

Sustainable Production of Microbial Polysaccharide Xanthan Gum From Supplemental Substrate.



Engineering

KEYWORDS : Xanthan gum; white biotechnology; substrate; starch; glucose

Magar Subhash B

Associate Professor, Chemical Engg. Depptt., Pravara Engg. College, Loni

Abhijit S Jadhav

Assistant Professor, Chemical Engg. Depptt., AISSMS COE Pune

Sumitkumar Jana

Assistant professor, Department of chemical engineering and Technology, BIT, Mesra, Ranchi

ABSTRACT

There are several pathways to produce bio-based chemicals and polymers. The pathways that are most important in terms of production volumes today and in the medium-term future are white biotechnology (industrial biotechnology) and the use of natural polymer (in particular xanthan gum, cellulose and starch). Increasing market price and demand suggest that glucose may no longer economics for the raw material, while using batch process may also limit the capacity. It is therefore the purpose of this review to investigate alternative to economically produce xanthan gum. The various researches studied that most supplemental substrate are best sole of carbon source. The scenario review shows properties of xanthan, various aspects of xanthan production including the various strains used for the production and various supplemental substrates used as alternate source of glucose. Substrate used for the production of xanthan were agro-industry or by products including spent malt grains, apple pomace, grape pomace citrus peels, which can be obtained at a very low cost. Also cheese whey hydrolyzed rice, barely, corn flour, and sugarcane molasses, Sugar beet molasses, olive mill wastewater etc. which causes several drawbacks on environment, when it is directly disposed in the environment. Utilization of these streams for the xanthan production increases economy of the process. With most substrate, xanthan yields were comparable to those obtained from conventional submerged cultivation. The basic characteristics of the process were studied on various supplemental substrate prepared with a solution of nutrients.

1.0 Introduction:

Xanthan gum is complex exopolysaccharides produced by the yellow pigmented gram -ve bacterium *Xanthomonas campestris* [1-2]. Xanthan is composed of pentasaccharide repeated units containing d-glucose, d-mannose, D-glucuronic acid [at a ratio 2:2:1] acetal linked pyruvic acid and d-acetyl groups [1]. Operational conditions include an adequate medium containing different nutrients, a temperature of 28°C pH 7 and adequate aeration [3]. Xanthan is a biopolymer with a high molecular weight between 2×10^6 and 20×10^6 Da. The polysaccharide has a relatively high solubility in water, and the size of the molecules and their interactions provide a very high viscosity to aqueous solution. [3]. Xanthan gum was discovered in 1950's at the northern regional research laboratories (NRRL) of the United States Department of Agriculture [4]. Extensive research was carried out in several industrial laboratories during 1960's culminating in semi commercial production as kalzin by Kelco. Substantial commercial production began in early 1964s [4]. Garcia-Ochoa F. in 2000 reported that the major producers of xanthan are Merck and Pfizer the United States, Hone Poulenc and Sanofi-EIF in France, and Jungbunzlauer in Austria. Currently the worldwide consumption of xanthan gum is approximately 23 million kg/y approximately 5 million kg/y are used as drillings fluid viscosity in the oil industry. Xanthan gum consumption in the United States has an estimated annual growth rate between 5 and 10%. The petrochemical industry uses other plant derived polysaccharide and synthetic polymers. Instead of xanthan gum based on the relative costs of xanthan gum to the other polymers. In the United States, the only commercially available xanthan gum is food grade [5]. Commercially available of xanthan gum is relatively expensive due to glucose or sucrose being used as sole carbon source and very stringent purity standards of the food and drug Administration for foods. For food grade xanthan gum, up to 50% of the production costs are related to downstream purification steps, many of which would not be necessary for non-food application. Several researches have investigated using less expensive carbon sources to produce xanthan gum [2,5-9]. For the efficient production of xanthan gum, *Xanthomonas campestris* needs several nutrients, including micronutrients (e.g. potassium iron and calcium salts) and macronutrients such as car-

bon and nitrogen. Glucose and sucrose are the most frequently used carbon sources [9]. Psomas, S.K., et al., in 2007 reported that production and properties of xanthan gum are influenced by bacterial strain, culture medium, substrate composition, temperature, pH, time of fermentation, agitation rate, impeller type, oxygenation. Xanthan gum is a major commercial biopolymer having worldwide production 30,000 tons of market price costs \$408 million. Due to its unique structure xanthan displays special pseudoplastic properties high viscosity and solubility, enhanced stability [8]. The viscosity of the solutions is stable over wide range of salt concentrations (up to 150 g/l NaCl), temperature (up to 90°C), pH (2-11). These solutions characteristics of xanthan gives rise to functional properties such as thickening and stabilizing ability, which are used in diverse range of applications [11]. Xanthan having high viscosity yield at lower shear rates, shear thinning ability are particularly important during and after many industrial application [12]. Sharp increase in viscosity (about hundred fold) when the pyruvate content increased above 3.0% by wt. [13]. The use of carbon to nitrogen ratios in the defined medium resulted in a major increase in the broth consistency index. Maximum production was achieved in nitrogen limitations, at a carbon to nitrogen ratios of 23. Mineral sources such as CaCO_3 and KH_2PO_4 proved to be important factors influencing polysaccharide production and quality. A maximum concentrations of 11.15 g/l of xanthan gum was obtained during the optimization of defined medium, an increase of xanthan gum production was accompanied by a decrease of exocellular protein [14]. The pentasaccharide repeating units is assembled on an isoprenoid lipid carrier by sequential addition of individual sugar residue that are denoted by sugar nucleotide diphosphate precursors. Each sugar addition is catalyzed by a specific glycosyltransferase enzyme. The mannose residue of the repeating units are specifically acetylated and pyruvylated. The repeating unit is polymerized, and the polymer is subsequently secreted [15]. Xanthan gum mixture shows a synergistic behavior, increasing the viscosity and forming gels. Xanthan molecular structure affects to xanthan/galactomannose mixture solution viscosity. Stronger interaction between galactomannose and xanthan gum was observed in mixtures containing deacetylated or native xanthan than in those containing depyruvated xanthan. Xanthan fermentations by *Xanthomonas campestris* is well studied with respect to aeration effects on growth and production as well as,

the rheological characteristics of the fermentations broths[17].

2.0 Growth Rate of Xanthan Gum.

Annual Average Growth Rate			
Polysaccharide	Food	Petroleum Industry	Cosmetics
Starches	2.5	1.5	~
CMC	3.5	0.5	3
MC	3.5	~	3.7
HEC	~	0.5	~
HPG	~	1.5	~
Xanthan	8.3	2	5.9
Pectin	5.2	~	~

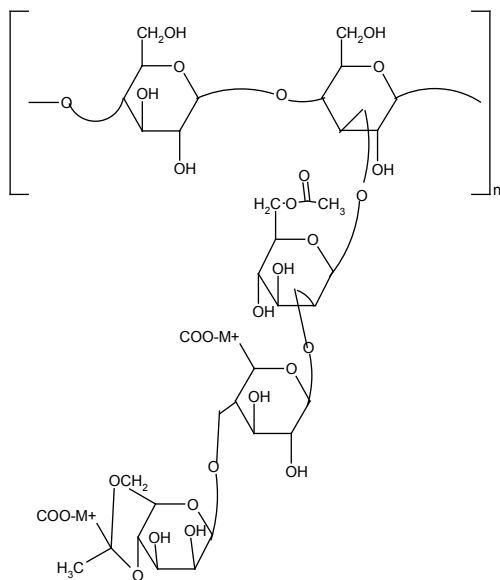
CMC: Carboxymethyl Cellulose MC: Methyl Cellulose

HEC: Hydroxyethyl Cellulose

HPG: Hydroxypropyl Guar

3.0 Chemical Structure

Xanthan gum is natural biopolymer is repeated unit of five sugars and varying amount of acetate and pyruvate i.e. two-D-Glucose, Two-D-Mannose, One-D-Glucuronate. Molecular formula- $C_{32.34}H_{48.58}O_{27.36}Na_{1.38}$ [12].



M+= Na, K, 1/2Ca

Fig. 1: Chemical structure of Xanthan Gum

4.0 Growth Medium

All the Media Used for *Xanthomonas Campestris* growth are complex media. The most commonly used are the YM Medium[4]. Cultivations on agar medium surface will be performed using 100 mm Petri dishes filled with 25 ml of 2% agar medium incubated in a water saturated atmosphere at 30 °C for either 4 or 8 hours.[6].

4.1 Growth Temperature

Xanthomonas Campestris has been cultured at different temperatures ranging from 25 to 30 °C [12-14]. Several authors studied the influence of temperature on growth in the temperature range of 22-35 °C; 28 °C is the optimal growth temperature in the media used[10-11].

5.0 Analytical methods

4.2 Xanthan production by *xanthomonas Campestris* by different supplemental substrate

Author	Substrate	Substrate Composition (g)	Xanthan gum yield (g/l)
Stredansky M., et.al., [6]	Apple pomace	1000	281
Stredansky M., et.al., [6]	Grape pomace	--	10
Garcia-ocha et.al., [4]	glucose	--	75
Yoo S.D., et.al., [5]	waste sugar beet pulp	2.29	0.88
Lopez M.J., et.al., [7]	olive mill wastewater	20	07
Kalogiannis s., et.al., [24]	sugar beet molasses	175	53
Kalogiannis s., et.al., [24]	sugar cane molasses	25 %	70.5
Kurbanoglu E.B., et.al., [25]	Ram horn hydrolysate	03 %	25
Ashraf S., et.al., [2]	whey	--	10
Silva M.F., et.al., [9]	cheese whey	--	25

6.0 Chemistry of xanthan gum

Xanthan gum is a complex microbial exo-polysaccharide industrially produced from glucose via fermentation by the plant-pathogenic bacterium, *Xanthomonas pv. campestris*. The molecular weight of xanthan gum is approximately 2 million but it can go as high as 13–50 million. As shown in "FIG.1" xanthan gum consists of d-glucosyl, d-mannosyl, and d-glucuronyl acid residues in a molar ratio of 2:2:1 and variable proportions of O-acetyl and pyruvyl residues. Xanthan gum is an acidic polymer made up of pentasaccharide subunits, forming a cellulose backbone with trisaccharide side-chains composed of mannose (β1,4) glucuronic acid (β1,2) mannose attached to alternate glucose residues in the backbone by α-1,3 linkages. On approximately half of the terminal mannose residues is a ketal linkage joined by a pyruvic acid moiety. Acetyl groups are often present as 6-O substituents on the internal mannose residues. Some external mannoses contain a second 6-O-acetyl substituent. [18].

7.0 Conclusion

Many attempts have been reported for optimizing variables of the xanthan gum fermentation i.e. nutrient composition and feeding technique, temperature, pH, agitation, adding antifoam, use of immobilized-cell cultures, recombinant DNA for the production of xanthan gum. All shows some improvement in the area studied. Other substrates were also tested and but glucose is still the best in term of the product yield, supply, and the product quality. Most of the previous works are not attempted to give a huge impact on the price of xanthan gum but more on the fine tunings on the particular areas studied, conclusion is that it initiate a new strategy on the xanthan gum processing technology that could improve the quality, increase the productivity and also reduce the cost. Most of the works are carried out to recover xanthan from highly viscous broth on which 50% of the total cost of production is utilized. But up to 80 % of the cost of recovery and energy is reduced.

8.0 Future scope

Study hydrodynamic and mass transfer in fermentation with rheologically complex culture medium. The determination of media nutrient in order to reach high product yields at low cost. Attainment of optimal conditions for production is a time-consuming task with many variable. it is possible to undertake a rational study by using adequate experimental statistical designs which avoids a great amount of tedious work by reducing the number of experiments and broadening the range of informa-

tion about the system. Xanthan gum currently dominates the microbial gum market, which is growing at between 6 and 7 % per year. It has been suggested that global production exceeds 50000 tons per year. The world wide xanthan market is valued between \$600 and \$800 millions per year. Development and improvement of xanthan production, membrane processes have been increasingly used for concentrating of high viscous broth .the axial-flow hollow fibre cell culture bioreactors, fibrous- bed bioreactors for continuous production ,ceramic membrane reactor possibly help in development of the continuous xanthan gum production.

REFERENCE

1. Ielpi I.,couso R.,dankert M., lipid-linked intermediate in the biosynthesis of xanthan gum.febs letter. 1981; 130(2):253-256. | 2. Ashraf S.,Souidi M.R.,Sodeghizabeh M.,isolation of a novel mutated strain of xanthomonas campestris for xanthan production using whey as the sole substrate .Pakistan journal of biological sciences .2008;11(3):438-442. | 3. Garcia-ochoaF.,Casas J.A.,Apparent yield stress in xanthan gum solution at low concentrations. The Chemical Engineering Journal.1994;53:B41-B46 . | 4. Garcia-ochoa F.,Santos J.A.,Gomez E., Research review paper of xanthan gum:production recovery and properties.Biotectnology Advances. 2000;18:549-579. | 5. YooS.D., Harcum S.W., Xanthan gum production from Waste sugar beet pulp.Bioresourse Technology.1999; 70:105-109. | 6. Stredansky M.,Conti E., Xanthan production by solid state fermentation.process biochemistry .1999;34:581-587. | 7. Lopez M.J., Moreno J., Ramos- cormenzano ., Xanthomonas Campestris strain selection for xanthan production from olive mill waste waters.water research. 2001;35:1828-1830. | 8. Kalogiannis S., Iakovidou G., Kyriakides M., Kyriakidis D., A and Skaracis G., N., Optimization of xanthan gum production by xanthomonas campestris grown in molasses. Process Biochemistry. 2003;39: 249-256. | 9. Silva M. F., Fornari R. C.G., Mazutti M. A.,Oliveira D. D., Padilha F.F., Cichoski A. J., Cansian R. L., Luccio M. D., Treichel H ., Production andcharacterization of xantham gum by Xanthomonas campestris using cheese whey as sole carbon source. Journal of Food Engineering. 2009; 90: 119-123. | 10. Psomas S.K., Liakopoulou-Kyriakides M., Kyriakidis D.A., Optimization study of xanthan gum production using response surface methodology. Biochemical Engineering Journal. 2007;35: 273-280. | 11. Candia Flores ., Deckwer W.D., xanthan gum. In flickinger mc,Drewsw Encyclopedia of bioprocess technology fermentation. Biocatalysis and bioseparation.5,NewYork Wiley.1999; 2695-2709. | 12. Casas J.A.,Santos. V. E., Ochoa Garcia-F., Xanthan gum production under several operational conditions .Enzymes and microbial technology.2007; 26(2-4):289-289. | 13. Roseiro,J.C., Esgalhado,M.E., Amaral C.M.T., EmeryA.N.,Medium development for xanthan production.process biochemistry.2006; 27:167-175. | 14. Hassler R.A., Doherty D.H., Genetic Engineering of polysaccharide struture:production of variants of xanthan gum in xanthomonas campetris. Biotechnology progress.1990;6:182-187. | 15. Casas J.S., Santos V.E., Garcia-Ochoa F., Xanthan gum production under several operational condition: Molecular structure and rheological properties. Enzyme and microbial technology. 2000;26:282-291. | 16. Papagianni M., Psomas S.K., Batsilas L., Paras S.V., Kyriakidis D.A., Kyriakides M.L.,Xanthan production by xanthomonas Campestris in batch culture. Process Biochemistry. 2001; 37:73-80. | 17. Rosalam S., England R., Review of xanthan gum production from unmodified starches by xanthomonas campestris sp. tecnology Enzymes and microbial.2005;39(2):197-207. | 18. Pons A., Dussap C.G.,Gros J.B.,Modelling Xanthomonas campestris Batch fermentations in bubble columns.Biotechnology and Bioengineering.1989;33:394-405. | 19. Lo Y.M., Yang S.T., Min D.B., Ultrafiltration of xanthan gum fermentation broth:process and economic analyses.Journal of food engineering.1997;31:219-236. | 20. Xuewu Z.,Xin L., Dexiang G., Wei Z., Tong X.,Yonghong M., Rheological models for xanthan gum.Journal of food engineering.1996;27:203-209. | 21. Albitar V., Torres L.G., Galindo E., Recovery of xanthan from fermentation broth by precipitation in stirred tank . Process Biochemistry.1994;29:187-196. | 22. Amanullah A., Serrano A.L.C., Galindo E., NienowA.W., Reproducibility of pilot scale xanthan fermentation . Biotechnology progress. 1996; 12: 466-473. | 23. Rein L., Obimdike N., Towards practical optimal control of batch reactors. Chemical engineering journal .1999;75:1-9. | 24. Kalogiannis S., Iakovidou G., Kyriakides M.L., Kyriakidis D.A., Skaracis G.N., Optimization of xanthan gum production by xanthomonas campestris grown in molasses. process Biochemistry.2003;39(2):249-256. | 25. Kurbanoglu E.B., Kurbanoglu N.L., Ram horn hydrolysate as enhancer of xanthan production in batch culture of xanthomonas campestris EBK-4 isolate Process Biochemistry 2007;42:1146-1149.