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Research Article
Impact of Amino Acids, Nitrogen Source and Buffering System on Xanthan Yield Produced on Hydrolyzed Whey Lactose

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Abstract

Background and Objective: Huge amount of whey and permeate are produced annually and can be used as substrate for production of valuable products. The purpose of this work was to employed hydrolyzed lactose of whey as a cheaper carbon source for reducing the cost of production of xanthan gum on submerged culture and solid agar medium. **Methodology:** Whey basal medium supplemented with different growth nutrient involved carbon, nitrogen (organic and inorganic) and amino acids and were used for xanthan production. Lactose of whey was acid hydrolyzed or partially hydrolyzed through preculturing process with *Lactobacillus rhamnosus* for 24-48 h before inoculating the production medium with *Xanthomonas campesteris* pv. *campestris* for xanthan production. Data were statistically analyzed by SAS software. **Results:** Acid hydrolyzed whey supplemented with 1% sucrose supported production of 28 g L^{-1} of xanthan gum. The precultured lactose mineral medium with *Lactobacillus rhamnosus* produced 17.6 g L^{-1} of xanthan on a medium precultured for 24 h. Diammonium phosphate was the best inorganic nitrogen source whilst peptone was the best organic nitrogen source supported production of 20 and 36 g L^{-1} of xanthan gum, respectively in submerged culture compared with 4.2 and 4 g L^{-1} of xanthan on solid agar medium, respectively. Cystein, alanine and histidine yielded a good yield of xanthan especially cystein which gave a yield reached 35 g L^{-1}. The buffering system exhibited profound effect on xanthan yield which reached its maximal value at pH 7. **Conclusion:** The hydrolyzed lactose can be used successfully for production of xanthan gum with employing microbial biotechnology techniques including acid pretreatment of lactose medium and preculturing of production medium with lactic acid bacteria which contribute for reduction of pollution resultant from dairy waste disposal.

Key words: Microbial polysaccharides, xanthan gum, lactose basal media, acid pretreatment, preculturing technique

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Xanthan gum is an extracellular hetero polysaccharide excreted by *Xanthomonas campestris*[1]. It is preferable microbial polysaccharide actually used in many industrial applications due to its superior rheological behavior. *Xanthomonas campestris* produced low level of β-galactosidase and therefore cannot use lactose as an appropriate carbon source. Thus *X. campestris* exhibits poor growth and low yield of gum in medium comprising lactose as a sole carbon source[2,3]. Many attempts have employed for production of xanthan gum in media containing lactose or whey medium, e.g., by using genetically modified *X. campestris* involved genetic engineering techniques. In other approach lactose was hydrolyzed into glucose and galactose or precultured with lactic acid bacteria for partial hydrolyzing of lactose into lactic acid and galactose which could be fermented producing xanthan gum[4].

Xanthan gum can be used as thickening, suspending, stabilizing and emulsifying additive material, in food and other industries[1]. Xanthan can be used safely in food products and has no inhibitory effect on organism's growth. It is nonsensitizing and non-irritable for skin and eyes. Accordingly, xanthan gum was approved by the United States Food and Drug Administration (FDA) to be used as food additive without any limitations[5]. It has been reported that the molecular structure of xanthan gum is highly affected by the composition of the production medium. In this trend, several studies have so far devoted for studying the impact of variety of nutrients, specially the carbon and nitrogen sources, including sucrose and glucose as the most carbon sources frequently used for xanthan production[6,7]. Invert sugars or glucose are the common carbon sources used for the commercial production of xanthan gum with preferring the batch cultures processes than continuous processes[8]. Sugar cane molasses, hydrolyzed rice, sucrose, barley, corn flour, acid whey , coconut juice, etc were used as other substrates. but glucose is still superior in supporting higher yield, and good product quality[9-11]. Many factors affect xanthan production. The bacterial strain used for xanthan production has marked effect on the yield and properties of the produced xanthan gum[12,13], culture medium[8,14,15], temperature[16,17], pH[18], time of fermentation[19] and agitation rate[14,20].

The aim of this study was to optimize the xanthan gum production in lactose basal media as whey medium by *Xanthomonas campestris* in submerged cultures compared with solid agar medium upon using different carbon, nitrogen and amino acids sources.

MATERIALS AND METHODS

This study was carried out on June 2, 2016 in the laboratories of Dairy Department, National Research Center, Egypt.

Microorganism and inoculum preparation: *Xanthomonas campestris* pv. *campestris* was obtained from Agricultural Biotechnology Laboratories, National Chung Hsing Univ., Taichung, Taiwan and used throughout this study. The culture was maintained on nutrient agar slant containing (g L^{-1}) glucose 10, malt extract 3, yeast extract 3 and peptone 5, pH = 7 grown at 30°C for 24 h 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 28-30°C for 24 h in an incubator shaker (New Brunswick Scientific Co., New Brunswick, N.J. USA). The liquid culture was used to inoculate the final fermentation medium.

Lactic acid bacteria: *Lactobacillus rhamnosus* NRRL B-442 was obtained from Microbial Genomic and Bioprocessing USDA, ARS, NCAUR USA (Peoria, Ill.).

Whey fermentation medium: Whey was hydrolyzed using different concentration of hydrochloric acid (0.1 N) (0.5, 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5%). Whey was used as carbon source with 1% sucrose in the basal fermentation medium.

Lactose fermentation medium: Lactose was used a carbon source in basal fermentation media in addition to 5 g L^{-1} sucrose, 1 g L^{-1} peptone and 1 g L^{-1} yeast extract. In this experiment lactose and sucrose were autoclaved separately and then add to the fermentation medium before inoculation with microorganisms. Two microorganisms were inoculated, the first organism was *Lactobacillus rhamnosus* which was first inoculated to the fermentation medium (2% v/v) then incubated at 37°C for 24 h or 48 h and the pH of the medium was adjusted at 7.0 with 0.1 N of NaOH . The medium was heated for 5 min at 100°C to stop growth of *L. rhamnosus* and cooled to 27°C prior to inoculation with 3% (v/v) with active viable culture of *Xanthomonas campestris*. The fermentation experiment was carried out in 250 mL conical flask containing 100 mL of fermentation medium and rotated at 150 rpm on a rotary shaker (New Brunswick Scientific Co., New Brunswick, N.J. USA) at 28±1°C for 4 days.

Fermentation medium: The production of xanthan gum was tested on liquid or agar media. The composition of the

fermentation medium without nitrogen or carbon sources in (g L^{-1}) was as follows: $3K_2HPO_4$, 0.25 $MgSO_4.7H_2O$ or 1 $CaCO_3$. The effect of carbon sources on xanthan gum production were tested by using different types of sugar as sucrose, glucose, fructose or maltose. The effects of different kinds of nitrogen sources either organic (yeast extract, meet extract, casein hydrolysate and peptone) or inorganic (NH_4Cl, $NaNO_3$, KNO_3 and $(NH_4)_2HPO_4$) were tested. The effects of amino acids including alanine, methionine, histidine and glycine were tested as will. The carbon sources were autoclaved separately and added to the fermentation medium before inoculation (in liquid medium). The media pH was adjusted to 7.0 with 0.1 N sodium hydroxide solution. The Fermentation experiments were carried out in 250 mL conical flasks containing 50 mL of fermentation medium in submerged culture and 15 mL for solid agar medium and were inoculated with 3% v/v with active viable culture of *Xanthomonas campestris* and rotated at 150 rpm on a rotary shaker at 28±1°C for 4 days (for liquid medium) and overnight for agar medium.

Isolation of crud xanthan: The main steps for the recovery process were deactivation, removal of microbial cells, precipitation of the biopolymer, dewatering, drying and milling. In case of the liquid medium xanthan was determined by precipitation with ethanol after removing cells by the method of Jeanes *et al.*[21] While, in case of agar medium xanthan was determined by scratching with distilled water according to the method of Ceranola *et al.*[22]. The precipitate was dried at 50°C in an oven until constant weight, to determine xanthan gum yield.

Statistical analysis: Statistical analysis was performed using the General Linear Model (GLM) procedure using SAS software (Version 8)[23]. Duncan's multiple comparison procedure was used to compare the means[24]. A probability of ($p \leq 0.05$) was used to establish statistical significance.

RESULTS

Xanthan gum production by using acid hydrolyzed whey supplemented with sucrose: *Xanthomonas campestris* is usually the most employed microorganism for producing Xanthan gum; it is an aerobic bacterium which is able to grow in both a complex and in synthetic medium[7]. The low level of β-galactosidase produced by *X. campestris* retards it from using lactose as an appropriate carbon source. Thus *X. campestris* exhibits poor growth and low yield of gum in medium comprising lactose as a sole carbon source[2,3]. Several

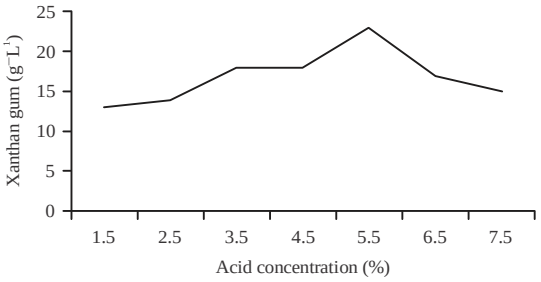

Fig. 1: Xanthan gum production by using acid hydrolyzed whey supplemented with sucrose by *X. campestris*

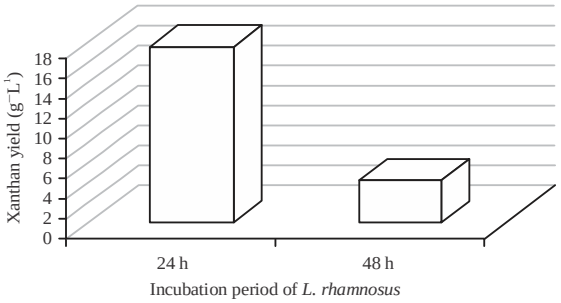

Fig. 2: Xanthan gum production on lactose mineral medium pre cultured with *Lactobacillus rhamnosus*

attempts as previously mentioned have employed for production of xanthan gum in media containing lactose or whey. In the current study different concentrations of hydrochloric acid were used for hydrolysis of lactose in whey to glucose and galactose to be fermented into xanthan gum. The results (Fig. 1) showed that concentration 5.5% of hydrochloric acid resulted in effective hydrolysis of whey and gave xanthan gum yield reached 23 g L^{-1}.

Xanthan gum production on lactose mineral medium pre cultured with *Lactobacillus rhamnosus*: Another approach to solve the problem of low level of β-galactosidase present in *X. campestris* involved using lactose mineral medium pre cultured with *Lactobacillus rhamnosus*. This microorganism could be produce β-galactosidase to convert lactose into glucose and galactose which are suitable for *X.campestris* to produce xanthan gum in high yield. The results (Fig. 2) showed that by increasing the incubation period of *Lactobacillus rhamnosus*, the yield was decreased.

Effect of different carbon sources on the production of xanthan gum: In the current study (Fig. 3) the best carbon source for production of xanthan gum was found to be

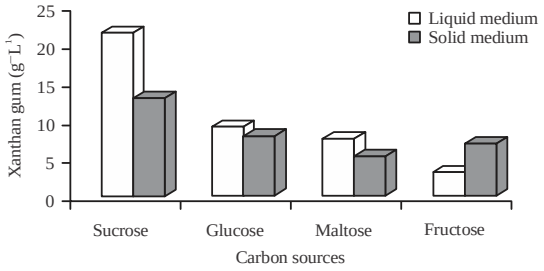

Fig. 3: Effect of different carbon sources on the production of xanthan gum by *X. campestris*

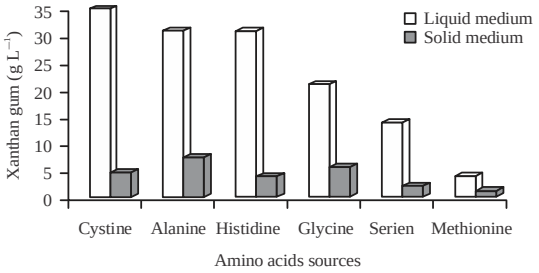

Fig. 6: Effect of different amino acids sources on the production of xanthan gum by *X. campestris*

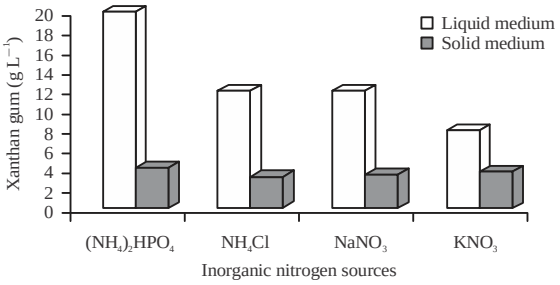

Fig. 4: Effect of different inorganic nitrogen sources on the production of xanthan gum by *X. campestris*

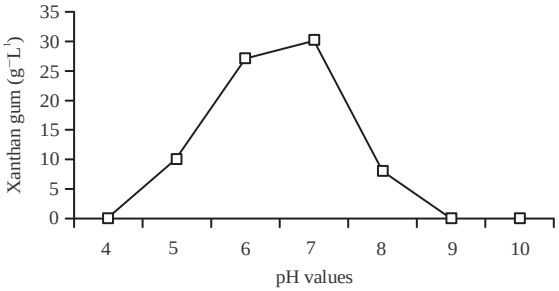

Fig. 7: Effect of buffering systems on the production of xanthan gum by *X. campestris*

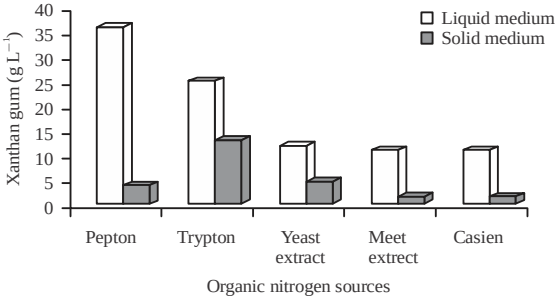

Fig. 5: Effect of different organic nitrogen sources on the production of xanthan by *X. campestris*

sucrose in both liquid and solid agar medium supported production of 21.6 and 13 g L^{-1}, respectively at optimum temperature.

Impact of different inorganic and organic nitrogen sources on the production of xanthan gum: Nitrogen is an essential element can be supplied as an organic ingredient or as inorganic compound. The best inorganic nitrogen source in the present study (Fig. 4, 5) was $(NH_4)_2HPO_4$ for both liquid and agar medium.

Effect of different amino acids sources on the production of xanthan gum: The simpler peptides and amino acids are responsible for increased exopolysaccarides (EPS) synthesis as well as to sustain catabolism process of lactose into glucose and galactose and EPS synthesis as depicted by De Vuyst and Degeest[25]. The results (Fig. 6) showed that using of amino acid cysteine in a medium containing sucrose and peptone increased the yield of xanthan gum to reach 35 g L^{-1} in liquid medium comparing with the other amino acids under the same conditions. On the other hand amino acid alanine with sucrose and peptone in agar medium gave a yield of xanthan gum reached 7.5 g L^{-1}.

Effect of buffering systems on the production of xanthan gum by *X. campestris*: The results in Fig. 7 indicated that by using phosphate buffer to adjust pH at 6 and 7, the yield was the highest as reached 27 and 30 g L^{-1}, respectively.

DISCUSSION

In the current investigation the hydrolyzed whey lactose as an inexpensive substrate was used for production of xanthan gum. Supplementation of production medium with appropriate nitrogen source as peptone and amino acids especially cyctein with adjusting pH at neutrality gave a good yield of xanthan gum. Lin and Nickerson[26] studied acid hydrolysis of lactose in whey and reported that acid hydrolysis

of concentrated whey with sulfuric acid produced a more browning reaction and off-flavor. This may be due to using a high concentration of acid in hydrolysis process and this led to decreasing of xanthan yield. Thus optimal concentration of acid was needed in hydrolysis process to produce a high yield of xanthan. Coughlin and Nickerson[27] reported that lactose in fresh cottage cheese whey and aqueous solutions was successfully hydrolyzed by using 0.5, 1, 2, 3 normal hydrochloric acid at 50, 60 and 70°C and reported that un desirable side reactions were minimized by conducting the hydrolysis at temperatures below 100°C. Similar hydrolysis process for other substrate involved rice straw with acid and alkali pretreatments were achieved by Jazini *et al*.[28] to use the hydrolyzates as substrates for xanthan gum. They recommended using NaOH at 100°C for 120 min to attain the highest xanthan yield.

By increasing the incubation period of *Lactobacillus rhamnosus*, the yield was decreased. These results may be attributed to the conversion of the majority of lactose into lactic acid and consumption of the librated sugars with *Lactobacillus rhamnosus*. Carevic *et al*.[29] studied optimization of β-galactosidase production from lactic acid bacteria. They screened various lactic acid bacteria and reported that the highest enzyme yield was obtained from *Lactobacillus acidophilus* ATTCC 4356 after 2 days by using shake flask culture containing modified MRS broth with using 2.5% lactose as sole carbon source.

Effect of different carbon sources on the production of xanthan gum: Sucrose gave the highest yield compared with the other tested carbon sources. Souw and Demain[30] mentioned that sucrose is a good substrate for xanthan gum. Production and indicated that succinate and 2-oxoglutarate enhanced xanthan gum production in sucrose-based medium. In other reports, Saied *et al*.[31] stated that sucrose supported production the highest yield followed by glucose. However, Leela and Sharma[32] reported that glucose was the best carbon source gave the highest yield (dry weight) 14.7 g L^{-1} followed by sucrose (13.234 g L^{-1}) and maltose (12.3 g L^{-1}). Low grade grape juice concentrate was used by Ghashghaei *et al*.[33] as a carbon source for xanthan production. They indicated the potential use of the mentioned substrate as aneconomical carbon source for xanthan production. Mudoi *et al*.[34] reported that the potentiality of using a low-cost renewable residual molasses as a substrate for xanthan production by *X. campestris* seems to be advantageous from the view point of economy. Under optimal conditions a maximum of 5.23 g L^{-1} xanthan could be produced using such feedstock. In other study, Kedar and Bholay[35] mentioned that the agro industries waste as rice husk produced 10 g L^{-1} of xanthan gum after 72 h and seems to be a better source of carbon for xanthan production. For direct utilization of lactose Ramezani *et al*.[36] indicated that *X. campestris* at lactose strains demonstrated a good level of xanthan production. Amongst all, NIGEBK37 consuming produced the greatest (14.62 g L^{-1}) amount of xanthan gum in experimental laboratory conditions. In another approach Niknezhad *et al*.[37] investigated production of xanthan gum using immobilized cells of *Xanthomonas campestris* and *Xanthomonas pelargonii* grown on glucose or hydrolyzed starch as carbon sources. Brandao *et al*.[38] studied production of Xanthan gum from crude glycerin primary by-product of the biodiesel industry. They obtained yield reached 7.2 g L^{-1}. Regenhardt *et al*.[39] hydrolyzed lactose through immbolization process in which β-galactosidase enzyme from *Kluyveromyces fragile* was employed for xanthan gum production. They indicated that the best immobilization conditions providing best results were: 5% (v/v) glutaraldehyde with the addition of 0.03 M galactose to the enzyme solution to conduct immobilization. Solid state fermentation was used for xanthan production from agro industrial wastes by Vidhyalakshmi *et al*.[40]; they yielded xanthan of 2.9/50 g of substrate when fermented by *Xanthomonas citri*. Several processes have been developed to utilize residues such as cassava bagasse green coconut shells[41], residue of apple juice, bark cocoa or whey, whey sugar cane, olive mill waste waters, sugar beet pulp residue, citrus waste, glycerin and vegetable leftovers for production of Xanthan gum[42].

Impact of different inorganic and organic nitrogen sources on the production of xanthan gum: Higher yields of xanthan were produced upon using nitrate as the nitrogen source. Qadeer and Baig[43] reported that sodium nitrate and ammonium phosphate were more effective nitrogenous nutrients in xanthan gum formation. Similar results were also reported by Murad[4]. Letisse *et al*.[8] indicated that NH_4Cl or $NaNO_3$ showed a poor cell growth rate at 0.07 h^{-1} than ammonium chloride at 0.13 h^{-1}. Ammonium chloride was therefore considered as a better substrate for biomass formation. On the other hand (Fig. 5) peptone was the best organic nitrogen source which agreed with Mohan and Babitha[44]; reported that peptone showed the highest polymer production compared with other nitrogen sources. Other experiments with peptone and yeast extract clearly showed that peptone was more effective as nitrogen source only on rheological properties of the xanthan producing culture[45]. Bhatia *et al*.[46] used hydrolyzed starch as a substrate for production of xanthan gum. They indicated that yeast extract

supported production of the highest xanthan yield reached 10.0 g L^{-1}. To increase the cell density to high level before reaching the stationary phase Lo *et al.*[47] used a moderately high yeast extract concentration in the medium. Non-limiting nitrogen concentration is required for rapid cell growth[38]. Letisse *et al.*[8] stated that the growth initiation was poor upon using inorganic nitrogen sources and this could be improved by using organic nitrogen (e.g., soybean flour hydrolysate).

Effect of different amino acids sources on the production of xanthan gum: Supplementation of production medium with some amino acids especially cystein inhanced production of the highest yield of xanthan gum. Behravan *et al.*[48] studied the optimization of dextran production by *Leuconostoc mesenteroides* NRRL B-512 using wheat bran as a cheap and local source of carbohydrate and nitrogen and contains amino acids and vitamins. The highest dextran production was observed in cultures with an initial concentration of 15 g of wheat bran extract/100 mL of culture medium. Souw and Demain[30] concluded that the glutamate at a concentration of 15 mM was the best nitrogen source for production of xanthan gum. The higher concentrations inhibited growth. Grobben *et al.*[49] reported that the majority of amino acids were required for growth of *L. delbrueckii* subsp., *bulgaricus* NCFB2772. For example, a single omission of glutamic acid, aspartic acid or glycine led to a good growth of the strain, whereas growth was scanty when these group of amino acids were all absent. The omission of single or multiple amino acids had no effect on the production of EPS relative to cell growth. The induced growth and the increase in production of xanthan noted upon using molasses as substrate may be attributed partly to the good availability of amino acids in molasses particularly glutamate[50]. Whey also has nutrients and growth factors, which can serve to activate the synthesis of the final product as xanthan gum[51].

Effect of buffering systems on the production of xanthan gum by *X. campestris*: The pH control at neutrality has a decisive effect on xanthan production. Psomas *et al.*[52], Kerdsup *et al.*[53], Silva *et al.*[54] and Gumus *et al.*[55] indicated that neutral pH is the optimum for growth of *X. campestris* during xanthan production. Esgalhado *et al.*[18] stated that the optimum pH for culture growth was 6-7.5 and optimum pH for xanthan production was ranged from 7-8[56]. These findings indicated that the neutral pH is a decisive factor in xanthan gum production.

CONCLUSION

Hydrolyzed lactose as a cheap substrate can be used successfully for production of xanthan gum in a good yield with employing microbial biotechnology techniques including acid pretreatment of lactose medium and preculturing of the production medium with lactic acid bacteria. Diammonium phosphate was the best inorganic nitrogen source whilst peptone was the best organic nitrogen source. Cystein, alanine and histidine yielded a good yield of xanthan especially cystein which gave a yield reached 35 g L^{-1}. buffering system exhibited profound effect on xanthan yield which reached its maximal value at pH 7. This studty also contributes for reduction of pollution resultant from dairy waste disposal.

Implications, applications, recommendations and limitations of this study in the current study searching for utilization of dairy by-products which considered as environmental pollutants for production of beneficial products as xanthane gum contribute will in economical, technological and environmental aspects. Xanthane gum has important applications in food, chemical, pharmaceutical and textile industries. Production of xanthan according to the methods involved in this search may require special experiences.

SIGNIFICANCE STATEMENTS

This study discover the possibility of using cheaper substrate for producing the valuable product xanthan gum to reduce production cost since the other substrates like glucose and sucrose are expensive. Cheese whey and permeate are produced in large amounts in dairy industry. They are cheaper substrates and can be used as a fermentation medium for xanthan production after some simple pretreatments. Thus this work may contribute in reduction of the production cost of xanthan and on the same time contribute in reducing pollution resultant from disposal of such dairy by-products.

REFERENCES

1. Sutherland, I.W., 1996. Extracellular Polysaccharides. In: Biotechnology: Products of Primary Metabolism, Volume 6, Rehm, H.J. and G. Reed (Eds.). 2nd Edn., Wiley-VCH Verlag GmbH, Weinheim, Germany, pp: 613-657.
2. Frank, J.F. and G.A. Somkuti, 1979. General properties of beta-galactosidase of *Xanthomonas campestris*. Applied Environ. Microbiol., 38: 554-556.
3. Fu, J.F. and Y.H. Tseng, 1990. Construction of lactose-utilizing Xanthomonas campestris and production of xanthan gum from whey. Applied Environ. Microbiol., 56: 919-923.

4. Murad, H.A., 1993. Comparative study for production of xanthan gum from the locally strain *Xanthomonas campestris* NRRL. B-1459. Egypt J. Applied Sci., 8: 545-552.
5. Kennedy, J.F. and I.J. Bradshaw, 1984. Production, properties and applications of xanthan. Prog. Ind. Microbiol., 19: 319-371.
6. Garcia-Ochoa, F. and E. Gomez, 1998. Mass transfer coefficient in stirred tank reactors for xanthan gum solutions. Biochem. Eng. J., 1: 1-10.
7. Garcia-Ochoa, F., V.E. Santos, J.A. Gasas and E. Goemz, 2000. Xanthan gum: Production, recovery and properties. Biotechnol. Adv., 18: 549-579.
8. Letisse, F., P. Chevallereau, J.L. Simon and N.D. Lindley, 2001. Kinetic analysis of growth and xanthan gum production with *Xanthomonas campestris* on sucrose, using sequentially consumed nitrogen sources. Applied Microbiol. Biotechnol., 55: 417-422.
9. Rosalam, S. and R. England, 2003. Review of xanthan gum production from unmodified starches by *Xanthomonas comprestris* sp. Enzyme. Microbiol. Technol., 39: 197-207.
10. Abd El-Salam, M.H., M.A. Fadel, H.A. Murad, 1994. Bioconversion of sugarcane molasses into xanthan gum. J. Biotechnol., 33: 103-106.
11. Kongruang, S., M. Thakonthawat and R. Promtu, 2005. Growth kinetics of xanthan production from uneconomical agricultural products with *Xanthomonas campestris* TISTR 1100. J. Applied Sci., 4: 78-88.
12. Rodriguez, H. and L. Aguilar, 1997. Detection of *Xanthomonas campestris* mutants with increased xanthan production. J. Ind. Microbiol. Biotechnol., 18: 232-234.
13. Moreira, A.S., J.L.S. Vendruscolo, C. Gil-Turnes and C.T. Vendruscolo, 2001. Screening among 18 novel strains of *Xanthomonas campestris* pv pruni. Food Hydrocolloids, 15: 469-474.
14. Amanullah, A., S. Satti and A.W. Nienow, 1998. Enhancing xanthan fermentations by different modes of glucose feeding. Biotechnol. Prog., 14: 265-269.
15. Garca-Ochoa, F., V.E. Santos and A.P. Fritsch, 1992. Nutritional study of *Xanthomonas campestris* in xanthan gum production by factorial design of experiments. Enzyme Microbiol. Technol., 14: 991-996.
16. Shu, C.H. and S.T. Yang, 1990. Effects of temperature on cell growth and xanthan production in batch cultures of *Xanthomonas campestris*. Biotechnol. Bioeng., 35: 454-468.
17. Shu, C.H. and S.T. Yang, 1991. Kinetics and modeling of temperature effects on batch xanthan gum fermentation. Biotechnol. Bioeng., 37: 567-574.
18. Esgalhado, M.E., J.C. Roseiro and M.T.A. Collaco, 1995. Interactive effects of pH and temperature on cell growth and polymer production by *Xanthomonas campestris*. Process Biochem., 30: 667-671.
19. Cacik, F., R.G. Dondo and D. Marques, 2001. Optimal control of a batch bioreactor for the production of xanthan gum. Comput. Chem. Eng., 25: 409-418.
20. Zhang, Z. and H. Chen, 2010. Fermentation performance and structure characteristics of xanthan produced by *Xanthomonas campestris* with a glucose/xylose mixture. Applied Biochem. Biotechnol., 160: 1653-1663.
21. Jeanes, A.R., S.P. Rogovin, M.C. Cadmus, R.W. Silman and A.C. Knutson, 1976. Polysaccharide (xanthan) of *Xanthomonas campestris* NRRL B-1459: Procedures of culture maintenance and polysaccharide production purification and analysis. ARS-NC-51. Agricultural Research Service, US Department of Agriculture, Peoria, Illinois.
22. Cerantola, S., N. Marty and H. Montrozier, 1996. Structural studies of the acidic exopolysaccharide produced by a mucoid strain of *Burkholderia cepacia*, isolated from cystic fibrosis. Carbohydr. Res., 285: 59-67.
23. SAS., 1999. SAS/STAT User's Guide, Version 8. SAS Institute Inc., Cary, NC., USA..
24. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
25. De Vuyst, L. and B. Degeest, 1999. Heteropolysaccharides from lactic acid bacteria. FEMS Microbiol. Rev., 23: 153-177.
26. Lin, A.Y. and T.A. Nickerson, 1977. Acid hydrolysis of lactose in whey versus aqueous solutions. J. Dairy Sci., 60: 34-39.
27. Coughlin, J.R. and T.A. Nickerson, 1975. Acid-catalyzed hydrolysis of lactose in whey and aqueous solutions. J. Dairy Sci., 58: 169-174.
28. Jazini, M.H., E. Fereydouni and K. Karimi, 2017. Microbial xanthan gum production from alkali-pretreated rice straw. RSC Adv., 7: 3507-3514.
29. Carevic, M., M. Vukasinovic-Sekulic, S. Grbavcic, M. Stojanovic, M. Mihailovic, A. Dimitrijevic and D. Bezbradica, 2015. Optimization of β-galactosidase production from lactic acid bacteria. Hemijska Industrija, 69: 305-312.
30. Souw, P. and A.L. Demain, 1979. Nutritional studies on xanthan production by *Xanthomonas campestris* NRRL B1459. Applied Environ. Microbiol., 37: 1186-1192.
31. Saied, E.L., S.A. Gabr, A.S. Hamed and H.T.M. Hefnawy, 2002. Production of xanthan gum by *Xanthomonas campestris*. Proceedings of the IFT Annual Meeting and Food Expo, June 15-19, 2002, Anaheim, California.
32. Leela, J.K. and G. Sharma, 2000. Studies on xanthan production from *Xanthomonas campestris*. Bioprecess. Eng., 23: 687-689.
33. Ghashghaei, T., M.R. Soudi and S. Hoseinkhani, 2016. Optimization of xanthan gum production from grape juice concentrate using Plackett-Burman design and response surface methodology. Applied Food Biotechnol., 3: 15-23.
34. Mudoi, P., P. Bharali and B.K. Konwar, 2013. Study on the effect of pH, temperature and aeration on the cellular growth and xanthan production by *Xanthomonas campestris* using waste residual molasses. J. Bioprocess Biotech., Vol. 3. 10.4172/2155-9821.1000135.

35. Kedar, J.A. and A.D. Bholay, 2014. Ecofriendly biosynthesis of xanthan gum by *Xanthomonas campestris*. Word J. Pharm. Pharm. Sci., 3: 1341-1355.
36. Ramezani, A., S.M. Alavi, A.H. Salmanian and M. Jafari, 2013. The screening of xanthomonas lactose-positive strains among bacterial populations of citrus canker disease isolated from Iran. Biol. J. Microorganism, 2: 19-30.
37. Niknezhad, S.V., M.A. Asadollahi, A. Zamani and D. Biria, 2016. Production of xanthan gum by free and immobilized cells of *Xanthomonas campestris* and *Xanthomonas pelargonii*. Int. J. Biol. Macromol., 82: 751-756.
38. Brandao, L.V., D.D.J. Assis, J.A. Lopez, M.C.A. Espiridiao, E.M. Echevarria and J.I. Druzian, 2013. Bioconversion from crude glycerin by *Xanthomonas campestris* 2103: Xanthan production and characterization. Braz. J. Chem. Eng., 30: 737-746.
39. Regenhardt, S.A., E.J. Mammarella and A.C. Rubiolo, 2013. Hydrolysis of lactose from cheese whey using a reactor with β-galactosidase enzyme immobilised on a commercial UF membrane. Chem. Process Eng., 34: 375-385.
40. Vidhyalakshmi, R., C. Vallinachiyar and R. Radhika, 2012. Production of xanthan from agro-industrial waste. J. Adv. Scient. Res., 3: 56-59.
41. Nery, T.B.R., A.J.G. da Cruz and J.I. Druzian, 2013. Use of green coconut shells as an alternative substrate for the production of xanthan gum on different scales of fermentation. Polimeros, 23: 602-607.
42. Lopes, B.D.M., V.L. Lessa, B.M. Sliva, M.A.D.S.C. Fillho, E. Schnitzler and L.G. Lacerda, 2015. Xanthan gum: Properties, production conditions, quality and economic perspective. J. Food Nutr. Res., 54: 185-194.
43. Quader, M.A. and S. Baig, 1989. Effect of nitrogen source on the production of extracellular polisaccharide by *Xanthomonas campestris* NRRL B 1459. Sci. Int., 14: 262-264.
44. Mohan, T.S. and R. Babitha, 2010. Influence of nutritional factors on xanthan production by *Xanthomonas malvacearum*. Arch. Applied Sci. Res., 2: 28-36.
45. Roseiro, J.C., M.E. Esgalhado, M.T.A. Collaco and A.N. Emery, 1992. Medium development for xanthan production. Process Biochem., 27: 167-175.
46. Bhatia, S.K., N. Kumar and R.K. Bhatia, 2015. Stepwise bioprocess for exopolysaccharide production using potato starch as carbon source. 3 Biotech, 5: 735-739.
47. Lo, Y.M., S.T. Yang and D.B. Min, 1996. Kinetic and feasibility studies of ultrafiltration of viscous xanthan gum fermentation broth. J. Membrane Sci., 117: 237-249.
48. Behravan, J., B.S.F. Bazzaz and Z. Salimi, 2003. Optimization of dextran production by *Leuconostoc mesenteroides* NRRL B-512 using cheap and local sources of carbohydrate and nitrogen. Biotechnol. Applied Biochem., 38: 267-269.
49. Grobben, G.J., I. Chin-Joe, V.A. Kitzen, I.C. Boels and F. Boer *et al*., 1998. Enhancement of exopolysaccharide production by *Lactobacillus delbrueckii* subsp. *Bulgaricus* NCFB 2772 with a simplified defined medium. Applied Environ. Microbiol., 64: 1333-1337.
50. Kalogiannis, S., G. Iakovidou, M. Liakopoulou-Kyriakides, D.A. Kyriakidis and G.N. Skaracis, 2003. Optimization of xanthan gum production by Xanthomonas campestris grown in molasses. Process Biochem., 39: 249-256.
51. Pirog, T.P., M.O. Ivakhniuk and A.A. Voronenko, 2016. Exopolysaccharides synthesis on industrial waste. Biotechnol. Acta, 9: 7-18.
52. Psomas, S.K., M. Liakopoulou-Kyriakides and D.A. Kyriakidis, 2007. Optimization study of xanthan gum production using response surface methodology. Biochem. Eng. J., 35: 273-280.
53. Kerdsup, P., S. Tantratian, R. Sanguandeekul and C. Imjongjirak, 2011. Xanthan production by mutant strain of *Xanthomonas campestris* TISTR 840 in raw cassava starch medium. Food Bioprocess Technol., 4: 1459-1462.
54. Silva, M.F., R.C.G. Fornari, M.A. Mazutti, D. de Oliveira and F.F. Padilha *et al*., 2009. Production and characterization of xantham gum by *Xanthomonas campestris* using cheese whey as sole carbon source. J. Food Eng., 90: 119-123.
55. Gumus, T., A.S. Demerci, M. Mirik, M. Arici and Y. Aysan, 2010. Xanthan gum production of *Xanthomonas* spp. isolated from different plants. Food Sci. Biotechnol., 19: 201-206.
56. Gomashe, A.V., P.G. Dharmik and P.S. Fuke, 2013. Optimization and production of xanthan gum by *Xanthomonas campestris* NRRL-B-1449 from sugar beet molasses. Int. J. Eng. Sci., 2: 52-55.