Study of Production of Hyaluronic Acid from Streptococcus zooepidemicus in a Fermentor and Its Reheological Properties

賴韋光、吳建一

E-mail: 9708313@mail.dyu.edu.tw

ABSTRACT

Hyaluronic acid (HA) is a very high molecular weight polymer made of repeating units of glucuronic acid and N-acetyl-glucosamine which is bound by alternating β-1,3 and β-1,4 bonds. HA is a high-value biopolymer with a wide variety of medical and cosmetic applications. HA can be extracted from rooster combs or produced in microbial fermentation. In rooster comb, HA is complexed with proteoglycans,making the isolation of high purity, high molecular weight HA costly. Moreover, the use of animal-derived biochemicals for human therapeutics is being met with growing opposition because of the risk of cross-species viral infection and other adventitious agents. Hence, microbial production is gradually replacing extraction as the preferred source of HA. The main aim of the research was to determine the effects of temperatures, aeration and agitation on the production of HA by S. zooepidemicus var. HAWU. In addition, the rheological properties of HA broth and solution were studied. The description of this is divided into four sections. The first section is focused on the effect of different culture variables on the production and molecular weight properties of HA by S. zooepidemicus var. HAWU in the flask and 5-L jar fermentor. In the flask, the optimal temperatures was 37°C, which led to highest HA production (0.78g/L). In a 5-L jar fermentor, we achieved maximum HA productivity (0.6 g/h/L) when the aeration rate and agitation speed increased simultaneously. HA concentration reached 6.7 g/L when agitation rate and aeration rate were controlled at 300 rpm and 1.0 vvm, respectively. Moreover, by reproducing these conditions in a 20-L jar fermentor, we were able to get the same result. The second section is focused on the empirical kinetic model for the batch production of HA from S. zooepidemicus var. HAWU. By using Monod and Michaelis- meten models, it was found that substrate inhibition for HA production when glucose was greater than 20 g/L. Moreover, a model involved with S. zooepidemicus var. HAWU growth, and HA accumulation combined non- growth- associated and growth- associated contributions, and consumprion of glucose and oxygen based on the logistic and Luedeking- Piret equation was developed. The results predicted by the model were good agreement with the experimental observations. The third section is focused on the rheological properties of different amounts of HA broths and solutions using a rotational viscometer at several temperatures (4-70 °C), pH (1-11) and rotational speed (10- 250 rpm). The modified of power law model were found to be the good agreement with the rheological properties of HA broths and purified HA solutions. Activation energy was determined using the Arrhenius equation and it was found that the activation energy of HA increased with the addition of HA. The last section is focused on the hydrolytic degradation of HA by kinetic measurements. The first-order rate constants of the hydrolytic degradation of HA at different pH and temperature were obtained on the basis of various concentration of HA. Around pH < 5, the decrease of viscosity is shown, and it is attributed to cooperative interchain interactions due to the reduction of polymer net charge and may be the protonation of the acetamido groups; for pH > 5, the decrease of viscosity is mainly attributed to a reduction of the stiffness of the polymeric backbone in alkaline conditions due to the partial breakage of the H- bond network. In addition, from the rate constant, we obtained the activation entropy (△S) and enthalpy (△H) of hydrolytic degradation of HA.

Keywords : S. zooepidemicus var. HAWU ; Hyaluronic acid ; Fermentation kinetics ; Rheological

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