

# POLYHYDROXYALKANOATES: BIODEGRADABLE POLYMERS AND PLASTICS FROM RENEWABLE RESOURCES

## POLIHIDROKSIALKANOATI: BIORAZGRADLJIVI POLIMERI IN PLASTIKE IZ OBNOVLJIVIH VIROV

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Polyhydroxyalkanoates (PHAs) attract considerable attention as sustainable "green plastics" with a real potential to replace their petrol-based competitors in some applications in the not-too-distant future. To reach this goal PHAs must be able to compete with the established petrol-based plastics in both technical and economic terms. The current PHA production is based on prized substrates of high nutritional value such as sucrose, starch or vegetable oils. An alternative, carbon-rich industrial waste can be used as a suitable feedstock. This would contribute to making PHAs economically competitive and would avoid the conflict with human nutrition or animal feeding.

Consequently, the decision about the location of the PHA-production facilities depends on the preferable in-house availability of such waste streams. The issue of competitive, large-scale PHA production in Europe was the topic of the ANIMPOL and the WHEYPOL projects. Both intended to develop novel processes for the transformation of abundant, locally available, renewable wastes. In the ANIMPOL case, waste lipids from slaughterhouses are converted to glycerol and a mixture of saturated and unsaturated fatty acid esters (FAEs), better known as biodiesel. The production of saturated FAEs is 50 000 t per year in Europe, decreasing the biodiesel performance as an engine fuel. However, within the project it was demonstrated that they can be efficiently metabolized to PHAs. In the WHEYPOL project attention was focused on  $1.4 \times 10^8$  t per year of whey from dairies. This waste is of limited use and causes environmental concern. However, lactose, the main carbohydrate found in whey, can be used as a substrate in the WHEYPOL bioprocesses. These strategies demonstrate the feasibility of making "green plastics" competitive by integrating their manufacturing directly into the existing production lines, where the convertible waste streams accrue.

Keywords: animal waste, biodiesel, biopolymers, lipids, polyhydroxyalkanoates, whey

Polihidroksialkanoati (PHA) vzbujajo posebno pozornost kot trajnostna "zelena plastika" z realno možnostjo, da nadomestijo svoje tekmece, ki temeljijo na nafti, za določene namene uporabe v bližnji prihodnosti. Za doseg tega cilja morajo biti PHA sposobni tekmovati tako po tehničnih kot po ekonomskih vidikih z uveljavljenimi plastikami na osnovi nafte. Sedanja proizvodnja PHA temelji na dragih podlagah z veliko hranilno vrednostjo, kot so sahara, škrob ali rastlinska olja. Kot alternativna surovina se lahko uporabi z ogljikom bogate industrijske odpadke. To bi prispevalo k bolj ekonomični izdelavi PHA in bi preprečilo konflikt s prehrano ljudi in krmo živali.

Posledično je odločitev o lokaciji izdelave PHA odvisna od razpoložljivosti in virov takih odpadkov. Vprašanje konkurenčne proizvodnje PHA v velikih količinah v Evropi je bila tema projektov ANIMPOL in WHEYPOL. Oba sta imela namen razviti nove postopke predelave obilnih in lokalno razpoložljivih obnovljivih odpadkov. V primeru ANIMPOL-a so bili odpadni lipidi iz klavnic pretvorjeni v glicerol in mešanico estrov (FAE) nasičenih in nenasičenih maščobnih kislin, bolj poznani kot biodizel. Proizvodnja nasičene FAE v Evropi je 50 000 t na leto in zmanjšuje zmogljivost biodizla kot pogonskega goriva. Projekt je pokazal, da jih lahko pretvorimo v PHA. Pri projektu WHEYPOL je bila pozornost usmerjena na  $1,4 \times 10^8$  t na leto sirotke iz mlekar. Ta odpadki ima omejeno uporabo in povzročajo okoljske skrbi. Vendar pa se laktoza, glavni ogljikovodik v sirotki, lahko uporabi kot osnova pri WHEYPOL-bioprocseh. Te strategije kažejo, da je mogoča proizvodnja konkurenčne "zelene plastike" z vključitvijo njihove izdelave neposredno v proizvodne linije, kjer se vredni odpadki pojavljajo.

Ključne besede: živalski odpadki, biodizel, biopolimeri, lipidi, polihidroksialkanoati, sirotka

## 1 INTRODUCTION

### 1.1 "Green plastics" today

Currently, we are experiencing a remarkably dynamic biopolymer market with an impressive increase in the volume and range of the products: in 2010, the market value reached a magnitude of  $10^{10}$  US dollars with an obvious upward trend. Only from 2008 to 2015, the global production of biopolymers is estimated to increase from 180 kt to 1710 kt (Plastic Additives and Compounding 2008). This positive development is unfortunately associated with an emergence of various

plastics that are labeled with the stylish attribute "green plastic" by the manufactures, but display properties that do not match the strict definitions by which they could be classified as *biobased*, *biodegradable*, *compostable* or *biocompatible*. On the other hand, polyhydroxyalkanoates (PHAs) are natural, thermoplastic, aliphatic biopolyesters that fully comply with these requirements.<sup>1</sup> Among all bio-based plastics, they are unique by being entirely produced and degraded by living cells.<sup>2</sup> The spectrum of their potential applications ranges from simple packaging to high-quality materials for niche uses.

### 1.2 General aspects of PHAs

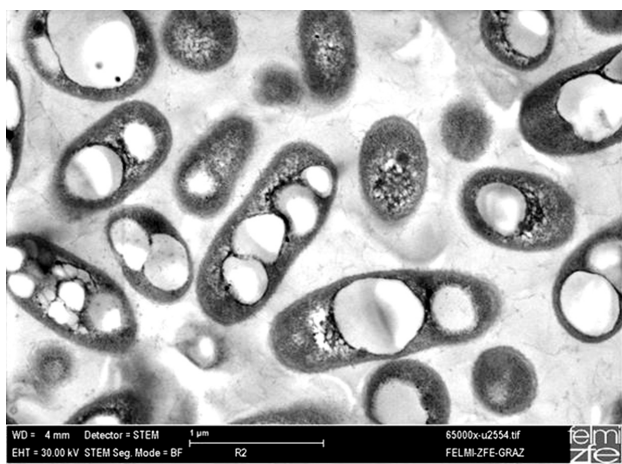
PHAs are synthesized and stored as intracellular granules by a wide range of prokaryotic genera using various renewable feedstocks. Predominately, the conversion of hexoses, pentoses, starch, sucrose, lactose, maltose, lipids, alcohols, or methane is reported in literature.<sup>3-5</sup> **Figure 1** provides a scanning transmission electron microscopy (STEM) picture of *Cupriavidus-necator* cells cultivated on carbohydrates in a continuous process. PHA inclusions are well visible as bright, refractive granules amounting to the mass fraction 52 % of the cell mass.

Principally, a high intracellular energy charge, characterized by an elevated pool of acetyl-CoA, NAD(P)H, ATP and others, promotes a PHA formation. Such conditions result from a sufficient availability of an external carbon source and a restricted supply of the growth-essential substrates such as nitrogen, phosphate, dissolved oxygen, or certain micro-components.<sup>3,6,7</sup> PHAs carry out important biological tasks, mainly acting as intracellular-energy and carbon reserves that can be re-utilized under the conditions of carbon starvation.<sup>8</sup> They are crucial for the regulation of the intracellular energy flow, routing the carbon compounds to the metabolic pathways and acting as protective factors against the environmental pressures like osmotic shock, UV irradiation, desiccation, heat and oxidative stress.<sup>9</sup>

### 1.3 Material properties and downstream processing

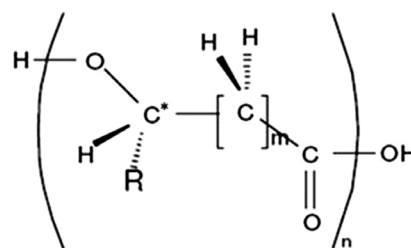
PHAs are polyesters consisting mainly of enantiomerically pure *R*-configured 3-hydroxyalkanoate (3HA) monomers; the general chemical structure is shown in **Figure 2**.

Depending on the microbial production strain, the nutrient supply and the process parameters during a biosynthesis, PHA-polymer chains contain 100 to 100



**Figure 1:** STEM picture of *Cupriavidus-necator* cells harboring PHA biopolymers (magnification 65.000x)

**Slika 1:** STEM-posnetek celic *Cupriavidus necator*, ki zadržujejo PHA-biopolimere (povečava 65000-kratna)



**Figure 2:** General chemical structure of PHAs. The asterisk (\*) indicates the chiral center,  $n$ : number of building blocks in the polyester chain (for natural PHAs:  $n = 100$  to  $10000$ ),  $m$ : number of carbon atoms in the monomer backbone; in the case of 3HAs,  $m = 1$ ,  $R$ : side chain of the building blocks; in the case of 3HB,  $R = -CH_3$ .

**Slika 2:** Splošna kemijska struktura PHA. Zvezdica (\*) označuje kiralni center,  $n$ : število gradnikov v poliestrski verigi (pri naravni PHA:  $n = 100$  do  $10000$ ),  $m$ : število ogljikovih atomov v monomerni hrbtenici; v primeru 3HAs,  $m = 1$ ,  $R$ : stranska veriga gradnikov; v primeru 3HB,  $R = -CH_3$ .

000 3HA units. Among all known PHAs, the homopolymer of *R*-3-hydroxybutyrate (3HB)-poly(*[R]*-3-hydroxybutyrate) (PHB) is most intensely studied and understood. Unfortunately, PHB exhibits a rather high crystallinity that hampers its processability. The low difference between the decomposition temperature (around  $270\text{ }^{\circ}\text{C}$ ) and the high melting point (around  $180\text{ }^{\circ}\text{C}$ ) provides a "window of processability" that is too narrow for many processing techniques, e.g., a melt extrusion or blowing of plastic films. This can be overcome by interrupting the crystalline PHB matrix with an incorporation of additional PHA building blocks, mainly 3-hydroxyvalerate (3HV) or an achiral building block of 4-hydroxybutyrate (4HB). The resulting co-polyesters display enhanced material properties in a broader range of applications. The exact material properties are strongly dependent on the monomer composition of co-polyesters. This composition can be triggered during a PHA biosynthesis by feeding the precursor substrates to obtain the desired building blocks. For example, 3HV building blocks are produced by many PHA-accumulating strains supplying odd-numbered fatty acids as precursors that lead to 3HV in the synthesized PHAs.<sup>8</sup>

After a production of the PHA-rich cells, efficient downstream processing is needed. This includes separating the biomass from the liquid phase, a PHA recovery from the biomass, and product refining. A PHA recovery from the cells may include a solvent or a supercritical fluid extraction, a chemical or enzymatic digestion of the cell wall, using high cell fragility of special microbial species, or a mechanical cell disruption.<sup>10</sup> The choice of the best method mainly depends on the required product purity that will satisfy the envisaged application. The recovered biopolyesters can be processed with the conventional equipment well established in the polymer industry. This technological path leads to proper biodegradable and biobased substitutes for a variety of "classical" petrol-based plastics such as thermoplastics, elastomers, and even latex rubbers.<sup>11-14</sup>

## 2 CHALLENGES IN THE PRODUCTION OF PHAs AND THE ATTEMPTS TO OVERCOME THEM

### 2.1 Global PHA production

Despite the enormous efforts during the last decades dedicated to the improvements in the PHA production, characterization and processing on the laboratory and pilot scale, their ultimate market penetration has not yet taken off. However, serious activities undertaken in the past by ICI-Marlborough (UK), Chemie Linz (Austria), and, more recently, by Monsanto, Metabolix (both USA), PHBISA (Brazil), TephA (USA) and Tianan or Shandong (PR China) constitute laudable exceptions. Due to an obvious gap between the often highly enthusiastic public announcements made by the manufacturers citing their capacities, and the actual PHA production, it is challenging to collect reliable data on the quantities of PHAs that are currently produced on the (semi)industrial scale. Realistically, it can be estimated that today PHAs cover about 5 % of the world's biolastic market, and less than 0.05 % of the overall global plastic production.

### 2.2 Economics of the PHA production: decisive factors

The major part, up to a half, of the entire PHA production costs is attributed to the cost of carbon substrates. On the one hand, from the ethical point of view, the explosive nutritional situation of the mankind in many global regions strongly discourages the utilization of various renewable feedstocks that can be used for food, for the production of chemicals, plastics or fuels; starch or edible oils are the prime examples. On the other hand, there are a number of diverse industrial carbon-rich wastes that do not interfere with the nutrition chain, and, at the same time, cause disposal problems and costs. Their utilization as carbon substrates for the microbial processes is a viable strategy for overcoming this ethical conflict and can be considered as the most promising approach in making PHAs economically competitive. Waste renewable resources like lignocellulosics, carbohydrates, waste lipids or alcohols, are mainly produced in agriculture and connected industrial branches.<sup>8,12,15,16</sup>

In addition to introducing the substrates, costs have to be reduced by optimizing the downstream processing required for the PHA recovery after a cell harvest. As intracellular products, PHAs have to be separated from the surrounding non-PHA cell mass (NPCM, also known as the residual biomass) that mainly consists of proteins, lipids, nucleic acids and polysaccharides. Here, a high usage of hazardous solvents and enormous energy demand still constitute the state-of-the-art, somewhat contradicting the patterns of sustainability and economic feasibility.<sup>3,17</sup>

Apart from the selection of raw materials and the improvements in downstream processing, enhanced productivity reached through the design of an optimal

engineering set-up is indispensable for the final breakthrough of PHAs on the market. Batch and fed-batch discontinuous fermentation modes are currently most commonly used techniques for the microbial PHA production.<sup>18–20</sup> In contrast, the continuous production mode is a well-known tool for achieving high productivities, lower production costs and a constant product quality in biotechnological processes. Therefore, an increasing research is focusing on investigating and assessing the potential of continuous PHA-production processes.<sup>21–23</sup> Recently, based on the kinetic considerations regarding biomass growth and PHA accumulation, the continuous production of PHB in a five-stage bioreactor cascade (5-CSTR) was investigated.<sup>23</sup> This 5-CSTR acts as a device-related engineering substitute for a tubular bioreactor that is theoretically the best match for the process-engineering requirements for an efficient PHA production from a kinetic point of view. As the main results, the authors report on a high PHB productivity of 1.85 g/(L h), and a constant and superior product quality concerning the mechanical properties and molecular masses.<sup>23</sup>

## 3 CURRENT ROUTES IN PHA RESEARCH

Current research in the field of PHAs focuses on several key topics. The application of growth additives that shorten the time for the production of a catalytically active biomass is a pre-requisite for enhancing the entire volumetric productivity of the process. Inexpensive growth additives can be found in agriculture, e.g., proteinaceous materials or side streams from the cultivation of green grass land, and were already tested successfully on the laboratory scale.<sup>24</sup>

Efforts in the field of genetic engineering mainly intend to increase the volumetric PHA productivity and achieve higher molar masses of the biopolymers. This can be accomplished with a knock-out of enzymes responsible for the intracellular PHA degradation. In addition, metabolic bottle necks that can limit a fast and complete substrate conversion can be overcome by genetic modifications. Enhancement of the microbial oxygen uptake may be improved by inserting the genes encoding catalase or peroxidases.<sup>25</sup>

In the field of downstream processing, together with lower energy consumption, environmentally safe and efficient solvents are investigated in order to enhance the recovery of PHAs from the cells. This goes in parallel with an examination of the novel biological lysis methods and enhanced strategies for a mechanical cell disruption. For an efficient polymer recovery, an increase in both the intracellular polymer content as well as the PHA granule size is beneficial. These factors are determined during the PHA biosynthesis.<sup>26</sup> In addition, the remaining NPCM has to be converted in a sustainable, value-adding way. Currently, the research in this direction is devoted to the anaerobic digestion of NPCM in



biogas plants, its application in agriculture as a "green fertilizer", or to the chemical or enzymatic digestion of NPCM into a nutrient-rich cocktail to be used for subsequent microbial cultivations. Downstream processing can also be facilitated by genetic modification; boosted nuclease excretion after a cell disruption results in decreased amounts of nucleic acids in the medium, leading to a lower viscosity that facilitates the separation of the PHA granules from the surrounding liquid phase.

The technological drawbacks of the bio-production itself can be handled by an application of the robust, microbial production strains that remain genetically stable for a long time under continuous cultivation conditions. At the same time, they should resist the contamination with the microbial competitors that, by endangering the whole production set-ups, represent a high risk.<sup>8</sup> Using extremophilic species, like the halophilic archaeon *Haloferax mediterranei*, minimizes the normally indispensable, energy-demanding sterilization precautions for the PHA production set-ups.<sup>27,28</sup>

In future, continuous PHA production should not only aim to increase the volumetric productivity, but also to open the door for tailor-made material properties by fine-tuning the polyester composition. This can be accomplished with the formation of block-copolymers, where a sequential arrangement of softer and harder polymer segments can result in well-adjusted novel polymeric materials. Here, a multistage bioreactor cascade for the PHA production is presented with potentially adequate process engineering equipment.<sup>23</sup>

During the last decades, the preparation of nanocomposites and composites involving natural fibers has become one of the key segments in the biopolymer science. To enhance material properties, PHAs can be processed together with a variety of compatible matters, resulting in the creation of novel materials. For this purpose, polymeric materials like poly(vinyl alcohol), poly(lactic acid), poly( $\epsilon$ -caprolactone), synthetic analogues of PHAs (e.g., atactic PHB), inorganic (e.g., clays or calcium carbonate) and organic (bagasse, wheat flour, fruit peels, fruit fibers, saw dust and straw) fillers of agricultural origin were already tested.<sup>29,30</sup> Nanocomposites have a potential to improve special polymer properties, such as gas permeability and thermal and mechanical characteristics. Here, only rather small amounts of a filler (mainly modified clay) are needed to improve the material performance.<sup>29</sup> Natural-fiber composites often display excellent mechanical properties, and decrease the density of the final product. In most cases, the fibers from agricultural residues are used as fillers; their application additionally enhances the biodegradability and reduces the production cost of the final product.<sup>30</sup>

#### 4 CASE STUDY 1: FROM SURPLUS WHEY TO PHA BIOPOLYESTERS

Whey is a surplus product in the cheese industry. Casein precipitates from milk enzymatically or by

acidification. This transformation results in curd cheese (mainly caseins) and full-fat whey that undergoes a degreasing and concentration procedure. From the resulting whey concentrate, whey retentate (protein fraction) is removed; the remaining whey permeate (carbohydrate fraction) contains about 80 % of the lactose originally included in the milk.

In 2008,  $1.6 \times 10^8$  t of whey were produced according to the estimates from OECD and FAO. The production has increased continually in the past and this trend is expected to continue in the future. The highest quantities of whey are available in the northern hemisphere with roughly  $4 \times 10^7$  t in the USA,  $2.2 \times 10^5$  t in Canada, and  $5 \times 10^7$  t in the EU-25.<sup>31</sup>

Due to its quantities, whey is not only an excess raw material, but it also causes disposal problems because of its high biochemical oxygen demand.<sup>18,32</sup> It accrues in the volumes almost equal to the processed milk, which means that the processing of one million liters of milk into cheese, almost one million liters of whey has to be treated. This exemplary quantity contains up to 50 t of lactose as the main carbon ingredient in whey. Just in the northern Italy, where a number of large dairies are located, about 1 million liters of whey have to be disposed off daily. This is often accomplished with a direct release of untreated whey into the aquatic environments.

Hence, it was reasonable to design an integral process for the PHA production from the surplus whey including the following steps:<sup>24</sup>

- Set-up of the optimized upstream technology for the feedstock whey
- Set-up of the optimized PHA-formation conditions with the selected microbial strains using the whey lactose as a carbon source
- Set-up of the biotechnological strategy for triggering the polymer composition as a tool for fine-tuning PHA properties
- Optimization of the downstream strategy for a PHA recovery
- Detailed product characterization
- Economic and ecological appraisal of the entire process and its steps
- Evaluation of the applications of the products

It is obvious that the discussed points can only be achieved, if there are intense interactions between the experts in different scientific fields: microbiology (a search for the ideal microbial production strain), biotechnology (the fermentation technology as the core part), chemical engineering (tailor-made downstream processing, design of industrial plant), polymer science (an on-going characterization of the products), and life-cycle assessment (LCA).

Within the FP5 Growth Program of the EU, the WHEYPOL project realized these ideas between 2001 and 2004. The demanded interactions between different research areas were put into practice with the formation

of a network consisting of seven scientific groups and three industrial partners each specialized in a certain field needed for the process development.

In a study that was accomplished within the WHEY-POL project, Koller and colleagues<sup>27</sup> compare the potential of three prokaryotic wild-type strains for the utilization of whey as carbon feedstock for the PHA production. In this work the archaeon *Haloferax mediterranei* and the eubacterial strains *Pseudomonas hydrogenovora* and *Hydrogenophaga pseudoflava* were investigated in bioreactors on the laboratory scale.

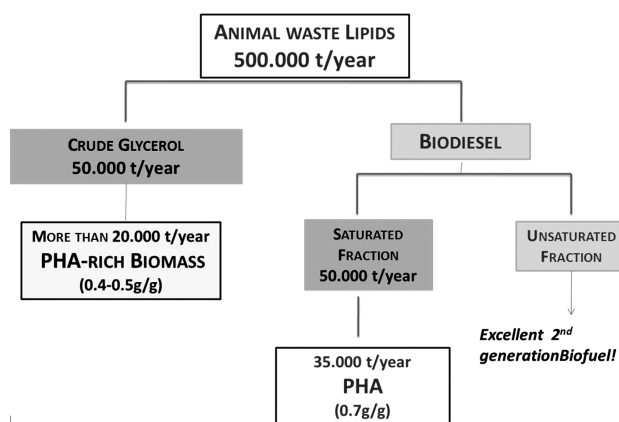
Among these organisms, *Haloferax mediterranei* turned out to be the most promising candidate for an eventual industrial-scale PHA production using whey. This is due to the strain's high robustness and stability; the risk of microbial contamination during the cultivation is restricted to the absolute minimum, thus a lot of energy can be saved with lower sterility demands. Additionally, the strain produces a P(3HB-co-8%-3HV) co-polyester directly from the 3HV-unrelated carbon-source whey, avoiding the costs for odd-numbered acids as the 3HV-related precursors.<sup>33</sup> The strain grew well on hydrolyzed whey permeate with the maximum specific growth rate  $\mu_{\max}$  of 0.11 1/h. PHAs were accumulated at the maximum specific production rate of 0.08 g/(g h). The conversion yield for whey sugar to PHA amounted to 0.33 g/g. After a further optimization of the production conditions, the productivity of this strain on hydrolyzed whey permeate was increased to 0.09 g/(L h) (the specific rate of 0.15 g/(g h); 16.8 g/L of biomass containing 73 % of PHAs were finally obtained. By co-feeding the precursors for 3HV and 4HB production (pentanoic acid and  $\gamma$ -butyrolactone, respectively), together with whey permeate as the main carbon source, a high value P-(3HB-co-21.8%-3HV-co-5.1%-4HB) terpolyester was completed by *Haloferax mediterranei*. A detailed characterization of thermal properties and molecular-mass distribution of the materials was accomplished; the results for polymer characterization indicate that the materials might be of special interest for application in the medical field. The partial conversion of whey sugars to 3HV units and the excellent polymer characteristics (low melting temperature, high molecular masses within a narrow distribution), together with a simple recovery of PHAs from the cells, make the strain especially interesting. The estimated production price amounted to € 2.82 per kg of PHAs. For a further process improvement, the recycling of the highly saline side streams should be tested and optimized.<sup>27,28</sup>

*P. hydrogenovora* features the disadvantages of low final polymer contents, low productivity and product yields due to a redirection of the carbon flux towards unwanted by-products such as various organic acids. Using this organism, the final PHB homopolyester content amounted to the mass fraction 12 % (qp: 2.9 mg/(g h)). By co-feeding the 3HV-related precursor pentanoate, the strain accumulated 12 % of poly-3(HB-co-21%-HV) (qp: 2.0 mg/(g h)).<sup>28,34</sup>

The research showed that *H. pseudoflava* produces PHAs of a rather good quality (high molar masses of  $M_w = 859$  MDa and low polydispersities of 3.3) from whey at acceptable specific production rates and yields, but is not competitive with *H. mediterranei* in terms of strain stability and robustness. In detail, using this strain, 40 % of poly-3(HB-co-5%-HV) in the cells with an addition of pentanoic acid (qp: 12.5 mg/(g h)) was obtained. Without the pentanoic acid, the strain accumulated 30 % of the homopolyester PHB in the cells (qp: 16.0 mg/(g h)).<sup>28</sup>

## 5 CASE STUDY 2: SLAUGHTERING WASTE FOR THE PHA PRODUCTION

Animal lipids from slaughtering and animal-processing industries in Europe amount to more than 500000 t per year. It is estimated that using this waste stream for biodiesel production through transesterification results in 50000 t of saturated biodiesel (SFAE) fraction. SFAE causes problems in fuel mixtures due to its precipitation at a low temperature, which may result in filter plugging. Using SFAE as a carbon feed stock, 35000 t of PHAs can theoretically be produced annually (a yield of 0.7 g of PHAs per g of SFAE). Considering the entire amount of biodiesel that is currently produced in the EU-25 (about 22 Mt), approximately 2 Mt of glycerol phase (CGP) are available as the major by-product of the conversion of lipids to biodiesel. This is in considerable excess over the quantities of the glycerol needed for its various classical applications. If applied in the production of microbial PHA-accumulating biomass, one can expect more than 0.4 g of biomass or PHAs per gram of glycerol. Regarding the entire quantity of glycerol from the animal-lipid transesterification, one can estimate that more than 20000 t of PHA-rich biomass can be produced annually. These amounts are shown in **Figure 3**.



**Figure 3:** Amounts of available animal-waste lipids, and the potential PHA production

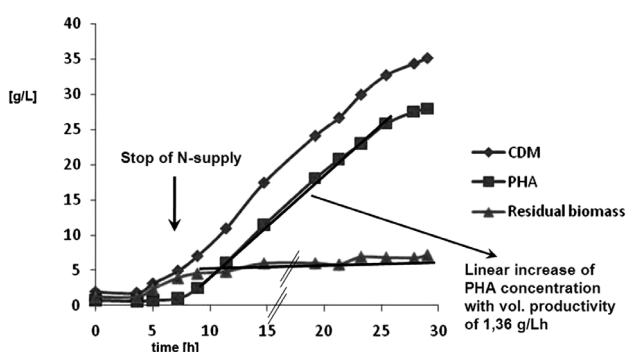
**Slika 3:** Količina razpoložljivih živalskih lipidov in mogoča proizvodnja PHA iz njih

In 2010, the project "Biotechnological conversion of carbon-containing wastes for eco-efficient production of high-added value products", acronym ANIMPOL, was launched and funded through the FP7 of the EU.

Within this project, a sound industrial process for the conversion of lipid-rich animal waste from the meat processing industry into biodiesel is being developed using innovative methods. Saturated biodiesel fractions have a negative effect on biodiesel fuel properties, are separated and, subsequently, used as feedstock for the biotechnological production of PHAs. The remaining unsaturated biodiesel fraction performs as an excellent 2<sup>nd</sup> generation biofuel. The significance of the project is obvious considering the high quantities of animal-derived waste in Europe. The project brings together partners managing waste resources from slaughterhouses, the rendering industry, biodiesel production, as well as the polymer-processing industry.

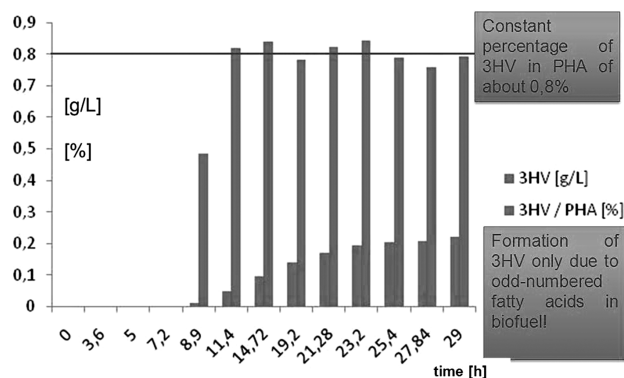
Experimentally, the most promising results for the PHA production from the animal-waste-derived SFAE are obtained by using *Cupriavidus necator*, a well-known wild-type strain from the Burkholderiaceae family. Fast microbial growth, satisfying cell densities, and high volumetric productivities of PHA homo- and co-polyesters, together with promising material characteristics, were obtained.

**Figure 4** illustrates the fermentation pattern of the laboratory bioreactor-scale production of PHB with *C. necator* using SFAE as the main carbon source. This fermentation was accomplished by using SFAE as the sole carbon source for both the biomass growth and the PHA accumulation in the fed-batch cultivation mode (a repeated supply of SFAE according to its conversion by the cells). The maximum of 28.0 g/L of PHAs was obtained, corresponding to the PHA share in a cell's dry mass that is 80.3 %. The specific growth rate of the



**Figure 4:** Fermentation pattern for the poly-(3HB-co-0.84 %-3HV) production with *C. necator* on SFAE. The arrow at  $t = 7$  h indicates the limitation of the nitrogen supply. Parallel lines at the time axis and the time curve for residual biomass indicate the end of the microbial growth after the nitrogen depletion, provoking the redirection of the intracellular carbon flow towards the PHA accumulation.

**Slika 4:** Model fermentacije za poli-(3HB-co-0,84 %-3HV) proizvodnjo s *C. necator* na SFAE. Puščica pri  $t = 7$  h kaže omejitev dobave dušika. Vzporedne linije na časovni krivulji za preostalo biomaso kažejo na konec mikrobne rasti po izčrpanju dušika, kar spodbudi preusmeritev toka medceličnega ogljika proti kopičenju PHA.



**Figure 5:** Poly-(3HB-co-0.84 %-3HV) production with *C. necator* on SFAE: composition of the polyester (small bars) and 3HV concentration (high bars) during the process

**Slika 5:** Proizvodnja poly-(3HB-co-0,84 %-3HV) s *C. necator* na SFAE: sestava poliestra (mali stolpci) in koncentracija 3HV (veliki stolpci) med postopkom

production strain amounted to 0.17 L/h. Especially the yield of the biomass production from SFAE of more than 0.6 g of CDM per g of SFAE is exceptionally high if compared to the well-known PHA substrates like sugars, where the theoretical yield does not exceed 0.48 g/g. This is due to the metabolic background of fatty acid catabolism of the cells. Considering the early stage of the process development, the high volumetric PHA productivity of 0.94 g/(L h) for the entire process can also be considered very promising if compared to the available data for the industrial PHA production from expensive substrates. Taking into account only the phase of the predominant PHA accumulation after nitrogen limitation, volumetric productivity was as high as 1.36 g/(L h). Regarding the specific volumetric productivity during nitrogen-limited conditions, the value of 0.19 g/(g h) was calculated. This is significantly higher than the values reported before for the utilization of whey lactose. During this phase, residual biomass (NPCM) concentration remained constant at about 7 g/L (see **Figure 4**). In addition, the produced PHA was a poly-(3HB-co-0.84 %-3HV) copolyester; here, odd-numbered fatty acids in SFAE acted as 3HV-related precursor substrates. The composition of the polyester during the fermentation and the concentration of 3HV in the fermentation broth are illustrated in **Figure 5**. It is well visible that, due to the identical composition of the added SFAE during the entire process, the share of 3HV in PHA (about 0.8 %) is constant during the phase of nitrogen limitation.

## 6 CONCLUSIONS

The study presents strategies for upgrading industrial wastes like surplus whey and residues from the slaughtering and biodiesel industries to feedstocks for biopolymer production. This approach can be regarded as a promising route for making the entire PHA-biopolymer-production process economically competitive. The selection of the discussed raw materials



is especially important to provide European industry with an advantage, on the one hand, to handle tremendous waste streams through a value-added conversion, and, on the other hand, to break new ground in developing and commercializing bio-polymeric materials.

Uniting the potential enhancements of each process step, one can make substantial progress towards an environmentally benign and cost-efficient technology. The development of efficient biopolymer-production processes needs a close cooperation between the experts from industry and different scientific fields; hence, a multidisciplinary approach is required. Decision makers from companies, chemical engineers, microbiologists, enzymologists, polymer scientists, genetic engineers, and the experts involved in LCA and the Cleaner Production studies have to join their respective expertise. Such an approach has a potential to close the existing gaps between the success achieved on the laboratory scale that, in most cases, affects only singular aspects of the biopolymer production, and the final success of competitive bio-plastics on the market.

Regardless of the selected microbial production strain, the facilities for the PHA production from whey and animal-derived waste should be integrated into the existing process lines of large dairies or biodiesel companies, where the raw material actually accrues. This can be considered as a viable strategy for minimizing the production costs by taking profit through the synergistic effects.

Due to the environmental considerations and expected shortage of fossil reserves, the increasing global demand for bio-plastics in the future is generally undisputed. Available data about the laboratory-scale developments shows a significant progress in combining the environmental benefit of the future-oriented bio-polyesters with the economic viability of their production. This development will hopefully facilitate the decisions of responsible policy-makers from various renewable waste-generating industries and from the polymer industry to break new ground in a sustainable production. If successfully implemented, these seminal biopolymers will be decoupled from the manufacturing, where biopolyester and biofuel syntheses compete with human nutrition, frequently lacking consideration of environmental requirements and acceptable human working conditions.

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