Journal of Chromatography B 1100–1101 (2018) 65–75

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/jchromb

Direct recovery of malate dehydrogenase from highly turbid yeast cell homogenate using dye-ligand affinity chromatography in stirred fluidized bed

Kuei-Hsiang Chen[a], Sze-Ying Lee[b], Pau-Loke Show[c], Shih-Cheng Hong[a], Yu-Kaung Chang[a,*]

[a] Department of Chemical Engineering, Graduate School of Biochemical Engineering, Ming Chi University of Technology, New Taipei City 24301, Taiwan
[b] Department of Chemical Engineering, Faculty of Engineering and Science, Universiti Tunku Abdul Rahman, Sungai Long Campus, Kajang 43000, Selangor, Malaysia
[c] Department of Chemical and Environmental Engineering, Faculty of Engineering, University of Nottingham Malaysia Campus, Jalan Broga, Semenyih 43500, Selangor, Malaysia

ARTICLE INFO

Keywords:
Dye-ligand affinity chromatography
Stirred fluidized bed
Recovery
Malate dehydrogenase
baker's yeast cell

ABSTRACT

Dye-ligand affinity chromatography in a stirred fluidized bed has been developed for the rapid recovery of malate dehydrogenase (MDH) from highly turbid baker's yeast cell homogenate in a single step. The most suitable dye, namely Reactive Orange 4, in its optimal immobilized concentration of 8.78 mg/mL was immobilized onto high-density STREAMLINE matrix. To further examine optimal adsorption and elution conditions, the enzyme recovery operation was carried out using unclarified cell homogenates in stirred fluidized bed system. Aiming to develop a non-specific eluent, namely NaCl, to effectively elute the MDH adsorbed, direct recovery of MDH from highly turbid cell homogenate (50% w/v) in a stirred fluidized bed adsorption system was performed. The proposed system successfully achieved a recovery yield of 73.6% and a purification factor of 73.5 in a single step by using 0.6 M NaCl as an eluent at a high liquid velocity of 200 cm/h.

1. Introduction

Dye-ligand chromatography has been one of the most popular techniques for protein purification [1–7]. The use of reactive dye as a ligand in protein purification technique is highly selective, inexpensive and has binding capacities that are 10 to 100 times greater than that of immobilized nucleotide matrices [8–11]. Therefore, dye-ligand affinity chromatography has appeared as a promising alternative for large-scale enzyme purification. The reactive dye-ligand adsorbents have complex but specific interactions with the active sites of protein molecules, including electrostatic, hydrophobic, hydrogen bonding, and van der Waals forces [1,2,5]. Although a highly substituted dye-ligand adsorbent has higher capacity to bind with more protein molecules, the elution of the target protein might be getting difficult [1,2,5]. Therefore, the selection of reactive dye and eluent are equally essential in determining the purification efficiency of the dye-ligand chromatography technique.

On the other hand, conventional expanded bed adsorption (EBA) and fluidized bed adsorption (FBA) have high potential to bind efficiently with the target protein from unclarified feedstock and promote high potential to simplify the purification processes [12–14]. Several studies have demonstrated that these methods can work as primary recovery operation, to allow solid-liquid separation and the extraction of protein of interest selectively from the crude biological feedstock in a single step [3,15–19]. However, the application of these techniques is limited by its drawbacks that the small sized pores of the support nets might be easily get clogged if the crude feedstock contains large solid particulates. This unfavorable phenomenon disturbs the liquid flow distribution, and thus affecting the purification efficiency [20–22]. In light of these constraints, stirred fluidized bed adsorption (SFBA) has been introduced whereby the liquid distribution system in the fluidized bed column was equipped with a specially designed stirrer device. The stirring motion inside the SFBA column promotes the even distribution of the process liquid and avoids of the instability of the fluidized bed due to the channeling or bypassing the liquid [20,21,23,24]. Additionally, the SFBA column is equipped with the bottom distributor

Abbreviations: ATP, adenosine triphosphate; CC-CPC, continuous counter-current protein chromatography; CIP, cleaning-in-place; EBA, expanded bed adsorption; FBA, fluidized bed adsorption; MDH, malate dehydrogenase; NADH, β-nicotinamide adenine dinucleotide; NADP, β-nicotinamide adenine dinucleotide phosphate; PVA, poly (vinyl alcohol); SFBA, stirred fluidized bed adsorption

* Corresponding author.
E-mail address: ykchang@mail.mcut.edu.tw (Y.-K. Chang).

https://doi.org/10.1016/j.jchromb.2018.09.039
Received 29 July 2018; Received in revised form 20 September 2018; Accepted 28 September 2018
Available online 02 October 2018
1570-0232/

plate and support net to prevent the clogging by the large-sized solid particulates in crude extract. All the operation processes such as adsorption, washing, elution and cleaning-in-place (CIP), can be carried out inside the column [24]. Our earlier work has reported the application of SFBA technique for direct recovery of lysozyme from crude chicken egg white with the recovery yield of 98% and purification factor of 11 achieved in a single step [24].

Malate dehydrogenase (MDH) has been frequently used as an indicator enzyme for the measurement of malic acid in the tissue and food samples. MDH from different microbial sources can be recovered and purified using anion exchange [25], affinity [26], and size exclusion [27] chromatographic methods. Besides, the purification of MDH from yeast cell homogenate using dye-ligand affinity adsorbent in an expanded bed contactor has been reported [15]. However, the study involved the dilution of sample which might lead to a decrease in the productivity of MDH. The purification process is more complex, since the concentration of MDH in the crude extract is relatively low [15,28]. Additionally, the loss of enzyme activity is frequently reported due to the lengthy downstream processing [29–31]. Therefore, this work focused on the evaluation of the SFBA using dyed adsorbent as a primary recovery operation for direct recovery of MDH from highly turbid yeast *Saccharomyces cerevisiae* cell homogenate. The proposed technique eliminates the need for the dilution of highly viscous feedstock and removal of the biomass particulate materials. Since the interaction between target protein and immobilized dyes occurs at protein's active sites, an affinity elution buffer might be a good candidate to recover the target enzyme selectively from the immobilized dye [2,5]. However, affinity eluents such as adenosine triphosphate (ATP), β-nicotinamide adenine dinucleotide (NADH), and β-nicotinamide adenine dinucleotide phosphate (NADP) are usually very costly [2]. Therefore, the present work aims to develop a suitable reactive dye that can be immobilized onto the STREAMLINE support matrix to effectively capture MDH and allow the rapid elution of the adsorbed MDH with a non-specific eluent like NaCl from the dyed adsorbent bed. Their optimal operating conditions were investigated.

2. Experimental

2.1. Materials and apparatus

STREAMLINE matrix (100–300 μm, 1.2 kg/m^3) adsorbent and ÄKTAprime chromatographic system were purchased from GE Healthcare (Uppsala, Sweden). Baker's yeast *S. cerevisiae* was acquired from a local supplier (Taipei, Taiwan). Reactive dyes were supplied by First Chemical Manufacture Co., Ltd. (Taipei, Taiwan). Two Watson-Marlow peristaltic pumps (Model 302S) were obtained from Waterson-Marlow Ltd. (Falmouth, UK). The SFBA column with inside diameter of 25 mm (see Fig. 1) was tailor-designed and fabricated in our laboratory (Chemist Scientific Corp., New Taipei City, Taiwan). The features of the SFBA column were discussed in detail in our previous works [24].

2.2. Dye immobilization

The reactive dye was covalently immobilized onto a STREAMLINE matrix via the nucleophilic reaction under alkaline conditions, as shown in Fig. 1. Immobilized Procion Orange MX-2R dye, or known as Reactive Orange 4 (RO4), was dissolved in distilled water to prepare at concentration of 0.25–15.0 mg/mL, and transferred to NaCl solution (2.0 M, 10 mL). The adsorbent suspension containing the dye was equilibrated for 1.5 h. After that, 2.12 g of sodium carbonate was added, and the mixture was heated to 60 °C and then mixed using a rotary mixer at 25 rpm for 6 h. After the completion of immobilization process, unreacted dye was washed out from the adsorbent carrier with distilled water and 1.0 M NaCl in 25% (v/v) ethanol. The immobilized dye concentration was determined by measuring the absorbance value at wavelength of 490 nm using a UV–vis spectrophotometer (Model Ultrospec 3100 pro, GE Healthcare). Likewise, large-scale STREAMLINE Orange 4 dyed adsorbent was prepared using the same protocols but with the large volume of 500 mL.

2.3. Yeast cell disruption

50% (ww/v) (wet weight, g/volume, mL) of yeast cell suspension in phosphate buffer solution (20 mM, pH 8) was homogenized using a high-pressure homogenizer (APV-2000, Invensys, Denmark) that equipped with a cooling system to control the temperature in the range of 4 to 8 °C. The cooling system is important to prevent localized heating during homogenization process that might lead to enzyme denaturation. Because of the relatively high rigid structures of yeast cell walls, mechanical method such as high-pressure homogenization was used, allowing the effective liberation of intracellular components from the yeast cells. The cell suspension was homogenized at a pressure of 1000 bar and were passed through the homogenizer up to six cycles. After each pass, the cell homogenate was collected for the measurement of the total proteins and MDH activity.

2.4. Total proteins assay

The total proteins assay was determined using the Bradford's method [32], and measured by a UV–vis spectrophotometer at 595 nm, and a standard calibration curve was established using standard protein bovine serum albumin. The protocols were performed as described elsewhere [33,34].

2.5. MDH activity assay

The MDH activity was assayed by adding 33.3 μL of enzyme solution to the substrate solution containing 0.1 M potassium phosphate buffer (pH 7.4, 0.87 mL), 6 mM oxaloacetate acid (0.06 mL), and 14.3 mM NADH (0.03 mL) [15]. MDH activity was determined by measuring the decrease in absorbance at 340 nm and monitoring the oxidation of NADH to NAD^+. One unit (U) of MDH activity was defined as the amount of enzyme required to convert 1 μmol of substrate to product in 1 min at 25 °C [35]. All experimental data were the mean value of the triplicate measurements, where the standard deviation was within 5% of the mean value. MDH activity in the samples was calculated using Eq. (1) [15]:

$$\mathrm{MDH}(U/ml) = \frac{\Delta \mathrm{OD}_{340}/\mathrm{min}}{6.22 \times 0.0333} \times DF \tag{1}$$

where OD_{340} is the optical density at 340 nm, 6.22 is the millimolar extinction coefficient of β-NADH at 340 nm, 0.0333 is the volume of sample used (mL), and DF is the dilution factor.

2.6. Optimal adsorption of MDH by immobilized dyes

1 mL of 1:1 (v/v) of dyed adsorbent in buffer (20 mM sodium phosphate buffer, pH 7) and 1 mL cell homogenate (5% ww/v, MDH of 15.83 U/mL, total protein of 2.45 mg/mL) were mixed by a rotatory mixer at 25 rpm and 25 °C for 1 h. The mixture was then allowed to settle to reach equilibrium, and the supernatant was collected for the total proteins and MDH activity assays.

The binding capacity of MDH (q_A, U/mL) on the dyed adsorbent in a batch mode was determined using Eq. (2):

$$q_{\mathrm{A}} = \frac{\mathrm{M}_i V - M_f (V + \nu)}{V_s} \tag{2}$$

where q_A (U/mL) is the binding capacity of MDH in the adsorbent phase (U/mL), M_i and M_f are the activity of MDH before and after adsorption in the liquid phase (U/mL), respectively, V is the initial volume of liquid phase before adsorption (mL), V_s is the volume of the adsorbent phase (mL), and ν is the volume of liquid phase in the 50% (1:1) adsorbent-