ISSN 1330-9862
(FTB-1693)

review

Scleroglucan: Fermentative Production, Downstream Processing and Applications

*Shrikant A. Survase, Parag S. Saudagar, Ishwar B. Bajaj and Rekha S. Singhal**

Food Engineering and Technology Department, Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai 400 019, India

Received: April 11, 2006
Accepted: November 6, 2006

Summary

Exopolysaccharides produced by a variety of microorganisms find multifarious industrial applications in foods, pharmaceutical and other industries as emulsifiers, stabilizers, binders, gelling agents, lubricants, and thickening agents. One such exopolysaccharide is scleroglucan, produced by pure culture fermentation from filamentous fungi of genus *Sclerotium*. The review discusses the properties, fermentative production, downstream processing and applications of scleroglucan.

Key words: scleroglucan, exopolysaccharide, *Sclerotium glucanicum*, *Sclerotium rolfsii*, fermentation

Introduction

Exopolysaccharides produced by a variety of microorganisms are chemically well defined and have attracted worldwide attention due to their novel and unique physical properties. These exopolysaccharides find multifarious industrial applications in foods, pharmaceutical and other industries as emulsifiers, stabilizers, binders, gelling agents, lubricants, and thickening agents. These are rapidly emerging as new and industrially important source of polymeric materials, which are gradually becoming economically competitive.

Microbial polysaccharides serve different functions in the microbial cells and are distinguished into three main types:

1. Intracellular polysaccharides, which provide mechanisms for storing carbon or energy for the cell;
2. Structural polysaccharides, which are components of the cell structure or are integral parts of the cell wall;
3. Extracellular polysaccharides or exopolysaccharides, which, depending on the microbial system, (*i*) form capsules outside the cell, thereby becoming a part of the cell wall, or (*ii*) form slimes that accumulate outside the cell wall and which subsequently diffuse in the liquid phase during the fermentation.

Microorganisms that produce a large amount of slime have the greatest potential for commercialization, since these exopolysaccharides can be recovered from the fermentation broth. A list of such biopolymers is shown in Table 1 (*1*). One such exopolysaccharide is scleroglucan.

Scleroglucan – producers and world market

The production of scleroglucan was first reported by Halleck (2) who observed *Sclerotium glucanicum* to secrete this extracellular polysaccharide. Pillsbury Co. introduced scleroglucan in the market under the trade name Polytran®, and in 1976 it was commercialized by CECA S.E. (France) under the name Biopolymer CS®. Subsequently, Satia, a division of Mero-Rousselot (France), produced scleroglucan under the trade name of Actigum CS6®. Sanofi Bio-Industries (Carentan, France), which obtained the rights from Satia and CECA, were the main scleroglucan producers, trading scleroglucan under the commercial names Polytran® and Actigum®, respectively (*3*,*4*). Sanofi Bio-Industries were acquired by Degussa

*Corresponding author; Phone: ++91 22 24 145 616; Fax: ++91 22 24 145 614; E-mail: rekha@udct.org

Table 1. Various exopolysaccharides of industrial importance

Product	Substrate	Microorganism	Yield*/%
Alginate	Sucrose	*Azotobacter vinelandii* NCIB 9068	5
Curdlan type	Glucose 5 %	*Alcaligenes faecalis* var. *myxogenes* 10C3 IFO 13140	50
Levan	Sucrose 2 %	*Zymomonas mobilis* NCIB 8938	<2
Levan	Lactose 6 %	*Alcaligenes viscosus* NRRL B-182	2.5
Scleroglucan	Glucose 3 %	*Sclerotium rolfsii* ATCC 15206	1.5–2.2
Pullulan	Sucrose 5 %	*Aureobasidium pullulans* S-1	50–60
Phosphomannan	Hydrolyzed whey, sugars 4.4 %	*Hansenula holstii* NRRL Y-2448	20
Xanthan gum	Lactose 6 %	*Xanthomonas campestris* NRRL B-1459	38.3
Galactoglucan	Lactose 6 %	*Zoogloea ramigera* NRRL B-3669	55.6
Gellan gum (S-60 polysaccharide)	Carbohydrate	*Pseudomonas elodea* ATCC 31461	5

*Yield based on conversion of the substrate to the product

Food Ingredients (Germany) in 1995, who manufactured scleroglucan under the brand name Actigum™ CS. In 2006, Cargill (Germany) acquired Degussa Food Ingredients and continued trading scleroglucan under the same brand name.

Initial application of scleroglucan was in the oil recovery where it showed greater efficiency and stability than xanthan over a wide range of temperature and pH. In oil recovery, scleroglucan increases the viscosity, and hence the hydraulic pressure of (sea) water or brine used to extract oil. Presently, scleroglucan is not as economical as xanthan, but the potential use of crude scleroglucan (biopolymer cells) could become economical for use in oil recovery and drilling**.**

The use of scleroglucan as an antitumor, antiviral and antimicrobial compound has also been investigated. Scleroglucan has shown immune stimulatory effects compared with other biopolymers, and its potential contribution to the treatment of many diseases should be taken into account in therapeutic regimens. Recently, the attractive properties of the polysaccharide in controlled drug release and especially in immunopharmaceutical applications have created a demand in the medical market, though in terms of quantity this market is comparatively limited. Despite its thickening and stabilizing abilities that could be exploited in several foodstuffs, currently scleroglucan is not used due to cost constraints. Genetic engineering techniques could improve the biopolymer yields and reduce the production costs, permitting much wider use of the biopolymer as a replacement for xanthan.

Chemical structure

Scleroglucan is a branched neutral homopolysaccharide that gives only D-glucose after the complete hydrolysis. The polymer consists of a main linear chain of β-D--(1–3)-glucopyranosyl units; there is a β-D-glucopyranosyl unit (1–6) linked to every third unit. This structure was first elucidated by periodic oxidation analysis, and later verified by methylated sugar analysis and ^{13}C nuclear magnetic resonance (NMR) (*5*). The chemical structure of the tetrasaccharide repeating unit of scleroglucan as established by NMR analysis is depicted in Fig. 1.

The length of the polymer chain, and hence the molecular mass vary according to the microbial cultures used. Generally, these polymers have average molecular masses ranging from $(1.3–3.2)\cdot10^5$ to $(0.3–6.0)\cdot10^6$ Da (*6,7*). The molecular mass of scleroglucan was determined by low-angle light scattering (LALS) and found to be between 5 and $12\cdot10^6$ Da (*5,8*). However, Lecacheux *et al.* (*9*) estimated the molecular mass of scleroglucan by size exclusion chromatography (SEC) coupled with LALS and found it to be $5.7\cdot10^6$ Da (±5 %). These authors also concluded that this polysaccharide had low polydispersity

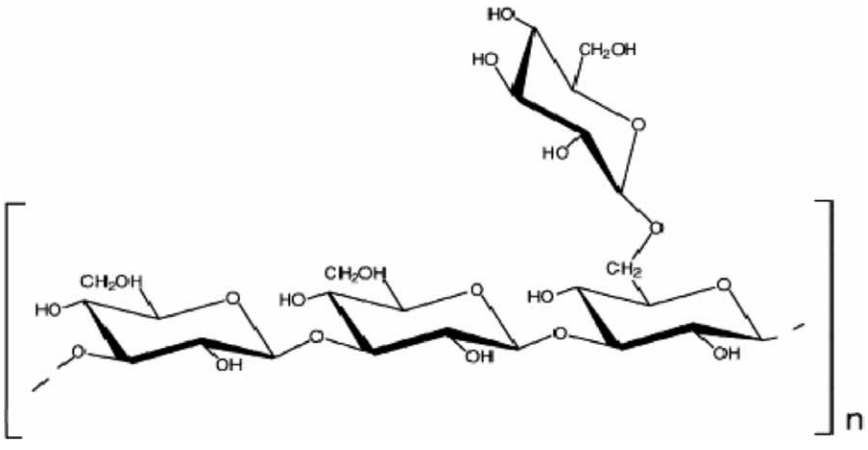

Fig. 1. Repeating unit of scleroglucan

and stable molecular mass under different fermentation conditions. When dissolved in water, scleroglucan forms linear triple helices (*9*), where the side glucose groups protrude and prevent helices from aggregating. This stabilizes the polysaccharide, but reduces its gelling ability. The triple helix structure is quite thermostable, but dissolves into single random coils when dispersed in dimethyl sulphoxide (DMSO) or at pH over 12.5. Scleroglucan has a structure that is identical to schizophyllan, which is produced by *Schizophyllum commune.* Schizophyllan has slightly higher molecular mass than scleroglucan (*10*).

Properties of Scleroglucan

The properties of scleroglucan may be influenced by the molecular mass and by recovery methods. In addition, the solution properties of scleroglucan may also be different, depending on the grade used. Biopolymer CS-6 contains 60–70 % of scleroglucan, whereas biopolymer CS-11 is a refined product containing 85–90 % polysaccharide.

Dissolution

Scleroglucan disperses rather easily in water at room temperature due to the presence of β-D-(1–6)-glucopyranosyl groups that increase the solubility of the polysaccharide and decrease the ability to form the gels (*3,11*). Refined grades of scleroglucan dissolve readily in hot and cold water to form pseudoplastic solutions with shear thinning characteristics that tolerate high temperature, broad range of pH, and a variety of electrolytes, whereas the crude isolate from the fermentation broth produces low viscosity solutions. Mixing, temperature, pH, and concentration influence the rate at which viscosity develops. The viscosity of scleroglucan solutions is affected only slightly by temperature variations. At 0.5 and 2.0 %, it remains practically constant between 10 and 90 °C. At low temperatures, close to 7 °C, solutions of scleroglucan form thermoreversible gels that may be caused by weakly interacting triple helix cross linking mechanism (*10*). The viscosity of scleroglucan is unaffected over a pH range of 1 to 11. In dimethyl sulphoxide, in aqueous solutions of pH=12.5 or higher, or at temperatures above 90 °C, the reduced viscosity, specific rotation, and sedimentation coefficient indicate disruption of the triple helical structure to a single random coil (*12*). Among 140 polymers tested for use in polymer flooding in the North Sea oil reservoirs, scleroglucan was the most stable, retaining more than 90 % of its viscosity after 500 days at 90 °C in seawater (*5*).

Scleroglucan forms stable gels in the presence of chromium salts and borax at pH=10–11, and can be precipitated by the addition of quaternary ammonium salts under alkaline conditions.

Compatibility

Prehydrated scleroglucan is compatible with electrolytes such as 5 % sodium chloride, 5 % sodium sulphate, 20 % calcium chloride, and 10 % disodium hydrogen phosphate (*11*)**.** However, when the electrolyte concentrations are very high, solutions may gel and flocculate. Scleroglucan is compatible, without synergism, with most other thickeners such as locust bean gum, alginates, xanthan, and carrageenan and cellulose derivatives. While scleroglucan remains soluble in the mixtures containing 50 % of glycols or polyols, solutions have high viscosity only when the polyol concentration is above 20 % (*5*).

Rheology

Pseudoplasticity, or shear thinning, is the salient characteristic of scleroglucan solutions. Pseudoplasticity is evident in the gum solutions of 0.2 % or lower, but the flow becomes progressively more Newtonian as the concentration decreases below 0.2 %. Solutions containing less than 0.8 % of scleroglucan are not significantly thixotropic, except at temperatures dropping to 10 °C and below. Above 20 °C, as determined by the Brookfield-type viscometer, the hysteresis loops traced by the response of shear stress to increasing and decreasing shear rate are of negligible proportions (*7*).

Due to high degree of pseudoplasticity, gel states are not always clearly defined. Thus, 1.2–1.5 % solutions of purified gum form self-supporting sliceable gels at approximately 25 °C, but at temperatures below 10 °C, even very diluted solutions form diffusely structured gels that tend to shrink and undergo syneresis when left undisturbed for long periods of time. Such diffused gels disperse quickly with mild agitation.

Suspending properties

A pseudoplastic flow system inherently combines a capacity for suspending fine particles with good pourability of suspension. Purified scleroglucan at 0.1–0.2 % effectively stabilizes 5–10 % aqueous suspensions of fine powders such as zinc oxide, reprecipitated calcium carbonate, and sulphamerazine. The viscosity of combinations of scleroglucan with bentonite suspensions is markedly synergistic. Thus, while the apparent viscosities of 0.15 % purified gum and 5 % bentonite are around 200 and 300 cps, respectively, a combination of the two yields the viscosity of >4000 cps. Although not a primary emulsifier in the sense of a surfactant, scleroglucan enables very low energy dispersion during the formation of stable oil-in-water emulsions. In addition to the suspending action of the pseudoplastic system, prevention of coalescence seems to underlie this kind of stabilization (*3,5*).

Physiology

Short and long term feeding studies with rats and dogs have not shown any toxicity, blood abnormalities, or significant tissue pathology. Eye and skin tests involving guinea pigs, rabbits and humans have not demonstrated significant adverse reactions or sensitization. With chicks and dogs, scleroglucan in the diet lowered the cholesterol levels and increased the excretion of lipids (*13*). Like other β-glucans, scleroglucan displays antitumor activity, but it is more effective than other polysaccharides such as curdlan and β-glucan.

Microbial Strains Producing Scleroglucan

Scleroglucan is synthesized extracellularly by species of the genus *Sclerotium, i.e. Sclerotium glucanicum, Sclerotium rolfsii* and *Sclerotium delphinii. Corticium rolfsii* and

Schizophyllum commune produce other polysaccharides that are structurally very similar to scleroglucan (*2*,*14*). The two main species for its production are *Sclerotium glucanicum* (*15–18*) and *Sclerotium rolfsii* (*6*,*19–23*).

Sclerotium glucanicum and *Sclerotium rolfsii* are heterotrophic filamentous fungi, which are characterized as plant pathogens and parasites. They possess enzymes including cellulases, phosphatidase, arabinase, exogalactanase, polygalacturanase, galactosidase and exomannase. These organisms also produce oxalic acid, which facilitates plant cell lysis. *Sclerotium* species have brown or black sclerotia (aggregated bodies of hyphae) or light-coloured mycelia, and do not sporulate (*24*). Sclerotia are more resistant to biological or chemical degradation than mycelia. In liquid media the organism forms pellets with central capsules from which hyphal residues extend. On solid media, aerial hyphae are formed and organized in mycelia. The role of scleroglucan in the life of the organism is mainly to assist in attachment to plant surfaces and the protection of sclerotia against unfavourable environmental conditions such as desiccation (*25*). In addition, scleroglucan may have a role as energy source. The hydrolytic enzymes synthesized by *Sclerotium* species degrade scleroglucan into glucose molecules, indicating that the microorganisms may utilize the biopolymer when other carbon sources are depleted (*26*).

Farina *et al.* (*27*) measured the colony radial growth rate (Kr) on solid medium of colonies of *Sclerotium rolfsii* Proimi F-6656 for the evaluation of scleroglucan production medium and other different media, incubation temperature and tolerance to diverse concentrations of sucrose and NaCl. The optimum growth temperature observed was 30 °C. The fungus tolerated concentrations of sucrose from 0.15 to 1.17 M on both Czapek and production medium. Growth was limited by the highest concentrations of sucrose tested (0.88 and 1.17 M), as indicated by a slower increase in colony size. Addition of 0.86 M NaCl to the production medium and yeast extract-malt extract agar (YMA) did not inhibit the growth completely, but decreased the radial growth rate considerably (80 and 70 %, respectively).

Biosynthetic Pathway

Sutherland (*28*,*29*) suggested a general pathway for the biosynthesis of extracellular polysaccharides in three major steps: (*i*) substrate uptake, (*ii*) intracellular formation of polysaccharide, and (*iii*) extrusion from the cell.

There is very little information available on the biosynthesis for scleroglucan formation in *Sclerotium glucanicum* and *Sclerotium rolfsii*, but generally this should resemble the biosynthetic steps encountered in the production of other glucans. First, glucose is transferred into the cells *via* a hexokinase and is then phosphorylated by the action of phosphoglucomutase (PGM) and phosphoglucoisomerase (PGI). Pyrophosphorylase (UGP) catalyses the formation of uridine diphosphate glucose (UDP-glucose), which reacts with lipid carrier and initiates polymerisation. This proposed pathway for the production of scleroglucan is depicted schematically in Fig. 2 (*7*).

Fermentative Production of Scleroglucan

In general, factors affecting scleroglucan production include inoculum preparation, growth medium, environmental conditions, and the formation of byproducts. Increasing the rate and extent of polysaccharide synthesis, eliminating undesirable enzyme activities or trans-

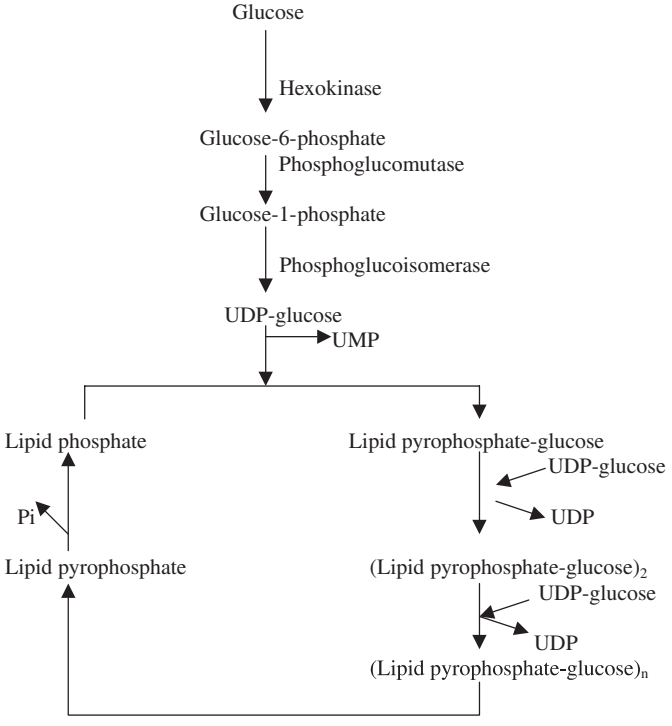

Fig. 2. Biosynthetic pathway for scleroglucan synthesis

ferring the genetic determinants of polysaccharide synthesis to more amenable host organisms can improve the polysaccharide production.

Effect of composition of culture medium

The amount of carbon substrate converted by the cell to polymer depends upon the composition of growth medium and under certain conditions the product may not be produced at all. Generally, media containing high carbon to limiting nutrient ratio (often nitrogen) is favoured for polysaccharide production. Conversion of 60–80 % of the utilized carbon source into crude polymer is commonly found in high yielding polysaccharide fermentations. Care must be taken in the interpretation of such yields because crude products often contain cells, other organic materials and salts which are co-precipitated with the polymer when it is recovered from fermentation broth (*1*).

The optimal design of the medium is very important in the growth of microorganisms, stimulating the formation of products and providing the necessary energy for metabolic purposes. The nutrients required by a fungus include macronutrients such as carbon, oxygen, nitrogen, phosphorus, sulphur, potassium and magnesium, which comprise an average 98 % of dry cell mass of fungi.

Carbon source

Usually, glucose and sucrose are used as carbon sources for biopolymer production, although other carbohydrates can also be utilized. Most studies on scleroglucan report a glucose or sucrose concentration of either 30 g/L (*18*) or 35 g/L (*21*). Under these conditions, a maximum yield of 8.5–10 g/L was obtained for scleroglucan, which is much lower than the highest reported concentration of 27 g/L of xanthan, the main rival of scleroglucan. Sucrose concentrations above 45 g/L have been found to inhibit growth of *Sclerotium glucanicum*, and further limit the scleroglucan production (*30*). In contrast to this, Farina *et al*. (*19*) studied the effect of high sucrose concentrations on the scleroglucan production by *S. rolfsii* and concluded that an increase in sucrose led to a clear improvement in glucan yield. While only 7 g/L of scleroglucan were produced with 30 g/L of initial sucrose, a threefold increase (21 g/L) of product occurred when 150 g/L sucrose were supplied to the culture medium. Despite this improvement, residual sucrose at the end of fermentation was as high as 100 g/L, thus questioning the economic benefits of this strategy. Survase *et al.* (*22*) reported the maximum production of 16.5 g/L at a sucrose concentration of 80 g/L.

Nitrogen source

Nitrogen comprises 8–14 % of the dry cell mass of bacteria and fungi. It is a component of proteins and enzymes, and it is necessary in cell metabolism. A wide range of inorganic and organic compounds such as inorganic salts of NH_4^+ and NO_3^-, or more complex, natural products such as yeast extract, casein hydrolyzate, soya hydrolyzate and corn steep liquor can be utilized to satisfy the requirement of this element (*31*). Generally, the addition of extra nitrogen favours the biomass concentration, but diminishes glucan formation. In the case of a glucan such as pullulan, the production of polysaccharide is stimulated by depletion of nitrogen source. Similarly, high concentrations of nitrogen in the form of ammonium sulphate have been reported to reduce the scleroglucan production (*19*). Also, nitrate rather than ammonium sulphate give better glucan levels (*19,22*). Ammonium was reported to inhibit the glucan-synthesizing enzymes.

Miscellaneous

Phosphorus is an important element for secondary metabolism, and it also regulates lipid and carbohydrate uptake by the cells. Phosphate salts, such as K_2HPO_4 or KH_2PO_4, also serve as a pH buffer in the fermentation medium (*32,33*). Farina *et al.* (*19*) indicated an increase of total phosphorus from 0.12 to 0.28 g/L to improve scleroglucan production (from 4 to 5 g/L). Although a clear explanation for this was not given, it is possible that phosphorus might increase glucose uptake and metabolism.

Potassium is the principal inorganic cation in the cell; it is usually added as an inorganic salt (*e.g.* K_2SO_4, K_2HPO_4, or KH_2PO_4). Potassium is a cofactor for some enzymes, required in the carbohydrate metabolism and in many transport processes. Magnesium is also required by fungi, it functions as an enzyme cofactor, and is present in cell walls and membranes. It is usually supplied as $MgSO_4{\cdot}7H_2O$.

Pilz *et al.* (*34*) noticed that thiamine and zinc addition in a defined mineral medium could replace yeast extract. Their results indicate the importance of meeting the zinc requirement of the microorganisms. *S. rolfsii* needs Zn^{2+} for its primary metabolism and for the production of scleroglucan in a mineral medium.

Effect of precursors

Addition of precursor molecules is of considerable importance in the polysaccharide synthesis in terms of metabolic driving force. In case of polysaccharides, higher intracellular levels of nucleotide phosphate sugars under nitrogen-limited conditions enhance metabolite flux of exopolysaccharide synthesis. Higher intracellular levels of UMP under nitrogen-limited conditions enhance metabolite flux of curdlan synthesis in *Agrobacterium* species (*35*). Gellan precursors were detected by enzyme assays, and they were found to be nucleotide phosphate sugars (*36*). Amino acids have been used by some researchers as nitrogen source or as stimulator for improving biopolymer yields such as gellan gum production (*37*). Although there is inadequate information available on the use of amino acids as precursors for scleroglucan production, L-threonine was used in the optimized medium, but it did not show an increased yield (*19*). Survase *et al.* (*23*) used sugar nucleotides and amino acids and concluded that sugar nucleotides such as UMP, UDPG and amino acid such as L-lysine could serve as metabolic precursors for the scleroglucan production. Addition of precursors significantly improved the yield, but not the molecular mass of scleroglucan.

Effect of environmental factors

Microorganisms respond rapidly to environmental changes in many different ways, such as induction and repression of protein synthesis, change in cell morphology, and enzyme inhibition or stimulation. In a bioreactor, the main environmental parameters of interest are pH, temperature, dissolved oxygen, and stirrer speed.

The physiology of *Sclerotium glucanicum* has been studied by many people in order to understand how the microorganism functions and responds to the controlled environment, and to optimize process conditions for scleroglucan production.

Temperature

The internal temperature of the microorganism must be equal to that of its environment. Like many chemical reactions, the microbial activity is sensitive to environmental temperature. Temperature is crucial parameter that affects both culture growth and polysaccharide production. However, optimal temperature for exopolysaccharide production (20–37 °C) (*2*) is different from that for culture growth (28 ºC) (*15*). Below 28 °C, oxalic acid formation is enhanced, which has an adverse effect on scleroglucan production.

pH

pH influences the physiology of a microorganism significantly by affecting nutrient solubility and uptake, enzyme activity, cell membrane morphology, byproduct formation and oxidative reductive reactions. Culture pH can have profound effects on both the rate of production and the synthesis of polysaccharides. As with temperature, the appropriate pH for maximum production of the polysaccharide can differ from that for optimum growth. In case of xanthan production, Moraine and Rogovin (*38*) observed that culture pH influenced polysachharide production more than cell growth. Kang and Cottrell (*39*) reported fungal biopolymer synthesis to be optimal in the range of pH=4.0–5.5. Based on these observations, researchers have developed two-stage processes, with the first stage designed for optimal culture growth and the second stage for maximum polysaccharide production. Lacroix *et al.* (*40*) conducted fermentations for pullulan, another fungal glucan, wherein at the first stage, the pH of 2.0 was maintained for the best biomass and growth rate. Once high levels of biomass were achieved, the pH was adjusted to 5.5 for maximum pullulan production. In a similar mode, Wang and McNeil (*16*) reported an improved scleroglucan production *via* a two-stage process. In the first phase, pH was controlled at 3.5 for optimal growth, after which pH=4.5 was used to promote polysaccharide synthesis. The increased production of scleroglucan achieved under these conditions was combined with a 10 % reduction of byproduct formation. This probably indicates that at pH levels higher than those for optimal growth (4.5), carbon flux to biopolymer synthesis is increased. In some studies, in order to simplify the process and reduce the cost, pH was not controlled after an initial adjustment, but scleroglucan production was comparatively low. To keep an optimum pH for the high production of the polysaccharide, it is necessary to control the pH during fermentation, especially in a view of the fact that *S. rolfsii* responds differently to the changes in a single process variable depending on whether cultivation is carried in shake flasks or in stirred tank reactors.

Dissolved oxygen

Oxygen occupies a key role in the life cycle of aerobic microorganisms by inducing or repressing several enzyme systems of primary or secondary metabolism and enables oxidative reactions for nutrient utilization and energy generation. It can also have negative functions in cell metabolism such as production of peroxide and superoxide radicals. The effect of dissolved oxygen on biopolymer production by *S. glucanicum* and *S. rolfsii* was studied, and it was observed that while a high oxygen supply increased the cell growth, it decreased the glucan formation. However, in the production of some polysaccharides such as pullulan (*41*) and gellan (*42*), oxygen is both stimulatory and vital to polymer synthesis. In contrast, the *Sclerotium* culture responds to limited oxygen supply with limited growth and specific stimulation of glucan formation (*20,43*). This is unexpected of an aerobic microorganism, but it is possible that a reduction in dissolved oxygen affects the fungal morphology and broth rheology, which in turn affects cell growth and metabolism. Another reason could be enhanced respiration at high dissolved oxygen, thereby converting more carbon to carbon dioxide and reducing it for scleroglucan production (*44*). Wang and McNeil (*17*) suggested that the stimulation of glucan synthesis at low dissolved oxygen could be due to limited cell growth. Under these conditions, more carbon of the substrate was utilized for scleroglucan formation. In addition, low dissolved oxygen causes a decrease in byproduct production repressing the synthetic enzyme glycolate oxidase (*21*), thus favouring the flow of carbon towards glucan production.

Aeration and agitation

Aeration and agitation determine the availability of nutrients and dissolved oxygen to the cultures, and control the rate of metabolite release from the cells, including biopolymers, byproducts and carbon dioxide. In polysaccharide production the fermentation medium becomes very viscous and exhibits non-Newtonian (pseudoplastic) behaviour. With filamentous fungi, apart from the concentration of the biopolymer, biomass may also contribute significantly to broth rheology. These phenomena restrict mixing in the bioreactor, and change the culture morphology. At high rates fungal hyphae may become fragmented, reducing the viscosity of the broth. On the other hand, the formation of mycelial pellets may occur at low agitation rates.

Vigorous agitation and aeration are usually beneficial for polysaccharide production, although contradictory reports are also available. McNeil and Kristiansen (*45*) indicated that increased agitation improves glucan synthesis by *A. pullulans* as well as polymer quality (high molecular mass), and influences culture morphology by promoting the formation of yeast type (instead of filamentous) cells, which seems to produce more glucan. For *S. glucanicum*, a higher growth rate was achieved

with increased aeration rate at an agitation level of 600 rpm, but more glucan was produced as aeration was reduced (*42*). The same authors reported that at high stirring rates, mechanical degradation of glucan and cell damage may occur; but if the shear rate applied in the bioreactor is not adequate, only low molecular mass scleroglucan is released from the cells, while larger remain attached to the cell surface. Schilling *et al.* (*20*) confirmed that high stirring rates yield scleroglucan of low molecular mass as compared to that obtained after moderate agitation.

Other factors

Some operational strategies can influence the production of polysaccharides. Rau *et al.* (*43*) reported fed-batch cultivation to result in higher rates of glucan formation than batch process. Fed-batch cultivation had previously been reported by Shin *et al.* (*46*) for pullulan production, where the addition of part of the carbon source at a later stage could improve pullulan formation. Taurhesia and McNeil (*47*) found that in the production of scleroglucan by *S. glucanicum* the addition of sugar in the non-growth phase might enhance the polysaccharide formation. But, sometimes the single shot addition of the supplementary carbon source may risk the sudden increase of the broth sugar concentration, causing some degree of inhibition.

The growth of microorganism and the release of the metabolites may also be affected by other factors such as light, radiation, and hydrostatic pressure. Miller and Lierta (*48*) observed blue or white light to stimulate β-1,3-glucan accumulation in *S. rolfsii.*

Byproduct formation

Oxalic acid is the main byproduct in the scleroglucan production. Its production is undesirable. Oxalic acid is a common metabolic product of fungi, and its production is linked to the culture conditions and nutrient source. The amount of oxalic acid varies with the fungal isolate, the carbon and nitrogen source, the initial pH, and is also influenced by the presence of buffers or other chemicals in the growth medium. The most favourable conditions for the oxalate formation include high carbohydrate concentration and adequate aeration, limited supply of inorganic nutrients, and relatively high pH (*49,50*). Maxwell and Bateman (*49*) proposed the enzyme glyoxalate dehydrogenase to be involved in the biosynthesis of oxalate by *S. rolfsii*, and suggested pH of external medium to be a major factor determining oxalate accumulation. Production of oxalate is also strongly affected by culture medium constituents. The addition of L-threonine to the medium reduced the level of oxalic acid secreted. Oxalate formation also depends on the nature of the carbon source that is used in the medium. Maxwell and Bateman (*49*) also found no growth or oxalate accumulation to occur when D-gluconate, pyruvate, citrate, fumarate, glycolate, glyoxylate, L-aspartate, glycine or glycerol was used as sole source of carbon.

Many researchers reviewed mechanisms of oxalate biosynthesis by microorganisms. They concluded that oxalate biosynthesis probably occurs by a number of diverse pathways such as the hydrolytic cleavage of oxaloacetate to oxalate and acetate, or the oxidation of glyoxalate to oxalate. This pathway of oxalate formation is shown in Fig. 3 (*51*).

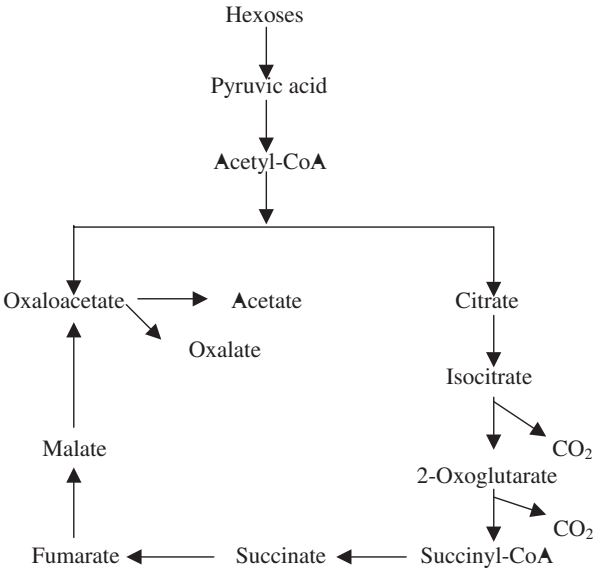

Fig. 3. Proposed route of glucose catabolism and oxalate synthesis in *S. rolfsii* (*51*)

Oxalate formation during fermentation presents a two-fold difficulty. First, it presents a diversion of carbon source away from exopolysaccharide formation; and second, there is a need to separate an oxalate from the product. Herve (*52*) investigated methods to decrease the oxalate formation, and found that adjusting the initial pH and controlling the addition of NH_4Cl during fermentation could reduce oxalic acid formation without reducing the yield. Low pH reportedly activates oxalate decarboxylase, which breaks down oxalate to formate and carbon dioxide. Schilling *et al.* (*21*) proposed low pH to reduce the synthetic enzymes, and hence recommended a pH of 2.0 to minimize carbon losses to byproducts. These authors also found oxygen limitation to decrease oxalate production, as the synthetic enzyme glycolate oxidase is repressed under anaerobic conditions and suggested that early low oxygen supply could be beneficial for glucan production.

Downstream Processing of Scleroglucan

Optimization of fermentation parameters alone is not enough to ensure a high yield of scleroglucan. The next crucial step after the completion of successful fermentation is the recovery of scleroglucan. The method used for recovery of the exopolysaccharide depends on characteristics of the producing organisms, the type of polysaccharide and desired grade of purity. Crude products may be obtained by drying entire fermentation broth. Unattached exopolysaccharide may be separated from the cells either by differential centrifugation or by filtration. Spray or drum drying or addition of water-miscible non-polar solvents such as acetone, ethanol, or isopropyl alcohol can precipitate a polymer and accomplish the removal of water. Often the addition of electro-

lytes helps in precipitation by neutralizing the charges on the polysaccharides. Recovery of solvents is essential for the economic reasons. If desired, the precipitate can be further purified by dissolving it in water and then dewatering, drying and milling (*31,53*).

There are three different methods of recovery reported in the literature, which are schematically shown in Fig 4. Pretreatment of fermentation broth in all the three is common. After obtaining the cell-free broth, the procedures for recovery differ. The common pretreatment scheme is as follows: fermentation broths are neutralized with NaOH or HCl, as required, diluted 3- to 4-fold with distilled water, heated at 80 °C for 30 min, homogenized and then centrifuged (10 000 × *g*, 30 min). The pellet so obtained is washed with distilled water and dried at 105 °C. The supernatant is then used for recovery of scleroglucan.

In the first method, the clear supernatant is cooled at 5 °C and precipitated by adding an equivalent volume of ethanol (96 %) or isopropanol. This mixture is allowed to stand at 5 °C for 8 h to complete exopolysaccharide precipitation, after which it is recovered with a fine sieve and then redissolved in distilled water. This crude exopolysaccharide can be purified twice by ethanol (96 %) reprecipitation. Finally, the precipitated polymer is either dried at 55 °C for 8 h or freeze-dried and milled to whitish glucan powder (*6*).

In the second method, divalent cations such as calcium, magnesium, manganese, iron, copper, cobalt and nickel with the water-miscible organic solvent are used. Calcium chloride at 0.5–2.0 % is the preferred divalent cation. Addition of calcium chloride results in insoluble precipitate of calcium oxalate, which is removed by centrifugation or filtration. A water miscible organic solvent, such as isopropyl alcohol or ethanol is then added to the solution at 20–40 %. The precipitate is separated by centrifugation or filtration. The polysaccharide can be further purified by rehydration and reprecipitation (*54*).

In the third method, recovery of glucan is done by employing 0.5–2.0 % calcium chloride, and then adjusting the solution to an alkaline pH by addition of metal hydroxides. Addition of calcium chloride precipitates the calcium oxalate, which is subsequently removed by centrifugation or filtration. Then the solution is made alkaline to about pH of 10–12 by addition of metal hydroxides such as sodium hydroxide or potassium hydroxide. The precipitated water-soluble polysaccharide is collected by centrifugation or filtration. The purity can be increased by repeated precipitation and varying the pH (*54*).

Applications of Scleroglucan

Oil industry

The initial application of scleroglucan was in the oil recovery, where it showed better stability than xanthan over a wide range of temperature and pH (*5,55*). In oil recovery, scleroglucan increases the viscosity, and hence the hydraulic pressure of sea water or brine used to extract oil. In watered-out reservoirs where sea water

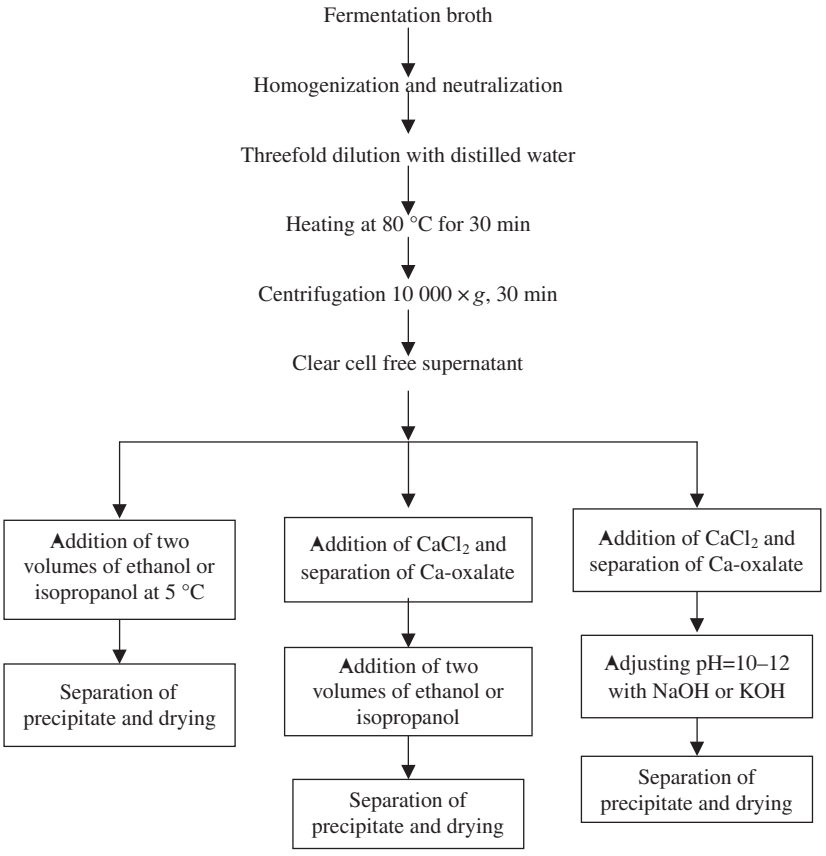

Fig. 4. Different recovery methods of scleroglucan from the fermentation broth

pressure is no longer sufficient to recover the oil, the addition of scleroglucan can improve the process significantly. In addition, scleroglucan (either in the crude form of dehydrated fermentation broth, or as clean, precipitated polymer) lubricates the drill and controls the backpressures created during drilling (*56*). Scleroglucan is very useful as an oil mud thickener and stabilizer; it increases the viscosity of very thin oil muds, which cannot otherwise be drilled. In comparison with xanthan, scleroglucan has several advantages, for example low sensitivity to shear stress and temperatures, which makes it suitable for drilling fluid applications. The rheology of scleroglucan-oil muds remains unchanged between 20 and 80 °C, and the scleroglucan-based system has higher tolerance to field contaminants (*57*).

Improved formulations of scleroglucan for the above uses have been developed. Zirconium citrate has been used as a deflocculant and dispersing agent in scleroglucan–oil muds, and was found to improve field performance by its thinning properties. It reduced mud dilution requirements, and improved the total fluid costs by up to 44 %. In addition, scleroglucan exhibited high thermostability in drilling fluids when it was associated with polyglycols through intermolecular interactions.

Food industry

The food industry worldwide uses 70 000 tonnes of polysaccharides per year as thickening agents, stabilizers and texturizers. As the emerging food products become more complex and diverse, the requirement for new and versatile additives is stronger. Presently, different polysaccharides are used to modify food viscosity and texture. Additionally, polysaccharide gums constitute non-fat alternatives that may serve as a source of soluble dietary fibre with beneficial health effects at quite low levels. They are currently obtained from plants (starch, cellulose, pectin, guar gum), seaweed and crustaceans (alginate, carrageenans, chitosan) or microbial sources (xanthan gum) but the exploration for novel candidates still continues (*58*).

Scleroglucan in food may be used as a thickener, gelling or stabilizing agent. However, xanthan has similar properties and applications, and at present it dominates the market. If the problem of high cost and low productivity of scleroglucan could be overcome, then it could replace xanthan in many foods such as jams and marmalades, soups, confectionery products and water-based gels, frozen foods, dairy products such as yogurt or ice-cream, low calorie or non-fat products, or in fabricated/structural foods (*2,7,59–64*). Scleroglucan could be especially useful in food manufacturing where a heating process is involved, because of thermal stability that it exhibits.

Vinarta *et al.* (*65*) investigated the ability of exopolysaccharides EPS I (after 48-hour cultivation) and EPS II (after 72-hour cultivation), produced by *Sclerotium rolfsii* ATCC 201126, to minimize the liquid separation (syneresis) that occurred during refrigeration of cooked starch pastes. After comparing different techniques, the extent of syneresis was finally estimated by daily measurements of the liquid phase length (Δh) separated above the sedimented phase throughout the storage at 5 °C. The degree of syneresis was represented by $\Delta h/h_0$, where h_0 stands for the initial height of the sample dispersion. Proportions varying between 9.90/0.10 and 9.00/1.00 mass ratio for 2 % mass per volume of corn starch/EPS aqueous blends were evaluated against 2 % mass per volume of corn starch (CS) as control. Up to 20 days of refrigeration and for the highest tested proportion (9.00/1.00), syneresis could be completely inhibited or reduced 91 % by EPS II and EPS I. EPS II was thereby selected as the optimal syneresis preventive and subsequent analysis of its rheological behaviour in distilled water, skimmed and whole milk confirmed the ability to increase viscosity with a non-Newtonian, pseudoplastic, behaviour. Rheology of CS/EPS II blends, when compared to the separated CS and EPS II, also evidenced a desirable synergistic effect in the aforementioned solvents, as witnessed by the increase in viscosity, higher consistency coefficients and lower flow behaviour indices. Additionally, EPS II was able to prevent syneresis without affecting pH, gelling properties, hardness or colour. These results revealed that scleroglucan might become a hydrocolloid approved in food with prospective use as food stabilizer and for prevention of water loss.

Immunostimulator and antiviral

Some complex polysaccharides stimulate immunity and increase resistance to neoplastic and/or microbial disease. These immunotherapeutics are classified as biological response modifiers (BRMs), and β-glucans are the most significant BRMs among the carbohydrate BRMs (*8*). Scleroglucan is more effective than other exopolysaccharides that also have antitumor activity. The antitumor activity of the scleroglucan may be *via* macrophage participation. Water-soluble β-glucans enhance both the number and function of macrophages. Scleroglucan has the effect of increasing macrophage function *in vivo*. Scleroglucan has higher immune stimulatory, antineoplastic, and antimicrobial activity than any other β-D-glucans (*66*). High molecular mass and the presence of (1,6)-β-D-glucosyl residue appear to be important for the immunostimulatory activity.

Scleroglucan also has antiviral effect. The mode of action against the herpes virus (*67*) and rubella virus (*68*) has been investigated. The binding of the polysaccharide on the host cell membrane may prevent or reduce the attachment and entry of the virus into the cell. This inhibitory effect occurs only at the early stages of infection. The key reaction seems to be the binding of scleroglucan with glycoproteins of the cell membrane, which impedes the interaction between the virus and the host cell plasma. Another possible explanation for antiviral activity can be that after the virus enters the cell, scleroglucan is also internalized in the cell and encapsulates the virus, thus inhibiting its activity. However, host cell penetration is unlikely for the case of high molecular mass polysaccharides such as scleroglucan.

Pharmaceutical industry

Pharmaceutical applications include the use in tablet coatings, ophthalmic solutions, injectable antibiotic suspensions and calamine lotion. Another important use of scleroglucan is in the form of carboxylated derivative

for use as a matrix for drug delivery in the form of tablets or films. For this purpose, hydrogels obtained by the crosslinking reaction between the polycarboxylated derivative of scleroglucan and alkane dihalides were evaluated for the diffusion experiments and water uptake (*69*). Here scleroglucan offers advantages of controlled release as well as compatibility, biodegradability, and bioadhesiveness and thermal and chemical stability (*70*). The peculiar physicochemical properties of scleroglucan suggested its suitability as a slow release matrix. Tablets prepared with the polymer show a remarkable swelling process that can slow down the diffusion of molecules previously loaded in the system. Furthermore, during the hydration process, the formation of a swelled layer slows down the penetration of the dissolution medium. This layer therefore represents the rate-limiting step of water penetration, which is very important for the release of model drugs. Coviello *et al.* (*71*) reviewed the use of scleroglucan and some derivatives in the field of pharmaceutics and in particular for the formulation of modified release dosage forms. The native scleroglucan can be used for the preparation of sustained release tablets (*72*) and ocular formulations; oxidized and crosslinked scleroglucan can be used as a matrix for dosage forms sensitive to environmental conditions (*73*); co-crosslinked scleroglucan/gellan can also be used for the drug delivery (*74*). Furthermore, a novel hydrogel obtained with this polysaccharide and borate ions is described for the controlled drug delivery (*75*).

Other applications

In cosmetic industry, scleroglucan applications may be used in the formulations for hair sprays and in various skin care preparations such as creams, protective lotions, emollients, demulcents and antisoilants (*2,76*).

In agriculture, scleroglucan is a useful antisettling agent for phytosanitary products; it facilitates the preparation of spraying mixtures and particularly improves the contact of the droplets sprayed onto leaves. It may also be used in pesticides, defoliant sprays and seed coatings (*5,7*).

Other suggested uses include porcelain and ceramic glazes, extruded refractory products, integrated circuit chips, water-based paints, printing inks, liquid animal feed concentrates, source of gentiobiose, and as a ceramic binder (*5,7,77*).

Conclusions

It is now clear that this polymer has received great attention from both oil and pharmaceutical industries. Continued research has also led to the formulation of many variations of the original product, thereby altering its properties and extending its applications. From the biotechnological and engineering perspective, the production process can be further improved by optimizing the fermentation conditions and by producing genetically engineered, highly productive mutants of *S. glucanicum* and *S. rolfsii* in order to achieve better yields and reduce the cost.

References

1. A. Margaritis, G.W. Pace: Microbial Polysaccharides. In: *Advances in Biotechnology, Vol. 2*, M. Moo-Young, C.W. Robinson (Eds.), Pergamon Press, New York, USA (1985) pp. 1005–1044.
2. F.E. Halleck, Polysaccharides and methods for the production thereof. *US patent 3,302,848* (1967).
3. P.A. Sandford, Extracellular microbial polysaccharides, *Adv. Carbohydr. Chem. Biochem. 36* (1979) 265–312.
4. T.E. Ouriaghli, J. Francois, D. Sarazin, N.T. Dinh, Influence of nonionic surfactant on aggregation state of scleroglucan in aqueous solution, *Carbohydr. Polym. 17* (1992) 305–312.
5. G. Brigand: Scleroglucan. In: *Industrial Gums*, Academic Press, New York, USA (1993) pp. 461–472.
6. J.I. Farina, F. Sineriz, O.E. Molina, N.I. Perotti, Isolation and physicochemical characterization of soluble scleroglucan from *Sclerotium rolfsii* – Rheological properties, molecular weight and conformational characteristics, *Carbohydr. Polym. 44* (2001) 41–50.
7. N.E. Rodgers: Scleroglucan. In: *Industrial Gums*, Academic Press, New York, USA (1973) pp. 499–511.
8. H.A. Pretus, H.E. Ensley, R.B. McNamee, E.L. Jones, I.W. Browder, D.L. Williams, Isolation, physicochemical characterization and preclinical efficacy evaluation of a soluble scleroglucan, *J. Pharmacol. Exp. Ther. 257* (1991) 500–510.
9. D. Lecacheux, Y. Mustiere, R. Panaras, Molecular weight of scleroglucan and other extracellular microbial polysaccharides by size exclusion chromatography and low angle laser scattering, *Carbohydr. Polym. 6* (1986) 477–492.
10. T.L. Bluhm, Y. Deslands, R.H. Marchessult, S. Perz, M. Rinaudo, Solid state and solution conformations of scleroglucan, *Carbohydr. Res. 100* (1982) 117–130.
11. I.A. Cottrel: Industrial Potential of Fungal and Bacterial Polysaccharides. In: *Fungal Polysaccharides, ACS Symposium Series, Vol. 126*, P.A. Sandford, I. Matsuda (Eds.), ACS, Washington, USA (1980) pp. 251–270.
12. R. Nardin, M. Vincendon, Isotopic exchange study of scleroglucan chain in solution, *Macromolecules, 22* (1989) 3551–3554.
13. P. Griminger, H. Fisher, Anti-hypercholesterolemic action of scleroglucan and pectin in chickens, *Proc. Soc. Exp. Biol. Med. 122* (1966) 551–553.
14. R.B. Ferguson, J.D. Westover, S. Paul, Fungal polysaccharide composition and method for making the same. *US patent 3,436,311* (1969).
15. Y. Wang, B. McNeil, Effect of temperature on scleroglucan synthesis and organic acid production by *Sclerotium glucanicum, Enzyme Microb. Technol. 17* (1995) 893–899.
16. Y. Wang, B. McNeil, pH effects on exopolysaccharide and oxalic acid production in cultures of *Sclerotium glucanicum, Enzyme Microb. Technol. 17* (1995) 124–130.
17. Y. Wang, B. McNeil, Dissolved oxygen and scleroglucan fermentation process, *Biotechnol. Lett. 17* (1995) 257–262.
18. Y. Wang, B. McNeil, Production of the fungal exopolysaccharide scleroglucan by cultivation of *Sclerotium glucanicum* in an airlift reactor with an external loop, *J. Chem. Technol. Biotechnol. 63* (1995) 215–222.
19. J.I. Farina, F. Sineriz, O.E. Molina, N.I. Perotti, High scleroglucan production by *Sclerotium rolfsii*: Influence of media composition, *Biotechnol. Lett. 20* (1998) 825–831.
20. B.M. Schilling, U. Rau, T. Maier, P. Fankhauser, Modeling and scale up of unsterile scleroglucan production process with *Sclerotium rolfsii* ATCC 15205, *Bioprocess Biosyst. Eng. 20* (1999) 195–201.
21. B.M. Schilling, A. Henning, U. Rau, Repression of oxalic acid biosynthesis in the unsterile scleroglucan production process with *Sclerotium rolfsii* ATCC 15205, *Bioprocess Biosyst. Eng. 22* (2000) 51–55.

22. S.A. Survase, P.S. Saudagar, R.S. Singhal, Production of scleroglucan from *Sclerotium rolfsii* MTCC 2156, *Bioresour. Technol. 97* (2006) 989–993.
23. S.A. Survase, P.S. Saudagar, R.S. Singhal, Enhanced production of scleroglucan by *Sclerotium rolfsii* MTCC 2156 by use of metabolic precursors, *Bioresour. Technol. 98* (2007) 410–415.
24. H.J. Willets: Sclerotium Formation. In: *The Filamentous Fungi, Vol. 3*, J.E. Smith, D.R. Berry (Eds.), Edward Arnold Publishers Ltd., London, UK (1978) pp. 197–221.
25. D. Backhouse, A. Stewart, Anatomy and histochemistry of resting and germinating sclerotia of *Sclerotium cepivorum*, *Trans. Brit. Mycol. Soc. 89* (1987) 561–567.
26. P. Rapp, 1,3-β-glucanase, 1,6-β-glucanase and β-glucosidase activities of *Sclerotium glucanicum:* Synthesis and properties, *J. Gen. Microbiol. 135* (1989) 2847–2858.
27. J.I. Farina, F. Sineriz, O.E. Molina, N.I. Perotti, Determination of radial growth rate of colonies of *Sclerotium rolfsii* F-6656 for the evaluation of culture medium, optimum incubation temperature, osmo- and halotolerance, *Rev. Arg. Microbiol. 28* (1996) 190–196.
28. I.W. Sutherland: Microbial Exopolysaccharide Synthesis. In: *Extracellular Microbial Polysaccharides*, P.A. Sanford, A. Laskin (Eds.), American Chemical Society, Washington, DC, USA (1977) pp. 40–57.
29. I.W. Sutherland: Biosynthesis of Extracellular Polysaccharides. In: *Industrial Gums*, R.L. Whistler, J.N. BeMiller (Eds.), Academic Press, Inc., San Diego, CA, USA (1993) pp. 69–85.
30. S. Taurhesia, B. McNeil, Physicochemical factors affecting formation of biological response modifier scleroglucan, *J. Chem. Technol. Biotechnol. 59* (1994) 157–163.
31. Y. Wang, B. McNeil, Scleroglucan, *Crit. Rev. Biotechnol. 16* (1996) 185–215.
32. H.C. Dube: Nutrition of Fungi. In: *An Introduction to Fungi*, H.C. Dube (Ed.), Vicks Publishing House Pvt. Ltd., India (1983) pp. 481–507.
33. G.M. Dunn: Nutritional Requirements of Microorganisms. In: *Comprehensive Biotechnology, Vol. 1*, M. Moo-Young (Ed.), Pergamon Press, Oxford, New York, USA (1985) pp. 113–125.
34. F. Pilz, G. Auling, D. Stephan, U. Rau, F. Wagner, A high-affinity Zn^{2+} uptake system controls growth and biosynthesis of an extracellular branched β-1,3-β-1,6-glucan in *Sclerotium rolfsii* ATCC 15205, *Exp. Mycol. 15* (1991) 181–192.
35. M.K. Kim, I.Y. Lee, J.H. Ko, Y.H. Rhee, Y.H. Park, Higher intracellular levels of uridine monophosphate under nitrogen-limited conditions enhance the metabolic flux of curdlan synthesis in *Agrobacterium* species, *Biotechnol. Bioeng. 62* (1998) 317–323.
36. I. Giavasis, L.M. Harvey, B. McNeil, Gellan gum, *Crit. Rev. Biotechnol. 20* (2000) 177–211.
37. K.M. Nampoothiri, R.R. Singhania, C. Sabarinath, A. Pandey, Fermentative production of gellan using *Sphingomonas paucimobilis*, *Process Biochem. 38* (2003) 1513–1519.
38. R.A. Moraine, P. Rogovin, Xanthan biopolymer production at increased concentration by pH control, *Biotechnol. Bioeng. 8* (1971) 381–391.
39. K.S. Kang, I.W. Cottrell: Polysaccharides. In: *Microbial Technology*, H.G. Peppler, D. Perlmen (Eds.), Academic Press, New York, USA (1979) pp. 417–481.
40. C. Lacroix, A. LeDuy, G. Noel, L. Choplin, Effect of pH on the batch fermentation of pullulan from sucrose medium, *Biotechnol. Bioeng. 27* (1985) 202–207.
41. D. Rho, A. Mulchandani, J.H.T. Luong, A. LeDuy, Oxygen requirement in pullulan fermentation, *Appl. Microbiol. Biotechnol. 28* (1988) 361–366.
42. E. Dreveton, M. Frederic, L. Jacqueline, B. Daniel, C. Lionen, Effect of mixing and mass transfer conditions on gellan production by *Auromonas elodea*, *J. Ferment. Bioeng. 77* (1994) 642–649.
43. U. Rau, E. Gura, E. Olezewski, F. Wagner, Enhanced glucan formation of filamentous fungi by effective mixing, oxygen limitation and fed batch processing, *Ind. Microbiol. 9* (1992) 19–26.
44. I.W. Sutherland: Microbial Polysaccharides: Control of Synthesis and Acylation. In: *Microbial Polysaccharides and Polysaccharases*, R.C.W. Berkeley, G.W. Gooday, D.C. Ellwood (Eds.), Academic Press, London, UK (1979) pp. 1–28.
45. B. McNeil, B. Kristiansen, Influence of impeller speed upon the pullulan fermentation, *Biotechnol. Lett. 9* (1987) 101–104.
46. Y.C. Shin, Y.K. Kim, H.S. Le, Y.N. Kin, S.M. Byun, Production of pullulan by a fed batch fermentation, *Biotechnol. Lett. 9* (1987) 621–624.
47. S. Taurhesia, B. McNeil, Production of scleroglucan by *Sclerotium glucanicum* in batch and supplemented batch cultures, *Enzyme Microb. Technol. 16* (1994) 223–228.
48. R.M. Miller, A.E. Lierta, Effects of light on acid-soluble polysaccharide accumulation in *S. rolfsii*, *Can. J. Microbiol. 22* (1976) 967–972.
49. D.P. Maxwell, D.F. Bateman, Influence of carbon source and pH on oxalate accumulation in culture filtrates of *S. rolfsii*, *Phytopathology, 58* (1968) 1351–1355.
50. Y. Wang, B. McNeil, Scleroglucan and oxalic acid formation by *Sclerotium glucanicum* in sucrose supplemented fermentation, *Biotechnol. Lett. 16* (1994) 605–610.
51. A. Hodgkinson: *Oxalic Acid in Biology and Medicine,* Academic Press, New York, USA (1977) pp. 1–129.
52. C. Herve, Microbial manufacture of polysaccharide. *Patent GB178437A* (1985).
53. I.H. Smith, W. Pace, Recovery of microbial polysaccharides, *J. Chem. Technol. Biotechnol. 32* (1982) 119–129.
54. S.S. Johal, G.M. Coleman, Recovery of glucan by employing a divalent cation at an alkaline pH. *US patent 4,950,749* (1990).
55. B. McNeil, L.M. Harvey, Viscous fermentation products, *Crit. Rev. Biotechnol. 13* (1993) 275–304.
56. S.A. Williams, Enhanced oil recovery. *US patent 3,373,810* (1968).
57. G. Holzworth, Xanthan and scleroglucan: Structure and use in enhanced oil recovery, *Dev. Ind. Microbiol. 26* (1985) 271–280.
58. E. Gimeno, C.I. Moraru, J.L. Kokini, Effect of xanthan gum and CMC on the structure and texture of corn flour pellets expanded by microwave heating, *Cereal Chem. 81* (2004) 100–107.
59. L.M. Kayser, Aerated food products. *US patent 3,495,990* (1979).
60. Improvement of frozen food quality with sclerogum, *Japanese Kokai Tokkyo Koho, 57,163,451*, San-Ei Chemical Industries Ltd., Osaka, Japan (1982a).
61. Improvement of quality of Japanese cake with sclerogum, *Japanese Kokai Tokkyo Koho, 57,163,442*, San-Ei Chemical Industries Ltd., Osaka, Japan (1982b).
62. Improvement of steamed food quality with sclerogum, *Japanese Kokai Tokkyo Koho, 163,163,441*, San-Ei Chemical Industries Ltd., Osaka, Japan (1982c).
63. Rice cracker quality improvement with sclerogum, *Japanese Kokai Tokkyo Koho, 57,163,440*, San-Ei Chemical Industries Ltd., Osaka, Japan (1982d).
64. Quality improvement of bakery products with sclerogum, *Japanese Kokai Tokkyo Koho, 57,163,432*, San-Ei Chemical Industries Ltd., Osaka, Japan (1982e).

65. S.C. Vinarta, O.E. Molina, L.I.C. Figueroa, J.I. Farina, A further insight into the practical applications of exopolysaccharides from *Sclerotium rolfsii*, *Food Hydrocolloids*, *20* (2006) 619–629.

66. P.P. Singh, R.L. Wisler, R. Tokuzen, W. Nakahara, Scleroglucan, an antitumor polysaccharide from *Sclerotium glucanicum*, *Carbohydr. Res. 37* (1974) 245–247.

67. M. Marchetti, S. Pisani, V. Petropaolo, L. Seganti, R. Nicoletti, A. Degener, N. Orsi, Antiviral effect of polysaccharide from *Sclerotium glucanicum* towards herpes simplex virus type I infection, *Planta Med. 62* (1996) 303–307.

68. P. Mastromarino, R. Petruzziello, S. Macchia, S. Rieti, R. Nicoletti, N. Orsi, Antiviral activity of natural and semisynthetic polysaccharides on early steps of rubella virus infection, *J. Antimicrob. Chemother. 39* (1997) 339–345.

69. T. Coviello, M. Grassi, G. Rambone, E. Santucchi, M. Carafa, E. Muratas, F. Ricciers, F. Alhaique, Novel hydrogel systems from scleroglucan: Synthesis and characterization, *J. Control. Release*, *60* (1999) 367–378.

70. P. Sheth, L. Lachman, The coating of tablets. *FR patent 1,480,874* (1967).

71. T. Coviello, A. Palleschi, P. Matricardi, G. Bocchinfuso, M. Grassi, F. Alhaique, Scleroglucan: A versatile polysaccharide for modified drug delivery, *Molecules*, *10* (2005) 6–33.

72. S. Rizk, C. Duru, D. Gaudy, M. Jacob, F. Ferrari, M. Bertoni, C. Caramella, Physico-chemical characterization and tabletting properties of scleroglucan, *Int. J. Pharm. 112* (1994) 125–130.

73. T. Coviello, M. Grassi, G. Rambone, F. Alhaique, A crosslinked system from scleroglucan derivative: Preparation and characterization, *Biomaterials*, *22* (2001) 1998–2004.

74. T. Coviello, M. Dentini, G. Rambone, P. Desideri, M. Carafa, E. Murtas, F.M. Riccieri, F. Alhaique, A novel co-crosslinked polysaccharide: Studies for a controlled delivery matrix, *J. Control. Release*, *55* (1998) 57–63.

75. T. Coviello, M. Grassi, R. Lapasin, A. Marino, F. Alhaique, Scleroglucan/borax: Characterization of a novel hydrogel system suitable for drug delivery, *Biomaterials*, *24* (2003) 2789–2796.

76. F.E. Halleck, Cosmetic composition employing water-soluble polysaccharide. *US patent 3,659,025* (1972).

77. F.E. Halleck, Paint composition containing polysaccharides. *US patent 3,447,940* (1969).