CHAPTER 2
REVIEW OF LITERATURE

In recent years, the increased demand for natural polymers for various industrial applications has led to an interest in exopolysaccharides or extracellular polymeric substances (EPS) production by microorganisms. This chapter deals with the review of pertinent literature on microbial production, isolation, purification, structural elucidation and applications of exopolysaccharide including welan gum in various industries.

2.1 Synthetic Polysaccharides

Synthetic polymers like nylon, polyethylene, and polyurethane have great impact on our daily life. But increased dependence on synthetic polymers has also generated environmental and human health concerns. Plastic materials derived from non-renewable resources are not biodegradable and remain in environment. Synthesis of some polymeric material involved the use of toxic compounds as well as toxic byproducts which has harmful effects on living system. Also monomeric units of polymers have many possibilities to combine because of the presence of hydroxyl groups of similar reactivity and glycosidic linkages to be formed stereospecifically (α- or β-anomers). Due to the complex structures, most of the polysaccharides have been found to be difficult to synthesize through conventional synthetic methods. Since, the stereospecificity and the regiospecificity of these products have found difficult to control and thus limit efficient chemical synthesis. In comparison to natural polysaccharides, the synthesis of synthetic polysaccharides also has other
disadvantages including high cost of the essential chemicals and their hazardous effects towards health and environment (Khan et al., 2007). These problems have diverted increased attention towards polymers derived from biological precursors or produced using biotechnological tools.

2.2 Natural Polysaccharides

Polysaccharides currently employed for industrial use were obtained from plants and seaweeds. Production of biopolymers from plants and seaweed origin suffers from certain geographical and climatic constraints, and has confined their production to certain parts of world only. Microbial synthesis of polysaccharides has recently emerged as an important source of novel biopolymers and considered economically competitive with natural gums produced from marine algae and other plants (Bajaj et al., 2007). Production of microbial polysaccharides starts by providing specific conditions without any restriction of regional and climatic conditions. Genetic engineering has been played very important role to alter the microbial exopolysaccharide production (Vartak et al., 1995; Fialho et al., 2008). The type of microorganism and its polysaccharide have been given in Table 2.1. The crucial properties possessed by microbial EPSs (dextran, xanthan, gellan, pullulan, yeast glucans and bacterial alginates) like viscofying, stabilizing and emulsifying agents have been well recognized in the food industry. Moreover microbial polysaccharides have the ability to replace as immunostimulatory, immunomodulatory, antitumor, antiviral, anti-inflammatory and antioxidant agents in various medical and pharmaceutical industries. Microbial EPSs have been more suited for EPS production than microalgae and plant (Wang et al., 2008; Ismail and Nampoorthiri, 2010; Liu et al., 2011).
Table 2.1 Types of microorganism producing polysaccharides

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Type of polysaccharide</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gram positive</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus polymyxa</em></td>
<td>Neutral polysaccharide</td>
<td>Madden <em>et al.</em> (1986)</td>
</tr>
<tr>
<td><em>Leuconostoc mesenteroides</em></td>
<td>Neutral polysaccharide</td>
<td>Sarwat <em>et al.</em> (2008)</td>
</tr>
<tr>
<td><em>Lactobacillus delbrueckii</em></td>
<td>Neutral polysaccharide</td>
<td>Grobben <em>et al.</em> (1997)</td>
</tr>
<tr>
<td>subsp. <em>Bulgaricus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Lactobacillus delbrueckii</em></td>
<td>Neutral polysaccharide</td>
<td>Sanchez-Medina <em>et al.</em> (2009)</td>
</tr>
<tr>
<td>subsp. <em>Bulgaricus</em> RR</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bifidobacterium adolescentis</em></td>
<td>Anionic polysaccharides</td>
<td>Hosono <em>et al.</em> (1997)</td>
</tr>
<tr>
<td><em>Bacillus sp. DP-152</em></td>
<td>Anionic polysaccharides</td>
<td>Suh <em>et al.</em> (1998)</td>
</tr>
<tr>
<td><strong>Gram negative</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Alcaligenes cupidus</em> KT201</td>
<td>Anionic polysaccharides</td>
<td>Toeda and Kurene (1991)</td>
</tr>
<tr>
<td><em>Xanthomonas campestris</em></td>
<td>Anionic polysaccharides</td>
<td>Tao <em>et al.</em> (2012)</td>
</tr>
<tr>
<td><em>Enterobacter sp. BY-29</em></td>
<td>Anionic polysaccharides</td>
<td>Yokoi <em>et al.</em> (1997)</td>
</tr>
<tr>
<td><em>Paecilomyces sp. 1-1</em></td>
<td>Cationic polysaccharide</td>
<td>Takagi and Kadowaki (1985)</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aurobasidium pullulans</em></td>
<td>Neutral polysaccharides</td>
<td>Jakovljevic <em>et al.</em> (2001)</td>
</tr>
<tr>
<td><em>Pestalotiopsis sp.</em></td>
<td>Anionic polysaccharides</td>
<td>Kwon <em>et al.</em> (1997)</td>
</tr>
<tr>
<td><em>Lentinus edodes</em></td>
<td>Cationic polysaccharide</td>
<td>Crestini <em>et al.</em> (1996)</td>
</tr>
<tr>
<td><em>Aspergillus sp. JS-42</em></td>
<td>Anionic polysaccharides</td>
<td>Nam <em>et al.</em> (1996)</td>
</tr>
<tr>
<td><em>Boletus aereus</em></td>
<td>Neutral polysaccharides</td>
<td>Zheng <em>et al.</em> (2014)</td>
</tr>
</tbody>
</table>
2.3 Exopolysaccharides

Microbial polymers are the polysaccharides synthesized by many microorganisms that either remain attached to the cell surface or are found in the extracellular medium in the form of amorphous slime (Banik et al., 2000; Nwodo et al., 2014). These polysaccharides are called as exopolysaccharides (EPS). The exopolysaccharides produced by many microorganisms were found beneficial for their adherence to solid surfaces, and surviving adverse conditions like protection against toxins and antibiotics, pathogenesis and symbiosis, protection from engulfment by predatory protozoa and white blood cells (phagocytes), protection from perennial effects of drying or desiccation in certain soil bacteria or from attack by the antimicrobial agents of plant or animal origin and the communal life of biofilm, cryoprotection for growth at low temperatures and high salinity with reference to sea ice microbial community and bacteria of other marine environments like antarctic and soda lakes among others (Gerba and Bitton, 1984; Cortes et al., 2002; Lin et al., 2003; Junge et al., 2004; Nichols et al., 2005; Dogsa et al., 2005; Lin et al., 2006; Singha, 2012; Donot et al., 2012; Prasad et al., 2014).

Technological progress has led to gain in knowledge about the usefulness of bacterial biopolymers to the human, consequently a numerous industrial and medical applications ensured. The large numbers of microbial EPSs have been studied over the last decades, to describe their structural composition, biosynthesis and functional properties, only some of them approved for commercial use (Freitas et al., 2011).

Fermentative production of biopolymers has a long history and Dextran was the first glycopolymer synthesized in 1880 and the prokaryote responsible for its production was identified as *Leuconostoc mesenteroides* (Linker and Jones, 1966; Rehm, 2010). EPS have played an extensive role as biopolymers in environment, by
replacing synthetic polymers, as they were considered degradable, nontoxic polymers and produced by microorganisms. Microbial polysaccharides have been classified into three groups, namely-the cell wall polysaccharides, the intercellular polysaccharides and the exocellular polysaccharides (Poli et al., 2011; Orsod et al., 2012; Mostefaoui et al., 2014).

First two groups (capsular polysaccharide) were the integral parts of cell and their extraction from cell biomass was difficult and uneconomical commercially. The exo-cellular polysaccharides translocate through cell wall into the cell culture medium making it slimy and viscous. These were easy to isolate from the culture media, free from protein and cell debris. Yeast glucan, was an example of capsular polysaccharide while xanthan, dextran, curdlan, welan and gellan were typical exocellular polysaccharides. Microbial polysaccharides could be considered either homopolysaccharide (e.g. pullulan, levan, curdlan, dextran and cellulose) or hetropolysaccharides (Table 2.2). The basic structure of exopolysaccharides included long-chain polysaccharides containing repeating units of sugars or sugar derivatives such as glucose, fructose, mannose and galactose etc (Ismail and Nampoothiri, 2010). The backbone chain could be linear or branched. As studied in previous reports, the structural composition and branching length affected the properties such as rheology of exopolysaccharides (Vincent et al., 2001; Tao et al., 2006; Surrenjav et al., 2006; Kreisman et al., 2007).

Most of the polysaccharides used in food have been derived from plant origin e.g. cellulose, starch, pectin, alginate and carrageenan. These were used in modified form either chemically or enzymatically to improve their rheological properties. Polysaccharides of microbial origin possessed unique rheological properties with
pseudoplastic nature and were effective at low concentrations (Kodali et al., 2009; Patel and Prejapati, 2013).

**Table 2.2 Types of microbial polymers containing heteropolysaccharides**

<table>
<thead>
<tr>
<th>Type</th>
<th>Microorganism</th>
<th>EPS structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial alginates</td>
<td>Algal</td>
<td>Copolymer rof (1-4)-linked β-D-mannuronic acid and its C(5) epimer, l-α-glucuronic acid</td>
<td>Crescenzi (1995)</td>
</tr>
<tr>
<td>Emulsion and related</td>
<td><em>Azotobacter</em> sp.</td>
<td>Mainly of rhamnose, mannose, glucose and glucuronic acid.</td>
<td>Sutherland (1990)</td>
</tr>
<tr>
<td>Emulsion and related</td>
<td><em>Pseudomonas</em> sp.</td>
<td>Several composed of D-galactosamine aminouronic acid and amino sugar, molecular mass about $5 \times 10^5$</td>
<td></td>
</tr>
<tr>
<td>Emulsion and related</td>
<td><em>Acinetobacter calcoaceticus</em></td>
<td>Monosaccharide of β-D-glucose, β-D-glucuronic acid and α-(1→4)-L rhamnose in molar ratios of 2:1:1, high molecular mass anionic polysaccharides</td>
<td>Banik et al. (2000)</td>
</tr>
<tr>
<td>Emulsion and related</td>
<td><em>Enterobacter aerogenes</em> type-54</td>
<td>Closely EPS from Klebsiella aerogenes type-54, composed of same tetrasaccharide unit</td>
<td>Sutherland (1990)</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Disaccharide unit, 1,4- linked disaccharide of D-glucuronosyl-1,3 β-N acetyl-D-glucosamine, high molecular mass size from 5000 to 20000000, insoluble water</td>
<td>Crescenzi (1995)</td>
</tr>
<tr>
<td>Rhizobium Heteroglycan</td>
<td><em>R. trifolii</em>, <em>R. meliloti</em> and <em>R. leguminosarum</em></td>
<td>Heteropolysaccharide of D-glucose, D-galactose and D-mannose in molar ratio 1:3:2, forming a hexasaccharide repeat unit, insoluble water</td>
<td>Sutherland (1996)</td>
</tr>
<tr>
<td>Xanthan</td>
<td><em>Xanthomonas campestris</em></td>
<td>Closely cellulose, the terminal β-D-mannosyl residue replaced by an L-rhamnosyl, high molecular mass about $4.7 \times 10^7$ Da</td>
<td>Sutherland (1996)</td>
</tr>
</tbody>
</table>
2.3.1 Commercial Exopolysaccharides and their Applications

Microbial exopolysaccharides have gained importance because of their potential applications in industrial sector. The exopolysaccharides from microbial source have important functional role because of their potential applications in food industries such as gelling agent, texturizers, viscosifiers, emulsifiers and water binding capacity to improve the texture, quality and functionality in different food preparations (Kodali et al., 2009; Patel and Prejapati, 2013) as given in Table 2.3. In food formulations, the functionality of polysaccharides usually included the prevention of phase separation in emulsions, modification of food texture/ mouthfeel, retardation of ice crystal formation in frozen foods and replacement of fats in reduced calorie products. The applications of different bacterial and fungal strains have been given in Table 2.3 and applications based on different properties have given in Figure 2.1.

Table 2.3 Applications of microbial exopolysaccharides in food industry

<table>
<thead>
<tr>
<th>EPS</th>
<th>Applications</th>
<th>Reference (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td>Yoghurt, icecream, bakery, pudding, desert gels</td>
<td>Brownlee et al. (2009)</td>
</tr>
<tr>
<td>Cellulose</td>
<td>Desserts, weight reduction thickners, artificial meat, sausage and meat casings</td>
<td>Bertocchi et al. (1997)</td>
</tr>
<tr>
<td>Curdlan</td>
<td>Heat-resistant gels, jellies, mayonnaises</td>
<td>Sutherland (1990)</td>
</tr>
<tr>
<td>Dextran</td>
<td>Baked foods, beverages, sweets, ice creams, fruit syrup</td>
<td>Whistler and Daniel (1990)</td>
</tr>
<tr>
<td>Gellan</td>
<td>Dairy products, Jams, Pie fillings and puddings</td>
<td>Bajaj et al. (2007)</td>
</tr>
<tr>
<td>Pullulan</td>
<td>Airtight and edible film or foils, food coatings</td>
<td>Lin et al. (2007)</td>
</tr>
<tr>
<td>Xanthan</td>
<td>Pudding, soft drinks, alcoholic beverages, fruit beverages, fruit juice, jellies, canned food</td>
<td>Sutherland (1998)</td>
</tr>
</tbody>
</table>
Figure 2.1 Applications of exopolysaccharides based on different properties
Typical examples of commercially important bacterial exopolysaccharides are presented below:

### 2.3.1.1 Dextran

Dextran was the first commercial microbial polysaccharide and approved for food applications by FDA (US Food and Drug Administration). Dextran was fermentatively produced from two specific strains *L. mesentecosides* and *L. dextranicum*. It was commercially used in confectionary to improve moisture retention, viscosity and inhibit sugar crystallization. In ice cream it has played a role as a crystallization inhibitor, and in pudding mixes it provided the desirable body and mouth feel. Now days, it has been commonly used as a blood plasma extender in blood transfusions. It has been reported that dextran (SephadexTm) was used as matrices in size-exclusion chromatography and also used in the gel filtration columns (Rehm, 2010; Patel *et al.*, 2011; Freitas *et al.*, 2011; Donot *et al.*, 2012; Prajapati *et al.*, 2013).

### 2.3.1.2 Xanthan Gum

The polysaccharide B-1459, or xanthan gum, a commercial exopolysaccharide fermentatively produced from *Xanthomonas campestris* NRRL B-1459. It was discovered in the 1950s at the Northern Regional Research Laboratories (NRRL) of the United States Department of Agriculture (Margaritis and Zajic, 1978). It was extensively studied because of its properties that would allow it to supplement other known natural and synthetic water-soluble gums. Today, the commercial producers of xanthan are Merck and Pfizer the United States, RhoAne Poulenc and Sanofi-Elf in France, and Jungbunzlauer in Austria. Xanthan’s primary structure consisted of repeated pentasaccharide units formed by two glucose units, two mannose units, and one
glucuronic acid unit, in the molar ratio 2.8:2.0:2.0. Xanthan’s backbone consisted of (1→4) β-D-glucopyranosyl units (Garcia-Ochoa et al., 2000). Because of its unique structure xanthan gum exhibited marked rheological properties, high solubility, and enhanced stability over a wide range of pH values and temperature, as well as compatibility with many salts, food ingredients and other polysaccharides used as thickening agents. These properties of xanthan gum, contributed to its use in a wide range of applications especially in the food industry as a thickening and stabilizing agent, in cosmetics, in the paper milling, textiles and the pharmaceutical sector and also used in enhancing oil recovery (Davidson, 1978; Donot et al., 2012; Prajapati et al., 2013; Kalogiannis et al., 2003).

2.3.1.3 Curdlan

Curdlan, discovered by Harada et al. (1966) was considered an extracellular polysaccharide produced by *Alcaligenes* sp. and *Agrobacterium* sp. It has been given its name curdlan because of its ability to “curdle” when heated. Curdlan was characterized by repeating glucose subunits joined by a β-linkage between the first and third carbon of the glucose rings (Patel and Patel, 2011). Takeda Chemical Ind., Ltd. (Osaka, Japan) in 1989, supplied commercialized curdlan powder for food industry (Miwa et al., 1993). It could be used as food additive to improve, modify and stabilize the physical properties of the products like texture modifier in noodles and fish paste products such as kamaboko, Water-holding agent in meat products such as sausages, hams and hamburgers, etc. As an food ingredient to develop the new products like noodle-shaped tofu; tofu for retorting, freezing or freeze-drying; frozen noodle-shaped konjac-like gel food; low-fat sausage (coarse-cut), substituting curdlan gel containing vegetable oil for pork fat; non-fat
whipped cream (analogue), as a milk fat substitute etc. (Divyasri et al., 2014; Poli et al., 2011).

2.3.1.4 Cellulose

*Acetobacter xylinum* strains were well known for oxidizing alcohols to acids and ketones, especially for the production of vinegars. Cellulose pellicle was produced on the upper surface of the supernatant film during the process. Production of cellulose by green algae (*Valonia*) and some bacteria, principally of the genera *Acetobacter*, *Sarcina* and *Agrobacterium* has been reported (Wielgus et al., 2012).

Due to its high purity, hydrophilicity, structure forming potential, chirality and biocompatibility, it was used as thickener to maintain viscosity in food, cosmetics, as food additives and others. It has several important applications in human and veterinary medicine such as use of cellulose films as a temporary substitute for human skin in the case of burns, ulcers, decubitus and others. Microbial cellulose products such as Biofill®, Bioprocess® and Gengiflex® have been used for its applications in surgery and dental implants (Jonas and Farah, 1998; Keshk, 2014).

2.3.1.5 Sphingans

Sphingans were considered as a group of series of structurally closely related exopolysaccharides secreted by members of genus *Sphingomonas* (Fialho et al., 2008). Sphingan group has similar backbone structure but differs in location of side chains. This group has in common linear tetrasaccharide backbone structure (-X-glucose-glucuronic acid-X, where X is either L-rhamnose or L-mannose) to which distinct side groups have attached (Pollock, 1993). The presence of distinct side group provided them different physical properties. The Sphingan group included the following bacterium (and
exopolysaccharide secreted by them) as such: *Sphingomonas paucimobilis* ATCC 31461 (gellan), *Sphingomonas* sp. ATCC 31555 (welan), *Sphingomonas* sp. ATCC 31961 (rhamson), *Sphingomonas* sp. ATCC 53159 (diutan), *Sphingomonas* sp. ATCC 53154 (S-88), *Sphingomonas* sp. ATCC 31853 (S-198), *Sphingomonas* sp. ATCC 21423 (S-7).

This group was named as sphingan group based on the presence of glycosphingolipids in their outer membranes instead of lipopolysaccharide as in Gram-negative bacteria (Kawasaki *et al*., 1994). Among these biopolymers, currently more attention has been paid on welan gum because of its recent addition to the group of exopolysaccharide known as sphingan group with novel properties and potential applications.

### 2.3.1.5.1 Gellan Gum

*Sphingomonas elodea* (ATCC 31461) previously referred as *Pseudomonas elodea* was reported to produce fermentatively commercial gellan gum. The polymer was discovered in the laboratory of the Kelco Division of Merck and Co., California, USA in 1978 and had previously been referred to by the code names S-60 or PS-60. It was consisted of repeating unit of β-D-glucose (D-Glc), L-rhamnose (L-Rha), and D-glucuronic acid (D-GlcA). The US FDA approved gellan gum for use as food additive in 1992 (Pszczoła, 1993). Gellan gum has applications in diverse fields such as, in microbiological media, tissue-culture media, foods and pet foods, deodorant gels, films and coatings and capsules, bakery products, photographic emulsions and microcapsules (Fialho *et al*., 2008; Prajapati *et al*., 2013).

### 2.3.1.5.2 Welan Gum

Welan gum was produced by *Sphingomonas* sp. ATCC 31555 (also known as *Alcaligenes* sp. ATCC 31555) a Gram-negative microorganism (Kang and Veeder, 1982;
O’Neill et al., 1986; Wei et al., 2012). It was structurally (Figure 2.2) similar to gellan which has the same tetrasaccharide repeating units, except the side chains (Jansson et al., 1985). Both the gellan and welan gum have different aqueous solution properties. Gellan reported to form gel in aqueous solution whereas welan did not form gel (Sandford et al., 1984). Welan gum has been considered as an anionic polysaccharide and therefore, has polyelectrolyte properties due to the presence of D-glucuronic acid in its chemical structure.

![Diagram of tetrasaccharide backbone structure of sphingan family showing different sidechains and their linkage positions for gellan, welan, rhamsan, diutan](image)

<table>
<thead>
<tr>
<th>Name of sphingan</th>
<th>$R_1$</th>
<th>$R_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gellan</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Welan</td>
<td>H</td>
<td>$\leftarrow HC_{1}$-$\alpha$-L-Rha or $\leftarrow HC_{1}$-$\alpha$-L-Manp</td>
</tr>
<tr>
<td>Rhamsan</td>
<td>$\leftarrow HC_{1}$-$\alpha$-GlcP-$\leftarrow HC_{1}$-$\beta$-GlcP</td>
<td>H</td>
</tr>
<tr>
<td>Diutan</td>
<td>H</td>
<td>$\leftarrow HC_{1}$-$\alpha$-L-Rha-$\leftarrow HC_{1}$-$\alpha$-L-Rha</td>
</tr>
</tbody>
</table>

**Figure 2.2 Basic tetrasaccharide backbone structure of sphingan family showing different sidechains and their linkage positions for gellan, welan, rhamsan, diutan**

Welan gum consisted of an acidic hetero-polysaccharide having approximate molecular weight (MW) $1.0 \times 10^6$ g/mol (Kang and Veeder, 1982; Lopes et al., 1995; Vandamme et al., 2002; Plank et al., 2010). The extracellular polysaccharide welan gum contained of L-mannose, L-rhamnose, D-glucose, and D-glucuronic acid in the molar
ratios 1.0:4.5:3.1:2.3. The structure has been determined by partial acid hydrolysis and base-catalyzed β-elimination to form a series of oligosaccharides. These oligosaccharides were isolated as their alkylated alditol derivatives by reverse-phase HPLC and characterized by fast atomic bombardment mass spectrometry (FABMS), proton nuclear magnetic resonance (1H NMR) spectroscopy and gas liquid chromatography (GLC), resulting in a structure with D-glucose, D-glucuronic acid and L-rhamnose units with singular side chains containing either L-rhamnose or L-mannose substituted on C3 of every 1,4-linked glucose repeating unit (O’Neill et al., 1986; Jansson et al., 1985; Kumar et al., 1996; Lapasin and Pricl, 1999).

Although it has been several years since welan gum was first discovered, the process for the production has not been thoroughly studied. Many researchers have focused their efforts on the structure and characteristics of welan gum (given in section 2.3.1.5.2.1) as well as the development of its applications (Lachemi et al., 2004). Welan gum has commercial application in the area of construction using cement systems and also has potential applications in oil-well drilling (Kang and Veeder, 1982; Talashek, 1992; Khayat and Saric-Coric, 2000; Li et al., 2010).

Microbial exopolysaccharides such as dextrans, xanthan, gellan, pullulan, are potentially used in food industries as food additive (Wang et al., 2008). Although welan gum have same backbone structure as that of gellan gum with difference in single monomer unit, the work on its application in food industry has not been focused.

2.3.1.5.2.1 Conformational Characterization of Welan Gum

Biopolymers namely, gellan, welan, and rhamsan, share the same four-sugar backbone repeating-unit. Circular-dichroism spectra of welan gum (S-130) (in water)
suggested that it might be present in a stiff conformation similar to that assumed by
gellan gum (S-60) in aqueous Me₄NCl (Crescenzi et al., 1987). Dilute aqueous solution
behavior of gellan gum (S-60) and welan gum (S-130) were compared (Crescenzi et al.,
1986). Gellan gum was found to be unbranched, whereas welan and rhamsan displayed
comb like branching. The effect of chain branching on backbone conformation of these
polymers (gellan, welan, rhamsan) was reported (Talashek and Brant, 1987). The range of
conformational freedom of the welan backbone (in comparison to gellan) was limited by
Van der Waals’ repulsive interactions of side chain and backbone. The differences in the
physical properties of these polymers were not only of Van der Waals’ interaction but
also might be due to other contributions to the side chain-backbone interaction, e.g.,
hydrogen bonding. S-130 (welan) and S-657 (diutan) displayed similar behavior in dilute
aqueous solution (Urbani and Brant, 1989). X-Ray fiber diffraction analysis and computer
modeling of welan, S-657, and rhamsan showed half-staggered, double-helical
conformations as in case of gellan gum (Lee and Chandrasekaran, 1991). The presence of
side chains was considered responsible for diminishing gelling behavior of welan, S-657,
and rhamsan. Flow behavior and dynamic visco-elasticity properties of welan gum and its
rheological properties with respect to its association characteristic were studied and
comparison was made with deacylated gellan gum by Tako and Kiriaki (1990).
Correlative studies on the primary and secondary structures of gellan, welan, rhamsan,
and succinoglucon in solution (dilute and semidilute) with their rheological properties has
been reported. Chain conformation ranged from random coil (galactoglucon) to that of
the stiff double helix of gellan, welan and rhamsan in dilute solution was reported by
Cesaro et al. (1992). The rheological behavior of the above microbial polysaccharides was mainly determined by their conformation in solution.

In aqueous solution welan showed “weak gel” properties similar to xanthan. When the polymer was dissolved in dimethyl sulphoxide (DMSO), it showed total loss of gel behavior. The polymer showed typical order-disorder and disorder-order transitions in DMSO. The native structure of welan undergoes dissociation in DMSO, which on rapid renaturation from the disordered state gave shorter helices, and formed a stable cross-linked network. Disordered welan on addition of salt caused rapid re-ordering and resulted in the development of a ‘true’ gel network by cation-mediated helix-helix aggregation (Hember et al., 1994; Member and Morris, 1995; Morris et al., 1996). On exposure to excess water, the gels swelled but remained intact. The effect of dimethyl sulfoxide on the conformation of gellan, welan and rhamsan by circular dichroism (CD) was demonstrated by Edward and Eugene (1996), which was previously reported on the basis of light-scattering and viscosity studies.

A comparison has been made among the stiffness parameters. The reports on the weak polyelectrolyte character and high stiffness of the welan chain agreed with the previous investigation. Stoke et al. (1986) also revealed stiffness in the chains of polysaccharides (welan and xanthan) of their solutions using electron microscopy investigation. Due to the presence of D-glucuronosyl residues in its primary structure, welan gum has presented polyelectrolyte properties and high stiffness (Filho et al., 1996; Campana et al., 1990).
2.3.2 Production of Exopolysaccharides

The environment has contained an enormous variety of microorganisms in various ecological sources, from which EPS producing microorganisms could be isolated. The sources like effluents from the sugar, paper or food industries as well as wastewater plants, with high carbon/nitrogen ratio were known to support the growth of microorganisms (Morin, 1998; Singha, 2012). In general, the environment that offers high amount of organic substances is the main source of EPS producing bacteria.

Fermentation is a very important process technology for manufacturing value added products such as microbial biopolymers. Various factors influenced the production of EPS were the composition of the medium, especially carbon and nitrogen sources, and conditions like pH, temperature, and incubation time, agitation (Sutherland, 2007; Nicolaus et al., 2010; Kazak et al., 2010). Based on fermentation conditions maintained, type of strain used, influenced the chemical structural composition, physicochemical and rheological properties of the final product. Therefore control of fermentation conditions, using feasible substrate, and high-level producer strains are crucial from industrial point of view to obtain product with desired specifications.

In fermentation process, proper growth and cells to be reproductive, intake of nutrients is necessary to assemble membranes, proteins, cell walls, chromosomes and other components. The formulation of fermentation medium with optimized carbon and nitrogen source could improve the yield of EPS. Carbon source have great influence on chemical composition and yield of EPS. Some studies conducted by Breedveld (1993) reported that EPS composition remained same with different carbon substrate utilization while others like Cerning (1994) reported that EPS composition could change with
different carbon sources. EPS production by *Lactobacillus delbrueckii* subsp. *bulgaricus* (B3, G12) and *Streptococcus thermophilus* (W22) in the medium containing various carbon sources (glucose, sucrose, fructose, or lactose) were studied by Yuksektdag and Aslim (2008) reported that glucose was the most efficient carbon source and its higher concentration stimulated the EPS production and growth.

Similarly EPS yield was reported to vary based upon the nitrogen source (Datta and Basu, 1999). In one study different carbon sources (glucose, sucrose, maltose, lactose, glycerol and Corn Starch) and nitrogen sources (peptone, yeast extract, defatted soybean powder (DSP), cottonseed cake flour (CCF), ammonium nitrate and ammonium sulfate) have been used for the production of exopolysaccharide using *Alcaligenes faecalis* NX-3. The maximum production was reported in a medium containing optimal combinations of high carbon to low nitrogen content (Li et al., 2010).

Different microbial strains differ in their responses to the effect of change in environment. During fermentation, the limitation of the nitrogen, phosphorus or sulphur source in the presence of excess carbohydrate might have lead to an increased production of EPS, but the yield has been seen to be affected by the oxygen, pH and temperature requirements. Strains also differ in carbon source utilization, mineral requirements and temperature optima for exopolysaccharide synthesis (Patel et al., 2010). The effect of temperature (-2 °C, 10 °C, and 20 °C) on production of EPS in batch culture by CAM025 (a marine bacterium isolated from sea ice) has been reported where the yield of EPS at -2 °C and 10 °C was 30 times higher than at 20 °C (Nichols et al., 2005).

The synthesis of EPS and its molecular weight was reported to be affected by rate of aeration during the fermentation process. With the progress of fermentation process, the
broth viscosity increased due to the extracellular accretion of biopolymer, resulting in the significant decrease in oxygen mass transfer rate. The oxygen mass transfer was considered essential for the growth of microorganisms. The unavailability of oxygen might affect the functioning of enzyme causing decrease in the biosynthesis of desired product or even death of microorganism (Li et al., 2011).

2.3.3 Exopolysaccharide Extraction and Purification

2.3.3.1 Extraction

The extraction of extracellular microbial polysaccharides from the fermentation broth could be achieved by following: (i) cell removal, usually achieved by centrifugation or filtration; (ii) polymer precipitation from the cell-free supernatant by the addition of a precipitating agent that consisted of a water-miscible solvent in which the polymer was insoluble (e.g. methanol, ethanol, isopropanol or acetone); and (iii) drying of the precipitated polymer, namely by freeze drying (laboratory scale) or drum drying (industrial scale) (Hebber et al., 1992; Rosalam and England, 2006; Bajaj et al., 2007; Pena et al., 2008; Freitas et al., 2009b).

The extraction method depended on the nature and viscosity of exopolysaccharide. Removal of cell biomass using centrifugation from clear growth medium could be easy but highly viscous culture medium cause hindrance in cell deposition. The EPS was separated with ease using high speed centrifugation (Brown and Lester, 1980; Cerning, 1990). While for extraction of EPS at laboratory scale, ultracentrifugation could be used to separate most of the cell debris from the culture medium (Morin, 1998). Also for the separation of capsular EPS, dissociation of EPS from the
cells was required. The separation could be achieved depending on the nature of the association between the cells and the polysaccharides. The centrifugation could separate cell in weakly associated capsular EPS and for tightly associated capsular EPS with the cells required alkaline treatment using sodium hydroxide before centrifugation and alcohol precipitation (Jansson et al., 1985). The alkaline treatment caused charged groups to be ionized because their isoelectric points were generally lies in the range of 4-6. Another procedure involved heat treatment (upto 80-95 °C) of the fermentation broth at the end of the fermentation process, before cell removal (Nielsen and Jahn, 1999; Bajaj et al., 2007). Heat treatment step helped to kill the bacterial cells and inactivation of enzymes and thus could prevent polymer degradation in following steps. It also reduced the broth viscosity.

### 2.3.3.2 Purification

During the fermentation process, large range of low-molecular-weight compounds have been reported to be added or co-produced, which caused impurities (such as cell debris, salts and proteins) in the desired product (Kumar et al., 2007; Wang et al., 2007; Freits et al., 2009b). To purify the EPS, the following processes could be used: (i) re-precipitation of the polymer from diluted aqueous solution (<1.0 g/L); (ii) chemical deproteinization (salting out or protein precipitation with trichloroacetic acid) (Yang et al., 1999; Ayala-Hernandez, 2008) or enzymatic methods such as; use of proteases (Wang et al., 2007); and (iii) membrane processes (e.g. ultrafiltration and diafiltration).

Crude polysaccharide solution could be purified by treating with trichloroacetic acid for precipitation of proteins and centrifuged to obtain clear solution. Sevag method, involved the addition of chloroform and n-butanol into crude polysaccharide solution and
separating out the denatured protein from the junction of the water and solvent layer formed in the separating funnel (Feng et al., 2010).

In all the methods described by previous researchers (Abu et al., 1991; Vincent et al., 1994; Moriello et al., 2003), the bacterial cells were pelleted by centrifugation and the EPS in the supernatant was precipitated by addition of 95% ethanol. For further purification and characterization of the EPS, generally gel filtration chromatography and GC analysis were used.

2.3.4 Fermentation Kinetics

It is difficult to know the actual behavior of particular fermentation process as it is very complex system. The development of kinetic models is crucial for understanding, controlling, and optimizing fermentation processes. A model can determine the relationship between the key state variables of the fermentation system while explaining the later quantitatively. The information gained using kinetic models along with designed experiments can aid bioengineers to design and control microbial processes.

Two classes of fermentation models were usually followed: structured model where intracellular metabolic pathways were considered and unstructured models where the biomass was described by one variable. Applicability of structured models needed the basic information about cell structure, their function and composition whereas the unstructured models considered only total cellular concentration instead of physiological characterization of the cells. It has been reported that the former was much complicated for normal use than the later which was easier to use and had been applied to describe
various fermentation processes (Jian-Zhong et al., 2002; Wang et al., 2006; Baei et al., 2008).

Literature on fermentation variables of welan gum production is fragmentary, however many literature on EPS production has been documented. Gellan gum produced by *Sphingomonas paucimobilis* ATCC 31461 and kinetics of microbial growth, substrate consumption, and product formation has been reported. The production of gellan gum has been found to be largely growth associated in its batch fermentation process (Wang et al., 2006). It could be inferred that factors or process parameters that could enhance cell growth should enhance the production of gellan gum.

Kinetics of microbial growth, substrate consumption, and product formation has been evaluated using *Bacillus flexus* (Divyashree et al., 2009). The polyhydroxyalkanoates (product) formation process has been demonstrated with development of a simple kinetic model and modification of the logistic equation. The microbial growth was described by logistic equation. A simplified Monod's model as well as modification of the logistic equation for growth kinetics and Luedeking-Piret type model has been employed to predict product kinetics.

The kinetics of *Bifidobacterium animalis* subsp. *lactis* Bb 12 has been examined in uncontrolled batch fermentation (Jalili et al., 2010). The Monod and the Luedeking and Piret equations have been applied in terms of product inhibition which included toxic power terms. It showed the effect of media and coating materials on toxic power terms.

Gilani et al. (2011) also studied the growth kinetic parameters using unstructured model. Based on the Malthus and Logistic rate equations, the parameters like maximum specific growth rate (\(\mu_{\text{max}}\)), initial cell dry weight has been defined. The Luedeking-Piret
and Modified Luedeking-Piret models have been applied to predict the product formation and substrate consumption rates. Xanthan gum production and glucose consumption in the fermentation kinetic studies were found to be growth associated. Similarly, kinetic analysis for cell growth and polyhydroxyalkanoates production by *Azotobacter Beijerinckii* DSMZ 1041 has been reported by using Logistic model, Malthus model and Luediking-Piret (Pirouz *et al*., 2011).

*Gandoderma tsugae*, a medicinal mushroom has been used for exopolysaccharide production (Narkprasom *et al*., 2012). The biomass formation and exopolysaccharide production has been determined by using mathematical relationship (cube-root equation and Luedekin-Piret equation respectively), which was considered useful to control the fermentation process.

### 2.3.5 Exopolysaccharide Properties

Microbial polysaccharides possessed unique/superior physical properties and stand out from traditional plant polysaccharides (Sutherland, 1998). Apart from their intrinsic biodegradability, non-toxicity and biocompatibility characteristics, this type of natural polysaccharides can possess many properties that could be broadly used in industrial applications: as emulsion stabilizing and gelling agents in food products; as foam stabilizing agents in the beverage industry and as inhibitors of crystal formation (Kumar *et al*., 2007). Existing and new ingredients including EPS have been regularly incorporated into food systems to improve their rheological, physicochemical and nutritional properties.
2.3.5.1 Rheological Characterization

Fluid is a substance that undergoes continuous deformation when force is applied. It is generally classified as Newtonian (shear rate independent) or Non-Newtonian (shear rate dependent). An accurate knowledge of the rheological behavior of fluids is important in engineering design to determine their ability to perform certain functions. In food applications, understanding the rheology is crucial in optimizing product development efforts, processing methodology and final product quality.

Aqueous solutions of polysaccharides could be characterized as Newtonian or non-newtonian fluids (Verbeeten, 2010). Without any applied force, the EPS in the aqueous media were found to be randomly arranged and present no resistance. But, when a unidirectional shear stress was applied, an initial resistance could be observed, before the fluid starts to flow. Hence, when the biomolecules were tend to move, these entangle themselves with each other, resulting in an enhanced resistance to flow (McNeil et al., 1993; Gibbs et al., 2000). Depending on the imposed degree of shearing, the relationship between shear stress and shear rate was not found constant, (Seviuor et al., 2011).

Regarding shear stress, shear-thinning fluids appeared to be thinner (exhibiting lower viscosity) at higher shear stresses due to the increased alignment of the molecules, while at low shear stresses, they appeared to be thicker (exhibiting higher viscosity) as a result of the entanglements between their molecules. Shear-thinning fluids constituted the largest and probably most important class of Non-Newtonian fluids (Cross, 1965).

The flow behavior (in terms of viscosity, yield stress and thixotropy) of semi-dilute solutions of hydroxypropyl guar gum (HPGG), a derivative of naturally occurring guar galactomannan, was investigated showing shear-dependent viscosity behaviour
under various HPGG concentrations, added salts and temperature has been described by
the Cross viscosity model (Zhang et al., 2007). The power law function demonstrated
change in zero-shear-rate viscosity with HPGG concentration and decreased with the
increase of temperature according to an Arrhenius-type equation. Added salts (KCl and
CaCl₂) have lowered the zero-shear-rate viscosity and thixotropic property.

A range of microbial polysaccharides (exopolysaccharides) showed high water
solubility, producing aqueous solutions with interesting rheological properties that could
be used as viscosifying, thickening, stabilizing and/or gelling agents in several
applications. Exopolysaccharides are the natural polymers and have numerous
applications in industrial areas due to their rheological properties that allow the formation
of viscous solutions at low (0.05-1%) concentrations (Freitas et al., 2009a). The type of
EPS produced in the yoghurt has been known to influence the yoghurt texture, and also
some mechanisms involving it’s interaction with milk proteins has been proposed. The
study on the rheological properties of EPS produced by yoghurt cultures (S.
thermophilus) has been reported by Purwandari et al. (2010). Such information helped in
predicting possible interactions of EPS with milk component(s) that would influence the
texture of yoghurt.

Hydrocolloids have the property to prevent phase separation and could be added
as an ingredient in food preparation to improve the food attributes. The hydrocolloids,
xanthan and guar gum (0.3, 0.7 and 1.0%; w/w) have been added to yellow passion fruit
pulp to avoid phase separation (Moraes et al., 2011). The dynamic and steady-shear
rheological behavior has been evaluated using Herschel-Bulkley and Cross models. The
Arrhenius equation has been applied to study the dependence of the viscosity on
temperature. Xanthan dispersions showed a more pronounced pseudoplasticity and the presence of yield stress, which was not observed in the guar gum dispersions and an increase in temperature led to lower values for this parameter.

2.3.5.2 Emulsion Formation and Stabilization

In an emulsion, one liquid (the dispersed phase) is dispersed in other liquid (the continuous phase), presenting a two-phase system. Emulsifying compounds have been used to efficiently lower the surface tension and interfacial tensions or could bind tightly to surfaces thereby making it more effective at stabilizing water/oil emulsions (Abbasi and Amiri, 2008; Vianna-Filho, 2013). Many microbial exopolysaccharides have the ability to be used as bioemulsifier because they can stabilize emulsions between water and hydrocarbons.

The use of bioemulsifiers have been considered advantageous over to synthetic emulsifiers due to their biodegradable, non-toxic nature and high activity even with wide varied conditions of temperature, pH, salinity (Freitas et al., 2009b). Biobased emulsions could be utilized for targeted delivery of specialized bioactive agents and functional foods, to combat diseases and to promote and sustain good health. Hence, biobased emulsions could play a role in wide variety of natural and manufactured materials used in many fields, including the pharmaceutical, cosmetics and food industries (Lin and Mei, 2000; McClements, 2005; Imam et al., 2013; Camacho-Chab et al., 2013).

2.3.6 Structural Characterization of Exopolysaccharides

The isolation and subsequent structural characterization of EPS produced by bacterial species is essential to understand the role played by this class of molecules in a
wide range of functional applications. As previously explained in Section 2.3 and taking into consideration, the chemical composition, EPS could group into homopolysaccharides and heteropolysaccharides (Ruas-Madiedo et al., 2002). Homopolysaccharides contained only one type of monomer unit i.e. monosaccharide while heteropolysaccharides could included repeating units, varying in size from disaccharides to heptasaccharides.

The monomeric unit may be present in either $\alpha$ or $\beta$ configuration, in their pyranose ($p$) or furanose ($f$) forms or in either D- or L- absolute configuration that could cause structural variation in heteropolysaccharides. The composition of the EPS material produced varied widely from species to species (Neihaus et al., 1993; Rabha et al., 2012; Prasad et al., 2014). The diversity of bacterial exo-polysaccharides has led to classify them based on chemical structure, functionality, molecular weight and linkage bonds.

The chemical analysis of exopolysaccharide can be done to get assess on the components of exopolysaccharide. The total carbohydrate content of EPS could be assayed using the phenol sulfuric acid method of Dubois et al. (1956), using glucose as standard. Uronic acids could be determined by method of Dische (1962) with glucuronic acid as standard. Methyl pentoses assay could be done by the method of Dische and Shettles (1948). Sulfated sugars could be determined by analyzing sulfates according to the method of Terho and Hartiala (1971) after hydrolysis of the EPS. Similarly glycosidic linkages of the repeating unit structures of EPS could be determined. The most readily employed method involved the derivatization of the polysaccharide to alditol acetates which were then analysed by gas chromatography (GC) and in combination with mass spectrometry (GC-MS).
Analytical determination of sugar composition of the hydrolyzed EPS could be carried out with GCMS using standard sugars as reference. The chemicals used for hydrolysis were sulphuric acid (Kang and Veeder, 1982), trifluoroacetic acid (Robinjn et al., 1995; Verhoef et al., 2003). Kang and Veeder (1982) performed the hydrolysis of exopolysaccharide (S-130) with sulphuric acid (2N) at 100 °C for 4 h, to release the monomers by breaking glycosidic bonds. Similarly hydrolysis of polysaccharide using trifluoroacetic acid at 105 °C for 2 h has been reported (Chen et al., 2011).

The EPS could be derivatized to alditol acetate derivatives (Zhang et al., 2011), acetonitrile derivatives (Yan et al., 2008), trimethylsilyl (TMS) derivatives (Ismail and Nampoothiri, 2010). An alditol acetate derivative of EPS obtained from *Pseudoalteromonas ruthenica* (SBT 033) has been prepared after hydrolysis using HCl for 2 h at 100 °C and gas chromatogram has been obtained after derivatization of EPS. Similarly sugar standards have been derivatized for comparison with the chromatogram of EPS (Saravanan and Jayachanran, 2008).

The methylated and hydrolyzed sugar has been derivatized to partially methylated alditol acetates by reduction with NaBH₄/NaBD₄, followed by acetylation with acetic anhydride. The derivatized sugar residues has been extracted with dichloromethane and evaporated to dryness, and dissolved again in dichloromethane. The products were analyzed by gas chromatography-mass spectrometry (GC-MS). The peaks on the chromatogram were identified from their retention times (Robinjn et al., 1995; Chen et al., 2011). In another method polysaccharide was methylated (with 0.625M methanolic HCl at 80 °C for 16 h) and silylated with 1:1 pyridine-BSTFA for 16 h at 80 °C. Isobutanol was added to the mixture and then dried under a stream of nitrogen. The TMS
derivatives were analyzed by GC-MS for determination of monosaccharides (Duenas-Chasco et al., 1998).

A Glycosyl composition of EPS produced by Lactobacillus plantarum MTCC 9510 has been determined by GC-MS analysis and in this trimethylsilyl (TMS) glycoside derivatives were prepared, dissolved in cyclohexane. 2 N trifluoroacetic acid has been used to carry out hydrolysis at 110 °C for 4 h. A gas chromatogram can be obtained with an OV1 capillary column and a flame ionization detector (Ismail amd Nampoothiri, 2010).

For linkage analysis, methylation of the EPS must be carried out before hydrolysis. In this methanolysis with methanolic 1 M HCl solution for 18 h at 85 °C was carried out and the resulting glycosides were hydrolyzed and derivatized for GC-MS analysis (Robinjn et al., 1995). Methylation analysis could also be performed by the method of Hakomori (1964). In this method polysaccharide in dimethyl sulfoxide was methylated using sodium hydride (NaH) and iodomethane (CH$_3$I). The completeness of methylation was confirmed by using infrared spectroscopy (Verhoef et al., 2003; Chen et al., 2011).

NMR was applied to determine the overall secondary structure of the EPS by providing information on ring size (pyranose/furanose) and anomic configuration of the individual monomers. In 1H NMR spectrum of the polysaccharide, the spectrum regions could generally be divided into three major regions: the anomic region ($\delta$H 4.5-5.5), the ring proton region ($\delta$H 3.1-4.5) and the alkyl region ($\delta$H 1.2-2.3). Chemical shifts were expressed in ppm. These signals were considered as signatures for differentiating complex carbohydrate structures (Ismail et al., 2010). Vliegenthart et al. (1983) reported
that 1H NMR spectrum obtained should be split and viewed as having ‘structural reporter signals’ (up field and down field) and a ‘bulk region’. This break down of the spectra facilitated the easy identification of individual sugars along with their structural features and linkage compositions. The up field (high field) reporter region contained resonances from structural motifs, that included ring substituents such as acyl, alkyl and acetal, ring substitutions such as N-acetylamino groups and H6 signals of 6-deoxy sugars (e.g. rhamnose). The anomeric proton resonances were located in the down field (low field) reporter region of the spectra between 4.4-5.5 ppm. Integration of the anomeric resonances can be used to estimate the number of different monosaccharide units present in the repeat unit structure. The remaining ring protons were situated in the ‘bulk region’ located between 4.3-3.0 ppm.

FT-IR has been used effectively as an analytical instrument, to detect the presence of type of functional groups and characterization of covalent bonding. Basically the technique worked on the principle that bonds and groups of bonds vibrate at characteristic frequencies. The information provided on FTIR spectrum was considered essentially a molecular fingerprint for organic, polymeric substances and in some case inorganic materials. To predict the information provided on the FTIR spectrum of the unknown material, comparison can be made for “best matches” with libraries of spectra that have been recorded for known materials.

The absorption in the region 1,200-950 cm\(^{-1}\), that related to the so-called fingerprint region for carbohydrates where the position and intensity of the bands were specific for every polysaccharide, allowing its possible identification (Filippov, 1992; Ismail \textit{et al}., 2010; Kalapathy and Proctor, 2001; Cerna \textit{et al}., 2003; Singthong \textit{et al}.,
2005). The spectrum band in the range 1,030-944 cm\(^{-1}\), with minimum at 998 cm\(^{-1}\) could attributed the presence of glucose (Cerna \textit{et al.}, 2003). \(\alpha\) and \(\beta\)-type glycosidic linkages in polysaccharide structure showed absorption bands at 812, and 879 cm\(^{-1}\) (Barker \textit{et al.}, 1954). In the anomeric region (950-700 cm\(^{-1}\)), the appearance of characteristic absorption band at 812.03 cm\(^{-1}\) corresponded to the existence of mannose (Mathlouthi and Koenig, 1986). The presence of peaks near 1,000-1,200, indicated that the polysaccharide contained \(\alpha\)-pyranose (Chi \textit{et al.}, 2007).

A comparative study on FT-IR of EPS and spectrum of other polysaccharides were reported by Ismail \textit{et al.} (2010). A characteristic absorption band appeared at 1658.78 cm\(^{-1}\) for carboxyl group (C = O), while another absorption band at 2,937.59 cm\(^{-1}\) of methylene group (-CH\(_2\)-), were reported for hexoses (glucose or galactose) or deoxyhexoses (rhamnose or fucose). The absorption peak at 1,056.99 cm\(^{-1}\) has been assigned to carbohydrate C-O stretching vibrations. The spectrum peak around 1530 cm\(^{-1}\) and 879.54 cm\(^{-1}\) corresponded to an amino group and the existence of \(\beta\)-glycosidic bond respectively (Braissant \textit{et al.}, 2009).

### 2.3.7 Applications of Welan Gum

Till date literature on application of welan gum in food has not been well documented however applications of exopolysaccharide obtained from sphingan secreting bacteria has been given in Table 2.4. The Welan gum, a member of sphingan family, has applications mainly in non-food systems.
Table 2.4 Sphingan secreting bacteria, exopolysaccharide and their applications

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>EPS</th>
<th>Applications</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sphingomonas</em> sp.</td>
<td>Gellan</td>
<td>To improve the texture of food products</td>
<td>Kang and Veeder (1982);</td>
</tr>
<tr>
<td>ATCC 31461</td>
<td>(S-60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>To enhance the water-holding capacity during cooking and storage</td>
<td>Banik <em>et al.</em> (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sustained release of drugs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Replace agar for the culture of</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>thermophilic microbial species</td>
<td></td>
</tr>
<tr>
<td><em>Sphingomonas</em> sp.</td>
<td>Rhamsan</td>
<td>Used in suspension fertilizers</td>
<td>Peik <em>et al.</em> (1983)</td>
</tr>
<tr>
<td>ATCC 31961</td>
<td>(S-194)</td>
<td>Pharmaceutical suspension</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Food systems</td>
<td></td>
</tr>
<tr>
<td><em>Sphingomonas</em> sp.</td>
<td>Diutan</td>
<td>Used in the field of petroleum and water well treating fluids and muds</td>
<td>Peik <em>et al.</em> (1992)</td>
</tr>
<tr>
<td>ATCC 53159</td>
<td>(S-657)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sphingomonas</em> sp.</td>
<td>S-88</td>
<td>Utility in field of petroleum and water-well drilling muds</td>
<td>Kang and Veeder (1985)</td>
</tr>
<tr>
<td>ATCC 53154</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sphingomonas</em> sp.</td>
<td>S-198</td>
<td>Used in pigment clay coating</td>
<td>Peik <em>et al.</em> (1985)</td>
</tr>
<tr>
<td>ATCC 31853</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sphingomonas</em> sp.</td>
<td>S-7</td>
<td>Used in oil well drilling</td>
<td>Moorhouse and Shim (1980)</td>
</tr>
<tr>
<td>ATCC 21423</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

The use of welan gum in cement compositions has a distinct significance. The use of low viscosity welan gum in cement compositions reduced fluid loss of the cement
compositions, increased the suspension properties of cement suspension, and was effective in low concentration (Allen et al., 1990; Allen et al., 1991). Rapidly hydrating welan gum has been reported in improving the cement workability (Rakitsky and Richey, 1992). Welan gum acted as a liquefied viscosity agent with superplasticizer. When the welan gum was added into a superplasticizer and agitated well, welan gum particles swelled in the superplasticizer, resulting in stable suspension without much viscosity increase (Sakata et al., 2003).

The admixture was useful for proportioning underwater concrete, post-tensioning grout and self-consolidating concrete (Khayat and Saric-Coric, 2000; Wei et al., 2012). The attempts have been made to extend the application of welan gum in other areas. It has a wide range of uses in oilfield operations including hydraulic fracturing, wellbore cleanup, cementing and drilling for viscosity enhancement and friction pressure reduction purposes. It has utility as a spacer fluid in oil well drilling (Sandford et al., 1984; Seheult and Grebe, 1988; Whistler and Bemiller, 1993).

The good thermal insulating properties and rheology of the gel make them useful as an insulating material for pipeline bundles and pipeline riser calsssons that helped in maintaining temperature (above 30 °C) of oil streams at a level considerably above sea level.