Microbial production of Green polymer Xanthan Gum from Xanthomonas Campestris

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Abstract: Microbial polysaccharides are biothikners mostly added in wide variety of products as a viscosifying, Stabilizing, emulsifying, gelling agent. In conventional cultivation method, air is used as source of oxygen for the fermentation process. During xanthan gum fermentation, formed gum increases viscosity of fermentation broth. Novel cultivation methods have been successfully developed in laboratory with hydrogen peroxide as oxygen source to eliminate gas-liquid mass transfer resistance in the xanthan gum fermentation broth. The experiments were carried out using wild type cell and HOCL treated cells. The experiments shows productivity increases from 0.126 g of xanthan gum per g. of cell for wild culture with H_2O_2 based cultivation (110%).Viscosity of xanthan gum increased about folds from 65 cP (wild culture with aeration) to 122.5 cP (HOCL treated with H_2O_2 based cultivation).Thus experimental results shows that the improvement in xanthan gum quality and yield can be obtained using HOCL treatment and oxygen supply strategy.

Keywords: Xanthan gum, Microbial polysaccharides, cultivation, viscosity

1. Introduction

Xanthan Gum is the first microbial polysaccharide produced by the culture fermentation of xanthomonas campestris on a polysaccharide backbone. This polymer consist of pentasaccharide repeating units

containing D-Glucose, D-Mannose, D-Glucuronic acid, Acetyl linked pyruvic acid

and d- Acetyl groups [1]. Xanthan gum biopolymer is different than other microbial exopolysaccharides not only in its chemical

structure but also in its excellent rheological properties in aqueous solutions. Number of

strains used to produce xanthan gum like X. campestris, X.phaseoli, and X. malvacearum. Among these Xanthan produced by X.Campestris is having the good rheological properties which are having commercial significance. Gum is used in many industries like food, cosmetics and medicine etc, because of its physical, chemical, rheological and functional properties. Many of the researchers studied the effect of share rate, temperature and concentration on the viscosity of xanthan gum [2].The gum also has unusual stability to temperature, pH, shear, enzyme degradation and excellent compatibility with

salts over a wide pH range. Xanthan gum having much application due to its rheological properties. It is used as suspending, stabilizing and thickening agent in the food industry. It is also used as emulsifier, lubricant, thickening agent or mobility control agent in enhanced oil recovery [1].Many of the xanthan gum production processes are energy-intensive and costly because the high viscosity of xanthan solution limits the final xanthan

concentration in the fermentation broth below 3 % (W/V) [3].The worldwide market of xanthan is estimated at between 600 to 800 million dollars per year and growing at a annual rate of 6-7 %. To maximize given amount of xanthan gum in minimum time. Optimal control procedure that uses the model proposed by Shu and Yang to compute a temperature operating policy [4]. Xanthan have been used in food and pharmaceutical industries due to its toxicological and safety properties. Xanthan is non-toxic and does not inhibit growth [5]. The main objective of this work is to study effects of various cultivation

methods on the rheology and quality of xanthan gum . The main work will involve the testing of the novel liquid phase H_2O_2 based oxygen supply strategy and cell treatment method and its effect on production and rheological properties of the gum. However, use of H_2O_2 based oxygen supply strategy and cell treatment method and its effect on productivity and rheological properties of the gum [6]. However, use of H_2O_2 may cause reactive oxygen species (ROS) formation. To overcome the ill effects of ROS, cultivations will be performed with antioxidants such as ascorbic acid (Vitamin C) and tocopherol (Vitamin E). In previous study (Manjula 1999) it was observed that ROS has impact on productivity and quality of gum. Attempt will be made to study the effect of this intracellular free radical (ROS) level on xanthan gum productivity and rheology. This work is mainly focused on oxygen supply limitation decreases the cell

growth and results in poor quality xanthan gum [8]. So a liquid phase oxygen supply strategy using H_2O_2 as a oxygen source, will be employed to study the effect of various cultivation methods on the rheology of the gum.

2. Material and Methods

2.1 Microorganism and inoculum preparation:

The microorganism used for the study was Xanthomonas campestris (MTCC 2286,
Chandigarh, India) producing Xanthan gum. The cultures were maintained at 4^o C on agar slants containing 20 g L^{-1} glucose, 20 gL^{-1} agar and 10 gL^{-1} $CaCO_3$ and 10 gL^{-1} yeast extract. It was observed for maintaining the slants, if we add magnesium and Potassium salts (K_2HPO_4 and $MgSO_4.7H_2O$) in the medium, it significantly inhibits the growth of Xanthomonas campestris. The growth medium consisted of 40 gL^{-1} Glucose, 8 gL^{-1} yeast extract, 5 $gL^{-1}K_2HPO_4$, 4 gL^{-1} $MgSO_4.7H_2O$ and 0.4 gL^{-1} urea in tap water (Manjula 1999). The medium was sterilized by autoclaving without glucose before inoculation. The PH of the glucose solution was adjusted to 4.0 by addition of acid. The glucose solution was autoclaved and added .
to medium under aseptic conditions in laminar hood.

2.0 Analytical Methods

2.1. 1HOCl Treatment:

The HOCl treatment procedure for Xanthomonas campestris, was adopted from
Manjula (1999) cells in the exponential growth phase, at a concentration of 2 g/L, were resuspended in 0.05 M phosphate buffer freshly prepared hypochlorous acid the concentration of which was determined
by iodometry, were added to cultures in three stages. Each stage consisted of a 60
seconds HOCL exposure after which cell was removed from the HOCl containing treatment medium by
centrifugation. Then the cell were incubated in normal growth Medium for 40 min dark at 30^o C with shaking The concentration of HOCl used for exposure at the beginning of each stage were: 1st stage: 0.66 m mole/g cell, 2nd stage: 4.65 m mole/g cell,3^{rd} stage: 20 m mole/g cell After third incubation the
free chlorine left in the flasks was quenched by using sterile sodium thiosulphate culturable bacteria were assayed by plating on agar slants.

2.2 Cell Concentrations:

The cell concentration was measured using a spectrophotometer (JASCO, v-530 UV/VIS
spectrophotometer) through cell scatter at 630 nm, using calibration curve of cell concentration versus optical density. The liquid samples were centrifuged to separate cell from the liquid medium. The centrifugation is done at 8000 rpm for 15 minutes and cells are washed with saline and resuspended in saline for OD measurement. Cell density was determined from optical density was determined from optical density
at 630 nm (OD_{630}) as measured with 1.5 ml cuvette and a spectrophotometer.

2.3 Xanthan Gum Concentration:

The Xanthan concentration in fermentation broth was measured by using gravimetric technique described by flahive et al (1994) and Amanllha et al (1996). The gum concentration in broth was determined through dry weight measurement.
The cell in known volume of fermentation broth was separated by centrifugation at 8000 rpm for about 15 minutes. Then the supernatant containing Xanthan gum was isolated. Two
volumes of isopropyl alcohol and 10% V/V of 2% W/V of KCl solution were added to supernatant to separate out Xanthan gum. The sample was centrifuged at 9000 rpm for about 45 minutes to separate Xanthan gum.

The precipitated Xanthan gum was then dried in dryer at 60 ^{o}C for 24 hours and dry mass of Xanthan gum was measured and concentration was calculated.

2.4 Rheology:

The rheological behavior of the gum produced under different cultivation methods was studies by measuring viscosities of the gum at different shear rates. Viscosities of Xanthan gum was measured using digital Brookfield LVT viscometer equipped with a number 18 spindle and chamber 13 R at 3, 6, 12, 30 and 60 rpm. The concentration of 0.1 % was used for the measurement at 30 ^{o}C for viscosity measurement by Brookfield

viscometer. After addition 3.0 gm within 45 s to 90 s into 250 ml of a 12 gL^{-1} solution of KCL R in 500 ml beaker stirred with low-pitch propeller-type stirrer rotating at 800 rpm 44 ml of water R was added to rinse any adhering residue from the walls of the beaker. Preparation was stirred about 2 h whilst maintaining the temperature at 24±1 ^{o}C viscosity is determined by viscometer. The results of the viscosity measurement of Xanthan gum produced under different cultivation condition are reported in next chapter. These conditions employed are similar to those employed by Randal et al. (1990) for standardization purpose viscosity of 99.9% glycerol solution was measured at 30 ^{o}C and 1.5 and 3 rpm.

2.5 Fermentation:

Experiments were carried out in a bioreactor under different cultivation method with wild type and HOCl treated culture using conventional as well as H_2O_2 based oxygen supply strategy. In previous study (Manjula 1999) it was postulated that the reason for less that expected increase in Xanthan gum productivity during H_2O_2 based cultivation is due to deleterious effect of free radical generated when H_2O_2 reacts with metals such as iron in the cell. So we have carried out experiments with well known antioxidants ascorbic acid and tocopherol, which can alter free radical levels in the cells so that the effect of these antioxidants on the productivity and properties of Xanthan gum can be studied. Also the antioxidant chosen in this study namely ascorbic acid is water soluble and can be effective against free radicals cytoplasm and in medium tocopherol is lipid soluble and can be effective against free radical in cell membrane region.

3.0 Experimental Set-up:

Experimental set-up mainly consists of two parts, one is the lab scale, a three necked round bottom 500 ml capacity borosil glass flask was used as a reactor and other is data acquisition system is used as data recoding. A temperature sensor was inserted in a thermo well with mercury for intimate contact. A stirrer passing through central wide neck was provided with a glass turbine impellers and controller for impeller speed and reactor temperature.
Airflow rate was maintained at 3 rpm (for conventional cultivation method) and 300 rpm impeller speed. For H_2O_2 based cultivation, the addition of hydrogen peroxide was done manually or by using feed pump. The air is passed through the alcohol bottles, which was used for sterilization purpose. The dissolved oxygen (D.O.) data were obtained online using probes through data acquisition and control system. For the calibration of D.O. probe, in the first step, nitrogen was passed through the medium. It droves out the dissolved oxygen from the medium. After attaining a steady state lowest value the system was set to read zero. In the second step air at the same rate and at the same rpm (the same conditions for the cultivations) was passed through the medium. The change in D.O. level was recorded with data acquisition system at specified time interval. After attaining steady value (after saturation of medium with air) the system was set to read 100%.

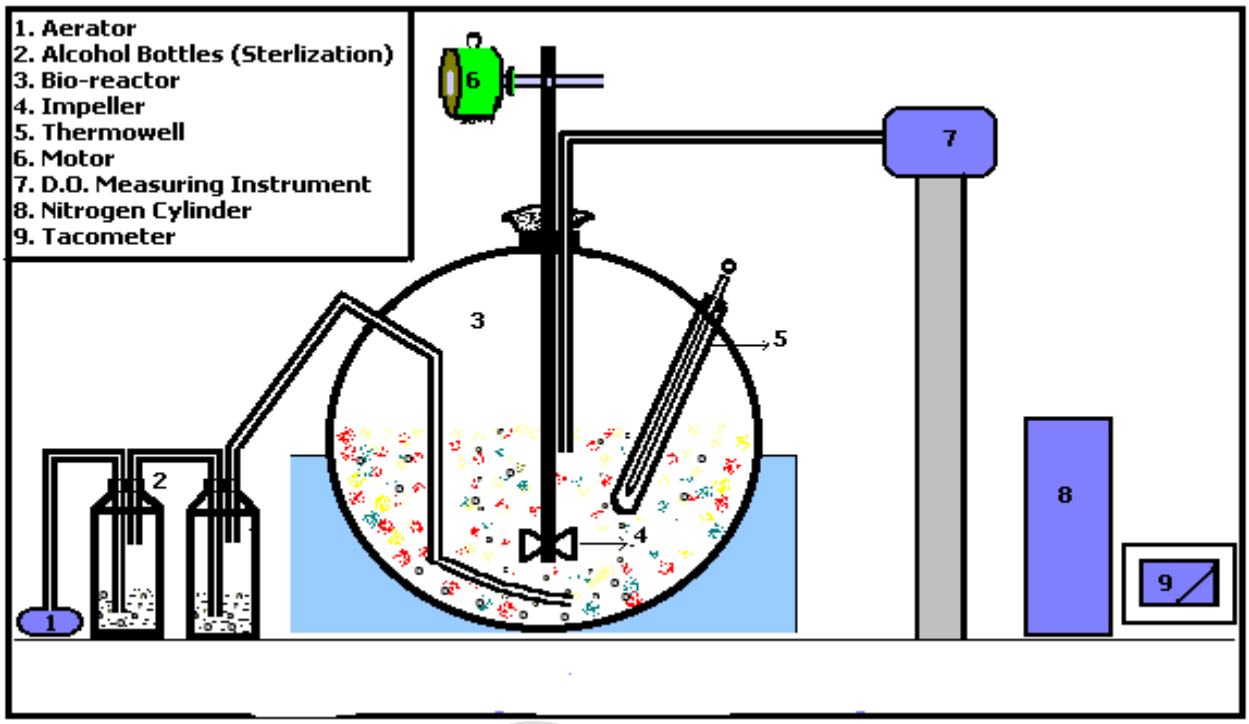

Fig no:1 Experimental Setup

4.0 . Results and discussion

4.1 Reproducibility:

The reproducibility of the experimental results is an essential prerequisite for a successful systematic study of the influence of different cultivations methods and conditions on the fermentation performance and subsequently on the product properties and quality. The lack of reproducibility in xanthan gum fermentation has been commented up earlier and arises mainly due
to from inconstancies in inoculum

preparation and strain instability (Jeans et.al. 1976, Kidby et.al. 1977; Galindo et.al. 1994). Quality is particularly important in the production of xanthan gum which can be readily measured by its rheological character. Since the end application of the gum is mainly dependent on this parameter. It is important to be able to obtain reproducible gum rheological characteristics.

4.2 Effect of share rate and cultivation conditions on xanthan gum viscosity

Table no 1 and 2 gives the viscosities of xanthan gum obtained with wild type culture in conventional and H_2O_2 based cultivation
respectively, repeated with same physiological conditions. It can be observed
from the figure 1 and 2 and table 1 that variations in viscosities of xanthan gum obtained from the repeat runs, is more pronounced at low shear rate where as at high shear rate viscosity of gum is virtually same. The viscosities of gum obtained from conventional cultivation of wild culture are 120.0 cP and 150.6 cP (Run 1 and Run 2 respectively) at a shear rate of 3.96 S^{-1} and
17.25 cP and 18.9 cP (run 1 and Run 2 respectively) at a shear rate of 79.2 S^{-1}. The

variation in viscosities of the gum a shear rate of 3.96 S^{-1} is about 11% and that of at 79.2 S^{-1} similarly, the viscosities of the gum obtained from H_2O_2 based cultivation of wild culture are 170.0 cP and 198.3 cP (Run 1 and Run 2 respectively) at a shear rate of 3.96 S^{-1} and 18.5 cP and 22.3 cP (Run 1 and Run 2 respectively) at shear rate of 79.2 S^{-1}. The variation in viscosities of the gum at 3.96 S^{-1} is about 8.0% and that of at 79.2 S^{-1} is 9.0%.

Table 1. Viscosities of the xanthan gum obtained from wild type culture with conventional and H_2O_2 based cultivation under the same conditions.

RPM	**3**	**6**	**12**	**30**	**60**
Shear Rates (S^{-1})	3.96	7.92	15.84	39.6	79.2
Cultivations	Viscosity (CP)				
Wild (aeration) Run 1	120	65.0	39.5	22.5	17.25
Wild (aeration) Run 2	150.6	70.6	42.6	26.6	18.9
Wild (H_2O_2) Run 1	170	90	35.5	22.5	18.5
Wild (H_2O_2) Run 2	198.3	104.3	39.3	30.3	22.3

4.3 Effect of cultivation conditions on cell growth and concentration

Figure 2 gives results of cell growth for wild type culture in conventional and H_2O_2 based cultivation, repeated under the same conditions. It can be seen from the figure that the results of cell growth for repeat experiments are reproducible. The variations in final cell concentration, final xanthan gum concentration and growth rate constant is given in Table 2 & 3 respectively. The final cell concentration for wild type with aeration is 7.02 g/L (Run 1) and 6.33 g/L (Run 2). So variation in results 15 $\pm$ 5% for wild type with H_2O_2 based cultivations the final cell concentration is 3.5 g/L (Run 1) and 3.03 g/L (Run 2) so variation in results is about $\pm$7%.]

Table 2: Reproducibility of productivities, Gum concentration and final cell concentration obtained from different cultivations.

Cultivation	Final Cell Conc. (g/L)	% Variations	Gum Conc. (g/L)	% Variations
Wild + Air Run 1	7.02		0.88	
		Approx.		Approx.
Wild + Air Run 2	6.33	5%	0.93	3%
Wild + H_2O_2 Run 1	3.50		2.13	
		Approx.		Approx.
Wild + H_2O_2 Run 2	3.03	75	1.93	5%

Table 3: Reproducibility of growth rate obtained for different cultivations.

Cultivation	Kh^{-1}	% variations
Wild + Air Run 1	0.28	--
Wild + Air Run 2	0.28	
Wild + H_2O_2 Run 1	0.13	Approx.
Wild + H_2O_2 Run 2	0.16	10%

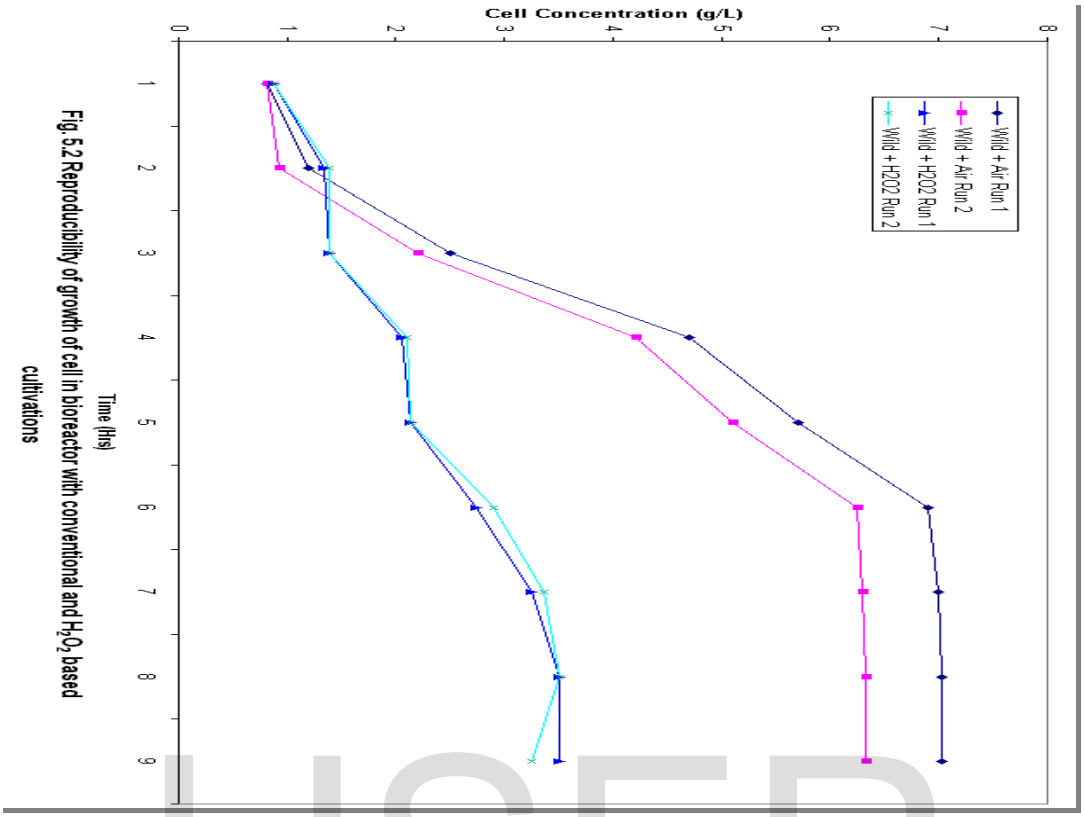

Fig. 5.2 Reproducibility of growth of cell in bioreactor with conventional and H_2O_2 based cultivations

5.0 Conclusion

A novel hydrogen peroxide based oxygen supply strategy was successfully employed for the production of Xanthan gum from Xanthomonas campestris.HOCL treatment of culture makes it more tolerant towards hydrogen peroxide so that more hydrogen peroxide can be used for Cultivation. Hydrogen peroxide based cultivation of wild culture improves productivity of Xanthan gum from 0.126 g of Xanthan gum/g of cells to 0.609 Xanthan gum per gm of cells (4 fold increase). HOCL treated culture with hydrogen peroxide based cultivation showed highest growth rate constant (K=0.34 h^{-1}) as well as increase in Xanthan gum production. Hydrogen peroxide based cultivation of HOCl treated culture improved viscosity of Xanthan gum from 65 cP to 122.5 cP (about 2 fold increase). Also gum produced from this cultivation showed highest shear thinning behavior and consistency index which is reflected by lowest value of n (n=0.1) and highest value of k (k=727.1 M pascal S^n) respectively.

6.0 References

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