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Editorial

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Linking Food Industry to “Green Plastics” – Polyhydroxyalkanoate (PHA) Biopolyesters from Agro-industrial By-Products for Securing Food Safety

Martin Koller

University of Graz, Institute of Chemistry, NAWI Graz, Heinrichstrasse 28/III, A-8010 Graz, Austria, Tel: +43-316-380-5463;

Email: martin.koller@uni-graz.at

Introduction

This special issue of *Applied Food Biotechnology* is dedicated to two major threats of our today´s world: First, humankind needs secure access to food, which indispensably demands the preservation of food and feed resources instead of converting them towards energy carriers, polymers, or other materials. Second, the current global plastic situation is increasingly aggravating considering the growing piles of plastic waste stemming from exploitation of fossil resources, the tremendous effects of global warming, which is also caused by incineration of crude oil-derived materials like plastic waste, and the dramatic pollution of marine and other aquatic environments especially by microplastics. Microplastics in particular have a direct effect on food security; they easily travel through the entire food supply chain and are finally accumulated in the top of the food chain, hence by the human metabolism [1-3]. Food production using equipment with plastic parts and food storage in plastic bottles and containers is another direct source of microplastic contamination of the food, mainly by abrasion [4]. The identification and assessment of potential effects on the human health, which can nowadays guessed at best, are currently only in their infancy. Just recently (autumn 2018), microplastics of a total of nine plastics, mainly polypropylene and poly (ethylene terephthalate) (PET), in the size of 50 to 500 µm were unambiguously identified by Austrian researchers in the intestine of humans, from where they can potentially be transported to the blood and lymph system and diverse organs [5]. Another direct link between exhaustive use of established plastics and endangering the security of food supply is obvious by the global warming; already now, it is expected that the temperature rise will impede agriculture in different global areas, which will provoke shortage in agricultural products needed for human nutrition, and in parallel in an increase of costs of these products which makes them unaffordable for a growing number of people [6,7].

Biopolymers with plastic-like material features, such as poly(lactic acid), thermoplastic starch, or, as the topic of this special issue, microbial polyhydroxyalkanoate (PHA) polyoxoesters, are a viable alternative to established plastics originating from petrochemistry [8]. Being based on renewable resources, these biomaterials do not contribute to the depletion of fossil resources and, beyond that, they are embedded into nature´s closed carbon cycle, which means that their degradation does not cause an increase of the atmospheric CO_2 level. The latter can easily be visualized by the fact that raw materials classically used for production of PHA and other biopolymers are renewables like carbohydrates or lipids, which are products and substrates of the natural catabolism and anabolism of carbonaceous materials. These renewable materials were not enclosed within our planet´s interior since millions of years, as crude oil, the raw material for production of established plastics, does! After their use, biodegradation of biopolymers like PHA generates biomass and CO_2 as part of the active carbon cycle, which is fixed again by green plants; now, the cycle of generation of renewable feedstocks, microbial biopolymer synthesis and biodegradation starts again. This is in clear contrast to the production and incineration of petrochemical plastics, where carbon fixed in the Earth is suddenly released into the atmosphere as surplus CO_2 [9].

Now, at this point one has to consider the holistic aspects of ethics and sustainability when choosing the adequate raw material for biopolymer production. For sure, the use of edible carbohydrates like glucose or lipids of nutritional value is a convenient and more or less optimized technology for biotechnological PHA production, being exhaustively described in the scientific literature and already successfully implemented on a pilot and industrial scale since decades. However, this strategy is not feasible considering the number of people starving globally; using edible resources provokes the well-known "plate-versus-plastics" contr-oversy, similar to the "plate-versus-tank" debate caused by the production of bioethanol starting from sucrose or starch [10]. In a nutshell, simply replacing established plastics by bio-alternative without taking into account the entire process chain and life cycle of the biopolymer cannot be considered the one and only panacea for the plastic problem [11]. A sustainable process for biopolymer production needs to encompass aspects of economics, environmental protect-tion, optimized engineering, and ethics [10]. As a real exit strategy to escape this dilemma, it is possible to directly link the food production sector with biopolymer production in a way suitable of generating synergism for both parties. This becomes possible by using carbon-rich waste streams of food and feed production and -processing as raw materials for biopolymer manufacturing [12,13]. To underline the significance and justness of this now emerging mindset, one should consider that per year an estimated amount of about 10^{12} kg of food is simply discarded [14]. Replacing pure carbon sources by such carbon-rich organic food waste streams offers the change to safe about half of the entire biopolymer production costs. Of course, one needs to understand the composition of such complex substrates and their impact on the applied microbial production strains; as the case arises, it might be needed to develop adequate methods of upstream processing to make such waste stream suitable biotechnological feedstocks, hence, it might be required to hydrolyze polymers into smaller, convertible compounds [15,16], or to detoxify complex hydrolysis cocktails by removing inhibiting compounds [17]. Apart from using food and agricultural waste as feedstocks to cultivate whole cell biocatalysts, it is more and more recognized that several waste streams from agriculture and food production, mainly lignocelluloses, can be applied to develop new biopolymer composite materials which unprecedented, triggered properties and performance features making them suitable to be applied in different sectors of the plastic market, *inter alia* the production of food packaging materials [18-23].

Hence, there are two distinguished investigative directions followed by the authorships of the individual contributions to this special issue; said authorships were carefully selected among the leading research groups currently active in the development of such production processes for PHA and follow-up products, which are independent from the depletion of food resources but, in contrast, focus on the value-added integration of food and feed by-products into the biopolymer production chain. The achieved synergism upgrades waste streams of food production to raw materials for production of "green plastics", and even helps food industry to overcome waste disposal problems [24]. Gratefully appointed guest editor of this journal issue, I was more than enthusiastic to bring together a number of eight differently focused teams of researchers, among them microbiologists, genetic engineers, biotechnologists, and polymer scientists stemming from different global regions, all of them well recognized by their peers in the scientific community, and ready to participate in this inspiring publication project. To make a long story short, the following paragraphs invite the respected reader on a journey into the individual chapters, highlighting the most significant progress and take-home messages of each paper.

Individual chapters

In their review article, Brigham and Riedel summarize prime examples of the current endeavors to utilize the most important carbohydrate-rich side streams of food processing industry, namely whey from cheese production, molasses from sugar industry, or saccharified starch waste, e.g., from potatoes processing for production of PHA biopolyesters of different composition and properties. Special focus of this chapter is also dedicated to the use of lipid waste fractions associated to the food sector, such as waste lipids from the slaughtering and rendering industry, or lipids extracted from the incredible amounts of spent coffee ground, a still strongly neglected waste stream. Another intriguing approach is described by the new two-stage process of anaerobically converting food waste to volatile fatty acids, which, in a second step, can be supplied to aerobic cultivation processes of PHA-producing microbes towards biopolyesters. The authors of this review also frankly discuss the challenges associated to collection of food waste from households, and emphasize the necessity to implement PHA production facilities into industrial food production facilities, where the raw materials directly accrue as waste streams in sufficient quantity. This saves transportation costs and makes the biopolymer production process even more viable in economic terms. This integration should go in parallel with the downstream processing of PHA-containing biomass, which is needed to recover the biopolymers from microbial cells; downstreaming should be operated on-site, too, in order to save the shipment of biomass. Finally, successful attempts for utilization of hydrolyzed residual biomass, which remains after PHA recovery, to feed microbial cultures in subsequent cultivation setups for PHA production are demonstrated;

this step contributes another brick in closing material cycles in biopolymer manufacturing [25].

Cinelli and colleagues provide a comprehensive study on the use of agricultural and food processing by-products to develop novel PHA-based biocomposite materials. These biocomposites were designed by melt extrusion and injection molding of mixtures of PHA copolyesters with biobased and biocompatible plasticizers, inorganic fillers and adequate organic fillers such as wood fibers or fibers of pea shells, which constitute a typical waste product from food production. After optimization of the processing conditions, novel biocomposites with remarkable material properties were obtained; these biocomposites match the material performance features requested for products to be used as rigid thermoplastic food packaging materials, tableware, or agricultural pots for single use. Not only do these materials match the performance of concurring petro-plastics in these applications; beyond that, they feature the additional benefit of being biodegradable and compostable. Moreover, this paper provides a mathematic model describing the interaction between the PHA matrix and the organic filler phase in order to understand and further optimize the exact biocomposite composition [26].

Chee *et al.* describe a completely new route of research to link PHA biosynthesis with food security and food production. These authors present the most prominent bacterial PHA production strain, *Cupriavidus necator*, as a source of single cell protein. Starting from heterotrophic PHA and biomass production by this strain based on conversion of agricultural and food waste under optimized biotechnological conditions, this organism can be harvested as biomass pellet with PHA fractions up to 90% of their cell dry mass. Recovery of the intracellularly stored PHA normally requires the exhaustive use of solvents, which, in most cases, are highly flammable and deleterious to human health and the environment. In this article, the authors suggest the use of the mealworm *Tenebrio molitor*, an insect digesting the non-PHA part of the biomass pellets, and excreting native, astonishingly pure PHA biopolyesters pellets as feces, which can directly be processed without further purification at least for non-medical applications. Now, mealworms farmed and multiplied by feeding the bacterial biomass are considered a potential source of human nutrition in various global regions, or to feed animals used for meat production. While the latter does not pose any conflicts, the direct use for human nutrition is candidly discussed by the authors in an ethical, religious, consumer-acceptance, and cultural context [27].

Favaro and associates investigated a broad range of lipid-rich waste streams as substrates for PHA biosynthesis, such as crude glycerol phase from tallow-based biodiesel production, biodiesel obtained from lipid waste, waste oil, and bacon rind, udder or tallow as typical abundant waste materials from animal processing in slaughterhouses. Several new microbial isolates and already previously known PHA producing strains have been screened to assess, on the one hand, their lipolytic activities needed to utilize the lipophilic substrates, and, on the other hand, their PHA biosynthesis potential. The authors found out that particularly soil provides a rich source to isolate new intriguing microbial strains, an even better source than the *a priori* expected pre-destined ecological niches such as slaughterhouses. Among the investigated strain collection species, *C. necator* and *Pseudomonas oleovorans* (a.k.a. *Pseuomonas putida*) displayed proficient PHA production potential, especially on biodiesel, glycerol, and enzymatically hydrolyzed waste oil [28].

A similar approach to use waste lipids as feedstocks was followed by Kumar and Kim, who exploited waste cooking oil, a typical waste material from households and gastronomy, for PHA copolyester biosynthesis by the halophilic production strain *Paracoccus* sp. LL1, an own isolate of this research group. These experiments were carried out under controlled aerobic conditions in a laboratory bioreactor to evaluate the kinetics of microbial growth and PHA biopolyesters production. Beside the production of about 1 g l^{-1} PHA biopolyesters, which contained mainly 3-hydroxybutyrate and some 3-hydroxyvalerate building blocks, the strain also turned out as a proficient producer of a high carotenoid fraction rich in astaxanthin. Astaxanthin is a valuable pigment used for farming of salmonids in order to provide these fish with the typical salmon coloration desired by the customers. Hence, the work demonstrates the viability of using a waste product from food production to co-produce biopolymers and nutrient additives for aquaculture [29].

Bustamante and colleagues studied the use of another inexpensive plant oil not interfering with food and feed purposes, namely oil extracted from the seeds of *Camelina sativa*, a member of the *Brassicaceae* family that can be cultured without major efforts under various challenging environmental conditions. In this study, *Pseudomonas resinovorans* was used as production strain. This organism was able of accumulating an elastomeric *mcl*-PHA from this substrate without the need for any pretreatment such as hydrolysis or saponification. In bioreactor cultivation setups operated under phosphate-limited conditions and fed-batch pulse feeding of the substrate, a high *mcl*-PHA con-centration exceeding 13 g l^{-1}, and a *mcl*-PHA fraction in biomass of more than 40% was obtained, which is among the top-productivities up to now reported for *mcl*-PHA biosynthesis on inexpensive carbon sources. The composition of this *mcl*-PHA (building blocks ranging from 3-hydroxybutyrate to 3-hydroxytetradecanoate), a compost-able “bio-latex”, and its molecular mass data are similar to those obtained by other expensive strain-substrate combinations described for production of *mcl*-PHA [30].

Apple pomace, a waste generated by the fruit processing industry at huge amounts, was tested by Rebocho and associates as the sole carbon source for *mcl*-PHA biosynthesis in bioreactor cultivation setups using the previously underexplored bacterial strain *Pseudomonas citronellolis* NRRL B-2504. Apple pulp was separated into a solid fraction and a soluble fraction rich in glucose, fructose, and some quantity of sucrose; these sugars acted as substrates in the bioprocess, which produced biomass with an intracellular *mcl*-PHA fraction of 0.3 $g\ g^{-1}$. The *mcl*-PHA was composed predominately of 3-hydroxydecanoate and 3-hydroxyoctanoate, displayed thermomechanical and physico-chemical features typical for *mcl*-PHA “bio-latex”, and was processed by the authors to transparent polymeric films. These films were investigated in details, and turned out to be dense, ductile and permeable to O_2 and CO_2, hence, they hold promise for future application as food packaging materials. This work provides an expedient example how to close the cycle from using a waste stream from food production to generate new functional materials to be used for food packaging [31].

The last article, contributed by Pernicova *et al.*, demonstrates the use of a rather unexpected waste stream from food production, namely hydrolyzed chicken feathers. Chicken feathers are an abundant side product of the poultry processing industry, which up to now has no reasonable use and has to undergo disposal or simple anaerobic digestion to biogas in the myriad of global regions, wherever poultry is farmed. *Pseudomonas putida* KT2440 was cultivated on chicken feathers. The organism readily degraded this proteinaceous matter by excreting the extracellular enzyme keratinase. Harvesting the active bacterial biomass, separating keratinase and transferring the biomass into nitrogen-deficient nutrient media resulted in accumulation of 0.6 g/g *mcl*-PHA “bio-latex” in cell mass, composed of 3-hydroxyoctanoate and 3-hydroxyhexanoate. This article provides a new strategy to convert a real waste stream from food production into active biomass for PHA biopolyesters biosynthesis and, in parallel, into keratinase, an enzyme exhaustively used, e.g., for production of drain cleaners [32].

Conclusion

Based on these contributions, I hope the respected readers get a new perspective of possibilities to link waste streams from food production to production of PHA biopolyesters of different composition and properties, which shall find use in different areas of application. These strategies combine the use of waste with new approaches of “White Biotechnology”, and are characterized by closed material cycles, waste reduction, and target-oriented development of new bioproducts. It would be very desirable if the studies presented in this special issue act as the ignition spark to inspire further researchers all over the world to get active in linking measures to support the food supply chain, to upgrade food processing and agricultural waste and to produce sustainable “bioplastics” and, at the same time, to preserve food resources.

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