Application of Activated Charcoal in the Downstream Processing of Bacterial Olefinic Poly(3-hydroxyalkanoates)

Bruno Wampfler, Thomas Ramsauer, Karl Kehl, Manfred Zinn, and Linda Thöny-Meyer*

Abstract: Medium chain length poly(hydroxyalkanoates) (mcl-PHAs) are bacterial thermoplastic elastomers with a large potential in medical applications. The present study provides a novel process to isolate and purify poly([*R*]-3-hydroxy-ω-undecenoate-*co*-3-hydroxy-ω-nonenoate-*co*-3-hydroxy-ω-heptenoate) (PHUE) and poly([*R*]-3-hydroxy-ω-undecenoate-*co*-3-hydroxy-ω-nonenoate-*co*-3-hydroxyoctanoate-*co*-3-hydroxy-ω-heptenoate-*co*-3-hydroxyhexanoate) (PHOUE) from *Pseudomonas putida* species. Three different types of activated charcoal were compared with regard to their capability to selectively remove impurities. The product 'Charcoal activated, powder, pure' from Merck was found to be most suitable. Using ethyl acetate as solvent, the polyesters were extracted from freeze-dried biomass at room temperature and simultaneously purified by addition of activated charcoal at the beginning of the extraction. The period of extraction was one hour and the ratio solvent to biomass was 15:1 (vol/wt). After extraction, the solids were separated by pressure filtration through a metallic lace tissue. The filtrate was again passed through the previously accumulated filter cake, followed by a second filtration through a 0.45 μm membrane to remove finest coal particles. The resulting filtrate was concentrated, thus yielding polyesters whose quality and yield depended on the quantity of activated charcoal applied. For highly pure PHUE and PHOUE with low endotoxin levels, the optimum ratio of activated charcoal to solvent for extraction (V/V) was found to be 0.5 for PHUE and 0.25 for PHOUE. The yields with regard to the raw polymers amounted to 55 wt% for PHUE and 75 wt% for PHOUE, which are acceptable for polymers that can be used for medical applications.

Keywords: Activated charcoal · Downstream processing · PHA · Polyhydroxyalkanoate · Purification

1. Introduction

Bacterial poly(hydroxyalkanoate)s (PHAs) whose monomers contain 6 to 14 carbon atoms are called medium chain length poly(hydroxyalkanoate)s (mcl-PHAs). Mcl-PHAs are thermoplastic elastomers. Length and functional groups of side chains can be controlled to some extent by feeding the cells with appropriate organic acids during biosynthesis in batch cultures,[1] or more precisely under multiple-nutrient-limited growth conditions in chemostat cultures.[2,3] When bearing carbon–carbon double bonds, mcl-PHAs can be used for chemical functionalization,[4,5] *e.g.* cross-linking to obtain a biodegradable rubber.[6–8] They also enable covalent binding of small molecules such as natural antifouling agents[9] or the synthesis of organic/inorganic hybrid polymers.[10] In order to separate PHA from other cell components such as cell walls, nucleic acids and peptides, appropriate extraction processes must be applied. Besides mechanical disruption, methods used most often are solvent extraction as well as chemical and enzymatic digestion.[11] The recovery by using organic solvents prevails over aqueous treatment if PHA of high purity is required. PHA with a purity of about 90% was obtained when the dry biomass from *Pseudomonas putida* was pre-treated with methanol for 5 min and subsequently extracted in a Soxhlet apparatus for 5 h, followed by precipitation of PHA in cold methanol.[12] For the large-scale production of poly([*R*]-3-hydroxyoctanoate-*co*-3-hydroxyhexanoate) (PHO), extraction or treatment of the biomass, respectively, using acetone, chloroform and sodium dodecyl sulfate was investigated.[13] Acetone was found to give the highest yield. Purity of 99% ± 0.2% was determined, when the PHO concentrate had been precipitated in a volumetrically prepared mixture of 35% methanol, 35% ethanol and 30% water. When bacterial biopolymers are isolated for medical applications, contaminating pyrogenic compounds must be removed. Such compounds in the form of endotoxins (lipopolysaccharides) are present in the outer cell membrane of Gram-negative production strains.[14] The United States Pharmacopeia (USP) approves maximum 20 endotoxin units (EU) per medical device, and 2.15 EU per device that contacts the cerebrospinal fluid.[15] Low endotoxin PHO was achieved by extraction of the freeze-dried biomass with *n*-hexane at 50 °C, followed by reducing the temperature of the extract to 0–5 °C after filtration.[16] The precipitate thus obtained was subjected to temperature controlled re-dissolution and precipitation in 2-propanol, which resulted in a purity of over 99 wt%. Yield and endotoxicity of mcl-PHA were investigated in dependence of the solvent used for extraction of the freeze-dried biomass.[17] The results revealed that ethyl acetate and acetone have the same power of extraction as methylene chloride, but their use led to a polymer containing significantly less endotoxin. Endotoxin could be removed from aqueous solutions by filtration through activated charcoal.[18] In the field of PHA, activated charcoal was also used

Correspondence: Dr. L. Thöny-Meyer
Empa, Swiss Federal Laboratories for Materials
Science and Technology
Laboratory for Biomaterials
Lerchenfeldstrasse 5
CH-9014 St. Gallen
Tel.: +41 71 274 7792
Fax.: +41 71 274 7788
E-mail: linda.thoeny@empa.ch

for the purpose of purification, for example to decolorize and detoxify growth media prior to fermentation,[19] to decolorize solutions of hydroxy acid methyl esters,[20] or to purify poly(hydroxybutyrate) dissolved in chloroform.[21]

In a recent study,[22] we developed a process to isolate PHO and poly([*R*]-3-hydroxy-ω-undecenoate-*co*-3-hydroxy-ω-nonenoate-*co*-3-hydroxy-ω-heptenoate) (PHUE) from freeze-dried biomass of *Pseudomonas putida* species. Hereby, after solvent extraction, the crude extract was subjected to a filtration through activated charcoal. The resulting PHO and PHUE were of high purity and contained less than one endotoxin unit per g (EU/g). However, purification with activated charcoal caused a loss of material of 50% which consisted not only of impurities but also of PHO and PHUE, respectively. The present study aimed at optimizing the purification with activated charcoal, especially in terms of yield. For this purpose, bacterial PHUE and poly([*R*]-3-hydroxy-ω-undecenoate-*co*-3-hydroxy-ω-nonenoate-*co*-3-hydroxyoctanoate-*co*-3-hydroxy-ω-heptenoate-*co*-3-hydroxyhexanoate) (PHOUE) (Fig. 1) were extracted from freeze-dried biomass and purified with three different types of activated charcoal. The quality of the resulting polymers was evaluated as a function of the quantity of the activated charcoal. Critical parameters were yield, content of endotoxin and content of impurities characterized by UV/VIS spectrometry and size exclusion chromatography (SEC). The goal was to develop a downstream process which consists of only few simple laboratory steps, so that mcl-PHAs of high quality can be produced in a time-saving manner.

2. Experimental Section

2.1 *Materials*

PHUE was produced from ω-undecenoate in a chemostat culture ($D = 0.1$ h^{-1}) of *Pseudomonas putida* GPo1 under dual (carbon, nitrogen) limited growth conditions (C/N = 12.5 g/g).[3] Gas chromatographic analysis revealed the following composition: 17.8 mol% of 3-hydroxy-ω-undecenoate, 63.2 mol% of 3-hydroxy-ω-nonenoate, and 19.0 mol% of 3-hydroxy-ω-heptenoate. PHOUE was produced from octanoic and ω-undecenoic acid in a chemostat culture ($D = 0.1$ h^{-1}) of *Pseudomonas putida* Gpo1 under dual (carbon, nitrogen) limited growth conditions (C/N = 12.5 g/g). According to gas chromatographic analysis, the polymer consisted of 6.5 mol% of 3-hydroxy-ω-undecenoate, 22.9 mol% of 3-hydroxy-ω-nonenoate, 58.2 mol% of 3-hydroxyoctanoate, 5.3 mol% of 3-hydroxy-ω-heptenoate, and 7.1 mol%
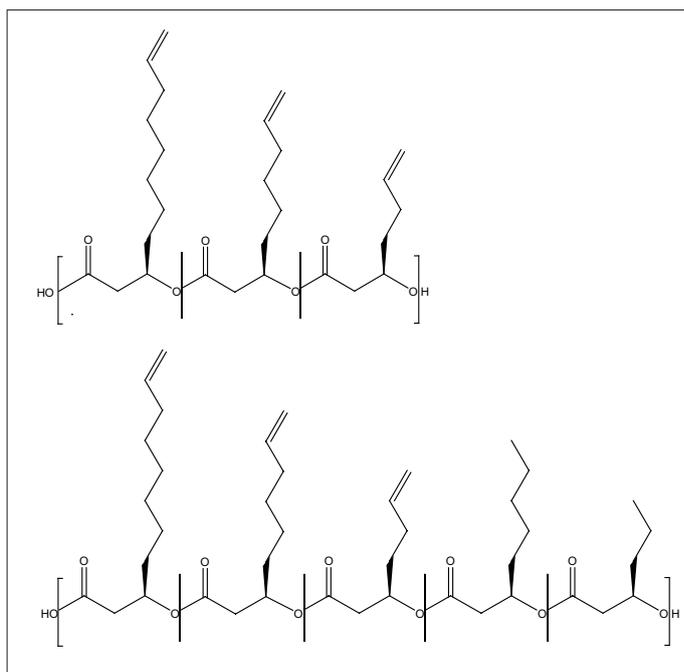

Fig. 1. Chemical structure of poly([*R*]-3-hydroxy-ω-undecenoate-*co*-3-hydroxy-ω-nonenoate-*co*-3-hydroxy-ω-heptenoate) (PHUE) produced by *Pseudomonas putida* GPo1 when cultured on ω-undecenoic acid (top), and of poly([*R*]-3-hydroxy-ω-undecenoate-*co*-3-hydroxy-ω-nonenoate-*co*-3-hydroxyoctanoate-*co*-3-hydroxy-ω-heptenoate-*co*-3-hydroxyhexanoate) (PHOUE) produced by *Pseudomonas putida* GPo1 when cultured on octanoic acid and ω-undecenoic acid (bottom).

of 3-hydroxyhexanoate. Ethyl acetate was of Ph. Eur. quality and was obtained from Hänseler AG, Herisau, Switzerland. Three types of activated charcoal were tested: i) charcoal activated, granular, about 1.5 mm, pure, food grade, from Merck, Darmstadt, Germany, hereinafter called 'Merck granules', ii) charcoal activated, powder, pure, from Merck, Darmstadt, Germany, hereinafter called 'Merck powder', iii) Norit®, from peat, steam activated, granular, 1–3 mm from Sigma Aldrich, Steinheim, Germany, hereinafter called Norit.

2.2 *Isolation of PHUE and PHOUE from Freeze-Dried Biomass*

A portion of 10 g of freeze-dried biomass was manually crushed to small pieces and mixed with 150 mL of ethyl acetate. The solids in the solvent were further reduced using a SilentCrusher M (Heidolph, Kelheim, Germany) equipped with a disperser 18 G/M. Afterwards, y mL of activated charcoal were added, where y was 9, 18, 37.5, 75, or 112.5 mL. The suspension was stirred for 1 h at room temperature. The phases were separated by pressure filtration (air, 2–5 bar) through a metallic lace tissue Duplex 15 (diameter of 47 mm, G. Bopp, Zürich, Switzerland) using a 200 mL pressure vessel (SM 16249, Sartorius, Göttingen, Germany). In order to remove particles that swept along with the solution, the filtrate was again passed through the previously accumulated filter cake. The

metallic lace tissue was replaced by a 0.45 µm membrane filter (RC 55, Whatman, Göttingen, Germany) and the solution was again filtered to remove finest coal particles. The filtrate was concentrated in a rotary evaporator at 40 °C and about 200 mbar. Residual solvent was removed by drying the gel-like solid in a vacuum dryer (VTR 5036, Heraeus, Hanau, Germany) for 24 h at 40 °C and 100 mbar. The pure polymer was weighed and afterwards stored at –20 °C to prevent autoxidation.[23] The raw PHA was obtained in the same manner, but without addition of activated charcoal.

2.3 *Polymer Characterization*

Molecular weight fractionation was performed by size exclusion chromatography (SEC) using a differential refractive index detector (SEC apparatus: Viscotek, Houston, TX, USA). About 30 mg of each polymer sample were dissolved in 10 mL of tetrahydrofuran (THF). Aliquots of 100 µL of the polymer solution were injected and separated on three sequentially coupled SEC columns (300 mm × 8 mm, pore sizes 10^3, 10^5, and 10^6 Å, PSS, Mainz, Germany) at 35 °C, applying a flow rate of 1 mL/min with THF. Calibration was performed with 10 narrow standard polystyrene samples from PSS (2×10^3 g/mol to 2.13×10^6 g/mol).

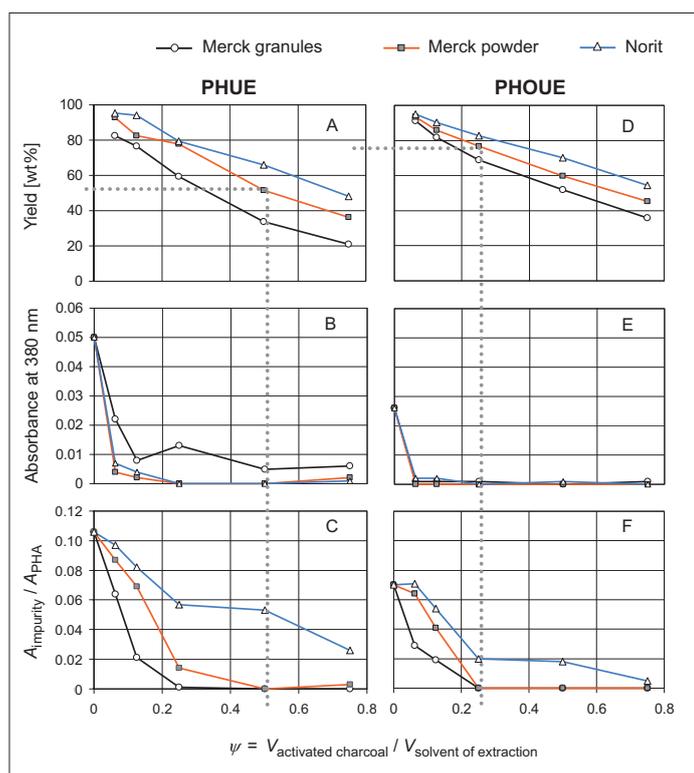The partitions of 3-hydroxyalkanoates in PHUE and PHOUE were determined by

Fig. 2. Charts for evaluating quantity and type of activated charcoal for the purification of PHUE and PHOUE. The yield of PHA (A and D) is related to the corresponding raw polymer. The light absorption at 380 nm (B and E) is a measure for the unwanted yellow tinge of the polymer. $A_{impurity}/A_{PHA}$ (C and F) means the ratio of the area of the impurity peak to the area of the adjoining PHA peak in chromatograms obtained by SEC. The values of the raw polymers can be seen at the volume ratio $\psi = 0$. The dotted lines show the optimum choice of an activated charcoal for PHUE (A to C) and PHOUE (D to F) when highest quality is required.

gas chromatography (GC) after conversion of the polymer into the methyl ester of the monomers using boron trifluoride in methanol.[24] The analyses of the resulting hydroxyalkenoic and hydroxyalkanoic acid methyl esters were performed with a gas chromatograph (GC Trace 2000 Series, CE Instruments, Rodano, Italy) equipped with a flame ionization detector and a capillary column DB-Wax (30 m × 0.32 mm, film thickness: 0.3 μm, Agilent, Santa Clara, CA, USA).

UV/VIS absorption spectra were taken from solutions of PHUE and PHOUE in chloroform at ambient temperature using a Synergy Mx UV/VIS spectrophotometer from Biotek Instruments GmbH, Luzern, Switzerland. Endotoxicity was determined by means of the Limulus Amebocyte Lysate (LAL) assay Endosafe-PTS (Charles River, L'Arbresle Cedex, France). Samples were prepared by dissolving 200 mg of polymer in 13 mL of chloroform and casting into depyrogenized glass Petri dishes (d = 4 cm), followed by drying at 40 °C and 100 mbar for 24 h. In order to extract endotoxins, samples were incubated with 3 mL of endotoxin-free water (LAL Reagent Water Endosafe) at 37 °C for 24 h.

Aliquots of 200 μL were withdrawn and each mixed with 200 μL of dispersing agent BD100 (Endosafe). Samples which contained more than 10 EU/g of endotoxin were diluted 20 to 50 times with dispersing agent BD 100. The resulting solutions were measured according to the manufacturer's instructions with the spectrophotometer Endosafe-PTS using cartridges PTS2001. PTS cartridges allow testing of sample and positive sample control in duplicates. When the endotoxin content of the raw polymers was determined, water extracts were diluted 10 times with endotoxin-free water and no dispersing agent was added. In this case, measurements were taken with the cartridge Endosafe PTS2005.

3. Results

3.1 *Comparison of Three Types of Activated Charcoal*

The PHAs were extracted from freeze-dried biomass with ethyl acetate and simultaneously purified by addition of different quantities of activated charcoal to the mixture of extraction. Afterwards, the mixture was filtered in three steps and the

filtrate was concentrated, thus yielding purified polymers. Three types of activated charcoal were compared: Merck granules, Merck powder, and Norit. Their suitability to purify PHUE and PHOUE was evaluated on the basis of their capability to remove impurities and of the resulting yield related to the raw polymers. In Fig. 2, the results for PHUE (A to C) and PHOUE (D to F) are depicted in function of the ratio ψ of activated charcoal to solvent used for extraction (V/V). The values of the raw polymers can be seen at $\psi = 0$. With increasing ψ, the yield decreased almost linearly to 20–50 wt% (PHUE) or to 35–55 wt% (PHOUE), depending on the type of activated charcoal used (Charts A and D). Charts B and E show the light absorbance of the raw and pure PHA at 380 nm. Since molar extinction coefficients differ over log scales, direct conclusions about the quantity of impurities cannot be drawn from UV/VIS absorbance without knowing the substances responsible for absorption. Nevertheless, the UV/VIS absorbance was judged to be critical, because it gradually decreased from 380 nm to longer wave length and came to zero in the middle part of the visible spectrum (see spectra a and b in Fig. 3). Such absorption spectra go along with a yellow tinge of the polymer, which is not acceptable for products of high quality. According to the results of the UV/VIS measurements (Fig. 2 and 3), compounds causing this tinge were selectively removed by all types of activated charcoals except for Merck granules in case of PHUE (Fig. 2B). In Charts C and F, the ratio $A_{impurity}/A_{PHA}$ is shown in function of ψ. It refers to the areas of the SEC peaks at 30–30.5 mL (impurity) and at 24–26 mL (PHA) (see Fig. 4), respectively, and is a measure for the removal of polymeric impurities being contained in PHUE and PHOUE. Their complete removal required a ψ of 0.5 for PHUE and of 0.25 for PHOUE (Fig. 2C and F). The selectivity of Norit for polymeric impurities was found to be low. These impurities were not even removed completely at $\psi = 0.5$, neither in case of PHUE nor of PHOUE.

3.2 *Molecular Weight Fractionation*

Fig. 4A shows the SE chromatograms of raw PHUE and PHOUE which have not been treated with activated charcoal. The weight average molecular weight M_w of PHUE was found to be 135 kDa accompanied by a polydispersity (PDI) of 3.3. For PHOUE, an M_w value of 201 kDa and a PDI of 3.6 were determined. As can be seen from Fig. 4A, both raw polymers contained polymeric impurities, indicated by a peak at 30.4 mL and corresponding to an M_w of about 9 kDa. The peaks overlap in both cases. It could be observed by SEC that not only impurities but also PHUE and
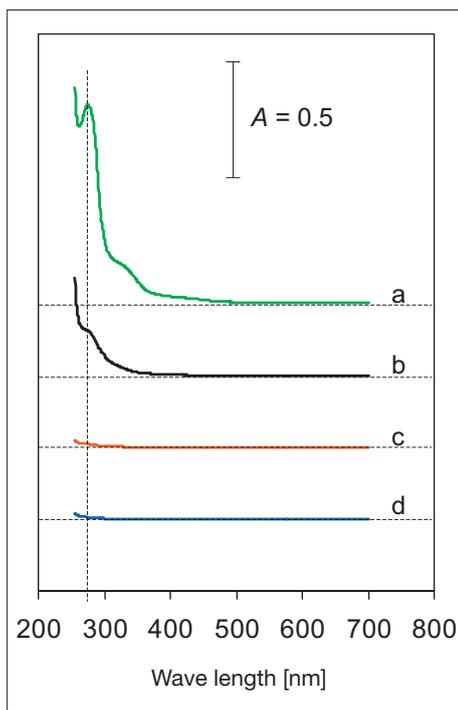
Fig. 3. UV/VIS spectra of raw PHUE (a) and PHUE purified with Merck granules (b), Merck powder (c), and Norit (d). Purification was performed applying the ratio $\psi = 0.25$ = volume of activated charcoal / volume of solvent for extraction. Spectra were taken from polymers dissolved in chloroform and diluted to identical concentration. A = absorbance.
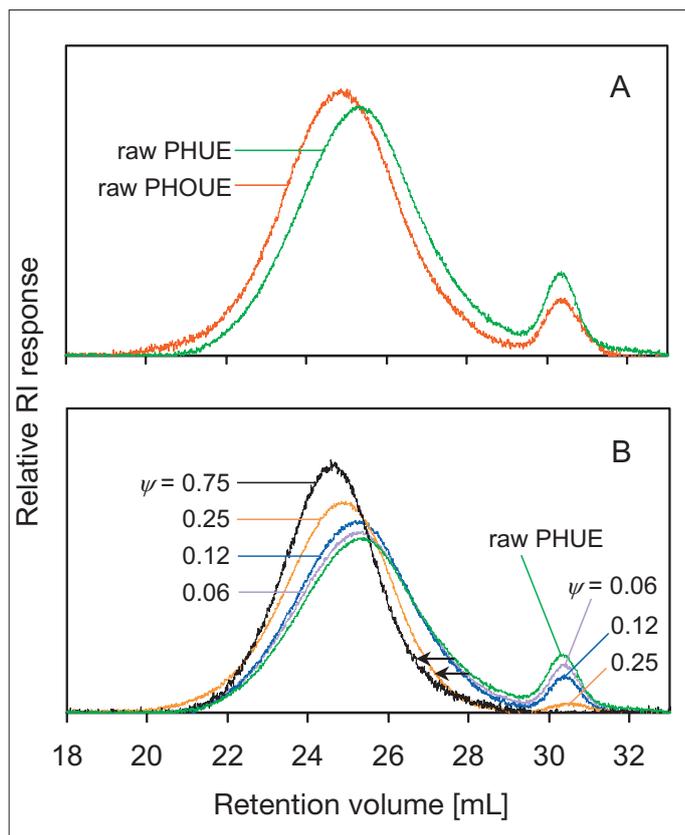

Fig. 4. Size exclusion chromatograms of raw PHUE and raw PHOUE which were extracted from freeze-dried biomass without addition of activated charcoal (A), and size exclusion chromatograms of PHUE extracted from freeze-dried biomass in the presence of different quantities of activated charcoal (B). The quantities of activated charcoal are given as a ratio ψ = volume of activated charcoal / volume of solvent used for extraction. All chromatograms are normalized to identical peak areas. The arrows point to the adsorption of low molecular PHUE, which occurred as soon as the impurities had almost been removed.

PHOUE were retained by activated charcoal. Fig. 4B shows an example in which PHUE was treated with different quantities of Merck powder during extraction. The common intersection point of three curves at 26.5 mL (raw PHUE, $\psi = 0.06$ and 0.12) can be explained by adsorption of impurities causing a decrease of the peak at 30.5 mL and a corresponding increase of the main peak due to the standardization to identical peak areas. Once the impurities had been removed to some extent, low molecular PHUE was additionally retained and the main peak consequently shifted towards lower retention volumes ($\psi = 0.25$ and 0.75, see arrows). The selective removal of low molecular PHUE caused a decrease of the polydispersity from 2.1 ($\psi = 0.25$) to 1.6 ($\psi = 0.75$), indicating a decrease of the broadness of the molecular weight distribution. When ψ was increased from 0 to 0.75, the M_w of PHUE continuously increased from 135 to 219 kDa while the PDI decreased from 3.3 to 1.6. Analogous effects were observed in case of the other charcoals and with PHOUE. However, the change of M_w and PDI caused by Norit was less pronounced, because this activated charcoal showed i) low selectivity in terms of impurities, especially for the ones of PHUE, and ii) rather low adsorption capacity for the material in question.

3.3 *Endotoxicity*

Both raw polymers were contaminated with more than 50 EU/g of endotoxin. Since medical devices such as vascular grafts, heart valves or catheters often weigh only few grams, and the limit approved by USP is 20 EU/device,[15] the basic materials require endotoxin contents of less than 1 to 2 EU/g. The capability of the three types of activated charcoal to adsorb endotoxins was examined by application of $\psi = 0.25$. After purification with Merck granules or Merck powder, the endotoxin content of PHUE was still 22 EU/g or 13 EU/g, respectively. When PHUE was purified with Norit, the amount of endotoxin could not be determined accurately, because the spike recovery was too low. Norit granules were accompanied by very fine powder. Some small particles probably passed through the filter and contaminated the polymer, where they could interfere with the measurement by partial adsorption of the spike. Removal of endotoxins from PHOUE was more successful: from PHOUE treated with Merck granules or Merck powder, endotoxins were almost completely eliminated; the contents were found to be 0.4 or 0.7 EU/g, respectively. A content of 3.1 EU/g was measured after purification using Norit. When applying Merck granules at $\psi = 0.5$ in a separate filtration step,[22] we obtained PHO and PHUE with an endotoxin level of less than 1 EU/g. Similar values were obtained in another project, where PHOUE was produced with the following molar ratios of octanoic to ω-undecenoic acid: 90/10, 75/25, and 50/50. Endotoxin contents of all samples decreased from 20 to 50 EU/g to values below 1 EU/g by purification with Merck granules (data not shown).

4. Discussion

We recently developed a downstream process which uses Merck granules to purify PHO and PHUE.[22] The process consisted of nine steps including a separate filtration of the PHA solution through a column filled with activated charcoal as well as subsequent precipitation of PHO and PHUE to remove remaining impuri-

ties. The filtration step lasted 5 h and had to be repeated if the filtrate was still yellowish. In the new process reported here, the two steps mentioned above have been eliminated. Purification with activated charcoal is now performed simultaneously with the extraction of PHA from freeze-dried biomass. After filtration, the PHAs are isolated by direct concentration of the filtrate without prior precipitation. The novel process allows the production of PHUE and PHOUE of high quality.

As can be learned from Fig. 2, Merck powder applied with $\psi = 0.5$ led to PHUE free from detectable polymeric impurities ($A_{impurity}/A_{PHA} = 0$) and yellow tinge ($A_{380} = 0$) (dotted line on the left). The resulting yield of 55 wt% might be acceptable for a polymer that can be used for medical purposes. A similar quality was achieved by analogous application of Merck granules, but the yield decreased to 35 wt%. PHUE of middle quality but in high yields was obtained by treatment with Merck powder at $\psi = 0.25$. The resulting polymer was colorless and contained only small amounts of polymeric impurities. In contrast, application of Merck granules at $\psi = 0.25$ led to 60 wt% of a yellowish PHUE free of polymeric impurities. In order to achieve low endotoxin levels, the ratio of activated charcoal had to be increased to $\psi = 0.5$ at the expense of yield. However, application of higher quantities of Merck powder ($\psi > 0.12$) caused unwanted loss of low molecular PHUE (Fig. 4B, arrows). Similar effects were observed in case of the two other activated charcoals and with PHOUE (data not shown). With Norit, PHUE could not be purified efficiently; the highest ψ value was still too low for complete removal of polymeric impurities (Fig. 2C). In case of PHOUE, application of Merck powder or Merck granules with a volume ratio $\psi = 0.25$ yielded a pure polymer (dotted line on the right) with an endotoxin content of less than 1 EU/g. The yield with respect to the raw polymer was found to be 75 or 70 wt%, respectively, which is acceptable for a product that can be used for medical applications. If high yield at the expense of the quality was required, Merck granules could be applied with a ratio of 0.12 or even of 0.06. In order to remove the polymeric impurities from PHOUE with Norit, a high volume ratio ψ of 0.75 was necessary, which entailed a yield of only 55 w%. In comparison to the downstream process recently developed,[22] the required quantity of activated charcoal to produce highly pure PHA could be halved in the case of PHOUE. On the basis of the investigated parameters, the following rating for the suitability to purify PHUE or PHOUE was found: Merck powder > Merck granules >> Norit. A disadvantage of these activated charcoals is their low adsorption capacity for organic acids and their salts. When the biomass of *Pseudomonas putida* GPo1 contained ω-undecenoate, for example, PHUE purified by activated charcoal was found to be contaminated by this educt. Therefore, it is of importance that all carbon substrates are metabolized to completion during biosynthesis of PHA. This can be achieved by continuous cultivation when the carbon to nitrogen ratio in the medium feed are controlled accurately.[25] Interestingly, PHUE required significantly more activated charcoal for the purification than PHOUE. One can argue that this fact is based on the higher content of impurities found in PHUE (see Fig. 2 at $\psi = 0$ and Fig. 4A). However, with increasing ψ, the decrease of the yield continued without changing the slope after complete adsorption of the impurities (Fig. 2A and D). This suggests that PHAs were strongly adsorbed by activated charcoals and hence were in competition with impurities for adsorption sites. Due to the pore size distribution of the activated charcoals used, low molecular PHAs were adsorbed preferentially (see Fig. 4B). Since PHUE has a lower M_r than PHOUE (Fig. 4A), more PHUE molecules could enter into the pores and hence the adsorption capacity for impurities became lower. This may be the explanation why different quantities of activated charcoal were needed to purify PHUE and PHOUE.

5. Conclusions

The present study provides an optimized downstream process that permits the isolation and purification of PHUE and PHOUE from freeze-dried biomass using activated charcoal. Three charcoals were compared in terms of yield of PHA and removal of impurities. Merck powder excelled at its specificity for impurities, whereas Norit was not suitable for the intended purpose. Extraction from biomass and purification from soluble impurities were performed simultaneously by addition of activated charcoal just at the beginning of the extraction process. Subsequent separation of the solids by filtration followed by concentration of the filtrate led to a pure polymer that is almost free of endotoxins and thus is suitable for medical applications.

In the present process, all steps involving separate filtration through activated charcoal or precipitation of PHAs to remove impurities could be eliminated. Thus, time, energy, solvent and non-solvent can be saved. The developed process is better suited to isolate and purify PHUE and PHOUE. However, the method needs further optimization to improve the yield of PHUE. Possible approaches include i) fine-tuning of the volume ratio ψ, ii) optimization of the period of extraction, and iii) the inclusion of further types of activated charcoal or of zeolite.

[1] Y. B. Kim, R. W. Lenz, R. C. Fuller, *J. Polymer Sci. Polymer Chem.* **1995**, *33*, 1367.
[2] R. Hartmann, R. Hany, T. Geiger, T. Egli, B. Witholt, M. Zinn, *Macromolecules* **2004**, *37*, 6780.
[3] R. Hartmann, R. Hany, E. Pletscher, A. Ritter, B. Witholt, M. Zinn, *Biotechnol. Bioeng.* **2006**, *93*, 737.
[4] B. Hazer, A. Steinbüchel, *Appl. Microbiol. Biotechnol.* **2007**, *74*, 1.
[5] E. Renard, V. Langlois, P. Guerin, *Corros. Eng. Sci. Technol.* **2007**, *42*, 300.
[6] K. D. Gagnon, R. W. Lenz, R. J. Farris, R. C. Fuller, *Polymer* **1994**, *35*, 4358.
[7] K. D. Gagnon, R. W. Lenz, R. J. Farris, R. C. Fuller, *Polymer* **1994**, *35*, 4368.
[8] G. J. M. de Koning, H. H. M. van Bilsen, P. J. Lemstra, W. Hazenberg, B. Witholt, H. Preusting, J. G. van der Galiën, A. Schirmer, D. Jendrossek, *Polymer* **1994**, *35*, 2090.
[9] R. Hany, C. Böhlen, T. Geiger, M. Schmid, M. Zinn, *Biomacromolecules* **2004**, *5*, 1452.
[10] R. Hany, R. Hartmann, C. Böhlen, S. Brandenberger, J. Kawada, C. Löwe, M. Zinn, B. Witholt, R. H. Marchessault, *Polymer* **2005**, *46*, 5025.
[11] N. Jacquel, C.-W. Lo, Y.-H. Wei, H.-S. Wu, S. S. Wang, *Biochem. Eng. J.* **2008**, *39*, 15.
[12] X. Jiang, J. A. Ramsay, B. A. Ramsay, *J. Microbiol. Meth.* **2006**, *67*, 212.
[13] Y. Elbahloul, A. Steinbüchel, *Appl. Environ. Microbiol.* **2009**, *75*, 643.
[14] D. Petsch, F. B. Anspach, *J. Biotechnol.* **2000**, *76*, 97.
[15] 'United States Pharmacopeia and National Formulary', (USP29-NF24) United States Pharmacopeial Convention, Rockville, **2005**, Chapter 161.
[16] P. Furrer, S. Panke, M. Zinn, *J. Microbiol. Meth.* **2007**, *69*, 206.
[17] P. Furrer, M. Schmid, A. Hinz, E. Pletscher, S. Panke, M. Zinn, *Eur. Cells Mat.* **2004**, *7 (Suppl. 2)*, 30.
[18] A. S. Pegues, S. S. Sofer, R. E. McCallum, L. B. Hinshaw, *Int. J. Artif. Organs* **1979**, *2*, 153.
[19] T. M. Keenan, J. P. Nakas, S. W. Tanenbaum, *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 616.
[20] W. He, W. Tian, G. Zhang, G.-Q. Chen, Z. Zhang, *FEMS Microbiol. Lett.* **1998**, *169*, 45.
[21] R. N. Reusch, H. L. Sadoff, *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4176.
[22] B. Wampfler, T. Ramsauer, S. Rezzonico, R. Hischier, R. Köhling, L. Thöny-Meyer, M. Zinn, *Biomacromolecules* **2010**, *11*, 2716.
[23] M. Schmid, A. Ritter, A. Grubelnik, M. Zinn, *Biomacromolecules* **2007**, *8*, 579.
[24] P. Furrer, R. Hany, D. Rentsch, A. Grubelnik, K. Ruth, S. Panke, M. Zinn, *J. Chromatogr., A* **2007**, *1143*, 199.
[25] M. Zinn, B. Witholt, T. Egli, *J. Biotechnol.* **2004**, *113*, 263.