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# INFLUENCE OF NITROGEN SOURCES AND AGITATION IN XANTHAN GUM PRODUCTION BY *XANTHOMONAS CAMPESTRIS*

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## ABSTRACT

Xanthan gum is a hetero polysaccharide produced by *Xanthomonas campestris*. Xanthan gum fermentation by a local isolate of *Xanthomonas campestris* NCIM 2961 using different nitrogen sources were studied in both batch and fed-batch fermentations. The production of polysaccharide was influenced by the nitrogen sources used. Yeast extract was found to be efficient nitrogen source for the production of the xanthan gum. The production of xanthan was 3.6 g/L in batch fermentation and 5.2 g/L in fed batch fermentation. Xanthan production increased with the increase of yeast extract concentration, probably due to the facilitated nitrogen uptake. Optimal pH was pH=7.0, and sufficient supply of oxygen was needed for the xanthan gum production.

**Keywords:** Polysaccharide, biopolymer, hydrolyzed rice, pseudoplastic.

## [I] INTRODUCTION

Xanthan gum is an extracellular hetero polysaccharide produced by *Xanthomonas campestris*. Because of its unique rheological behaviour, xanthan gum is one of the major microbial polysaccharide actually employed in many industrial processes. Solutions of xanthan gum are highly pseudoplastic and show very good suspending properties [1]. The polysaccharide is used as suspending, stabilizing, thickening and emulsifying agent, for food and non-food industrial applications [2].

Dextran, discovered in early 1940s, was the first microbial polysaccharide to be commercialized. The second microbial polysaccharide commercialized was xanthan gum. It is a natural polysaccharide and an important industrial biopolymer. It was discovered in 1963 at Northern Regional Research Center (Now called The National Center for Agricultural Utilization Research) of the United States Department of Agriculture (USDA) [3]. The polysaccharide B-1459, or xanthan gum, produced by the bacterium *Xanthomonas campestris NRRL B-1459* was extensively studied because of its properties that would allow it to supplement other known natural and synthetic water-soluble gums. Substantial commercial production began in early 1964. The toxicological and safety properties of xanthan gum for food and pharmaceutical applications have been extensively researched. Xanthan is non-toxic and does not inhibit growth. It is non-sensitizing and does not cause skin or eye irritation. On this basis, xanthan has been approved by the United States Food and Drug Administration (FDA) for use in food additive without any specific quantity limitations [4].

Xanthan has particularly complicated molecular structure. Its main chain consists of glucose molecules connected by β-1,4 glycosidic links and is similar to that of cellulose. Every second glucose unit carries a side chain which is composed of β-D-mannose, β-1,4-D-glucoronic acid and α-1,2-D-mannose together with a pyruvic acid unit [5]. Xanthan molecular structure is often reported to be heavily affected by the composition of the production medium. In this respect, several studies have so far focused

on a variety of nutrients, particularly the nitrogen and carbon sources, with glucose and sucrose as the most frequently used carbon sources [6,7].

Most commercial production method for xanthan gum uses glucose or invert sugars, and most industries prefer batch processes than continuous processes [8]. Other substrates have also been tested, such as sucrose, hydrolyzed rice, barley, corn flour, acid whey, sugar cane molasses, coconut juice, sugar cane, etc., but glucose is still the best in terms of product yield, supply, and product quality [5, 9-11]. It has been found that the production and the properties of xanthan gum are influenced by bacterial strain [12-13], culture medium [8, 14-15], temperature [16-17], pH [18], time of fermentation [19], and agitation rate [20-21].

The purpose of this study was to optimize the xanthan gum production by *Xanthomonas campestris* with respect to nitrogen sources and effect of agitation.

## [II] MATERIALS AND METHODS

### 2.1. Microorganism and inoculum preparation

*Xanthomonas campestris* NCIM 2961 was obtained from NCIM, Pune, and used throughout this study. The strain was maintained on nutrient agar slant containing (g/L) glucose 10; Malt extract 3; Yeast extract 3; and Peptone 5; pH=7 grown at 30 °C for 24 hours and stored at 4 °C. Actively growing cells from a newly prepared slant were inoculated into the liquid medium in 250 ml Erlenmeyer flask. The culture was incubated at 30-35 °C for 24 hours in an incubator shaker. The liquid culture was used to inoculate the final fermentation medium.

### 2.2. Fermentation experiments

All the fermentation experiments were conducted in a 2L bioreactor (Biostat-B, Sartorius, Germany). The production medium was composed of glucose (25 g/L), yeast extract (3.0 g/L), $KH_2PO_4$ (2.0 g/L), $MgSO_4$ (1.0 g/L) and antifoam agent (0.1 mL/L). The fermentation medium without the carbon source was sterilized in the fermentation vessel. The carbon source was sterilized separately and then aseptically introduced into the vessel. During fermentation runs, the dissolved oxygen concentration was maintained at 10-30 % of saturation value by increasing the stirrer speed as needed, while keeping constant 1 vvm (air volume/medium volume/minute) airflow rate. Temperature was maintained constant at 28 °C for 24 h. The pH of the medium was held at pH=7.0 by adding 1 M HCl/1M NaOH. During the process, the concentrations of the cells, glucose, and xanthan gum were measured in the culture medium in five samples of 50 mL each. All fermentation runs were conducted in triplicate.

### 2.3. Determination of biomass and xanthan gum concentration

The fermentation broth produced by the batch processes were diluted using distilled water to lower the viscosity, and then 20 mL aliquots were transferred into micro centrifuge tubes. The micro-centrifuge tubes containing aliquots were centrifuged at 10,000 rpm for 30 minutes at 4 °C. After centrifugation, two fractions were formed, supernatant containing xanthan gum, and biomass deposited as a pellet. The biomass pellet was resuspended with water for washing and then recentrifuged to reprecipitate the biomass. The biomass deposited at the bottom of tubes was dried in the oven at 60 °C for two hours and weighed to get the dry mass to show the relative performance of the cotton fibre in retaining the cells. Supernatants were mixed with 2/3 (v/v) isopropanol, re-centrifuged at 10,000 rpm for 45 minutes at 4 °C to completely precipitate xanthan gum before removing the solvent and water from the top portion. The precipitated xanthan gum collected from all samples was dried overnight in the oven at 50 to 60 °C in the pre weighed micro-centrifuge tube.

The concentration of xanthan gum was determined as the dry weight of xanthan gum per litre culture medium.

## [III] RESULTS AND DISCUSSION

### 3.1. Effect of nitrogen sources

*Xanthomonas campestris* NCIM 2961 was cultivated in the medium containing various nitrogen sources such as urid dhal, toor dhal, green gram, soybean meal and yeast extract. When the cells were grown in the yeast extract containing medium, the xanthan production (3.6 g/L) was the highest among those tested shown in **[Figiure-1]**. The xanthan produced (2.7 g/L) from urid dhal and the other nitrogen sources was much lower than that from Yeast extract shown in **[Table-1]**.

| Nitrogen sources | Xanthan gum yield (g/L) | Biomass production (g/L) |
|---|---|---|
| Urid dhal | 2.8 | 6.2 |
| Toor dhal | 2.1 | 6.3 |
| Green gram | 2.4 | 6.5 |
| Soybean meal | 2.5 | 5.3 |
| Yeast extract | 3.6 | 5.6 |

Table 1. Yield of xanthan gum from various nitrogen sources by batch fermentation

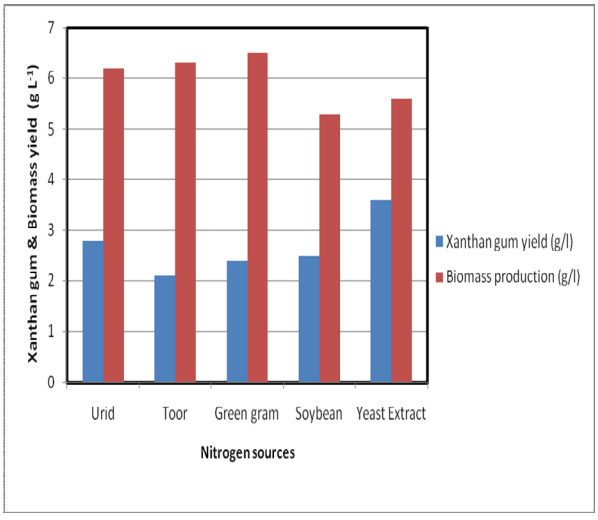


Fig. 1. Effect of nitrogen sources on xanthan gum production by Batch fermentation

We further examined the effect of nitrogen concentration on the xanthan production since there have been reported broad ranges of optimal concentration depending on the microorganisms. It is interesting to see that the maximum xanthan production obtained at 30 g/L as the initial yeast extract concentration increased from 10 to 40 g/L shown in **[Figure-2]**.

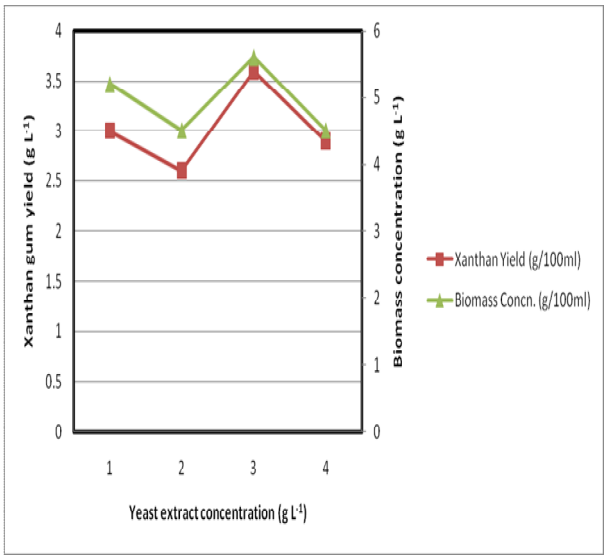


Fig. 2. Effect of Agitation on Xanthan gum production

### 3.2. Effect of agitation

The kinetics of growth and xanthan production by *X*anthomonas *campestris* NCIM 2961 in batch culture was studied in laboratory fermenter. Fermentations were carried out over a range of stirrer speeds from 100 to 600 rpm, which covered the entire range of stirrer speeds practical for the bioreactor used. **[Figure-3]** shows the fermentation carried out at 300 to 600 rpm. Cell dryweight, and xanthan concentration are presented over a time course of 72 h. Increased agitation levels resulted in higher production levels xanthan production almost doubled as the stirrer speed increased from 300 to 600 rpm and similar was the effect on cell growth. Growth and production were low and rather similar for the two lower speeds of 300 and 400 rpm. Xanthan production appeared slightly higher at the end of fermentation (72 h) at 400 rpm compared with

300 rpm production levels on increasing the stirrer speed beyond 400 rpm, xanthan production appeared to depend on agitation, with significantly increasing concentrations with increased stirrer speeds. The optimum agitation was found to be at 500 rpm and the yield of xanthan was 3.62 g/L.

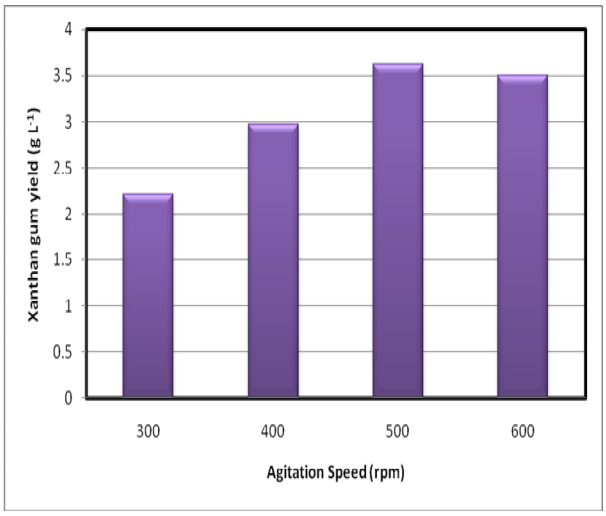


Fig. 3. Xanthan gum yield and biomass production from yeast extract

### 3.3. Batch fermentation

To examine the pattern of the xanthan production in detail, batch fermentations were carried out in a jar fermentor. **[Figure-1]** shows the yield xanthan gum from various nitrogen sources used in batch fermentation at 500 rpm. The yeast extract gave the maximum production of xanthan, 3.6 g/L at 500 rpm. Xanthan production showed the same profile with the cell growth. It was also noted that no further xanthan was produced after nitrogen sources were exhausted and the yeast extract concentration fell below 40 g/L shown in **[Table-2]**.

| **Yeast extract concentration (g/L)** | **Xanthan gum yield (g $L^{-1}$)** | **Biomass production (g/L)** |
|---|---|---|
| 1 | 3.0 | 5.2 |
| 2 | 2.6 | 4.5 |
| 3 | 3.6 | 5.6 |
| 4 | 2.9 | 4.5 |

Table 2. Yield of xanthan gum and biomass production from yeast extract

### 3.4. Fed-batch fermentation

Results obtained from the batch cultivation led us to carry out a fed-batch fermentation to promote the xanthan production by adding glucose and yeast extract before they declined at certain concentrations. Dissolved oxygen concentration was also maintained above 10 % air saturation during the xanthan production by shift-up the agitation speed. Cell concentration and xanthan concentration continued to increase when sufficient nitrogen and carbon were supplied. The yield of xanthan gum produced from fed batch fermentation was 5.2 g/L. In the fed batch mode of fermentation the addition of substrates at regular intervals improved the xathan production. This was due to the reduction/elimination of catabolite repression due to the controlled feeding of substrate which would otherwise had turned on the fermentative path way leading to fermentative products because of excess of glucose in the medium.

## [IV] CONCLUSION

The optimization and production of the xanthan gum with different nitrogen sources such as the urid dhal, toor dhal, green gram and yeast extract were compared and yeast extract showed highest xanthan gum production of 0.36 g/100 mL while Urid dhal gave 0.27g/100 mL, toor dhal gave 0.22g/100 mL, and green gram gave 0.21g/100 mL.

Different agitation speed such as 300 rpm, 400 rpm, 500 rpm, and 600 rpm was optimized for xanthan gum production with yeast extract and the optimum production was found at 500 rpm.

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