Internal contactor가 설치된 stirred tank를 이용하여 yeast suspension 내의 Protein (Bovine Serun Albumin) 의 1차 분리.

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Primary recovery of protein (Bovine Serun Albumin) using internal contactor in stirred tank in yeast suspension

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1. Introduction

Downstream process usually accounts for the largest part of the production costs of protein. Fast and efficient recovery steps for further purification of proteins forms the basis for a successful downstream process. Primary recovery comprises the first steps of downstream processing where some purification and broth volume reduction occurs. Several engineers have considered adsorptions of proteins using several kinds of adsorbents. Suspended-adsorbent adsorption systems include stirred tank (batch), expanded/fluidized bed etc. In this experiment, modified stirred tank with internal contactor was used to have both advantages of previous stirred tank system and EBA, fast adsorption and deletion of filtration step respectively. A *Hansenula* polymorpha was shown as a good yeast for recombinant protein nowadays. It was known that the primary recovery of this cell could be processed under 5% cell suspension using EBA. In this paper, the performance of primary recovery using internal contactor was shown pretty good with a simulated solution.

2. Materials and Methods

2.1 Model protein

The model protein was bovine serum albumin (BSA) (Sigma-Aldrich, Republic of Korea) of 99.9% purity.

2.2 Anion Exchanger

DEAE-STREAMLINE (Sepharose based ion exchanger, Pharmacia Biotech AB, Uppsala, Sweden) is weak anion exchanger. Particle size is 100 ~ 300 (㎛).

2.3 Fed-batch culture and cell washing for Yeast (*Hansenula* Polymorpha) preparation

To obtain subculture, the frozen strain was transferred to YPD agar plates containing 10.0 g/L yeast extract, 10.0 g/L peptone, 20.0 g/L glucose and 10.0 g/L agar. These were incubated at 30℃ for 24 hrs in an incubator. Subcultures were transferred to the seed culture medium (50mL) in 500mL Erlenmeyer flask, containing 1% yeast extract, 2% peptone and 2% glycerol. The medium was incubated at 37℃, 300 rpm for 24 hr to become 3.0 g/L DCW. Following the batch phase, a concentrated glycerol feeding phase (200g/L glycerol, 50g/L yeast extract and 100g/L bactopeptone.) was started using a computer controlled peristaltic pump. The process was finished after the cell density in broth became about 70g DCW/L. The pH of culture broth was controlled to 5.0 with 2 N H_2SO_4 and 2 N NaOH. Antifoam 204 (Sigma Chemical Co., St Louis, U.S.A) was added prior to batch and feeding solution. After cell cultivation, cell had to be cleaned to remove possible cell debris and remained proteins of medium. Cells were precipitated using a centrifuge at 3000rpm for 10min, then were suspended with distilled water. These steps were repeated several times until a supernatant of cells had no proteins.

2.4 **Buffers for simulated solutions**

A simulated solution in this paper was meant a BSA solution in buffer or a BSA solution in cell suspension buffer. The buffer in a simulated solution was made by Tris-HCl (pH7.6). An ionic conductivity was also adjusted with NaCl using ion conductivity meter.

2.5 Internal contactor and desorption chamber

Internal contactor was easily installed and uninstalled. BSA in cell suspension in a stirred tank was adsorbed onto anion exchanger in an internal contactor. The protein was then desorbed in the internal contactor using a desorption chamber with high salt solution (0.5M NaCl) to be concentrated.

3. Results and Discussion

The optimum values of ion conductivity and process time were determined to be about 7.3 mS/cm and 2 hr respectively from several trials and errors (Fig.1 & Fig.2). A screw-type impeller was chosen for a better adsorption performance rather than Rushton turbine type impeller in this system (Fig.3). The desorption step using a chamber showed a great performance (Fig.4). In a low cell concentration (2%), the adsorption process was done pretty well (Fig.5). In a high cell concentration (6.1%), the adsorption process showed that the internal contactor had a potential as a equipment for the primary recovery of proteins (Fig.6). In a high cell concentration, cell autolysis was thought a problem, so process time should be reduced.

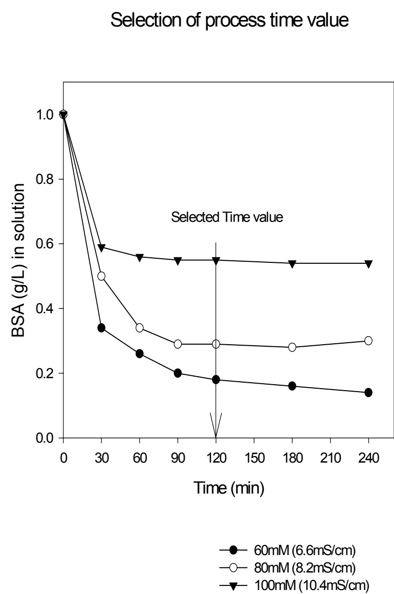


Fig.1 Selection of process time

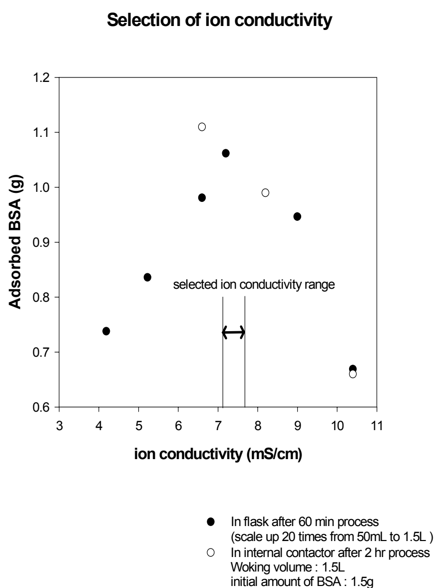


Fig.2 Selection of ion conductivity

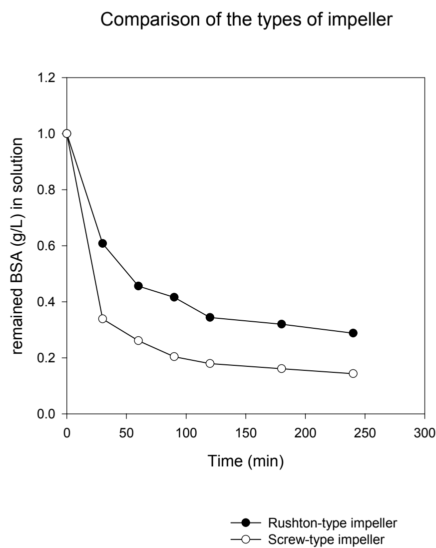


Fig.3 Determination of impeller type

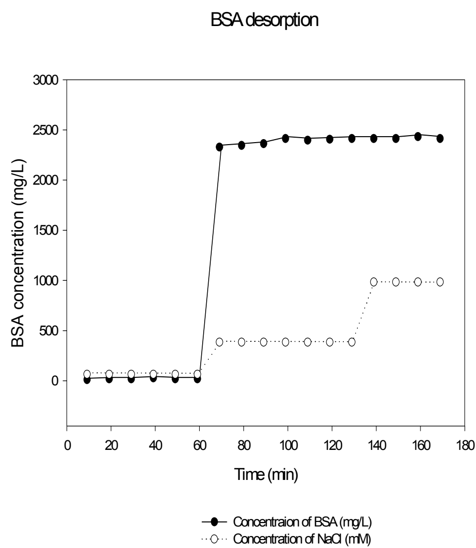
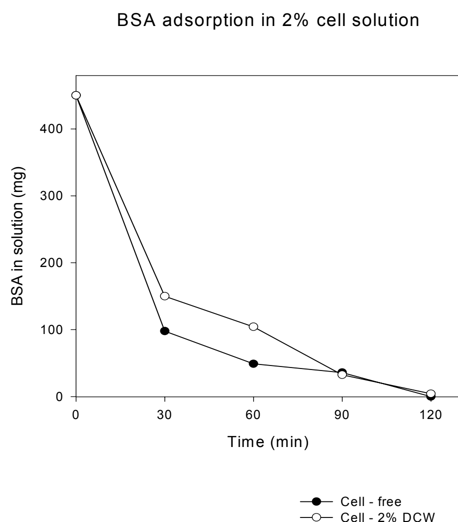


Fig.4 Desorption using a chamber



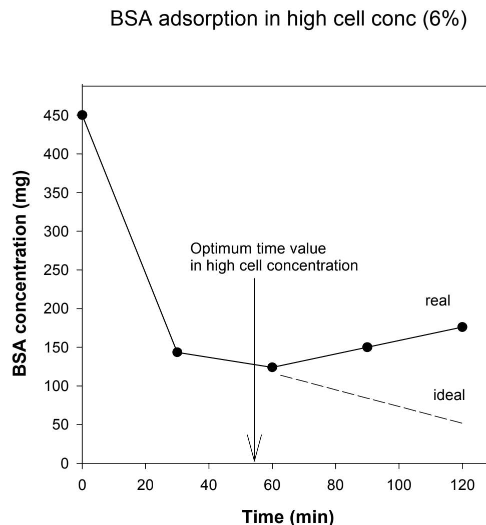


Fig.5 BSA adsorption in low (2%) cell suspension

Fig.6 BSA adsorption in high (6%) cell concentration

4. Reference

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