Claims

1. A method for the purification of lacto-*N*-*neo*tetraose from other carbohydrates, wherein the lacto-*N*-*neo*tetraose is obtained by microbial fermentation, characterized in that the method comprises the steps of:
   i. separating the biomass from the fermentation broth;
   ii. subjecting the cell-free fermentation broth to ion-exchange treatment for the removal of charged material, wherein the aqueous solution is treated with a cation exchange resin and an anion exchange resin;
   iii. subjecting the aqueous solution obtained in step ii. to a membrane filtration step to remove carbohydrates having a molecular weight smaller than that of lacto-*N*-*neo*tetraose, wherein the membrane has a molecular weight cut-off of between about 300 to about 500 Dalton;
   iv. subjecting the retentate obtained in step iii. to a membrane filtration step to remove carbohydrates having a molecular weight larger than that of lacto-*N*-*neo*tetraose, wherein the membrane has a molecular weight cut-off of between about 600 to about 800 Dalton; and
   v. increasing the concentration of the lacto-*N*-*neo*tetraose in the filtrate of step iv.

2. The method according to claim 1, wherein separating the biomass from the fermentation broth comprises at least one step of ultrafiltration, preferably two steps of ultrafiltration, more preferably a first ultrafiltration using a membrane having a molecular weight cut-off of about 500 kDa and a second ultrafiltration using a membrane having a molecular weight cut-off of about 150 kDa.

3. The method according to claim 1 or 2, wherein the aqueous medium is treated with a cation exchange resin in H^+ form, and an anion exchange resin in Cl^- form, more preferably before subjecting the aqueous solution to the membrane filtration steps.

4. The method according to any one of claims 1 to 3, wherein the method further comprises a step of dialysis, preferably of electrodialysis.

5. A method for the purification of lacto-*N*-*neo*tetraose from other carbohydrates, wherein the lacto-*N*-*neo*tetraose is obtained by microbial fermentation, characterized in that the method comprises the steps of

   i. separating the biomass from the fermentation broth;

   ii. subjecting the cell-free fermentation broth to ion-exchange treatment for the removal of charged material, wherein the aqueous solution is treated with a cation exchange resin and an anion exchange resin;

   iii. subjecting the aqueous solution obtained in step ii. to a membrane filtration step to remove carbohydrates having a molecular weight smaller than that of lacto-*N*-*neo*tetraose, wherein the membrane has a molecular weight cut-off of between about 300 to about 500 Dalton;

   iv. subjecting the retentate obtained in step iii. to a simulated moving bed chromatography to remove carbohydrates having a molecular weight larger than that of lacto-N-neotetraose; and

   v. increasing the concentration of the lacto-*N*-*neo*tetraose in the filtrate of step iv.

6. The method according to claim 5, wherein separating the biomass from the fermentation broth comprises at least one step of ultrafiltration, preferably two steps of ultrafiltration, more preferably a first ultrafiltration using a membrane having a molecular weight cut-off of about 500 kDa and a second ultrafiltration using a membrane having a molecular weight cut-off of about 150 kDa.

7. The method according to claim 5 or 6, wherein the aqueous solution is treated with a cation exchange resin in H^+ form, and an anion exchange resin in Cl^- form.

8. The method according to any one of claims 5 to 7, wherein the method further comprises a step of dialysis, preferably of electrodialysis.

9. The method according to any one of steps 1 to 8, wherein the purity of lacto-*N*-*neo*tetraose in the aqueous solution is ≤ 70 %, ≤ 60 %, ≤ 50 %, ≤ 40 %, ≤ 30 %, ≤ 20 %, ≤ 10 %, ≤ 5 % prior to the purification and /or the aqueous solution contains lacto-*N*-*neo*tetraose at a purity of ≥ 80 %, preferably of ≥ 85 % or more preferably ≥ 90 % after the purification.

10. The method according to any one of steps 1 to 9, wherein the method comprises the use of at least one glycosidase for hydrolyzing one or more carbohydrates other than LNnT in the fermentation broth.

11. The method according to any one of steps 1 to 10, wherein a second microorganism is added to the fermentation broth after fermentative production of LNnT, wherein said second microorganism expresses a β-galactosidase, preferably *E. coli* LacZ, and/or a *N*-acetylhexosaminidase, preferably Bbhl of *B. bifidum*.