Applications of dextranase in Thai sugar factories

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Abstract

High dextran contamination is a serious problem in the Thai cane and sugar industry because of the long cut-to-crush period from the cane field to the mill. Using dextran contamination data collected from raw syrups at five factories, the two factories with the highest dextran contamination (2,783 and 2,210 ppm) were selected for dextran removal experiments. Dextranase L ‘Amano’ was applied at these two factories to the fifth effect of their evaporator sets. The dextranase was tested to determine the most suitable conditions for its use in the evaporators. The dextranase kinetic parameters were also determined. The kinetic parameters of the enzyme, namely, Vmax and Km, were determined as 1,000 µg/mL min and 300 µg/mL, respectively. The optimal conditions for the enzyme application at 60°C were 20 ppm of enzyme with 30 minutes reaction time. For application in the selected factories, dextranase at only 2 ppm was sufficient to resolve dextran contamination in the factories. The retention time in the fifth effect was around 20 minutes, which was enough to reduce dextran by 50% in both factories (from 1,796 to 989 ppm, and from 1,712 to 858 ppm). The correlation between the amount of dextran and viscosity, as well as the dextran reduction and reducing sugar increase, were also established to elucidate the hydrolysis of dextran to reducing sugar by dextranase.

Key words Dextranase L ‘Amano’, dextran, Thailand, sugar factories, enzyme

INTRODUCTION

Sugarcane production in Thailand has increased in the last 5 years, with milling rising from 68 Mt of sugarcane in 2009/2010 to 103 Mt in 2013/14 (Office of the Cane and Sugar Board 2014). However, there are many problems in the sugar factories, for example, the loss of sugarcane during harvesting and transporting from the farms to the factories and the low quality of sugarcane and sugar processing. The process from the farm to the factory most commonly involves sugarcane being harvested by burning and then cutting. This is convenient for the cane cutters and harvesters, but causes significant damage to cane quality, particularly if the cane is left in the field awaiting transport for more than 24 h. The waiting time for picking up in the field and transporting to the factories (known as the cut-to-crush time) is usually quite long in Thailand.

During the cut-to-crush period, many microorganisms can grow using the sucrose in the sugarcane after harvesting to produce polymers such as dextrans (Sriroth et al. 2011). Dextrans are undesirable compounds produced by Leuconostoc mesenteroides, which is able to utilize a high proportion of the sugar present in the juice in a short time. Under favorable temperature and humidity conditions, dextranase catalyzes the hydrolyses of the sucrose, forming dextrans. These dextrans are extracted in the mill along with the juice and contaminate the sugar mill flow, reaching levels in the juice exceeding 10,000 ppm (1%) in very extreme cases (Cuddihy et al. 1998). In burnt sugarcane, a rapid increase in the level of dextrans of almost 10 times occurs from 12 to 48 h, reaching 3,200 ppm (Cuddihy and Day 1999). Dextrans are high molecular weight polysaccharides, with at least half their composition being α-1,6 linked glucose units, with α-1,3 branch linkages and may contain other branch linkages such as α-1,2 or α-1,4 (Khalikova et al. 2005). The presence of dextran in the sugar factory leads to a false high polarization, increased viscosity, slowing of filtration, lower evaporation rates, elongated crystals, longer wash and separation cycles in centrifugals, and an increase in the sugar loss to molasses (Kim and Day 2004). These result in economic losses for the sugar industry.

Physical methods such as ultrafiltration, dialysis and reverse osmosis are some techniques for removing dextran, but they are not yet technologically developed for their economical application in the factory processes. The only appropriate method in the sugar industry is the enzymatic hydrolysis of dextran. Dextranase (1,6-α-D-glucan-6-glucanohydrolase, EC3.2.1.11) is an enzyme that catalyzes endohydrolysis of α-(1-6)-d-glycoside linkages at random sites of the dextran molecule.

The aim of our work was to study the application of dextranase into the final effect to remove contamination by dextran.
MATERIALS AND METHODS

Materials

Dextranase L ‘Amano’ was obtained from Amano Enzyme Inc., Japan. It has an activity of 30,000 U/mL.

Optimization of dextranase activity

The optimum conditions for using dextranase for the removal of contaminated dextran in the syrup evaporator were determined. Syrup was prepared using 55°Brix white-sugar syrup as the sucrose solution and dextran (T500) was added at concentrations of 5,000 and 25,000 ppm at a constant temperature (60°C). Factors used to affect the conditions were the reaction time with values of 0, 5, 10, 15, 20, 30 and 60 minutes and the concentration of added dextranase at 2, 5, 10 and 20 ppm. The $K_m$ and $V_{max}$ values were determined from a Lineweaver-Burk plot. $V_{max}$ represents the maximum rate achieved by the system at maximum saturation of the substrate concentration. The Michaelis constant $K_m$ is the substrate concentration at which the reaction rate is half of $V_{max}$ (Lineweaver and Burk 1934).

Application of dextranase in the last syrup evaporator

The experiment was conducted in five Thai sugar factories (A, B, C, D and E) in the 2014/15 milling season. Duplicate daily composite samples were taken from raw syrup for 10 days. Dextran determination was undertaken using the modified haze method (Eggles ton and Monge 2005). Two of the five factories were chosen for the experiments due to their high dextran content and the potential to remove dextran using the optimum conditions for the addition of dextranase.

The dextranase was added continuously into the last evaporator. The amounts of dextran before and after the addition of dextranase were measured using the modified haze method and the amount of reducing sugars was determined using the Somogyi-Nelson method (Somogyi 1994).

Viscosity determination

The viscosity of samples was measured using a Brookfield (Middleboro, USA) DV-II+ rotational viscometer at 25°C with spindle no. 2 and a rate of 150 rpm.

RESULTS AND DISCUSSION

Amount of dextran contamination in sugar factories

The average amount of dextran contamination in the raw syrup from the five sugar factories (A, B, C, D and E) in the 2014/2015 milling season is shown in Figure 1. The two factories with the highest dextran contamination in raw syrup (factory C and factory A) were used in the experiment.

Optimum conditions of dextranase for removing dextran contamination

The effects of various concentrations of dextranase on the syrup containing dextran at 5,000 ppm are shown in Figure 2(A). At 20 ppm concentration of dextranase, all dextran was removed after 30 minutes. There was no significant difference amongst the 2, 5 and 10 ppm concentrations of added dextranase on dextran removal, resulting in 85.5, 86.3 and 93.5% removal, respectively, in 60 minutes. Figure 2(B) shows the effects of various concentrations of dextranase on the syrup containing added dextran at 25,000 ppm. 20 ppm of dextranase removed all the dextran in 60 minutes and 2, 5, 10 ppm concentrations of dextranase removed 21.3, 26.2 and 41.8%, respectively, of the dextran in 60 minutes.

The optimum conditions for dextranase were a dextranase concentration of 20 ppm, 60°C and 30 min reaction time. However, 2 ppm of dextranase was used for removing dextran in the selected sugar factories as it was sufficient to reduce the dextran concentration to an acceptable commercial level. Under the optimal conditions, the kinetic parameter values of $V_{max}$ and $K_m$ for 2 ppm dextranase were 1,000 µg/mL.min and 300 µg/mL, respectively.
Fig. 1. Amount of dextran contamination in raw syrup from five sugar factories in 2014/15.

Fig. 2. Effects of dextranase concentration and time on dextran removal.

The trends associated with different dextranase concentrations on the intrinsic viscosity at different times are shown in Figure 3. A dextran concentration at 5,000 ppm had little effect on the viscosity (Fig. 3A). A dextran concentration at 25,000 ppm showed higher viscosity (Fig. 3B), but after adding dextranase the viscosity decreased; viscosity was related to the percentage of dextran remaining (Fig. 2).

Fig. 3. Effects of dextranase concentration, viscosity and time on dextran removal.
Application of dextranases to the last effect evaporator

Dextranase L ‘Amano’ was used to remove dextran in the sugar process. The suitable addition point was at the cut over pipe between the 4th and 5th effects as illustrated in Figure 4. The operation temperature was 60-70°C at this location and the pH was around 5.5-6.5 and the concentration range was 50-60°Brix. The retention time in the evaporator was around 20 min.

We studied the effect of adding concentrated dextranase at 2 ppm to the last effect in factories A and C. The amount of dextran before and after adding dextanase L ‘Amano’ is shown in Figure 5, where the average amount of dextran in raw syrup at factory A before adding dextranase was 1,761 ppm. After dextranase treatment, the average amount of dextran in the raw syrup decreased to 975 ppm.

For factory C, the results in Figure 5 show the dextran concentration before and after adding 2 ppm concentration of dextanase. The average amount of dextran in the raw syrup in factory C before adding dextranase was 1,549 ppm and after adding dextranase in the last evaporator effect syrup, the average amount of dextran in the raw syrup decreased to 889 ppm.

After adding dextranase, the amount of reducing sugars increased (Fig. 6) with the average amount of reducing sugars in factory A increasing from 13,400 µg/mL to 16,000 µg/mL and in factory C the average amount of reducing sugar increasing from 12,700 µg/mL to 14,900 µg/mL. These results were related to the removal of dextran (Fig. 5) because the dextranases hydrolyzed dextran and released isomaltooligosaccharides, isomaltose or glucose (Khalikova et al. 2005).

After adding 2 ppm dextranase to the last evaporator effect, the average viscosity in factory A decreased from 239 cP to 155 cP and the average viscosity in factory C decreased from 171 cP to 111 cP (Fig. 7), with these results again being related to the removal of dextran (Fig. 5).
Fig. 5. Effect of dextranase addition on dextran removal from syrup in factories A and C.
Fig. 6. Reducing sugars on removal of dextran from syrup in sugar factories A and C.
CONCLUSIONS

We found that the optimum dextranase conditions for the removal of dextran in 55 brix syrup were 20 ppm at 60°C for 30 min. However, 2 ppm of dextranase was used to reduce the effects of dextran in the selected sugar factories as it was sufficient to result in commercially acceptable levels of dextran concentrations. Under optimal conditions, the $K_m$ and $V_{max}$ kinetic parameter values of dextranase were 1000 $\mu$g/mL×min and 300 $\mu$g/mL, respectively. Applications of 2 ppm of dextranase were carried out in two factories at the last evaporator. The results showed that the dextran concentration and the viscosity decreased after the addition of dextranase and the amount of reducing sugars increased. The experiments showed the effectiveness of the enzyme in the removal of dextran in the raw syrup under conditions of high sucrose concentration (65-70°Brix) and high temperature (60-70°C).

Fig. 7. Effect on viscosity with dextran removal from syrup in factories A and C.
REFERENCES


Applications de dextranase dans les sucreries Thai

Résumé. Les fortes concentrations de dextran sont un problème grave dans l’industrie thaïlandaise à cause des longs délais entre la coupe des cannes et le broyage. On a déterminé les concentrations de dextran dans les sirops de cinq usines; les deux usines avec les plus fortes concentrations (2 783 et 2 210 ppm) ont été sélectionnées pour des expériences afin de réduire la présence du dextran. La dextranase «Amano» a été appliquée à ces deux usines, dans le cinquième corps des évaporateurs. La dextranase a été testée afin de déterminer les conditions optimales pour son utilisation dans les évaporateurs. Les paramètres cinétiques de la dextranase ont été également déterminés. Pour les paramètres cinétiques de l’enzyme Vmax et Km, on a trouvé des valeurs de 1 000 µg/mL.min et 300 µg/mL, respectivement. Les conditions optimales pour l’application de l’enzyme à 60°C étaient 20 ppm d’enzyme avec un temps de réaction de 30 minutes. Pour l’application dans les usines sélectionnées, seulement 2 ppm de dextranase étaient suffisant pour réduire la concentration de dextran. Le temps de rétention dans le cinquième effet était environ 20 minutes, ce qui a été suffisant pour réduire le dextran par 50 % dans les deux usines (de 1 796 à 989 ppm et de 1 712 à 858 ppm). On a aussi étudié la corrélation entre la quantité de dextran et la viscosité, la réduction du dextran, la réduction des sucres réducteurs et l’augmentation du sucre, pour élucider l’hydrolyse du dextran en sucres réducteurs par la dextranase.

Mots-clés: Dextranase “Amano”, dextran, Thaïlande, sucreries, enzyme

Aplicación de dextranasa en fábricas de azúcar tailandesas

Resumen. La contaminación alta de dextrana es un problema serio en la caña e industria azucarera tailandesa debido a la gran demora entre cosecha y molienia. Usando datos de contaminación por dextrana colectados en jarabes crudos de cinco fábricas, fueron seleccionadas dos de ellas con las más altas contaminaciones (2.783 y 2.210 ppm) para experimentos de remoción de dextrana. Se aplicó Dextranasa L “Amano” en estas dos fábricas en el quinto efecto del tándem de evaporadores. Se probó la dextranasa para determinar las condiciones más apropiadas de uso en los evaporadores. Se determinaron también los parámetros cinéticos de la dextranasa. Los parámetros cinéticos de la enzima, llamados Kmax y Km, se calcularon como 1,000 µg/mL.min y 300 µg/mL, respectivamente. Las condiciones óptimas para la aplicación de la enzima a 60°C fueron 20 ppm de enzima con un tiempo de reacción de 30 minutos. En la aplicación en las fábricas seleccionadas, 2 ppm de dextranasa fueron suficientes para resolver la contaminación en ellas. El tiempo de retención en el quinto efecto fue de alrededor de 20 minutos, suficiente para reducir la concentración de dextrana en 50% en ambas fábricas (de 1.796 a 989 ppm, y de 1.712 a 858 ppm). La correlación entre la cantidad de dextrana y viscosidad, así como la reducción de dextrana y el incremento de azúcares reductores, fueron también establecidos para elucidar la hidrólisis de dextrana a azúcares reductores por la dextranasa.

Palabras clave: Dextranasa L “Amano”, dextrana, Tailandia, fábricas de azúcar, enzima