Concentration of cell-free fermentation broth containing poly (γ-glutamic acid) by ultrafiltration

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Abstract

After cell removal from fermentation broth the supernatant containing poly (γ-glutamic acid) (γ-PGA) was concentrated by ultrafiltration in order to reduce the amount of organic solvents such as methanol, ethanol, propanol required for the recover of γ-PGA with precipitation. The concentration and volume reduction of cell-free solution by ultrafiltration could reduce 3 times the amount of ethanol for the recover of γ-PGA from cell-free fermentation broth.

Introduction

γ-PGA was generally recovered by organic solvent precipitation so a large amount of solvent was required to recover γ-PGA from large-scale fermentation (Ito et al., 1996). As the high concentration of γ-PGA is advantageous for precipitation of γ-PGA by organic solvent, the volume reduction and concentration of cell-free fermentation broth containing γ-PGA by ultrafiltration was reasoned to be cost-effective process in the recovery of γ-PGA. Unlikely general protein concentration the fermentation broth containing γ-PGA was highly viscous. The solution with high viscosity was not easy to treat in membrane process. So the pretreatment for the reduction of viscosity should be performed. The high viscosity of fermentation broth containing γ-PGA was mainly due to the ionization of γ-PGA. Therefore the de-ionization of γ-PGA could reduce the viscosity of broth. In this study we have lowered pH of cell-free fermentation broth up to 2 for the de-ionization of γ-PGA.

Material and Methods

Fermentation broth The fermentation broth was obtained by batch cultures of *bacillus licheniformis* ATCC 9945A in E-medium (Leonard et al, 1958)

Concentration of cell-free fermentation broth Concentration of fermentation broth was performed by ultrafiltration using flat-sheet membranes (area of 0.1 m^2) including MWCOs of 30,000, 100,00, 500,000 and Sartoflow Alpha (Sartorius system Enginnering, Geottgen, Germany)

Results and discussion

Rejection of γ-PGA by membrane was dependent on not only MWCO of membrane but also pH of cell-free fermentation broth. The γ-PGA was perfectly rejected by the membrane having MWCO of 30,000, however the water flux was very low. In the case of membrane having MWCO of 100,000 only 1% of γ-PGA was passed through it and the water flux was comparable to the membrane having MWCO 500,000. Therefore the membrane having MWCO of 100,000 was chosen for the concentration of cell-free fermentation broth containing γ-PGA. When the concentration of γ-PGA in retentate exceeded 80 g/L there was little flux at pH 6. But the water flux was kept relatively high until 80 g/L of γ-PGA at pH 2. This was resulted from the reduction of viscosity in cell-free fermentation broth by de-ionization of γ-PGA at low pH. The loss of γ-PGA was higher at pH 2 than pH 6. This was reasoned that the conformation change of γ-PGA from random coil to helix, which is to some extent compact form, occurred at low pH. The γ-PGA could be successfully recovered with 50 - 70 %(v/v) of alcohol concentration from the cell-free fermentation broth containing γ-PGA of 80 g/L.

References

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