

## Towards medium-chain-length polyhydroxyalkanoate for medical applications

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**INTRODUCTION:** Medium-chain-length polyhydroxyalkanoate (mclPHA, monomers from C<sub>6</sub>-C<sub>14</sub>) is a water insoluble, biodegradable, and biocompatible elastomer and hence it is well suited for medical applications [1]. It is a promising material for the production of porous scaffolds like recent publications of PHA copolymers indicate [4, 5].

PHA is intracellularly accumulated in Gram negative production strains and is currently extracted by chlorinated solvents. However, it has been observed that PHA is contaminated by lipopolysaccharides (lps) from the outer cell membrane during recovery. For medical purposes the polymer needs to be highly pure, especially with respect to pyrogenic compounds. Lps are considered to be the main source of pyrogenic contamination.

Unfortunately only few methods for the depyrogenation of PHA have been described in literature, for instance the treatment with ozone, peroxides or NaOH [2, 3]. Generally the molecular weight of PHA is decreased by using lps-degrading agents. Therefore another non-PHA degrading approach for depyrogenation is required. It is known that appropriate extraction steps may minimize the co-extraction of lps.

**METHODS:** MclPHA produced with *Pseudomonas putida* GPo1 was recovered as follows: The fermentation broth was centrifuged (10'000xg) and the resulting wet biomass was freeze dried. The dry biomass was grinded and extracted with various organic solvents at room temperature for 24 h. The suspension was filtrated (1µm reg. cellulose filter) to obtain a clear PHA solution. One third of the solution was evaporated and finally dried under vacuum at 40°C for 48h to obtain the rough PHA. The remaining parts were concentrated and precipitated in water and methanol, respectively.

Test-tubes (10 ml) were coated by rotation with about 200 mg mclPHA, dried and exposed to endotoxin-free water for 36 h at 37°C. The endotoxin-containing water was removed and diluted 1:100. The chromogenic *Limulus*

amebocyte lysate (LAL) test (Cambrex QCL-1000) was applied to determine the amount of endotoxins in these solutions. For calibration a 29 EU standard (strain 0111:B4) was used. The final values were expressed as endotoxin units (EU) per gram PHA.

The molecular weights were determined by GPC-RALS (Viscotek, TDA 302).

**RESULTS:** The capacity to extract mclPHA from freeze-dried *P. Putida* GPo1 was investigated for various solvents as shown in Fig. 1.

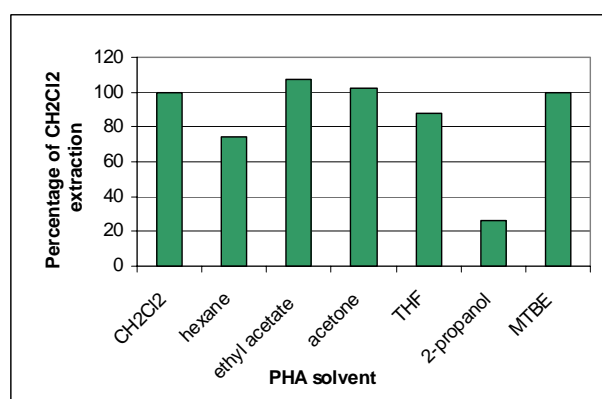


Fig. 1: Efficiency of PHA extraction from biomass for various solvents in comparison to standard extraction (methylene chloride).

GPC measurements revealed that the molecular weight was equal for all extracts ( $M_w=170$  kDa,  $M_n=75$  kDa) within the measurement uncertainty (<10%), except for the 2-propanol extract ( $M_w=92$  kDa,  $M_n=55$  kDa).

Significant differences in endotoxicity could be observed (Fig. 2). Ethyl acetate, acetone, tetrahydrofuran (THF), 2-propanol and *tert.*-butyl-methyl ether (MTBE) extracts showed a much lower endotoxin content than the extracts with methylene chloride (CH<sub>2</sub>Cl<sub>2</sub>) and hexane.

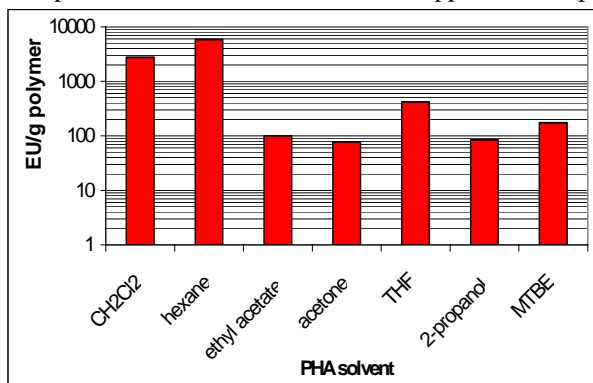


Fig. 2: Endotoxicity of differently extracted PHA. The exposure time of PHA samples to water was 36 h.

Whether the precipitation of solubilized PHA has an influence on the endotoxicity was assessed for methanol and water (Fig. 3).

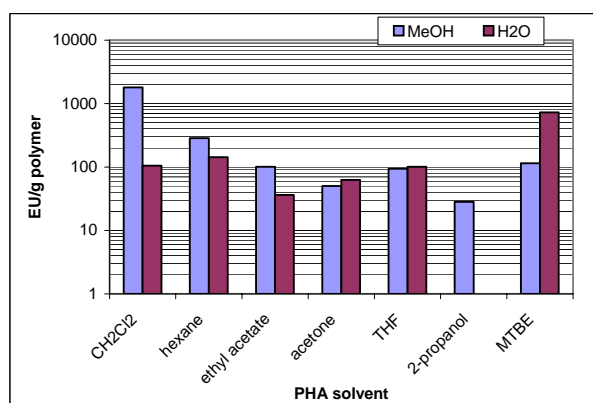


Fig. 3: Endotoxicity of PHA precipitated in either methanol or water.

**DISCUSSION & CONCLUSIONS:** The standard extraction solvent (CH<sub>2</sub>Cl<sub>2</sub>) has to be replaced by a more environmentally friendly one. All solvents except hexane and 2-propanol showed similar extraction yields like methylene chloride. A significant decrease in the molecular weight could only be seen for 2-propanol. Based on these criteria ethyl acetate, acetone, THF and MTBE are valid alternatives.

However, ethyl acetate and acetone extracts contain the lowest amount of endotoxin and will be considered for further, more detailed analysis.

Finally, precipitation with water or methanol strongly decreased the endotoxicity of nearly all extracts.

It has to be noted that special attention has to be paid to the test methods for samples of mclPHA, because Ips may still be enclosed in the polymer matrix. Therefore the porosity and the surface roughness of PHA and the extraction time with water are crucial for analysis.

**REFERENCES:** <sup>1</sup>B. Witholt and B. Kessler (1999) *Curr. Opin. Biotechnol.* **10**:279-285. <sup>2</sup>S.F. Williams et al. (1999) *Int. J. Biol. Macromol.* **25**:111-121. <sup>3</sup>S.Y. Lee et al. (1999) *Appl. Environ. Microbiol.* **65**:2762-2764. <sup>4</sup>Y. Deng et al. (2003) *Biomaterials* **24**:4273-4281. <sup>5</sup>Y.-W. Wang et al. (2004) *Biomaterials* **25**:669-675.

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