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Supersaturation and Crystal Behavior during Crystallization of Difructose Anhydride III (DFA III) in Batch Cooling Crystallization System

Umi Laila*, Sri Pudjiraharti

**Research Centre for Chemistry, Indonesian Institute of Sciences,
Jl. Cisitu Sangkuriang Bandung 40135, Indonesia**

***Corres. author: umilaila38@yahoo.com**

Abstract: Difructose Anhydride III (DFA III) can be recovered in crystal form, through cooling crystallization. Supersaturation and crystal behavior of DFA III was studied in batch cooling crystallization system in which the used temperature profile was controlled cooling temperature. The measured parameters in this study were supersaturation ratio and crystal size. Crystalization process was conducted for 21 hours. Supersaturation ratio raised until certain value, afterwards supersaturation decreased until the end of crystallization. The longer crystallization time, the higher amount and the bigger size of crystal was observed.
Keywords: Difructose Anhydride III, crystallization, supersaturation, crystal size

Introduction

One of process to get purity of substances is crystallization. Crystallization is one part of industrial operation unit which involve with solid-liquid separation. Crystallization allows the molecule is converted from a solute dissolve in a liquid phase to a solid phase which the steps of crystallization comprise: nucleation and crystal growth. Nucleation and crystal growth can be achieved by supersaturation[1]. The term supersaturation refers to solution that contains 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 can be conducted using evaporation or cooling crystallization technique. Cooling crystallization has been widely used in industrial operation especially sugar processing. Cooling crystallization is a simple technique and by using that technique the experimental data is easily obtained.

Difructose Anhydride III (DFA III) is one of stable saccharides, which have role to enhance calcium adsorption in human[3]. It is produced through enzymatic reaction of inulin by Inulin Fructotransferase (IFT)[4]. IFT can be produced by fermentation process using *Nonomuraea* sp.[5] or *Arthrobacter* sp.[6]. The production DFA III can be produced in ultrafiltration membrane bioreactor system[7], besides in traditional mixed reactor system[8]. The physical properties of DFA III such as melting point, solubility in water, are almost similar to sucrose[9]. Therefore, DFA III can be recovered in crystal form through similar technique used to crystallizing sucrose. The crystal form is more resistant against microbial contamination, thus DFA III crystal is more preserved.

This research was conducted to study supersaturation and crystal size behavior during DFA III crystallization by using batch cooling crystallization process. It is hypothesized that supersaturation and crystal size will alter during crystallization.

Materials and Methods:

Preparation of Difructose Anhydride III solution

Inulin (ORAFTI, Belgium) was dissolved 20 mM citric acid-NaOH buffer (pH 5.5) to a final concentration of 10% (w/v), mixed with *Nonomuraea* sp. ID 06A0189 Inulin Fructotransferase with ratio 1:1, and incubated at 65 ^{o}C for 24 hours. Afterwards, commercial baker's yeast (FERMIPAN, Indonesia) was added to enzymatic reaction product in concentration of 1% (w/v) and incubated at 30 ^{o}C with agitation of 150 rpm for 1-2 days[10]. After that, separation was conducted between DFA III solution and biomass by centrifugation.

Preparation of supersaturated Difructose Anhydride III

Purified DFA III solution was concentrated until exceeded saturated concentration. Evaporation was conducted in an oven provided with a blower at temperature under 50 ^{o}C to avoid substance destruction. Solution concentration was measured as % Brix based on off-line refractometer (ATAGO PAL-a, Japan).

% 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 a paddle with 2 blade impellers with diameter (D_l) of 4.2 cm. To ensure the homogeneity of the solution, stirring velocity was selected. Water bath was used to control operation temperature. The profile of operation temperature was controlled cooling temperature which was figured as follows:

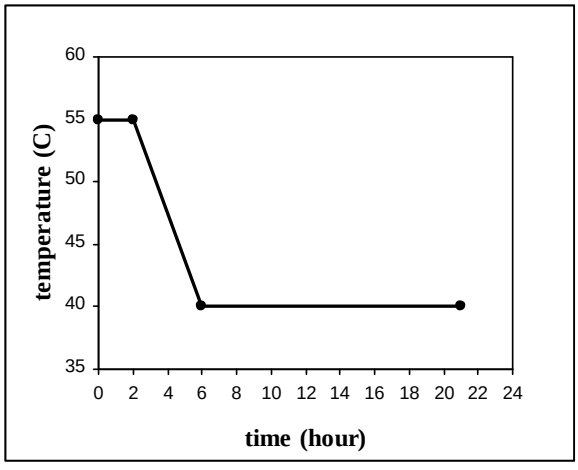

Fig 1. Experimental temperature profile.

Initial concentration of DFA III applied to the crystallization system was 70.3% Brix (31.6 ^{o}C). Some of seed crystal was added to the solution to accelerate nucleation. Sampling of DFA III massecuite was conducted by a one ml micropipette (EPPENDORF, Germany) for supersaturation or crystal size analysis at hour-4, hour-5, hour-6, and hour-21.

Analysis of DFA III concentration and DFA III crystal

DFA III concentration was determined by a refractometer (ATAGO PAL-a, Japan). 1 ml of DFA III massecuite was vacuum filtered with a filter paper (WHATMAN Grade 41, United States). The DFA III crystal size was measured by using millimeter block paper. Determination by other offline methode like coulter counter or sieving analysis could not be conducted because of limited amount of the crystal. Determination of amount and size of crystal in filter paper was done by dividing area into two subareas which were as follow.

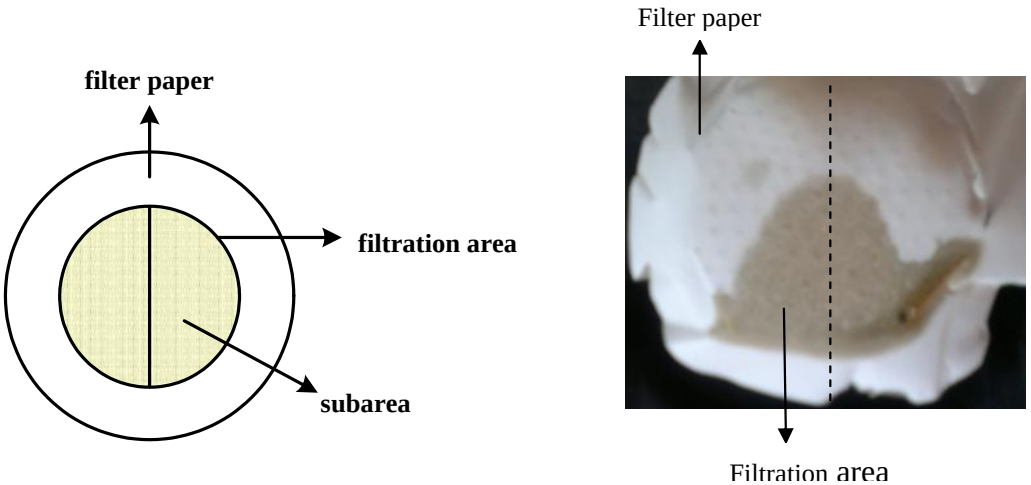

Fig 2. Crystal calculation area in filter paper

Determination was conducted by calculating size and amount of crystal for one subarea. The overall amount of crystal was assumed by multiplying its value by 2 as long as the size of crystal was almost similar. For crystal size at the end of process (hour-21), calculation was conducted by sieving method because the amount of crystal was high and pipetting was difficult.

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)[11], the relevant relations are given as follow:

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

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

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

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[12] which solubility at given temperature is defined:

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

Where T is temperature (^oC).

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

Results And Discussion

The behavior of supersaturation ratio during crystallization can be seen at Figure 3. As can be seen in figure 3, alteration of supersaturation ratio (S) during crystallization was occured. Supersaturation ratio rose at early crystallization time (until hour-6). It was because evaporation occurred at early time therefore concentration of DFA III liquid raised. Moreover at this period, crystal formation and growth was occurred later than the evaporation. But after hour-6, supersaturation ratio decreased until the end of crystallization which caused concentration of DFA III liquid also decreased. It was occurred because crystal formation and growth was high.

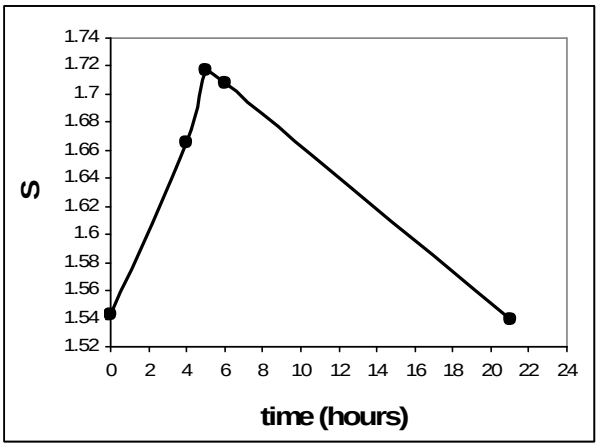

Fig 3. Behavior of supersaturation ratio during crystallization

The result of crystallization process can be seen at Table 1. From hour-4 and hour-5, we can see that there was different number of crystal especially at small crystal size (< 0.2 mm). There was significant increasing of crystal number at that size. But in other crystal sizes, the increasing was not significant. It means that at that period, nucleation was higher than crystal growth. From hour-21 data, it can be seen that the amount of small size crystal (< 0.25 mm) was smaller than that of the bigger sizes. It is predicted that at the end of crystallization, growth of crystal was occured instead of crystal nucleation.

Table 1. Crystal size and number per ml of massecuite during crystallization process

Crystallization Time	Crystal size	Number (amount) of crystal / ml of massecuite
Hour-4	1 mm	2
	0.5 mm	1
	0.2 mm	3
	< 0.2 mm	18
Hour-5	1.5 mm	1
	1 mm	4
	0.5 mm	6
	< 0.2 mm	180
Hour-21	> 1.19 mm	46.66 % (w/w)
	1.19 – 0.25 mm	53.11 % (w/w)
	< 0.25 mm	0.23 % (w/w)

Conclusion

There was alteration of supersaturation and crystal behavior during crystallization process in batch cooling crystallization system. The supesaturation increased until hour-6, afterwards the supersaturation decreased until the end of crystallization process (hour-21). At initial crystallization, nucleation of crystal was higher than crystal growth; therefore, the crystal size was small. Meanwhile, close to the end of crystallization; crystal growth was dominant and crystal size was high.

Acknowledgments

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