CHAPTER I

INTRODUCTION

The occurrence of natural gums in all forms of our life is normal. Sticking of postal stamps and sealing of envelopes are usually done by using gum arabic. Gelatin or dextran provides better consistency to ice-cream. China grass (agar and sea weed) is used to mould the delicious jams and jellies into attractive and stunning shapes. We can produce various types of gums from the sources of plant. We need skilled laborers and more money to collect plant sources. Another problem with the plant source gums is that the quantity and quality of the gums are subjected to the changes in climate and season.

The awareness in environmental friendly natural polysaccharide has increased in recent years due to their huge potential in varied sectors, possessing several advantages like biodegradability, nontoxicity and biocompatibility over synthetic or chemical polymers which are non-ecofriendly. Pens, computers, cars, food packaging, and clothing are all examples of products which contain plastic. Plastic is a material made up of one or more polymers. The main sources of the chemicals needed to manufacture plastics are fossil fuels. Due to innovations and advancements in material science and chemistry, many novel varieties of synthetic polymers have come up during the last 100 years. With the arrivals of synthetic polymers like polyurethane, polyethylene and nylon, our day to day life has witnessed a great transformation. This tendency of increasing dependence on synthetic polymers has become the source of pollution and health issues.

Almost all plastic materials are derived from non-renewable resources. They are also not biodegradable. Though the properties of plastics specifically like durability and strength make it so desirable and useful, the very same qualities ensure the complication of their disposal due to their persistence in the environment. Besides this,
the production of some of the polymeric materials involves either the use of toxic compounds or generates toxic byproducts. Also the degradation intermediates are also highly pollutants. With the advent of modern bio-technology, there has been an increased attention and focus on polymers that could be derived from the sources of biological precursors using modern biotechnology.

The polysaccharides for scientific and industrial applications are obtained more conveniently from microbial sources due to several factors. They can be produced under controlled conditions from selected species using renewable sources and are biocompatible and biodegradable. These factors have accelerated the use of microbial gums such as pullulan, curdlan, scleroglucan, dextran and xanthan. Gums chemically are known as polysaccharides or carbohydrate polymers. The only exceptional case is gelatin which is a protein. Normally polysaccharides’ presence is felt in all life forms. The physical and chemical characteristics of polysaccharides are very many and unique. They are utilized as a structural material to the plant kingdom as adhesives and energy reserves. They also work as information transfer agents. The composition of polysaccharides are of repeating, regular and simple units of sugars like fructose, mannose, glucose etc., these polysaccharides may also be called as exopolysaccharides or slime (biopolymers).

Polysaccharides are synthesized biologically by microbes such as bacteria, fungi, yeasts and eukaryotic algae. Such biopolymers may prove to have a variety of environmental benefits. Various types of naturally occurring biopolymers are listed in Table1.1.
Table 1.1 Biopolymer Family

<table>
<thead>
<tr>
<th>Name of the Polysaccharides</th>
<th>Sources</th>
</tr>
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<tbody>
<tr>
<td>Starch (amylase / amylopectin)</td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
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<tr>
<td>Agar</td>
<td></td>
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<tr>
<td>Alginates</td>
<td></td>
</tr>
<tr>
<td>Carrageenan</td>
<td>Plant / Algal</td>
</tr>
<tr>
<td>Pectin</td>
<td></td>
</tr>
<tr>
<td>Konjac</td>
<td></td>
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<tr>
<td>Various gums (e.g., guar)</td>
<td></td>
</tr>
<tr>
<td>Chitin/chitosan</td>
<td>Animal</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td></td>
</tr>
<tr>
<td>Xanthan</td>
<td></td>
</tr>
<tr>
<td>Dextran</td>
<td>Bacteria / fungi</td>
</tr>
<tr>
<td>GelIan</td>
<td></td>
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<tr>
<td>Levan</td>
<td></td>
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<tr>
<td>Curd Ian</td>
<td></td>
</tr>
<tr>
<td>Polygalactosamine</td>
<td></td>
</tr>
<tr>
<td>Cellulose (bacterial)</td>
<td></td>
</tr>
<tr>
<td>Pullulan</td>
<td></td>
</tr>
<tr>
<td>Silks</td>
<td></td>
</tr>
<tr>
<td>Collagen/gelatin</td>
<td>Proteins</td>
</tr>
<tr>
<td>Elastin</td>
<td></td>
</tr>
<tr>
<td>Resilin</td>
<td></td>
</tr>
<tr>
<td>Polyamino acids</td>
<td></td>
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<tr>
<td>Wheat gluten, Casein</td>
<td></td>
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<tr>
<td>Serum albumin</td>
<td></td>
</tr>
<tr>
<td>Polyhydroxyalkanoates</td>
<td>Polyesters</td>
</tr>
<tr>
<td>Polylactic acid</td>
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</table>
“Xanthan” is taken up as the topic of our research and discussion amongst all other biopolymer families in this transcript. Xanthan may also be called as xanthan gum. The principle functions of polysaccharides are of two. No.1, its function as starch stores energy for cell activities and No.2 as cellulose it works as a structural material for living systems. Polysaccharides are one of the most complex diverse groups of polymers. It is due to the fact that the bonds linking the sugar monomers on the sugar units. By a simple linking process of glucose monomers together at various positions, different polymers with different properties can be produced. We have identified around 20 different sugars among a variety of polysaccharides from the biological sources. So we can create a great range of polymer structures. By adding other molecules, many polysaccharides containing branched structures could be chemically modified. As the monomeric or repeat units are generally made up of more than one sugar molecule, they are little complex. There are five sugars containing in the xanthan gum repeat unit. Neither the assembly of polysaccharide repeat units nor the associated polymerization processes are based on a template or genetic blue print but are specified by the enzymes (biological catalysts) involved in an organism’s “biosynthetic pathway” (Davidson, 1978).

1.1. Xanthan

Xanthan gum is an important and natural biopolymer used in industries. The second microbial polysaccharide commercialized as well as one of the most extensively investigated polysaccharide was xanthan. It was discovered in 1963 at Northern Regional Research Centre, United States Department of Agriculture, (USDA) (Margaritis and Zajic, 1978). The bacterium *Xanthomonas campestris* produced xanthan was studied extensively for the properties that would allow it to supplement other known synthetic and natural water-soluble gums. Commercial production began substantially in early 1964. The safety properties of xanthan gum for its toxicological limit of pharmaceutical and food applications have been extensively researched. Xanthan is non-toxic and non-sensitizing so it doesn’t inhibit growth or cause eye or skin irritation. So United States Food and Drug Administration (FDA) has approved xanthan for use in food additive without any specific quantity limitations (Kennedy and Bradshaw, 1984). Xanthan is the only food grade in the USA that meets very stringent purity standards of the FDA. It is relatively expensive due to the use of glucose or
sucrose as the only carbon source. For the production of food-grade xanthan gum, the downstream purification steps cost up to 50% and for non food applications we can do away with these expenses. Further the costs could be reduced by using low cost substrates like waste agricultural products.

1.2. Chemistry and Structure of Xanthan Gum
Repeating pentasaccharide units consisting of two D-mannopyranosyl units, two D-glucopyranosyl units and one D-glucopyranosyluronic acid are the constituents of the primary structure of xanthan (Figure 1.1). 1-4 linked β-D-glucopyranosyl units are the backbone of xanthan. A trisaccharide side chain with a D-glucuronosyl unit is attached between two D-mannosyl units at the O-3 position. At the O-4 position the β-D-glucopyranosyluronic acid unit links the terminal β-D-mannopyranosyl unit. Consequently a α-D-mannopyranosyl unit glycosidically links to the O-2 position. (Kang and Pettitt, 1993).

Fig. 1.1 Structure of xanthan gum.
1.3. Properties

Xanthan is a free flowing powder in white to cream coloured, soluble in both hot and cold water. It hydrates very quickly if dispersed. Due to it highly viscosity solutions at low concentration, it binds water. Over the temperature ranging from freezing to near boiling, it solution provides uniform viscosity with excellent thermal stability (Pelletien et al., 2001).

Xanthan's properties of stability and solubility with both alkaline and acidic conditions are excellent. Xanthan has become one of the top food and industrial polymers because of its stability with salts and its resistance to common enzyme. It is able to produce very good sensory qualities like mouth-feel and flavor release in food by its rheological behaviour. The property of xanthan is to control the rheology of water based systems on the basis for its demand in the industry. The quality of the xanthan gum solutions’ high viscosity, even at low concentrations when compare to other polysaccharides makes it a very effective stabilizer and thickener (Lachke, 2004).

Xanthan gum solutions have the property of rebuilding its initial viscosity even after high shear rates. This property is otherwise known as “highly pseudo-plastic”. Shear thinning and recovery are instantaneous that is “No evidence of hysteresis”. This property of pseudo-plasticity in the final products enhances sensory qualities (flavor release, mouth-feel etc.,) and ensures high degree mix, pump and pourability.

As xanthan gum solutions are stable in both alkaline and acidic, they are highly resistant to pH variations. The viscosity of xanthan solution remains practically constant between pH 1 to 13. With very little effect on its solution properties, xanthan gradually deacetylates at pH 9 or above. Over a wide range of temperatures, the viscosity of aqueous solution of xanthan remains nearly unchanged. The temperature of pure water from the freezing point to the boiling point virtually does not affect the viscosity of a xanthan solution. So, the final products being stored either in room temperature or in a refrigerator, the rheological properties of xanthan remains stable.

The electrolytes’ effects on xanthan solutions are depending upon the gum concentration. The addition of an electrolyte (NaCl) with a gum concentration below
0.15% reduces viscosity slightly but the electrolyte has the opposite effect with higher gum concentrations. The viscosity attains its peak at a concentration level of 0.02-0.07% NaCl. Additional salt has hardly any effect on viscosity. Only calcium and magnesium salts have a similar influence on viscosity. Xanthan gum has the tendency to form gels in the presence of divalent cations only at high pH levels (pH >10). Trivalent cations like iron or aluminium form gels at neutral or acid pH levels. High levels of monovalent metal salts may prevent gelling (Andrew, 1977).

1.4. Xanthan Gum in Food Applications

1.4.1. Food applications

Xanthan gum as a thickening and suspending agent for fruit pulp and chocolates is widely used in the food industry. On the basis of toxicology tests on xanthan for using it in human food, The United States Food and Drug Administration has approved the usage of xanthan in food industry. Xanthan improves many of the unique requirements of foods of today like texturization, viscosity, appearance, flavor release and water-control properties besides controlling in the reology of the final food product. The formation of weak gel like structure resulting from an unusually high “low shear rate viscosity” at low polymer concentrations that could be utilized to thicken aqueous samples and permits stabilization of foams, emulsions and particulate suspensions. Xanthan has pseudoplastic properties in solutions and also less ‘gummy’ mouth feel than gums with highly Newtonian characteristics. Finally xanthan allows manipulation and control of processes like pumping, pouring, spreading and spraying due to its reversible shear thinning behaviour (Sharma et al., 2006).

1.4.2. Bakery

Xanthan gum increases water binding during baking and storing of bakery products. The shelf life of bakery products and refrigerated doughs can be increased by xanthan. We can use xanthan gum as an egg replacer particularly the white content of the egg can be reduced without affecting the taste and appearance in soft baked goods. It also provides air incorporation and retention, smoothness and recipe tolerance to batters for cakes, biscuits, muffins and bread mixes. The texture and volume of gluten-free breads and reduced-calorie baked goods can be improved by xanthan gum. The texture and flavour release of either hot or cold processed bakery and fruit pie fillings
can be improved by adding xanthan. The extended shelf stability, syneresis control and freeze–thaw stability are the added benefits of xanthan in cream and fruit fillings (Sharma et al., 2006).

1.4.3. Beverages

Xanthan can be used as bodying agent in squashes and beverages. With the addition of xanthan in drinks containing particles of fruit pulp, a good texture and product appearance is achieved by maintaining the suspension of fruit pulp. Xanthan contributes to pleasing mouth-feel with excellent suspension of insolubles and compatibility and complete solubility at low pH with most components. Along with rapid viscosity development, enhanced body and quality to the reconstituted drink is provided by xanthan in dry-mix beverage bases.

1.4.4. Dairy

For milk shakes, water ices, sherbet, ice milk and ice cream, the blends of xanthan gum, LBG, galactomannans, guar and carrageenan are excellent stabilizers. For frozen dairy, xanthan with methyl-carboxy methyl cellulose works very well whereas for directly acidified yogurts xanthan with carboxy methyl cellulose works nicely. We use similar blends for acidified milk gels, dessert puddings and others. Ice-crystal control, heat-shock protection, enhanced flavor release, improved heat transfer during processing, long-term stability and optimal viscosity are provided by these economical blends (Sharma et al., 2006; Rosalam and England, 2006). The xanthan, LBG and guar blends are important to sliceability, firm body and flavor release of cream cheese. Cottage cheese dressings are thickened by xanthan by providing good drainage control.

1.4.5. Dressings

The properties of xanthan as the ideal stabilizer for pourable no-oil, low-oil and regular salad dressing are made possible by its stability to acid and salt, its effectiveness at low concentrations and its property of highly pseudo-plastic rheology. Relatively excellent long term constant viscosity and emulsion stability over a wide temperature range is achieved by doing dressing with xanthan gum as xanthan pours easily but clings well to the salad. As xanthan gum produces desirable body, texture and freeze–thaw stability as well as improved flavour release and eating sensation, it
works as a partial substitute for starch in regular and compact calorie spoonable dressings (Sharma et al., 2006).

1.4.6. Pet food

A homogeneous gelled product for using blood chunks or semi-moist pet foods is produced by xanthan along with guar or Locust Bean Gum (LBG). We use xanthan as replacers for calves and piglets in liquid milk to stabilize the suspension of insoluble substances. So mostly we use xanthan combined with guar and LBG gum as stabilizer and binder in the production of canned gravy based pet foods.

1.4.7. Syrups and toppings

Some of the unique properties of xanthan solution are used in syrups and toppings. Xanthan brings about an excellent consistency and flow properties in buttered syrups and chocolate toppings. Its properties of high viscosities, appetizing and appear thick greatly help on products like pancakes, cooked meats and ice cream (Sharma et al., 2006; Rosalam and England, 2006). Xanthan also brings about a firm texture, excellent freeze–thaw stability and high over run on Frozen non dairy whipped topping concentrates.

1.4.8. Relish

Adding of xanthan gum to relish completely eliminates the loss of liquor and also improves the drained weight during handling. Xanthan ensures uniform distribution of the relish and liquor during filling in portion pack relish. Spattering is also prevented by xanthan.

1.4.9. Sauces and gravies

High viscosity is provided by the low levels of xanthan in gravies and sauces with both acid and neutral pH. The properties of xanthan’s extremely stable condition of viscosity to temperature variations help maintain a variety of long-term storage conditions. It makes the sauces and gravies clinging to hot foods and brings about appearance and excellent flavour release.
1.5. Industrial applications

Industrial application of xanthan in the chemical industry is very wide. In deodorant gels, we use the mixture of LBG and xanthan. We can also form a special gel of xanthan with borate present in it. With this combination, we can prepare explosives. The right consistency to the tooth paste is achieved through the properties of xanthan to impart the required dispersion stability at rest and low viscosity under application.

1.5.1. Oil industry

For the ‘Enhanced Oil Recovery’ (EOR), the unique rheological properties of xanthan are technologically most suitable one. Xanthan gum has a wide range of applications in the petroleum industry like oil drilling (Katzbauer, 1998), pipeline cleaning and work-over, fracturing and completion. It works as a useful additive in drilling fluids as xanthan gum has an excellent compatibility with salt and has good resistance to thermal degradation. In the application of micellar-polymer flooding operation, xanthan is used to recover tertiary oil. We use polymer thickened brine to drive the slug of the surfactant through porous reservoir rock to mobilize residual oil. Bypassing of the drive water through the surfactant band is prevented by the polymer and good area sweeping is ensured (Byong, 1996).

1.5.2. Agricultural products

The usage of xanthan in agriculture industry improves the flow ability in herbicides, fungicides and insecticides formulations by suspending the solid component (Flickinger and Draw, 1999). The contact time between the pesticide and the crop is increased by controlling the spray drift and clinging.

1.5.3. Cleaners

Xanthan’s properties of flow ability and broad pH stability made it a choice thickener in both alkaline and acidic solutions. It is used in highly alkaline products to clean tile, drain and grout. Xanthan in acidic solutions removes rust and metal oxides and also used as graffiti removers, toilet bowl cleaners, aerosol oven cleaners and metal cleaning compounds. Xanthan gum provides cling to vertical surface, as well as easy removal.
1.5.4. Coatings

The pseudo plastic properties of xanthan gum provide excellent texturing in ceiling-tile coating, ensuring in-can stability, paints with high-solids content and ease of application to the wall and retention of the texture finish. Xanthan gum thickens latex paints and coatings, and suspends zinc, copper and other metal additives in corrosion coatings are uniformly suspended by xanthan gum.

1.5.5. Paper

Xanthan gum can be used as a stabilizer or suspension aid for the manufacture of paper and paperboard.

1.5.6. Personal care applications

The flow properties of shampoos and liquid soaps are improved by the addition of xanthan gum and also it facilitates a stable, rich and creamy lather (Rosalam and England, 2006). It functions as an excellent binder for all toothpastes, including gel and pumpable types. It also improves the ribbon quality and easy extrusion quality.

1.5.7. Pharmaceutical applications

The suspensions of a variety of insoluble materials like barium sulphate (X-ray diagnoses), complexed dextromethorphan (for cough preparations) and thiabendazole are stabilize by xanthan.

1.6. Objectives of This Research Work

With these in back ground, the objective of this research work is to enhance the xanthan gum production in submerged fermentation using microorganisms. The specific objectives are:

[1] To isolate the bacterial strain to be used for this study

[2] To optimize the xanthan gum production in submerged fermentation by *Xanthomonas campestris* ATCC (29497) and *Enterobacter oryzae* (isolated) using glucose as a synthetic substrate

[3] To screen the nutrients by Plackett–Burman design for the production of xanthan gum using the microorganisms
[4] To optimize the selected nutrients for the maximum production of xanthan gum using the microorganisms for various substrates by Response Surface Methodology (RSM) using Central Composite Design

[5] To optimize the effect of process variables namely, initial pH, temperature, fermentation time and microbial load on xanthan gum production by response surface methodology (RSM) using Central Composite Design

[6] To study the xanthan gum production using Artificial Neural Network (ANN) and to compare the experimental values with RSM and ANN predicted values

[7] To pretreat agro industrial wastes

[8] To use agro industrial wastes as the cost effective carbon substrates to produce xanthan gum using the strain Enterobacter oryzae (isolated)

[9] To study the different solvents to extract the xanthan gum using Enterobacter oryzae (isolated)

[10] To study the kinetics of xanthan gum production

1.7. Organization of the Thesis

The thesis is presented in six chapters.

Chapter I - Contains introduction about xanthan gum and also highlights on the scope of the present work.

Chapter II - A comprehensive literature review is presented. It Contains the factors influencing the production of xanthan gum, various organisms producing xanthan gum, different methods to produce xanthan gum, methods to extract, recovering and analyzing xanthan gum, use of agro residues as carbon substrate, optimisation of medium for xanthan gum production, characterisation and applications of xanthan gum.

Chapter III - Explains about the materials used and methods adopted for producing xanthan gum from the bacterial strains. Morphological identification and biochemical characteristics of newly isolated strain were studied. Screening of medium components using Response Surface Methodology using Plackett-Burman has been explained. Optimisation of components and concentrations using Response Surface Methodology...
using Central Composite Design has been described. Artificial neural network modeling is used to optimize the production of xanthan gum. ANN predicted and RSM predicted values are compared with the experimental values of RSM. This chapter deals with how the xanthan gum was produced in lab scale and the way they were recovered and analysed.

Chapter IV - Presents the results obtained in batch studies. It also presents a detailed discussion on the findings of this work. Kinetics and simulation of the experiments conducted for the production of xanthan gum using various substrates through Enterobacter oryzae (isolated) was explained by implementing kinetic models in this chapter.

Chapter V - This chapter offers the conclusions from this work and future scope of the present study.