

CLAIMS

1. A process for the purification of LNnT (lacto-N-neotetraose) from a fermentation broth, the process comprises:
 - 5 - providing a fermentation broth which contains LNnT, biomass, medium components, contaminants and carbohydrates other than LNnT;
 - subjecting the fermentation broth to at least one membrane filtration step using nanofiltration membranes, thereby providing a filtrated solution containing LNnT;
 - 10 - subjecting the filtrated solution to at least one simulated moving bed chromatography step, thereby providing a purified solution which contains LNnT;
 - subjecting the purified solution to at least one crystallization step, thereby providing a crystal mass; and
 - 15 - subjecting such crystal mass to at least one step of homogenization, thereby providing a homogenized and dried purified of LNnT;
- wherein the purified preparation contains the LNnT with a purity of $\geq 90\%$.
2. The process according to claim 1, wherein the membrane filtration step is nanofiltration, using a membrane which has a molecular weight cut-off of
20 between 0.2 and 3.5 kDa.
3. The process according to any of the preceding claims, wherein the membrane has a molecular weight cut-off of between 0.2 and 2.0 kDa.
4. The process according to any of the preceding claims, wherein the membrane has a molecular weight cut-off of between 0.2 and 1.0 kDa.
- 25 5. The process according to any of the preceding claims, wherein the filtrated solution obtained from the membrane filtration step contains LNnT with a purity of more than 60%, more than 65%, or more than 70%.

6. The process according to claim 1, wherein the simulated moving bed chromatography step comprises
- i) at least 4 columns, preferably at least 8 columns, more preferably at least 12 columns, wherein at least one column comprises a weak or strong cation exchange resin, preferably a cation exchange resin in the H⁺-form, Na⁺-form, K⁺-form or Ca²⁺-form; and/or
 - ii) four zones I, II, III and IV with different flow rates; and/or
 - iii) an eluent comprising or consisting of water, preferably ethanol and water, more preferably 5-15 vol.% ethanol and 85-95 vol.% water, most preferably 9-11 vol.% ethanol and 89-91 vol.% water,
 - iv) an operating temperature of 15 to 60°C, preferably 20 to 55°C, more preferably 25 to 50°C
7. The process according to claim 1 and 6, wherein the simulated moving bed chromatography step comprises
- i) four zones I, II, III and IV with different flow rates, wherein the flow rates are preferably 22-32 ml/min in zone I, 17-23 ml/min in zone II, 18-25 ml/min in zone III and/or 14-20 ml/min in zone IV; and/or
 - ii) a feed rate of 0.5-4 ml/min, preferably 2 ml/min; and/or
 - iii) an eluent flow rate of 6-12 ml/min, preferably 8 ml/min; and/or
 - iv) a switching time of 14-20 min, preferably 16-18 min, more preferably 17 min. Preferably, at least one of the columns comprises 0.1 to 5000 kg of cation exchange resin, preferably 0.2 to 500 kg of cationic exchange resin, more preferably 0.5 to 50 kg of cation exchange resin, most preferably 1.0 to 20 kg of cation exchange resin.
8. The process according to any of the preceding claims, wherein the purified solution obtained from the simulated moving bed chromatography contains LNNt with a purity of more than 75% or more than 80%.

9. The process according to claims 1, 6 to 8, wherein the purified solution obtained from at least one simulated moving bed chromatography step can be subjected to at least one other further purification step using simulating moving bed chromatography, wherein a purified solution comprising LNnT with a purity of >85 %, preferably >90%; more preferably > 93% is provided.
10. The process according to claim 1, wherein the crystallization step comprises
- obtaining a mixture of at least two oligosaccharides, containing at least LNnT in a concentration of at least about 1% DSC, preferably at least 10% DSC, preferably at least 30% DSC, and more preferably at least about 50% DSC and such LNnT purity of this mixture is of at least about 15%, preferably at least about 25%, preferably about 40% and more preferably at least about 60%.
 - crystallizing the purified solution obtained from the simulated moving bed chromatography step from a starting temperature of at least about 20-30°C, preferably at least about 30-40°C, preferably at least about 40-50°C, and more preferably at least about 50-60°C;
 - letting the saturated mass crystallize by cooling down to at least 40-50°C, preferably at least about 30-40°C, preferably at least about 20-30°C, preferably at least about 10-20°C, and more preferably at least about 0-10°C to obtain a homogeneous crystal mass,
 - treating the crystal mass with a mixture of an alcohol, solvent, or water-miscible alcohol or solvent and water, wherein the alcohol or solvent content of the solution is between 10 and 90 vol.%, preferably between 30 and 80 vol.% and more preferably between 50 and 70 vol.%, to obtain a mixture of the alcohol or solvent-containing solution and the crystal mass; and
 - at least one washing of the alcohol or solvent-containing or sugar-containing fraction of the crystal mass in order to reduce the content of lower saccharides from the crystal mass.
11. The process according to claims 1 and 10, wherein washing of the alcohol or solvent-containing or sugar-containing fraction of the crystal can be repeated at least two times in order to reduce the content of lower saccharides.

12. The process according to claims 1, 10 and 11, wherein the content of lower saccharides is less than 10%, preferably less than 5% and the more preferably less than 3%.
- 5 13. The process according to any of the preceding claims, wherein the crystals obtained from the crystallization step contain LNnT with a purity of more than 85%, more than 90% and more than 95%.
- 10 14. The process according to claim 1, wherein the homogenization step can be selected from freeze-drying, spray-drying, roller or drum drying and/or band or vacuum band drying.
- 15 15. The process according to any one of the preceding claims, wherein the purity of the LNnT in the purified preparation is of $\geq 80\%$, preferably of $\geq 85\%$, more preferably of $\geq 90\%$, with respect to the dry matter of the purified preparation.

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