Chapter 3

Whey Lactose as a Raw Material for Microbial Production of Biodegradable Polyesters

Martin Koller, Anna Salerno, Alexander Muhr, Angelika Reiterer, Emo Chiellini, Sergio Casella, Predrag Horvat and Gerhart Braunegg

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/48737

1. Introduction

Whey, a by-product of diary and cheese industry, constitutes the watery portion after the separation of fat and caseins from whole milk. Cheese whey is a surplus material produced in volumes almost equal to the milk processed in cheese manufactories, therefore its disposal as a waste causes serious pollution problems in the surrounding environment where it´s discarded. This is due to its enormous biochemical oxygen demand that is mainly caused by its high lactose content; as a consequence a large amount of industrial capital is requested for whey disposal. During the last years, the amounts of whey increased to such an extent that they cannot be simply used as animal feed as the most common application. To overcome these problems a sustainable alternative is to upgrade whey and its derivates to a resource for many value added industrial products, making whey not only a waste but also a valuable resource.

The article presents a future-oriented, alternative strategy to use surplus whey, namely its upgrading to the role of a raw material for cost-efficient production of polyhydroxyalkanoate (PHA) biopolyesters. PHAs are a group of bio-based, bio-compatible and compostable bio-plastics of increasing significance for numerous industrial applications. Data for PHA production from whey on different production scales and techniques by various microbial species are compared and embedded in the entire scientific field of biopolymers. The review shows how the smart solution of an industrial waste disposal problem can be combined with enhanced cost efficiency in production of "green plastics".

2. General: The need for sustainable utilization of whey

Whey is the liquid remaining after the coagulation of milk casein in cheese making or casein manufacture. Cheese whey, representing about the 85-95% (Guimarães et al., 2010) of the

milk volume, constitutes a waste- and surplus material from dairy and cheese industries in many regions of the world (Illanes, 2011). The reported amounts of whey that are produced globally vary from $1.15*10^8$ tons (Peters, 2006) to $1.40*10^8$ tons (Audic, 2003) per year. OECD-FAO estimations for 2008 even report $1.60*10^8$ tons with annual increase of 1-2% (reviewed in Guimarães et al., 2010). Mainly in North America and Europe, huge quantities of whey are available; in 2008, the estimated accruing values are reported with $4*10^7$ tons for the USA, and $5*10^7$ tons for the European Union. Reliable data for Canada, another important whey producing country, are valid for the year 1997; here $2.2*10^5$ t are reported annually (Ghaly & El-Taweel, 1997).

Bovine whey is not only a cheap raw material, but also causes severe disposal problems because of the huge amounts generated and its high organic matter content. During cheese production, whey accrues in almost equal volumes to the processed milk. It can be estimated that, to make 1 kg of cheese, about 9L of whey are produced (Kosikowki, 1979). Whey shows a high biochemical oxygen demand (BOD, 40,000 –60,000 ppm) and chemical oxygen demand (COD, 50,000–80,000 ppm), making the disposal of surplus whey rather expensive (Kim et al., 1995; Viñas et al., 1994); lactose, the most representative compound of whey, is the main responsible component causing these high values for BOD and COD. The major part of whey is discarded as waste in the surrounding environments, causing dramatic pollution problems. Its disposal can be accomplished *via* different strategies: piping it into lagoons, rivers, lakes or oceans, funnelling in caves, feeding to ruminants and pigs, and spreading over fields. The negative impact of disposing whey in water bodies is visible by a quick reduction of aquatic life due to the depletion of the dissolved oxygen. In case of releasing on fields, the physical and chemical structures of soil are severely affected, with a consequent decrease in crop yield (Gonzales-Siso, 1996).

It has to be considered that the processing of one million litres of milk causes the challenging task of disposing of nearby one million litres of whey. This exemplary quantity contains up to 50 t of whey main carbon ingredient, namely lactose. The resulting problem becomes especially obvious considering the fact that today surplus whey is very often disposed of just by being poured into the sea. For example, in the Northern Italian Po-region, where a variety of well-known dairies is located, about 1 million litres of whey has to be disposed daily.

The market of whey products for human nutrition, e.g. for sweets, whey powder as nutritional supplement in body building formulations for increase of muscle size, whey beverages, additives for food processing (e.g. ice cream, meat products), baby food, or for application as pharmaceutical matrices, is also restricted. This is due to the high number of people suffering from lactose intolerance due to lacking activity of the enzyme lactase in their metabolism (Heyman, 2006). It is estimated that 75% of adults worldwide show some decrease in lactase activity (hypolactasia), resulting in lactose intolerance still during adulthood. The phenomenon "lactose intolerance" is mainly found in African, Asian, South American and South European regions (Bulhões et al., 2007). Unfortunately, this frequently occurring of lactase activity deficiency hampers a broader application of whey for nutrition

of mankind. It has to be considered that whey is a material of substantial nutritional value due to its high contents of carbohydrates, proteins, lipids, and precious minerals like calcium (see Table 1). The consumption of 100 g of whey results in the considerable uptake of more than 100 kJ of nutritional energy.

The facts discussed above clearly underline the necessity to convert whey in a safe and, most favourably, value-adding way. During the last couple of years, the generated amounts of whey increased to such an extent that they cannot be simply used as porcine feed as it's nowadays most common application. It has to be emphasised that e.g in Italy this applications is not allowed any more for the production of "*Denominazione Origine Protetta*" (DOP, protected origin) porcine meet (ham) because the related disciplinary regulation does not admit the feeding of pigs with whey.

Considerable efforts are undertaken worldwide to upgrade surplus whey to a carbon feed stock for bioconversion towards various value-added products. The disaccharide lactose, as it´s major carbohydrate, can function as a carbon substrate for growth and product formation in numerous biotechnological processes. In literature, the production of bioethanol (Ghaly & El-Taweel, 1997; Zafar & Owais, 2006), vinegar (Parrondo et al., 2003), antibiotics, e.g. the bacteriocin Nisin, (Hickmann Flôres & Monte Alegre, 2001), yeasts for yeast extract production (de Palma Revillion et al., 2003), surface active compounds like sophorolipids (Daniel et al., 1999), single-cell protein (Schultz et al., 2006), "green bioplastics" like Polyhydroxyalkanoates, PHAs, (Ahn et al., 2000; Ahn et al., 2001; Kim, 2000; Povolo & Casella, 2003; Koller et al., 2007 a,b), and lactic acid that is of importance as food additive (E 270), for pharmaceutical matrices, and as monomer for the production of polylactic acid (PLA) is described (Kim et al., 1995). In addition, the induction of high-cell-density production of recombinant proteins can be accomplished by providing whey (Viitanen et al., 2003). Chemically, whey lactose can be converted to the artificial sweetener lactitol (E 966) or the laxative lactulose (Illanes 2011). Table 1 collects the value-added products that can be produced starting from whey.

Products from Whey	**Production Mode**	**Strains**	**References**
PHA	Biotechnological (direct conversion or upstream processing of substrate and conversion into PHA)	Recombinant *Escherichia coli, Hydrogenophaga psedoflava,* Azotobacter spp (direct conversion). Lactobacilli (from whey to lactic acid), (e.g.) *Cupriavidus necator* (from lactic acid to PHA). *Haloferax mediterranei, Pseudomonas hydrogenovora* (from hydrolysed lactose	Ahn et al., 2000; Ahn et al., 2001; Koller et al., 2007; Povolo & Casella, 2003

Products from Whey	Production Mode	Strains	References
		to PHA).	
Lactic Acid (PLA)	Biotechnological (anaerobic conversion) and chemical polymerization (PLA)	Lactobacilli (from whey to lactic acid)	Koller et al., 2010
Bioethanol	Biotechnological conversion	*Saccharomyces cerevisiae, Klyveromyces lactis, Klyveromyces marxianus, Candida pseudotropicalis*	Guimarães et al., 2010
SCP (single cell proteins)	Biotechnological conversion	*Klyveromyces lactis, K. fragilis, Torulopsis bovina, Candida intermedia*	Guimarães et al., 2010, Siso et al., 1996
Yeast	Biotechnological conversion	*K. marxianus*	de Palma Revillion, 2003
Vinegar	Biotechnological conversion (2 steps)	*K. marxianus (*alcoholic fermentation*) Acetobacter pasteurans (*acetic fermentation*)*	Parrondo et al., 2003
Artificial Sweeteners	Chemical reduction or enzymatic hydrolysis	*Aspergillus* and *Kluyveromyces* spp. (enzymes)	Gänzle et al. ,2008
Antibiotics	Biotechnological conversion	*Lactococcus lactis* (bacteriocin); *Listeria monocytogenes* and *Clostridium botulinum*	Hickmann, Flores & Monte Alegre, 2001
Sophorolipids	Biotechnological conversion (2 steps)	*Cryptococcus curvatus* (single cell oil, SCO), *Candida bombicola* (SCO converted in sophorolipids)	Daniel et al., 1999

Table 1. Products accessible from whey lactose

3. Polyhydroxyalkanoates: The only family of "Green plastics" completely synthesized by microbes

3.1. "Green plastics"

Nowadays, a variety of manufactures claim the fashionable labelling "*green plastic*" for polymeric materials they commercialize on the market. In most cases, the *de facto* properties of these products are not really in accordance with the generally valid definitions for classifying them as "*biobased*", "*biodegradable*", "*compostable*", or "*biocompatible*" (CEN/TR

15391, CEN/TR 15932), thus making them "*green*". Such attributes do only apply to plastics if they come along with the strict requirements defined by standardized norms and certificates.

For instance, the norm EN-13432 that deals with biodegradation and composting of plastic packaging materials clearly postulates that a (plastic) material can be termed "*biodegradable*", if 90% of its carbon is metabolized within 180 days. According to the same norm, the material is "*compostable*", if not more than 10% of the material remains in a sieve of 2 mm pore size after 180 days of composting.

The classification „*biocompatible*" refers to the fact that, using standardized methods for assessing the ecotoxicity of the (plastic) material, it must not feature any negative impact on living organisms or the involved environment. This is described in the certification according to a standardized norm ISO 10993. Together with biodegradability, biocompatibility is of special significance for *in vivo* applications of polymers such as implants for surgery or other medical applications.

In addition, a polymer can be classified as „*biobased*", if the production of the building block monomeric units is based on renewable resources; afterwards, the polymerization of the monomers may occur chemically (e.g.: polymerization of biosynthesized lactic acid to PLA) or biologically (e.g.: in vivo polymerization of hydroxyacyl-CoAs by PHA synthases towards PHAs).

3.2. Particularities and significance of PHAs

PHAs constitute a family of biodegradable intracellular polyesters synthesized by a wide range of prokaryotic genera starting from renewable feedstocks (Koller et al., 2010). Among all known classes of bio-based polymers with plastic-like properties, PHAs are the only ones that are entirely produced and degraded by living cells. Figure 1 provides a Scanning Transmission Electron Microscopy (STEM) picture of *Cupriavidus necator* cells cultured on glucose as carbon source in a continuous cultivation process. The accumulated PHA inclusions are well visible as bright, refractive granules.

For the producing microbial cells, PHAs fulfil important biological functions; most prominently, they act as reserve and storage materials for energy and carbon (Steinbüchel & Hein, 2001). Under conditions of starvation due to lacking extracellular carbon source, these reserves can be mobilized and utilized as carbon and energy substrates. PHAs are key compounds for the regulation of balanced intracellular energy flow, e.g. for cell motility, and the target-oriented distribution and deviation of carbon reserves to different metabolic pathways. In addition to their role as reserve materials, a variety of important functions of PHAs in various ecosystems was elucidated especially during the last couple of years, such as cell protection against environmental stress conditions like osmotic shock, UV irradiation, desiccation, or thermal or oxidative stress. Further, they are involved in special metabolic on-goings in different microbial species, such as sporulation, cyste formation, germination,

control of exopolysaccharide excretion, and, considering diazotrophic species, in the energy flow during nitrogen fixation (reviewed by Koller et al., 2011). In general, PHA accumulation is favoured by a sufficient availability of carbon source together with a restricted supply with macro-components like nitrogen, phosphate or dissolved oxygen, or micro-components like magnesium, sulphate, and various metals (Kim & Lenz, 2001; Helm et al., 2008, reviewed by Koller et al., 2010).

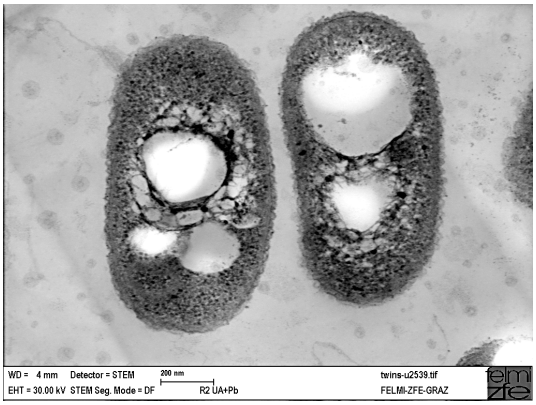

Figure 1. STEM picture of *Cupriavidus necator* cells harbouring about 37 wt.-% of PHA granules. Magnification: 1/65000.

PHAs mainly consist of 3-hydroxyalkanoates (3HAs) as monomeric building blocks. The general chemical structure is provided in Fig. 2.

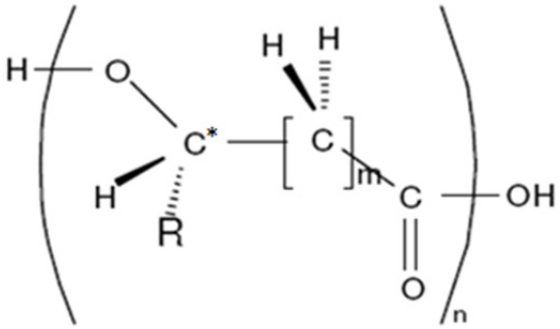

Figure 2. General chemical structure of Polyhydroxyalkanoates (PHAs). The chiral center is indicated by an asterisk (*).

3.3. Composition and production of PHAs

Depended on various factors like the type of microbial production strain, the feeding regime for nutrient supply, and the process parameters during the biosynthesis, PHA polymer chains can contain a magnitude of 10^2 to 10^5 3-hydroxyalkanoate (3HA) monomers. These

3HAs normally are enantiomerically pure, *R*-configurated chiral compounds. Among all known PHAs, Poly([*R*]-3-hydroxybutyrate) (PHB) is the most widely investigated and best characterized one. PHB is a homopolyester consisting merely of 3-hydroxybutyrate (3HB) building blocks; this material features a rather high degree of crystallinity and restricted processability. The low difference between the decomposition temperature (typically around 270°C) and the high melting point (typically around 180°C) provides a too small window of processability for many processing techniques, e.g. in melt extrusion technology or production of polymeric films. This drawback can be overcome by interrupting the crystalline PHB matrix by incorporation of additional building blocks like 3-hydroxyvalerate (3HV) or the achiral building blocks 4-hydroxybutyrate (4HB) and 5-hydroxyvalerate (5HV). This results in co-polyesters with enhanced material properties, opening the door for a broader range of applications. The exact material properties strongly depend on the monomeric composition of the co-polyesters; this composition can be triggered during the PHA biosynthesis by co-feeding of precursor substrates in order to obtain the desired monomeric building blocks. For example, 3HV building blocks are produced by many PHA producing strains if they are supplied with 3HV related precursors, namely odd-numbered fatty acids, such as propanoic or pentanoic acid (Braunegg et al., 1998).

By methods of industrial (white) biotechnology, PHAs can be biosynthesized starting from renewable resources. For this purpose, monosaccharides, lipids, methanol, agroindustrial wastes like the hydrolyzates of various (ligno)cellulosics, starch, beet sugar, cane sugar, maltose, glycerol from biodiesel production, or, as it is the focus of the review at hands, whey lactose from dairy industry, are available in sufficient quantities (Lee, 1996; Braunegg et al., 2007; Koller et al., 2010).

After production of PHA-rich bacterial biomass, appropriate methods of downstream processing are needed, encompassing cell harvest, product isolation and product refining. The biopolymers can be recovered from the microbial cells by extraction, chemical or enzymatic digestion of the cell wall, or by mechanical cell disruption (Kunasundari & Sudesh, 2011). After the release from cells, they can be processed to a marketable form, e.g. to granulates, and used as sustainable biodegradable substitutes for a variety of "classical" petrochemical plastics such as poly(ethylene) (PE), poly(propylene) (PP), poly(ethlyleneterephtalate) (PET) and many others (Reddy et al., 2003; Khanna & Srivastava, 2005; Ren et al., 2005; Chen, 2009). According to calculations accomplished by Akiyama et al. (2003), Harding et al. (2007), Pietrini et al. (2007), and Titz et al. (2012), the production of PHAs should be more beneficial if considering full cradle-to-gate life cycle analysis (LCA) than the production of the petrochemical plastics mentioned before.

4. The potential of Life Cycle Assessment (LCA) in the field of biopolymers

Life cycle analysis (LCA) is an excellent method to quantify the sustainability of a product or a process, encompassing all factors from raw materials, transportation, product manufacture, end use, to disposal (Gonzalez et al., 2010; Champrateep, 2010).

The energy use and water pollution, as well as the hazards of the chemicals are assessed and compared to alternativee products or solutions. The total impact on the environment is quantified for each step (Patel et al., 2005).

As indicated in the ISO-14040 series, LCA methodology distinguishes four phases: goal and scope definition, inventory analysis, impact assessment, and interpretation as reported in Figure 3.

The Goal and Scope Definition involves LCA application, type, reason, and audience together with the geographical and temporal scope. The system boundaries and functional units (FU) are also determined.

The Inventory Analysis (LCI) creates a flow chart for the process where the inputs (energy, materials) and the environmental releases of each process phase are determined.

The Impact Assessment (LCIA) is a consequence of LCI and it represents the environmental impacts related to the functional unit. The results can be grouped and weighted.

The Interpretation is the discussion of the data obtained in previous steps of LCA.

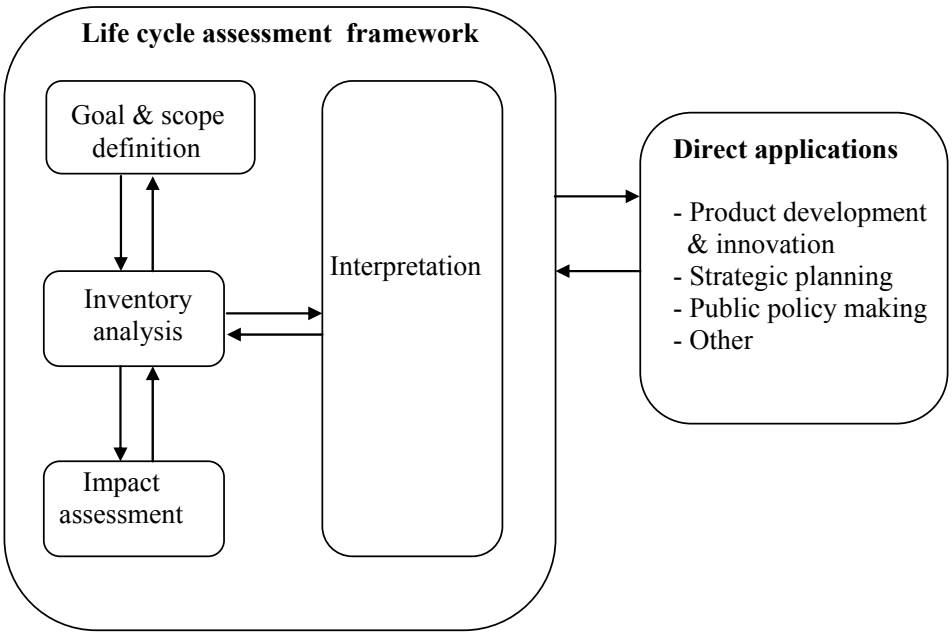

Figure 3. General Scheme for Life Cycle Assessment Studies.

The goal of LCA is the prediction of the environmental benefits that could be reached by the replacing conventional petrochemical polymers with bio-based polymer composites, such as

PHB or PLA. The evaluation of the indicators NREU and GWP100 permits to understand how each step of the LCA and the material properties can influence the final environmental performance of the end products.

In full-scale LCA studies, the impact categories taken into account are eutrophication (EP), acidification (AP), ozone layer depletion (ODP), ecotoxicity (ETP), and human toxicity (HTP).

The functional units are typical commodities made of petrochemical polymers taken into account to perform a comparison with the same items made of materials from renewable resources.

The investigation of all material properties is useful to collect the information on which property has to be improved to maximize the material performance in the application.

The system boundary is a simplified flow chart describing the commodity production in terms of processing technique and production process of all components included in the composite.

The polymer production process considers all the steps from the agricultural cultivation up to down-stream processing.

After the product´s life span, the products post-consumer have to submit to a waste management method where the NREU for transport and waste treatment has been considered 1% of NREU of polymers production. The GWP100 impacts have been calculated by assuming that all the carbon fixed in the petrochemical polymers is converted to carbon dioxide during the combustion process.

In order to reflect energy recovery during incineration of the composites after their useful life, credits are introduced in the calculation. One can assume that for each joule of incinerated waste there is a credit of 0.12 J of electricity and 0.12 J of heat (Patel et al., 2002; Pietrini et al., 2007).

The inventory analysis involves the energy used for the polymer production. For example, when bagasse is used as filler for a PHA based material, the final impact of PHA production must consider the obtainment of the biomass from the renewable resource, as well as a large amount of this can be combusted producing electricity (Pietrini et al., 2007).

For the utilization phase of the commodity, we can consider the correlation between the lightness of the PHB based product for the substitution of the interior car panel, and the car economy in terms of kilometres driven per kilogram of fuels.

The impact assessment is correlated with the indicators NREU and GWP100, from which values is possible to make a comparison between the new products and the conventional ones.

The interpretation is a collection of the previous data, where their complete analysis permits to have an improved application due to the PHB based material (Table 2).

Usually the production of materials from natural resources requires a relatively small amount of fossil energy, because the main contribution comes from solar energy. As a

consequence, also GWP emissions are lower if compared with materials coming from non-renewable resources. In addition the score of other categories could be greatly affected by the cultivation of the renewable resources and complicated processes, possibly involving toxic compounds. Pesticides, needed for the cultivation of sugar cane and corn, can release in the environment a considerable amount of phosphates and nitrates, increasing the final score in HTP, ETP, and EP indicators.

PHA Type or Product	Renewable Resource	Compared Commodity or Process	Petro-Based Polymer	Balance Energy (NREU, GWP100)	Reference
PHAs	Corn grain, corn stover	Film	Polystyrene (PS)	Favorable	Kim et al., 2005
[P3HB-*co*-5mol% 3HHx)]	Soybean oil, glucose	-	LDPE, HDPE, PP, PS, PET	Favorable	Akiyama et al., 2003
-	Genetically engineered corn	-	PE	-	Kurdikar et al., 2001
PHB, PHV	Corn plant, bacterial fermentation	-	HDPE, PET, PS	Favorable	Patel et al., 2002; Shen & Patel, 2008
PHB	Sugar beet, starch, fossil methane, fossil-based methanol	Film	PE, PS	Favorable	Harding et al., 2007; Heyde, 1998
-	Corn crop	-	LDPE, HDPE, PP, PVC,PET, PC	-	Tabone et al., 2010
Mixed culture PHA	Waste stream	-	PS	-	Guerrif et al., 2007
-	Corn grain, corn stover	Ethanol production system	-	Favorable	Anastas et al., 2000
-	Corn plant, bacterial fermentation	bottles	HDPE, PET, PS	Favorable	Kim & Dale, 2004
PHB-SCB PHB-OMMT composites	Sugar cane, corn starch	Cathode ray tube monitor housing, internal panels of an average car	High-impact PS, glass-fibers-filled polypropylene	Favorable	Patel et al., 2002
PHB	Sugar cane crop and waste	Ethanol and sugar production	PE, PP	-	Pietrini et al., 2007
PHB	Beet or cane molasses, plant oils, plant derived fatty acids, alkanes, steam, sucrose, corn	-	PE and PP production	Favorable	Nonato et al., 2001;
PHB	Corn-derived glucose	-	-	Unfavorable	Kendall, 2010
PHB	Corn grain	-	-	Favorable	Kim & Dale, 2008

Table 2. Types of PHA, Originating Renewable Resource, Compared Petrochemical Commodity, the balance energy (NREU, GWP100) and the source reference.

Another very important aspect is represented by the feasibility of using biodegradable materials for durable products. The lifetime of products such as monitor housings or car panels is expected to be in the order of some years. In this view, it is of fundamental importance that the biodegradation of the product does not take place during this useful period.

The life cycle interpretation considers all contributions in terms of energy for each production step for the new PHB based commodity to NREU.

Then it becomes important to know the renewable resource used for PHA production and what the item made of petrochemical polymers it has to compete with in order to evaluate a comparison with the new PHA based material.

4.1. Life Cycle Assessment of PHB Based Composites

A cradle-to-grave environmental life cycle assessment (LCA) of some relevant PHB based composites was performed, with the purpose of assessing the potential environmental benefits that could be reached with the application of these new biodegradable materials instead of petrochemical polymers.

The investigation was pointed out on two functional units, specifically the cathode ray tube (CRT) monitor housing that actually is made of high impact polystyrene (HIPS) with an average weight of 2.2 kg and on the internal panels of an average car with a total distance travelled of 150000 km, that is made of glass fibres filled polypropylene and compatibilizers in the percent of 30/63/7. The used key environmental parameters were the non-renewable energy use (NREU) and global warming potential over a 100 year's time horizon (GWP100).

In particular the assessment was carried out on composites reinforced with 10 and 20 wt-% of BNTf and 5 and 10 wt-% of OMMTSi, because of their enhanced mechanical properties.

Figure 4 shows a simplifying flow chart describing a conventional production of the CRT monitor housing and the internal car panels (Pietrini et al., 2007).

For the collection of cradle-to-factory gate data, all the steps involved in the production of the components mixed in our composites have to be taken into account. So clay production included extraction, ion exchange, hydro-cycloning, spray drying, organic modification, filter pressing, heating and milling (Roes et al., 2007).

The PHB production process involved agricultural cultivation and sugar production, the bio-fermentation and the downstream processing. The sugar used to feed the micro-organisms can be extracted from sugar cane or corn starch. The residual biomass produced along with the sugar was considered to be combusted for electricity production, with an assumed power generation efficiency of about 35%.

The sugar cane milling gives 1-3% of SCB that can be used as PHB filler in correlation with the composition of PHB composite.

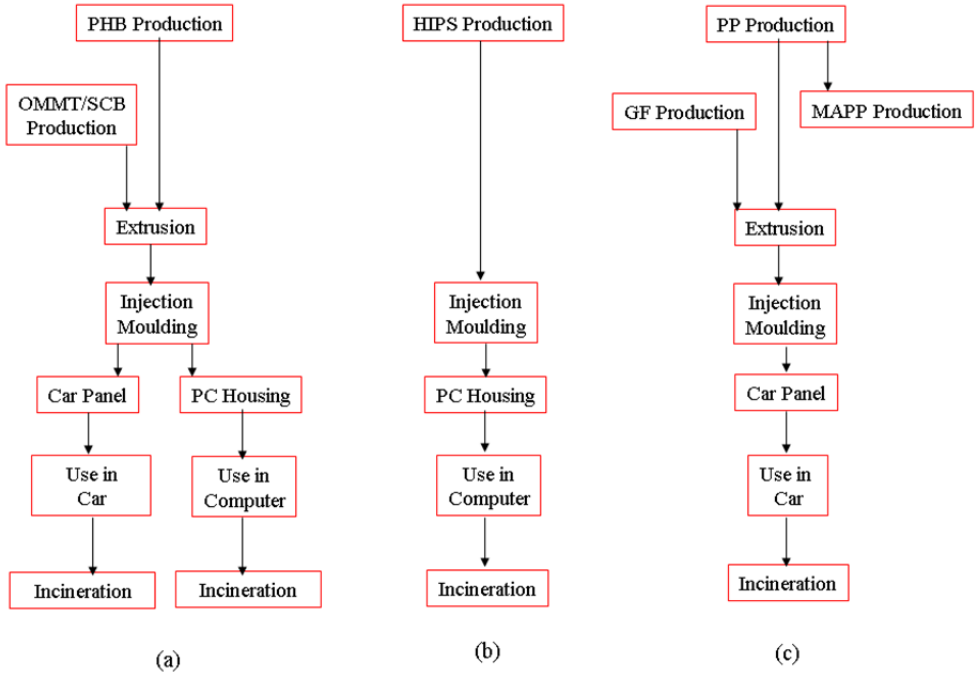

Figure 4. Simplified flow chart for the production process of both functional units: PHB based composites CRT monitor housing and internal car panels (a), HIPS CRT monitor housing (b), PP-GF internal car panels (c).

The maleic acid anhydride (MAPP), a product obtained by the PP and maleic anhydride extrusion in presence of a peroxidic agent, transfers a better adhesion between PP and the fillers inside the PP-GF formulation.

The use phase of internal car panels was represented by the fuel consumption of the car, that is strongly affected by the type of car and the car panels weights.

At the end-life, for both products, an energy recovery from the incineration of the municipal solid waste was assumed.

The NREU for transport and waste treatment was considered 1% of NREU of polymer production, while the GWP100 impacts were calculated assuming that all the carbon fixed in the petrochemical polymers is converted to CO_2 during the combustion process.

For the materials originated from renewable resources (PHB, SCB), this contribution was not considered, because the CO_2 originating from the incineration of the composites is equal to that extracted from the atmosphere during plant growth, creating a closed cycle.

The energy and CO_2 bonus coming from the incineration was calculated on the basis of the calorific value of the polymers and SCB and then deducted from the total NREU and GWP100 impacts.

For the inorganic materials (GF, OMMT), these parameters are neutral, if correlated with the energy and greenhouse gases (GHG) emissions in the waste incineration end stage.

4.2. Inventory analysis

The sugar used for the PHB fermentation process is produced from sugar cane or corn starch.

Both process create also biomass, combusted for the electricity production: a large amount of bagasse can be used for power generation in the first case, while the amount of biomass is smaller if the material used for the PHB fermentation is corn starch.

The indicators NREU and GWP100 have negative values calculated for the final impacts of PHB production in the first process and positive ones for the second case.

For injection moulding, data published by PlasticEurope were used (Boustead et al., 2005).

According to PlasticEurope, the NREU for PP production pellets and injection moulded PP items are 73.0 GJ/t and 113.2 GJ/t respectively.

During the use phase of the car, the share of fuel that can be assigned to the weight of the internal car panels was calculated following these assumptions:

Weight of the car without the panels: 1222 kg
Car weight: 1110 kg and passengers weight:112 kg. The second value is obtained considering an average of 1.6 passengers of 70 kg for each car.
Car life distance: 150000 km
Fuel economy for a conventional car: 15.13 km/kg of fuel, value calculated taking into account that for 1 driven km, 0.0125 kg of diesel and 0.0536 kg of petrol are combusted on average.

Table 3 shows the results of the calculations.

Composite	Panels weight (Kg)	Car fuel consumption (Kg)	Panels fuel use (Kg)
PP-GF	20.0	15.13	115.2
5-OMSi	25.5	15.08	147.0
10-OMSi	25.7	15.08	147.8
10-BNTf	24.6	15.09	141.6
20-BNTf	23.4	15.10	134.9

Table 3. Data used in the calculation of the use phase for internal car panels.

The fuel consumption of the car decreases with increasing weight of panels: the best result for PHB composites is observed for 20BNTf (17% more than PP-GF panels), that is the lightest PHB based product, whereas the larger value of fuel consumption is attributed to 10-OMSi composite (28% more than the conventional panel).

4.3. Impact assessment

Table 4 reports the NREU and GWP100 impacts for all selected products (Pietrini et al., 2007).

	CRT housing		Internal car panels	
	NREU (MJ/FU)	**GWP100 ($kgCO_2eq$/FU)**	**NREU (MJ/FU)**	**GWP100 ($kgCO_2eq$/FU)**
HIPS	200.0	15.1	-	-
PP-GF	-	-	8.2	569.9
PHB1-5-OMSi	8.3	0.5	8.4	602.0
PHB1-10-OMSi	22.4	1.2	8.6	612.1
PHB1-10BNTf	1.3	0.1	8.1	576.3
PHB1-20BNTf	6.8	0.5	7.7	552.6
PHB2-5-OMSi	160.2	8.2	9.9	677.2
PHB2-10-OMSi	167.0	8.6	10.0	683.7
PHB2-10BNTf	139.8	7.2	9.4	645.0
PHB2-20BNTf	124.9	6.4	8.9	610.7

Table 4. Estimation of NREU and GWP100 for the cradle-to-grave system of conventional and PHB based CRT monitor housings and internal car panels.

For CRT monitor housing, all PHB relevant composites scored better if compared to their conventional counterpart made of HIPS. Both indicators were lower both for PHB produced from sugar cane (PHB1) and PHB derived from corn starch (PHB2).

PHB1-10BNTf and PHB1-20BNTf composites showed a reduction for both indicators by about 99% and 97% respect to conventional HIPS housing.

On the other hand, clay filled composites showed savings ranging from 97% (GWP100 of PHb1-5-OMSi) to 89% (NREU of PHB-10-OMSi).

For internal car panels, best results were found for PHB produced from sugar cane (PHB1), even if there were not relevant savings for both indicators, in comparison with the conventional PP-GF panels.

The composite PHB1-20BNTf showed a lower impact for both indicators, with a reduction of around 5% and 3% for NREU and GWP100 respectively.

4.4. Life cycle interpretation

For HIPS, the total NREU was 200 MJ/housing, while the value associated with PHB production is -22.7 GJ/t for PHB produced from sugar cane (PHB1) and 38.6 GJ/t for PHB produced from corn starch (PHB2)(Pietrini et al., 2007).

For PHB based composites, the contribution of injection moulding was higher than for HIPS (from 60 to 66 MJ/housing against 54 MJ/housing for HIPS) because of the higher weight of PHB based monitor housing.

In this process, also the extrusion and the production of the filler must be taken into account.

The energy credit from post-consumer waste incineration was higher for HIPS (44 MJ/housing) compared to PHB composites (from 26 to28 MJ/housing) because of the higher calorific content of HIPS. The use phase for this end product had no contribution, because the electricity use of the monitor did not depend on the weight, the shape or the material of the housing. These properties could influence the transportation of the monitors (to and from retail and to waste management), but this parameter was not included in the calculation, because it was typically negliable.

So the life cycle stage that makes PHB composites so environmentally competitive in this application is the polymer production.

In fact for the HIPS monitor housing, polymer production accounted for 190 MJ/housing, that represents about 80% of the total positive contribution to NREU.

This indicator for the production of PHB1 was negative so the overall NREU connected to PHB1 production decreases by around 43 MJ/housing (PHB1-20BNTf composite) to 56 MJ/housing (PHB1-5-OMSi).

On a qualitative base, analogous considerations could be made in the case of GWP100 for PHB1 based CRT monitor housings. The only substantial difference between the contributions to the two indicators was represented by the incineration step.

CO_2 emissions originated from PHB and SCB incineration stem from renewable resources, so they do not pollute the environment, while the HIPS emissions coming from of fossil polymers were considered polluting.

The contribution to GWP100 due to the incineration was calculated assuming the complete conversion of the carbon contained in the composite to CO_2: this consideration gives CO_2 emissions of around 7 and 5 kg for HIPS and PHB based monitor housings respectively, due to the higher carbon content calculated for HIPS respect to PHB and SCB.

However, an equivalent CO_2 amount was decreased from the GWP100 emissions related to PHB and SCB production, in order to reflect the neutrality of bio-based materials with regard to CO_2 emissions.

The internal car panels showed very different results: the main difference compared to the display housing was the large contribution of the use phase in the case of the car panels.

Considering a cradle-to-factory gate system for the LCA data collection related to the car panels, the results would be quite similar to the display housing (Pietrini et al., 2007).

The NREU of PHB based internal car panels represented only approximately 15% (PHB1-10BNTf) to 25% (PHB1-10-OMSi) of NREU value of PP-GF panels.

When the use phase was included the NREU for the end products from PHB composites scored much worse than the product made from conventional polymer. The reason is the higher weight of the composites that leads to an higher fuel consumption.

While for the CRT monitor the housing have not any influence on electricity consumption of the monitor, in this case the fuel consumption of the car is related to the weight of the internal panels.

The energy recovery without incineration represented 84% of total NREU for PP-GF service phase, while is 100% of the total impact for PHB based composites.

Hence, the low mechanical properties and high densities of the composites nullify all the savings provided by the environmentally favourable PHB production process. The same considerations are valid for the GWP100 impact of the panels, although the contribution of the use phase to the final value of this indicator was higher if compared to NREU.

The GWP100 value was influenced by the large amount of carbon dioxide produced during fuel combustion.

4.5. Sensitivity analysis on internal car panels

The relation between the relative impacts of both NREU and GWP100 and PHB1 based composite for car internal panels is represented by an exponential trend (Pietrini et al., 2007).

So it becomes possible to evaluate the critical values of Young modulus that should be reached by PHB based composites in order to render these materials environmentally attractive in the case of the car panels application.

PHB1-20BNTf was the only composite that showed lower values for both relative indicators at the measured Young modulus (1.73 GPa) if compared with the conventional panels.

The composite PHB1-10BNTf showed values of relative NREU and GWP100 of 0.99 and 1.01, indicating that the environmental performances of the panels made with this composite can be considered equivalent to those of the panels made with PP-GF composite.

The higher values of relative impacts were found for clay filled composites: these impacts were found to be from 3% (NREU of PHB1-5-OMSi) to 7% (GWP100of PHB1-10-OMSi) higher that the impact calculated for the conventional PP-GF panels.

So PHB1-5-OMSi and PHB1-10-OMSi composites should reach the values of Young modulus of 2.1 and 2.3 GPa respectively.

5. The broad field of potential applications of PHAs

Concerning the potential applications, PHAs constitute very versatile materials that raise the attention of different industrial branches. As the best-known and most simple application, these biopolymers are of interest for packaging purposes, especially in such areas where compostable packaging is wanted, e.g. in the food producing industry. Especially in the field of packaging of easily spoiling food, the high oxygen barrier of PHA films is very beneficial. In addition, bottles for shampoos (Wella, Germany) made of PHAs were commercially available in the past. PHAs can be used for paper coating, production of daily commodity items like razors, diapers, hygiene products, or cups and dishes (Metabolix, USA; BASF, Germany). For these applications, PHAs can be processed by techniques of injection moulding or film blowing using the same equipment as known from the well-established processing of petrochemical plastics.

In the medical field, PHAs were already investigated as bone implant materials, for tissue engineering, for *in-vivo* application as implants, surgical pins, screws, meshes and sutures, and as carrier matrices for controlled drug release. Also the production of highly sophisticated surgical articles such as artificial blood vessels and vein valves, spinal fusion cages, bone marrow scaffolds, and meniscus regeneration devices is reported (Chen and Wu, 2005). Especially the possibility to change the composition of PHA allows the manufacture of materials with tailor-made mechanical properties and a fine-tuned degradation rate under *in-vivo* conditions. In fact, one can expect the fast increase of the number of medical applications for PHAs and its composites; a first step in this direction was done recently by approval of Poly-4-hydroxybutyrate (P4HB) as implant material (http://www.tepha.com).

Hydrolysis of PHA to the monomers generally results in a rich source of chiral synthons that can be used as starting materials for synthesis of fine chemicals and marketable products such as pheromons, aromatics, vitamins or antibiotics, or can even be used as pharmaceutically active compounds (Ren et al., 2005). It was demonstrated that 3HB and its oligomers reveal therapeutic effects. They promote cell proliferation and prevent necrotic cell death. Some of these chiral acids also display biological activity against pathogenic bacteria or viruses (Ruth et al., 2007).

Further, PHAs harbouring special building blocks can be applied as so-called "functional materials" for different niche applications. Here, they can act as heat sensitive adhesives, latex materials, or smart gels (reviewed by Chen et al., 2010a)

A completely new field of application for PHA is their conversion towards alkyl esters by means of transesterification. Here, the conversion leads to 3-hydroxyalkanoate methyl esters (3HAME) (Zhang et al., 2009). Chemically, 3HAME have a composition very similar to esters stemming from the alkaline transesterification of vegetable oils or tallow, the so called "biodiesel". In fact, PHA-stemming alkyl esters of 3HAs were successfully tested as engine fuels; similar combustion heats were determined for 3HAME if compared with gasoline. The conversion of PHA to "biofuels" seems to be reasonable for such biopolymer fractions that

show modest material properties, e.g. PHA blends stemming from mixed cultures grown in sewage water. PHA produced by such microbial consortia often contain building blocks with a rather long carbon side chain, so called medium chain length (mcl) PHAs. Examples for such mcl-building blocks are 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate (3HO), 3-hydroxydecanoate (3HD), and 3-hydroxydodecanoate (3HDD). The longer the carbon chain, the higher the expected combustion heat for the deriving 3HAMEs should be.

6. Economics challenges in the production of PHAs

Despite the huge efforts globally devoted to biopolymer research, PHAs are still not really competitive to petrochemical plastics mainly considering production costs and, to a certain extent, also regarding the material properties (Choi & Lee, 1999; Sudesh & Iwata, 2008; Koller et al., 2010). A major share of up to half of the entire production costs is related to the carbon substrates. Hence, the reduction of these expenses by utilizing cheap carbon-rich raw materials available in huge quantities is the first pre-condition to make the PHA production process economically competitive. Considering the increasing amounts of carbon-rich whey as discussed above, it is easily understandable that this surplus material has attracted and still attracts the attention of several research groups active in the field of PHA biopolymers.

In addition to the substrate expenses, costs have to be saved by optimizing the downstream processing for PHA recovery and refining after cell harvest. As intracellular products, PHAs have to be separated from the surrounding non-PHA cell mass, mainly consisting of proteins, lipids, nucleic acids and special polysaccharides. Here, high input of (often hazardous!) solvents and enormous energy demand still constitute the state-of-the art in PHA recovery, compromising the demanding claims of these bio-plastics to be ecologically sound materials (Koller et al., 2010).

Apart from the selected raw materials and the downstream processing, the increasing of productivity by designing of the optimal engineering set-up is indispensable for the final break-through of these biopolymers on the market. Batch and fed-batch discontinuous fermentation mode are up to date the most common techniques for microbial PHA production (Kim et al., 1994; Ryu et al., 1997; Ahn et al., 2000; Nonato et al., 2001; Atlić et al., 2011). In contrast, continuous biotechnological production mode is well known as a precious tool for achieving high productivities, lower costs and constant product quality. Due to these facts, a growing number of research activities were accomplished during the last couple of years, investigating and assessing the potential of continuous PHA production processes (Zinn et al. 2003; Sun et al. 2007; Atlić et al., 2011). Recently, the continuous production of PHB in a five-stage cascade of stirred bioreactors was investigated. Here, the biopolymer was produced on glucose by eubacterial producing strains belonging to *Cupriavidus necator* species. As a main results, the authors report high productivities of 1,85 g/L h for PHB and a constant and satisfying product quality (Atlić et al., 2011).

Figure 4 illustrates schematically the principle process line for PHA biopolymer production from the feedstock to the final commercial bioplastic product (Chen et al., 2010b).

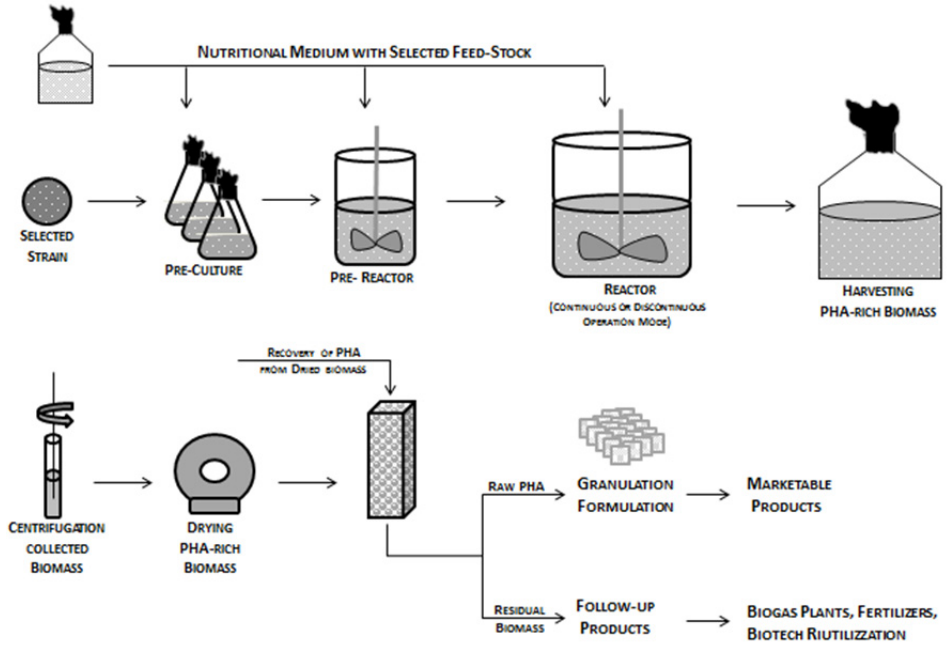

Figure 5. General process scheme for PHA biopolymer production

7. Current routes of march in PHA research

Nowadays, research in the field of PHAs focuses on several key topics. The application of growth additives that shorten the time for production of catalytically active biomass is a pre-requisite to enhance the entire volumetric productivity of the process. Such cheap growth additives can be found in agriculture, e.g. side streams from the cultivation of green grass land, and were already tested successfully on laboratory scale (Koller et al., 2005b). Concerning the raw materials, simple "unrelated" carbon sources that are available at low prices, or even constitute waste streams, should act as sole feedstocks for production of high-value PHA co-polyesters. This provides the possibility to safe costs for precursor compounds normally needed for co-polyester production.

Efforts done in the field of genetic engineering mainly aim at the increase of volumetric PHA productivity and at higher molecular masses of the biopolymers. This can be accomplished *via* the knock-out of the genes encoding for the enzymes responsible for intracellular PHA degradation, so called depolymerases. Such a strategy could also face other metabolic bottle necks that can hamper a fast and complete substrate conversion by the selected strain. For instance, *Cupriavidus necator*, a well-known PHA producer, but unable to grow on lactose, has been genetically modified in order to construct a recombinant strain that can use lactose-containing waste material such as cheese whey, and one of the intracellular PHA depolymerases (*phaZ1*) was chosen to insert the *lacZ*, *lacI* and *lacO* genes of *Escherichia coli* (Povolo et al., 2010)

This would have the effect to allow polymer production on lactose and, at the same time, to remove part of the PHA intracellular degradation system.

The enhancement of the microbial oxygen uptake by inserting genes encoding catalase or peroxidase was also attempted. This should enhance microbial growth kinetics and higher final biomass concentrations (Ouyang, 2007).

In the field of downstream processing, environmentally safe and efficient solvents are investigated for enhanced recovery of PHA from the cells. This goes in parallel with the examination of novel biological lysis methods and enhanced strategies for mechanical cell disruption. In any case, enhanced downstream processing has to feature lower energy demands if compared to the contemporary methods. For efficient polymer recovery, the increase of the intracellular polymer content as well as the increase of the PHA granule size is of importance; these factors are determined during the PHA bio-production (Chen, 2010a). Of course, also the remaining non-PHA cell mass (NPCM) has to be converted in a sustainable, value-adding way. Research in this direction is devoted to the anaerobic digestion of NPCM in biogas plants, or to the chemical or enzymatic hydrolysis of NPCM to a rich carbon- and nitrogen source for subsequent microbial cultivations. As an alternative, NCPM can be applied in agriculture as "green fertilizer". Also downstream processing can be facilitated by genetic modification; an excretion of high quantities of nuclease enzymes after cell disruption results in decreased amounts of nucleic acids in the medium, leading to lower viscosities that facilitate the separation of PHA granules from the surrounding liquid phase by centrifugation or flocculation. With this aim, a nuclease-encoding gene from *Staphylococcus aureus* was integrated into the genomes of a number of PHA-producing bacterial species, including *Ralstonia eutropha* (now renamed as *C. necator*). Here, the staphylococcal nuclease was functionally integrated into the chromosome and readily expressed in *Pseudomonas* strains, directed to the periplasm and occasionally to the culture medium, without affecting PHA production or strain stability (Boynton et al., 1999).

The technological drawbacks of the bio-production itself can be handled by the application of robust microbial production strains that remain genetically stable for a long time period under continuous cultivation conditions, and, at the same time, can resist the risk of contamination by microbial competitors. Here, extremophilic species like the highly salt requiring archaeon *Haloferax mediterranei* might be a viable solution in order to minimize the normally indispensible, highly energy demanding sterility requirements for PHA production set-ups (Koller et al., 2007a,b). In future, continuous PHA production should not only aim at the increase of volumetric productivity, but should also open the door for tailor-made material properties by fine-tuning the polyester composition. This can be accomplished by the formation of block-copolymers, where the sequential arrangement of softer and harder polymer parts can result in well-adjusted novel polymeric materials. Here, a multistage bioreactor cascade for PHA production as presented by Atlić and colleagues (2011) might be the adequate process engineering equipment.

During the last two to three decades, the preparation of composites and blends has become one of the key research fields in biopolymer science. For enhancement of the material properties, PHAs can be processed together with a variety of compatible matters, resulting in the creation of novel PHA-based blends and composites. For this purpose, the utilization of polymeric materials like Poly(vinyl alcohol) (PVA), poly-ε-caprolactone (PCL) etc., including synthetic analogues of PHA (e.g. atactic PHB), inorganic fillers (clays, sepiolites, Montmorillonite, or calcium carbonate), and organic fillers of agricultural origin was already tested (Chiellini et al., 2004; Pietrini et al., 2007). Concerning fillers from agriculture, the application of surplus materials such as lignocelluloses like sugar cane bagasse, wheat flour, fruit peels, crop fruit fibres, saw dust and wheat straw is reported in literature (Chiellini et al., 2004). In general, nanocomposites and natural fibers composites can be distinguished. Nanocomposites have the potential to improve special polymer properties, such as gas permeability and thermal and mechanical characteristics. For creation of nanocomposites, rather small amounts of filler, commonly an organophilic modified clay, are needed for efficient enhancement of the properties. Natural fibers composites often display excellent mechanical properties, and, as desired for many applications, they lower the density of the final product. Due to the fact that in most cases fibres constituting agricultural residues are used as fillers, the biodegradability of the final product is enhanced. Because such fibers do not feature a considerable price, this normally goes in parallel with a reduction of the entire production cost of the marketable product (reviewed by Pietrini et al., 2007).

8. From the feedstock milk to whey to PHA biopolyesters

Cheese whey is a surplus product in the dairy industry. Figure 6 illustrates the process line from the feed stock milk via whey towards PHA biopolyester production. From the feed stock milk, casein is precipitated enzymatically or by acidification. This so called "transformation" results in the generation of solid curd cheese (predominately consisting of caseins), and liquid full fat whey. After removing the major part of lipids from full fat whey by skimming, skimmed whey remains. Sweet skimmed whey is subjected to a concentration step, removing 80% of its water content. This whey concentrate is separated via ultra-filtration in whey permeate (carbohydrate fraction) and whey retentate (protein fraction with considerable lactose residues).

Whereas whey permeate that contains about 80% of the lactose originally included in milk can be used as carbon source for fermentative production of e.g. PHA, special proteins of the retentate like lactoferrin and lactoferricin are of significance for pharmaceutical application. The predominant proteins in whey retentate, namely α-lactalbumin and β-lactoglobulin, are potential candidates for food- and feed supplements. Biotechnologically, they can be applied as nitrogen source for enhanced cultivation of microbial PHA production strains. More recently, plastic films coated by whey proteins are developed; also here, classical polymers are replaced by recyclable, bio-based materials featuring low oxygen and moisture permeability, making them especially interesting for food packaging (http://www.wheylayer.eu/project.html). Table 5 summarizes the composition of sweet whey, fermented whey, whey permeate and retentate.

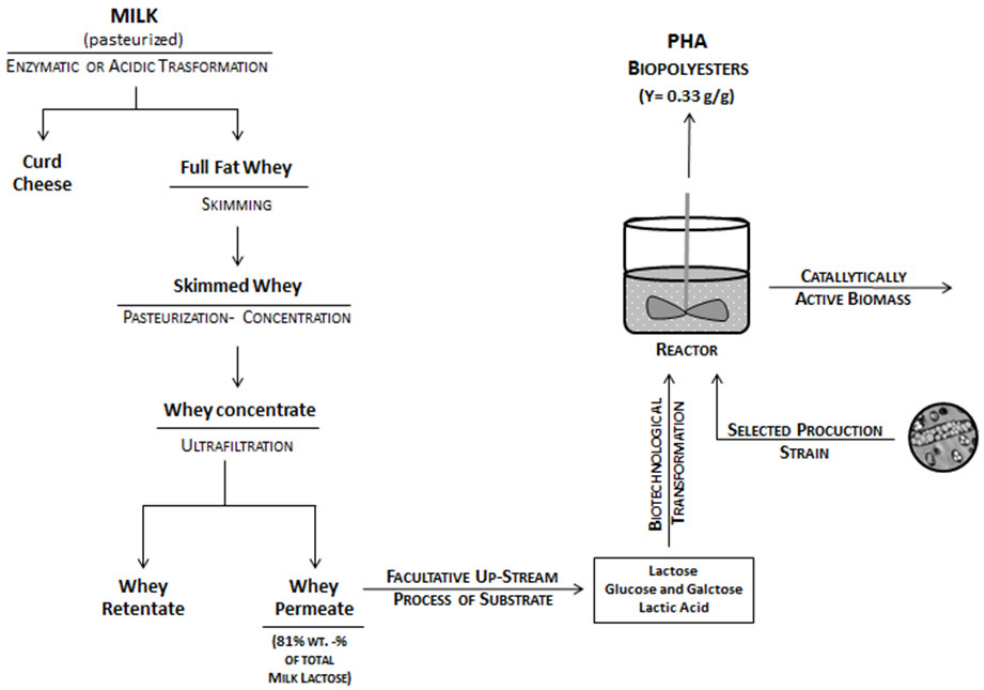

Figure 6. From the feedstock milk to whey to the biopolyester including a rough estimation of the material balances. The necessity for hydrolysis and/or desalting of the raw material whey depends on the PHA production strain (see text)

Compound [% (w/w)]	Sweet Whey	Fermented Whey	Whey Permeate	Whey Retentate
Lactose	4.7–4.9	4.5–4.9	23	14
Lactic acid	traces	0.5	-	-
Proteins	0.75–1.1	0.45	0.75	13
Lipids	0.15–0.2	traces	-	3-4
Inorganic compounds (minerals like e.g. calcium)	ca. 7	6-7	ca. 27	ca. 7

Table 5. Composition of different types of whey (Braunegg et al., 2007)

9. Reported PHA production from whey by different microbial strains

Converting the pollutant whey into valuable products combines the creation of economic benefit with abating of inherent ecological concerns. Biotechnological production of PHAs from different sugars via condensation of Acetyl-CoA units stemming from the catabolic breakdown of simple sugars such as hexoses is well known and exhaustively described in literature (Braunegg et al., 1998; Lee, 1996; Steinbüchel, 1991; Steinbüchel & Valentin, 1995; Sudesh et al., 2008). Nevertheless, only a rather restricted number of prokaryotic wild-type microorganisms directly convert the disaccharide lactose into PHAs (Young et al., 1994; Koller et al., 2008a,b). Reports for PHA biopolyester production starting from whey lactose as the main or even sole carbon source is reported in literature for microbial wild-type species as well as for genetically modified strains, mainly for engineered *Escherichia coli*. Volumetric productivities for PHA of more than 4 g/L h can be obtained by optimized experimental set-ups (Ahn et al., 2001).

In 1994, Young and co-workers delivered the very first report for PHB production from the whey-related carbon source lactose by a wild-type strain, namely *Pseudomonas cepacia* (now renamed to *Burkholderia cepacia*). Here, the authors asses the strain´s potential for growth and PHB production on shaking flask scale on the sugars lactose, glucose and xylose. Focusing on lactose, the organism was able to accumulate up to 56% of PHB in cell dry mass that amounted to nearby 5 g/L. Some years later, an Austrian research group reports further experiments dealing with PHA production by this promising organism on an artificial carbon substrate consisting of an equimolar mixture of glucose and galactose, corresponding to a hydrolysed lactose solution. This experiment was carried out under controlled conditions on bioreactor scale; the results obtained exceeded by far the data obtained from lactose on shaking flask scale (volumetric productivity for PHB: 0.18 g/Lh, final PHB concentration 4.0 g/L). The authors concluded that the application of hydrolysed lactose (mixture of the monomeric sugars) for this organism is more beneficial in terms of specific growth rates, and propose the application of enzymatically hydrolysed whey lactose for cost efficient PHA production based on the surplus material whey (Wallner et al., 2001).

From a *Bacillus megaterium* strain isolated from the sludge of a sewage treatment plant, the production of a rather low amount of 26% PHB in cell dry mass from lactose was reported on shaking flask scale (Omar et al., 2001).

The principal possibility of direct conversion of whey lactose towards PHA using different wild type bacterial strains was first investigated on shaking flask scale. Yellore and Desai (1998) isolated a *Methylobacterium* sp. ZP24 from a local pond in India. This organism grew on pure lactose and, on shaking flask scale, produced PHB at a final concentration of 3.1 g/L polymer, corresponding to a PHA content in biomass of about 59%. Using whey as substrate and optimization of the nitrogen supply, the PHB yield amounted to up to 2.6 g/L, corresponding to a final percentage of PHB in biomass of 44%. These findings were extended later on bioreactor scale, where Nath and colleagues (2008) cultivated this organism on cheese whey. In batch mode, final PHB concentrations of 2.07 g/L with 67% of PHB in biomass and 0.06 g/L h of volumetric PHB productivity are reported.

Povolo & Casella (2003) reported the production of PHA from lactose by *Paracoccus denitrificans* DSM 413, *Sinorhizobium meliloti* 41 and *Hydrogenophaga pseudoflava* DSM1034. The latter two strains were also able to produce the polymer directly from cheese whey permeate and especially *Hydrogenophaga pseudoflava* turned out to be a promising candidate for PHA production from whey. The same research group constructed a genetically engineered *Cupriavidus necator* strain harbouring the *lacZ*, *lacI* and *lacO* genes of *Escherichia coli* for lactose conversion. The recombinant strains obtained here were able to produce the polymer directly from lactose and from whey permeate. In addition, as reported above, the insertion of the lac operon within *phaZ* gene may reduce the amount of PHA depolymerised by the cell, thus improving the final polymer yield (Povolo et al., 2010). Catalán and co-workers (2007) constructed a modified strain of *Herbaspirillum seropedicae* Z69 carrying the *lacZ*, *lacI* and *lacO* genes of *Escherichia coli* for lactose conversion in plasmid pHM3. They obtained production of 1.8 g/L PHB with 5 g/L biomass in shaking flasks experiments.

Recombinant *Escherichia coli* strains harbouring PHA synthesis genes were also well studied for directly converting lactose to PHAs. In contrast to the work presented by Povolo et al., (2010), these activities start from a direct lactose converter (*E. coli*) that is later equipped with the required genes for PHA production. These works are of special interest due to high volumetric productivities found, opening a route for industrial scale PHA production from lactose (Lee, 1997; Wong & Lee, 1998). These high productivities are mainly due to the fact that only the genetic information for PHA production is inserted into the cells, but not the information for PHA degradation. Moreover, also the direct utilization of whey permeate as carbon source for growth and PHA production by recombinant *E. coli* strains is well investigated (Ahn et al., 2000; Ahn et al., 2001; Kim, 2000). The until today most promising results for PHA biosynthesis on whey by recombinant *E. coli*, namely a final PHA concentration of 168 g/L and a volumetric PHA productivity of 4.6 g/L h are reported by Ahn and colleagues (2001). In this study, the authors employed a cell-recycle system in order to overcome the typical problems arising from the continuous addition of the whey feed in fed batch cultures, i.e. a rapidly increasing volume of fermentation broth in the bioreactor.

If the natural *ß*-galactosidase activity featured by an investigated production strain is not sufficiently high for efficient conversion of the substrate lactose, additional processing of lactose prior to the application as substrate is needed. Here, the disaccharide can be hydrolyzed by enzymatic or chemical means to equimolar mixtures of the monomeric sugars D-(+)-glucose and D-(+)-galactose. Compared to lactose these monosaccharides are converted by a much higher number of organisms together with typically higher conversion rates (Koller et al., 2008b).

A third, more complex possibility arises from the anaerobic conversion of lactose to lactic acid in a first process step using lactobacilli capable of producing lactic acid with high yields (more than 0.9 g of lactic acid per gram of carbon source). In a subsequent aerobic cultivation lactic acid is metabolized to acetyl-CoA and further to PHAs by numerous strains, e.g. most common PHA producers like *Cupriavidus necator* or *Alcaligenes latus* or *Azotobacter vinelandii*. Alternatively, lactic acid can be converted to polylactic acid (PLA), if wanted.

Based on the above, for PHA production from whey, the decision for applying whey lactose, hydrolysed whey lactose or first-step fermentation towards lactic acid mainly depends on the production strain (Fig.7).

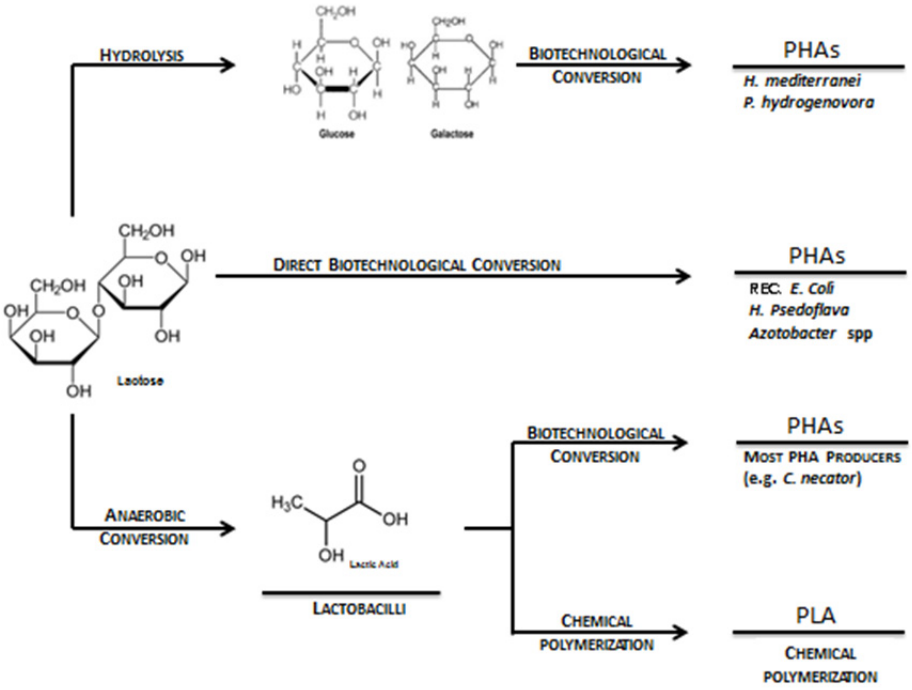

Figure 7. Three different routes from whey lactose to PHA; alternative products like PLA and follow-up products of PHA are included

In a recent study, Koller and colleagues (2007a) compare the potential of three prokaryotic wild type strains for utilization of whey as carbon source for PHA production. In this work, the archaeon *Haloferax mediterranei*, and the eubacterial strains *Pseudomonas hydrogenovora* and *Hydrogenophaga pseudoflava* were investigated in laboratory scale bioreactors. Among these organisms, *H. mediterranei* turned out to be the most promising candidate for eventual industrial scale PHA production starting from whey. This is due to the strain's high robustness and stability; the risk of microbial contamination during cultivation is restricted to an absolute minimum, thus a lot of energy can be saved by the lower sterility demands. Additionally the strain produces a P(3HB-*co*-8%-3HV) copolyester directly from the 3HV-unrelated carbon source whereby the normally high costs for propanoic acid or pentanoic acid as precursors can be saved. The strain grew well on hydrolyzed whey permeate with a maximum specific growth rate μ_{max} of 0.11 1/h. PHA was accumulated at a maximum specific production rate of 0.08 g/g h. The conversion yield for whey sugars to PHA was calculated with 0.33 g/g. After further optimizing of the production conditions, the productivity for this strain on hydrolysed whey permeate was increased to 0.09 g/L h

(specific rate 0.15 g/g h); 16.8 g/L biomass containing 73% PHA were obtained (Koller et al., 2007b). By co-feeding of precursors for 3HV and 4HB production (pentanoic acid and γ-butyrolactone, respectively) together with hydrolysed whey permeate as main carbon source, a high value P-(3HB-*co*-21.8%-3HV-*co*-5.1%-4HB) terpolyester was produced by *H. mediterranei*. Also in this case, the polymer was recovered from the cells and underwent a detailed characterization of thermal properties and molecular mass distribution. The promising results for polymer characterization indicate that the material might be of special interest for application in the medical field (Koller et al., 2007b). The partial conversion of whey sugars to 3-hydroxyvalerate (3HV) units and the excellent polymer characteristics (low melting temperature, high molecular masses within narrow distribution) together with a viable cheap and simple downstream processing (Munoz et al., 1994) make the strain especially interesting. The estimated production price amounted to € 2.82 per kg PHA. For further improvement, the recycling of the highly saline side streams has to be tested and optimized. Additionally, high salinity requires special material demands for the bioreactor equipment and the probes (Hezayen et al., 2000).

P. hydrogenovora features the disadvantage of low final polymer contents, low productivities and product yields due to redirection of the carbon flux towards unwanted by-products such as organic acid. Using this organism, the final PHB homopolyester content amounted to 12 wt.-% (q_P: 2.9 mg/g h). By co-feeding of pentanoic acid, the strain accumulated 12 wt.-% of poly-3(HB-*co*-21%-HV) (q_P: 2.0 mg/g h) (Koller et al., 2007a; Koller et al., 2008a, b).

H. pseudoflava produces biopolyesters of rather good quality (high molecular masses and low polydispersities) directly from whey lactose at acceptable specific production rates and yields, but is not competitive with *H. mediterranei* in terms of strain stability and robustness. In details, using this strain, 40 wt.-% of poly-3(HB-*co*-5%-HV) in cells with addition of pentanoic acid (q_P: 12.5 mg/g h) were obtained. Without pentanoic acid, the strain accumulated 30 wt.-% of the homopolyester PHB in cells (q_P: 16.0 mg/g h)(Koller et al., 2007a).

A number of very recent reports describe the production of PHA by other bacterial species, suggesting that the occurrence of these traits in new isolates from various environmental samples is underestimated and has still to be deeply investigated.

For instance, the thermophilic bacterium *Thermus thermophilus* HB8 (DSM 579) was found to be able to utilize lactose from whey-based media, using both glucose and galactose, for the biosynthesis of polyhydroxyalkanoates under nitrogen limitation (Pantazaki et al., 2009) PHA was accumulated up to 35% (w/w) of its biomass and revealed a novel heteropolymer consisting of the short chain length 3-hydroxyvalerate (3HV; 38 mol%) and the medium chain length 3-hydroxyheptanoate (3HHp; 9.89 mol%), 3-hydroxynonanoate (3HN; 16.59 mol%) and 3-hydroxyundecanoate (3HU; 35.42 mol%).

Moreover, *Azohydromonas lata* DSM 1123 was reported to produce poly-3(HB-*co*-27%-HV) from whey hydrolysate (Baei et al., 2010), *Bacillus megaterium* CCM 2032 was shown to accumulate more than 50% of its biomass (w/w) in optimized whey media (Obruca et al., 2011).

The subsequent Table 2 collects the data for PHA production from lactose, whey and whey-derived substrates available in literature as discussed above. Here, the applied microbial production strains, the pre-treatment of the raw material whey, PHA productivities, final concentrations of cell mass and PHA biopolymer, production scale and information about post synthetic polymer characterization are provided.

Production strain	Applied carbon source	Type of PHA produced	Vol. productivity for PHA [g/L h]	CDM final [g/L]	PHA final [g/L]	Information about PHA quality available	Production scale	Reference
Pseudomonas cepacia ATCC 17759	Lactose	PHB	0.03	4.9	2.75	Yes: Molecular mass characterization	Shaking flask scale	Young et al., 1994
Pseudomonas cepacia ATCC 17759	Equimolar mixtures of glucose and galactose	PHB	0.18	16.7	4.0	no	Shaking flask scale	Young et al., 1994
rec. E. coli GCSC 4401 (pSYL107)	bovine whey powder solution	PHB	n.r.	6.4	5.2	no	Shaking flask scale	Lee et al., 1997
rec. E. coli GCSC 6576 (pSYL107)	bovine whey powder solution	PHB	n.r.	5.7	4.5	no	Shaking flask scale	Lee et al., 1997
rec. Cupriavidus necator mRePT	lactose	PHB	0.04	8.1	0.21	no	Shaking flask scale	Povolo et al., 2010
rec. Cupriavidus necator mRePT	Hydrolyzed whey permeate	PHB	0.05	8.0	2.4	no	Shaking flask scale	Povolo et al., 2010
rec. Cupriavidus necator mRePT	Not hydrolyzed whey permeate	PHB	0.03	6.5	1.4	no	Shaking flask scale	Povolo et al., 2010
Sinorhizobium melioti 41	Whey permeate	PHB	0.00018	0.483	0.017	no	Shaking flask scale	Povolo & Casella, 2003
Hydrogenophaga pseudoflava DSM 1034	Whey permeate	PHB	0.00018	0.375	0.017	no	Shaking flask scale	Povolo & Casella, 2003
rec. Escherichia coli CGSC 4401 harbouring pJC4	Processed bovine whey powder solution; controlled DOC	PHB	1.15	83.1	46.8	no	6.6-liter jar fermentor; fed-Batch	Ahn et al., 2000
rec. Escherichia coli CGSC 4401 harbouring pJC4	Processed bovine whey powder solution; higher concentration of whey feed	PHB	1.42	102.9	59.6	no	6.6-liter jar fermentor; fed-Batch	Ahn et al., 2000

Production strain	Applied carbon source	Type of PHA produced	Vol. productivity for PHA [g/L h]	CDM final [g/L]	PHA final [g/L]	Information about PHA quality available	Production scale	Reference
rec. Escherichia coli CGSC 4401 harbouring pJC4	Processed bovine whey powder solution; controlled DOC	PHB	2.57	119.5	96.2	no	6.6-liter jar fermentor; fed-Batch	Ahn et al., 2000
rec. Escherichia coli CGSC 4401 harbouring pJC4	Processed bovine whey powder solution;	PHB	4.6	194	168	no	6.6-liter jar fermentor; Fed-Batch with cell recycle membrane module system	Ahn et al., 2001
Hydrogenophaga pseudoflava DSM 1034	Whey permeate + 3HV precursor pentanoic acid	P(3HB-*co*-4,6%-3HV)	0.05	6.7	2.7	yes: Thermal analysis characterization, Molecular mass characterization	Bioreactor, 10L; Fed-Batch	Koller et al., 2007a
Pseudomonas hydrogenovora DSM 1749	Enzymatically hydrolyzed whey permeate	PHB	0.03	10.6	1.27	yes: Thermal analysis characterization, Molecular mass characterization	Bioreactor, 2L; Fed-Batch	Koller et al., 2007a, Koller et al., 2008
Pseudomonas hydrogenovora DSM 1749	Enzymatically hydrolyzed whey permeate + 3HV precursor pentanoic acid	P(3HB-*co*-21%-3HV)	0.05	11.7	1.44	yes: Thermal analysis characterization, Molecular mass characterization	Bioreactor, 2L; Fed-Batch	Koller et al., 2008
Haloferax mediterranei DSM 1411	Enzymatically hydrolyzed whey permeate	P(3HB-*co*-8%-3HV)	0.05	11.0	5.5	yes: Thermal analysis characterization, Molecular mass characterization	Bioreactor, 42L; Fed-Batch	Koller et al., 2007a
Haloferax mediterranei DSM 1411	Enzymatically hydrolyzed whey permeate	P(3HB-*co*-6%-3HV)	0.09	16.8	12.2	yes: Thermal analysis characterization, Molecular mass characterization	Bioreactor, 42L; Fed-Batch	Koller et al., 2007b
Haloferax mediterranei DSM 1411	Enzymatically hydrolyzed whey permeate + 3HV precursor pentanoic acid + 4HB precursor γ-butyrolactone	P-(3HB-*co*-21.8%-3HV-*co*-5.1%-4HB)	0.14	16.8	14.7	yes: Thermal analysis characterization, Molecular mass characterization	Bioreactor, 10L; Fed-Batch	Koller et al., 2007b
Methylobacterium sp. ZP24	Lactose	PHB	0.08	5.25	3.1	no	Shaking flask scale	Yellore & Desai, 1998

Production strain	Applied carbon source	Type of PHA produced	Vol. productivity for PHA [g/L h]	CDM final [g/L]	PHA final [g/L]	Information about PHA quality available	Production scale	Reference
Methylobacterium sp. ZP24	Whey supernatant	PHB	0.017	3.8	0.8	no	Shaking flask scale	Yellore & Desai, 1998
Methylobacterium sp. ZP24	Whey supernatant + nitrogen	PHB	0.054	5.9	2.6	no	Shaking flask scale	Yellore & Desai, 1998
Methylobacterium sp. ZP24	Whole whey	PHB	0.023	5.1	1.1	no	Shaking flask scale	Yellore & Desai, 1998
Methylobacterium sp. ZP24	Whole whey + nitrogen	PHB	0.008	7.1	0.4	no	Shaking flask scale	Yellore & Desai, 1998
Methylobacterium sp. ZP24	Processed cheese whey	PHB	0.06	3.34	2.07	no	semiautomatic jar fermenter, 2 L Batch	Nath et al., 2008
Methylobacterium sp. ZP24	Processed cheese whey	PHB	0.09	5.53	3.54	no	semiautomatic jar fermenter, 2 L Fed-Batch	Nath et al., 2008
Methylobacterium sp. ZP24	Processed cheese whey	PHB	0.056		3.91	no	semiautomatic jar fermenter, 30 L Fed-Batch	Nath et al., 2008
Bacillus megaterium	lactose	PHB	0.01	1.8	0.47	no	Shaking flask scale	Omar et al., 2001
Bacillus megaterium SRKP-3	Dairy waste	PHB	0.31	n.r.	11.32	no	Bioreactor, 3 L; Fed-Batch	Pandian et al., 2010
Bacillus megaterium CCM2037	whey	PHB		2.87	1.46	no	Shaking flask scale	Obruca et al., 2011
Azohydromonas lata DSM 1123	whey	P(3HB-co-12.7%-3HV)		9.2	1.66	no	Shaking flask scale	Baei et al, 2010
Herbaspirillum seropedicae Z69 (pHM3)	lactose	PHB		5	1.8		Shaking flask scale	Catalan et al., 2007

Table 6. PHA production from lactose, whey and whey-derived substrates: an overview about literature data

10. Mathematical models for value-added conversion of whey

Due to the complex nature of the regulatory mechanism in PHA production, mathematical modelling of the processes, based on experimental results, becomes more and more interesting. This is especially true for multi-substrate carbon sources like whey. Such models provide a precious tool to shorten the number of needed experiments during biotechnological process development. The first, early developed mathematical models for PHA synthesis (Sonnleitner et al., 1979; Heinzle & Lafferty, 1980) have postulated a "two

compartment" strategy which is applicable and valid till today. Biomass (*X*) was structured as having two components:

1. The catalytically active fraction consisting of proteins, glycolipids, glycoproteins and nucleic acids (known as residual biomass);
2. The intracellular, catalytically inert fraction which is the biopolymer (PHB).

In addition, it was experimentally proved that:

3. Nitrogen (N) or phosphorus (P), on the one hand, act as limiting substrates affecting the growth kinetics, but N or P starvation are inductivity factors provoking PHA synthesis;
4. TIn some strains, the production of PHB is uncoupled from the biomass growth (not growth associated PHA production), whereas in some other strains PHB synthesis can be evidenced in both, growth and stationary phase respectively. In the last case, PHB synthesis occurs with significantly different rates (partially associated PHA production to biomass growth).

In above citied works, the specific growth rate was defined as function of C-source and N-source concentration respectively. Limitation by N-source was expressed as linear sum of two different kinetics: Monod saturation type and sigmoidal Hill type. Above postulates are also useful tools for modelling of processes based on whey as C-source. In literature a lot of different types of mathematical models (formal kinetic, low and high structured models) are available arranged for microbia1 PHA production, but predominantly dealing with glucose, fructose, sucrose and glycerol as the C sources (Sonnleitner et al., 1979; Mulchadani et al., 1988; Raje & Srivastava, 1998; Leaf & Srienc, 1998; Katoh et al., 1999; Patwardhan & Srivastava, 2004; Franz et al., 2011). In contrast, mathematical models of processes with whey itself or with whey sugars are still rare.

Different model types can be considered as suitable for this purpose, depending on chemical composition of whey (raw native whey or whey permeate, partially fermented whey, hydrolysed whey or permeate) and on PHA accumulating microorganism (growth associated or partially growth associated synthesis of PHA). Native whey contains lactose (C-source), casein (complex N-source), phosphorus compounds, and, due to partial fermentation, lactic acid and some other metabolites (see also Table 5). Due to its high N- and P-content, this substrate is not suitable for microbial PHA synthesis, especially not for partially growth associated PHA producers with week lactase expression (able to activate PHA synthesis under N/P starvation). In contrast, hydrolysed casein-free whey permeate containing hydrolysed lactose (i.e. glucose and galactose) is more promising for this purpose. However, if any type of whey will be used as the C-source, the multisubstrate kinetic models will be necessary for successful modelling of biomass growth. Recently, unstructured mathematical models for production of PHB from whey and sucrose by *Azotobacter vinelandii* were developed using kinetic patterns from batch experiments (Dhanasekar & Viruthagiri, 2005). The mentioned organism is able to use sucrose, cane molasses and cheese whey as a C-source. It is a growth associated PHB producer which

synthetizes product also in the non-growth stationary phase. The authors have applied Logistic equation and modified Logistic equation (Mulchadani et al., 1988) for the modelling of sigmoidal growth curves (to compensate a certain lag-phase and to reduce it to the desired length) combined with Leudeking-Piret specific growth rate/specific production rate relation (Leudeking & Piret, 1959).

For the biosynthesis of P(3HB-*co*-3HV-*co*-4HB) terpolyesters from whey plus cosubstrates by *H. mediterranei*, Koller et al. (2006) compiled a formal kinetic model as well as a low structured model for PHB synthesis from whey by *P. hydrogenovora*. Batch and feed-batch processes with *H. mediteranei* were modelled by these authors using the subsequent assumptions:

a. Residual biomass (non-PHA biomass) is synthesized from all three main carbon sources i.e. glucose, galactose and γ-butyrolactone (independent growth on each substrate according Monod relation was introduced!) and from yeast extract as an obligate complex nitrogen source.
b. There is no direct influence of the consumption of one C-source on the consumption rate of two other C-sources (simultaneous independent consumption of C-sources).
c. Both 3HB and 3HV were supposed to be synthesized from both glucose and galactose, 4HB is synthesized only from γ -butyrolactone.
d. PHA synthesis is inversely influenced by the increase of the intracellular mass fraction of PHA (known as steric disturbing effect). The equation according to Luong (1985) was applied for this purpose.

Metabolic pathways for the applied microorganism were not well known (except a few basic metabolic routes!). This is especially valid for the strain's galactose degradation pathway. An insufficient metabolic knowledge was the reason why authors have chosen a formal-kinetic modelling instead of low structured or high structured metabolic flux model as available for *Escherichia coli* (Van Aalst et al., 1997; Van Wegen et al., 2001). Simulated values of process variables were in good but not in excellent agreement with the experimental data. This is especially true for PHB concentration in the time period of late exponential phase and for glucose concentration in the transient time period before stationary phase of growth.

Additionally, based on experiences and results of above formal-kinetic model a low structured metabolic mathematical model for fed-batch cultivation of *Pseudomonas hydrogenovora* was established. *P. hydrogenovora* is a typical strain having 3HB synthesis provoked by nitrogen limitation of growth. The total uncoupling of growth phase and 3HB synthesis phase (''non-growth-associated PHA production'') was evidenced. Here, the production of PHB from glucose and galactose (C-sources) using ammonia and casein hydrolyzate as N-sources was studied.

The following assumptions were necessary to establish a useful mathematical model:

Ac-CoA (originated from the central metabolic pathways - that mean from the metabolism of both sugars) is chosen as the ubiquitary precursor for PHB. This was based on the fact

that all sugar degrading pathways in microorganisms (Entner–Doudoroff, pentose phosphate, Leloir, DeLey–Doudoroff, and EMP pathway) lead to EMP substrates (i.e. pyruvate, consecutively transformed to Ac-CoA!). Ac-CoA was chosen because it constitutes the substrate for the thiolase reaction (first step in the PHB synthesis), and for the citrate synthase reaction (substrates for biomass synthesis). Furthermore, the breakdown of the complex nitrogen source (deamination and degradation of casamino acids) leads to the Ac-CoA. An independent Monod kinetic of Ac-CoA synthesis from both sugars was applied as the modelling strategy.

i. Negative feedback control mechanism of Ac-CoA synthesis is built in as strategy for its own regulation /Luong type of inhibition pattern; Luong, (1985)/.
ii. Ac-CoA is foreseen for consumption toward biomass formation, energy supply including maintenance energy (NADPH generation in TCA cycle), 3HB accumulation (thiolase substrate), α-ketoglutarate excretion, for one chemically unknown excreted compound production and for PHB-polymerase synthesis.
iii. A small quantity of biomass is assumed to be synthesized directly from the complex nitrogen source (casamino acids could be used as a separate C/N source).
iv. PHB synthesis rate is proportional to the intracellular biocatalyst concentration (PHB-polymerase complex). Its synthesis rate from Ac-CoA and nitrogen sources (multiple Michaelis-Menten kinetic) with desired degradation rate (protein turnover) was assumed.
v. Ac-CoA synthesis from sugars is inhibited (but not stopped) by complex nitrogen (Jerusalimsky type of inhibitory influence applied).
vi. The PHB-polymerase complex synthesis is inhibited by the presence of high level of complex nitrogen source (Jerusalimsky type of kinetic equation), its activity started after complex nitrogen sources was almost completely depleted.
vii. Inorganic nitrogen source consumption is inhibited by complex nitrogen source (no consumption of ammonia in the first part of growth phase when casamino acids were provided at sufficient amounts).
viii. The excreted metabolite α-ketoglutarate is assumed to be a negative feedback controller of own synthesis as well as for unknown metabolite. Ammonia and PHB polymerase were adopted as inhibitory agents of metabolites production (Jerusalimsky type of equation).

The authors emphasise that the developed models provide feasible tools for better understanding the complex intracellular on-goings during PHA production from multi-substrate carbon sources without a specific analysis of the involved enzymatic reactions.

Despite of the complex intracellular regulatory mechanisms of PHA metabolism and despite of complexity of whey as the substrate, mathematical modelling of PHA synthesis could be a powerful tool to predict different situations and to reduce the number of needed experimental set ups. Significant progress has been achieved during the last two decades in the field of metabolic flux and metabolic control modelling (Ghadkar et al., 2003;

Thilakavathi et al., 2007; Franz et al., 2011). Cited cybernetic, kinetic, dynamic and structured metabolic modelling methods have not been applied yet in the analysis of PHA production from whey. The developed models are structured kinetic models, simulating the effects of enzyme and metabolite concentrations on the rate of PHB synthesis in microorganisms (Franz et al., 2011). They suggest how the physiological state of the cell, intracellular concentrations of metabolites, and enzyme levels affect the rate of PHB synthesis.

Extensive experimental tools, suitable computational methods, a detailed kinetically-metabolic knowledge of metabolic fluxes are necessary for the development of high structured models. That's why for practical laboratory and industrial purposes respectively, a certain degree of simplification of the model structure is desired and welcome. A typical example for this type of simplification was done by Visser and colleagues (2004) dealing with the metabolic modelling of *Saccharomyces cerevisiae*. Compared to more detailed models, the simulations with simplified models are less accurate. This simplified approach in modelling is perhaps a promising tool in the case when metabolic pathways and/or rate control mechanisms are insufficiently known, and therefore the building of metabolic flux models is not possible.

11. Conclusion

The study presents a strategy how industrial waste streams like surplus whey can be upgraded to the role of substrates for production of high-value bio-products. The selection of waste materials like surplus whey from dairy industry can be regarded as the most promising route to make the entire PHA biopolymer production process economically competitive to the petrochemical competitors like PE and PP. Improvements in the fermentation strategy by switching from discontinuous to continuous mode can be considered as another decisive step for achieving enhanced productivities and to obtain tailor-made bioplastics under constant qualities. Beside the raw material costs and the fermentation process itself, downstream processing for polymer recovery from the surrounding cells is a decisive cost-determining factor in biopolymer production.

Uniting the potential enhancements of each process step, one can definitely make substantial progress towards a cost-efficient technology. In any case, the development of really efficient biopolymer production processes needs the narrow cooperation of experts from different scientific fields. Chemical engineers, microbiologists, enzymologists, polymer scientists, genetic engineers and experts in the fields of mathematical modelling of bio-processes, LCA and Cleaner Production studies have to concentrate their special expertise and know-how in order to close the existing gaps between promising data from the laboratory scale and industrial realization.

Independent from the selected production strain, an industrial plant for PHA production from whey should be integrated into existing process lines of large dairies, were the raw material whey directly accrues. This can be considered as a viable strategy to minimize

production costs by taking profit of synergistic effects. Regarding the already broad amount of available data from literature, one can conclude that important progress has been achieved in terms of combining the environmental benefit of future-oriented biopolyesters with a cost efficiency that makes them interesting for potential industrial partners.

Author details

Martin Koller*
Graz University of Technology; Institute of Biotechnology & Biochemical Engineering, Graz, Austria
ARENA Arbeitsgemeinschaft für Ressourcenschonende & Nachhaltige Technologien, Graz, Austria

Anna Salerno, Alexander Muhr and Angelika Reiterer
Graz University of Technology; Institute of Biotechnology & Biochemical Engineering, Graz, Austria

Emo Chiellini
Laboratory of Bioactive Polymeric Materials for Biomedical and Environmental Applications via Vecchia Livornese, Loc. S. Piero a Grado (PI), University of Pisa, Department of Chemistry and Industrial Chemistry, Pisa, Italy

Sergio Casella
DAFNAE, Department of Agronomy Food Natural Resources Animals and Environment, Università degli Studi di Padova, Legnaro, Padova, Italy

Predrag Horvat
Department of Biochemical Engineering, Faculty of Food Technology and Biotechnology, University of Zagreb, Croatia

Gerhart Braunegg
ARENA Arbeitsgemeinschaft für Ressourcenschonende & Nachhaltige Technologien, Graz, Austria

Acknowledgement

The authors thank for financial support by the European Commission, granting the FP5 and FP7 projects WHEYPOL (GRD2-2000-30385) and ANIMPOL (Contract No: 245084) and the Austrian project FFG-Laura Bassi, centre of expertise "BRIC – BioResobable Implats for Children". In addition, the authors are thankful for the electron microscopic picture of *C. necator* cells that was provided by Dr. Elisabeth Ingolić, FELMI-ZFE Graz.

12. 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 Environmental Microbiology*. 66(8): 3624–3627.

* Corresponding Author

Ahn, W.S., Park, S.J. & Lee, S.Y. (2001). Production of poly(3-hydroxybutyrate) from whey by cell recycle fed-batch culture of recombinant *Escherichia coli*, *Biotechnological Letters* 23: 235-240.

Akiyama, M., Tsuge, T. & Doi, Y. (2003). Environmental Life Cycle Comparison of Polyhydroxyalkanoates Produced from Renewable Carbon Resources by Bacterial Fermentation, *Polymer Degradation and Stability* 80: 183-194.

Anastas, P.T. & Lankey, R.L. (2000). Life Cycle Assessment and Green Chemistry: the Yin and the Yang of Industrial Ecology, *Green Chemistry* 2: 289-295.

Atlic, A., Koller, M., Scherzer, D., Kutschera, C., Grillo Fernandes, E., Horvat, P., Chiellini, E. & Braunegg, G. (2011). Continuous Production of Poly([(R]-3-hydroxybutyrate) by *Cupriavidus necator* in a Multistage Bioreactor Cascade, *Applied microbiology and biotechnology* 91: 295-304.

Audic, J.L., Chaufer, B. & Daufin, G. (2003). Non-food applications of milk components and dairy co-products: A review, *De Lait* 83(6): 417-438.

Baei, M.S., Najafpour, G.D., Lasemi, Z., Tabandeh, F., Younesi, H., Issazadeh, H. & Khodabandeh, M. (2010). Optimization PHAs production from dairy industry wastewater (cheese whey) by *Azohydromonas lata* DSMZ 1123, *Iranica Journal of Energy and Environment* 1(2): 132-136.

Boustead, I. (2005). Eco-Profiles of the European Plastic Industry: Polypropylene (PP). *Plastics Europe*, Brussels.

Boynton, Z.L., Koon, J.J., Brennan, E.M., Clouart, J.D., Horowitz, D.M., Gerngross, T.U. & Huisman, G.W. (1999). Reduction of Cell Lysate Viscosity during Processing of Poly(3-Hydroxyalkanoates) by Chromosomal Integration of the Staphylococcal Nuclease Gene in *Pseudomonas putida*, *Applied and Environmental Microbiology* 65(4): 1524–1529.

Braunegg, G., Koller, M., Hesse, P.J., Kutschera, C., Bona, R., Hermann, C., Horvat, P., Neto, J. & Dos Santos Pereira, L. (2007). Production of Plastics from Waste Derived from Agrofood Industry, *in*: Graziani, M. & Fornasiero, P. (eds.), *Renewable resources and renewable energy: a global challenge*, Taylor and Francis Group, Boca Raton, New York, London, pp. 119 – 135.

Braunegg, G., Lefebvre, G. & Genser, K.F. (1998). Polyhydroxyalkanoates, biopolyesters from renewable resources: physiological and engineering aspects, *Journal of Biotechnology* 65(2,3):127-161.

Bulhões, A.C., Goldani, H.A.S., Oliveira, F.S., Matte, U.S., Mazzuca, R.B. & Silveira, T.R. (2007). Correlation between lactose absorption and the C/T-13910 and G/A-22018 mutations of the lactase-phlorizin hydrolase (LCT) gene in adult-type hypolactasia, *Brazilian Journal of Medical and Biological Research* 40 (11): 1441–6.

Catalán, A.I., Ferreira, F., Gill, P.R. & Batista, S. (2007). Production of polyhydroxyalkanoates by *Herbaspirillum seropedicae* grown with different sole carbon sources and on lactose when engineered to express the lacZlacY genes, *Enzyme and Microbial Technology* 40: 1352–1357.

Champrateep, S. (2010). Current trends in biodegradable Polyhydroxyalkanoates, *Journal of Bioscience and Bioengineering* 110 (6): 621-632.

Chen, G.Q. & Wu, Q. (2005). Polyhydroxyalkanoates as tissue engineering materials, *Biomaterials* 26:6565-6578.

Chen, C.Q. (2009) A microbial polyhydroxyalkanoates (PHA) based bio- and materials industry, *Chemical Society Review* 38:2434–2446.

Chen, G.Q. (2010) Plastics completely synthesized by bacteria: Polyhydroxyalkanoates, *in*: G.-Q. Chen (ed.), *Plastics from Bacteria.*, Alexander Steinbüchel, Series (ed.) *Microbiology Monographs*, Springer-Verlag, Berlin, Heidelberg, pp. 17-37.

Chen, G.Q. (2010), Industrial Production of PHA. *in*: G.-Q. Chen (ed.), *Plastics from Bacteria. Microbiology Monographs,* Alexander Steinbüchel, Series (ed.) Springer-Verlag, Berlin, Heidelberg, pp. 121-132.

Chiellini, E., Cinelli, P., Chiellini, F. & S.H. Imam (2004). Environmentally Degradable Bio-Based Polymeric Blends and Composites, *Macromolecular Biosciences* 4 (3): 218-231.

Choi, J. & Lee, S.Y. (1999). Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation, *Applied Microbiological Biotechnology* 51:13-21.

Dhanasekar, R. & Viruthagiri, T. (2005). Batch kinetics and modeling of poly-β-hydroxy butyrate synthesis from *Azotobacter vinelandii* using different carbon sources, *Indian Journal of Chemical Technology* 12(*3*): 322-326.

Daniel Ferrari, M., Bianco, R., Froche, C. & Loperena, M.L. (2001). Baker's yeast production from molasses/cheese whey mixtures, *Biotechnology Letters* 23: 1–4.

Daniel, H.J., Otto, R.T., Binder, M., Reuss, M. & Syldatk, C. (1999). Production of sophorolipids from whey: development of a two-stage process with *Cryptococcus curvatus* ATCC 20509 and *Candida bombicola* ATCC 22214 using deproteinized whey concentrates as substrates, *Applied Microbiology and Biotechnology* 51(1):40-45.

De Palma Revillion, J.P., Brandelli, A. & Záchia Ayub, M.A. (2003). Production of yeast extract from whey using *Kluyveromyces marxianus*, *Brasilian Archives of Biology and Technology* 46(1): 121-127.

Franz, A., Song, H., Ramkrishna, D. & Kienle, A. (2011). Experimental and Theorethical analysiys of Poly (b-hydroxybutyrate) formation and consumptions in *Ralstonia eutropha*, *Biochem Engineering Journal* 55:49-58.

Gadkar, K.G., Doyle, F.J., Crowley, J.T. & Varner, J.G. (2003). Cybernetic Model Control of a continuous Bioreactor with Cell Recycle, *Biotechnology Progress* 19: 1487-1497.

Gänzle, M.G., Haase, G. & Jelen, P. (2008). Lactose: crystallization, hydrolysis and value-added derivatives, *International Dairy Journal* 18:685–94.

Ghaley, A.E. & El-Taweel, A.A. (1997). Kinetic modelling of continuous production of ethanol from cheese whey, *Biomass and Bioenergy* 12(6), 461-472.

Gonzales Siso, M.I. (1996) The biotechnological utilization of cheese whey. A review. Bioresearch Technology, 57: 1-11.

Gonzalez, C.J. & Woodley, J.M. (2010). Bioprocesses: Modelling needs for process evaluation and sustainability assessment, *Computers and Chemical Engineering* 34: 1009-1017.

Gueriff, N. & Lant, P. (2007). Comparative Life Cycle Assessment and Financial Analysis of Mixed Culture Polyhydroxyalkanoate Production, *Bioresource Technology* 98: 3393-3403.

Guimarães, P.M.R., Teixeira, J.A. & Domingues, L. (2010). Fermentation of lactose to bio-ethanol by yeasts as part of integrated solutions for the valorisation of cheese whey, *Biotechnology Advances* 28:375–384.

Guo-Qiang, C. & Qiong, W. (2005). The application of polyhydroxyalkanoates as tissue engineering materials, *Biomaterials* 26 : 6565–6578.

Harding, K.G., Dennis, J.S., Blottnitz, H.V. & 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 Biothechnology* 130: 57-66.

Heinzle, E. & Lafferty, R.M. (1980). A mathematical model for growth and synthesis of poly-b-hydroxybutyric acid (PHB) in *Alcalygenes eutrophus* H16, *European Journal of Applied Microbiology and Biotechnology* 11: 8-16.

Helm, J., Wendlandt, K.D., Jechorek, M. & Stottmeister, U. (2008). Potassium deficiency results in accumulation of ultra-high molecular weight poly-beta-hydroxybutyrate in a methane-utilizing mixed culture, *Journal of applied microbiology* 105(4):1054-61.

Heyde, M. (1998). Ecological Considerations on the Use and Production of Biosynthetic and Synthetic Biodegradable Polymers, *Polymer Degradation & Stability* 59: 3-6.

Heyman, M.B., (2006). Lactose Intolerance in Infants, Children, and Adolescents, *Pediatrics* 118(3): 1279–1286.

Hezayen, F.F., Rehm, B.H., Eberhardt, R. & Steinbuchel, A. (2000). Polymer production by two newly isolated extremely halophilic archaea: application of a novel corrosion-resistant bioreactor, *Applied Microbiology and Biotechnology* 54(3): 319-25.

Hickmann Flôres, S. & Monte Alegre, R. (2001). Nisin production from *Lactococcus lactis* A.T.C.C. 7962 using supplemented whey permeate, *Biotechnology and Applied Biochemistry* 34: 103–107.

Illanes, A. (2011). Whey upgrading by enzyme biocatalysis, *Electronic Journal of Biotechnology* 14(6): 15.

Katoh, T., Yuguchi, H., Shi, H. & Shimizu, K. (1999) Dynamics and modeling on fermentative production of poly (β-hydroxybutyric acid) from sugars via lactate by a mixed culture of *Lactobacillus delbrueckii* and *Alcaligenes eutrophus*, *Journal of Biotechnology* 67(2-3): 113-134.

Khanna, S., Srivastava, A.K. (2005). Recent advances in microbial Polyhydroxyalkanoates, *Process Biochemistry*. 40, 607–619

Kendall, A. (2010). A life cycle assessment of biopolymer production from material recovery facility residuals, *Resources, Conservation and Recycling* 61: 69-74.

Kim, B.S., Lee, S.C., Lee, S.Y., Chang, H.N., Chang, Y.K. & Woo S.I. (1994). Production of poly(3-hydroxybutyric acid) by fedbatch culture of *Alcaligenes eutrophus* with glucose concentration control. *Biotechnology and Bioengineering* 43:892-898.

Kim, H.O., Wee, Y.J., Kim, J.N., Yun, J.S., Ryu, H.W. (1995). Production of lactic acid from cheese whey by batch and repeated batch cultures of *Lactobacillu*s sp. RKY2. Applied Biochemestry and Biotechnology 129-132:694-704

Kim, B.S. (2000) Production of poly(3-hydroxybutyrate) from inexpensive substrates, *Enzyme and Microbial Technology* 27: 774–777.

Kim, S. & Dale, B.E. (2005)Life cycle assessment of integrated biorefinery-cropping systems: all biomass is local," in *Agriculture as a Producer and Consumer of Energy*, JL Oulaw, KJ Collins, and JA Duffield (Eds.), CAB International, Wallingford, Oxfordshire, UK, 2005, conference proceedings of Farm Foundation and USDA Office of Energy Policy and New Uses, Arlington, VA, June 24-25, 2004.

Kim, S. & Dale, B.E. (2005). Life Cycle Assessment Study of Biopolymers (Polyhydroxyalkanoates) Derived from No-Tilled Corn, *International Journal of Life Cycle Assessment* 10(3): 200-210.

Kim, S. & Dale B.E (2008). Energy and greenhouse gas profiles of polyhydroxyalkanoates derived from corn grain: a life cycle perspective, *Environmental Science and Technology* 42: 7690-7695.

Kim, Y.B. & Lenz, R.W. (2001). Polyesters from microorganisms, *Advances in Biochemical Engineering/Biotechnology* 71:51-79.

Koller, M., Bona, R., Braunegg, G., Hermann, C., Horvat, P., Kroutil, M., Martinz, J., Neto, J., Varila, P. & Pereira, L. (2005a). Production of Polyhydroxyalkanoates from Agricultural Waste and Surplus Materials, *Biomacromolecules* 6: 561-565.

Koller, M., Bona, R., Hermann, C., Horvat, P., Martinz, J., Neto, , Pereira, L., J., Varila, P. & Braunegg, G. (2005b).Biotechnological production of poly(3-hydroxybutyrate) with *Wautersia eutropha* by application of green grass juice and silage juice as additional complex substrates, *Biocatalysis and Biotransformation* 23(5): 329–337.

Koller, M., Hesse, P.J., Bona, R., Kutschera, C., Atlic, A. & Braunegg, G. (2007a). Various Archae- and Eubacterial Strains as Potential Polyhydroxyalkanoate Producers from Whey Lactose, *Macromolecular bioscience* 7: 218 – 2266.

Koller, M., Hesse, P.J., Bona, R., Kutschera, C., Atlic, A. & Braunegg, G. (2007b). Biosynthesis of High Quality Polyhydroxyalkanoate Co- and Terpolyesters for Potential Medical Application by the Archaeon *Haloferax mediterranei*, *Macromolecular symposia* 253: 33-39.

Koller, M., Atlic, A., Gonzalez-Garcia, Y., Kutschera, C. & Braunegg, G. (2008a). Polyhydroxyalkanoate (PHA) Biosynthesis from Whey Lactose, *Macromolecular symposia* 27: 287-92.

Koller, M., Bona, R., Chiellini, E., Grillo Fernandes, E., Horvat, P., Kutschera, C., Hesse P.J. & Braunegg, G. (2008b). Polyhydroxyalkanoate production from whey by *Pseudomonas hydrogenovora*, *Bioresource technology* 99: 4854-4863.

Koller, M., Salerno, A., Miranda de Sousa Dias, M., Reiterer, A. & Braunegg, G. (2010). Modern Biotechnological Polymer Synthesis: A Review, *Food technology and biotechnology* 48 (3), 255 – 269.

Koller, M., Gasser, I., Schmid, F. & Berg, G. (2011). Linking ecology with economy: insights into polyhydroxyalkanoate-producing microorganisms, *Engineering in life sciences* 11(3):222-237.

Kosikowski , F.V. (1979) Whey utilisation and whey products, *Journal of Dairy Science* 62: 1149-60

Kunasundari, B. & Sudesh, K. (2011). Isolation and recovery of microbial polyhydroxyalkanoates, *eXPRESS Polymer Letters* 5(7): 620–634.

Kurdikar, D., Fournet, L., Slater, S.C., Paster, M., Gerngross, T.U. & Coulon, R. (2001). Greenhouse Gas Profile of a Plastic Material Derived from a Genetically Modified Plant, *Journal of Industrial Ecology* 4(3): 107-122.

Leaf, T.E.A. & Srienc, F. (1998). Metabolic modeling of polyhydroxybutyrate biosynthesis, *Biotechnology and Bioengineering* 57(5): 557.

Lee, S.Y., Middelberg, A.P.J. & Lee, Y.K. (1997). Poly(3-hydroxybutyrate) production from whey using recombinant Escherichia coli, *Biotechnology Letters* 19(10):1033–1035.

Lee, S.Y. (1996). Bacterial Poly- b-hydroxyalkanoates, *Biotechnology and Bioengineering* 49: 1-14.

Luedeking, R. & Piret E.L. (1959). Transient and steady states in continuous fermentation. Theory and experiment, *Biotechnology and Bioengineering* 1(*4*): 431-459.

Luong, J.H.T. (1985). Kinetics of ethanol inhibition in alcohol fermentation, *Biotechnology and Bioengineering* 27(*3*):

Mulchandani ALuong, J.H.T. & Groom, C. (1988). Substrate inhibition kinetics for microbial growth and synthesis of PHB by *Alcaligenes eutrophus* ATCC 17697, *Applied Microbiology and Biotechnology* 32:639–646.

Munoz, E.A., Rodriguez-Valera, F. & Marcilla Gomis, A. (1994). Procedure for the extraction of polyhydroxyalkanoates from halophilic bacteria which contain them, European Patent 0622462.

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 cultures of *Methylobacterium* sp. ZP24. *Bioressource Technology* 99(13):5749-5755.

Nonato, R.V., Mantelatto, P.E. & Rossell, C.E. (2001). Integrated Production of Biodegradable Plastic, Sugar and Ethanol, *Applied Microbiology and Biotechnology* 57: 1-5.

Obruca, S., Marova, I., Melusova, S. & Mravcova, M. (2011). Production of polyhydroxyalkanoates from cheese whey employing *Bacillus megaterium* CCM 2037, *Annals Microbiology* 61: 947–953.

Omar, S., Rayes, A., Eqaab, A., Viss, I. & Steinbuchel, A. (2001). Optimization of cell growth and poly(3-hydroxybutyrate) accumulation on date syrup by a *Bacillus megaterium* strain, *Biotechnology Letters* 23:1119–1123.

Ouyang, S.P., Liu, Q., Fang, L. & Chen, G.Q. (2007). Construction of pha-Operon-Defined Knockout Mutants of *Pseudomonas putida* KT2442 and their Applications in Poly(hydroxyalkanoate) Production, *Macromolecular Bioscience* 7(2): 227–233.

Pandian S.RK., Deepak, V., Kalishwaralal, K., Rameshkumar, N., Jeyaraj, M. & Gurunathan, S. (2010). Optimization and fed-batch production of PHB utilizing dairy waste and sea water as nutrient sources by *Bacillus megatrium* SRKP-3, Bioresource Technology 101: 705-711.

Pantazaki, A.A., Papaneophytou, C.P., Pritsa, A.G., Liakopoulou-Kyriakides, M. & Kyriakidis, D.A. (2009). Production of polyhydroxyalkanoates from whey by *Thermus thermophilus* HB8, *Process Biochemistry* 44: 847-853.

Parrondo, J., Herrero, M., García, L.A. & Díaz, M. (2003). A Note Production of Vinegar from Whey, *The Journal of the The Institute of Brewing&Distilling* 109(4):356–358.

Shen, L. & Patel, M.K. (2008). Life Cycle Assessment of Polysaccharide Materials: A Review, *Journal of Polymers and the Environment*, 16(2): 154-167.

Patel, M.K., Bastioli, C., Marini, L. & Wurdinger, G.E. (2005). Life-cycle Assessment of Bio-based Polymers and Natural Fiber Composites Environmental Assessment of Bio-based Polymers and Natural Fibres, *Biopolymers online* 21-24.

Patwardhan, P.R., Srivastava, A.K. & Luong J.H.T. (1985). Kinetics of ethanol inhibition in alcohol fermentation, *Biochemical Engineering Journal* 20:21–28.

Peters, D. (2006). Carbohydrates for fermentation, *Biotechnology Journal* 1(7-8): 806-814.

Phylipsen, D., Kerssemeeckers, K., Blok, K., Patel, M.K. & de Beer, J. (2002). Clean Technologies in the Materials Sector- Current and Future Environmental Performance of Material Technologies, Report Commissioned by European Commission's Institute for Prospective Technological Studies (IPTS), Ecofys, Department of Science, Technology and Society, Utrecht University, Utrecht, 2002.

Pietrini, M., Roes, L., Patel, M.K. & Chiellini, E. (2007). Comparative Life Cycle Studies on Poly(3-hydroxyalkanoate)-Based Composites as Potential Replacement for Conventional Petrochemical Plastics, *Biomacromolecules* 8: 2210-2218.

Povolo, S. & Casella, S. (2003). Bacterial production of PHA from Lactose and cheese whey permeate, *Macromolecular symposia* 197:1-9.

Povolo, S., Toffano, P., Basaglia, M., Casella, S. (2010). Polyhydroxyalkanoates production by engineered *Cupriavidus necator* from waste material containing lactose, *Bioresource Technology* 101: 7902–7907

Reddy, C.S.K., Ghai, R., Rashmi, K.V.C. (2003). Polyhydroxyalkanoates: an overview, *Bioresource Technology* 87:137–146.

Ren, Q., Grubelnik, A., Hoerler, M., Ruth K., Hartmann, R., Felber, H., Zinn M (2005). Bacterial poly(hydroxyalkanoates) as a source of chiral hydroxyalkanoic acids, *Biomacromolecules* 6:2290–2298.

Roes, L., Marsili, E., Nieuwlaar, E. & Patel M.K. (2007). Environmental and Cost Assessment of a Polypropylene Nanocomposite, *Journal of Polymers and the Environment* 15(3):212-226.

Ruth, K., Grubelnik, A., Hartmann, R., Egli, T., Zinn, M. & Ren, Q. (2007). Efficient Production of (R)-3-Hydroxycarboxylic Acids by Biotechnological Conversion of Polyhydroxyalkanoates and Their Purification, *Biomacromolecules* 8:279-286.

Ryu, H.W., Hahn, S.K., Chang, Y.K. & Chang, H.N. (1997). Production of poly(3-hydroxybutyrate) by high cell density fed-batch culture of *Alcaligenes eutrophus* with phosphate limitation, *Biotechnology and Bioengineering* 55:28-32.

Schultz, N., Chang, L., Hauck, A., Reuss, M. & Syldatk, C. (2006). Microbial production of single-cell protein from deproteinized whey concentrates, *Applied Microbiology and Biotechnology* 69(5): 515-520.

Siso, M.I.G., Ramil, E., Cerdan, M.E. & Freire Picos, M.A. (1996). Respirofermentative metabolism in *Kluyveromyces lactis*: ethanol production and the Crabtree effect, *Enzyme and Microbial Technology* 18:585–91.

Sonnleitner, E., Heinzle, E., Braunegg, G. & Lafferty, R.M. (1979). Formal kinetics of poly-hydroxy butyric acid (PHB) production in *Alcaligenes eutrophus* H16 and Mycolplasma

rubra R14 with respect to dissolved oxygen tension in ammonia limited batch cultures, *European Journal of Applied Microbiology and Biotechnology* 7:1–10.

Spielmann, M., Kägi, T., Stadler, P. and Tietje, O. (2004). Life Cycle Inventories of Transport Services. Final report, ecoinvent 2000 No. 14. Swiss Centre for Life Cycle Inventories, Dübendorf, 246.

Steinbuchel, A. (1991). Recent advances in the knowledge of the metabolism of bacterial polyhydroxyalkanoic acids and potential impacts on the production of biodegradable thermoplastics, *Acta Biotechnologica* 11: 419-427.

Steinbüchel, A. & Hein, S. (2001). Biochemical and molecular basis of microbial synthesis of polyhydroxyalkanoates in microorganisms, *Advances in Biochemical Engineering/ Biotechnology* 71:81-123.

Steinbüchel, A. & Valentin, H.E. (1995). Diversity of bacterial polyhydroxyalkanoic acids, *FEMS Microbiology Letters* 128: 219-228.

Sudesh, K. & Iwata, T. (2008). Sustainability of biobased and biodegradable plastics, *Clean* 36(5-6):433-442.

Sun, Z., Ramsay, J.A., Guay, M. & Ramsay, B.A. (2007). Fermentation process development for the production of medium-chain-length poly-3-hydroxyalkanoates, *Applied Microbiology and Biotechnology* 75:475-485.

Tabone, M.D., Cregg, J.J., Beckman, E.J. & Landis, A.E. (2010). Sustainability Metrics: Life Cycle Assessment and Green design in Polymers, *Environmental Science & Technology* 30(20): 8264–8269.

Thilakavathi, M., Basak, T. & Panda, T. (2007). Modelling of enzyme production kinetics. *Applied Microbiology and Biotechnology* 73: 991-1007.

Titz, M., Kettl, K.H., Shahzad, K., Koller, M., Schnitzer, H. & Narodoslawsky, M. (2012). Process Optimization for Efficient Biomediated PHA Production from Animal-Based Waste Streams, *Clean technologies and environmental policy* DOI: 10.1007/s10098-012-0464-7.

Van Aalst-Van Leeuwen, M.A., Pot, M.A., Van Loosdrecht, M.C.M. & Heijnen, J.J. (1997). Kinetic modeling of poly(b-hydroxybutyrate) production and consumption by *Paracoccus pantotrophus* under dynamic substrate supply, *Biotechnology and Bioengineering* 55:773–782.

Van Wegen, R.J., Lee, S.Y. & Middelberg, A.P.J. (2001). Metabolic and kinetic analysis of poly(3-hydroxybutyrate) production by recombinant *Escherichia coli*, *Biotechnology and Bioengineering* 74:70–80.

Viitanen, M.I., Vasala, A., Neubauer, P. & Alatossava, T. (2003). Cheese whey-induced high-cell-density production of recombinant proteins in *Escherichia coli*, *Microbial Cell Factories* 2:2.

Viñas, M., Borzacconi, L. & Martínez, J. (1994). Anaerobic treatment of yeast manufacturing wastewater in UASB reactors, *Environmental technology* 15: 79–85.

Viñas, M., Borzacconi, L., Martínez, J., Mallo, M. & Galisteo, M. (1994). Simultaneous degradation of carbohydrates and proteins in anaerobic treatment, *VII International Symposium on Anaerobic Digestion, January 23–27*, Cape Town, South Africa, pp. 345–348.

Visser, D., van Zuvlen, G.A., van Dam, J.C., Eman, M.R., Pröl, A., Ras, C., Wu, L., van Gulik, W.M. & Heijnen, J.J. (2004). Analysis in vivo kinetics in aerobic *Saccharomyces cerevisiae*

by application of glucose and ethanol pulses, *Biotechnology and Bioengineering* 20: 157-167.

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* 50 (*1*), 30-33.

Wallner, E., Haage, G., Bona, R., Schellauf, F. & Brauengg, G. (2001). The production of poly-3-hydroxybutyrate-co-3-hydroxyvalerate with *Pseudomona cepacia* ATCC 17759 on various carbon sources, in E. Chiellini et al (ed.), *Biorelated polymers Sustainable Polymer science and Technology* Kluwer Academic / Plenum Publishers, New York, Boston, Dordrecht, London, Moscow 2001, pp. 139-145.

Yellore, V. & Desai, A. (1998). Production of poly-3-hydroxybutyrate from lactose and whey by *Methylobacterium* sp. ZP24, *Letters in Applied Microbiology* 25:391–394.

Young, F.K., James, R., Sheldon, K. & May, W. (1994). Microbial Production of Poly-β-Hydroxybutyric Acid from d-Xylose and Lactose by *Pseudomonas cepacia.* Applied and Environmental Microbiology 60(11): 4195-4198.

Zafar, S. & Owais, M. (2006). Ethanol production from crude whey by *Kluyveromyces marxianus*, *Biochemical Engineering Journal* 27(3): 295-298.

Zhang, X., Luo, R., Wang, Z., Deng, Y. & Chen, G.Q. (2009). Application of (R)-3-Hydroxyalkanoate Methyl Esters Derived from Microbial Polyhydroxyalkanoates as Novel Biofuels, *Biomacromolecules* 10(4): 707-711.

Zinn, M., Weilenmann, H.U., Hany, R., Schmid, M. & Egli, T. (2003). Tailored synthesis of poly([R]-3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB/HV) in *Ralstonia eutropha* DSM 428, *Acta Biotechnolica* 23:309-316.