Kirk-Othmer Encyclopedia of Chemical Technology.

MICROBIAL POLYSACCHARIDES

Produced by virtually all microbes, carbohydrate polymers serve as intracellular energy stores and cell wall components, among other roles (see Carbohydrates). They are perhaps most apparent when present as extracellular capsules, sheaths, or slime secretions. Extracellular polysaccharides may confer a survival advantage to microbes by protecting against desiccation, acting as buffers against environmental changes, preventing invasion by bacteriophages, and by helping cells adhere to surfaces and to one another. Technological exploitation of microbial polysaccharides has been limited mostly to those produced extracellularly in substantial quantities. Applications of these exopolysaccharides can be based on the unique chemical functionalities present, or on the bulk physical properties of the biopolymer. For example, vaccines against meningitis caused by *Haemophilus influenzae* are made from the capsular polysaccharide of the bacterium, and rely on its immunochemical properties. By contrast, the usefulness of gellan results from its ability to form clear, firm gels at low concentrations. The physical properties of polymers, including polysaccharides, result from their chemical structures. This article deals with the chemical structures of some microbial exopolysaccharides having actual or potential commercial applications, and describes how the chemical structures confer certain useful properties on those materials (see also Biopolymers).

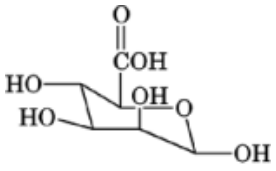
Microbial polysaccharides may be categorized into groups based on the types of monomer units present. Two of the most important types of microbial polysaccharides are neutral homopolysaccharides and anionically charged heteropolysaccharides. Other groups also exist, such as charged homopolysaccharides, but are of limited occurrence and not commercially significant as of this writing. Homopolysaccharides consist of a single type of monosaccharide, some examples being pullulan and dextran, both of which are polymers of D-glucose [50-99-7]. Some important anionic heteropolysaccharides are xanthan, which contains both neutral sugar and uronic acid residues within its structure, and alginic acid, which consists of two different types of uronic acid. Structures can be complicated by the presence of noncarbohydrate groups attached to the carbohydrate chains. Such groups include *O*-acetyl esters, and 1-carboxyethylidene (or pyruvate) ketals.

Polysaccharides can be classified by structure, biological origin, or mode of biosynthesis. Most polysaccharides are synthesized by an elaborate sequence of steps catalyzed by cytosolic and membrane-bound enzymes. The usual precursor is a nucleotide phosphate–sugar glycosidic ester. This activated sugar is transferred to a membrane-bound lipid phosphate, and the repeating units are assembled in a blockwise sequence, ie, each repeating unit is added as a single group (1–4). Once assembled, the polysaccharide is released. Another simpler mechanism is used for the synthesis of a few types of neutral homopolysaccharides. In these instances, monosaccharide units from sucrose or a similar glycosyl donor are incorporated directly into the polysaccharide; the glycosidic linkage of the donor contains sufficient stored energy to allow the reaction to proceed. No lipid–phosphate intermediates exist, and usually only a single enzyme is required. The best known examples of this latter mechanism are synthesis of dextran, catalyzed by dextransucrase, and synthesis of levan, catalyzed by levansucrase.

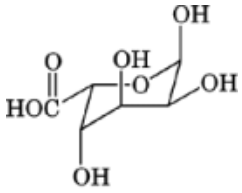
For some applications, microbial polysaccharides have supplemented or replaced those derived from plants or algae; in other instances, microbial polysaccharides have been developed for specific applications that cannot be met by other polysaccharides. Further information is available (5–24).

1. Alginates

The term alginate refers to the salt forms of alginic acid [9005-32-7], a copolymer of D-mannopyranosyluronic acid [56687-62-8] **1** and L-gulopyranosyluronic acid [56688-68-7] **2** residues.



(**1**)



(**2**)

For many years, alginates were derived solely from marine algae, hence the origin of the term algin. However, in 1964 it was reported that a strain of *Pseudomonas* isolated from the sputum of a cystic fibrosis patient produced an extracellular capsular polysaccharide essentially identical to algal alginate (25). Later it was demonstrated that the common nitrogen-fixing soil bacterium *Azotobacter vinelandii* also produces extracellular capsules of alginate (26). The phenomenon is known to be common among strains of *A. vinelandii*, as well as *A. chroococcum* (27, 28) and *A. beijerinckii* (29). Many other bacteria have been found to produce extracellular alginate, most notably strains of the opportunistic human pathogen *Pseudomonas aeruginosa* (30), the nonpathogenic *P. mendocina* (31), and numerous plant pathogenic species of *Pseudomonas* (32). Most of these organisms also produce other extracellular polysaccharides, and the relative yields of the different polymers often depend on strain, growth conditions, carbon source, and other such factors. Much has been made of the possibility of using bacterial fermentations to produce alginates on a large scale, but marine algae remain the only significant commercial source. However, a great deal of the research done on bacterial alginate has led to a better understanding of alginate structure and biosynthesis.

Alginic acid is a copolymer of β-D-mannopyranosyluronic acid **1** and α-L-gulopyranosyluronic acid **2** units linked $1 \longrightarrow 4$, with no detectable branching. Bacterial alginates contain *O*-acetyl residues linked to the sugar units. The sequences and proportions of the constituent units can vary, and much research has focused on understanding the factors determining these structural variations. Particularly important is the arrangement of the D-mannuronate and L-guluronate residues within the alginate chains. Sequences of poly-β-D-mannuronate tend to be soluble and nongelling. The introduction of blocks of α-L-guluronate sequences into the chain changes the conformation, so that the polymer is able to complex with divalent cations to form rigid gels. The so-called egg-box model has been used to describe the complexes between polyuronates and cations that result in gel formation (33). To obtain an alginate with good gelling properties it is necessary to grow the bacteria under conditions that favor the biosynthesis of a product with the proper ratio and arrangement of mannuronate and guluronate sequences, which implies a need for understanding the biosynthetic mechanism. It is generally accepted that bacterial alginate, like the algal product, is first assembled as a polymer of

poly-β-D-mannuronate, and that a key enzyme, polymannuronic acid C-5 epimerase, then epimerizes some of the D-mannuronate residues to L-guluronate residues (34–36). The extracellular epimerase from *A. vinelandii* has been purified and characterized (37), and is active on alginates from algae as well as bacteria. The enzyme is calcium dependent, and the concentration of calcium can determine the degree and pattern of epimerization. This knowledge has been used to select growth conditions influencing the constituent ratio in alginate produced by *A. vinelandii* fermentations (38, 39). *O*-Acetyl groups in bacterial alginate are also believed to affect structure–property relationships. The C-5 epimerase is incapable of acting on mannuronate residues bearing *O*-acetyl groups (40, 41); acetylation therefore prevents blocks of poly-β-D-mannuronate residues from becoming converted to L-guluronate. Regulation of *O*-acetylation, which apparently occurs intracellularly, is not fully understood. The chain length, or molecular weight, of the alginate molecule also plays a role in determining its physical properties. Bacterial alginate chains often begin to be broken down at some point during their biosynthesis and subsequent accumulation. This breakdown is usually attributed to the presence of alginate lyase, an enzyme that cleaves the polyuronate chains by an elimination, rather than hydrolysis type of reaction (42). Evidence seems to suggest that these enzymes can arise from infecting bacteriophages within the host organism (43, 44), although more recent evidence indicates that the presence of endogenous alginases may be common among strains of *Azotobacter* (45). The depolymerization of alginate causes a decrease in its viscosity and gelling ability.

1.1. Production and Utilization

Uses proposed for bacterial alginate have been the same as those for the algal-derived material, and are based on its gelling, viscosifying, film-forming, and suspension-stabilizing properties. The topic has been reviewed extensively (21, 46–49). Much of the potential for bacterially produced alginate has not yet been realized, owing mainly to the difficulty in competing with algal-derived products. Most research has been aimed at developing more efficient, higher yielding fermentations (38, 39, 50, 51) and at strain improvement (52) (see Fermentation). The main difficulties in competing with algal alginates are of two types. On the commercial side, there are the usual problems associated with bringing a new process to market, ie, building facilities, finding inexpensive and reliable feedstocks (qv), etc. On the technical side, there are still some unsolved problems. *Azotobacter* possess extremely high rates of respiration. This translates into a need for energy-intensive rates of aeration in fermentations. It also means that much of the carbon feedstock is diverted to metabolic products other than alginate (50). There is also the problem of product degradation by endogenous alginases. One example of the microbial product having unique advantages utilizes cultures of *A. vinelandii* in an *in situ* process to coat ceramic particles for enhanced aqueous dispersion (53) (see Ceramics). Another suggested application of *A. vinelandii* is the use of bacterially derived polymannuronate C-5 epimerase to improve the gelling qualities of alginates from traditional sources (48).

2. Bacterial Cellulose

Although cellulose [9004-34-6] is usually thought of as a plant-derived polysaccharide, there does exist one well-known example of cellulose (qv) production by a bacterium. In 1886, a bacterial isolate from what was referred to as the vinegar plant was described. This bacterium, *Acetobacter xylinum*, produced a tough, membranous pellicle in liquid cultures. Using the best methods then available, it was concluded that the pellicular material was cellulose (54), which was later confirmed by chemical methods and x-ray diffraction (55). *A. xylinum* has become a useful model in the study of cellulose biosynthesis (56, 57). The extracellular pellicle is composed primarily of microfibrils of cellulose, a $\beta\ (1 \longrightarrow 4)$-linked D-glucan. These microfibrils, which consist of parallel chains of polysaccharide molecules, form ribbon-like arrays, which in turn make up the pellicle itself (56, 58). Values for the average molecular weight vary, but are typically in the range of 350,000 to 975,000, corresponding

to average chain lengths of approximately 2000 to 6000 glucose residues. The polymer chains are synthesized by a membrane-associated enzyme complex. The precursor is the nucleotide phosphate–sugar ester uridine disphosphate–glucose (UDP–glucose), and synthesis seems to proceed through a lipid-linked intermediate (56), although there is still some disagreement as to the exact mechanism of biosynthesis. Part of the problem with studying cellulose biosynthesis in *A. xylinum* is that the bacteria also produce other polysaccharides (59), some of which also contain β-inked D-glucose units. These include $\beta(1 \longrightarrow 2)$-linked D-glucans (60) and heteropolysaccharides such as acetan (61, 62). Acetan appears to be structurally related to xanthan as well as to cellulose in that it consists of a $\beta(1 \longrightarrow 4)$-linked D-glucan backbone with side-chain units containing D-glucose, D-mannose, and D-glucuronic acid, as well as terminal L-rhamnose residues (62). Because it is possible that these polysaccharides are all produced by similar mechanisms, the difficulty in separating the enzymes and intermediates involved in the biosynthesis of each is considerable.

2.1. Production and Utilization

Although bacterial cellulose has been known since the late 1800s, there had been little commercial interest for many years, owing to the abundance and low cost of plant-derived cellulose. However, the ability of bacterial cellulose to form tough, uniform membranes has suggested applications, for example, in ultrafiltration membranes. Bacterial cellulose is used in speaker diaphragms for personal stereo headphones (57), which are reported to have excellent acoustical properties (63). Other applications have been proposed for bacterial cellulose, including nonwoven fabrics, coatings (qv), and suspending agents. In addition, it is claimed that the sheared material possesses good thickening properties (64). Research into improved methods for the production of bacterial cellulose continues (65). Processes have been developed which are said to yield cellulose at a cost of approximately $11–26/kg ($5–$12/lb) (57).

3. Dextrans

Dextran [9004-54-0] is a term that has traditionally been applied to any extracellular bacterial α-D-glucan synthesized from sucrose [57-50-1] in which $\alpha(1 \longrightarrow 6)$ linkages predominate. Dextrans have been more strictly defined as D-glucans containing chains of D-glucopyranosyl residues consecutively $\alpha(1 \longrightarrow 6)$-linked, with various degrees of branching through $\alpha(1 \longrightarrow 2)$, $\alpha(1 \longrightarrow 3)$, or $\alpha(1 \longrightarrow 4)$ linkages (66). Dextran has been known for many years, and was recognized as a polymer of dextrose (D-glucose) by the latter half of the nineteenth century, hence the origin of the name. Dextran research has been reviewed (66–73), and a bibliography has been published (74). A number of lactic acid bacteria produce dextrans (75), the most notable being *Leuconostoc mesenteroides* and certain *Streptococcus* species. Much of the early interest in dextrans arose as a result of its occurrence in sugar (qv) refineries. Infection of sugar cane, and to a lesser extent, sugar beets, by *L. mesenteroides* during harvesting and processing leads to the production of dextran. Large amounts can give rise to sticky, gummy solutions that foul processing equipment, whereas lesser concentrations inhibit proper crystallization of sugar (71). Food preparations that contain sucrose can also become contaminated by growths of dextran-producing bacteria, leading to sliminess, gumminess, or "ropy" solutions.

Interest in dextrans became intense after 1945, when it was shown that solutions of the polymer were suitable for use as artificial blood plasma substitutes (76, 77) (see Blood, artificial). In the late 1940s an investigation into the production and properties of dextran was undertaken. This work led to the characterization of dextrans produced by nearly 100 strains of bacteria (78), and to several patents for methods of producing dextran and dextran fractions (79–87). There is great structural diversity among dextrans from different bacterial sources, and some strains of bacteria produce more than one type of dextran (78, 88). Among those strains of bacteria that produce two or more types of dextran are *L. mesenteroides* strains (Northern Regional Research Laboratory (NRRL)) B-742, B-1299, B-1355, B-1498, and B-1501, and *Streptobacterium dextranicum* B-1254.

Dextrans produced by these bacteria may be separated into two or more fractions by precipitation with varying concentrations of ethanol, or other water-miscible solvents, in water. Designation of the fractions is based on their differing solubilities in water–alcohol mixtures. Those precipitated with lower alcohol concentrations are referred to as *L*-fractions (less soluble); those precipitated at higher alcohol concentrations are referred to as *S*-fractions (more soluble). The most important factor in determining the properties of the various types of dextrans is the percentage of non-$\alpha(1 \longrightarrow 6)$ linkages (78, 89); the nature and distribution of the non-$\alpha(1 \longrightarrow 6)$ linkages have been the subject of many studies (90–103).

Much of our understanding of the structure of dextrans is a result of studies carried out during the 1970s and 1980s using gc/mass spectrometry of methylated dextran derivatives (104–107), ^{1}H and ^{13}C-nmr spectrometry (108–117), and Fourier-transform infrared spectroscopy (ftir) (118). According to one proposal (116), dextrans may be classified into three types: class 1 dextrans, which contain a main chain of $\alpha(1 \longrightarrow 6)$-linked D-glucopyranosyl units, with branching through carbon positions 2, 3, or 4 of the D-glucopyranosyl ring; class 2 dextrans, which contain 3-mono-*O*-substituted D-glucopyranosyl units in nonconsecutive positions, as well as 6-mono- and 3,6-di-*O*-substituted residues; and class 3 dextrans, which contain consecutive 3-mono-*O*-substituted D-glucopyranosyl units in addition to 6-mono- and 3,6-di-*O*-substituted residues. The vast majority of polysaccharides that have been broadly defined as dextrans fall into class 1, and in most of these the branching is through position 3. Only three glucans from the NRRL strains are considered class 2 dextrans, and these are the fraction *S*-glucans from strains B-1355, B-1498, and B-1501 (112). These three glucans contain alternating sequences of $\alpha(1 \longrightarrow 3)$ and $\alpha(1 \longrightarrow 6)$-linked D-glucopyranosyl residues. Because they do not contain significant linear, consecutive sequences of $\alpha(1 \longrightarrow 6)$-linked D-glucopyranosyl residues, these glucans do not fit the strict definition of dextrans as $(1 \longrightarrow 6)$-α-D-glucans. For this reason, the name alternan has been proposed for this class of polysaccharides (119). Alternan from *L. mesenteroides* strain NRRL B-1355 has been studied in more detail than the others (92, 120–122), and continues to be of interest because of its unique immunochemical (123) and physical (124) properties. *L. mesenteroides* strains NRRL B-523 and B-1149, as well as many strains of *Streptococcus*, produce glucans that fall into the category of class 3 dextrans. These dextrans contain linear sequences of $\alpha(1 \longrightarrow 3)$-linked D-glucopyranosyl units, and tend to be insoluble or only slightly soluble in water. The distinction between these α-D-glucans and true dextrans has been noted by most workers in the field, and the name mutan has been proposed for polysaccharides consisting mainly of $\alpha(1 \longrightarrow 3)$-linked D-glucose chains (125). Streptococcal α-D-glucans play an important role in the adhesion of the bacteria to oral surfaces and the subsequent formation of carious lesions (cavities) in the teeth (126), and may also be an important factor in bacterial endocarditis (127). Because of this, much chemical and microbiological research has been done on streptococcal α-D-glucans (75, 126, 128).

Class 1, or true dextrans, are a structurally diverse group. The percentage of non-$\alpha(1 \longrightarrow 6)$ branch linkages ranges from as low as 3% in *L. dextranicum* NRRL B-1146 (78) to as high as 39% in some preparations of *L. mesenteroides* NRRL B-742 *S*-fraction dextran (107). Studies done on this latter strain between 1937 and 1986 have resulted in findings that seem to differ significantly (78, 89, 91, 93, 105–107, 111, 116, 129–134). Some of these differences can be explained by the fact that strain B-742 makes two distinct dextrans, the proportions of which can vary, depending on fermentation (qv) conditions. Fraction *L* contains approximately 15% $\alpha(1 \longrightarrow 4)$ branch linkages, whereas fraction *S* contains a much higher percentage of branch linkages, primarily $\alpha(1 \longrightarrow 3)$ (107). The percentage of $\alpha(1 \longrightarrow 3)$ linkages can vary, depending on the conditions of biosynthesis.

L. mesenteroides strain NRRL B-512F produces a water-soluble dextran with 95% $\alpha(1 \longrightarrow 6)$ main-chain linkages and 5% $\alpha(1 \longrightarrow 3)$ branch linkages (78). This strain was subcultured from NRRL strain B-512, isolated in 1943. Strain NRRL B-512F is the strain used for commercial dextran production in the United States and most other countries. Nearly all of the studies done on the industrial production and utilization of dextran have used this strain.

6 MICROBIAL POLYSACCHARIDES

3.1. Biosynthesis

Unlike most microbial exopolysaccharides, dextrans are enzymatically synthesized directly from sucrose. The enzymes, classified as glycosyltransferases, are known generically as dextransucrase (Enzyme Commission (EC) 2.4.1.5), or α-1,6-glucan:D-fructose 2-glucosyltransferase (see Enzyme applications). Dextransucrase is quite specific in its requirement for sucrose as the glucosyl donor substrate. The only other known glucosyl donors that give rise to dextran are α-D-glucopyranosyl fluoride (135), lactulosucrose (136), and *p*-nitrophenyl-α-D-glucopyranoside (137). The latter compound yields dextran at less than 1% of the rate for sucrose. Dextransucrase usually occurs as a soluble enzyme in the cell-free culture fluid of bacteria grown in liquid media (138). The streptococcal enzymes are constitutive (126, 128), whereas *L. mesenteroides* requires the presence of sucrose to induce enzyme production. Many methods have been reported for the isolation and purification of dextransucrases. The enzymes from *L. mesenteroides* B-1299 have been investigated, and several different isozymic forms isolated and partially characterized (139–142). Interest in alternan from *L. mesenteroides* B-1355 has led to the isolation and partial purification of the enzyme responsible for alternan synthesis (119, 143, 144); this enzyme, named alternansucrase, is distinct from the dextransucrase also elaborated by this organism (119).

Because of its commercial importance, *L. mesenteroides* B-512F dextransucrase has been studied most extensively. Several methods have been reported for its purification (145–150), and high yields of electrophoretically pure dextransucrase have been obtained from a mutant of B-512F that produces enhanced levels of enzyme activity (151). The purified enzyme had a molecular weight of 158,000, and was reportedly derived from a larger native protein of 177,000 molecular weight by a specific proteolytic cleavage (150, 151). The pH optimum of this enzyme is in the range of 5.0–5.5, and its Michaelis constant, K_m, for sucrose is approximately 12–16 mM (150).

In addition to polymerizing glucosyl units to form dextran, dextransucrases also catalyze the transfer of α-D-glucopyranosyl units to a wide variety of hydroxyl-bearing acceptor molecules (152–154). These transfers result in the formation of di- and oligosaccharides when sugars are the acceptors, and various glycosides when the acceptor is a noncarbohydrate. A number of sugars and sugar derivatives have been tested for their ability to act as acceptors in reactions catalyzed by B-512F dextransucrase (155–158). These studies have shown that maltose [69-79-4], the most effective acceptor sugar, gives rise to large amounts of the trisaccharide panose and diverts glucosyl transfer away from dextran synthesis. The kinetics of this reaction have been described (159). The acceptor reactions catalyzed by alternansucrase (160) and streptococcal glucansucrases (161–164) have also been investigated. In each case, it was shown that the acceptor substrate and product specificity depend on the enzyme source. One important acceptor reaction to note involves fructose [57-48-7]. Because fructose is released from sucrose during the glucosyl transfer reaction, it is always present during the enzymatic synthesis of dextran. Reactions of fructose catalyzed by B-512F dextransucrase give rise to one major and one minor product. The main product is 5-*O*-α-D-glucopyranosyl-D-fructose, known by the trivial name leucrose (165, 166), and the minor product is 6-*O*-α-D-glucopyranosyl-D-fructose (167), known as isomaltulose or palatinose.

The molecular mechanism by which dextransucrase synthesizes dextran has been a matter of considerable interest. It was long assumed that dextran synthesis occurred by the transfer of glucosyl residues from sucrose to the 6-hydroxyl group at the nonreducing ends of growing dextran chains (168). An alternative mechanism was proposed in which α-D-glucopyranosyl residues were inserted between the enzyme and the reducing end of the growing dextran chain (169). Experimental evidence has been provided for this type of mechanism (170–174). The mechanism by which acceptor reactions occur can be explained within this context (175), and there is evidence for separate sucrose and acceptor-binding sites on the enzyme (176). It is believed that acceptor reactions play an important role in the formation of branch linkages by dextransucrase (177–180). According to the insertion–mechanism model, branches are formed when glucosyl units or dextranosyl chains are transferred to secondary hydroxyl positions on the dextran chains. Differences in acceptor binding among dextransucrases from various sources account for the differences in branching among the dextrans.

Although sucrose is generally the only natural substrate that bacteria can use to synthesize dextran, there is one notable exception to this rule. *Acetobacter capsulatum* and certain related bacteria are known to produce dextrans from amylodextrins (maltodextrins) [9050-36-6], via an enzyme referred to as dextrandextrinase (EC 2.4.1.2: dextrin, α-1,6-glucan 6-glucosyltransferase). This enzyme synthesizes a typical $\alpha(1 \longrightarrow 6)$-linked dextran from $\alpha(1 \longrightarrow 4)$-linked D-glucans (181, 182). Some glucans, such as native starch, glycogen, and unhydrolyzed amylose or amylopectin, are very poor substrates, whereas starch hydrolyzates such as corn syrup or isolated amylodextrins are readily converted to dextran. The conversion efficiencies are not nearly so high as for dextransucrase. Typically, the reaction reaches equilibrium at a 1:1 mixture of amylodextrin and dextran (183). Several patents have been issued covering dextran production by dextran–dextrinase (184–188), and studies have been done on the properties (189, 190) and mechanism (191) of the enzyme. This enzyme has thus far found no commercial applications, probably because of the low conversion efficiencies. The finding that reduced maltodextrins serve as more efficient glucosyl donors (190) suggests that this drawback may not be insurmountable.

3.2. Derivatives

Derivatives of dextran may be classified into two categories: those in which the dextran chain has been covalently modified, and those in which the structure is unchanged, but the molecular weight has been lowered, either by alteration of biosynthetic conditions or by depolymerization of high molecular weight dextran. Under the usual conditions of biosynthesis, dextran quickly reaches molecular weights of 10^6 or greater. In some applications, for example blood plasma extenders, dextran of a lower size is required because of its osmotic and rheological properties, and because dextrans of higher molecular weight are not cleared from the bloodstream as quickly. The recommended molecular weight range for blood plasma extenders is $75,000 \pm 25,000$. These low molecular weight fractions are thus often referred to as clinical dextrans. Several approaches can be used to obtain dextrans of the desired molecular weight range. The method used most often involves hydrolysis with dilute acid under mild conditions, followed by separation of the variously sized fractions by graded precipitation with ethanol (qv). Drawbacks include the formation of unwanted side-products, eg, colored material; oligosaccharides arising from reversion reactions; and significant amounts of glucose. Nevertheless, the method is relatively inexpensive, and the ethanol precipitation step can remove nearly all of these contaminants. Other routes for obtaining clinical-sized dextrans have been developed, and may be more suitable for some applications. Biosynthesis in the presence of acceptors gives rise to dextran of lowered molecular weight (192); a systematic study of the biosynthetic parameters affecting molecular weight has been done (193). It has been found that at higher sucrose concentrations, overall dextran yield is diminished, and the average molecular weight lower. The addition of such exogenous acceptors as maltose, glucose, or preformed clinical dextrans has been found to be particularly useful for the synthesis of clinical dextrans. Conditions for producing clinical dextrans of suitable molecular weights have been established (194), and several methods have been patented (79, 81–83). The clinical dextrans produced by hydrolysis and synthesis in the presence of acceptors are similar in terms of their structures and viscosity behavior (195). Besides acid hydrolysis, there are other ways to degrade dextran to lower molecular weight fractions. Preparations of endodextranases (EC 3.2.1.11), enzymes that split dextran chains in an *endo*-hydrolytic fashion, have been described (196). Several types of Penicillium mold produce large quantities of extracellular endodextranase, and a method for the production of clinical dextrans using endodextranase to hydrolyze dextran to a suitable molecular weight has been patented (86). Co-fermentation of *L. mesenteroides* and a dextranase-producing organism has also been used to synthesize clinical-sized dextran (197). Other methods have been used in the laboratory to produce clinical-sized dextrans, including depolymerization by ultraviolet radiation, ultrasonic treatment (198, 199), dry heating (85), and synthesis by a streptococcal species known to produce dextrans of lower molecular weight (87). None of these methods has been used commercially. In the 1990s research has focused on the use of acceptor reactions for the synthesis of clinical dextrans (200–202). Although clinical dextrans are still used in some applications,

artificial polymers such as poly(vinylpyrrolidone) are replacing dextran as blood plasma extenders, because dextran tends to elicit an immune response in sensitive individuals.

Many covalently modified derivatives of dextran have been described. Of these, the most important are dextran sulfate [9042-14-2] and cross-linked dextran. The uses and properties of these and other dextran derivatives have been reviewed (66, 69, 71–73, 203).

3.3. Production and Utilization

Dextran sulfate displays anticoagulant properties, and has been investigated as a substitute for heparin (73) (see Blood, coagulants and anticoagulants). It has been shown that dextran sulfate can inhibit HIV binding to human T-lymphocytes (204), and is being studied for its potential in the treatment of AIDS and other viruses. Dextran, chemically cross-linked with epichlorohydrin (Sephadex), is useful in gel-filtration chromatography (qv). Derivatives such as diethylaminoethyl–Sephadex and carboxymethyl–Sephadex are used in ion-exchange chromatography.

Cross-linked dextran known as dextranomer (Debrisan), which is similar to Sephadex, has been used in treating wounds. Fluids and small molecules are absorbed into the gel particles, and proteins and cellular material are excluded (205). Complexes of colloidal iron with dextran (206), known as iron–dextran [9004-66-4], are used in treating iron deficiency anemia. This use is limited mainly to animals, especially pigs, because iron–dextran has been listed as a suspected carcinogen. The ability of dextran to form stable complexes with metals is one of its more useful properties. One of the largest markets for dextran is in the manufacture of photographic and x-ray films, where it is used to stabilize silver halide emulsions. Another large market is in aluminum manufacturing, where dextran solutions are sometimes used in the recovery of aluminum from bauxite ores. Dextran has been used as a binder in tobacco products, and its use in shaving creams and other cosmetics (qv) has also been suggested. The U.S. FDA status of dextran as a food additive is not clear. Although GRAS approval of low molecular weight dextran as a direct food additive was dropped in 1977 (207), *L. mesenteroides* is approved for use in fermented foods (see Food additives). Because many foods of plant origin contain sucrose, it is virtually certain that any foods containing *L. mesenteroides* also contain dextran. In fact, some patents describing food applications of *L. mesenteroides* B-523 and similar proprietary strains rely on the production of insoluble gelling dextran for key properties as food ingredients (209–211). Other food uses have been proposed for unusual dextrans. Alternan and low molecular weight alternan fractions from *L. mesenteroides* B-1355 are being studied as bulking agents for reduced calorie foods (124), and dextran oligomers containing $\alpha(1 \longrightarrow 2)$-linkages have been proposed for similar applications (211).

Dextran is produced commercially by fermentation of sucrose with *L. mesenteroides* B-512F (212). Typical media consist of sodium or potassium phosphate, sucrose or a sucrose source such as molasses, and a nitrogen and nutrient source such as yeast extract or corn steep liquor. The initial pH is adjusted to ~ 7, but unless the pH is controlled, it may fall to below 5 by the time growth is complete, due to lactic acid formation. Sucrose concentrations up to 10% have been used (213), but anything over 2% gives such high viscosities in the final stages of synthesis that 2% is usually the preferred concentration. If a clean product is required, cells must first be removed, typically by centrifugation. The polysaccharide is recovered by precipitation with a suitable solvent, such as methanol, ethanol, or 2-propanol, and dried to a white powder. The only U.S. manufacturer as of this writing is Pharmachem Corp. The price is about \$11–13/kg (\$5–6/lb), and U.S. sales are on the order of approximately \$3 million per year. Another main supplier is Pharmacia–LKB (Sweden), which manufactures clinical-sized dextrans, Sephadex, and other derivatives, as well as industrial-grade dextran.

Although dextran is manufactured using traditional fermentation (qv) methods, there are advantages of using cell-free enzyme preparations to synthesize dextran (212). These advantages include better control over the synthetic process and greater ease of purification of the end product. The profit margin for dextran producers is not large, however, and it may be difficult to justify changes in processes requiring large capital investments. A systematic study of this subject has shown that existing plants could be adapted to separate

enzyme and dextran production (214). Scale-up of the enzymatic process can give high dextran yields in shorter reaction times (215) (see Enzyme applications, industrial). Researchers have looked to the use of immobilized enzymes for improved synthesis of dextran. Immobilized dextransucrase is especially well suited for the production of low molecular weight, low viscosity oligosaccharides and clinical-sized dextrans (216). Problems are encountered, however, when larger, high viscosity dextrans are synthesized. The problem is particularly severe if the enzyme is immobilized using a porous gel; the pores become filled with entrapped dextran, and synthesis of new polysaccharide slows down and eventually comes to a halt. Research into new supports and methods of immobilization may overcome these problems.

4. Emulsan and Liposan

Microorganisms that degrade hydrocarbons and utilize them as carbon sources usually possess some way of rendering the hydrophobic hydrocarbons water soluble, generally by secreting some type of surfactant or emulsifying agent (see Surfactants). Two of the best known compounds in this category are emulsan [80450-55-1] and liposan. Emulsan, first isolated from cultures of the oil-degrading bacterium *Acinetobacter calcoaceticus* strain RAG-1 (217), consists mainly of a heteropolysaccharide with amino sugars substituted by *O*-esterification with long-chain acyl groups (218). This combination of hydrophilic carbohydrate and hydrophobic fatty acid functionalities gives the emulsan molecule an amphiphilic character. The polymeric nature of the emulsan molecules causes the resultant emulsions (qv) to be very stable and not prone to phase separation. Emulsan can stabilize oil-in-water emulsions at concentrations as low as 1:1000 emulsan:oil (219). Many different strains of *Acinetobacter* produce emulsan-type compounds (220). Emulsan from strain RAG-1 (American Type Culture Collection (ATCC) strain 31012) has been shown to contain 2-amino-2-deoxy-D-galacturonic acid, 2,4-diamino-2,4,6-trideoxy-D-glucose, and 2-amino-2-deoxy-D-galactose (galactosamine). The amino groups are at least partially acetylated, and 3-hydroxybutyrate ester groups have been found. The fatty acyl chains are predominantly 2- and 3-hydroxydodecanoate, with one fatty acyl chain per trisaccharide repeating unit. The average molecular weight is approximately one million, corresponding to chain lengths of 3000 to 3500 monosaccharide units (219). In contrast, *A. calcoaceticus* strain BD4 secretes an emulsan composed of L-rhamnose, D-mannose, D-glucose, and D-glucuronic acid residues. Amino sugars and fatty acyl groups are conspicuously absent (21). It has been noted, however, that the purified polysaccharide from this strain possesses no emulsifying ability (221). It has been shown that both the yield and structure of emulsan can vary, depending on the carbon source and growth conditions (222).

Another microbial polysaccharide-based emulsifier is liposan, produced by the yeast *Candida lipolytica* when grown on hydrocarbons (223). Liposan is apparently induced by certain water-immiscible hydrocarbons. It is composed of approximately 83% polysaccharide and 17% protein (224). The polysaccharide portion consists of D-glucose, D-galactose, 2-amino-2-deoxy-D-galactose, and D-galacturonic acid. The presence of fatty acyl groups has not been demonstrated; the protein portion may confer some hydrophobic properties on the complex.

4.1. Applications

Proposed uses of emulsan have included scouring crude oil from tankers and other containers, cleaning oil-handling equipment, dispersing oil-soluble pigments (see Dispersants), enhanced oil recovery, and other emulsion-stabilizing applications. Field tests in the 1980s demonstrated its usefulness in forming easily pumped emulsions for the transport of crude oil (219). It was also found that oil-in-water emulsions of No. 6 fuel oil with emulsan could be used directly in combustion processes (219). Proposed consumer uses include cosmetics (qv), cleaning compounds, and as a food ingredient emulsifier. Emulsan has been marketed by Petroferm (United States).

5. Gellan

Gellan [71010-52-1] was successfully introduced as a replacement for agar in the early 1980s. Synthesized by the bacterium *Pseudomonas elodea* (*Sphingomonas elodea*, *Auromonas elodea*) (225–227), this anionic heteropolysaccharide consists of D-glucopyranosyl (Glc*p*), L-rhamnopyranosyl (Rha*p*), and D-glucopyranosyluronic acid (Glc*p*A) residues linked in repeating units:

$$[\longrightarrow 3)-\beta-\text{D}-\text{Glc}p-(1\longrightarrow 4)-\beta-\text{D}-\text{Glc}p\text{A}-(1\longrightarrow 4)-\beta-\text{D}-\text{Glc}p-(1\longrightarrow 4)-\alpha-\text{L}-\text{Rha}p-(1\longrightarrow]_n$$

The polysaccharide also contains *O*-acetyl groups linked to C-6 of approximately half of the 3-linked D-glucopyranosyl units (228), and L-glyceric acid groups esterified to position 2 of the same residues (229). Native gellan forms soft, elastic gels, whereas gels formed by deacylated gellan are more rigid and brittle (225). Gels are formed only in the presence of cations, and the presence of a chelating agent such as EDTA can prevent gel formation (see Chelating agents). Divalent cations such as calcium or magnesium give far stronger gels than monovalent cations like sodium or potassium. Gellan gels are typically thermoreversible, with 1 wt %/vol gels melting at temperatures just below 100°C and gelling at lower temperatures, usually in the range of 35–50°C (230). The gel properties are strongly dependent on the degree of acyl substitution, type and concentration of cation present, and polysaccharide concentration (225, 230). Gellan was developed as a substitute for agar, and many of its properties are similar. The most notable differences are the dependence on cations for gel formation, and the fact that lower concentrations of gellan are required to give gels comparable to agar. For example, 1.5 wt %/vol agar is a typical concentration used in microbiological plate media. Similar gels can be made using gellan at concentrations of 0.5–0.8% in the presence of magnesium salts (225). Calcium–gellan gels are especially stable, and unlike agar gels show no syneresis (shrinkage and loss of water). As with agar, heating is required to achieve dissolution of the dried material. If gellan is dissolved in distilled ion-free water, no gel forms on cooling, but an increase in viscosity is observed (230). Fibers of gellan are formed by extruding heated solutions into a bath containing an aqueous solution of magnesium salts (231).

A left-handed double-helical structure has been proposed for gellan in the crystalline state, based on x-ray diffraction studies (227). The presence of acetyl groups presumably disrupts interchain aggregation, since these groups are postulated to be on the outside of the helices. The role played by acetyl and glyceryl ester groups and their influence on the double-helical structure has been studied using computer models (232).

5.1. Production and Utilization

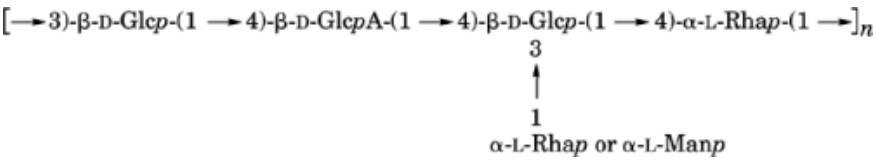
Unlike many newly discovered microbial polysaccharides, gellan has become a commercial success in a relatively short period. Discovered in the late 1970s, gellan was patented in 1982 (233, 234). It was first marketed as a replacement for agar in microbiological applications under the trade name GELRITE (Kelco Division, Monsanto (formerly with Merck & Co.)). The advantages of gellan over agar include higher purity, better clarity, and the ability to obtain strong gels at lower polysaccharide concentrations. Gellan is especially useful in marine microbiology, where agar-degrading microbes are often encountered. Gellan has also found biotechnological applications in plant tissue culture and in cell immobilization (14, 230). Another possible use is in air-freshener gels (16). In 1990 the U.S. FDA approved gellan for use as a food additive in icings, frostings, jams, jellies, and fillings, where it can be used as a replacement for agar and carrageenans (235, 236). Use of gellan as a general food additive for stabilizing, thickening, and gelling was approved by the FDA in 1992 (237). Additional applications include structured meat products, pet foods, candies, cheeses, yogurt, dressings, sauces, and ice cream and other frozen desserts (13, 238) (see Food additives; Meat products; Milk and milk products).

Gellan is made by fermentation in a medium containing glucose, salts, and a nitrogen source. The bacteria are grown for 2–3 days at 28–30°C, and the polysaccharide is recovered by precipitation with 2-propanol or

ethanol (239). Aeration of the culture medium becomes difficult as the gellan concentration builds up. This is one of the primary difficulties in achieving high yields of the product. Gelling of the culture medium also causes problems in product recovery; one way of overcoming this is to dilute the final cultures with water. A method for post-fermentation modification of gellan to give a product containing fewer ($<1\%$) acetyl groups and approximately 3–12% glyceryl groups has been patented (240). As for many commercially important microbial polysaccharides, attention has focused on cloning genes for gellan biosynthesis (241) (see Biotechnology; Genetic engineering).

6. Welan

Welan is produced by an *Alcaligenes* species (ATCC-31555) by aerobic fermentation, and marketed under the trade name BIOZAN (Merck and Co., Inc.); early reports also referred to it as S-130 (229). The polymer is structurally similar to gellan, sharing the same backbone sequence. It has an additional side group of an α-L-rhamnopyranosyl or an α-L-mannopyranosyl (Man*p*) unit linked ($1 \longrightarrow 3$) to a β-D-glucopyranosyl unit in the backbone of the polymer:



The presence of the L-form of mannose is unusual. The side-chain substitution is randomly distributed (242); approximately two-thirds of the side chains are rhamnose. The repeat unit may also contain an *O*-acyl group, but the distribution of these units has not been completely determined. The polymer is moderately soluble in water but is insoluble in isopropanol solutions, which are used to obtain the polymer from the culture medium. A method for producing a rapidly hydrating form of welan is available (243).

Solutions of welan are very viscous and pseudoplastic, ie, shear results in a dramatic reduction in viscosity that immediately returns when shearing is stopped, even at low polymer concentrations (230). They maintain viscosity at elevated temperatures better than xanthan gum; at 135°C the viscosity half-life of a 0.4% xanthan gum solution is essentially zero, whereas a welan gum solution has a viscosity half-life of 900 minutes (230). The addition of salt to welan solutions slightly reduces viscosity, but not significantly. It has excellent stability and rheological properties in seawater, brine, or 3% KCl solutions (see Rheological measurements).

6.1. Applications

The high heat tolerance and good salt compatibility of welan gum indicate its potential for use as an additive in several aspects of oil and natural gas recovery. Welan also has suspension properties superior to xanthan gum, which is desirable in oil-field drilling operations and hydraulic fracturing projects. It is compatible with ethylene glycol, and a welan–ethylene glycol composition that forms a viscous material useful in the formulation of insulating materials has been described (244).

7. Levan

Levan [9013-95-0] has been the subject of numerous studies, most of which have focused on structure and biosynthesis (245, 246). The term levulan first appeared in 1881 (247), and was used to describe a gum

consisting of fructose (levulose) units that had been formed by microbial action on molasses. It was named by analogy with the dextrose-containing gum known as dextran. By 1931, when the first detailed structural characterization was published (248), the name had been shortened to levan. Levan is a homopolysaccharide consisting of $\beta(2 \longrightarrow 6)$-linked D-fructofuranoside units, with branch chains linked to the main chain via $\beta(2 \longrightarrow 1)$ linkages. The β-linked fructose units impart a strongly levoratatory effect on polarized light. Levan is synthesized by a number of bacteria, some of the better known examples being *Erwinia herbicola* (formerly *Aerobacter levanicum*) (249), *Bacillus amyloliquefaciens* (250), *Bacillus subtilis* (251–253), *Bacillus polymyxa* (254), *Zymomonas mobilis* (255), *Actinomyces viscosus* (256), *Gluconobacter oxydans* (257), a *Serratia* species (258), and various species of *Arthrobacter* (259), *Corynebacterium* (260, 261), *Streptococcus* (262–264), and *Pseudomonas* (251, 265, 266). It is synthesized from sucrose by the extracellular enzyme levansucrase (β-2,6-fructan:D-glucose 6-fructosyltransferase, EC 2.4.1.10), which incorporates the fructosyl moiety of sucrose into levan and releases the glucose portion (249, 252). In this respect, levan is analogous to the dextran family of polysaccharides; however, levansucrase can also utilize the sucrose-containing trisaccharide raffinose [512-69-6] as a substrate for levan synthesis, releasing the disaccharide melibiose as a side-product. This reaction is often used to differentiate between dextransucrase and levansucrase in unknown microbial preparations.

Levans are water-soluble, nongelling, and generally of lower viscosity than most other gums and polysaccharides, despite their often high molecular weights. The average molecular weight can vary considerably, and depends on the conditions of biosynthesis (267), usually falling in the range between 10^6 and 10^7. The reasons for the variation can be best explained in terms of the mechanism of enzymatic biosynthesis (268, 269). Not only does the enzyme levansucrase transfer fructosyl units from sucrose to growing levan chains, it can also transfer fructosyl units from sucrose to a wide variety of carbohydrate acceptors, as well as to water. Moreover, it is also capable of transferring fructosyl residues from the ends of levan chains to water, albeit at a much slower rate. Therefore, the molecular sizes of levan chains depend on the presence or absence of other carbohydrates in the synthetic reaction mixtures and on the length of time the enzyme acts on the levan after all of the sucrose is consumed. The source and purity of the enzyme, reaction temperature, and other factors may also play a role (246).

Levan's chemical structure also varies, especially in the degree of branching. The levan produced by *E. herbicola* was shown by methylation analysis to contain 16–18% $\beta(2 \longrightarrow 1)$ branch linkages, whereas levan from a *Corynebacterium* species analyzed in the same manner contained only 3–6% branch linkages. The levan produced by *L. mesenteroides* strain B-512 as a minor fraction of the total extracellular polysaccharide contained between 10 and 22% branching (270). A strain of *Streptococcus salivarius* produces a levan with 11–14% $\beta(2 \longrightarrow 1)$ branch linkages (271); the branches have been shown to be at least 4–6 residues in length. A *Bacillus polymyxa* levan analyzed by ^{13}C-nmr spectroscopy and methylation was found to contain 12–13% branching (272). The levan from a species of *Serratia* has been reported to contain a much lower than average degree of branching, as determined by enzymatic and spectroscopic methods (258), but methylation data for this levan are not available. Branching in *Bacillus subtilis* levan has been shown to be dependent on the conditions of biosynthesis (273). It has been reported that levan sometimes bears a nonreducing sucrose group at what would otherwise be considered its reducing end. In the case of levan synthesized from raffinose, the presence of a raffinose end group has been reported. These are presumed to arise from the transfer of fructosyl units (or a levan chain) to sucrose or raffinose. Often such terminal residues are not detected; this may simply be a reflection of the difficulty in detecting a single residue within a large polymer, or it may be due to the fact that water and other molecules can also act as acceptors (246, 269), resulting in any one of these acceptors being present at the reducing terminus.

7.1. Production and Utilization

Although many uses have been proposed for levan (246), it is not being manufactured or used for any applications as of this writing. Low inherent viscosity and sensitivity to hydrolysis render it unsuitable as a

thickening or viscosifying agent, and many of the applications for which it has been proposed (274) are better served by the more stable and readily available dextran. However, some specialty application for which its unique properties are well-suited may be found. One proposal was to use levan as a blood plasma extender, in a manner similar to clinical dextran (275). The antigenicity of early levan preparations, and the widespread acceptance of dextrans, prevented levan from being more seriously considered for this purpose. A variety of food uses have been proposed for levan (246, 276). These include use as fillers (qv), bulking agents, and as a substitute for gum arabic, as well as a source of fructooligosaccharides analogous to those known commercially as Neosugar (Meiji Seika Kaisha, Ltd.).

Two approaches can be used to synthesize levan: classical fermentation (277) and direct enzymatic synthesis (278, 279). Both require an organism that produces high levels of levansucrase. A number of researchers have directed their efforts at improving the methods for manufacturing the polysaccharide. A new strain of *B. polymyxa* capable of producing elevated levels of levan in the culture broth (254), conditions for production of a high molecular weight levan by *Z. mobilis* (280), and culture conditions for enhanced secretion of the constitutive extracellular enzyme from *E. herbicola* has been reported (281). In order to produce levan by cell-free enzymatic synthesis, levansucrases from *Bacillus* spp. have been immobilized by a variety of methods, including binding to activated silica (282) and a ceramic matrix (283), and by adsorption on hydroxyapatite (284). Enzymes immobilized by the latter method were found to have increased levels of polymerase activity, and gave good yields of a high molecular weight product.

8. Pullulan

Pullulan [9057-02-7], first described in detail in 1959, is a water-soluble extracellular α-D-glucan elaborated by the fungus *Aureobasidium pullulans* (formerly *Pullularia pullulans*) (285). It is a linear polymer of maltotriose units linked from the reducing end of one trisaccharidic unit to the nonreducing end of the next trisaccharidic unit by $\alpha(1 \longrightarrow 6)$ linkages (286):

$$[\longrightarrow 6)\alpha\text{-D-Glc}p(1\longrightarrow 4)\alpha\text{-D-Glc}p(1\longrightarrow 4)\alpha\text{-D-Glc}p(1\longrightarrow]_n$$

Pullulan also contains some maltotetraose units within the chains, to approximately 5–7% (287, 288). Occasional reports of other types of linkages and units in pullulan preparations (71) may be attributable to contamination by other polysaccharides also known to be produced by *Aureobasidium pullulans* (289), although enzymatic studies have suggested that pullulan itself may contain a small percentage of atypical units or branch linkages (290). The molecular weight of pullulan varies from one preparation to the next, with values as low as 10,000 and as high as 25 million having been reported. It is an essentially linear polymer and the viscosity of aqueous solutions is often used as a reliable measure of pullulan molecular weight (291). The relationship between viscosity and molecular size indicates pullulan chains are flexible and coil-like in aqueous solutions (292). Molecular modeling suggests that much of this flexibility arises from the $\alpha(1 \longrightarrow 6)$ linkages, which interrupt the $\alpha(1 \longrightarrow 4)$ sequences and add an extra degree of rotational freedom (293).

Pullulan is generally produced in liquid fermentations, and its accumulation causes a marked increase in the viscosity of the medium. However, as cultures age, the viscosity usually drops off. This lowering of viscosity results from enzymatic cleavage of the maltotetraosyl regions by an endogenous amylase-like enzyme (287, 291). This phenomenon is a key factor in determining the physical properties of pullulan produced by fermentation. The fungus is capable of producing pullulan from a number of carbohydrate substrates (294, 295), but the ones generally used are glucose, sucrose, or starch hydrolyzates. Biosynthesis is accomplished via a lipid-linked intermediate (296), with UDP–glucose serving as the initial glucosyl donor. Experimental evidence suggests that UDP–glucose is incorporated into lipid-linked panosyl or isopanosyl intermediates, which are then assembled into pullulan (297).

8.1. Production and Utilization

There are many patents and publications covering applications for production of pullulan (71, 298–301). Hayashibara Biochemical Laboratories, Inc. (Japan) manufactures pullulan for a number of applications; the market price in 1990 was approximately $11.50/kg (21). Pullulan can be used in foods as a viscosifier and low calorie partial replacement for starch, and as a binder. Its unique film-forming and oxygen-barrier properties (299) make it especially useful in protective and adhesive edible coatings (qv) (301). Other applications have been suggested in degradable films and fibers, paper coatings and binders, cosmetics, pharmaceutical tablet coatings, and even in soluble contact lenses (qv) which contain slow-release bioactive medicines (301). Pullulan fractions of narrow molecular weight ranges are available for use as standards in gel-permeation chromatography.

Much of the research on pullulan is focused on improving methods for its production. A technique has been described for synthesis of pullulan using immobilized cells of *A. pullulans* (302). Other studies have led to improved fermentation conditions (303), bioreactor systems (304), and methods for recovering pullulan from fermentation broths (305, 306). It is generally acknowledged that there is a need for strains of the fungus capable of producing high yields of pullulan that has not been degraded by endogenous amylolytic enzymes, and that is free of contamination by the melanin pigments produced by most strains of *Aureobasidium*. Some progress has been made in this area with newly isolated color variants (307, 308) and mutant forms of the fungus (309, 310).

9. Scleroglucan

Scleroglucans [39464-87-4] are neutral, branched homopolysaccharides composed of glucose residues (311, 312). They are produced by fungi of the genus *Sclerotium*, which are plant parasites in the Basidiomycete family. The main chain of scleroglucan consists of β-D-glucopyranosyl residues linked $(1 \longrightarrow 3)$ with every third sugar bearing a single D-glycopyranyl residue linked $\beta(1 \longrightarrow 6)$ (313). The polysaccharide is insoluble in 2-propanol, which is used to isolate and concentrate the material.

Scleroglucan exists in a triple helical conformation that is highly stable (314). The D-glucopyranosyl side groups project to the outside of the helix (312) and prevent the aggregation of helices, which would result in insolubility, as in the case of curdlan (*vide infra*). The transition from helix to coil occurs at temperatures above 90°C or by increasing the pH to 12 (315, 316). Solutions cooled below 8°C form weak gels (317). The molecular weight of the polymer depends on the time the culture is harvested; scleroglucan from a 10-hour culture has an average molecular weight of 1.2×10^6, whereas polymer from a 20-hour culture has an average value of 2.6×10^6 (318). Solutions of scleroglucan show pseudoplastic flow behavior during shear; the viscosity is relatively temperature insensitive and remains virtually constant from 10 to 90°C (312).

9.1. Production and Utilization

The most important potential use for scleroglucan is as a mobility control agent for enhanced oil recovery. Many of its rheological properties, such as the production of highly viscous solutions at low concentrations, and excellent long-term stability at elevated temperature and salt concentrations, are similar to those of xanthan gum. In a study of 140 synthetic and native biopolymers, scleroglucan was found to be the most thermostable in a synthetic North Sea brine at 90°C for extended time periods (319). A strategy to improve the dispersibility and filterability of scleroglucan preparations utilized a precipitant to form a coagulum, followed by the addition of a surfactant prior to drying and grinding; the dried mixture was reportedly easier to dissolve than dried native material (320). A problem with native scleroglucan is that the polymer readily adsorbs to rock, which can lead to pore plugging and reduced flow (312).

Scleroglucan, like other β-glucans, can stimulate an immune response and repress the development of some forms of cancer when administered intravenously (312, 321). A scleroglucan derived from *Sclerotium glucanicum* stimulated *in vivo* murine macrophage phagocytic activity by 66%, and enhanced murine bone marrow proliferation by up to 300% (321) (see Immunotherapeutic agents). The polysaccharide is produced commercially by fermentation in a medium containing glucose, corn steep liquor, nitrate, and mineral salts, which is cultured at 28–30°C for approximately 60 hours (312). Two fermentation products are available: native scleroglucan, containing some residual fungal mycelia, and a refined-grade product that has been sheared and filtered to yield mycelium-free scleroglucan.

10. Curdlan

Curdlan [54724-00-4] is a neutral $\beta(1 \longrightarrow 3)$-linked D-glucan produced by several bacteria, primarily *Alcaligenes faecalis var. myxogenes*, as well as by *Agrobacterium radiobacter*, *Rhizobium meliloti*, and *R. trifolii* (322). Prior to 1968, when a mutant strain of *A. faecalis var. myxogenes* that produced only curdlan was isolated (323), the *A. faecalis* strain used by most investigators produced both curdlan and the acidic polysaccharide succinoglycan. The *Agrobacterium* and *Rhizobium* species that produced curdlan also produced succinoglycan (322). The isolation of the mutant producing only curdlan simplified preparation of the polymer for characterization of its physical properties.

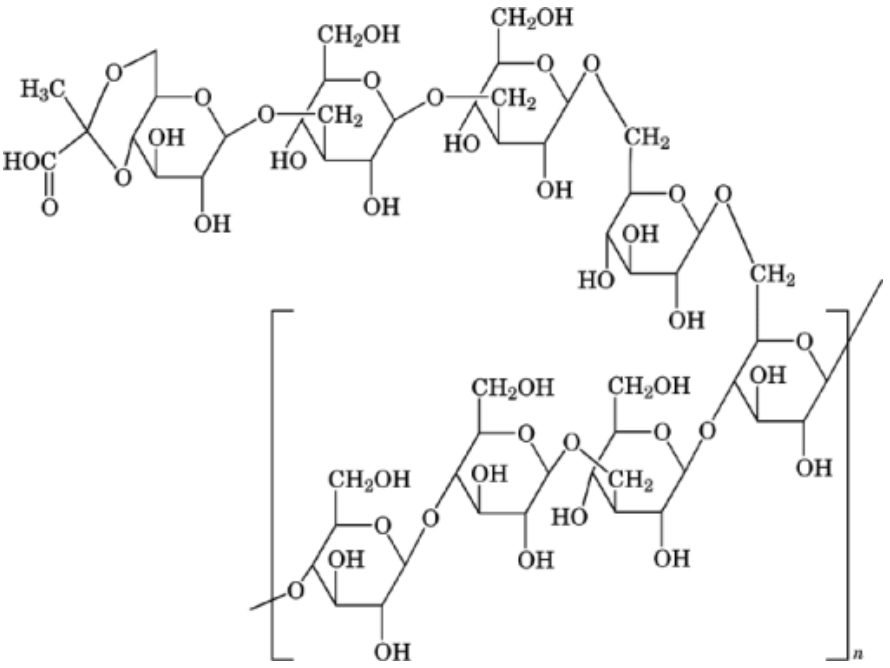
Curdlan is insoluble in water, but does absorb some water to become swollen. It can be dissolved in alkaline solutions, formic acid (see Formic acid and derivatives), and certain organic solvents such as dimethyl sulfoxide (322). Suspensions of curdlan heated above 54°C form a firm gel. A closely related polysaccharide, pachyman, which has a $\beta(1 \longrightarrow 3)$-D-glucan main chain and a small number of $\beta(1 \longrightarrow 6)$-linked D-glucose branches, does not form gels when heated, even at higher concentrations. The lack of side groups in curdlan apparently allows the polymer to form aggregates that are insoluble and to form gels at moderately warm temperatures. Curdlan forms a low set gel when heated to 55°C and allowed to cool (324); high set gels obtained by heating curdlan suspensions at higher temperatures have melting temperatures of 140–160°C (325). According to conformational studies, the solid and low set gelled polymer is a single-stranded helix (326); whereas the high set gelled polymer is a triple-stranded helix (322, 327, 328) with strands from several helices intertwining to form three-dimensional networks.

10.1. Applications

Several food uses have been proposed for curdlan including jellies, jams, noodles, and tofu (322). Its gelling properties make it useful for the preparation of instant puddings and multiple layer puddings. It has been suggested that it may be useful as a stabilizing agent in frozen desserts such as ice creams (322). Curdlan can be added to bind water, add stability, and improve the body and gloss of food products.

11. Succinoglycan

Succinoglycan is an acidic extracellular polysaccharide produced by several bacteria, including *Alcaligenes faecalis*, *Agrobacterium radiobacter*, *Rhizobium meliloti*, and *R. trifolii* (322). The polymer consists of a repeating octasaccharide, as shown for succinoglycan from *Alcaligenes faecalis var. myxogenes* (329).

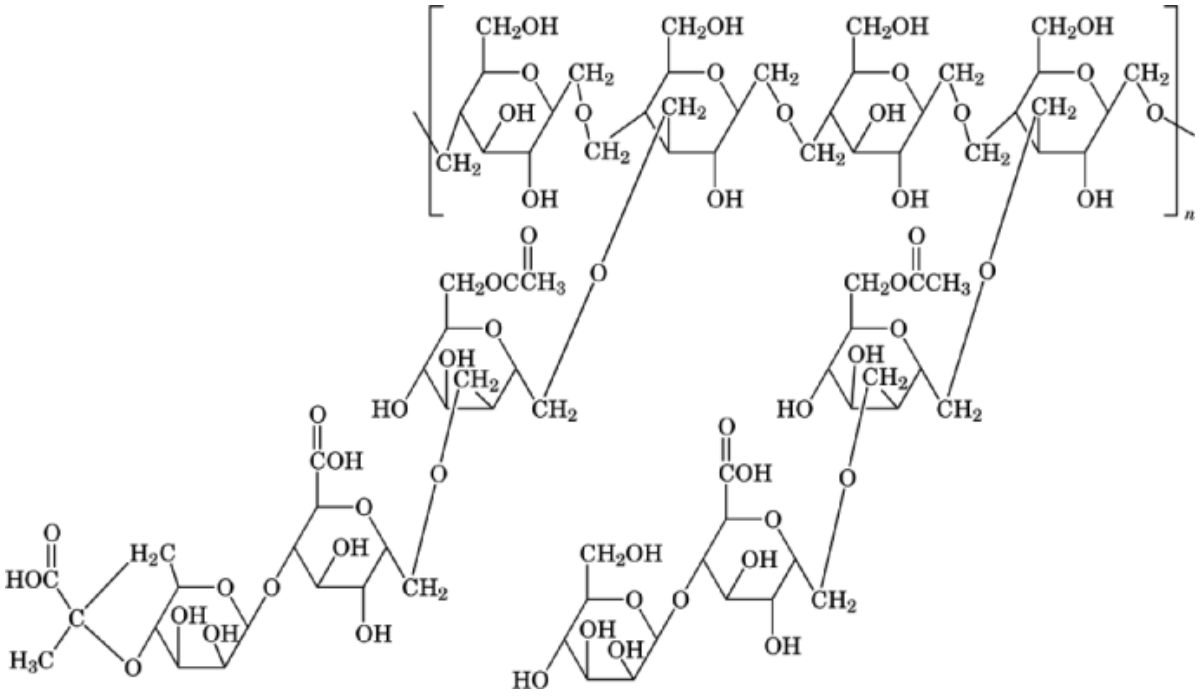


The name succinoglycan was derived from the presence of succinic acid covalently bound to the side chain of the polymer from *A. faecalis* (329, 330). In addition, the terminal D-glucosyl residue of the side chain contains pyruvic acid linked as a ketal to the glucopyranose ring (322). Structural determination of the polysaccharide produced by *R. meliloti* and several strains of *Agrobacterium* has shown that the ratio of succinate and pyruvate groups varies slightly between species, and that the succinoglycan produced by some strains also has some covalently bound acetate. Structural characterization by fast-atom bombardment mass spectrometry (qv) of succinoglycan oligosaccharide from *R. meliloti* has determined the location of succinyl and acetyl modifications (331).

Succinoglycan can form viscous solutions if in the free acid or calcium salt form, but produces low viscosity solutions if in the sodium salt form (332). The viscosity of solutions is quite temperature dependent; increasing the temperature leads to a reduction in viscosity, which approaches zero at approximately 60°C (322). The viscosity is relatively stable from pH 3–10 and is compatible with a number of inorganic salts other than sodium. The production of succinoglycan and its potential use in foods and industrial processes as a thickening agent has been described (322).

12. Xanthan Gum

Xanthan gum [11138-66-2] is an anionic heteropolysaccharide produced by several species of bacteria in the genus *Xanthomonas*; *X. campestris* NRRL B-1459 produces the biopolymer with the most desirable physical properties and is used for commercial production of *xanthan gum* (see Gums). This strain was identified in the 1950s as part of a program to develop microbial polysaccharides derived from fermentations utilizing corn sugar (333, 334). The primary structure of xanthan has been determined by chemical degradation and methylation analysis (335, 336); it is composed of repeating units consisting of a main chain of D-glucopyranosyl residues with trisaccharide side chains made up of D-mannopyranosyl and D-glucopyranosyluronic acid residues.



Approximately half of the terminal D-mannosyl residues have pyruvate present as 4,6-*O*-(1-carboxyethylidene) substituents. The pyruvate content has been shown to vary with culture conditions (337, 338) and strains of *X. campestris* (339). The internal D-mannosyl residue is acetylated at the O-6 position.

Xanthan gum has several desirable physical properties that explain the wide application range developed for this polysaccharide (6, 24). The viscosity of xanthan gum solutions is highly pseudoplastic. Relatively low concentrations of the biopolymer produce highly viscous solutions that maintain viscosity over wide ranges of temperature and pH. Mono- and divalent cations enhance the stability of solution viscosity to exposure to elevated temperatures (340). The addition of salt to solutions of xanthan results in essentially stable solution viscosity from pH 1.5 to 13; salt also increases the yield value, ie, suspending power, of xanthan gum solutions (230). There is a surprisingly small effect on viscosity over a relatively large range of salt concentrations even though xanthan gum is a polyelectrolyte. At a polysaccharide concentration of approximately 0.35%, there is essentially no change in solution viscosity between 0.01 and 1% KCl. At concentrations of polysaccharide lower than 0.35%, the presence of salt slightly lowers the viscosity, whereas at concentrations higher than 0.35% gum, the presence of salt results in a slight increase in viscosity (230).

Using a variety of measurement techniques, a relatively wide range of molecular weights have been reported for xanthan gum preparations ($\sim 2 - 50 \times 10^6$). Two preparations of native xanthan gum have molecular weights of 13 and 50×10^6, as determined by light scattering measurements (341). By measuring the contour length of the molecule from electron micrographs, an estimate of 20×10^6 has been made (342). Sedimentation studies have yielded an average value of 7.6×10^6 (343) and a range of values of $4 - 12 \times 10^6$ (344). Using size-exclusion chromatography, average molecular weights of 1.8 and 2.4×10^6 have been measured (345). Low angle laser light scattering has given molecular weight estimations in the range of $4.1 - 12.2 \times 10^6$ (346).

12.1. Structure and Conformation

The conformation of the biopolymer in solution has been a subject of debate. Several analytical methods (qv) have shown that the polymer goes through a phase transition at elevated temperatures, which depends on the concentration of salt. Increasing the temperature at low salt concentrations brings about a conformational shift from an ordered state to a disordered, random coil conformation (334). The change is associated with a decrease in solution viscosity and can also be characterized by changes in optical rotation and circular dicroism (347–349), nuclear magnetic resonance (348), and electron microscopy (342, 350, 351). The transition temperature increases with increasing sodium or calcium ion concentration, as well as with the acetate content of the polysaccharide, and decreases with increasing pyruvate content. The transition temperature is apparently independent of polymer concentration; this has been interpreted as evidence that the polymer is a single-stranded helix (352), and this conclusion has been supported by spectroscopic analysis of the polymer in solution (353–355). The crystalline structure of xanthan gum has been examined by x-ray diffraction (356) and reported to be a single-stranded helix with a diameter of 2 nm, but the possibility that the molecule could form a double-stranded helix has not been ruled out. Evidence that the native structure of xanthan is a double-stranded helix has been provided by light-scattering experiments (357). As of this writing, it appears that xanthan generally has a double-helical conformation but can be treated in a fashion, eg, prolonged dialysis at low ionic strength, which causes it to assume a single-helical conformation (349).

The structure of xanthan has also been investigated by electron microscopy. A series of micrographs of native and denatured xanthan preparations show stiff, relatively straight rod-shaped structures, ranging from 2 to 10 μm long and 4 nm in diameter (342). Xanthan gum prepared using several methods shows sections of single- and double-stranded structures within a single polymer strand (351); apparently the polymer becomes partly unwound under the particular conditions used to prepare the sample for analysis. The method of preparation and treatment of the sample is important in determining what structural features will be present in the polymer, and treatments exist to change the polymer from one conformation to another. The effect of salt on single- and double-stranded structures has been studied (358), and a model for salt-induced extension and dissociation of native xanthan gum involving a two-step mechanism proposed. Lowering the salt concentration results in an extended double-stranded polymer; at even lower salt concentrations, dissociation of the double helix into single strands occurs (359).

Studies utilizing enzymes capable of hydrolyzing the cellulosic backbone chain indicate that the polymer is incompletely substituted with trisaccharide side chains on alternating glucose residues. An excess of glucose in a high molecular weight fragment obtained by hydrolysis of native xanthan gum using a heat-stable xanthanese mixture (360), suggests that this hydrolytic fragment contains cellulosic regions lacking in side chains. When disordered xanthan gum was hydrolyzed using a fungal cellulase preparation the product had more glucose than the amount expected if the backbone chain were fully substituted (361).

12.2. Production and Utilization

The nutritional requirements of *X. campestris* have been studied in order to optimize the production of xanthan gum. Fermentations for the industrial production of xanthan gum are done at 28°C, and utilize glucose concentrations from 1–5% (362). Higher glucose concentrations do not result in higher levels of gum biosynthesis. Saccharides such as sucrose, starch, and maltodextrins can also be used for gum production. The use of a completely defined media for gum production has been described (230). It has also been shown that some organic acids including pyruvic, succinic, and α-ketoglutaric acids increase the production of xanthan gum. It is necessary to maintain a neutral pH during fermentation in order to obtain maximal yields; during polymer biosynthesis the medium becomes acidic, but can be neutralized by the addition of a suitable base.

A typical commercial production process starts with an innoculum of *X. campestris*, prepared in a yeast extract containing broth, which is added to the production medium consisting of 3% D-glucose, 0.5% potassium

phosphate buffer, 0.4% dried distillers solubles, and 0.01% magnesium sulfate (230). The stirred, aerated culture is held at 28°C and after 96 hours approximately 50% of the glucose is converted into polymer (230). The medium is highly viscous, and water is added to reduce the viscosity to improve the removal of cells. The fermentation broth is heated to nearly 100°C to pasteurize the liquid, and after cooling the polysaccharide is precipitated with ethanol or 2-propanol. The process of heating also causes the strands of polysaccharide to separate, and slow cooling allows the strands to reanneal between helices and form networks, improving the viscosity. The recovery of polymer is enhanced if potassium chloride is added to the mixture. The alcohol is removed and the material dried and packaged. Food-grade xanthan is tested during production and packaging to ensure that the material is not contaminated by microorganisms. It has been estimated that in 1989 the yearly production of xanthan gum for food markets was $18 - 22 \times 10^6$ kg ($8 - 10 \times 10^6$ lbs), with a value of $\$44 - 60 \times 10^6$ (230).

The unusual rheological properties of xanthan gum have led to its use in a wide variety of food and industrial applications. Uses of xanthan gum in food products have been reviewed (230). It is frequently the thickener of choice because of its ability to maintain solution viscosity (for emulsion stabilization) in salts and acids, such as are found in salad dressings. It has been used extensively in no oil or low oil salad dressing formulations, and has been shown to provide long-term emulsion stability. U.S. FDA Standards of Identity also permit its use in sauces, puddings, bakery and pie fillings, and dry mixes for beverages. Xanthan gum can be mixed with carrageenan and galactomannans such as locust bean gum to improve gelling and stabilization of frozen dairy products and desserts. The synergistic interactions of xanthan gum and the galactomannans can be used in situations where fast gelling time is desirable or to reduce costs of additives (230).

Large quantities of xanthan gum are used by the oil and natural gas industry in several aspects of hydrocarbon production (230, 349) (see Gas, natural; Hydrocarbons; Petroleum). The high viscosity achievable at low concentration and the high suspending power efficiently remove bit cuttings while reducing friction substantially throughout the drill string. Xanthan is compatible with many additives frequently used to prepare drilling fluids, and drilling fluids can be made up of fresh, brackish, or salt water and still maintain viscosity. It can effectively thicken hydraulic fracture fluids that are employed to improve porosity in subterranean formations, where viscosity is required to suspend a propping additive such as sand which is then pumped underground into newly formed fissures. The excellent heat stability and salt compatibility frequently result in its selection over lower cost viscosifiers. Xanthan is also added to solutions pumped underground that are used to displace oil toward a collection well. It improves the sweeping efficiency of these flooding fluids enough to make them cost effective.

Other industrial uses for xanthan gum include thickening textile and carpet printing pastes, suspending pigments in ceramic glazes to improve glaze dispersion, and ink and clay coating formulations in the printing and paper (qv) industries, respectively (230). Agrochemical producers blend herbicides (qv) and insecticides (qv) with xanthan in order to improve application to plants.

13. Other Microbial Polysaccharides

There are a number of other polysaccharides from fungi and bacteria which have actual or proposed practical applications. These include polysaccharides whose usefulness derives from particular structural features which are important not because of their viscosity or gelling ability, but because they elicit a particular biological response. The *Haemophilus influenzae* vaccine, composed in large part of the capsular polysaccharide of this organism, has prevented many deaths from meningitis since its introduction in the early 1980s. An earlier polysaccharide-based vaccine against *Streptococcus pneumoniae* was successful in preventing many types of bacterial pneumonia, but fell into disuse with the introduction of newer antibiotics (qv). The increased incidence of antibiotic-resistant bacterial infections has resulted in renewed interest in such vaccines, and the use of

pneumonia vaccines is on the rise. Other polysaccharide-based vaccines have also been developed, and their chemistry and biology reviewed (363, 364).

Some polysaccharides elicit a more general type of immune response. These immunomodulator polysaccharides often fall into two categories: sulfated polysaccharides and β-D-glucans. The biological activity of dextran sulfate has been discussed; other sulfated polysaccharides also show anticoagulant activity as well as immunomodulator activity. Some examples include curdlan sulfate, which exhibits anti-HIV activity (365), and sulfated yeast glucan, which enhances immune resistance against bacterial, viral, and fungal infections, and also shows antitumor activity (366). The β-D-glucans, are not only microbial in origin, but can also be found in plants, fungi, and macroalgae. Many of the studies regarding these polysaccharides have been reviewed (367). One compound of this type is Betafectin, derived from yeast cell wall β-D-glucan (368).

Many researchers continue to search for microbes that produce better polysaccharides, and higher yields (369–372). Many polysaccharides with exceptional qualities have been discovered and investigated, but few have been successfully commercialized (372). Because many of the proposed applications of new polysaccharides are as food ingredients, a primary obstacle to successful introduction of products is the need to obtain regulatory approval. This long and expensive process is a strong disincentive to introduce a product unless it has clear and overwhelming advantages over currently used materials. Some of these advantages might be a significantly lower price or functional properties not yet available. There appears to be a need for inexpensive carbohydrate-based bulking agents for use in artificially sweetened foods, and for a replacement for gum arabic [9000-01-5]. Gum arabic, a plant polysaccharide, is imported to the United States from Africa, and the price and availability are reported to fluctuate unpredictably. A microbially produced replacement could bring down the cost and create a more stable supply.

14. Notes

The USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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