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Downstreaming of Bacterial Polysialic Acid

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Introduction

For an artificial reproduction of tissue, e.g. for the use in reconstructive medicine a scaffold with a high biocompatibility and intense stability is needed. This scaffold material needs to be fully biodegradable and free of toxic remains. The human polysialic acid, a polysaccharide composed of 2,8-linked N-acetyl-neuraminic acid is believed to posses such properties. The bacterium E.coli K1 is encapsulated by a polysaccharide, whose structure is identical to the polysialic acid found in the human organism. During the biological growth of E.coli K1 polysialic acid is synthesized intracellular and transported in a complex process to the outer cell membrane. While culturing E.coli K1 most of the polysialic acid is separated from the cell surface and released into the culture media by the end of the growth phase.

Cultivation

- Use of the wild-type strain *E.coli* K1 B2032/82 for the biosynthetic production of polysialic acid.
- Cultivation was conducted for 14 h in a 10 L Biostat C using a glucose-based synthetic medium.
- Release of 60 % of the produced polysialic acid into the medium.
- Bacteria and supernatant were separated by continuous centrifugation at 4 °C.

Escherichia coli K1 B2032/82 → pre-culture in shaking flasks → reactor cultivation (10 L scale)

Downstreaming

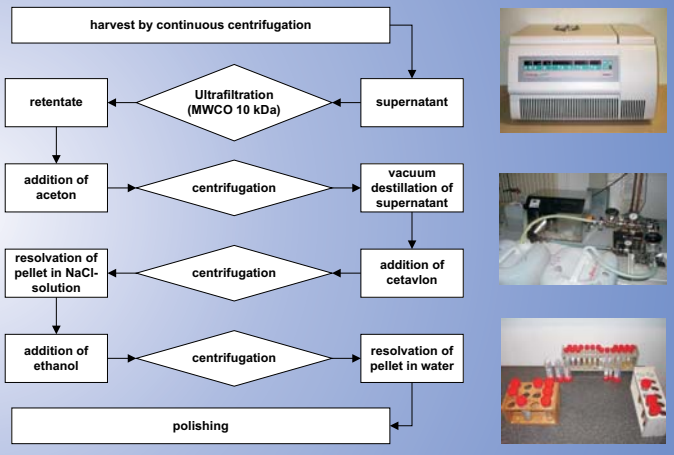
For a better handling during the downstream process the supernatant was concentrated using crossflow ultrafiltration.

- 20-fold concentration of supernatant by ultrafiltration
- 10 kDa MWCO results in a depletion of polymers with a DP > 35

Raw purification was achieved through a series of different precipitation reactions

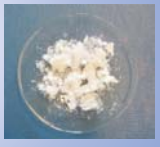
- Treatment with acetone results in a particulate denaturation of the contaminating proteins while polysialic acid is kept in solution.
- Precipitation of the polysialic acid with cetyltrimethylammonium-bromide (cetavlon).
- The suspension was centrifugated and the highly viscous pellet was resolved in NaCl-solution.

Finally the polysialic acid could be recovered from this solution by precipitating with ehanol.



Polishing

- Additional purification by SEC and dialysis
- Product recovery by lyophilisation
- K1-PSA loss during polishing < 10 %

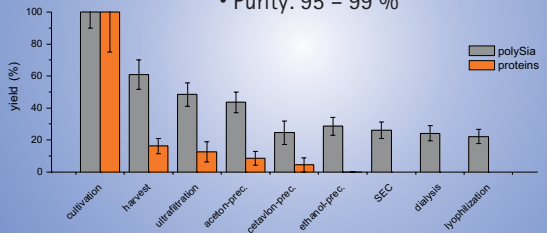


size exclusion chromatography *Sephacryl S-300* → dialysis (MWCO 10 kDa) → lyophilization → K1-PSA

Conclusion

An efficient downstream process with the following parameters could be established:

- Overall yield: 1,1 g (initial ~ 5 g) = 22 %
- Purity: 95 – 99 %



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