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Crystallization of Difructrose Anhydride III (DFA III) in Batch Cooling Crystallization System : The Influence of Initial Supersaturation

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ABSTRACT

Crystallization of Difructose Anhydride III (DFA III) was investigated in batch cooling crystallization system, in which profile of temperature was controlled cooling temperature. The measured experimental parameter were crystal yield and crystal size distribution (CSD). The influence of initial supersaturation on the crystallization of DFA III is discussed herein.

Keywords **- Difructose Anhydride III, crystallization, supersaturation, crystal yield, crystal size distribution.**

I. INTRODUCTION

Crystallization is important process to get purity of certain substances. The crystallization process comprises two steps : nucleation and crystal growth. Those steps affect mass of obtained crystal and crystal size distribution (CSD). Nucleation and crystal growth can be achieved by supersaturation [1]. The term supersaturation refers to solution that contain more dissolved material than can be dissolved by solvent under normal circumstance. Some factors influence the behavior of crystallization system like temperature, solute concentration, and impurities presence [2]. Crystallization process and its crystal product can not be separated with its downstream process such as separation and washing [3].

It has been known that Difructose Anhydride III have role in stimulating calcium adsorption in human bodies [4]. Difructose Anhydride III (DFA III) is one of saccharide which is produced through enzymatic reaction of inulin by Inulin Fructotransferase enzyme (IFT enzyme). IFT enzyme can be produced by fermentation process with *Nonomuraea* sp [5]. The physical properties of DFA III such as melting point, solubility in water, are almost similar to other saccharides such as sucrose [6]. So DFA III can be recovered in crystal form besides liquid form.

The aim of this research is to study the influence of initial supersaturation on DFA III crystallization which used batch cooling crystallization process. It is determined that initial supersaturation can affect crystal yield and crystal size distribution (CSD).

II. MATERIALS AND METHODS

Preparation of Difructose Anhydride III solution

Buffer inulin that consist of 10% (v/v) of 200mM citric acid - NaOH buffer (pH 5.5) and 20% (w/v) of the dissolved inulin (ORAFTI trademark) was mixed with *Nonomuraea* sp. ID 06A0189 Inulin Fructotransferase with ratio 1:1, so the final concentration of inulin in the reaction mix was 10% (w/v). The reaction mix was incubated at 65 ^{o}C for 24 hours. Afterwards, purification process was conducted to obtain pure DFA III solution, commercial baker's yeast (FERMIPAN trademark) was added to enzymatic reaction product in concentration 1% (w/v). Fermentation was conducted in shaker bath at 150 rpm with temperature 30 ^{o}C for 1-2 days [7]. After that, separation was conducted between DFA III solution and biomass.

Preparation of supersaturated Difructose Anhydride III

Purified DFA III solution was concentrated until exceeded saturated concentration. Evaporation was conducted in oven at temperature under 50 ^{o}C to avoid substance destruction. Solution concentration was measured as % Brix based on off-line refractometer Atago Pal-a.
% Brix is defined as:

$$\%Brix = \frac{w_d}{w_d + w_s} x100\%$$

where w_d is mass of dissolved material (g) and w_s is mass of solvent (g).

Crystallization of Difructose Anhydride III

The experimental set-up was based on 500 ml batch closed crystallizer made off glass with inlet diameter (D_i) 9.5 cm. Supersaturated solution was agitated using paddle with 2 blade impeller with diameter (D_l) 4.2 cm. To ensure the homogeneity of solution, stirring velocity was selected. Water bath was used to control operation temperature. The

profile of operation temperature was controlled cooling temperature which was as follow :

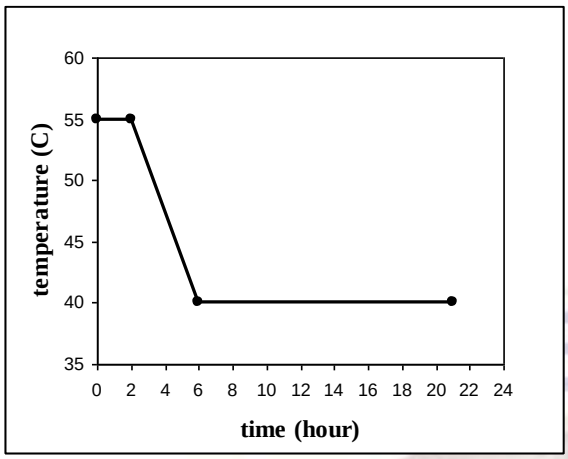


Fig 1. Experimental temperature profile.

Little of seed crystal was added to solution just to accelerate of nucleation. After crystallization process was accomplished, massecuite and crystal were separated by vacuum filter. Afterward, filtered crystal was washed by amount of water to avoid agglomeration and stickiness among crystals and residue of massecuite. Eventually, crystal was dried in oven at 40 ^{o}C prior further analysis.

Analysis of Difructose Anhyride solution and DFA III crystal

DFA III solution after purifying and obtained crystal of DFA III were analyzed by Thin Layer Chromatography (TLC). Mass of obtained crystal was used to determine crystal yield (%) which represent mass of obtained crystal from DFA III content in solution. Measurement and determination of crystal size was conducted by sieving method, US Standard Sieve Series ASTM was used with 14 mesh (1.19 mm), 60 mesh (250 mm), and 170 mesh (88 μm).

Definition of supersaturation

Supersaturation can be defined in several ways, as the supersaturation ratio (S), the concentration driving force (DC), and the relative supersaturation (s) [8]. The relevant relations are given as follow:

$$S = \frac{C}{C_{eq}}$$

$$\Delta C = C - C_{eq}$$

$$\sigma = \frac{\Delta C}{C_{eq}} = S - 1$$

where C and C_{eq} respectively represent the solution concentrations and equilibrium saturation at the given temperature, where :

$$C = \frac{w_d}{w_s}$$

Equilibrium saturation was obtained from Nagura et al. [9] which solubility at given temperature is defined :

$$\text{Solubility} = -0.00058\,T^2 + 0.39T + 48.8$$

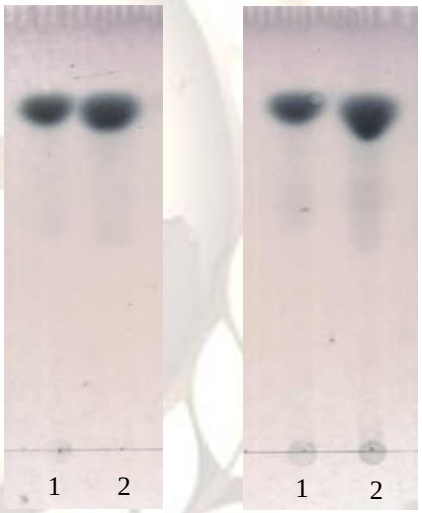
where T is temperature (^{o}C).

In this paper, supersaturation ratio is used to represent supersaturation.

III. RESULTS AND DISCUSSION

Thin Layer Chromatography (TLC) Analysis

The Thin Layer Chromatography (TLC) of concentrated DFA III solution (60% Brix) before crystallization and crystal of DFA III are shown in figure 2.



(a) (b)

Fig 2. TLC for (a) diluted DFA III (60% Brix) solution at concentration 4% (v/v) and 8 % (v/v), 1 and 2 respectively (b) dissolved crystal of DFA III at concentration 2% (w/v) and 4 % (w/v), 1 and 2 respectively.

As can be seen from figure 2 (a) and (b), either DFA III solution or crystal of DFA III have just one spot, which is DFA III spot. It means that there is no impurities like others saccharides, such as fructose, sucrose, etc which their spot is located under DFA III spot. It means that crystallization conducted for one of substance which was not affected by those impurities presence.

The influence of initial supersaturation ratio

Initial supersaturation gave significant effect to crystal yield which it can be seen in table 1.

Table 1. Effect initial supersaturation ratio on crystal yield

Initial Supersaturation Ratio (S)	Crystal Yield (%)
1.537	21.0691
1.636	29.8005
1.654	31.3699
1.722	37.7298
1.866	31.3903
2.214	24.1389

For more properly, it was shown in figure 3.

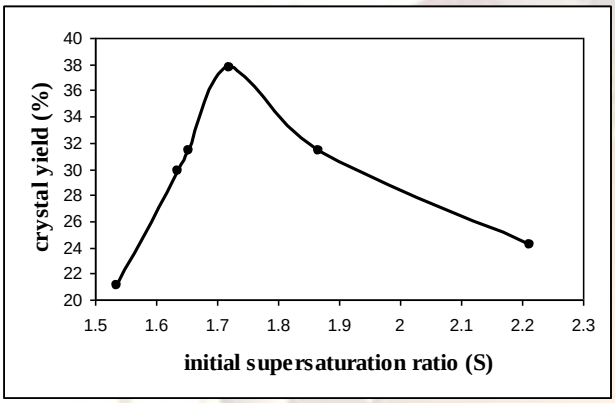


Fig 3. The influence of initial supersaturation ratio on crystal yield

As can be seen in table 1 and figure 3, initial supersaturation (S) 1.722 give highest crystal yield. The higher initial supersaturation, the more crystal yield that was obtained until it reached S (1.722). After that point, crystal yield decreased. Table 2 depict the influence of initial supersaturation ratio on crystal size distribution.

Table 2. Effect initial supersaturation ratio on crystal size distribution

S	mass fraction (w/w) L > 1.19 mm	mass fraction (w/w) L = 1.19 mm - 250 µm	mass fraction (w/w) L < 250 µm
1.537	63.46%	36.54%	0.00%
1.636	46.66%	53.11%	0.23%
1.654	22.05%	76.99%	0.96%
1.722	65.81%	33.98%	0.21%
1.866	6.33%	93.67%	0.00%
2.214	0.00%	2.16%	97.84%

Table 2 show that crystal size of DFA III was affected by initial supersaturation ratio (S). At lower S (1.537-1.722), formed crystal have high range in distribution, even the solution have tendency to form large crystal than that at higher S (1.866-2.214). It was might caused crystallization occurred at metastable zone, where it have tendency that nucleation rate was smaller than crystal growth rate. It is determined that after secondary nucleation formed because of seeding, the entire seed grew to be larger crystal. In contrast, at higher S (1.866-2.214) the crystal distribution was more uniform and the form of crystal was smaller. It was occurred because phase of these supersaturated solution was in labile zone where spontaneous nucleation occured although unpresence of seeding. It means that nucleation rate was higher than crystal growth rate. But as we know in table 1 and figure 3, crystal yield obtained in these supersaturation was smaller. It was might because there was dowstream process role that were washing and separation in reducing the amount of obtained crystal. Influence of those process on crystal yield at lower S (1.537-1.722) was low because larger crystal have low resistant to those downstream process.

IV. CONCLUSION

At lower initial supersaturation ratio (1.537-1.722), the formed crystal had tendency to have high range in distribution. At those supersaturation, the crystal yield was higher. Meanwhile, at higher initial supersaturation ratio (1.866-2.214), the crystal size was uniform in distribution and was smaller than that of lower supersaturation. But, at this region, the amount of crystal yield was lower. Study in kinetic of crystallization is needed to investigate optimum crystallization, and to investigate behaviour either crystal or supersaturation during crystallization.

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