Production of Poly(glutamic acid) Using the Isolated Strain Bacillus subtilis DYU1

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ABSTRACT

A newly isolated strain from soil which was identified taxonomically as Bacillus subtilis (called B. subtilis DYU1) with high extracellular poly-glutamic acid (PGA) productivity. PGA production by B. subtilis DYU1 was strongly dependent on the concentration of additional exogenous L-glutamic acid and carbon source in the fermentation medium. Most favorable carbon and nitrogen sources for PGA production were maltose and peptone, respectively. The maximum PGA production (61 g/L) was obtained when it was grown in a medium containing 70 g/L L-glutamic acid, 30 g/L maltose and 5 g/L peptone at 37°C for 96 h with shaking (150 rpm). The maximum yield and productivity rate of PGA were both markedly depended on the ratio of carbon to nitrogen (C/N). An optimal C/N ratio range from 0.6 to 7.5 and 6 to 10 were obtained for maltose-based (30 g/L) and peptone-based (5 g/L) culture, respectively. In addition, other effect factor such as pH, agitation speed, metal ions, NaCl and biotin was also discussed to found the optimization condition for PGA production. The analysis with nuclear magnetic resonance (NMR) spectrometry and amino acid identification shows that the purified biopolymer possesses the structure of a PGA. However, the product produced by B. subtilis DYU1 was characterized by amino acid analysis to be composed of solely glutamic acid (99%). The average molecular weight of purified PGA was over 1,000 kDa determined by gel permeation chromatography, and 98% of the purified PGA was D-glutamate indicating that the B. subtilis DYU1 possesses a metabolic pathway to produce D-glutamate from L-glutamate. Additionally, the fermentation kinetic of PGA by B. subtilis DYU1 were also investigated in this study. By using Monod and Michaelis-Meten models, it was found that substrate inhibition for PGA production when glutamic acid and maltose was greater than 70 and 50 g/L, respectively. Moreover, a model based on the logistic and Luedeking-Piret equation of B. subtilis DYU1 growth, PGA accumulation combined non-growth-assocaited and growth-assocaited contributions, and consumprion of maltose were developed. The results predicted by the model were good agreement with the experimental observations. The rheological properties of PGA broth were studied using a rotational viscometer at several pHs (1.0-13.0) and temperatures (5-75°C). The mathematical models were developed for determining the apparent viscosity of PGA broth as affected by temperature and PGA concentration. Additionally, carbon activated was used as an adsorbent for the removal of molasses wastewater. Batch adsorption studies were conducted to explore the effect of dulition times, adsorbate concentration, pH and temperature. Finally, using molasses wastewater without or with pretreatment as a substrate for the production of PGA by B. subtilis DYU1 and lessening the pollution of molasses wastewater.

Keywords : Bacillus subtilis ; poly(glutamic acid) ; kinetic model ; rheological property ; molasses wastewater

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