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(71) Applicant *(for all designated States except US)* **TATA CHEMICALS LTD.** [IN/IN], Bombay House, 24 Homi Modi Street, Mumbai 400001 (IN)

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* **AVALAKKI, Uday Kashinath** [IN/IN], Tata Chemicals Ltd , Innovation Centre, Ghotavde Phata, Urawde Road, Pirangut, Industrial Area, Gat No 1139/1, Mulshi, Pune 412108 (IN) **MAHESWARAN, Palamalai** [IN/IN], Tata Chemicals Ltd , Innovation Centre, Ghotavde Phata, Urawde Road, Pirangut, Industrial Area, Gat No 1139/1, Mulshi, Pune 412108 (IN) SARAVANAN, **Rengarajan** [IN/IN], Tata Chemicals Ltd , Innovation Centre, Ghotavde Phata, Urawde Road, Pirangut, Industrial Area, Gat No 1139/1, Mulshi, Pune 412108 **(IN)**

(74) Agents WILSON, **Neeti** et al , Anand And Anand, B-41, Nizamuddin East, New Delhi 11001 3 (IN)

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[Continued on next page]

(54) Title PRODUCTION OF HIGH PURITY GALACTOOLIGOSACCHARIDES

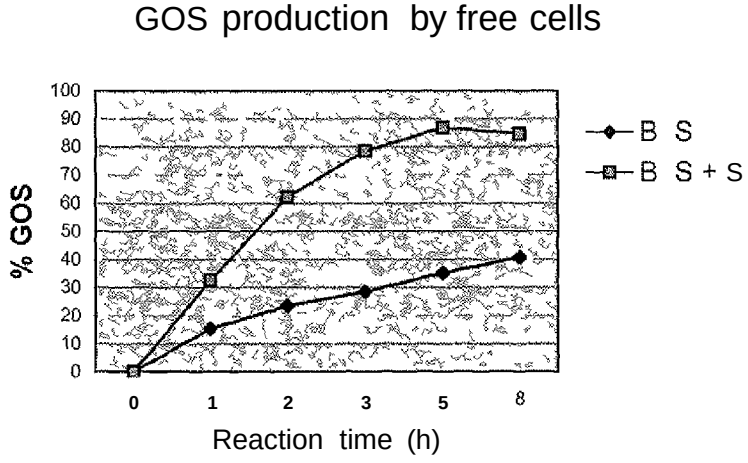

FIG. 2

(57) Abstract The present invention deals with an improved process for the production of high yield of pure Galactooligosaccha-ndes using microbial whole cells in a reactor with cross flow hollow fiber microfiltration system The process is economical as cell biomass is used repeatedly and eliminated the need to cany out downstream processing for the removal of mono and disaccha-ndes from the final product

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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(H))*

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AMENDED CLAIMS

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I Λ process for production of high purity galactooligosaccharides by free cells comprising:

a) growing microorganisms that produce enzyme for hydrolying sugar to oligosaccharide under optimum medium and conditions to obtain cell biomass of *B singnlans* and *Saccharomyce,s* Ν>.,

b) hydrolysis of lactose and utilization of produced glucose by mixed microbial culture,

c) seperating galactooligosaccharides from microbial culture using microfiltration membrane system or centrifugal ion,

d) filtering said galactooligosaccharides using a deep bed filter with cotton and activated carbon or carbon filter at a flow rale of l0-30ml/min,

e) concentrating galactooligosaccharides a(the temperature range of 40-60 ^{0}C in vacuum evaporator so as to obtain a syrup having 70-80% dissolved solids,

0 drying said syrup to obtain high purity of galactooligosaccharides in amorphous form powder.

g) crystallizing said amorphous powder to obtain crystalline galactooligosaccharides.

2. The process as claimed in claim I, wherein *B. singluuris* and *Saccharomyces sp* arc isolated from whey effluent and contaminated sugar solution, respectively.

3. The process as claimed in claim I, wherein Saccharomyces sp. is lac- glu+ or gal+.

4. The process as claimed in claim I. wherein said cell biomass is optionally prepared from β-galactosidase enzyme producing microbial cultures selected from Aspergillus Oryzae, Candida, Kluveromyces Sp., Bacillus Circulans, Lactobacillus Bulgaricus, Streptococcus thermophilic and Bifidobacterium sp.

5 The process as claimed in claim I wherein said growth of cell biomass is by shaker flask and fermentation

6. The process of claim I, wherein said cn/yme that hydrolyses lactose to oligosaccharide is /3-galactosidase. glucose isømcrasc, catalasc, lactate dehydrogenase, and combinations thereof.

7 The process as claimed in claim 1, wherein the ratio of the microbial cell is in the range of 1:1 to 1:2 on dry weight basis.

8 The process as claimed in claim 1, wherein hydrolyzation reaction is carried out in a bioreactor by using about 15 to about 45%, lactose solution of about 3 to about 10 pH, at about 1U to 60^{0}C in about 12 to 48 hours having agitation speed in the range of about 50 to 200 rpm.

9. The process as claimed in claim I, wherein hydrolysis cycles are repeated by adding said additional biomass Io compensate the desired conversion efficiency.

10. fhc process as claimed in claim 1, wherein fi Iteration of said galactooligosaccharidcs using a deep bed filter with cotton and activated carbon/carbon filter at a flow rate of 20 ml/min.

1I The process as claimed in claim I, wherein permeate is passed through carbon polisher followed by 0.2 micron micro filtration membrane system to remove the color and suspended carbon particles.

12. The process as claimed in claim I, wherein the carbon polished hydroly/ed solution is passed through a concentrator at 40 - 60 ^{0}C, in vacuum to obtain syrup of 70-80 % dissolved solid content.

13. The process as claimed in claim 1, wherein the membrane system is selected from PES, PTFE, regenerated cellulose or ceramic hollow fiber, TFF cassette membrane.

14. The process as claimed in claim I, wherein the dilute galactooligosaccharides is sprayed under pressure through the noz/.les in the temperature range of 100 to 135 °C.