Microbial Polysaccharides

Polysaccharides- Polysaccharides, or polycarbohydrates, are the most abundant carbohydrate found in food. They are long chain polymeric carbohydrates composed of monosaccharide units bound together by glycosidic linkages. This carbohydrate can react with water using amylase enzymes as catalyst, which produces constituent sugars.

Microbial Polysaccharides- The microorganisms can produce large amounts of polysaccharides in the presence of surplus carbon source. Some of these polysaccharides (e.g. glycogen) serve as storage compounds. The polysaccharides excreted by the cells, referred to as exopolysaccharides, are of commercial importance. The exopolysaccharides may be found in association with the cells or may remain in the medium.

The microbial polysaccharides may be neutral (e.g. dextran, scleroglucan) or acidic (xanthan, gellan) in nature. Acidic polysaccharides possessing ionized groups such as carboxyl, which can function as polyelectrolytes, are commercially more important.

Applications of Microbial Polysaccharides- Microbial polysaccharides have immense commercial importance. They are employed in the stabilization of foods, and production of several industrial and pharmaceutical compounds. The commercial value of a polysaccharide is based on its ability to modify the flow characteristics of solutions (technically known as rheology). Polysaccharides can increase the viscosity and, are therefore useful as thickening and gelling agents.

Microbial polysaccharides are of great importance in oil industry. By conventional methods, only 50% of the oil can be extracted. And the rest is either trapped in the rock or too viscous to be pumped out. It is now possible to recover such oils also by a technique called microbial enhanced oil recovery (MEOR). This can be done by injecting surfactants and viscosity decreasing biological agents (i.e. the microbial polysaccharides e.g. xanthan and emulsan).

Production of Microbial Polysaccharides- The synthesis of polysaccharides favorably occurs in the excess supply of carbon substrate in the growth medium while limiting nitrogen supply. A carbon/nitrogen ratio of around 10: 1 is considered to be favorable for optimal polysaccharide synthesis. The production process is mostly carried out by batch culture fermentation.

By manipulating the nutrient supply, differential synthesis of polysaccharides can be achieved. By limiting nitrogen supply in the medium, mostly neutral polysaccharides are produced. Whenmetal ions are limited, acidic polysaccharides are mainly synthesized.

Molecular oxygen supply of around 90% saturation is ideal for good growth and polysaccharide synthesis.

Biosynthesis of polysaccharides- Microorganisms are capable of producing a large number of polysaccharides. The pathways for their biosynthesis are comparable to the processes that occur for the formation bacterial cell wall. It is estimated that there are well over 100 enzymatic reactions, directly or indirectly involved in the synthesis of polysaccharides. Starting with glucose, appropriate sugars (by transforming glucose to others) are incorporated in the formation of polysaccharides.

Recovery of polysaccharides- As the polysaccharide production increases, there occurs a marked increase in viscosity of the culture broth. The polysaccharides can be precipitated by salts, acids or organic solvents, and recovered by employing appropriate techniques.

Microbial polysaccharides versus plant polysaccharides- There is a lot of competition between microbial and plant polysaccharides for industrial applications. Production of plant polysaccharides is relatively cheap, although it is uncontrolled and occurs for a short period in a year. In contrast, production of microbial polysaccharides is well controlled and can be continued throughout the year. However, fermentation processes for manufacture of cheap (from plant sources) polysaccharides is not advisable.

General Features of Microbial Polysaccharides- Of the several microbial polysaccharides, around 20 are of industrial importance. As already stated, the commercial value of a polysaccharide is mostly dependent on its rheological properties i.e. its ability to modify the flow characteristics of solutions. A selected list of commercially important polysaccharides, the microorganisms used for their production,

1. **Xanthan:** Xanthan or more frequently referred to as xanthan gum was the first polysaccharide available commercially. It is a well-studied and most widely used polysaccharides.

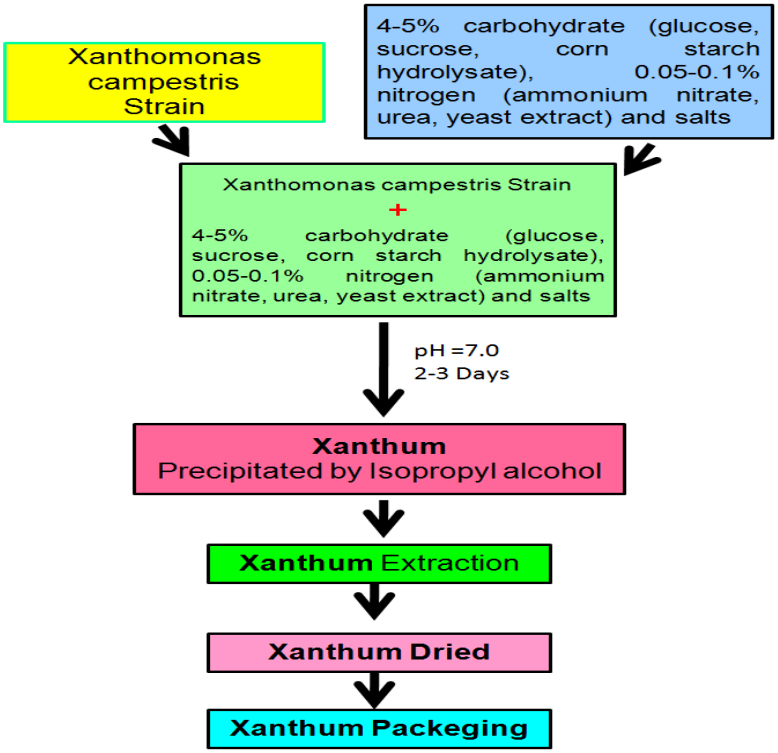
Basically, xanthan is a branched polymer with β (1 → 4) linked glucan (glucose polymer) backbone bound to a trisaccharide (Man, GIcA, Man) side chain on alternate glucose residues. The mannose has either acetate or pyruvate groups.

Applications- Xanthan gum is used as a food additive for the preparation of soft foods (ice cream, cheese). It is also used in oil industry for enhancing oil recovery. Further, xanthan is useful for the preparation of tooth pastes and water based paints.

Biosynthesis- For the biosynthesis of xanthan, the monomers are bound to a carrier lipid molecule and then transferred to a growing polymer chain. The activated monosaccharide nucleotides (e.g. uridine diphosphate glucose, UDP-glucose) supply energy for the formation of glycosidic bonds between adjacent units. The biosynthesis of other exopolysaccharides is comparable with that of xanthan. Dextran synthesis however is much simpler as described later.

Production: by following process

a) Xanthan is commercially produced by the Gram-negative bacterium, Xanthomonas campestris. The culture medium usually consists of 4-5% carbohydrate (glucose, sucrose, corn starch hydrolysate), 0.05-0.1% nitrogen (ammonium nitrate, urea, yeast extract) and salts.
b) The pH is maintained around 7.0,
c) Fermentation is carried out by batch culture for 2-3 days.
d) Xanthan in the culture broth is precipitated by isopropanol or methanol.
e) These agents also kill the microorganisms.
f) The precipitated xanthan can be dried and used for commercial purposes.



Genetic engineering of Xanthomonas campestris for xanthan production: Genetically engineered X. campestris have been developed that can utilize lactose (from whey) for the production of xanthan. For this purpose, the E. coli lazy genes (encoding the enzyme β-

galactosidase and lactose permease respectively) were cloned under the transcriptional control of X. campestris bacteriophage promoter. This construct was first introduced into E. coli, and then transferred to X. campestris.

The genetically engineered strains of X. campestris expressed the genes and produced high quantities of the enzymes β-galactosidase and lactose permease. These new strains utilize lactose or whey very efficiently for the industrial production of xanthan. This is a good example of successfully converting a waste product (whey) into a commercially important and valuable product (a biopolymer namely xanthan gum).

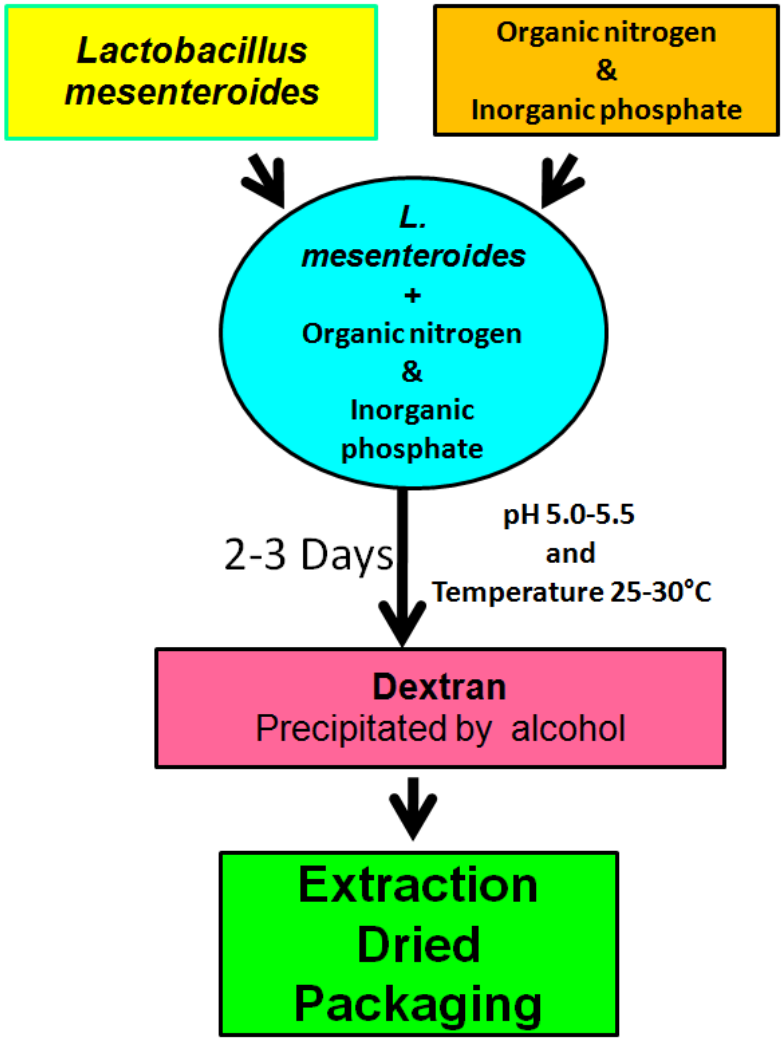
1. **Dextran-** Chemically, dextrans are glucans (polymers of glucose) containing 1→6 glycosidic linkages. Some dextrans also have α 1→2, α 1→3 and a 1→4 linkages. The molecular weights of dextrans are in the range of 15,000-500,000. It is used as blood plasma expanders, for the prevention of thrombosis and in wound dressing. In addition, dextrans are useful in the laboratory analytical techniques for purification of biomolecules.

   Production- Dextrans can be produced by a wide range of Gram-positive and Gram-negative bacteria e.g. Leuconostoc mesenteroides and Streptococcus mutans. In contrast to other exopolysaccharides (which are synthesized within the cells), dextrans are produced by extracellular enzyme in the medium. The enzyme is dextransucrase (a transglucosidase) which acts on sucrose and brings about polymerisation of glucose residues, and simultaneously liberates free fructose into the medium.

   The commercial production is carried out by using lactic acid bacterium, L. mesenteroides by a batch fermentation process. Besides sucrose, the culture medium contains organic nitrogen source and inorganic phosphate. The crude dextran produced is precipitated by alcohol and then subjected to acid hydrolysis.

   In recent years, the alcohol precipitated polymeric dextran is subjected to enzymatic hydrolysis by using exo- or endo-a dextranases to get dextrans of desired molecular weight. The resultant dextrans can be fractionated and dried.

   It is also possible to use a cell free system for the production of dextrans. The extracellular enzyme dextrasucrase can transform sucrose into dextran in a cell-free nutrient solution. This reaction is optimum at pH 5.0-5.5 and temperature 25-30°C.



Processing of dextron by lacobacillus

2. **Alginate-** Alginate is a linear polymer composed of mannuronic acid and glucuronic acid (both of them being uronic acids) in a proportion ranging from 4: 1 to 20: 1. Some of the mannuronic acid residues are acetylated. Alginate is commercially produced by gram-negative bacteria, Pseudomonas aeruginosa and Azobacter vinelandii.

   The type of organism used and the culture conditions determine the relative proportion of mannuronic acid and glucuronic acid residues and the degree of acetylation in alginate. Alginates with high contents of mannuronic acid are elastic in nature while those with high concentration of glucuronic acid are strong and brittle. Algal (seaweed) alginates are also polymers of mannuronic acid and glucuronic acid, and comparable in structure with bacterial alginates. However, algal alginates lack acetylation. For commercial purposes, seaweed alginates are more commonly used than bacterial alginates. This is mainly because bacterial

alginates are relatively unstable and get easily degraded. Alginates are useful as thickening agents in food industry, and for immobilization of cells and enzymes.

3. **Scleroglucan-** Scleroglucan is a glucose polymer (glucomer). It is a neutral polysaccharide with β 1→3 glucan backbone and single glucose (Glc) residue branches (β 1→6 linkage). The branching occurs at a regular sequence at every third glucose unit in the polymer backbone chain. Scleroglucan is a fungal heoxpolysaccharide. It is commercially produced by Sclerotium glucanicum, S. rolfsii and S. delphinii. Scleroglucan is useful for stabilizing latex paints, printing inks and drilling muds.
4. **Gellan-** Gellan is a linear heteropolysaccharide. The repeating unit of gellan is composed of two glucose, one glucuronic acid and one rhamnose molecules. Gellan is produced by Pseudomonas el odea. A deacetylated gellan which forms firm and brittle gels under the trade name Celrite has been developed by a reputed company in USA (Kalco Inc). Gellan is used in food industry. Even at a low concentration, it is a thicker.
5. **Pollulan-** Pollulan is an α-glucose polymer (α-glucan) with α 1→4, and a few α, 1→6 glycosidic bonds. Pollulan is produced by using the fungus, Aureobasidium pollulans. It is estimated that about 70% of glucose (the substrate) is converted to pollulan during fermentation, although the time taken is rather long (5-7 days). Pollulan is mainly used in food coating and packaging.
6. **Curdlan-** Curdlan is a β-glucose polymer (β-glucan). The glucose residues are held together by β 1 —>3 glycosidic bonds. The exopolysaccharide curdlan is commercially produced by employing Alcaligenes faecalis. Curdlan-like polysaccharides are also produced by other microorganisms such as Agrobacterium rhizogenes and Rhizobium trifolii. Curdlan forms strong gels when heated to above 55°C. Therefore, it is used as a gelling agent for cooked foods. In addition, curdlan is also employed for immobilization of enzymes.