PRODUCTION OF BACTERIAL CELLULOSE USING *Gluconacetobacter hansenii* ATCC 1431 STRAIN IN SYNTHETIC MEDIUM

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ABSTRACT

The production of bacterial cellulose (BC) by *Gluconacetobacter hansenii* ATCC 1431 in synthetic medium (20 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 1.15 g/L citric acid and 2.7 g/L sodium phosphate) was evaluated in this work. The parameters investigated on BC productivity were: cell growth, effect of inoculum biomass concentration, volume of medium and nitrogen source. The highest production was obtained using 3% of inoculum and 10 mL of medium after 5 days of fermentation using only yeast extract as nitrogen source.

INTRODUCTION

The bacterial cellulose (BC) is an extracellular polysaccharide produced by some species of microorganisms such as *Acetobacter xylinus* and *Gluconacetobacter hansenii* (Castro, 2011). This biopolymer has a cross-linked structure ultrafine naturally nanometric (diameter between 24 and 28 nm) and chemically pure, which distinguishes the cellulose obtained from vegetable sources, usually associated with lignin and hemicellulose.

Gluconacetobacter is a genus with a great number of species, and deploying a great variety of physiological traits, like organic acid production, nitrogen fixation, carbohydrate polymer synthesis, and others. Among the different members of the genus, *G. hansenii* has been identified as a species that is strictly aerobic, gram-negative, catalase positive, oxidase positive, not liquefy gelatin and not reduce nitrate and nitrite. Currently, this species is considered a model organism for studying cellulose synthesis (Lyer, 2010). Since each strain has genetic variations that influence the production capacity in response to variables (such as temperature of the medium, source of carbon and nitrogen), a study aiming at the optimization of BC production in the strain of work is essential.

RESULTS AND CONCLUSIONS

The production of BC by *G. hansenii* ATCC 1431 in synthetic medium (20 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 1.15 g/L citric acid and 2.7 g/L sodium phosphate) was evaluated. The parameters investigated were: cell growth and BC production kinetics, effect of inoculum concentration, volume of medium and nitrogen source. The cultures occurred at 30°C in medium with pH adjusted to 5, under static conditions.

Kinetic studies of growth and production of BC by *G. hansenii* ATCC 1431 in synthetic medium showed that after 5 days of culture, there was no significant variation in cell concentration, or in the production of BC. Therefore, other experiments were performed using 5 days as cultivation time.

The effect of inoculum concentration was evaluated starting with a solution containing approximately $40x10^4$ CFU/mL. The percentage of cells in the solution added was varied (1% to 20%, v/v) and the effect on production of BC was analyzed. Inoculum of 10, 15 and 20% (v/v) showed lower production, while inoculum at 1, 3 and 5% had higher production of BC, being 3% selected as the optimum biomass concentration.

In a 500 mL Erlenmeyer flask, 300 mL of this medium was inoculated and redistributed in 125 mL Erlenmeyer flasks, varying the amount added of 10 to 50 mL to study the effect of medium volume in BC production. It was observed that smaller volume promoted greater production. Due to the conical form of the flask, there is a variation in the oxygen transfer area when the volume was varied. Therefore, greater oxygen transfer area (obtained with smaller volumes of inoculated medium) resulted in higher BC production by *G. hansenii* ATCC 1431, in this tested conditions. The specific influence of the area was later confirmed in a test with cylindrical bottles, where the contact area liquid:air did not vary with the volume added. In this test no difference was observed in the production of BC, regardless of the volume of medium used.

Finally, we also investigated the effect of nitrogen sources on the production of BC by *G. hansenii* ATCC 1431. The C:N ratio was maintained at 15.5 and nitrogen sources were tested. The use of easily absorbed sources (ammonium sulfate, sodium nitrate and urea) or peptone showed no cell growth or BC production. Already the medium containing only yeast extract or corn steep liquor showed production of BC (0.87 and 0.16 g BC/L, respectively), and this value was close to that obtained with synthetic medium (0.92 g BC/L). As synthetic medium is a combination of yeast extract and peptone, for cost reduction, we can use only yeast extract as nitrogen source.

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