

## Waste Streams of the Animal-Processing Industry as Feedstocks to Produce Polyhydroxyalkanoate Biopolyesters

Martin Koller<sup>1\*</sup>, Khurram Shahzad<sup>2</sup>, Gerhart Braunegg<sup>3</sup>

1-Institute of Chemistry, University of Graz, NAWI Graz, Austria.

2-Center of Excellence in Environmental Studies (CEES), King Abdulaziz University, Saudi Arabia.

3-ARENA-Association for Resource Efficient and Sustainable Technologies, Graz, Austria.

### Abstract

**Background and objective:** Animal processing industry in the EU-28 states, encompassing slaughterhouses, rendering companies, and others, generates high quantities of waste streams containing about 500,000 t of lipids plus considerable amounts of offal material and meat and bone meal. These materials need to be utilized in a value-creating way, such as via bioconversion towards polyhydroxyalkanoate biopolyesters of diverse molecular composition and various plastic-like features. As a novelty, the present article summarizes for the first time previous and current efforts to utilize these animal-based waste streams for polyhydroxyalkanoate production in terms of selection of suitable microbial production strains, upstream processing of the raw material to generate accessible carbon sources, kinetics of the bioprocess, characterization of the produced biopolyesters of diverse molecular architecture, environmental process assessment, and economic feasibility.

**Results and conclusion:** The compared case studies clearly demonstrate that utilization of animal processing waste as a second generation feedstock for biopolyester production can definitely become an economically viable and sustainable process provided the utilization of optimized microbial strains, tailored feeding regime, short transportation distances, and clear business plans for commercialization of the final products. Most of all, using animal based waste for generation of second generation biopolyesters and second generation biofuel contributes to food security by preserving raw materials of nutritional value.

**Conflict of interest:** The authors declare no conflict of interest.

### Article Information

#### Article history:

Received 19 Sep 2017  
Revised 23 Aug 2018  
Accepted 25 Aug 2018

#### Keywords:

- Animal-processing waste
- Biodiesel
- Food security
- Lipids
- Polyhydroxyalkanoates

#### \*Corresponding author:

**Martin Koller,**  
University of Graz, Institute  
of Chemistry, NAWI Graz,  
Heinrichstrasse 28/III, A-  
8010 Graz, Austria.

Tel: +43-316-380-5463

E-mail:  
[martin.koller@uni-graz.at](mailto:martin.koller@uni-graz.at)

## 1. Introduction

Materials with plastic-like properties are ubiquitous in our today's society; they serve for diverse pivotal applications like food packaging, low density commodities, chemical resistant materials, or as special niche products, e.g., in electronic fabricates. To an increasing extent, their implementation in the medical field as scaffolds, implants, etc., attracts their interest in science and industry [1-4]. Established techniques for the production of plastics and follow up disposal of plastic after its life span provoke presently prevailing environmental threats: Depletion of fossil resources required for their production, growing piles of plastic waste when landfilling plastics, invasion of recalcitrant (micro-) plastics in marine environments, elevated CO<sub>2</sub> levels in the atmosphere and toxin generation by plastic

incineration [5]. Current efforts to develop alternatives refer to plastic-like "bio-materials" originating from renewable resources, and/or to such materials, which undergo complete biodegradation after disposal, e.g., via composting [6]. Microbial polyhydroxyalkanoates (PHA), a group of biopolyesters stored by various prokaryotes as intracellular reserve materials, are accessible from carbonaceous renewable resources, and undergo bio-mediated decomposition by the action of living organisms. This decomposition finally yields CO<sub>2</sub> and water as the only end products of their aerobic breakdown, or methane, respectively, after anaerobic decomposition, e.g., in biogas plants [1,6].

In dependence on the applied microbial production strain and the selected carbon source, thermoplastic short

chain length PHA (*scl*-PHA) or elastomeric medium chain length PHA (*mcl*-PHA) are distinguished. *Scl*-PHA are composed of hydroxyalkanoic (HA) monomers of three to five carbon atoms (most important examples: 3-hydroxybutyrate [3HB], 3-hydroxyvalerate [3HV], 4-hydroxybutyrate [4HB]), while *mcl*-PHA consist of monomers with at least six carbon atoms, e.g., 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate (3HO), 3-hydroxydecanoate (3HD) and their higher homologues [6,7].

Cost-efficient production and broad commercialization of microbial PHA and their follow-up products requires the optimization of all biotechnological process steps, encompassing the upstream processing to provide accessible carbon sources, the bioprocess in appropriate bioreactor facilities, and the downstream processing for sustainable PHA recovery from microbial biomass. As the currently most cost-decisive factor, selection of adequate, inexpensive carbon-rich raw materials is requested in order to develop economically efficient PHA production processes [1,6]. In principle, (poly)saccharides, alcohols, lipids, low molecular organic acids (acetate, butyrate, propionate, levulinic acid, etc.), methane and similar gaseous C1-substrates, or protein hydrolysates are available for organoheterotrophic PHA production by well-described prokaryotic species such as *Cupriavidus necator*, *Burkholderia sacchari*, *Azohydromonas lata*, *Haloferax mediterranei*, *Aeromonas caviae*, *Hydrogenophaga pseudoflava*, and a variety of *Pseudomonas* sp., just to mention those PHA producers most frequently occurring in today's literature [6-10]. As a technology still in its infancy, a growing number of cyanobacteria are currently in the status of investigation for solar mediated photolithotrophic PHA production starting from CO<sub>2</sub> emitted by industrial effluents. Apart from cyanobacteria, a number of facultative autotrophic microbes such as *C. necator*, the so-called "Knallgasbacteria", can be used for biotechnological conversion of CO<sub>2</sub> into PHA [11-13].

All the organoheterotrophic substrates mentioned above are abundantly available from agro-industrial surplus streams, which are by-products of the food and feed production; they often result in severe industrial disposal problems. Just to mention some examples, PHA production is described based on diverse lignocellulosics [8,14-16], surplus whey from dairy industry [17-19], crude glycerol phase from the biodiesel production [20-25], or various oils of no or only restricted significance for nutrition [26-28]. As a novel, emerging field, PHA production starting from lipid-rich waste streams of the animal processing industry is presented and discussed in the following paragraphs of the review at hand.

## 2. Waste streams of animal processing available for biotechnological polyester production

Lipid-rich waste streams of bovine, porcine, avian, ovine, etc., origin are produced by the European animal processing industry (slaughtering, meat converters, rendering) at estimated annual quantities of 500,000 annual tons [20]. As demonstrated by the research accomplished in the EU-funded project ANIMPOL (Biotechnological Conversion of Carbon Containing Wastes for Eco-Efficient Production of High Value Products), which was carried out during the 7<sup>th</sup> Framework Program by a consortium of seven academic and four industrial partners, the lipid fractions can be conveniently extracted from these waste streams, and subjected towards transesterification into fatty acid methyl esters (FAME), a well-known renewable biofuel ("biodiesel"). Not only the enormous quantities of animal based waste gave rise to develop this project; moreover, considering the "mad-cow-disease" (BSE) crisis some years ago, it was reasonable to find value-adding alternatives to the current incineration of animal-based waste materials, which finally supports food industry in economic terms.

Regarding the fatty acid pattern of animal based lipids, a considerable share of saturated fatty acids, predominately pelargonic (C9:0), pentadecylic (C15:0), palmitic (C16:0), margaric (C17:0), and stearic acid (C18:0), are present in animal based FAME [29]. This highly saturated share of biodiesel (SFAE) results in a high cold filter plugging point, thus hampering its application as an engine fuel at low temperature; solid particles of SFAE participate under these conditions and cause chocking in the engine. As a real alternative, the SFAE fraction can be separated from the unsaturated FAME fraction by simply precipitating it by cooling, followed by a filtration step. Subsequently, SFAE can be transformed biotechnologically to PHA by a number of powerful microbial production strains at a theoretical quantity of 35,000 annual tons of the biopolyester, if considering a theoretical conversion yield of 0.7 g PHA per g converted SFAE [20]. The unsaturated FAME fraction that remains after the separation can be commercialized as a high quality second-generation biofuel.

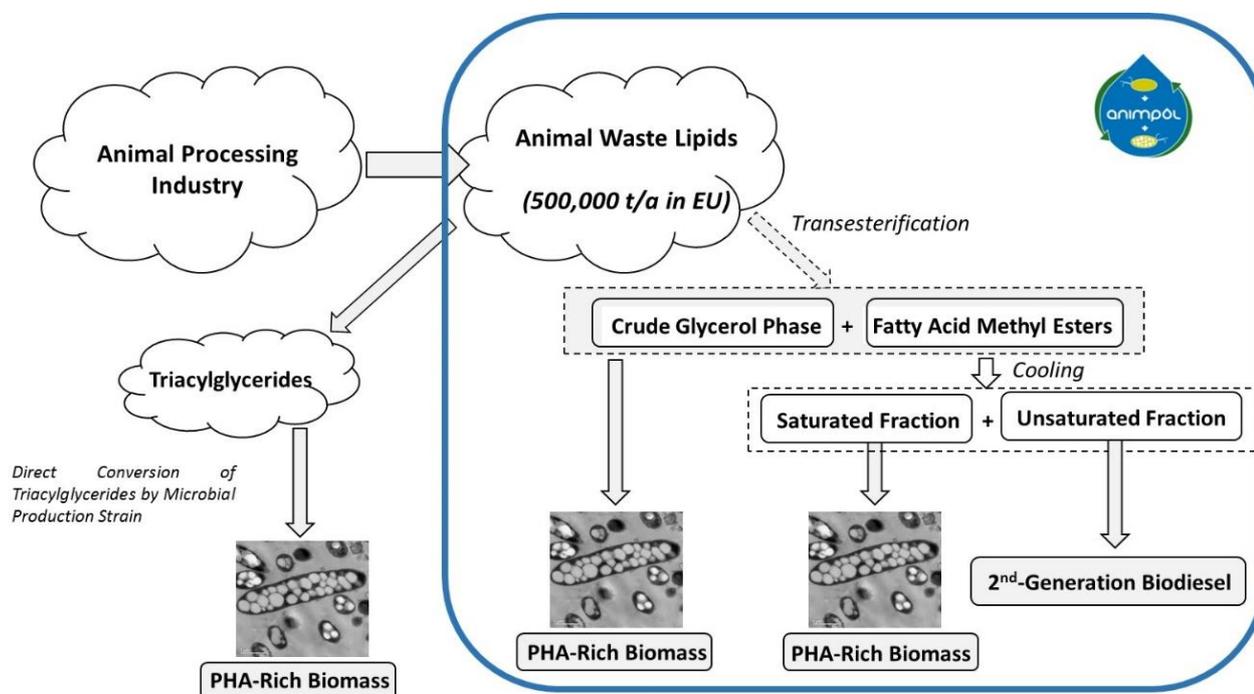
The described transesterification process applied to convert lipids (triacylglycerides) to FAME generates crude glycerol phase (CGP) as the major by-product at about 0.1 kg per kg of raw material (triacylglycerides) [21,24]. Considering the entire amount of biodiesel that is currently produced in the EU-28, which is about 20-30 metric tons (Mt) annually, more than 2 Mt of CGP are available as the major by-product of the conversion of lipids to biodiesel

[20]. This is in considerable excess over the quantities of glycerol needed for its various classical applications, e.g., in food industry, sweets production or cosmetics. As demonstrated before, a variety of microorganisms can thrive on glycerol as a carbon source, and are able to convert glycerol to PHA under adequate environmental conditions [23]. If applied for the production of microbial PHA-accumulating biomass, the theoretical yield amounts to more than 0.4 g cell dry mass (CDM; sum of biomass plus PHA) per gram of metabolized glycerol [30]. Stemming from the transesterification process, CGP contains some impurities, mainly methanol, which are inhibiting for the biotechnological process. Therefore, purification steps like demethanolization, e.g., via evaporation of this alcohol, are needed to provide glycerol in a microbiologically convertible form [23,24,31]. Here, one should add that some rather exotic microbial PHA-producers like *Methylomonas extorquens* exist, which thrive well on both glycerol and methanol, and convert both substrates to PHA. Unfortunately, these strains do not display sufficient volumetric productivity in terms of PHA accumulation [31]. Calculations based on the available amounts of waste lipids from the European animal processing industry estimate that more than 20,000 tons of PHA-rich biomass could be produced annually just from the CGP resulting from animal based biodiesel production [32,33]. Figure 1 provides an illustration of the quantities

of lipid-rich waste from the animal processing industry currently accruing in the EU, and the theoretical quantities of PHA-biopolymers to be produced thereof.

### 3. Case studies for using animal based lipids for PHA production

Both *scl*- and *mcl*-PHA can be produced starting from animal based waste lipids [20]. When studying the available literature, it becomes obvious that at the present only a restricted number of microbial strains were investigated in details to assess their potential for converting such substrates towards PHA. Whereas some representatives of the genus *Pseudomonas* are reported as *mcl*-PHA producers from animal-based lipids [34-37], the best-known PHA-producer *C. necator* was successfully used in the recent past as a powerful *scl*-PHA producer when supplied with such substrates [20,38]. The subsequent paragraphs summarize these R and D endeavors, comparing the kinetic outcomes of both *scl*- and *mcl*-PHA production by different microbial strains using lipophilic waste materials from animal processing, such as lipids in their native/non-transesterified form (tallow), or SFAE. In addition, the produced polymers are compared in terms of their thermoanalytical characteristics and molecular mass data.



**Figure 1.** Process schematic for polyhydroxyalkanoate (PHA) production from waste streams of the animal processing industry. Encircled in blue: ANIMPOL process (Biotechnological Conversion of Carbon Containing Wastes for Eco-Efficient Production of High Value Products) encompassing the transesterification of animal waste lipids to glycerol and fatty acid methyl esters (FAME).

**Table 1.** Case studies for PHA biosynthesis starting from animal processing waste streams

Production strain	Main carbon substrate	PHA produced	Production scale	Productivity	Polymer characterization available? [Y/N]	Ref.
<i>Pseudomonas citronellolis</i> DSM 50332	Tallow	<i>mcl</i> -PHA	Shaking flask	0.15 g g <sup>-1</sup> PHA in CDM	N	[34]
<i>Pseudomonas citronellolis</i> DSM 50322	Tallow	<i>mcl</i> -PHA	Shaking flask	1.35 g l <sup>-1</sup> PHA	Y	[35]
<i>Pseudomonas citronellolis</i> DSM 50332	SFAE	<i>mcl</i> -PHA (3HO and 3HD, minor amounts of 3HDD, 3HN), 3HHx, 3HHp, traces of unsaturated monomers	Bioreactor (2-5 l working volume)	0.050 g (l h) <sup>-1</sup> ; up to 0.27 g g <sup>-1</sup> PHA in CDM	Y	[36]
<i>Pseudomonas chlororaphis</i> DSM 50083	SFAE	<i>mcl</i> -PHA (predominantly 3HO and 3HD, minor quantities of 3HDD, 3HN, 3HHx, and 3HHp)	Bioreactor (5 l working volume)	0.14 g (l h) <sup>-1</sup> ; 0.23 g g <sup>-1</sup> PHA in CDM	Y	[37]
<i>Cupriavidus necator</i> DSM 545	SFAE	P (3HB- <i>co</i> -3HV) (3HV fraction in PHA < 1 mol%)	Bioreactor (5 l working volume)	0.94 g (l-h) <sup>-1</sup> ; 0.80 g g <sup>-1</sup> PHA in CDM	Y	[20]
<i>Cupriavidus necator</i> DSM 545	Ozonolytically oxidized SFAE	P (3HB- <i>co</i> -3HV) (3HV fraction in PHA 6 mol%)	Shaking flask	0.02 g (l-h) <sup>-1</sup> ; 0.41 g g <sup>-1</sup> PHA in CDM	Y	[40]
<i>Cupriavidus necator</i> DSM 545	CGP	PHB	Bioreactor (5 l working volume)	0.98 g (l-h) <sup>-1</sup> ; 0.65 g g <sup>-1</sup> PHA in CDM	Y	[20]
<i>Haloferax mediterranei</i> DSM 1411	CGP and hydrolyzed MBM	P (3HB- <i>co</i> -3HV) (3HV fraction in PHA ca. 10 mol%)	Bioreactor (42 l entire volume)	0.04 g (l-h) <sup>-1</sup> ; 0.75 g g <sup>-1</sup> PHA in CDM	N	[42]
<i>Haloferax mediterranei</i> DSM 1411	CGP	P (3HB- <i>co</i> -3HV) (3HV fraction in PHA ca. 10 mol%)	Bioreactor (10 l entire volume)	0.12 g (l-h) <sup>-1</sup> ; 0.76 g g <sup>-1</sup> PHA in CDM	Y	[22]
<i>Haloferax mediterranei</i> DSM 1411	CGP plus 4HV precursor GBL	P (3HB- <i>co</i> -3HV- <i>co</i> -4HB) (3HV fraction in PHA ca. 11 mol%, 4HB fraction ca. 5 mol%)	Bioreactor (10 l entire volume)	0.10 g (l-h) <sup>-1</sup> ; 0.66 g g <sup>-1</sup> PHA in CDM	Y	[22]
<i>Cupriavidus necator</i> DSM 428	Waste animal triacylglycerides	PHB	Shaking flask	0.3 g (l-h) <sup>-1</sup> ; ca. 0.8 g g <sup>-1</sup> PHA in CDM	N	[38]
<i>Cupriavidus necator</i> Re2058/pCB113	Waste animal triacylglycerides	P(3HB- <i>co</i> -3HHx)	Shaking flask	0.49-0.72 g g <sup>-1</sup> PHA in CDM	N	[38]
<i>Cupriavidus necator</i> Re2058/pCB113	Waste animal triacylglycerides	P(3HB- <i>co</i> -3HHx) (19 mol% 3HHx)	Bioreactor (2.5 l working volume)	0.40 g (l-h) <sup>-1</sup> ; 0.60 g g <sup>-1</sup> PHA in CDM	N	[38]

**Abbreviations:** 3HB: 3-hydroxybutyrate; 3HD: 3-hydroxydecanoate; 3HDD: 3-hydroxydocecanoate; 3HHp: 3-hydroxyheptanoate; 3HHx: 3-hydroxyhexanoate; 3HN: 3-hydroxynonanoate; 3HO: 3-hydroxyoctanoate; 3HV: 3-hydroxyvalerate; 4HB: 4-hydroxybutyrate; CDM: Cell dry mass; CGP: Crude glycerol phase; GBL:  $\gamma$ -butyrolactone; MBM: Meat and bone-meal; PHA: Polyhydroxyalkanoate; SFAE: Saturated fatty acid methyl esters

In the first reported study on PHA production on animal based waste lipids, the bacterial species *Pseudomonas* (*P.*) *oleovorans*, *P. resinovorans*, *P. putida*, and *P. citronellolis* were investigated about 20 years ago by Cromwick and colleagues [34]. These authors evaluated the ability of these strains to thrive and produce PHA using free fatty acids (FFA) from tallow and tallow-derived triglycerides as carbon sources. Using FFA from tallow, significant biomass formation (microbial growth) and PHA accumulation were observed for all four strains. Depending on the production strain, different mass fractions of PHA in bacterial CDM were observed: 0.18 g g<sup>-1</sup> (*P. oleovorans*), 0.15 g g<sup>-1</sup> (*P. resinovorans*), 0.19 g g<sup>-1</sup> (*P. putida*), and 0.03 g g<sup>-1</sup> (*P. citronellolis*). The direct utilization of non-hydrolyzed tallow for bacterial growth and PHA accumulation was only observed in the case of *P. resinovorans*; similar to the utilization of FFA, a mass fraction of 0.15 g PHA per g CDM was reported for this organism (Table 1). The authors carried out an in-depth investigation to elucidate these findings by investigating the lipase activity of extracts of all four strains when they were cultivated for 46 h on tallow as substrate. Among the four strains, *P. resinovorans* displayed the highest lipase activity of 12.80 U (μl·min)<sup>-1</sup>; the other cultures revealed lipase activity below 0.03 U (μl·min)<sup>-1</sup>. In order to investigate the monomer composition of the PHA accumulated by the individual strains from the described carbon sources, the polyesters were transformed into volatile methyl esters of hydroxyalkanoates via acidic methanolysis, which were subjected towards GC-analysis. For all four strains, the carbon chain lengths of the identified monomers ranged from C4 (3HB) to C14 (3-hydroxytetradecanoate [3HT]) with C8 (3HO) and C10 (3HD) repeat units being the predominant ones; in some longer carbon chains (C12 and C14), mono-unsaturation was detected; hence, both hydroxyalkanoates and hydroxyalkenoates were found as constituents of the produced PHA, in which hydroxyalkenoate biosynthesis originates from unsaturated fatty acids present in tallow [34].

Based on the auspicious results obtained with *P. resinovorans*, the same research group made follow-up experiments with this strain by comparing the effect of different triacylglycerides, both from animals and plants, as a carbon source. All investigated lipids resulted in quite promising CDM concentrations of more than 3 g l<sup>-1</sup>, with about 45% of the CDM being PHA. After GC analysis, supported by mass spectroscopy, it was evident that animal based lipids and most of the plant derived oils resulted in the formation of PHA with monomers of a carbon chain length of C4 to C14, with C8 and C10 presenting the predominant monomer fractions. Different substrates resulted in different degrees of unsaturation in the carbon chains of the monomers, dependent on the fatty acid

pattern of the applied substrate. Generally, triacylglycerides isolated from plants contain higher fractions of unsaturated fatty acids than those stemming from animals. It has been shown that the degree of unsaturation in the carbon chains tremendously changes the thermoanalytical properties of the products. Higher degrees of unsaturation were reflected by decreased melting temperature ( $T_m$ ), glass transition temperature ( $T_g$ ), and melting enthalpy ( $\delta H_m$ ). Molecular mass analysis of the polyester samples revealed relatively constant values for number-average molar masses ( $M_n$ ) between 65,000 and 101,000 Da, and rather low polydispersity values ( $P_i$ ) between 1.6 and 1.8, indicating a highly homogenous distribution of molecular masses of the individual PHA chains (Table 1) [35].

Instead of native tallow, animal-based SFAE was used as the sole carbon source to cultivate the *scl*-PHA producer *C. necator* under controlled conditions (temperature, pH-value, aeration) in a laboratory-scale bioreactor experiment carried out in the ANIMPOL project. The process consisted of two separate phases: In the first phase, cells were cultivated on a nutritionally balanced medium (ammonium ions were used as the nitrogen source, SFAE as the sole carbon source) until a PHA-free biomass concentration of about 7 g l<sup>-1</sup> was reached, which remained constant until the end of the process. After 23 h, the feed of nitrogen source was stopped, which provoked predominant conversion of SFAE to PHA instead of biomass. While the nitrogen source was added to the culture continuously in the first phase of cultivation in parallel to progressing microbial growth, SFAE was supplied to the microbial cells in a fed-batch cultivation regime during the entire process, hence, repeated SFAE feed pulses were carried out according to the SFAE conversion by the microbes. A maximum of about 28.0 g l<sup>-1</sup> PHA were generated in this process, which corresponds to an outstandingly high PHA fraction in CDM of 0.80 g g<sup>-1</sup> (Table 1). During the phase of microbial growth (first phase characterized by the availability of nitrogen source), the specific growth rate  $\mu_{max}$  amounted to 0.17 h<sup>-1</sup>, which is in the same range as *C. necator* cultivation processes on well-established substrates like glucose [20]. As a benefit of the applied substrate, the yield for biomass production from SFAE amounted to 0.6 g CDM per g SFAE, which is considerably higher than values obtainable on well-known PHA substrates like sugars or glycerol, where the metabolic pathway (breakdown of substrate to pyruvate via glycolysis or the 2-keto-3-desoxy-6-phosphogluconate pathway, and subsequent oxidative decarboxylation to acetyl-CoA) prevents conversion yields exceeding 0.48 g g<sup>-1</sup>. In the case of fatty acid conversion, the substrate is catabolized via the  $\beta$ -oxidation pathway, generating a number of acetyl-CoA units according to the carbon chain length of the fatty acid without carbon loss by oxidative decarboxylation. In all cases, acetyl-CoA directly serves as

biomass- and PHA precursor. For this process, volumetric PHA productivity was reported with  $0.94 \text{ g (l h)}^{-1}$ , which constitutes a value already competitive with industrial PHA production from expensive substrates of nutritional significance (Table 1). For example, the well-known semi-industrial PHA production process based on sugarcane sucrose, carried out at the Brazilian company PHB INDUSTRIAL S.A., delivers PHA (commercialized under the trademark Biocycle™) at a volumetric productivity of  $1.44 \text{ g (l·h)}^{-1}$  [39]. For the nitrogen limited phase (predominant PHA accumulation, no more biomass growth), the volumetric productivity even amounted to  $1.36 \text{ g (l·h)}^{-1}$  in the SFAE-based process. The specific volumetric productivity ( $q_p$ ) during this phase of predominant PHA accumulation was calculated with  $0.19 \text{ g (g h)}^{-1}$  and with  $0.14 \text{ g (g h)}^{-1}$  for the entire process. GC analysis revealed the monomer composition of the produced PHA; the material was a copolyester mainly consisting of 3HB monomers, and a minor fraction (0.84 mol%) of 3HV originating from odd-numbered fatty acids in the SFAE. Here, it should be pointed out that  $\beta$ -oxidation of odd-numbered fatty acids not only generates acetyl-CoA but, at the end of the  $\beta$ -oxidation, releases a propionyl-CoA moiety, which, after coupling with acetyl-CoA finally generates the 3HV-precursor 3-hydroxyvaleryl-CoA. Thermoanalysis results of the isolated PHA samples revealed values typical for *scl*-PHA with  $T_m$  of  $169.0^\circ\text{C}$ ,  $T_g$  of  $4.6^\circ\text{C}$ ,  $M_n$  of 203,740 Da, and a  $P_i$  of 1.50, but a rather low degree of crystallinity  $X_c$  of only 30.8 (Table 1) [20].

As a follow up, efforts were devoted to increase the share of odd-numbered moieties in the substrate, in order to achieve a higher molar 3HV fraction in the biopolyester, which should be beneficial in terms of decreased brittleness, crystallinity and melting temperature, hence factors facilitating its processibility. For this purpose, SFAE produced by transesterification of animal based tallow was reduced to fatty alcohols by using sodium. Catalyzed by aluminum oxide, these fatty alcohols were converted to the corresponding alkenes, which were finally oxidatively ozonolyzed, resulting in a mixture of odd-numbered carboxylic acids with 9-17 carbon atoms. This mixture was supplied as a carbon source to shaking flask cultures of *C. necator*. Using this carbon source cocktail as feedstock, the molar fraction of 3HV in PHA accumulated by *C. necator* exceeded  $0.06 \text{ mol mol}^{-1}$ . The isolated copolyesters displayed low  $X_c$  (22.5), low  $P_i$  (1.6), and low  $T_m$  ( $159^\circ\text{C}$ ), making them auspicious polymers for further processing (Table 1) [40].

This SFAE-based process was repeated using glycerol as the by-product of the transesterification process as the sole carbon source. Again, *C. necator* was cultivated in an aerobic fed-batch feeding regime with a stop of nitrogen supply after 23 h to terminate microbial growth and to

provoke predominant PHA formation. In this process,  $\mu_{max}$  and  $q_p$  amounted to  $0.11 \text{ l h}^{-1}$  and  $0.16 \text{ g (g·h)}^{-1}$ , respectively. After 30 h, the mass fraction of PHA in CDM amounted to  $0.65 \text{ g g}^{-1}$ , the volumetric PHA productivity to  $0.98 \text{ g (l·h)}^{-1}$ . With only  $0.29 \text{ g g}^{-1}$ , the yield of glycerol conversion to biomass was considerably lower than demonstrated by the same organism for SFAE conversion. Here, the homopolyester poly (3-hydroxybutyrate) (PHB) was produced by the cells. Results for thermo-analytical PHB characterization revealed data typical for PHB ( $T_m$   $173.0^\circ\text{C}$ ,  $T_g$   $5.6^\circ\text{C}$ ,  $X_c$  71.2%,  $M_n$  296,150 Da,  $P_i$  1.28) (Table 1) [20].

For the described *C. necator* mediated PHA production processes based on fed-batch addition of SFAE or glycerol, respectively, low structured mathematical models were established by the ANIMPOL project partners to further optimize the feeding strategies for supply with carbon and nitrogen sources; these models aimed to obtain enhanced PHA productivity in optimized follow-up cultivations. Developed models matched well the experimental results. Based on the models, it was demonstrated that in the case of SFAE as a substrate, it is necessary to adapt the culture to this substrate by a series of pre-cultures. Further, it was shown that the consumption rates for individual SFAEs (C14-C20) are proportional to the individual mass fraction; hence, they are utilized by the cells in a competitive nature. Finally, an optimal constant SFAE concentration of 10-12  $\text{g l}^{-1}$  was suggested based on the developed models, which should be maintained during the process by applying an optimized feeding regime [30].

As a follow-up of the experiments carried out by Cromwick and colleagues on shaking flask scale, Muhr and associates investigated the Pseudomonads *P. citronellolis* and *P. chlororaphis* in controlled bioreactor fed-batch cultivations, using tallow-based FAME as feedstock for *mcl*-PHA biosynthesis. The emulsifier Grinsted Citrem SP70 served for enhanced distribution of the substrate in the aqueous phase [36,37]. For *P. citronellolis*,  $\mu_{max}$  of 0.10 and  $0.08 \text{ l h}^{-1}$ , volumetric *mcl*-PHA productivity of  $0.036 \text{ g (l h)}^{-1}$  and  $0.050 \text{ g (l h)}^{-1}$ , and *mcl*-PHA mass fractions in CDM of 0.20 and  $0.27 \text{ g g}^{-1}$  were obtained in two parallel cultivation setups. GC-analysis revealed that the obtained *mcl*-PHA predominantly contained 3HO and 3HD monomers beside minor amounts of 3HDD, 3-hydroxynonanoate (3HN), 3HHx) and 3-hydroxyheptanoate (3HHp).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR further confirmed the presence of traces of unsaturated and, surprisingly, 3HV monomers. Results from thermo-analysis revealed a  $T_m$  of  $48.6^\circ\text{C}$  and  $53.6^\circ\text{C}$ ,  $T_g$  of  $-46.9^\circ\text{C}$  and  $-43.5^\circ\text{C}$ ,  $X_c$  of 12.3% and 10.4%,  $M_n$  = 35,000 and 196,000,  $P_i$  of 1.9 and 2.5 for the two parallel setups, which are typical values for *mcl*-PHA (Table 1) [36].

The bioreactor process for *mcl*-PHA production from animal based biodiesel using *P. chlororaphis* as production strain delivered a  $\mu_{max}$  of  $0.13 \text{ h}^{-1}$ , a  $q_P$  of  $0.006 \text{ g (g h)}^{-1}$ , a volumetric productivity of  $0.14 \text{ g (l h)}^{-1}$ , and a PHA mass fraction in CDM of  $0.23 \text{ g g}^{-1}$ . Also in this case, the supply of nitrogen source after 27 h provoked stop of biomass growth and predominant PHA biosynthesis. Similar to the previous processes with *C. necator* or *P. citronellolis*, the yield for SFAE conversion towards biomass was exceptionally high and amounted to  $0.62 \text{ g g}^{-1}$ . The produced *mcl*-PHA predominantly consisted of 3HO and 3HD monomers and minor quantities of 3HDD, 3HN, 3HHx, and 3HHp. Due to these characteristics, this material displayed a resin- like, highly amorphous product; no exact determination of  $T_m$  and  $X_c$  was possible. Similar to the *mcl*-PHA produced by *P. citronellolis*,  $T_g$  amounted to  $47.0^\circ\text{C}$ . Determination of molecular mass distribution revealed slightly higher values ( $M_n$  38,000,  $P_i$  1.93) than for the *P. citronellolis* based products (Table 1) [37].

Industrially rendered waste animal fats (triacylglycerides) were used by Riedel et al. without prior transesterification to FAME in shaking flask experiments for PHA production by the wild type strain *C. necator* DSM428 and its recombinant strain *C. necator* Re2058/pCB113 harboring the *Rhodococcus aetherivorans* genes encoding a *scl/mcl*-PHA-synthase which enables synthesis of P (3HB-*co*-3HHx) copolyesters. Waste animal fats were prepared by high-pressure thermolysis of animal waste of avian, bovine, porcine, and game origin; C16:0, C18:0, C18:1 and C18:2 constituted the predominant fatty acids. These materials were compared to tallow and waste frying oil when used as a carbon source. A simple emulsification strategy without any mechanical or chemical pre-treatment was developed to increase the bioavailability of solid hardly consumable fats. The wild type strain *C. necator* DSM428 produced PHB homopolymer at a mass fraction of  $0.79\text{--}0.82 \text{ g g}^{-1}$  CDM when cultivated on different lipids. Using tallow as sole carbon source, a volumetric PHB productivity of  $0.3 \text{ g (l h)}^{-1}$  and a PHB concentration of  $24 \text{ g l}^{-1}$  was obtained. The recombinant strain reached a mass fraction of the copolyester P (3HB-*co*-3HHx) of  $0.49\text{--}0.72 \text{ g g}^{-1}$  in dependence on the applied waste stream, with a molar 3HHx fraction of  $0.16\text{--}0.27 \text{ mol mol}^{-1}$ . Later, the recombinant strain was cultivated on low quality animal waste lipids under controlled conditions in a laboratory bioreactor, and reached a CDM of  $45 \text{ g l}^{-1}$  with a polyester mass fraction in CDM of  $0.6 \text{ g g}^{-1}$  P (3HB-*co*-3HHx), a volumetric PHA productivity of  $0.4 \text{ g (l h)}^{-1}$ , and a final 3HHx fraction in PHA of  $0.19 \text{ mol mol}^{-1}$  (Table 1) [38]. Considering a conversion yield of 0.6 to 0.7 g PHA per g triacylglycerides, one could estimate a theoretical quantity of roughly 300 kt PHA per year from the 500 kt of available animal based waste lipids (see also Figure 1).

#### 4. Economic and environmental considerations

Based on the experimental data described in the previous section, the preliminary process design for slaughterhouse waste based PHA production according to the ANIMPOL process, hence, transesterification of the waste lipids and application of the SFAE fraction as biotechnological substrate, has been published previously by Kettl et al. [41]. In these work, application of slaughtering waste from poultry, cattle, and pigs was taken into account, as well as the potential benefit to hydrolyze the slaughtering waste prior to extraction in order to generate higher lipid yields. These authors also suggest the application of hydrolyzed meat and bone meal (MBM) as an inexpensive nitrogen source, as it was successfully demonstrated in the past for PHA production by a halophilic strain, where a combined feeding of CGP and MBM was used for P (3HB-*co*-3HV) biosynthesis using the haloarchaeon *Haloferax mediterranei*. This halophilic organism produced a P (3HB-*co*-3HV) copolyester without addition of any additional precursor substrates structurally related to 3HV (Table 1) [42]. Later, further development and optimization of the process design regarding the system boundaries, material and energy streams was carried out by Titz et al. [32]. These authors summarized the whole ANIMPOL-process with special emphasize on optimized acidic hydrolysis of the waste materials, and, for the first time suggested to use hydrolyzed offal material as nitrogen source for the bacterial growth phase of the bioprocess. In this study, the process design was optimized in terms of minimizing waste stream generation and energy losses by Cleaner Production principles according to the ideas of Schnitzer and Ulgiati. Further, the authors carried out an ecological process evaluation via Life Cycle Assessment [32].

Later, a more in-depth ecological assessment of PHA production starting from slaughterhouse waste as starting material was carried out by Shahzad and colleagues, who compared *inter alia* the impact of diverse energy sources and the location of the production plant on the process sustainability and economic viability. This is a pivotal aspect considering the fact that the rendering process for lipid recovery is a highly energy demanding step, hence, the available energy mix (renewable, fossil, or nuclear) in different countries massively determined the ecological footprint of the PHA-production process starting from animal residues. Generally, transportation distances for the raw materials to the PHA production plant should be as short as possible to obtain higher economic efficiency. As major outcome, the authors assess a bandwidth for the ecological footprint area per t PHA between 373,000 and 956,000  $\text{m}^2$ , which is significantly lower than published data for the ecological footprint of poly(ethylene) of about

2.500,000 m<sup>2</sup> [33]. Subsequently, Narodoslowsky et al. published a study, where various additional factors affecting the sustainability of the ANIMPOL process and other PHA production processes based on inexpensive substrates were considered, such as the downstream processing for PHA recovery from biomass, co-substrate requirements, etc. This study was based on modern tools for calculating the ecological footprint of biopolymers, such as the sustainable process index (SPI). It was concluded that biopolymer production is not intrinsically more sustainable than production of well-established full carbon backbone polymers from petro-chemistry, but can become superior in ecological terms if all process steps (selection and preparation of raw materials, volumetric productivity, PHA recovery, closing of water, energy, and material cycles, energy supply, etc.) are considered and optimized [43].

Only recently, Shahzad et al. delivered a detailed economic analysis of PHA production from this animal waste bio-refinery concept based on the utilization of as many fractions of the animal based waste as possible. This encompasses the utilization of low quality biodiesel as main carbon source, and offal material as complex nitrogen sources, whereas MBM and the unsaturated FAME fraction are considered to have a market value as fertilizer or biofuel, respectively, hence, they should rather be sold for value generation than undergo bioconversion. Techno-economic analysis performed in this study revealed that PHA production cost varies from 1.41 € kg<sup>-1</sup> to 1.64 € kg<sup>-1</sup> when considering offal either as waste, or when its market price is considered, while fixed costs for unsaturated FAME fraction to be used as biofuel (0.97 € l<sup>-1</sup>) and for MBM (350 € t<sup>-1</sup>) were used for the cost assessment. The impact of market price fluctuations for offal materials, biodiesel, and MBM on the final PHA production cost and the investment payback time have also been assessed. As major outcome, the calculated investment payback time varies from 3.25 to 4.5 years in reliance to the current market situation [44].

## 5. Conclusion

The use of low quality waste animal fats as an inexpensive carbon feedstock displays high potential to make the ambitiously anticipated breakthrough of PHA on the plastic market finally a reality. Dependent on the applied microbial production strain, different types of PHA (*scl*-PHA and *mcl*-PHA) are accessible from the same substrates (animal waste lipids); these different types of PHA can be applied in various fields of the plastic market, starting from (food) packing materials, biodegradable (nano)carriers for active compounds, surgical application,

and other niches. Moreover, mathematical modeling provides a powerful tool for a better understanding of the bioprocesses on a metabolic level, and to identify and overcome eventual biotechnological bottlenecks in the process; this ultimately paved the way towards increased PHA production rates. As shown by the presented studies, it is pivotal to consider all process steps when designing a cost efficient biorefinery concept to convert animal residues to biopolyesters and marketable by products such as biofuels or MBM. Overall, considering the amounts of waste streams from the animal processing food industry currently available, which to date have to be disposed of in an expensive fashion, it appears likely that these surplus and waste streams will eventually be upgraded to the role of biotechnological feedstocks in a not too distant future, e.g., for biopolyester production, as demonstrated in the present review. In this context, it is essential to emphasize that a *de facto* costefficient technology for industrial scale PHA production from animal waste based lipids indispensably requires the integration of PHA production facilities into industrial production lines, where the raw material directly accrues; this will save transportation costs. This means that bioreactors dedicated to PHA production need to be located at rendering companies or large biodiesel producers [33]. As demonstrated in the ANIMPOL project, the lipid substrates, once produced, can conveniently be stored at cooling room temperature for extended periods without the need for additional precautions to prevent microbial decomposition.

Finally, it needs to be underlined that the ANIMPOL process displays an ethically clear technology, which only uses real waste and surplus streams as raw materials; neither does the process conflict with food production, nor will animals be farmed and killed for bioplastic production. In contrast, the process supports food industry by adding value to its waste streams! Considering the growing global demand for meat as essential part of human nutrition, the lipid-rich waste stream will accrue and rise anyhow in near future; hence, ANIMPOL does not contribute to the current “plate vs. plastic” controversy, but subsidizes food security by preserving resources of nutritional value, such as starch or sugars, which are normally used for PHA production.

## 6. Acknowledgements

The ANIMPOL project (“Biotechnological Conversion of Carbon Containing Wastes for Eco-Efficient Production of High-Value Products”, Grant no. 245084) was financed by the 7<sup>th</sup> Framework Program of the European Union.

## 7. Conflict of interest

The authors declare no conflict of interest.

## References

- Kourmentza C, Placido J, Venetsaneas N, Burniol-Figols A, Varrone C, Gavala HN, Reis MAM. Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production. *Bioeng.* 2017; 4 (2): E55. doi: 10.3390/bioengineering4020055
- Koller M. Poly (hydroxyalkanoates) for food packaging: Application and attempts towards implementation. *Appl Food Biotechnol.* 2014; 1 (1): 3-15. doi:10.22037/afb.v1i1.7127
- Koller M. Biodegradable and biocompatible polyhydroxyalkanoates (PHA): Auspicious microbial macromolecules for pharmaceutical and therapeutic applications. *Molecules.* 2018; 23 (2): 362. doi: 10.3390/molecules23020362
- Khosravi-Darani K, Bucci DZ. Application of poly (hydroxy-alkanoate) in food packaging: Improvements by nanotechnology. *Chem. Biochem. Eng. Q.* 2015; 29 (2):275-285. doi:10.15255/CABEQ.2014.2260
- Braunegg G, Bona R, Koller M. Sustainable polymer production. *Polymer-Plastics Technol Eng.* 2004; 43 (6): 1779-1793. doi: 10.1081/PPT-200040130
- Koller M, Marsalek L, de Sousa Dias MM, Braunegg G. Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. *New Biotechnol.* 2017; 37: 24-38. doi:10.1016/j.nbt.2016.05.001
- Tan GYA, Chen CL, Li L, Ge L, Wang L, Razaad IMN, Li Y, Zhao L, Mo Y, Wang JY. Start a research on biopolymer polyhydroxyalkanoate (PHA): a review. *Polymers* 2014; 6: 706-754. doi: 10.3390/polym6030706
- Obruca S, Benesova P, Marsalek L, Marova I. Use of lignocellulosic materials for PHA production. *Chem Biochem Eng Q.* 2015; 29(2): 135-144. doi:10.15255/CABEQ.2014.2253
- Khosravi-Darani K, Mokhtari ZB, Amai T, Tanaka K. Microbial production of poly(hydroxybutyrate) from C<sub>1</sub> carbon sources. *Appl Microbiol Biotechnol.* 2013; 97: 1407-1424. doi: 10.1007/s00253-012-4649-0
- Koller M, Hesse ., Fasl H, Stelzer F, Braunegg G. Study on the effect of levulinic acid on whey-based biosynthesis of Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by *Hydrogenophaga pseudoflava*. *Appl. Food Biotechnol.* 2017; 4(2): 65-78. doi: 10.22037/afb.v4i2.16337
- Koller M, Marsalek L. Cyanobacterial polyhydroxyalkanoate production: *Status Quo* and *Quo Vadis*? *Curr Biotechnol.* 2015; 4: 464-480.
- Drosg B, Fritz I, Gattermayr F, Silvestrini L. Photoautotrophic production of poly (hydroxyalkanoates) in cyanobacteria. *Chem Biochem Eng Q.* 2015; 29: 145-156. doi:10.15255/CABEQ.2014.2254
- Troschl C, Meixner K, Drosg B. Cyanobacterial PHA production-review of recent advances and a summary of three years' working experience running a pilot plant. *Bioengineering.* 2017; 4: 26. doi: 10.3390/bioengineering4020026
- Kucera D, Benesova P, Ladicky P, Pekar M, Sedlacek P, Obruca S. Production of polyhydroxyalkanoates Using hydrolyzates of spruce sawdust: Comparison of hydrolyzates detoxification by application of overliming, active carbon, and lignite. *Bioengineering.* 2017; 4(2): 53. doi: 10.3390/bioengineering4020053
- Cesario MT, Raposo RS, de Almeida MC, van Keulen F, Ferreira BS, da Fonseca MM. Enhanced bioproduction of poly-3-hydroxybutyrate from wheat straw lignocellulosic hydrolysates. *New Biotechnol.* 2014; 31(1): 104-113. doi: 10.1016/j.nbt.2013.10.004.
- Salgaonkar BB, Bragança JM. Utilization of sugarcane bagasse by *Halogeometricum borinquense* strain E3 for biosynthesis of poly (3-hydroxybutyrate-co-3-hydroxyvalerate). *Bioengineering.* 2017; 4(2): E50. doi: 10.3390/bioengineering4020050.
- Obruca S, Marova I, Melusova S, Mravcova L. Production of polyhydroxyalkanoates from cheese whey employing *Bacillus megaterium* CCM 2037. *Ann Microbiol.* 2011; 61: 947-953. doi: 10.1007/s13213-011-0218-5
- Koller M, Hesse P, Bona R, Kutschera C, Atlic A, Braunegg G. Potential of various archae-and eubacterial strains as industrial polyhydroxyalkanoate producers from whey. *Macromol Biosci.* 2007; 7: 218-226. doi: 10.1002/mabi.200600211
- Pais J, Serafim LS, Freitas F, Reis MA. Conversion of cheese whey into poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by *Haloferax mediterranei*. *New Biotechnol.* 2016; 33(1): 224-230. doi: 10.1016/j.nbt.2015.06.001.
- Koller M, Braunegg G. Biomediated production of structurally diverse poly (hydroxyalkanoates) from surplus streams of the animal processing industry. *Polimery.* 2015; 60: 298-308. doi:10.14314/polimery.2015.298
- Cavalheiro JM, de Almeida MCM, Grandfils C, Da Fonseca MMR. Poly (3-hydroxybutyrate) production by *Cupriavidus necator* using waste glycerol. *Proc Biochem.* 2009; 44: 509-515. doi:10.1016/j.procbio.2009.01.008
- Hermann-Krauss C, Koller M, Muhr A, Fasl H, Stelzer F, Braunegg G. Archaeal production of polyhydroxyalkanoate (PHA) co-and terpolyesters from biodiesel industry-derived by-products. *Archaea.* 2013; 2013(article ID). doi:10.1155/2013/129268
- Koller M, Marsalek L. Potential of diverse prokaryotic organisms for glycerol-based Polyhydroxyalkanoate production. *Appl Food Biotechnol.* 2015; 2(3):3-15.
- Koller M, Marsalek L. Principles of glycerol-based Polyhydroxyalkanoate (PHA) production. *Appl Food Biotechnol.* 2015; 2(4): 3-10.

25. Bhattacharya S, Dubey S, Singh P, Shrivastava A, Mishra S. Biodegradable polymeric substances produced by a marine bacterium from a surplus stream of the biodiesel industry. *Bioengineering* 2016; 3(4):34. doi: 10.3390/bioengineering3040034.
26. Obruca S, Marova I, Snajdar O, Mravcova L, Svoboda Z. Production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by *Cupriavidus necator* from waste rapeseed oil using propanol as a precursor of 3-hydroxyvalerate. *Biotechnol Lett.* 2010; 32: 1925-1932. doi: 10.1007/s10529-010-0376-8
27. Walsh M, O'Connor K, Babu R, Woods T, Kenny S. Plant oils and products of their hydrolysis as substrates for polyhydroxyalkanoate synthesis. *Chem Biochem Eng Q.* 2015; 29: 123-133. doi: 10.15255/CABEQ.2014.2252
28. Obruca S, Petrik S, Benesova P, Svoboda Z, Eremka L, Marova I. Utilization of oil extracted from spent coffee grounds for sustainable production of polyhydroxyalkanoates. *Applied Microbiol Biotechnol.* 2014; 98 (13): 5883-5890. doi: 10.1007/s00253-014-5653-3
29. Schober S, Seidl I, Mittelbach M. Ester content evaluation in biodiesel from animal fats and lauric oils. *Europ J Lipid Sci Technol.* 2006; 108: 309-314. doi:10.1002/ejlt.200500324
30. Spoljaric V, Lopar M, Koller M, Muhr A, Salerno A, Reiterer A, Malli K, Angerer H, Strohmeier K, Schober S, Mittelbach M, Horvat P. Mathematical modeling of poly [(R)-3-hydroxyalkanoate] synthesis by *Cupriavidus necator* DSM 545 on substrates stemming from biodiesel production. *Bioresour Technol.* 2013; 133: 482-494. doi: 10.1016/j.biortech.2013.01.126
31. Braunegg G, Genser K, Bona R, Haage G, Schellauf F, Winkler E. Production of PHAs from agricultural waste material. *Macromol Symp.* 1999; 144: 375-383. doi:10.1002/masy.19991440135
32. Titz M, Kettl KH, Shahzad K, Koller M, Schnitzer H, Narodoslawsky M. Process optimization for efficient biomediated PHA production from animal-based waste streams. *Clean Technol Environ Pol.* 2012; 14: 495-503. doi: 10.1007/s10098-012-0464-7
33. Shahzad K, Kettl KH, Titz M, Koller M, Schnitzer H, Narodoslawsky M. Comparison of ecological footprint for biobased PHA production from animal residues utilizing different energy resources. *Clean Technol Environ Pol.* 2013; 15: 525-536. doi: 10.1007/s10098-013-0608-4
34. Cromwick AM, Foglia T, Lenz RW. The microbial production of poly (hydroxyalkanoates) from tallow. *Appl Microbiol Biotechnol.* 1996; 46: 464-469. doi: 10.1007/s002530050845
35. Ashby RD, Foglia TA. Poly (hydroxyalkanoate) biosynthesis from triglyceride substrates. *Appl Microbiol Biotechnol.* 1998; 49:431-437. doi: 10.1007/s002530051194
36. Muhr A, Rechberger EM, Salerno A, Reiterer A, Schiller M, Kwiecien M, Adamus G, Kowalczyk M, Strohmeier K, Schober S, Mittelbach M, Koller M. Biodegradable latexes from animal-derived waste: Biosynthesis and characterization of mcl-PHA accumulated by *Ps. citronellolis*. *React Funct Polym.* 2013; 73: 1391-1398. doi:10.1016/j.reactfunctpolym.2012.12.009
37. Muhr A, Rechberger EM, Salerno A, Reiterer A, Malli K, Strohmeier K, Schober S, Mittelbach M, Koller M. Novel description of mcl-PHA biosynthesis by *Pseudomonas chlororaphis* from animal-derived waste. *J Biotechnol.* 2013; 165: 45-51. doi:10.1016/j.jbiotec.2013.02.003
38. Riedel SL, Jahns S, Koenig S, Bock MC, Brigham CJ, Bader J, Stahl U. Polyhydroxyalkanoates production with *Ralstonia eutropha* from low quality waste animal fats. *J Biotechnol.* 2015; 214: 119-127. doi:10.1016/j.jbiotec.2015.09.002
39. Nonato R, Mantelatto P, Rossell C. Integrated production of biodegradable plastic, sugar and ethanol. *Appl Microbiol Biotechnol.* 2001; 57(1-2): 1-5. doi: 10.1007/s002530100732
40. Koller M, Salerno A, Strohmeier K, Schober S, Mittelbach M, Illieva V, Chiellini E, Braunegg G. Novel precursors for production of 3-hydroxyvalerate-containing poly [(R)-hydroxyalkanoate]s. *Biocatal Biotransfor.* 2014; 32: 161-167. doi:10.3109/10242422.2014.913580
41. Kettl KH, Titz M, Koller M, Shahzad K, Schnitzer H, Narodoslawsky M. Process design and evaluation of biobased polyhydroxyalkanoates (PHA) production. *Chem Eng Trans.* 2011;25:983-988 doi: 10.3303/CET1125164
42. Koller M, Bona R, Braunegg G, Hermann C, Horvat P, Kroutil M, Martinz J, Neto J, Pereira L, Varila P. Production of polyhydroxyalkanoates from agricultural waste and surplus materials. *Biomacromol-ecules* 2005; 6(2): 561-565. doi: 10.1021/bm049478b
43. Narodoslawsky M, Shahzad K, Kollmann R, Schnitzer H. LCA of PHA production-identifying the ecological potential of bio-plastic. *Chem Biochem Eng Q.* 2015; 29: 299-305. doi: 10.15255/CABEQ.2014.2262
44. Shahzad K, Narodoslawsky M, Sagir M, Ali N, Ali S, Imtiaz Rashid M, Ismail IMI, Koller M. Techno-economic feasibility of waste biorefinery: Using slaughtering waste streams as starting material for biopolyester production. *Waste Manage.* 2017; 67: 73-85. doi:10.1016/j.wasman.2017.05.047

## پساب صنعت فرایند حیوانی به عنوان ماده اولیه برای تولید زیست‌بسپارهای پلی هیدروکسی آلکانوات‌ها

مارتین کولر<sup>۱\*</sup>، خارام شهزاد<sup>۲</sup>، گرهارت برانگ<sup>۳</sup>

۱- انستیتو شیمی، دانشگاه گراتس، ناوی (NAWI) گراتس، اتریش.

۲- مرکز عالی مطالعات محیطی، دانشگاه پادشاه عبدالعزیز، عربستان سعودی.

۳- آرن-انجمن فناوری های پایدار و منابع کارا، گراتس، اتریش.

### چکیده

**سابقه و هدف:** صنعت فرآوری حیوانات در ۲۸ کشور اتحادیه اروپا (the EU-28 states)، شامل کشتارگاه‌ها، شرکت های تبدیل مواد مازاد (ذوب چربی Rendering) و سایرین حجم زیادی از پساب حاوی حدود ۵۰۰,۰۰۰ تن چربی به همراه مقادیر قابل توجهی ضایعات لاشه و پودر استخوان و گوشت تولید می‌کنند. این مواد باید با روش‌هایی مانند زیست دگرگونی زیست‌بسپارهای پلی هیدروکسی آلکانوات (PHA) با ترکیب‌های مولکولی متنوع و ویژگی‌های شبه پلاستیک (Plastic-like features) ارزش بیشتری پیدا کنند. نوآوری مقاله حاضر این است که برای اولین بار پژوهش‌های پیشین و فعلی در خصوص تولید PHA از پساب‌های حیوانی خلاصه و انتخاب گونه‌های میکروبی تولیدکننده مناسب، فرآیندهای بالادستی مواد خام برای تولید منابع کربنی در دسترس، سینتیک زیست فرآیند، تعیین ویژگی‌های زیست‌بسپارها با ساختار مولکولی دگرگون شده، ارزیابی فرآیند محیطی و امکان‌سنجی اقتصادی بیان شده است.

**یافته‌ها و نتیجه‌گیری:** مطالعات مقایسه موردی به وضوح نشان می‌دهند استفاده از ضایعات فرآیند حیوانی به عنوان نسل دوم مواد اولیه برای تولید زیست‌بسپارها به دلیل استفاده از گونه‌های میکروبی بهینه، خوراک‌دهی مناسب، کوتاه بودن مسیر انتقال و طرح‌های کسب و کار شفاف برای تجاری‌سازی محصول نهایی به یقین می‌تواند فرآیندی مناسب و با ارزش اقتصادی باشد. بیشتر از همه، استفاده از پسماند حیوانی برای تولید زیست‌بسپارها و سوخت‌های زیستی نسل دوم، با حفظ ارزش تغذیه‌ای مواد اولیه می‌تواند در ایمنی مواد غذایی نقش داشته باشد.

### تاریخچه مقاله

دریافت ۱۹ سپتامبر ۲۰۱۷

داوری ۲۳ اگوست ۲۰۱۸

پذیرش ۲۵ اگوست ۲۰۱۸

### واژگان کلیدی

- ضایعات فرآیند حیوانی
- دیزل سوختی
- ایمنی مواد غذایی
- لیپیدها
- پلی هیدروکسی آلکانوات‌ها

### \*نویسنده مسئول

مارتین کولر، دانشگاه گراتس، انستیتو شیمی، ناوی گراتس، هنریش استراس A-8010، گراتس، اتریش.

تلفن: +۴۳-۳۱۶-۳۸۰-۵۴۶۳

پست الکترونیک:

[martin.koller@uni-graz.at](mailto:martin.koller@uni-graz.at)