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Review on production of Xanthan gum in batch and continuous reactors

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Abstract: Xanthan is the most fortunate industry level biopolymer with the highest yield of 30 g/l, and also has wide range of applications in pharmaceutical and food industries. Since substrate being the only costliest thing in its production many attempts of using alternative resources such as agricultural wastes as carbon source have been employed successfully and obtained a good range of yield. But still there are various problems out there, which are to be sorted out. Producing xanthan in a batch process gives higher yield but aeration is being the problem as increased rate of culture viscosity limits the availability of oxygen and nutrients. And opting for continuous or fed batch mode sterility maintenance and emergence of fast growing mutants are the major challenges to be faced.
Keywords: Xanthan gum, batch and continuous reactors.

Introduction

Exopolysaccharides are a heterogeneous matrix of polymers comprised of polysaccharides, proteins, nucleic acids and lipids, secreted by micro-organism into the surrounding environment. Microbes produce exopolysaccharides extracellularly, they get accumulated on the microbial cell surface and provide protection to the cells by stabilizing membrane structure from environmental shocks, heat, pH, etc., by this way they are useful for the producing organism. EPS production was first reported in the 1880's. Generally, EPS have often been reported in bacteria and cyanobacteria but they are also reported in marine micro algae, chroomonas species, *Dunaliella salina*, the medicinal mushroom *Phellinus linteus*, yeast, basidomycetes and marine micro-organisms.

Xanthan is a biopolymer synthesized with glucose as a main carbon source mostly by *Xanthomonas campestris*, a gram-negative plant pathogenic bacterium. Xanthan is a secondary metabolite produced by means of microbial fermentation in aerobic conditions. There are also other pathovars that efficiently produce exopolysaccharides; they are pathovar *phaseoli*, *malvacearum, carotae, citrumelo, juglandis* and some organisms belonging to genus *Xanthomonas* like *Xanthomonas fragariae*, *Xanthomonas oryzae*. Xanthan gum is a branched anionic acidic heteropolysaccharide ($>10^6$ Da), main chain comprising of glucose units (D-glucosyl, D-mannosyl and D-glucronyl acid residues in a molar ratio of 2:2:1) whereas side chain is a trisaccharide, consisting of α-D-mannose which contains an acetyl group, β-D-mannose unit linked with a pyruvate group is attached to alternate glucose residues of the cellulose backbone by α1-3 linkages but mostly the external mannose residues a pyruvic acid moiety is joined by a ketal linkage (1). Xanthan solubility in cold water is intensified by the anionic side chains and also soluble in almost acids and bases and scarcely affected by changes in pH. The great advantage is xanthan gum viscosity is unchanged even in the presence of large amounts of salts at high temperature, it is due to the branching make-up of xanthan confers unusual rheological properties than other natural gums like guar gum or locust bean gum. Pyruvic acid and acetyl substitution level varies with bacterial growth and operational conditions and also with various *Xanthomonas* species used. Xanthan molecular structure plays a vital role in its rheological properties.

Xanthan gum is the most fortunate industry level biopolymer with the highest yield of 30g/l and productivities of 0.7g/l are reported and also due to its excellent rheological properties, viscosity and pseudo elasticity, it has wide range of applications as foam enhancer, suspending agents, emulsion stabilizer in food industries, also used in foods such as sauces and dressings, bakery and pastry, meat products, beverages, ice creams, dairy products, toothpastes, cosmetics, cleaning products, oil drilling, coatings, paints and in fire extinguishers. Acetan, produced by *Acetobacter xylinum*is, a biopolymer that is structurally related to xanthan bacteria which also utilize glucose as the major carbon source. Xanthan gum has been preferred in pharmaceutical formulations as a gelling agent, binder and disintegrates. Controlled drug release is essential in order to increase the efficacy of the drug delivery to the target region without causing a toxic effect. Xanthan gum has the potential in retarding drug release to its gelling nature and ability of entrapping the drug within the gel. One of the major applications of xanthan gum is in drug release. Carboxymethylated xanthan is smaller than xanthan and enhance a faster rate of drug delivery and this carboxymethylated xanthan is found to have lower viscosity (2).

Substrate

Materialistic xanthan is reasonably expensive because of the carbon source used, usually glucose or sucrose and high level of naturalness is required for drug and food applications. Various agro-industry deplete can be used as substrate to reduce the production value such as,

- Citrus waste
- Carob extract
- Olive-mill waste waters
- Waste sugar-beet pulp
- Corn steep liquor
- Whey permeate(byproduct of dairy industry)
- Ram horn hydrolysate (waste from meat processing industry)

Table 1: Commercial substrates used for Xanthan production and its corresponding yields.

Substrate	Yield (g/l)	Reference
Glucose	14.744	(3)
Sucrose	13.234	(4)
Soluble starch	12.10	(4)

Table 2: Xanthan yield using Agro industrial wastes as substrates.

Substrate	Yield(g/l)	Reference
Cassava starch	1.86	(5),(6
Sugarbeet molasses	20	(7)
Ram horn hydrolysate	6.3	(8)
Date palm juice	43.35	(9)
Cheese whey	36	(10)
Apple pomace	52.1	(11)
Whey	17.3	(11)
Molasses	17.1	(12)
Cassava Baggase	14	(13)
Chestnut extract	33	(14)
Date extract	11.2	(15)

In industries 2-4 % (glucose or sucrose) is used as a carbon source whereas 0.05-0.1 % (ammonium nitrate, peptone, yeast extract, urea) is used as a nitrogen source. Some papers report sucrose as a best carbon source, whereas in case of nitrogen source there is lot of controversies going on as organic or inorganic source being the best nitrogen source. *Xanthomonas campestris* grow in nitrogen rich media whereas biomass concentration is limited by nitrogen source and xanthan production value is regulated by carbon application. Hence, the required nutrient are different in duo stages namely the growth stage and gum production stage. A new culture media was formulated for high biomass concentration to increase xanthan production that is high

concentration of sucrose (2.5%), glutamate (0.15%), acetate (0.05%), and yeast extract (0.5%) satisfactorily manufactured biomass for xanthan production and this medium was found superior to YM medium economically and technically. Another point to keep in mind is Production medium affects xanthan molecular structure.

Strains Used and Isolated

Xanthomonas campestris NRRL B-1459 belonging to the family Brassicaceae which is a plant pathogenic bacterium is reported to produce xanthan gum in high yield ,Some papers report that isolated strains likes *Xanthomonas axonopodis pv. Dieffenbachia, Xanthomonas arbicola pv. Juglandis, Xanthomonas axonopodis pv. Begonia, Xanthomonas axonopodis pv. vesicatoria* from various infected plant tissue can produce good yield of xanthan gum. Lilly et al.,1951 reported that *Xathomonas.sp* cause black rot disease in the stems leaves or fruits of various plants like cauliflower, lettuce, cabbage, and broccoli (16). And it becomes important to screen for high xanthan yielding species and to optimize the production conditions for best yield of xanthan in large scale.

Table 3: Isolated xanthan producing species and their yield:

Source	Organism	Yield (g/l)	References
Sugarcane leaf	*Xanthomonas albilineans*	1.3	(17)
Cruciferous Plant	*Xanthomonas campestris*	9.75	(18)
Cotton leaves	*Xanthomonas malvacearum*	6.5	(19)
Pepper plant	*Xanthomonas axonopodis pv vesicatoria*	1.3	(20)
Banana petioles	*Xanthomonas sp*	21.8	(21)

Effect of pH

The optimum pH for xanthan production is 7, and it is necessary to maintain the pH level due to organic acids formation during the fermentation time and there is lot of chances for the pH to fall below 5 and due to which there will be a drastic decrease in xanthan productivity. In industries pH level is maintained by adding buffers or base at the required time level by means of pH control probe. The ideal pH for xanthan production is 7 (20, 5), ideal pH for cell cultures are 6-7.5 (22), whereas ideal pH which is followed now for xanthan production is 7-8. pH control may enhance cell growth but not the xanthan production.

Effect of temperature

Many authors reported two different temperature range for growth of inoculums and gum production(23). *Xanthomonas* species are usually inoculated at 27^{o} and pH 6, whereas for gum production that is idiophase is enhanced at 32^{o} and pH 7. To manufacture xanthan with high molecular weight, employing at low temperature is necessary i.e., nearly 25^{o} such that xanthan with high acetate content will be manufactured, whereas during 34^{o}, low viscous xanthan with low acetate content and also a low molecular weight product will be manufactured. There is a conformational change in xanthan depending upon temperature change (24).

Table 4: The effect of pH and temperature in xanthan productivity is summarized below with maximum productivity:

Optimum pH	Optimum temperature	Mode of operation	Productivity(g/l)	Reference
6-7	30-32	Batch	18.6	(25)
7	28	Airlift	15	(26)
6.9	29	Bubble column	-	(24)
7	28	Plugging jet	20	(24)
7	30	Batch	-	(27)
7	30	Batch	4.31	(5)
7	30	-	-	(20)

Aeration rate

Aeration rate is an important desiring factor in xanthan production, as it's an aerobic process oxygen distribution has to be equal throughout the fermenting liquid. For a uniform oxygen distribution stirrer speed has to be optimized. It was found that around 800 rev/min enhances 100% oxygen distribution but xanthan productivity is low when compared to 300 and 500 rev/min stirrer speeds in a 1.5 liter batch fermenter employing a two disc turbine impellers, this may be due to the reason that during high stirrer speed there can be damage to cells decreasing the biomass concentration which in turn decrease the xanthan productivity.

Maintaining a uniform oxygen dispersion to enhance homogeneity of the fluid broth is difficult in batch cultures due to various reasons like shear stress, mutation stability, hence a varied approach of cultivating in immobilized reactors to enhance a larger surface area for oxygen and nutrient mass transfer and which appreciated 55 g/l production but it took a long fermentation time for about 130 hour.

Enhancers

Citrate (0.09-0.18%) stimulates xanthan production inspite of the desired pH range. Pyruvate content is very important in determining polymer performance. Kennedy et al., concluded that increase in nitrogen concentration increases the pyruvilation degree (28). Authors also report that there is more pyruvilation degree when nitrogen source is introduced as organic nitrogen source. Cadmus et al., concluded that $(NH_4)_2HPO_4$ is the best nitrogen source for increasing the pyruvate content (29).

Dissolved Oxygen concentration becomes the rate limiting step and this can be overcome by enhancing the mixing rate but shear stress of microorganism is becoming the major drawback here. Idea of mutating shear resistance strains is also not a good breakthrough as mutation stability is a fragile one. Hence, addition of hydrogen peroxide (H_2O_2) would intensify oxygen supply by breaking hydrogen bonds. Xanthan yield was improved to 2.8% when12.5 mm H_2O_2 was added in shaker flask level whereas in pilot scale level 4.2% (w/w) was achieved ,it also improved xanthan quality by increased pyruvate content (30).

S.Shehni et al., reported that using organic acids in the growth medium shows stimulatory effect on xanthan production by *Xanthomonas* species (31). It was found that using citric acid as chelating agents can prevent salt precipitation during heat sterilization which in turn improves xanthan productivity and acetic acid being the weak carboxylic acid and also xanthan being soluble in acetic acid, using acetic acid can improve xanthan solubility in the solution.

Types of reactors

Immobilized reactors

Yong et al., reported good results upon immobilizing *Xanthomonas campestris* on a support, but most of the produced xanthan is trapped in-between the immobilized beds and is difficult to be extracted and also as already stated mixing becomes the problem due to increasing viscosity in the fermentation media (32). A.Bono et al., did a work with immobilized xanthan producing cells in a packed cotton fiber inside silicon tubing with two modes of operation, i.e., batch and continuous and obtained 18 to 20.6 g/l xanthan yield in batch process, 17.2 to 18.7 g/l xanthan yield in fed-batch process mode (32). Using the fact that xanthan producing cells require different conditions for growth and production, the cells present in fermentation broth was absorbed by fibers present in the packed bed and hence the problem for purifying produced xanthan is reduced to a greater extent. Robinson and Wang in 1988 reported 35 g/l of xanthan production using free cells and 55 g/l yield using immobilized cells with a very long fermentation time up to 130 hours (26). Ju and Zhao used an oil/water dispersion technique to ensure larger surface area for *Xanthomonas* cultivation increasing oxygen transfer rate and obtained 65g/l xanthan yield (26). A.palaniraj et al., reported that attempting with different woven materials like cotton towel, cotton fabric, and 50% cotton and 50% polyester, the result showed that cotton with rough surfaces is the preferred material (28). Rosalam et al. also observed the xanthan gum purity from the continuous recycled bed reactor was found to be greater than 98% (32). Lo et al. also reported the same finding that the cells free xanthan gum broth by immobilizing in fibrous matrix supports did not significantly foul the membrane during the entire period (33).

Batch and continuous Reactors

Batch reactors require totally 2 days for cultivation and growing viscosity of the culture during fermentation affects availability of oxygen and nutrients which is the major problem.

Molecular weight of xanthan is 2×10^6 to 5×10^7 Da, but while using a batch reactor the intrinsic viscosity of xanthan is lesser at 35^o (34) and the variations in the fermentation condition in xanthan production

may influence its molecular weight as reported by F.Gracia-ochoa et al., (35). P.Prasertsan et al., studied the optimal cultivation parameters for exopolysaccharide production and concluded that batch cultivation gave better results than continuous or fed-batch mode in laboratory scale which can also be extended to industrial level production, they also suggest 3%w/v sucrose to be a better carbon source than glucose and the optimum growth conditions to be 30 degrees and pH 7 (36).

To maximize the cell production, continuous recycling system is opted since it increases the substrate conversion rate and followed in industries widely. In a continuous system there is a continuous supply of substrates and with drawl of fermentation broths hence, maintaining the reactor volume constant is one of the major problems faced in continuous reactors, maintaining sterility and the risk of emergence of fast growing mutants that do not produce desired products are other commonly faced problems.

M.Stredansky et al., studied xanthan production in solid state fermenter systems, various solid substrates were employed mostly of agriculture wastes such as spent malt grains, apple pomace grape pomace, citrus peels and the maximum productivity was observed with using apple pomace as substrate (54 g/l) but the pectin content in apple was found to be the contaminant and disadvantage of using this as substrate (8)

Reviewing the above works, batch mode is preferable for xanthan production even though it has slight disadvantage.

Influence of impellers

A.Amanullah et al., studied the influence of various type of impellers in xanthan bio production, designing a bioreactor for high viscous fermentation broth giving a high yield stress is the most challenging outcome in industries.(26) Especially in xanthan production problems of low oxygen mass transfer and poor bulk mixing is faced along with another major problem of purifying xanthan which comes under down-stream processing column, when yield stress fluids are subjected to agitation there are chances for a zone of significant motion called cavern around the impeller to be formed as reported by Elson et al.,(26). Hence the fluid properties, power input, agitation speed becomes the rate limiting factors for fermentation design. Xanthan is usually produced in stationary phase and Rushton turbines are preferred mostly in industry level because of their good gas dispersion rate, but due to its high power consumption rate large diameter impellers with low power number like axial flow Prochem Maxflo T operated in downward pumping mode and the radial flow Scaba 6SRGT impellers with turbulent power number of 1.8 were studied. Highest xanthan productivity of 0.86 g/l/hr. was obtained with SRGT impellers, but PMD was the best in low power consumption when compared with SRGT, power input was 14% lower than SRGT/unit.

Xanthan Recovery

The main steps in recovery are removal of cell lysis, precipitation of biopolymer, dewatering, drying and milling. Most important thing in these methods is it should be followed without degrading biopolymer. Various techniques like chemical method, mechanical method, enzymatic method can be employed each one with its own advantage and disadvantage. Precipitation is followed by acetone, ethanol, or methanol and also measurable amount of salts like calcium; ammonium can also be used in precipitation. Advanced technique in recovery process was reported by lo et al., he developed ultrafiltration technique as an alternative method for alcohol precipitation even in dilute fermentation broths, since UF didn't affect xanthan's rheological properties this method can be followed efficiently and this reduces the alcohol needed for precipitation by 80% (33). Enzyme treatment is the costliest process ever followed and not suitable for industry level recovery process, mechanical treatment also needs high power input which is not feasible, hence to conclude using simple salts and solvents, thermal treatments (80–130^{o}C, 10–20 min, pH 6.3–6.9) enhances xanthan dissolution without thermal degradation and disruption of cells is observed.

Advanced Applications

Xanthan is reported as GRAS by United States drug and food administration on the basis of toxicology tests in human foods. Xanthan gum improves the viscosity, water holding, flavor releasing like properties in food and this is a gum with non-Newtonian property that exhibits a good pesudoplastic behavior, hence used mainly in salad dressings(0.1-0.5%),bakery products to increase the water binding properties (0.05-0.3%), prepared foods (0.1-0.3%). Besides many food applications it has a vital role in industries to maintain long term emulsion and suspension stability in salt, acid and alkaline solutions, oil recovery processes. Xanthan also finds application in our day to day products like toothpastes, cosmetics, cleaning products, coating and paints. The most advanced application of xanthan gum is controlled drug delivery system in pharma industries.

Xanthan gum in soil strengthening

To improve soil properties like aggregate strength, erosion resistance and stability xanthan gum is used. Since being an eco-friendly product it is preferred largely, studies show that xanthan strengthens the high graded soil effectively and the strengthening effect depends upon hydration property of the soil. While forming Xanthan matrices, the xanthan gum fibres interact with charged clayey surfaces that resemble a hard plastic between uncharged particles (37).

Conclusion

Glucose is the best carbon source giving maximum yield of 14.74 g/l, other alternatives like sugarcane, cassava bagasse also gives good range of yield. Maintaining optimum pH and temperature is very important to obtain a reasonably good yield of xanthan. Aeration is another important factor which is to be monitored. Besides these important factors, some enhancers like citrate, hydrogen peroxide also improves xanthan yield to a greater extent. Xanthan has a wide range of applications in industries hence various techniques like using a starch rich waste as an alternative substrate, employing inducers, isolating various strains that can give higher yield than *Xanthomonas campestris* are some of the areas that have to be concentrated for producing xanthan in low budget range for making a good profit in industries.

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