applied and Environmental Microbiology*, Vol. 72, No. 6, (June 2006), pp. 3949-3954, ISSN 0099-2240
- Nikel, P.I.; Pettinari, M.J., Galvagno, M.A. & Méndez, B.S. (2006b). Poly(3-Hydroxybutyrate) Synthesis by Recombinant *Escherichia coli* *arcA* Mutants in Microaerobiosis. *Applied and Environmental Microbiology*, Vol. 72, No. 4, (April 2006), pp. 2614-2620, ISSN 0099-2240
- Nikel, P.I.; de Almeida, A., Pettinari, M.J. & Méndez, B.S. (2008a). The Legacy of HfrH: Mutations in the Two-Component System CreBC Are Responsible for the Unusual Phenotype of an *Escherichia coli* *arcA* Mutant. *Journal of Bacteriology*, Vol. 190, No. 9, (May 2008), pp. 3404-3407, ISSN 0021-9193
- Nikel, P.I.; Pettinari, M.J., Galvagno, M.A. & Méndez, B.S. (2008b). Poly(3-Hydroxybutyrate) Synthesis from Glycerol by a Recombinant *Escherichia coli* *arcA* Mutant in Fed-Batch Microaerobic Cultures. *Applied Microbiology and Biotechnology*, Vol. 77, No. 6, (January 2008), pp. 1337-1343, ISSN 0175-7598
- Nikel, P.I.; Zhu, J., San, K.Y., Méndez, B.S. & Bennett, G.N. (2009). Metabolic Flux Analysis of *Escherichia coli* *creB* and *arcA* Mutants Reveals Shared Control of Carbon Catabolism under Microaerobic Growth Conditions. *Journal of Bacteriology*, Vol. 191, No. 17, (September 2009), pp. 5538-5548, ISSN 0021-9193
- Nonato, R.V.; Mantelatto, P.E. & Rossell, C.E.V. (2001). Integrated Production of Biodegradable Plastic, Sugar and Ethanol. *Applied Microbiology and Biotechnology*, Vol. 57, No. 1-2, (October 2001), pp. 1-5, ISSN 0175-7598
- Oliveira, F.C.; Freire, D.M. & Castilho, L.R. (2004). Production of Poly(3-Hydroxybutyrate) by Solid-State Fermentation with *Ralstonia eutropha*. *Biotechnology Letters*, Vol. 26, No. 24, (December 2004), pp. 1851-1855, ISSN 0141-5492

- Olivera, E.R.; Carnicero, D., García, B., Miñambres, B., Moreno, M.A., Cañedo, L., DiRusso, C.C., Naharro, G. & Luengo, J.M. (2001a). Two Different Pathways Are Involved in the β -Oxidation of *n*-Alkanoic and *n*-Phenylalkanoic Acids in *Pseudomonas putida* U: Genetic Studies and Biotechnological Applications. *Molecular Microbiology*, Vol. 39, No. 4, (February 2001), pp. 863-874, ISSN 1365-2958
- Olivera, E.R.; Carnicero, D., Jodra, R., Miñambres, B., García, B., Abraham, G.A., Gallardo, A., Román, J.S., García, J.L., Naharro, G. & Luengo, J.M. (2001b). Genetically Engineered *Pseudomonas*: A Factory of New Bioplastics with Broad Applications. *Environmental Microbiology*, Vol. 3, No. 10, (October 2001), pp. 612-618, ISSN 1462-2920
- Ouyang, S.P.; Luo, R.C., Chen, S.S., Liu, Q., Chung, A., Wu, Q. & Chen, G.G.Q. (2007). Production of Polyhydroxyalkanoates with High 3-Hydroxydodecanoate Monomer Content by *fadB* and *fadA* Knockout Mutant of *Pseudomonas putida* KT2442. *Biomacromolecules*, Vol. 8, No. 8, (August 2007), pp. 2504-2511, ISSN 1525-7797
- Park, S.J.; Park, J.P. & Lee, S.Y. (2002). Production of Poly(3-Hydroxybutyrate) from Whey by Fed-Batch Culture of Recombinant *Escherichia coli* in a Pilot-Scale Fermenter. *Biotechnology Letters*, Vol. 24, No. 3, (February 2002), pp. 185-189, ISSN 0141-5492
- Park, W.H.; Lenz, R.W. & Goodwin, S. (1998). Epoxidation of Bacterial Polyesters with Unsaturated Side Chains. I. Production and Epoxidation of Polyesters from 10-Undecenoic Acid. *Macromolecules*, Vol. 31, No. 5, (March 1998), pp. 1480-1486, ISSN 0024-9297
- Philip, S.; Keshavarz, T. & Roy, I. (2007). Polyhydroxyalkanoates: Biodegradable Polymers with a Range of Applications. *Journal of Chemical Technology and Biotechnology*, Vol. 82, No. 3, (March 2007), pp. 233-247, ISSN 1097-4660
- Pohlmann, A.; Fricke, W.F., Reinecke, F., Kusian, B., Liesegang, H., Cramm, R., Eitinger, T., Ewering, C., Pötter, M., Schwartz, E., Strittmatter, A., Voß, I., Gottschalk, G., Steinbüchel, A., Friedrich, B. & Bowien, B. (2006). Genome Sequence of the Bioplastic-Producing "Knallgas" Bacterium *Ralstonia eutropha* H16. *Nature Biotechnology*, Vol. 24, No. 10, (October 2006), pp. 1257-1262, ISSN 1087-0156
- Povolo, S.; Toffano, P., Basaglia, M. & Casella, S. (2010). Polyhydroxyalkanoates Production by Engineered *Cupriavidus necator* from Waste Material Containing Lactose. *Bioresource Technology*, Vol. 101, No. 20, (October 2010), pp. 7902-7907, ISSN 0960-8524
- Pries, A.; Steinbüchel, A. & Schlegel, H.G. (1990). Lactose- and Galactose-Utilizing Strains of Poly(Hydroxyalkanoic Acid)-Accumulating *Alcaligenes eutrophus* and *Pseudomonas saccharophila* Obtained by Recombinant DNA Technology. *Applied Microbiology and Biotechnology*, Vol. 33, No. 4, (July 1990), pp. 410-417, ISSN 0175-7598
- Queiroz, A.U.B. & Collares-Queiroz, F.P. (2009). Innovation and Industrial Trends in Bioplastics. *Polymer Reviews*, Vol. 49, No. 2, (April 2009), pp. 65-78, ISSN 1558-3724
- Reddy, S.V.; Thirumala, M. & Mahmood, S.K. (2009). A Novel *Bacillus* sp. Accumulating Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate) from a Single Carbon Substrate. *Journal of Industrial Microbiology and Biotechnology*, Vol. 36, No. 6, (June 2009), pp. 837-843, ISSN 1367-5435
- Rehm, B.H.A. (2010). Bacterial Polymers: Biosynthesis, Modifications and Applications. *Nature Reviews Microbiology*, Vol. 8, No. 8, (August 2010), pp. 578-592, ISSN 1740-1526
- Reinecke, F. & Steinbüchel, A. (2009). *Ralstonia eutropha* Strain H16 as Model Organism for PHA Metabolism and for Biotechnological Production of Technically Interesting

- Biopolymers. *Journal of Molecular Microbiology and Biotechnology*, Vol. 16, No. 1-2, (October 2009), pp. 91-108, ISSN 1464-1801
- Sabirova, J.S.; Ferrer, M., Lünsdorf, H., Wray, V., Kalscheuer, R., Steinbüchel, A., Timmis, K.N. & Golyshin, P.N. (2006). Mutation in a "tesB-Like" Hydroxyacyl-Coenzyme A-Specific Thioesterase Gene Causes Hyperproduction of Extracellular Polyhydroxyalkanoates by *Alcanivorax borkumensis* SK2. *Journal of Bacteriology*, Vol. 188, No. 24, (December 2006), pp. 8452-8459, ISSN 0021-9193
- Sauer, U. (2006). Metabolic Networks in Motion: ¹³C-Based Flux Analysis. *Molecular Systems Biology*, Vol. 2, No. 1, (November 2006), pp. 62, ISSN 1744-4292
- Sawaya Jank, M. (2011). Company Production Rankings for Sugarcane, Sugar and Ethanol - South-Central Brazil. In: UNICA - Sugarcane Industry Association, 07.06.2011, Available from <http://english.unica.com.br/dadosCotacao/estatistica/>
- Scholz, C.; Fuller, R.C. & Lenz, R.W. (1994). Growth and Polymer Incorporation of *Pseudomonas oleovorans* on Alkyl Esters of Heptanoic Acid. *Macromolecules*, Vol. 27, No. 10, (May 1994), pp. 2886-2889, ISSN 0024-9297
- Scholz, C. (2010). Perspectives to Produce Positively or Negatively Charged Polyhydroxyalkanoic Acids. *Applied Microbiology and Biotechnology*, Vol. 88, No. 4, (October 2010), pp. 829-837, ISSN 0175-7598
- Serafim, L.S.; Lemos, P.C., Albuquerque, M.G.E. & Reis, M.A.M. (2008). Strategies for PHA Production by Mixed Cultures and Renewable Waste Materials. *Applied Microbiology and Biotechnology*, Vol. 81, No. 4, (December 2008), pp. 615-628, ISSN 0175-7598
- Silva-Queiroz, S.R.; Silva, L.F., Pradella, J.G.C., Pereira, E.M. & Gomez, J.G.C. (2009). PHA_{MCL} Biosynthesis Systems in *Pseudomonas aeruginosa* and *Pseudomonas putida* Strains Show Differences on Monomer Specificities. *Journal of Biotechnology*, Vol. 143, No. 2, (August 2009), pp. 111-118, ISSN 0168-1656
- Solaiman, D.K.Y.; Ashby, R.D., Foglia, T. & Marmer, W.N. (2006). Conversion of Agricultural Feedstock and Coproducts into Poly(Hydroxyalkanoates). *Applied Microbiology and Biotechnology*, Vol. 71, No. 6, (August 2006), pp. 783-789, ISSN 0175-7598
- Steinbüchel, A. (2001). Perspectives for Biotechnological Production and Utilization of Biopolymers: Metabolic Engineering of Polyhydroxyalkanoate Biosynthesis as a Successful Example. *Macromolecular Bioscience*, Vol. 1, No. (January 2001), pp. 1-24, ISSN 1616-5195
- Steinbüchel, A. & Lütke-Eversloh, T. (2003). Metabolic Engineering and Pathway Construction for Biotechnological Production of Relevant Polyhydroxyalkanoates in Microorganisms. *Biochemical Engineering Journal*, Vol. 16, No. 2, (November 2003), pp. 81-96, ISSN 1369-703X
- Steinbüchel, A. (2005). Non-Biodegradable Biopolymers from Renewable Resources: Perspectives and Impacts. *Current Opinion in Biotechnology*, Vol. 16, No. 6, (December 2005), pp. 607-613, ISSN 0958-1669
- Stephanopoulos, G. (1999). Metabolic Fluxes and Metabolic Engineering. *Metabolic Engineering*, Vol. 1, No. 1, (January 1999), pp. 1-11, ISSN 1096-7176
- Suriyamongkol, P.; Weselake, R., Narine, S., Moloney, M. & Shah, S. (2007). Biotechnological Approaches for the Production of Polyhydroxyalkanoates in Microorganisms and Plants - a Review. *Biotechnology Advances*, Vol. 25, No. 2, (March-April 2007), pp. 148-175, ISSN 0734-9750

- Taidi, B.; Anderson, A.J., Dawes, E.A. & Byrom, D. (1994). Effect of Carbon Source and Concentration on the Molecular Mass of Poly(3-Hydroxybutyrate) Produced by *Methylobacterium extorquens* and *Alcaligenes eutrophus*. *Applied Microbiology and Biotechnology*, Vol. 40, No. 6, (February 1994), pp. 786-790, ISSN 0175-7598
- Takagi, Y.; Hashii, M., Maehara, A. & Yamane, T. (1999). Biosynthesis of Polyhydroxyalkanoate with a Thiophenoxy Side Group Obtained from *Pseudomonas putida*. *Macromolecules*, Vol. 32, No. 25, (December 1999), pp. 8315-8318, ISSN 0024-9297
- Tyo, K.E.; Fischer, C.R., Simeon, F. & Stephanopoulos, G. (2010). Analysis of Polyhydroxybutyrate Flux Limitations by Systematic Genetic and Metabolic Perturbations. *Metabolic Engineering*, Vol. 12, No. 3, (May 2010), pp. 187-195, ISSN 1096-7176
- Wang, F. & Lee, S.Y. (1997). Production of Poly(3-Hydroxybutyrate) by Fed-Batch Culture of Filamentation-Suppressed Recombinant *Escherichia coli*. *Applied and Environmental Microbiology*, Vol. 63, No. 12, (December 1997), pp. 4765-4769, ISSN 0099-2240
- Wei, X.X.; Shi, Z.Y., Yuan, M.Q. & Chen, G.G.Q. (2009). Effect of Anaerobic Promoters on the Microaerobic Production of Polyhydroxybutyrate (PHB) in Recombinant *Escherichia coli*. *Applied Microbiology and Biotechnology*, Vol. 82, No. 4, (March 2009), pp. 703-712, ISSN 0175-7598
- Wiechert, W. (2001). ¹³C Metabolic Flux Analysis. *Metabolic Engineering*, Vol. 3, No. 3, (July 2001), pp. 195-206, ISSN 1096-7176
- Wong, H.H. & Lee, S.Y. (1998). Poly-(3-Hydroxybutyrate) Production from Whey by High-Density Cultivation of Recombinant *Escherichia coli*. *Applied Microbiology and Biotechnology*, Vol. 50, No. 1, (July 1998), pp. 30-33, ISSN 0175-7598
- Wu, Q.; Wang, Y. & Chen, G.G.Q. (2009). Medical Application of Microbial Biopolyesters Polyhydroxyalkanoates. *Artificial Cells, Blood Substitutes and Biotechnology*, Vol. 37, No. 1, (January 2009), pp. 1-12, ISSN 1073-1199
- Yang, S.T.; Zhu, H., Li, Y. & Hong, G. (1994). Continuous Propionate Production from Whey Permeate Using a Novel Fibrous Bed Bioreactor. *Biotechnology and Bioengineering*, Vol. 43, No. 11, (May 1994), pp. 1124-1130, ISSN 1097-0290
- Young, F.K., Kastner, J.R. & May, S.W. (1994). Microbial Production of Poly- β -Hydroxybutyric Acid from D-Xylose and Lactose by *Pseudomonas cepacia*. *Applied and Environmental Microbiology*, Vol. 60, No. 11, (November 1994), pp. 4195-4198, ISSN 0099-2240
- Yu, J. & Chen, L.X.L. (2006). Cost-Effective Recovery and Purification of Polyhydroxyalkanoates by Selective Dissolution of Cell Mass. *Biotechnology Progress*, Vol. 22, No. 2, (March 2006), pp. 547-553, ISSN 1520-6063
- Zhang, H.; Obias, V., Gonyer, K. & Dennis, D. (1994). Production of Polyhydroxyalkanoates in Sucrose-Utilizing Recombinant *Escherichia coli* and *Klebsiella* Strains. *Applied and Environmental Microbiology*, Vol. 60, No. 4, (April 1994), pp. 1198-1205, ISSN 0099-2240
- Zhong, Z.W.; Song, B. & Huang, C.X. (2009). Environmental Impacts of Three Polyhydroxyalkanoate (PHA) Manufacturing Processes. *Materials and Manufacturing Processes*, Vol. 24, No. 5, (March 2009), pp. 519-523, ISSN 1042-6914



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Biotechnology is the scientific field of studying and applying the most efficient methods and techniques to get useful end-products for the human society by using viable micro-organisms, cells, and tissues of plants or animals, or even certain functional components of their organisms, that are grown in fully controlled conditions to maximize their specific metabolism inside fully automatic bioreactors. It is very important to make the specific difference between biotechnology as a distinct science of getting valuable products from molecules, cells or tissues of viable organisms, and any other applications of bioprocesses that are based on using the whole living plants or animals in different fields of human activities such as bioremediation, environmental protection, organic agriculture, or industrial exploitation of natural resources. The volume *Advances in Applied Biotechnology* is a scientific book containing recent advances of selected research works that are ongoing in certain biotechnological applications. Fourteen chapters divided in four sections related to the newest biotechnological achievements in environmental protection, medicine and health care, biopharmaceutical producing, molecular genetics, and tissue engineering are presented.

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