Simulated moving bed (SMB) is a well-established technology based on simulating a countercurrent contact between the solid (stationary) phase and liquid phase. It is widely used in separation of sugars, monosaccharides and drugs in commercial scale. SMB has been shown to be more beneficial in terms of productivity such as product concentration as well as reduction of column backwash, while requiring less protein feed. Use of SMB in protein purification in biopharma industry is common. This is in spite of the fact that most of the work reported in the area of FPLC based protein purification is in separation of binary mixtures. Protein purification is ideally suited for SMB where two components are separated as effluent and× effluent. There are a couple of reasons on the SMB method of choice. The SMB method (Bind, Wash, Elute and Regenerate) using Nickel (HIS-Select) affinity chromatography and SMB, NdmB was chosen for the study of N-dimethylase activity. NdmB showed good activity towards 7,8-dihydro-7,8-dihydroxy-2-methylchromene (2H-1-Benzopyran-2-one) (Figure 4).

Introduction (NdmB)

We choose to compare purification of recombinant N-dimethylase (NdmB) using both batch column chromatography and SMB. NdmB was chosen for this study among several Rieske Oxygenases that cleave C-N bonds (carbazole with several Rieske Oxygenases that cleave C-N bonds (carbazole

Comparative Study on FPLC and SMB

Comparative purification study was carried out on FPLC and SMB using same column volume and same resin. In case of FPLC, XK20/30 column with 40 ml 4.6 Select Riotte resin was used. For SMB, 5-50 ml columns were used. The NdmB column used sample was prepared as described below under identical conditions.

* Anonym: of cell paste: 24g
* Lysis Buffer: 200mM of Binding Washing Buffer (25mM KPi, 3M NaCl, H1bM Imidazole, pH 7.5)
* Cell breaking method: MicroPelletization Two phases (15Krpm)
* Centrifuge: Beckman rotor no 14, 124K (73K 4°C)
*  Filter: The 50 ml load through 0 than 0. 05 membrane

Table 2: shows the recovery of NdmB from FPLC -column. Figure 6 is the elution profile from the Affinity chromatography . Table 3 shows the recovery of NdmB from SMB.

Results

Cell paste was obtained from 30-L fermentation of E. coli expressing NdmB. The cell paste was frozen at -80°C until processing. Initial column chromatography process was optimized using SMB (Octave 100, Semba Biosciences) and FPLC (Amersham). About 24 grams of cell paste was used for each method of purification. For SMB we used 8 columns of 5 ml each for FPLC, one work (XK20) columns. Figure 6 shows the Ni protein load was loaded on to both systems. After washing the column with Buffer (25mM KPi, 300mM NaCl, 30mM Imidazole, pH 7.5), NdmB was added with Elution Buffer (25mM KPi, 300mM NaCl, 30mM Imidazole, pH 7.5). The recovery of NdmB from SMB and FPLC was 30% and 25%, respectively. Based on band intensity on SDS-PAGE of NdmB, it was assessed that SMB achieved 95% purity whereas from FPLC it was 90%. Measuring the overall productivity in terms of (i) time taken for purification, 150 minutes vs. 350 minutes (SMB vs FPLC), (ii) buffer use, 1 vs 1.5 times (SMB vs FPLC), and (iii) column regeneration (no additional unit operation for SMB), SMB method was better than the FPLC (batch chromatography). SMB being a continuous process is more suitable for large scale manufacturing of proteins/therapeutics. SMB will continue to explore SMB application for protein purification and for cellular molecules separations with the intent of developing this technology for industrial applications.

Table 1: NdmB Purification on FPLC with and without regeneration

Table 2: NdmB Recovery on Akta FPLC

Table 3: NdmB Recovery on SMB (Octave 100)

References

3. Ryan E. Werners, Michael Louie, Chi迄今为止,纳米生物和纳米技术的研究和应用在生物医学、材料科学和环境工程等领域取得了显著进展。纳米晶体或纳米颗粒在许多应用中具有独特的性质，包括表面改性、生物活性和纳米材料的合成。纳米晶体的合成和表征是开发纳米材料性能的关键步骤。

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