The International Journal Of Engineering And Science (IJES)
||Volume||2 ||Issue|| 5 ||Pages|| 52-55||2013||
ISSN(e): 2319 – 1813 ISSN(p): 2319 – 1805

Optimization And Production Of Xanthan Gum By *Xanthomonas Campestris* NRRL-B-1449 From Sugar Beet Molasses

[1,]A. V. Gomashe, [2,]P.G. Dharmik , [3,]P.S. Fuke
[1,2,3], *Department of Microbiology , Shri Shivaji Science College, Congress Nagar, Nagpur-440012, M.S. India*

----------ABSTRACT----------
Xanthan gum is a natural polysaccharide having an industrial importance. It is commonly called as heteropolysaccharide which is produced by Xanthomonas campestris. In the present investigation Xanthan gum fermentation by local isolates of Xanthomonas campestris NRRL-B-1449 from sugar beet molasses using different nitrogen sources were studied in both batch and fed-batch fermentation. The results of the study revealed that the production of Xanthan gum is influenced by using different nitrogen sources. Xanthan production increased with the increased yeast extract concentration, probably due to facilitated nitrogen uptake. The optimum pH was found to be 7.0 and sufficient supply of oxygen was needed for the Xanthan gum production.

KEYWORDS: *Xanthan gum, Xanthomanas campestris*

Date Of Submission: 06 May 2013 Date Of Publication: 28,May.2013

I. INTRODUCTION

The polysaccharides or gum has novel and unique flow properties in food processing and in large number of industrial operations. The newly developed method for the production of polysaccharide is the fermentation process instead of their extraction from plant and animal sources. Biopolymers are of having great significance and one of them is extracellular polysaccharide. Extracellular polysaccharide can be separated into homopolysaccharide and heteropolysaccharides.

Most of the organisms produce gum due to variable physiological condition. Many of photogenic bacteria are resistance to desiccation and can survive under dry condition at normal temperature for more than 50 years due to protecting layer. This layer is chemically polysaccharide. *Xanthomonas campestris* is one of the example of gum producing organism which produce Xanthan gum, a producer of useful polymer affecting the physic mechanical condition of products through thickening, stabilizing, jellifying and emulsifying [Konicek J. et al.1993].It was approved for the use in foods after extensive animal testing for toxicity in 1968 and accepted as a safe food additive in USA.

Xanthan gum was discovered by an extensive research efforts by Allene Rosalind Jeans and her research team at Northern Regional Research Laboratories (NRRL) of the United State Department of Agriculture[Gills P.S.et al.2009].Xanthan gum derived its name from the strain of bacteria used during fermentation process i.e. *Xanthomonas campestris.* This bacteria form slimy substance which acts as a natural stabilizer. It is heteropolysaccharide having pentasaccharide units formed by 2 moles of glucose, 2 moles of mannose and 1 mole of glucuronic unit (2:8:2:2).

It can be used in pharmaceuticals. It is non-toxic, non-sensitizing and does not cause any skin and eye irritation. Xanthan gum produced by *Xanthomonas campestris* NRRL-B-1449 is widely studied because of its properties that allow it to be suitable alternative for other known natural and synthetic water soluble gums [Kurbanoglu E.B.et al.2007]. *Xanthomonas* is the genus of *Pseudomonas* family. It occurs as a single rod 0.4±0.7mm wide and 0.7±1.8mm long. It is motile, gram negative have single flagella. The bacterium cannot denitrify and it is catalase positive, oxidase negative. The colonies are yellow, smooth and viscid [Marzieh Moosavi et al.2010]. *Xanthomonas campestris* grow on standard lab media and several Strain variation have been observed in continuous and batch culture [Cadmus et al.1978][Jeans et al.1976][Kidby D et al.1977][Slodki ME et al. 1978].

They require oxygen as the terminal electron acceptor. The substrate used for the production in less expensive such as agricultural waste production like cotton seed wool, cabbage, corn steep liquor, penicillin etc. Sugar beet molasses is widely used in fermentation because it acts as growth factor like pantothenic acid, inositol etc [Antunes AEC et al.2003].

II. MATERIALS AND METHOD

- **Test organism:-** *Xanthomonas campestris* NRRL-B-1459 was obtained from NCL, Pune.
- **Malt extract glucose yeast extract peptone agar (Maintenance media):-** It consists of Malt extract, glucose, yeast extract, peptone, $CaCO_3$ and agar.
- **Seed culture media:-** It consists of yeast extract, tryptone, sucrose, Nacl and Distilled water.
- **Fermentation media:-** This medium consist of yeast extract, KH_2PO_4, $MgSO_4$ and Nacl.
- **Maintenance of organism on maintenance media:-** *X. campestris* NRRL-B-149 obtained from NCL, Pune was maintained on glucose yeast extract agar and incubated at 26^0c for 24 hours and then kept in refrigerator. The same medium without agar was used for stock culture in test tube and incubated at 28^0c. The pH was adjusted between 6.5 and 7.1.
- **Preparation of substrate:-** 10 gm of soluble solids of sugar beet molasses was dissolved into 100ml of distilled water and preheated in 90^0c water bath for 10 mins and again centrifuge at 8000 rpm for 20 mins.
- **Inoculums preparation:-** Flasks containing fermentation media were inoculated by 10 ml of culture. Incubate the flask in rotary shaker incubator (25^0c and 240 rpm) for 5 days and gum separation was carried out with 24 hours intervals.
- **Determination of pH variation:-**The changes in pH values of fermentation broth were measured before separation of gum in each interval.
- **Production of Xanthan gum:-**

1. **For sucrose fermentation medium:** The cell mass was separated by centrifugation. The amount of Xanthan gum produced was determined by precipitating the whole broth in 95% ethanol. Later on the dried mass obtained was the mass of Xanthan gum.
2. **For molasses media:** The same procedure was followed but the step of washing in water was avoided because Xanthan gum is water soluble and washing will removed it.
3. **Effect of different parameters on Xanthan gum production:** The variation in Xanthan gum production at different physical parameter and different nitrogen sources was studied and the amount of Xanthan gum produced was estimated by using above procedure.

III. RESULT AND DISCUSSION

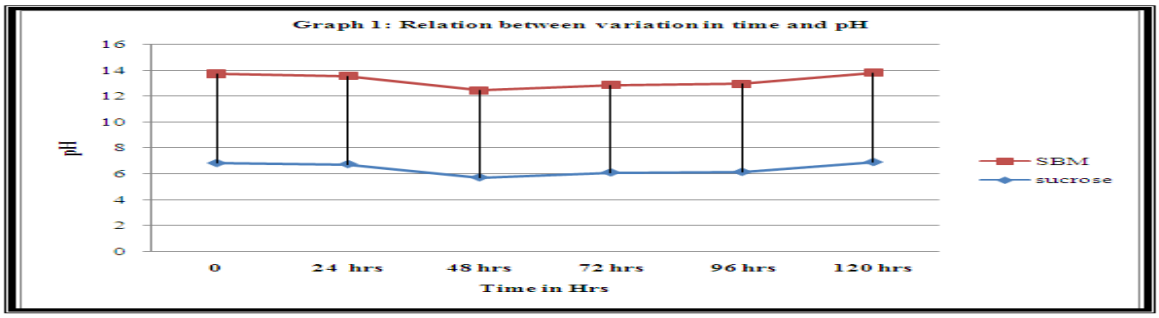
The current study deals with the production of Xanthan gum by *Xanthomonas campestris* from sugar beet molasses. For this *Xanthomonas campestris* was procured from NCL, Pune. Initially the organism was maintained on Glucose Yeast Extract Agar (Fig 1). Cultures were transferred at 2 weeks intervals and plates were incubated at 26^oC. Xanthan production is a strictly aerobic process which can be carried out in either semi-solid or liquid media. A fundamental understanding in the fermentation is parameter which is necessary in order to optimized production [Antunes AEC et al.2003].

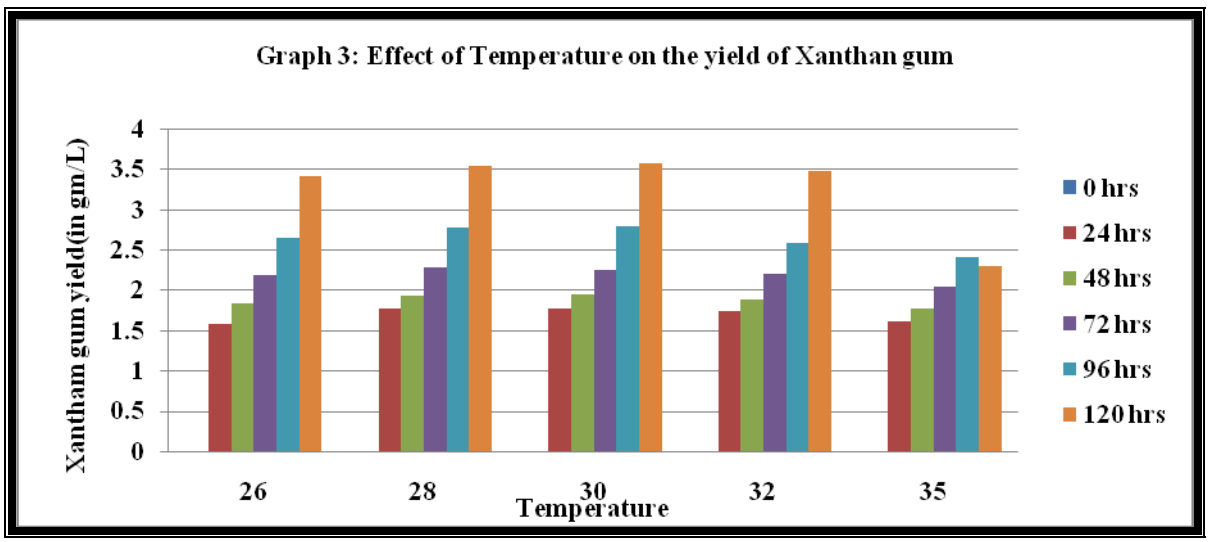
The current study aimed for the optimization and production of Xanthan gum from *Xanthomonas campestris* (NRRL-B-1449). The strain of *Xanthomonas* was obtained from NCL pune and was used throughout the study for production and optimization of Xanthan gum. Xanthan gum production was estimated at an interval of 24 hrs. Results showed that the maximum yield was obtained after 120 hrs of incubation (3.59fm/L) as shown in graph 2. Initially pH of the medium was maintained at neutral. After 24 hrs of incubation the pH was found to be 6.75 and it further decreased up to 5.73 after 48 hrs. (Graph 1) This was may be due to xanthan gum is acidic in nature. Own result coincide with the finding of Marzieh Moosavi et. al, 2010. The optimum pH for the production of xanthan gum was found to be 6.91 in the current investigation. Further Xanthan production with respect to temperature was investigated (Graph 3). The inoculated culture of *Xanthomonas campestris* was incubated within the temperature range of 25^0C to 30^0C, since the organism was known as mesophilic organism. Effect of temperature on Xanthan production has been investigated by other researcher, the optimum temperature was in the range of 28^0C to 30^0C [Tait MI et al.1986][Marzi Moosavi et al.2010][Gillani S.L.et al.2011].Similar types of findings were also shown by [Gilani S.L. et al. 2011]Lastly in the current study, the xanthan production was studied with different nitrogen sources (Graph 5) and at different agitation rate (Graph 4). The results showed that out of all the nitrogen sources tested, yeast extract gave the effective yield & that to 3.59 gm/L. As the optimum agitation rate was found to be 500rpm at which the yield obtained was 3.59gm/L. These above finding correlates with the finding of Aarthy Palaniray et. al, 2011.

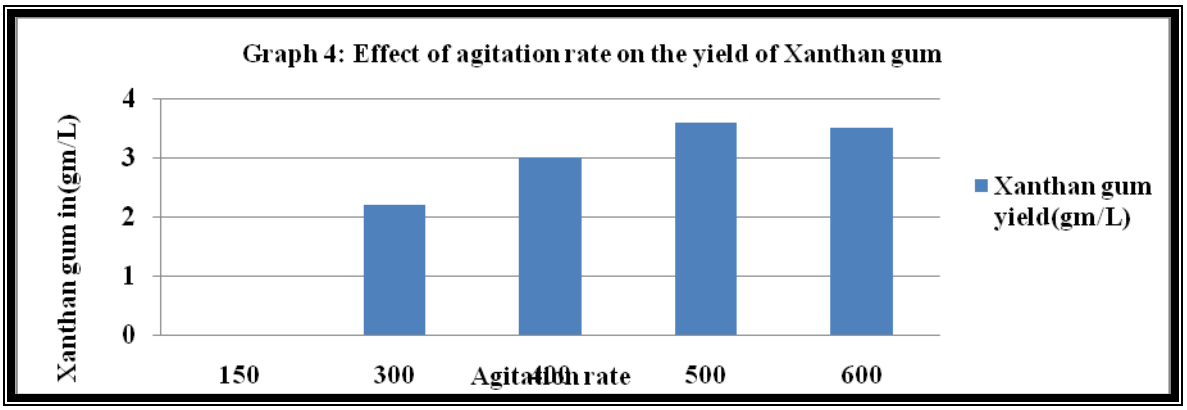
IV. CONCLUSION

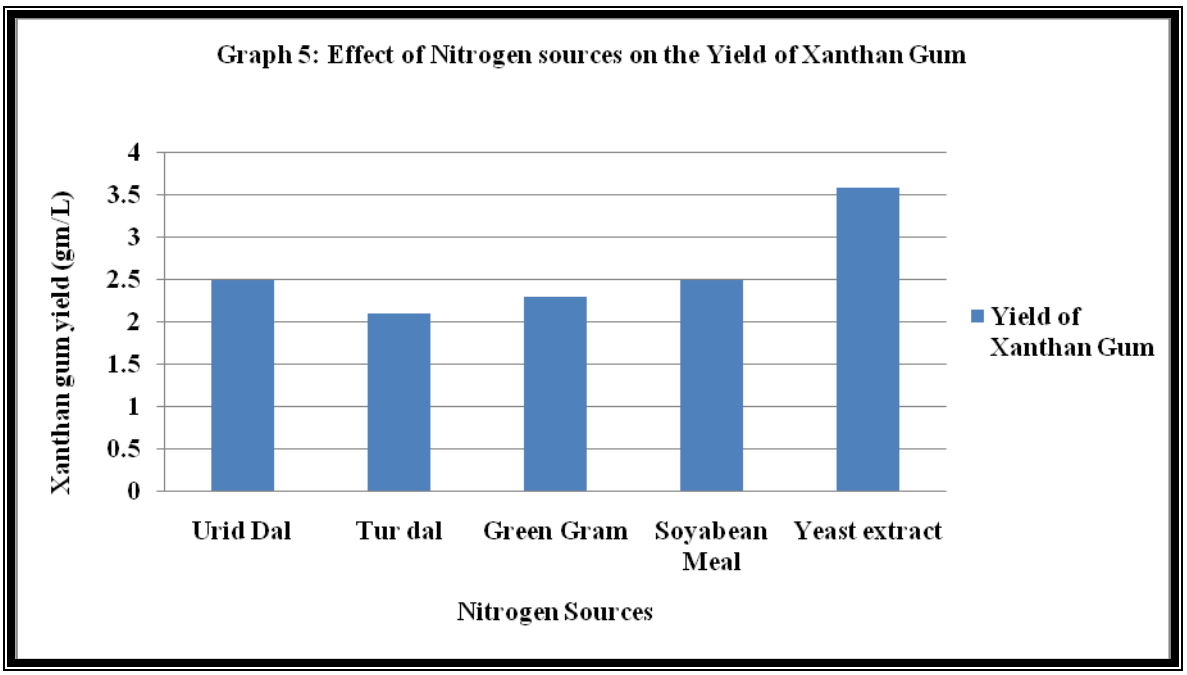
From the above discussion it can be concluded that, Xanthan gum was produced by *Xanthomonas campestris*(NRRL-B-1449) and its production yield can be further increased at suitable physical conditions i.e. temperature, pH, agitation and nitrogen sources.

Fig 1: colonies of *Xanthomonas campestris* on agar plate









REFERENCES

[1] Antunes AEC, Moreira AS, Vendruscolo CT. Screening of *Xanthpmonas campestris* pv pruni strains according to their production of Xanthan gum, viscosity and chemical composition. Braz J. Food Technol. 2003;6;317-22.

[2] Cadmus MC, Knuston CA, Lagodo AA, Pittsley JE, Burton KA. Synthetic media for production of quality Xanthan gum 120 litre fermenters. Biotechnollogy Bioeng 1978;20: 1003-14.

[3] Gilani S.L., Najafpour G.D, Heydrazadeh H.D, .Zare H kinetics models for Xanthan gum production using *Xanthomonas campestris* from molasses chemical industry and chemical engineering Quarterly 17(2)-179-187(2011).

[4] Gills P.S, Ray.D , Sahoo P.K, "scharacteristics of Xanthan gum based biodegradable super porous hydrogen",vol 45. International journal of biological micro molecules, 2009, 45, 364-371.

[5] Jeans A, Rovin P, Cadmus MC, Silman RW, Knutson CA. Polysaccharide(xanthan) *Xanthomonas campestris* NRRL-B-1459 : producers of culture maintenance and polysaccharide production, purification and analysis. ARS-NC-%!. Agriculturer research services, US Department of Agriculture, Peoria, Illinosis

[6] Kidby D, Sandford P, Herman A, Cadmus MC. Maintanance produce for the cultailment of genetic instability; *Xanthomonas campestris* NRRL-B-1459. Appl. environs microbial polysaccharides. Adv Appl Microbiol 1977; 33; 840- 5.

[7] Knonicek J, Lasik J, Safar H, Xanthan gum produce from whey by mutant strain of *Xanthomonas campestris* folia microbial 1993; 28; 403-5.

[8] Kurbanoglu E.B, Kurbanoglu N.I., "Rram horn hydrolysate a enhancer of Xanthan production in batch culture of *Xannthmonas campestris* EBK-4 isolate", vol. 42 process Biochemistry, 2007, pp. 1146-1149.

[9] Marzieh Moosavi- Nasab, Safoora Pashangeh, and Maryam Rafsanjani "Effect of Fermentation Time on Xanthan Gum Production from Sugar Beet Molasses",World Academy of Science, Engineering and Technology 68-2010.

[10] Slodki ME, Cadmus MC. Production of microbial polysaccharides. Adc Appl Microbiol 1978; 23:19-49.

[11] Sutherland IW, Extracellular polysaccharide. In: Dellwey H. Biotechnology. Weinhein, Verlag Chemie, 1983,p. 553-74

[12] Tait MI, Sutherland W, Clarke-Sturman A, Effect of growth condition on the production, composition and viscosity of *Xanthomonas campestris* exopolysaccharides. J Gen Microbiol 1986;132;1483-92.

[13] Arthy Palaniraj ,Vijayakumar Jayaraman , and Sekar Babu Hariram Dept. Of Biotechnology,'' Influence of nitrogen sources and agitation in xanthan gum production by *Xanthomonas campestries* '' International Journal of Advanced Biotechnology and Research ISSN 0976-2612, Vol 2,Issue 3,2011,pp 305-309.