oligosaccharide consisting of at least three monosaccharide moieties; cultivating the genetically engineered microbial cell in a culture medium which contains the oligosaccharide consisting of at least three monosaccharide moieties for the genetically engineered microbial cell to take up the oligosaccharide consisting of at least three monosaccharide moieties and to transfer at least one monosaccharide moiety to the oligosaccharide consisting of at least three monosaccharide moieties, thereby synthesizing the desired saccharide comprising more than three monosaccharide moieties; and retrieving the desired saccharide from the culture medium and/or the genetically engineered microbial cell.

In one aspect, the present disclosure refers to a process for the production of a desired oligosaccharide comprising more than three monosaccharide moieties, wherein the process comprises

- providing a genetically engineered microbial cell that possesses
  - a saccharide importer for the uptake of an intermediate oligosaccharide consisting of at least three monosaccharide moieties but less monosaccharide moieties than the desired oligosaccharide by the microbial cell, and
  - an enzyme which is able to transfer a monosaccharide moiety from a donor substrate to the intermediate oligosaccharide;
- cultivating the genetically engineered microbial cell in a culture medium which contains the intermediate oligosaccharide for the genetically engineered microbial cell to take up the intermediate oligosaccharide and to transfer at least one monosaccharide moiety to the intermediate oligosaccharide, thereby synthesizing the desired oligosaccharide; and

retrieving the desired oligosaccharide from the culture medium and/or the genetically engineered microbial cell.

In another aspect, the present disclosure refers to a process for the production of lacto-N-neotetraose, wherein the process comprises

- providing a genetically engineered microbial cell that possesses

a. a transporter which facilitates uptake of LNT-II, and

b. a β-1,4 galactosyltransferase;

- cultivating the genetically engineered microbial cell in a culture medium which contains LNT-II for the genetically engineered microbial cell to take up LNT-II

and to transfer a galactose moiety from UDP-galactose to LNT-II by said β-1,4 galactosyltransferase, thereby synthesizing LNnT; and
retrieving the LNnT from the culture medium and/or the genetically engineered microbial cell.

More specifically, the process may comprise production of larger oligosaccharides, i.e. of oligosaccharides consisting of at least four monosaccharide moieties, in a stepwise or sequential manner using at least two different genetically engineered microbial cells that are cultivated in separate compartments, wherein an oligosaccharide produced by one of the at least two different genetically engineered microbial cells serves as an educt for the production of another oligosaccharide, e.g. the desired oligosaccharide or another intermediate oligosaccharide, by the other of the at least two genetically engineered microbial cells.

When the process is performed in a sequential manner, a fermentation step builds on the product of a previous fermentation step. An oligosaccharide product - optionally containing undesired oligosaccharide by-products - is generated in an initial fermentation step. In a subsequent fermentation step, the oligosaccharide product of the initial fermentation step is further processed, either in that the undesired oligosaccharide by-products are degraded and/or in that the oligosaccharide of the product is used as an educt. At the end of the sequential fermentation a desired oligosaccharide is obtained at higher purity, i.e. accompanied by less undesired by-products, than by a process performing the production in a single compartment with different microorganism, or by using an individual microbial strain performing all enzymatic reactions required to obtain the desired oligosaccharide.

The sequential fermentation process utilizes specific biosynthetic compartments (comprising synthesis and/or specific degradation reaction) by employing different genetically engineered microbial cells (bacterial and/or eukaryotic microorganism