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Effect of Preparation Conditions on Xanthan Gum Production and Rheological Behavior using Cheese Whey by *Xanthomonas Campestris*

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Abstract: Xanthan gum production by *Xanthomonas campestris* PTCC 1473 using cheese whey as carbon source was studied. The pre-treatment of cheese whey and effect of agitation rate on the medium were investigated in order to improve xanthan gum production. The cheese whey is a plentiful residue obtained during the cheese processing and its removal in the environment causes several drawbacks. The bioconversion of this by-product in added value products is an important alternative to overcome this environmental problem. Maximum xanthan gum productions were observed after 72 h using cheese whey as sole carbon source at agitation rate 550 rpm, yielding approximately 16.5 g/l. This value is higher than some results presented in the literature using waste material as substrate. FT-IR studies also carried out for further conformations of compatibility.

Key words: Xanthan Gum; Cheese Whey; Xanthomonas *Campestris*; Broth Viscosity; FT-IR.

INTRODUCTION

Natural polysaccharides are essential materials for vital in vivo functions such as providing an energy source and as a structural material (Berg, J. M, *et al.*, 2006). In view of the fact that polysaccharides are naturally recycled carbon resources and looked at to be eco-friendly because of their biodegradability, it is estimated that the efficient use of polysaccharides will show the way to the production of environmentally benign materials. In addition, many polysaccharides like cellulose, starch, chitin, plants, bacteria, and seaweeds have been considered (Schuerch, C., *et al.*, 1986). For example, some polysaccharides are used as hydrocolloids for a stabilizer, a viscous agent, and a structure provider in food industries (Stephen, A. M, *et al.*, 1995).

Xanthan gum, a water soluble polysaccharide produced by *Xanthomonas campestris*, is a representative food hydrocolloid (Jansson, P. E, *et al.*, 1975). *X.campestris* is usually the most employed microorganism for producing xanthan gum; it is an aerobic bacterium which be able to grow both in a complex and in completely defined medium (Garcia-Ochoa *et al*, 2000). For the efficient production of xanthan gum, *X.campestris* needs several nutrients, including micronutrients (e.g. potassium, iron, and calcium salts) and macronutrients such as carbon and nitrogen (Garcia-Ochoa *et al.*, 2000; Rosalan and England, 2006).

Because of the relative high cost of xanthan gum in compare to synthetic polymers, many industries prefer to use other plant-derived polysaccharides and synthetic polymers instead of xanthan gum (Papagianni, M, *et al*, 2001). Reducing cost of the final product might result from using cheaper substrates instead of expensive ones like glucose or sucrose. Cheese whey is obtained in a large amount of dairy product. Inattention to this by-product and pouring it on ground may cause serious environmental defects. On the other hand, the cheese whey is a valuable culture medium that is why it has mostly 50% of milk nutrients (proteins, lactose, and minerals salts), therefore it is chosen as a cheap and worth substrate for xanthan gum production in this research.

In the present study, the main object of work the production of xanthan gum using cheese whey as substrate by batch fermentation is evaluated. In addition, the rheological properties of broth and the optimum condition of xanthan gum production are discussed.

MATERIALS AND METHODS

Microorganism and Inoculum Preparation:

Xanthomonas campestris PTCC 1473 was supplied by Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. The microorganism was grown on agar medium containing (g/l): yeast extract 5.0, peptone 5.0, glucose 10.0, and agar 20.0 (Merck, Germany). The propagated cells for the inoculums were grown in a medium (the stated compositions excluding agar) at neutral pH in a 250 ml flask with 100 ml of culture medium in an incubator shaker (Stuart, S1500 series, USA) for 24 h. The incubator shaker was set at 180 rpm and 28 °C to enhance oxygen mass transfer rate into the media. Thus, maximum cell growth was obtained and the revived fresh inoculum of the bacteria was used for each experimental run. The stock culture was stored on slant agar was maintained at 4°C and the subcultures were revived for every two weeks to avoid strain degradation (Hsu, C.H, *et al*, 2003, Babbar, S, *et al.*, 2006, Baba Hamed, S, *et al.*, 2009).

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Preparation of Cheese Whey:

Kaleh cheese whey was used as the source of carbon and nitrogen, provided by the Kaleh Dairy Factory (Amole, Mazandaran, Iran). The cheese whey was used in the liquid form, soon after collection. Pretreatment was done by cooling cheese whey to 0-5°C, adding calcium chloride, adjusting to neutral pH, warming to 50°C, and removing the insoluble precipitate that formed by centrifugation.

Xanthan Gum Production:

The production of xanthan gum from cheese whey was carried out in 1000 ml Erlenmeyer flasks with 300 ml of medium. It was containing (g/l): lactose 30.0, yeast extract 5.0, and additive solution 3.0 ml (consist of KH_2PO_4 (3 g/l), $MgCl_2$ (0.6 g/l), Na_2SO_4 (0.1 g/l), H_3BO_3 (0.006 g/l), ZnO (0.006 g/l), $FeCl_36H_2O$ (0.02 g/l), $CaCO_3$ (0.02 g/l)), that lactose was obtained from pretreated cheese whey.

The fermentations were commenced with inoculums size of 10% (v/v), experiments was conducted at various stirrer rate on a magnetic hot-plat stirrer (VELP, Italy) and at temperature 32 °C. Runs were terminated after 72 h of incubation. The pH was initially neutral and was not controlled by any titrants throughout the runs. All experimental runs were replicated and averaged values were reported in this work.

Analytical Methods:

During fermentation, for evaluating the xanthan gum, it was recovered by centrifuging at (10000 g) for 40 min at 4°C in order to sediment the cells. Xanthan gum in the supernatant was precipitated using ethanol, methanol or acetone (1:3 v/v) (Fulka, Germany). The solution was maintained at 4 °C for 24 h and re-centrifuged at 10000g, for 40 min at 4°C. The precipitate was diluted in distillated water and dried at 50°C in a conventional oven (Binder, Germany) until constant weight, to determine the xanthan gum content. Cell dry weigh was also determined using a cellulosic filter, 25mm diameter and 0.25 pore sizes (Wathman, USA) then the filter were dried in an oven at 80°C for 24h and weighed. The medium viscosity was measured by Siemens glassy viscometer. FT-IR spectra were recorded on the dried powder of xanthan was analyzed with Fourier Transform Infrared (FTIR, Bruker, Model Vector 22, Germany) to define the functional group of synthesized xanthan gum. The dry sample powder was mixed with KBr and pressed into pellets under reduced pressure. The FTIR spectra were obtained by scanning between 4000 and 500 cm^{-1}(Fernandes, M.S, *et al*, 2009, Kalogiannis, S. *et al*, 2003, Garcia-Ochoa, F, *et al*, 2000, Yoo, S.D, *et al*, 1999).

RESULTS AND DISCUSSION

In this research paper, xanthan gum production by cheese whey in several agitation rates was conducted. The broth viscosity of xanthan gum produced in different agitation rate was determined. Because xanthan production is strictly aerobic process, it was necessary in these experiments to increase the stirrer speed in order to enhance the oxygen dissolved concentration and so avoid anaerobic conditions. Five experiments were carried out maintaining the stirrer speed constant along the fermentation time. The results of this section were shown in Figure.1.

As can be seen in Fig. 1 with increasing in agitation rate to 550 rpm, xanthan gum production were enhanced but more increasing caused to decrease amount of xanthan gum. This must be due to the fact when stirrer speed increased two effects can be observed: an increase in the oxygen mass transfer rate and cell damage. When the agitation rate was too high (550 rpm) both biomass growth and xanthan production reached to a maximum amount. At higher stirrer speeds (850 rpm) both biomass growth and xanthan production descended. This must be due to the fact that high stirrer speed caused damage the cells and decrease the biomass growth rate by hydrodynamic stress; therefore xanthan gum production was very small. In addition, at lower stirrer speed (250 rpm) also lower biomass growth and xanthan production was reached due to oxygen transfer limitation.

Figure 2 demonstrates the apparent viscosities of the fermentation broth. The cultured broth viscosities measured during fermentation. The culture media apparent viscosity for cheese whey was gradually varied in increasing trend from 1 to 160 centipoise (cP). The increasing trend in viscosity of media was one of the indicating parameter to prove the production of xanthan gum. The apparent viscosity of the media for agitation rate equal to 550 rpm was the highest value therefore maximum amount of xanthan gum produced at this stirrer speed. The obtained results are quite compatible by illustrated data in the last figure as these are having similar trends.

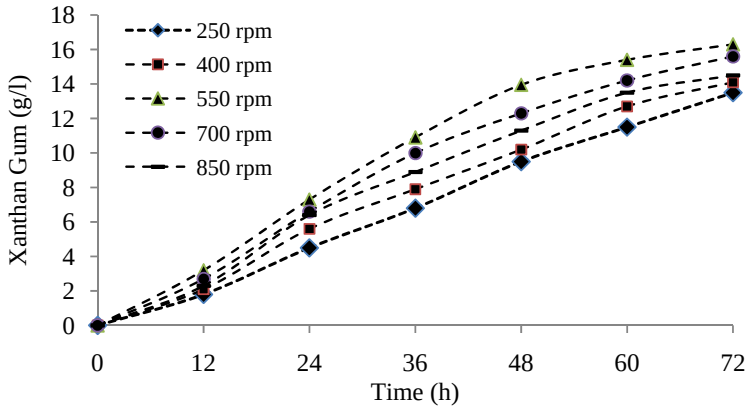

Fig. 1: Xanthan gum production with cheese whey by several agitation rates, at 32 °C, neutral pH and 72 hours.

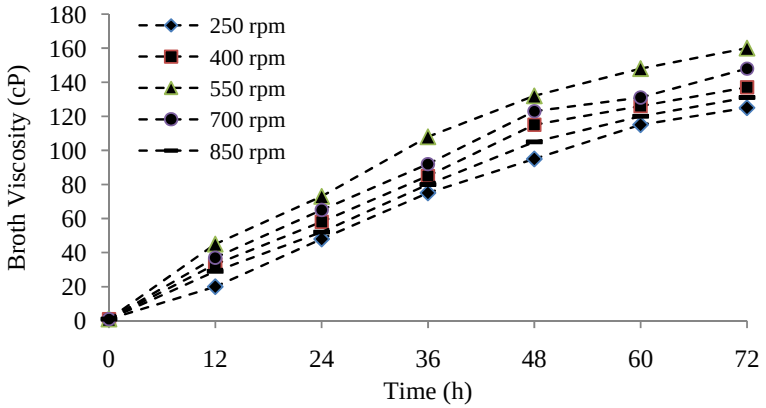

Fig. 2: The appearances viscosity of medium from cheese whey by several agitation rates, at 32 °C, neutral pH and 72 hours.

The yield of xanthan gum and mass of cell dry weight were obtained. Figure 3 shows the maximum amount of xanthan gum produced 16.3 g/l which was devoted to cheese whey as sole carbon source. Also, maximum yield of 0.543 g xanthan/g lactose was obtained. It was reported in the literatures that cheese whey was identified as the appropriate carbon source for xanthan gum production (Cacik, F, *et al*, 2001, Somas, S.K, *et al*, 2007). The treated cheese whey is enriched with proteins and minerals salts that lead to increase the yield of xanthan gum production. In addition, as the biopolymer was extracellular phase separation occurred with the aid of organic solvents such as acetone and methanol. The maximum cell dry weight with cheese whey was value 2.65 g/l.

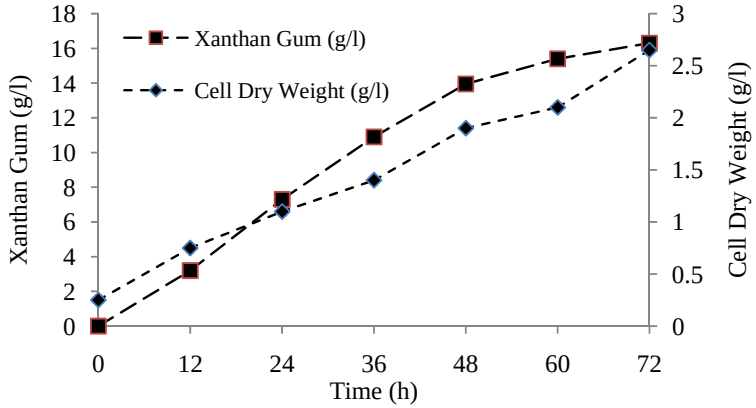

Fig. 3: Maximum xanthan gum production and mass of cell dry weight with 550 rpm, at 32°C, neutral pH and 72 hours.

FT-IR Spectra Of Xanthan Gum:

The FT-IR spectra of synthesized xanthan gum via fermentation and commercial xanthan gum recorded under the same conditions. The FTIR spectrum of the produced xanthan sample showed that the carboxyl, carbonyl, acetal, with quite similar value with the commercial sample functional groups as reported in Table.1. Figure 4 summarized and compared the wavelength spectra of commercial and produced xanthan gum with cheese whey. These results indicate the accommodating between synthesizes and commercial xanthan gum.

Table. 1: FTIR spectral data (cm^{-1}) of standard and product samples.

FUNCTIONAL GROUP	CARBONYL	CARBOXYL	ACETAL
Commercial Xanthan Gum	1627	1529	1160
Xanthan Gum From Cheese Whey	1604	1569	1081

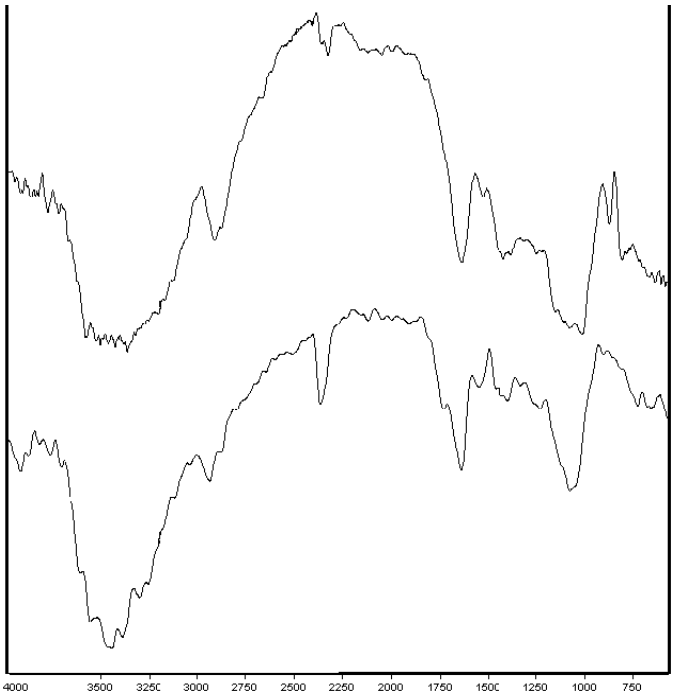

Fig. 4: FT-IR spectrum of commercial (a) and synthesized xanthan gum (b).

Conclusions:

Xanthan gum was produced from cheese whey using *Xanthomonas campestris*. The fermentation conditions were kept the same while the amounts of liberated biopolymer with different agitation rates were compared. The functional groups of bioproduct were compared with the commercial xanthan by FTIR spectra. The maximum amount of xanthan was obtained from cheese whey at 550 rpm of stirrer speed.

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