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Effect of air and stirring on the synthesis of xanthan by *Xanthomonas campestris* pv pruni strain 06

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Xanthan is the more important bacterial polysaccharide from a commercial point of view, with a consumption of approximately 30,000 ton/year (Stredansky & Conti, 1999). It is produced by bacteria of the genus *Xanthomonas* under strict aerobic conditions (Swings et al., 1993). *Xaanthomonas* may be grown in liquid, semisolid or solid media. During growth the synthesis of the biopolymer increases the viscosity of the culture medium hampering the availability of dissolved oxygen to the cells and limiting its use. Stirring favours the distribution of oxygen and other nutrients. Stirring speed and air supply are intimately correlated, and they may be regulated to optimize productivity. Stirring speed depend on fermentor design and capacity. The more important characteristic of xanthan is its viscosifying ability, that can be defined as the viscosity generated per unit of gum concentration, and can be used to compare the quality of xanthans obtained by different procedures (Torrestiana et al. 1990). Rheological properties of biopoymers depend on their chemical composition. The objective of this study was to evaluate the influence of stirring and air supply on yield, chemical composition and rheology of xanthan produced by *Xanthomonas campestris* pv strain 06 at different times of fermentation. *Xanthomonas campestris* pv pruni strain 06 was recovered by Olinda Martins (EMBRAPA-CPACT, Centro de Pesquisas de Clima Temperado, EMBRAPA, Pelotas, RS, Brazil) and preserved by liophylization and subculture. Two hundred and fifty mL Erlenmeyer flasks containing 50 mL of YM medium were inoculated with 10^9 CFU mL^{-1} *per flask* and incubated at 28 ºC and 150 rpm for 24 hours in an orbital shaker. These cultures were used to inoculate 3 L of production medium contained in a 5 L fermentor (Biostat). Fermentations were done under two conditions of aeration and stirring: (A) 250 rpm and 1,5 vvm, and (B) 350 rpm and 2,0 vvm, both at 25°C for 72 h with uncontrolled pH. Samples were collected at 18, 24, 42, 48, 66 and 72 h of culture to evaluate cell production, yield, rheological properties and chemical compoisition of xanthan. The samples were centrifuged at 16,000 x g during 45 min to separate cells and other debris. The exopolysaccharides were precipitated from the supernatant with 95% ethanol in a proportion of 1:4, washed 3 times with ethanol and dried at 50°C. Dry products were weighed, and means expressed in g L^{-1} of medium. All the experiments were made in triplicate. Microbial concentrations were estimated through

serial dilution and counting. The means were expressed in colony formers units per mL^{-1} (CFU mL^{-1}).One percent of not dialysed polymers (w/v) were putted into deionised water containing 2.2% of nipagin and 1.1% of nipazol and shaked during 1 h. Ten days after, measurements were made in a RS 150 Haake rheometer with cup dispositive, at 25 ºC and from 0,01 s^{-1} to 60^{-1}, during 100 s. In order to purify the polymers, 2% (v/v) aqueous solution of polysaccharides were dialysed against ultra pure water during 72 hours, dried at 50ºC and finally made into a powder. Samples were hydrolysed with 2N HCl and tested by comparative thin-layer chromatography (TLC). The spots were visualised by spraying the sheets with sulphuric- anisaldehyde and heating at 100.ºC for 5 min (Moreira, Souza & Vendruscolo 1998). In the work here reported xanthan production, cell growth and pH were measured during the culture of *Xanthomonas campestris* pv pruni strain 06 in a 3 L fermentor under two different combinations of stirring and air supply, treatment A, 250 rpm and 1,5 vvm and treatment B, 350 rpm and 2,0 vvm. Our results showed that during the first 48 h of culture, the rate of aeration did not influence the yield of xanthan, suggesting that stirring of 250 rpm and air supply of 1,5 vvm were enough for the demands of the bacterial population. At 66 h, however, higher air supply and stirring produced better yields. The polymers produced at condition A, under lower aeration rate and stirring spead had higther viscosities at all analysed fermentation times. The influence of aeration on the quality of the product obtained was more expressive that influence of time. At both oxygenation conditions, the viscosity of polymers produced was decreasing when the fermentation was increasing. Glucose, mannose, rhamnose and glucuronic acid, how expected for polymers produced by *Xanthomonas campestris* pv pruni, were detected in the polymers produced under both condiitions of aeration and fermentation time. Polymers obtained under conditon A showed higher mannose content and viscosity (Moreira et al., 2001). The stirring speed and air supply well as fermentation time had influence on yield and quality of xanthan produced by Xanthomonas campestris pv pruni. The highest aeration resulted in increasing on yield and decreasing on viscosity of obtained polymers.

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