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SEPARATION OF CYCLODEXTRINS (CDs) USING AQUEOUS TWO-PHASE SYSTEM

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ABSTRACT

Separation of three different types of cyclodextrins (CDs) [α-,β-, and γ- CD] were investigated in the polyethylene glycol (PEG) 20000/ dextran T500 aqueous two-phase system (ATPS) with an aim to study the partitioning behavior of CDs in the absence of the CDs producing enzyme, cyclodextrin glycosyltransferase (CGTase). The ATPS developed (7.7% (w/w) PEG 20000, 10.3% Dextran T500, with volume ratio, V_R of 4.0 was the optimum ATPS for the sago starch bioconversion of *Bacillus cereus* CGTase. This preliminary study was useful for the further kinetic studies of the sago starch conversion into CDs by the CGTase in an ATPS. The study showed that all types of CDs exhibit the PEG-rich phase preference with the high partition coefficient (K_{CD}) over time. However, difference in the partition coefficients between these 3 CDs was observed, suggesting that their partitioning behaviour in the ATPS were different as a result of the difference in the hydrophobic interactions with the phase components and solubility of CDs. The exclusive partition of CDs into the PEG-rich phase is beneficial for the extractive bioconversion of starch in ATPS.

Keywords: *Separation, cyclodextrin; Bacillus cereus; cyclodextrin glycosyltransferase; aqueous two-phase*

INTRODUCTION

Aqueous two-phase system (ATPS) is gaining attention in the biochemistry and biotechnology field due to the low cost for construction, minimization of denaturation of proteins and most importantly the extractive bioconversion in ATPS can be easily scaled-up to industrial scale and is able to retain a steady state continuously [1-2]. In addition, ATPSs are environmentally friendly as compared to other existing separation technologies [3]. ATPS is a liquid-liquid extraction technique that formed by mixing two immiscible liquid above a critical concentration where two phases were observed [4]. ATPS have been widely applied in separation of various biomaterials [5] and process integration (e.g. extractive fermentation and extractive bioconversion) [2].

Cyclodextrin (CD) is produced when the enzyme CGTase reacts with the substrate (e.g. starch or other related sugars) through cyclization, an intramolecular transglycosylation reaction [6]. CDs are the most useful products for CGTase enzyme with high commercial values due to the diversified applications in industries. α-, β- and γ-CDs are among the most common types of CDs which possess 6, 7, and 8 glucose residues respectively [7-8]. In addition, β-CD is the most highly produced CDs in large scale due to its low enzymatic activity [9]. The unique structure of CDs (i.e. hydrophobic internal cavity and a hydrophilic external surface) enables them to form inclusion complexes with a variety of foreign molecules. The formation of this inclusion complex will exert some physicochemical effect on the foreign molecules, results in modification of physical and chemical properties of the guest molecules. This unique feature of CDs allows them to act as a stabilizing agent, emulsifier or antioxidant in various industries such as food, pharmaceutical, cosmetic, and medical industries [10]. Moreover, CDs is utilized as a separating media in the separation of enantiomers [11]. In view of the commercial importance of CDs, it is vital to produce and separate them from the production mixture with maximum recovery and low cost. ATPS has been implemented in the separation of CDs in this study. This study

is useful for further investigation of simultaneous CDs production and separation in ATPS. The partition coefficients of three main types of CDs (i.e. α-, β-, and γ-CD) in PEG 20000/dextran ATPS were evaluated.

MATERIALS AND METHODS

Materials

Polyethylene glycols (PEG) with mol. wt. of 20000 g mol-1; phenolphthalein and potassium phosphate were obtained from Merck (Darmstadt, Germany). α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), γ-cyclodextrin (γ-CD), Dextran T500 (with average mol. wt. of 500000 g mol^{-1}) were supplied by Sigma-Aldrich (St. Loius, USA). Other chemicals were all of analytical grade.

Methods

Analytical procedure:

Concentrations of α-, β-, and γ-CDs were analyzed by a Shimadzu HPLC system (Liquid Chromatograph LC-10AT, Diode Array SPD-M10A, and RID 6A) equipped with a Pack Polyamine II column (250 mm x 4.6 mm, YMC Co., Ltd., Japan). Samples of CDs were eluted isocratically with eluent (water-acetonitrile 45:55) at a flow rate of 1.0 ml min^{-1}.

Experimental procedure:

50% (w/w) of PEG solutions, 20% (w/w) of dextran solutions and 15mg/mL CDs mixture solutions (i.e. α-, β-, γ-CD in a ratio of 1:1:1) were prepared prior to the partition experiments. 10 g of ATPS was prepared in a 15 mL centrifuged tubes by adding polymer or dextran stock solutions. 20% (w/w) mixtures of standard CDs (15 mg/mL) were then added into the ATPS. The established ATPS was then left in room temperature for phase separation and the partition coefficients of three types of CDs were determined at a certain time. Volumes of top and bottom phases were determined and withdrawn separately for HPLC CDs concentrations analysis.

Calculations:

The partition coefficient (K) of α-, β-, and γ-CD was denoted as K_α, K_β and K_γ respectively and was generally calculated as the ratio of their concentration in top and bottom phase respectively using the Eq.1:

$$K = \frac{C_T}{C_B} \quad (1)$$

where C_T and C_B are the CD concentration in the top and bottom phases respectively.
Yield of CD in the top phase (Y_T) was determined using Eq. 2:

$$Y_T\,(\%) = \frac{100\,C_T\,V_T}{C_I V_I} \quad (2)$$

where V_T and V_I are the volumes of the top phase and the volume of CDs subjected to ATPS extraction, respectively. C_T and C_I are the concentrations of CD in the top phase and the initial CDs mixture, respectively.
Selectivity (S) was defined as the ratio of the CD partition coefficient K_i (i.e. i = K_α, K_β, and K_γ) to the total CDs mixture partition coefficient (K_{CD}) using the Eq. 3:

$$\text{Selectivity} = \frac{K_i}{K_{CD}} \quad (3)$$

Volume ratio, V_R was defined as the ratio of volume in the top phase (V_T) to that in the bottom phase (V_B) and calculated by using Eq. 4:

$$V_R = \frac{V_T}{V_B} \quad (4)$$

RESULTS AND DISCUSSIONS

Phase diagrams of PEG/Dextran ATPSs were referred and adopted in this study [12-13]. The partitioning behaviour of CDs over time was investigated where V_R of the ATPSs were kept constant (V_R = 4.0). Fig. 1 shows the concentrations of various CDs in top phase over time. Results show that all the partition coefficients (i.e. K_α, K_β, K_γ, and K_{CD}) change over time. This indicates that the mass transfer of CD between the phases are signigficantly changing over time as a result of differ in the solubility and surface properties of the CDs. However, the overall K_{CD} was steadily increases over time, suggesting the interactions between each type of CD are ever changing while the phase capacity is about constant (Fig.1) where the transfer of CDs into top phase will attain a maximum point. At this point, there is no free capacity to accommodate CDs solutes in top phase of the ATPS.

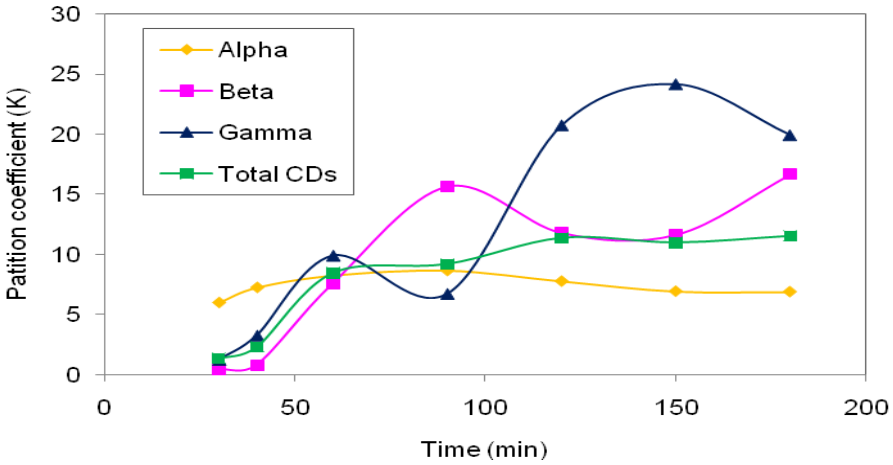
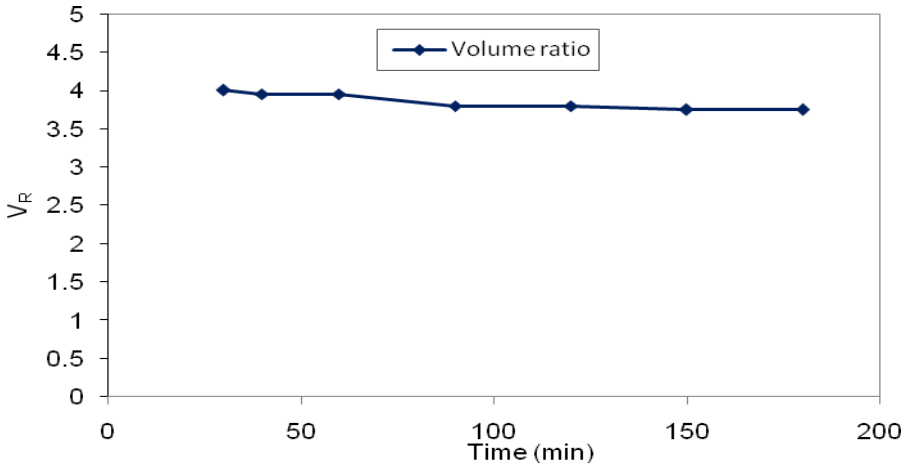


Fig. 1: Partitioning behaviour of different types of CDs over time.

Besides, the V_R of ATPS was determined at each regular time interval (Fig. 2). Results show there is no significant decrease in the V_R of the ATPS. It may assume that the phase composition of ATPS is independent of the presence of CDs over time. CDs mixture is inert towards the phase components and no significant reaction occurred between the CDs and phase components, as a result phase composition remained unaltered. This indicates the suitability of PEG and dextran T500 to act as the starch bioconversion media for the subsequent process integration of CDs using ATPS where the continuous production of CDs in ATPS is applicable.



Fig, 2: V_R of ATPS over time

CONCLUSIONS

ATPS can be a potential recovery technique for the separation of CDs in extractive bioconversion of starch. The partition behaviour of different types of CDs is differing as a result of their difference in structure and surface properties. The loaded CDs in an ATPS will not significantly alter the overall phase compositions of an ATPS, facilitating an efficient mass transfer of CDs between the phases over time.

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REFERENCES

[1] Z.D.V.L. Mayerhoff, I.C. Roberto, T.T. Franco (2004) Purification of xylose reductase from Candida mogii in aqueous two-phase systems, *Biochemical Engineering Journal*, 18 (pp.217-223).
[2] G.M. Zijlstra, C.D. de Gooijer, J. Tramper (1999) Extractive bioconversions in aqueous two-phase systems, *Current Opinion Biotechnology*, 9 (pp.171-176).
[3] A. Karakatsanis, M. Liakopoulou-Kyriakides (2007) Comparison of PEG/fractionated dextran and PEG/industrial grade dextran aqueous two-phase systems for the enzymic hydrolysis of starch, *Journal of Food Engineering*, 80 (pp.1213-1217).
[4] P.A. Albertsson (1986) *Partition of cell particles and macromolecules*, third ed., Wiley, New York.
[5] S. Bradoo, R.K. Saxena, R. Gupta (1999) Partitioning and resolution of mixture of two lipases from Bacillus stearothermophilus SB-1 in aqueous two-phase system, *Process Biochemistry*, 35 (pp.57-62).
[6] S. Jemli, E.B. Messaoud, D. Ayadi-Zouari, B. Naili, B. Khemakhem, S. Bejar (2007) A β-cyclodextrin glycosyltransferase from a newly isolated Paenibacillus pabuli US132 strain: Purification, properties and potential use in bread-making, *Biochemical Engineering Journal*, 34 (pp.44-50).
[7] R.F. Martins, R. Hatti-Kaul (2002) A new cyclodextrin glycosyltransferase from an alkaliphilic Bacillus agaradhaerens isolate: purification and characterisation, *Enzyme Microbiology Technology*, 30 (pp.116-124).
[8] K.L. Larsen, L. Duedahl-Olesen, H. Jørgen, S. Christensen, F. Mathiesen, L.H. Pedersen, W. Zimmermann (1998) Purification and characterisation of cyclodextrin glycosyltransferase from Paenibacillus sp. F8, *Carbohydrates Research*, 310 (pp.211-219).
[9] X. Cao, Z. Jin, X. Wang, F. Chen (2005) A novel cyclodextrin glycosyltransferase from an alkalophilic Bacillus species: Purification and characterization, *Food Research International*, 38 (pp.309-314).
[10] C. Moriwaki, G.L. Costa, R. Pazzetto, G.M. Zanin, F.F. Moraes, M. Portilho, G. Matioli (2007) Production and characterization of a new cyclodextrin glycosyltransferase from Bacillus firmus isolated from Brazilian soil, *Process Biochemistry*, 42 (pp.1384-1390).
[11] W.J. Chang, Y.M. Koo, S.S. Park (1997) Production of cyclodextrin homologues using aqueous two-phase system, *Biotechnology and Bioprocess Engineering*, 2 (pp.97-100).
[12] A.D. Diamond, J.T. Hsu (1992) Aqueous two-phase systems for biomolecule separation, *Advances in biochemical engineering/biotechnology*, 47 (pp.89-135).
[13] D. Forciniti, C.K. Hall, M.R. Kula (1991) Influence of polymer molecular weight and temperature on phase composition in aqueous two-phase systems, *Fluid Phase Equilibria*, 61 (pp.243-262).