Factory trials to optimize the application of dextranase in raw sugar manufacture: Part I

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Abstract

Application of commercial dextranase to hydrolyze dextran in U.S. sugar manufacture is still not optimized, partly because of confusion about which enzyme to use, and how and where to add the enzyme. Until recently, there was no uniform method to measure the activity of dextranases, but several factories are now successfully using the Eggleston factory titration method to 1) compare economically equivalent activities of different dextranases, 2) measure the activity of delivered batches, and 3) monitor the changing activities on factory storage. An approximate 14 to 20-fold difference in activity existed between the "non-concentrated" and "concentrated" forms of commercial dextranases used in 2004. Factory trials to optimize dextranase applications were conducted in the 2004 Louisiana processing season. As a previous laboratory study showed dextranase applications to syrup were uneconomical, only juice applications were studied. Results are reported from a factory that applies dextranase to a 5 min retention time tank adjacent to a mixed juice tank receiving juice from the 1st and 2nd mills. Higher dosages of dextranase were required in the factory than in laboratory. Working solutions of "concentrated" dextranase were required to improve contact between the enzyme and substrate (dextranase/dextran), and are more cost-effective than applying a "non-concentrated" dextranase undiluted. Working solutions can be easily prepared with tap or distilled water and are stable up to 24 h maximum; if prepared with a 24 °Brix raw sugar solution they are stable for 140 h. Greater levels of dextran improve hydrolysis by dextranase because of lower enzyme/substrate contact ratios. The factory had relatively high levels of antibody dextran (>1000 ppm/°Brix) in juice, and the application of 6 ppm (normalized to the original enzyme activity) of 2 or 5-fold working solutions of "concentrated"dextranase (52,000 DU/ml) were successful in hydrolyzing 70-94% dextran.

Pruebas de evaluación en la fábrica para optimizar la aplicación de dextranasa en la manufactura de azúcar crudo: Parte I

La aplicación de dextranasa comercial para hidrolizar dextranas en la manufactura de azúcar en los Estados Unidos aún no está optimizada, en parte por la confusión acerca de cuál enzima usar, además de cómo y cuándo se debería agregar la enzima. Hasta hace poco tiempo no existía un método uniforme para medir la actividad de las dextranasas, aunque en varias fábricas ya se está usando exitosamente el método Eggleston de titulación, o análisis volumétrico, en fábrica para 1) comparar las actividades económicamente equivalentes de las diferentes dextranasas, 2) medir la actividad de los lotes suministrados, y 3) controlar el cambio en la actividad durante el almacenamiento en la fábrica. Se encontró que existe una diferencia de 14 a 20 veces en la actividad entre las formas comerciales de dextranasa "no-concentrada" y "concentrada" utilizadas en 2004. Los ensayos de fábrica para optimizar las aplicaciones de dextranasa fueron llevados a cabo durante la temporada de procesamiento del 2004 en Louisiana. Tal como lo demostró un estudio previo en el laboratorio, las aplicaciones de dextranasa al jarabe resultaron poco económicas; sólo se estudiaron aplicaciones de jugo. Los resultados provienen de una fábrica que aplica dextranasa a un tanque de retención de 5 min., adyacente a un tanque de jugo mixto que recibe el jugo del primer y segundo molino. En la fábrica fue necesario utilizar dosis más altas que en el laboratorio. Para mejorar el contacto entre la enzima y el substrato (dextranasa / dextrana) fue necesario utilizar soluciones de trabajo de la dextranasa "concentrada" que son más económicas que la utilización de una

Werksversuche zur Optimierung der Anwendung von Dextranase bei der Rohzuckerherstellung: Teil I

Handelsübliche Dextranase wird in der US-amerikanischen Zuckerproduktion noch nicht optimal zur Hydrolisierung von Dextran eingesetzt – zum Teil aufgrund von Unsicherheit darüber, welches Enzym verwendet und wie und wo es hinzugefügt werden sollte. Bis vor kurzem gab es keine einheitliche Methode zur Messung der Aktivität von Dextranasen, aber heute benutzen mehrere Fabriken mit Erfolg die Titrationsmethode der Eggleston-Fabrik, um 1) wirtschaftlich äquivalente Aktivitäten verschiedener Dextranasen zu vergleichen, 2) die Aktivität der gelieferten Partien zu messen und 3) Aktivitätsveränderungen bei Fabrikslagerung zu überwachen. Es besteht ein zirka 14- bis 20-facher Unterschied der Aktivität zwischen „nicht-konzentrierten" und „konzentrierten" Formen handelsüblicher Dextranasen, die 2004 verwendet wurden. Werksversuche zur Optimierung der Dextranaseverwendung wurden in der Louisiana-Verarbeitungssaison 2004 durchgeführt. Da eine vorherige Laborstudie gezeigt hatte, dass Dextranaseverwendungen bei Sirup unwirtschaftlich waren, wurden nur Saftverwendungen studiert. Berichtet werden die Ergebnisse von einer Fabrik, die Dextranase in einem 5-Minuten-Verweilzeittank neben einem gemischten Saftank einsetzt, der Saft von der 1. und 2. Fabrik erhält. In der Fabrik waren höhere Dosen von Dextranase als im Labor erforderlich. Zur Verbesserung des Kontakts zwischen Enzym und Substrat (Dextranase/Dextran) wurden Arbeitslösungen von „konzentrierter" Dextranase benötigt, die gleichzeitig kosteneffektiver als der unverdünnte

dextranasa "no concentrada" sin diluir. Las soluciones de trabajo se pueden preparar fácilmente con agua corriente o agua destilada y son estables por hasta 24 horas máximo. Si se preparan con una solución de azúcar crudo con 24 oBrix, la estabilidad se mantiene por 140 h. Niveles de dextrana más altos mejoran la hidrólisis con dextranasa como consecuencia de los más bajos índices de contacto entre la enzima y el substrato. La fábrica tenía niveles relativamente altos de anticuerpo de dextrana (>1000 ppm/oBrix) en el jugo y la aplicación de 6 ppm (normalizada a la actividad original de la enzima) de 2- o 5-veces de soluciones de trabajo de dextranas "concentrada" (52,000 DU/ml) había hidrolizado exitosamente 70-94% de la dextrana.

Einsatz einer „nicht-konzentrierten" Dextranase sind. Arbeitslösungen lassen sich einfach mit Leitungs- oder destilliertem Wasser zubereiten und sind maximal 24 Stunden lang stabil. Wenn sie mit einer 24 oBrix Rohzuckerlösung zubereitet werden, bleiben sie 140 Stunden lang stabil. Größere Anteile von Dextran verbessern die Hydrolyse durch Dextranase aufgrund niedrigerer Enzym/Substrat-Kontaktverhältnisse. Die Fabrik hatte relativ hohe Anteile von Dextran-Antikörpern (>1000 ppm/oBrix) im Saft, und der Einsatz von 6 ppm (normalisiert zur ursprünglichen Enzymaktivität) von 2- oder 5-fachen Arbeitslösungen „konzentrierter" Dextranase (52.000 DU/ml) hydrolisierte erfolgreich 70-94% Dextran.

Introduction

The major contributor to sugarcane deterioration in the U.S., particularly Louisiana (LA) where humid conditions prevail, is *Leuconostoc mesenteroides* bacterial infection. Factors affecting infection in the sugarcane plant and juice are ambient temperature and humidity, level of rainfall and mud, length of cane billet, degree of burning, billet damage, delays between burning and cutting and subsequent processing, and (to a lesser extent) mill hygiene. *L. mesenteroides* species produce dextran polysaccharides (α-(1→6)-α-D-glucans), that in moderate and severe cases can interrupt normal processing operations. Dextrans not only cause expensive microbial losses of sucrose, but can distort factory pol readings and raise losses of sucrose in molasses. The high viscosity associated with the high molecular weight (HMW) portions (> 1000 KDa) of dextran especially affects boiling house operations, often reducing rates of evaporation and crystallization. Moreover, the factory is penalized by refineries based on dextran in the raw sugar. Although clarification processes remove some dextran (Eggleston et al, 2003), commercial dextranases (1→6-α-glucan hydrolases, EC 3.2.1.11) have been used in sugarcane factories to break down dextran by hydrolyzing α-(1→6) linkages endogenously, into a range of smaller, more managable molecules. Dextranase is mostly added to improve boiling house operations.

Although the application of dextranases in the sugar industry was pioneered by Australians in the 1970s (see Inkerman, 1980), their application in U.S. sugar manufacture is still not optimized. This is partly because of confusion about which enzyme to use, and how and where to add the enzyme. The problem has been exacerbated by the lack of any comprehensive U.S. dextranase studies, and those that have been undertaken most never stating the dextranase (see Eggleston and Monge, 2005). This has led to enormous confusion concerning what is the appropriate dosage to apply for each commercial dextranase. Furthermore, until recently there was no uniform method to measure the activity of commercial dextranases by vendors, which meant that direct comparison of activities at the factory was not possible (Eggleston and Monge, 2004). To solve this problem, Eggleston (2004) identified and modified a simple method to determine the relative activity of dextranases in DU/ml units, which is now being successfully used by several U.S. factories. Most commercial dextranases in the U.S. are from *Chaetomium gracile* or *erraticum*, and generally recognized as safe (GRAS). Eggleston and Monge (2004) reported that U.S. dextranases are available in a very wide range of activity which they classified into "non-concentrated" (<25.000 DU/ml but usually <6.000 DU/ml) or "concentrated" (25.000-58,000 DU/ml but usually between 48.000-58.000 DU/ml) forms. An approximate 8-10 fold difference in activity existed between the two forms in 2003, and variations existed within each form. Even more recently, similar differences in the activities of commercial dextranases available in South Africa have been reported (Anon, 2004).

At the request of the LA raw sugar industry, studies were undertaken to optimize the application of dextranase in U.S factories. The efficiency of dextranases in the factory depends on the pH, °Brix, temperature, retention time, agitation, and type, activity, and dosage of the dextranase applied (Inkerman, 1980). In LA there is often insufficient retention time. An initial, comprehensive laboratory study (Eggleston and Monge, 2004, 2005) showed that both "non-concentrated" and "concentrated" dextranases at juice pH 5.4-5.8, had similar maximum activity ~120 °F (50 °C), and activities decreased after 25-30 °Brix (Eggleston and Monge, 2005). The optimum pH range for dextranase activity is pH 5.0-6.0, with the lower end of the range more preferable and this, fortunately, coincides with the typical pH operating range of juices before clarification in sugarcane factories. Up until 2004 only "non-concentrated" dextranases were applied in LA sugarcane factories to either last evaporator syrup or juice. However, Eggleston and Monge (2005) unequivocally showed that juice applications were more efficient and economical than applying them to syrups, because there is less available water reactant in syrup, and temperatures and pH's are also sub-optimum. For every U.S. dollar spent on applying "non-concentrated" dextranase (5,999 DU/ml) to juice, $14.29 has to be spent for the equivalent dextran hydrolysis in syrup. This is only slightly better for a "concentrated" dextranase (52,000 DU/ml), where for every U.S. dollar spent on applying it to juice, $11.62 has to be spent for syrup application. Eggleston and Monge (2005) also showed that heating cane juice to 50 °C in the presence of all dextranases, dramatically removed more dextran than at the current ambient temperature of application (32 °C) and was more economical.

Although the initial laboratory study gave a useful indication of the conditions and dosage for applying dextranase to cane juice, results were gained under ideal conditions compared to those at factories where dextranase is added to much larger volumes of juice in tanks and pipes with fluctuating flow rates and agitation. Consequently, factory trials were undertaken to further optimize factory dextranase applications. The laboratory study did, however, give a solid foundation from which to start the factory trials, and allowed the decision not to study dextranase application to evaporator syrup. Trials were conducted at two LA factories across the 2004 processing season. This paper, Part I, reports results at one LA factory, and Part II (Eggleston et al, 2006) reports results at another LA factory.

Materials and methods

Dextranase activity

The modified titration method of Eggleston (2004) was used to measure the activity of dextranases. One dextranase unit (DU/ml) is the amount of enzyme which hydrolyzes dextran T2000™ to produce reducing sugar corresponding to the reducing power of one micromole of sodium thiosulfate in one min at 99 °F (37 °C) and pH 5.8. Averages of duplicates are reported.

Monoclonal antibody method for dextran

The Rapid Dextran Test or SucroTest™ (Midland, U.S.) was used (Rauh et al, 1999; Anon, 2003). A conversion factor was calculated for each batch of antibody used.

°Brix

The mean °Brix of triplicate samples was measured using an Index Instruments TCR 15-30 temperature controlled refractometer accurate to ± 0.01°Brix.

Storage characteristics of factory working solutions of dextranase

Working solutions were created by diluting a "concentrated" (52,000 DU/ml) dextranase 1:1 (2-fold) or 1:4 (5-fold) with either a 24 °Brix raw sugar solution, distilled water, or tap water. The raw sugar solution contained no dextran and was prepared with tap water. The tap water was obtained from New Orleans, LA and had approximately 3.1 ppm chlorine (Anon., 2005). Working solutions were prepared in beakers (500 ml), covered with aluminum foil, and then stored at room temperature ~77 °F (25 °C). The dextranase activity of the working solution was measured periodically over an approximate 48-140 h period.

General sampling

Juices before and after the addition of dextranase in the factories, were carefully stored to prevent further degradation reactions and/or microbial growth. Each sample was collected in a container (250 ml) containing 5 drops of biocide (Bussan 881TM, Buckman Labs. U.S.). Juices were immediately placed in dry ice before transportation to the Southern Regional Research Center laboratory in New Orleans, then stored in a -80 °C laboratory freezer subsequent to analyses.

Factory conditions

Because of previous results (Eggleston and Monge, 2004), in 2004 the factory installed a 5 min retention time tank that received mixed juice from the 1st and 2nd mills, to optimize dextranase application in the factory. Season av. flow rate was 400 short tons/h, and mixed juice flow was ~1600 gallons/min. Approx. 98% cane was billeted; 70% of the billeted cane was green (unburnt) and 30% burnt. Filtrate was not added to the tank. Dextranase was added continuosly in proportion to the juice flow rate, using a pulsatron pump (Series EPlus, Model LPG5SA, U.S.).

Factory sampling

Factory addition of "non-concentrated" dextranase

The factory added a "non-concentrated" dextranase (2,750 DU/ml) from 6 to 18 Oct. 2004. Eight random samples were collected of juice entering and exiting the tank, taking into account the 5 min retention time.

Factory trials on the effect of applying different working solutions of "concentrated" dextranase

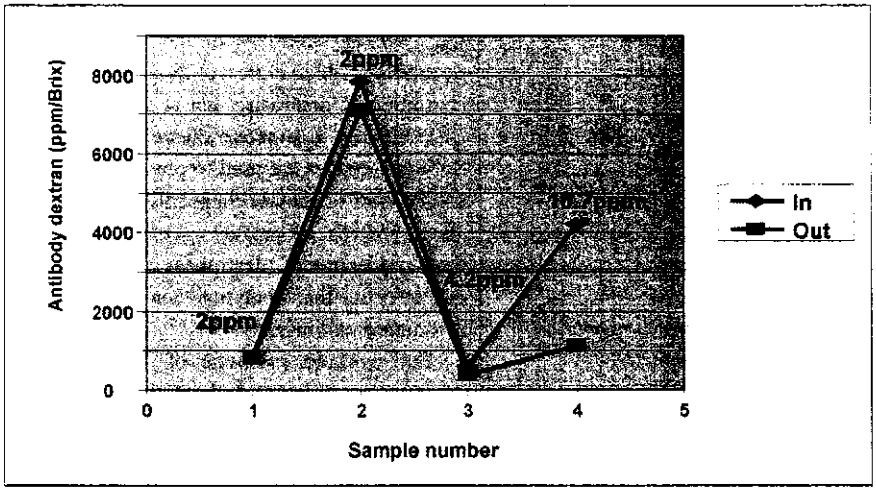
The first trial occurred on 22 Oct. 2004, after the factory had been shut-down for 24 h because of mechanical failure. Delivered cane had been stored in the factory yard for 24 h at high temperatures (30°C) and humidity levels, that caused the formation of very high levels of dextran (~2500-8000 ppm/°Brix). A working solution of "concentrated" dextranase (52,000 DU/ml) was prepared as a 2-fold (1:1) dilution with a 24 °Brix raw sugar solution. This solution was applied to the tank (from 8:00 – 9:40 a.m.) at a dosage of 6.4 to 10.7 ppm (i.e, normalized to the original enzyme activity by doubling the volume of the 2-fold working solution) on volume of juice. This initial dosage was determined by factory staff after measuring the dextran content of mixed juice. The dosage was then increased to a maximum of 21 ppm to improve dextran hydrolysis. Samples were collected just before entering and exiting the juice tank, taking into account the 5 min retention time, and occurred every hour from 10:00 a.m. to 5:00 p.m. Juice temperature was ~ 90 °F (32 °C).

A second trial occurred on 4 Nov. 2004. A working solution of the "concentrated" dextranase was first prepared as a 5-fold (1:4) dilution with distilled water, and added at 6 ppm (normalized to the original enzyme activity) to the juice. Samples were collected just before entering and exiting the tank, taking into account the 5 min retention time. This sampling was repeated seven times, every 15 min, to constitute a sampling period. A second sampling period was then undertaken, but this time a 2-fold working solution of the "concentrated" dextranase was prepared with distilled water, and added at the same dosage of 6 ppm to the juice. The juice temperature was ~ 32°C for both sampling periods.

Factory trial on the effect of different juice temperatures on dextranase efficiency

This trial occurred on 27 Oct. 2004 and began at 3:00 a.m. A working solution of the "concentrated" dextranase was prepared as a 2-fold dilution with a 24 °Brix raw sugar solution, and was first applied at 6 ppm (normalized to the original enzyme activity) to juice at ambient temperature (~92 °F or 33 °C), as the factory staff had been measuring 60-70% dextran hydrolysis at dextran levels <2000ppm/°Brix. Samples were collected just before entering and exiting the juice tank, taking into account the 5 min retention time. Composite samples of 240 ml (three 80 ml juice samples collected a minute apart) were collected. Sampling was repeated three times, every 20 min. After three sets of samples were taken, factory staff measured extremely high dextran levels of ~2220 ppm/°Brix. Consequently, the dosage was increased to 10 ppm and four more sample sets were collected. Juice temperature in the tank was then increased to 113-122 °F (45-50 °C) by opening the valve on the heated juice pipe that enters the tank. The system was allowed to equilibriate for 20 min before the last sample set was collected with the same 2-fold working solution of "concentrated" dextranase also added at 10 ppm.

Figure 1. Factory measurements of dextran hydrolysis at the factory in early October, 2004 using "non-concentrated" dextranase (2,750 DU/ml). Conditions: ~90 ºF (32.2 ºC). Factory ppm additions are noted directly on the graph



batches. The importance for reason (1) is highlighted in Table 1, which shows that the "non-concentrated" dextranases were being sold with lower activities in 2004 than in 2003, at only a slightly lower price. The monitoring of activity on storage is extremely important, as Eggleston and Monge (2005) recently reported that storage characteristics of commercially available dextranases vary widely. Under factory storage conditions (ambient temperatures of ~77 °F or 25 °C) over the typical length of a LA processing season (~90 days), the activity of a "concentrated" dextranase decreased only slightly at ~9%, whereas the activity of a "non-concentrated" dextranase decreased by 50% and was not even stable when stored under refrigeration. Furthermore, because "non-concentrated" dextranases lose their activity rapidly, transport conditions can affect their activity, e.g., they have arrived at the factory without any activity (A. Monge, personal communication). Therefore, the activity of delivered batches to the factory should be checked.

Results and discussion

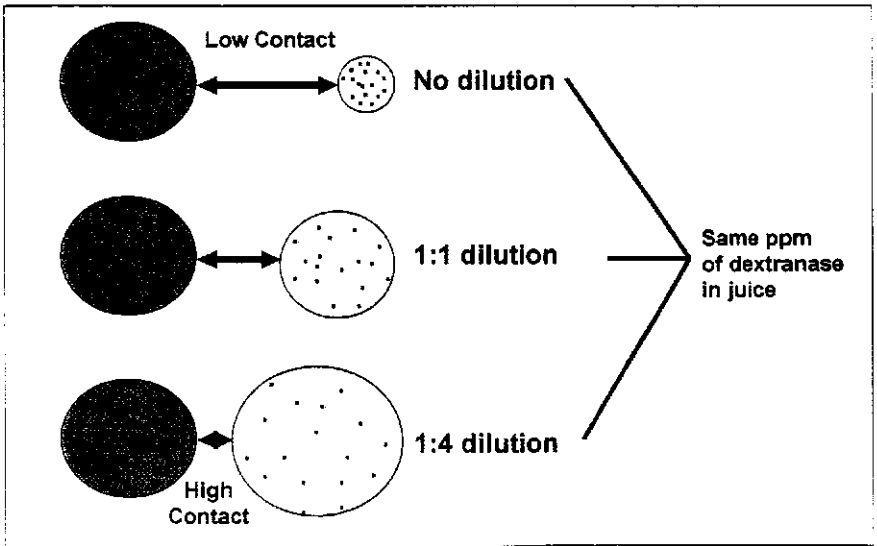
Activity of dextranases available in the U.S.

The activity of some dextranases commercially available in the U.S. (and used in LA factories) was measured (Table 1) using the factory titration method of Eggleston (2004). There are three important reasons to measure the dextranase activity at the factory: (1) to compare the economically equivalent activities of different commercial dextranases as vendor dextranase units differ, and activities and prices change regularly, (2) to monitor the changing activities of dextranases on factory storage, and (3) to measure the activity of delivered

Addition of "non-concentrated" dextranase to juice

To optimize juice application of dextranase, the factory installed a new 5 min retention time tank. The factory had traditionally applied "non-concentrated" dextranases to the last evaporator, and in the last 2 years had been using a monoclonal antibody method (Rauh et al, 1999) to monitor dextran hydrolysis. Fig. 1 shows typical results obtained by the factory applying a "non-concentrated" dextranase (2,750 DU/ml) to juice at the start of the 2004 processing season. Dextran levels were extremely high in juice (Fig. 1), as well as in syrups, massecuite, and raw sugar. This was because of higher than average temperatures (max. air temperature was 88 °F or 31 °C [Baton Rouge weather station data]) of unusually long duration (2 months) that, in conjunction with high humidity levels (max. 102.5 %) and above average rainfall (Waguespack, 2005), were ideal conditions for *L. mesenteroides* growth and dextran formation. As seen in Fig. 1, the addition of 2.0-4.2 ppm (typical dosage added previously in LA factories) of "non-concentrated" dextranase (2,750 DU/ml) had negligible effect on dextran hydrolysis. Even increasing the dextranase dosage to 10.7 ppm did not hydrolyze enough dextran to levels that would alleviate the boiler station operation (Fig. 1). For these reasons, the staff of Factory 1 decided to subsequently apply a "concentrated" dextranase (52,000 DU/ml).

Figure 2. A diagram of the contact between dextran (substrate) and dextranase (enzyme) in factory working solutions of "concentrated" dextranase. Working solutions represent the same ppm concentration of enzyme molecules (shown as squares), but larger volumes (represented by circles) to improve contact. The preparation of working solutions of "concentrated" dextranases is much more cost effective than adding "non-concentrated" dextranases directly



Adequate contact between substrate and enzyme (dextran and dextranase)

Before the application of "concentrated" dextranase (52,000 DU/ml) to industrial juice streams containing dextran was investigated, contact between the enzyme and substrate had to be con-

Figure 3. Stability of working solutions of "concentrated" dextranase (52,000 DU/ml) in (a) a 1:4, 24 ºBrix raw sugar solution, (b) distilled water, and (c) tap water

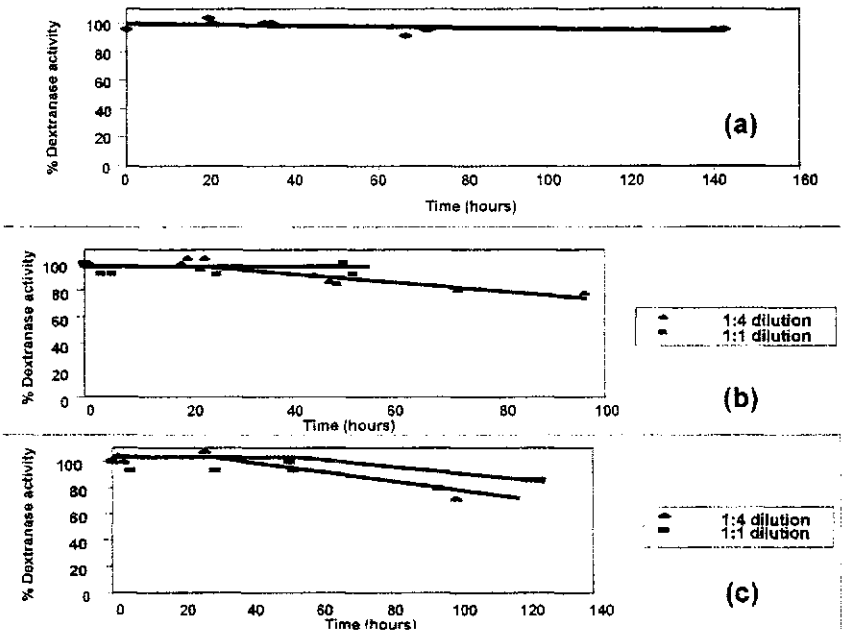
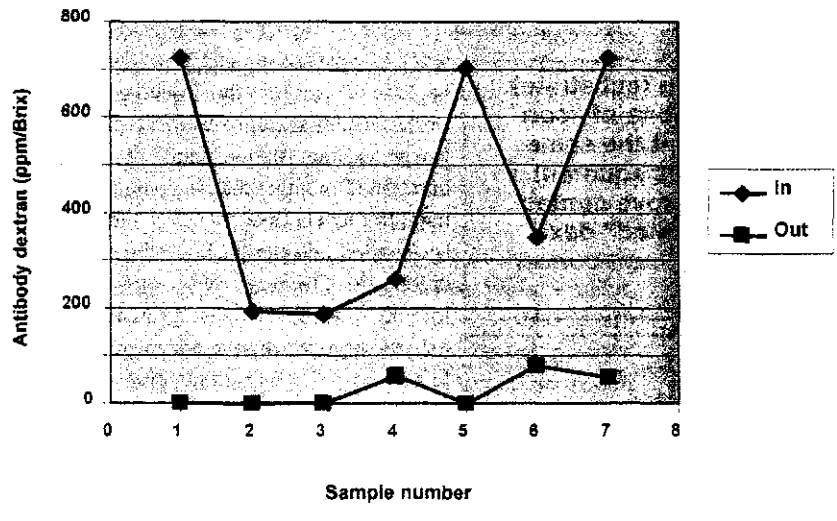


Figure 4a. Effect of applying a 2-fold working solution of "concentrated" dextranase (52,000 DU/ml) at the factory on 4 Nov, 2004. Conditions: 6 ppm; ~90 ºF (32.2 ºC)



sidered. Smaller volumes will take longer to disperse in the juice tank and Fig. 2 also illustrates the problem of low contact between dextranase and dextran when only small volumes of a "concentrated" dextranase are added. To solve this, working solutions of the "concentrated" dextranase, which represent the same final concentration of dextranase but at larger volumes were applied to improve contact (see Fig. 2). Staff at factories are used to preparing working solutions of flocculant chemicals for the clarification process. Moreover, working solutions of the "concentrated" dextranase is much more cost effective than applying "non-concentrated" dextranase undiluted.

For working solutions of "concentrated" dextranases to be useful, they need to be stable. As a consequence, stability studies were conducted. Sucrose is a known enzyme stabilizer (Davidson, 2001) and, fortunately, it is readily available at the factory, with its purest form in raw sugar (~99.5 % sucrose). Therefore, a 24 ºBrix raw sugar solution was used to dilute the "concentrated" dextranase 5-fold. The raw sugar solution effectively stabilized the dextranase activity over a 5 day period, i.e., the activity decreased by only ~2% after ~140 h, because of its lower water activity (Fig. 3a). However, for raw sugar solutions to be wholly effective, they must not contain dextran otherwise there is an unnecessary increase in substrate concentration. This is not always possible at the factory, especially in the first weeks of the season when cane deterioration frequently occurs and raw sugar stored in the warehouse often contains dextran. Raw sugar solutions may also be susceptible to microbial contamination if biocide is not added (Eggleston and Monge [2005] observed that dextranase works in the presence of dithiocarbamate biocide), and there is the possibility of inoculating the juice with *L. mesenteroides*. Furthermore, some factory staff complained of the extra time and effort that was required to prepare the raw sugar solution and then the working solution. As a consequence, the stability of working solutions of "concentrated" dextranase, diluted 2- or 5-

Table 1. Relative activities of dextranases in 2003 and 2004

Commerical Dextranase	Dextranase activity DU/ml 2003[a]	2004	Classification
(1)	52,000	51,920	"concentrated"
(2)	57,687	ND[b]	"concentrated"
(3)	5,499	3,500	"non-concentrated"
(4)	4,786	2,750	"non-concentrated"

[a] From Eggleston and Monge (2005)

[b] ND = not determined

Table 2. Hydrolysis of antibody dextran by a 2-fold working solution of "concentrated" dextranase (52,000 DU/ml) at the factory on 22 October, 2004. Conditions: ~90 °F (32.2 °C); 5 min retention time (Rt); working solution diluent was a 24 °Brix raw sugar solution

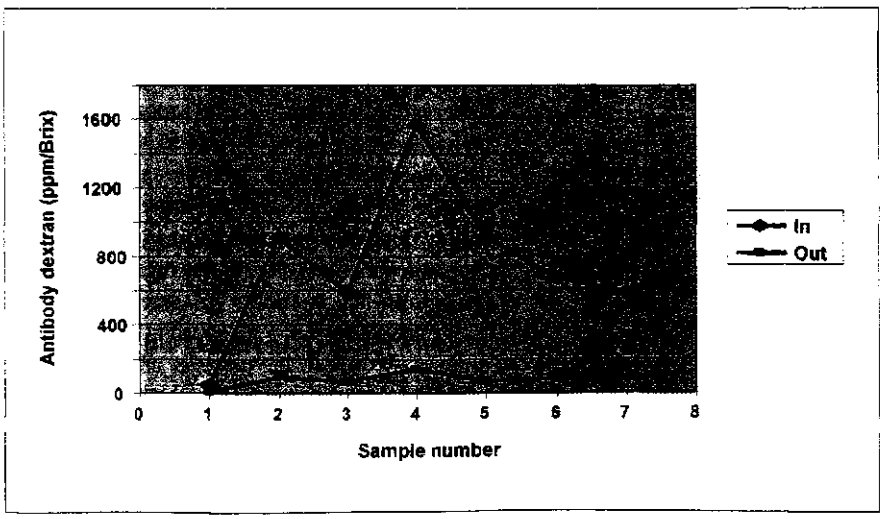
Time[a]	Factory Dosage (ppm)	AntibodyDextran (ppm/ °Brix)		% Dextran Hydrolysis
		$Tank_{In}$	$Tank_{Out}$	
8:10 a.m.	6.4	2855	3400	16.0
9:00 a.m.	6.4	2664	1500	43.7
9:40 a.m.	10.7	4075	3183	21.9
10:00 a.m.	21	6383	0	100.0
11:00 a.m.	21	6097	89	98.6
12:00 p.m.	21	6221	32	99.5
1:00 p.m.	21	7629	80	95.2
2:00 p.m.	21	4014	3525	12.2
2:30 p.m.	21	4239	151	96.4
4:00 p.m.	10.7	4863	554	88.6
5:00 p.m.	10.7	6062	1250	79.4

[a] $Tank_{OUT}$ samples were collected 5 min after $Tank_{IN}$ samples to take into account the 5 min R_t.

fold with distilled water, were investigated. The dextranase diluted 2-fold in distilled water was stable up to 48 h (Fig. 3b), and even a 5-fold dilution was stable for 24 h (Fig. 3b). The 5-fold working solution lost its activity more rapidly because the extra water can progressively de-activate/denature the enzyme protein structure and increase conformational mobility and unfolding of the protein.

The cheapest and most readily available source of water at the factory is tap water. Tap water (~3.1 ppm chlorine) behaved similarly to distilled water (Fig. 3c), with 2- and 5-fold dilutions stable for 48 h and 24 h, respectively. Tap water from the New Iberia area of Louisiana had no different effect than New Orleans, Louisiana tap water. This suggests that the more variable and higher chlorine and other impurity levels in tap water than distilled water were not sufficient to impede the stability of the working solutions. From these results, it is conservatively recommended that working solutions be prepared with tap water at the factory and stored for 12 to 24 h maximum. If factory staff prefer, they can store working solutions for longer if it is prepared with a 24 °Brix raw sugar solution treated with sodium dithiocarbamate biocide.

Figure 4b. Effect of applying a 5-fold working solution of "concentrated" dextranase (52,000 DU/ml) at the factory on 4 Nov, 2004. Conditions: 6 ppm; ~90 ºF (32.2 ºC)



Factory trials: The application of working solutions of "concentrated" dextranase

On 22 Oct. the factory had an acute dextran problem. The staff applied a 2-fold working solution of "concentrated" dextranase (52,000 DU/ml), and results of juice dextran hydrolysis during the day are listed in Table 2. From 8.00 – 9.40 a.m., dosages of 6.4 to 10.7 ppm of the working solution (normalized to the original enzyme activity) were applied with unacceptable results (Table 2). The factory staff increased the dosage to 21 ppm to obtain, in general, >95% hydrolysis of dextran (Table 2) that allowed for continual operation of the boiler house. Later in the day, when dextran levels decreased, the dosage was reduced to 10.7 ppm (Table 2). Although the consistent application of a 2-fold working solution of "concentrated" dextranase at 21 ppm across the whole season would not be economical, these results highlight that in, acute, emergency situations when dextran levels are substantially more severe than those during chronic periods of dextran occurrence in Louisiana, such dosages can be very useful. Furthermore, the factory staff were certain "that the concentrated dextranase allowed the factory to keep running [otherwise boiling operations would have ceased]" as compared to using a "non-concentrated" dextranase (D. Stewart, factory manager).

Further studies were conducted on the application of working solutions of "concentrated" dextranase during a chronic dextran period (Fig. 4), when the dextran levels were much lower than the acute situation just described, but still high enough to affect processing and cause penalty levels of dextran in raw sugar. The effect of applying 6 ppm of a 2-fold working solution (normalized to the original enzyme activity) of "concentrated" dextranase (52,000 DU/ml) is illustrated in Fig. 4a. Av. dextran hydrolysis was very acceptable at 94% and was very good even when the dextran levels fluctuated widely (Fig. 4a). This is in dramatic comparision to only ~0-20% dextran hydrolysis measured at the factory over the previous 5 years (results not shown),

Table 3. Effect of temperature on the ability of "concentrated" dextranase (52,000 DU/ml) to hydrolyze dextran at the factory. Conditions: 5 min Rt; 2-fold working solution diluent was a 24 °Brix raw sugar solution; composite samples

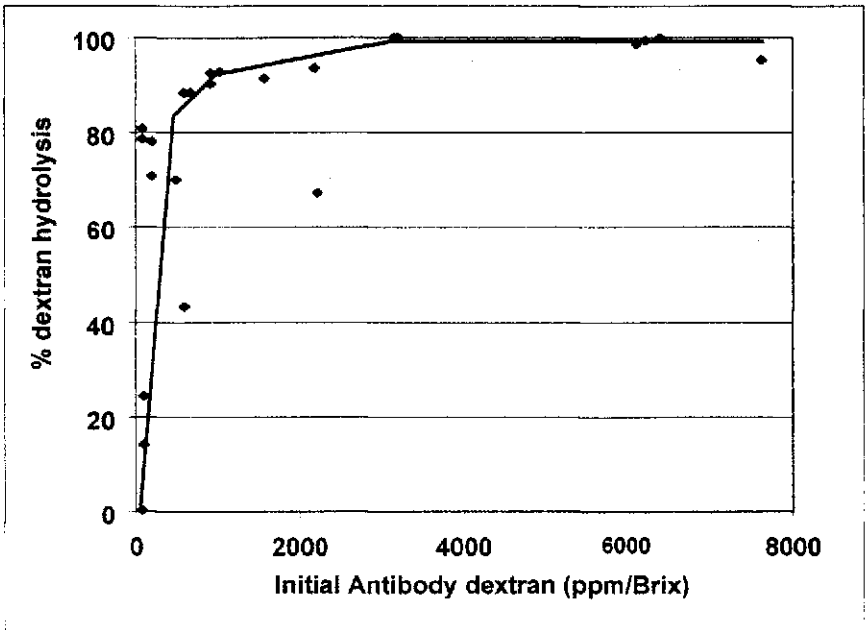
Time (a.m.)	Dosage (ppm)	Antibody Dextran (ppm/°Brix) $Tank_{In}$	$Tank_{Out}$	% Dextran Hydrolysis
		Juice Temperature ~92 °F (33.3 °C)		
3:20	6	80.7	0.0	100.0
3:40	6	98.1	84.0	14.4
4:00	6	2226.5	730.0	67.2
4:20	10[a]	80.4	80.1	0.4
4:40	10	3174.6	0.0	100.0
5:00	10	2179.9	138.5	93.6
5:20	10	1020.7	72.4	92.9
		Juice Temperature ~118 °F (47.8 °C)		
5:50	10	0.0	0.0	NA[b]
6:10	10	3209.2	0.0	100.0
6:30	10	0.0	0.0	NA
6:50	10	492.2	70.1	85.8
7:10	10	181.2	78.2	56.8
7:30	10	81.6	80.9	0.1

[a]The dosage was increased from 6 to 10 ppm because very high dextran levels were being measured

[b]NA=not applicable as there was no initial dextran in the juice entering the tank

when 3-10 ppm of a "non-concentrated" dextranase was applied to the last evaporator. The effect of applying 6 ppm of a 5-fold working solution of the "concentrated" dextranase under the same factory conditions is illustrated in Fig. 4b; av. dextran hydrolysis was slightly less at 85 % than for the 2-fold working solution (Fig. 4a). Although both dilutions of the two working solutions were efficient in hydrolyzing dextran, the difference in % hydrolysis was not large enough to draw any significant conclusions about their difference, and warrant further studies.

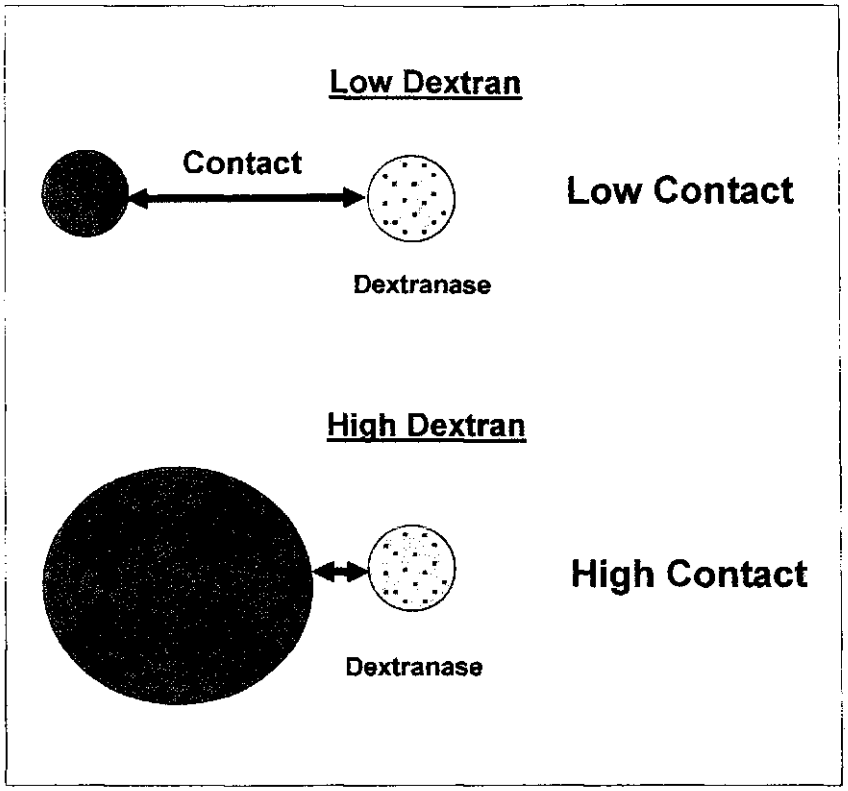
Figure 5. Effect of initial dextran concentration on the % dextran hydrolysis by dextranase at the factory



Factory trials: The effects of factory juice temperature and initial dextran levels

Current factory applications of dextranase to juice occur at ambient temperatures ~90 °F (32 °C), but Eggleston and Monge (2005) recently showed that the maximum activity of many U.S. available dextranases in juice is ~120 °F (50 °C). They also reported that in the laboratory, both "concentrated" and "non-concentrated" dextranases performed markedly better at hydrolyzing dextran in a juice at 120 °F (50 °C) than 90 °F (32 °F). For a "concentrated" dextranase (52,000 DU/ml) at 120 °F (50 °C), even after only 5 min at 4 ppm/juice, most of the dextran had been hydrolyzed (Eggleston and Monge, 2005). Heating the juice at the factory to ~120 °F (50 °C) may, therefore, markedly improve dextranase efficiency and the economics of application and, to some extent, overcome the limited availability of retention time in many U.S. factories. As a consequence, a juice temperature-effect trial was undertaken. Previous factory results had indicated that 4 ppm dosage was not sufficient, therefore, 6 ppm of a 2-fold working solution of the "concentrated" dextranase were applied. When this trial was undertaken there were no persistant factory dextran occurrences during the day, so the trial was conducted during the night (start time: 3:00 a.m.) when the factory was processing "sleeper" loads that had been delivered to the factory at or before 6:00 p.m. the previous day. The longer storage time of the cane in the yard usually causes dextran to form. As seen in Table 3, samples were first collected at normal, ambient juice temperatures measured at ~92 °F (33 °C), and many sam-

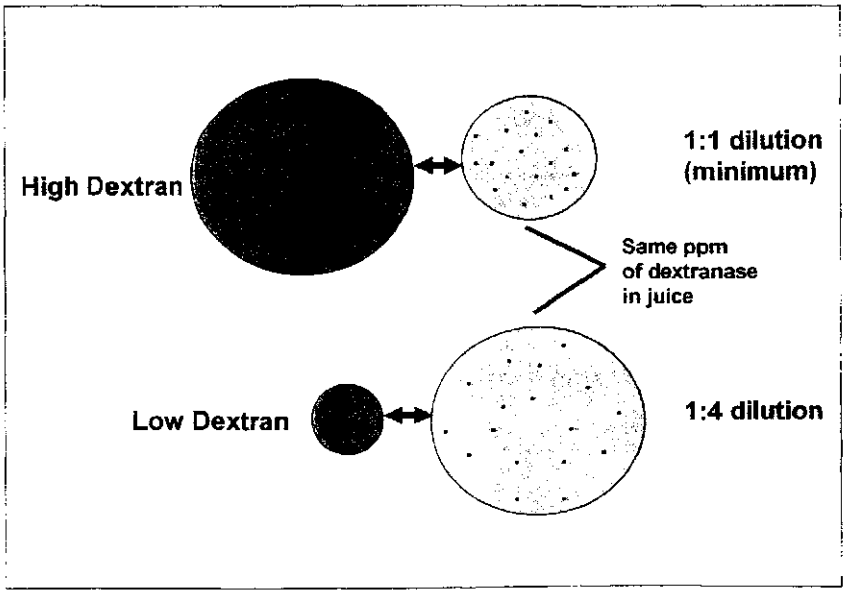
Figure 6a. Diagram of the contact between dextranase (enzyme) and different concentrations of substrate. Circles depict volumes and squares depict enzyme molecules



ples had high levels of dextran, i.e., >1000 ppm/°Brix. Av. hydrolysis at ~92 °F (33°C) was 70 %. Unfortunately, when the juice temperature was increased to ~48 °C (50 °C could not always be maintained) the samples collected, generally, had no or little initial dextran, particularly after 6:10 a.m (Table 3). This indicated that the sleeper loads had been processed and fresh cane was entering the factory. Even though the complete hydrolysis of 3209 ppm/°Brix dextran at ~118 °F (48 °C) in the 6:10 a.m. sample (Table 3) suggests that heating the juice improved dextranase efficiency, not enough samples of initial high levels of dextran were studied to draw firm conclusions. Therefore, further factory studies on temperature effects are required.

Although the temperature effect trial was inconclusive, it highlighted an important problem with respect to the optimization of factory dextranase applications – the effect of initial dextran levels on dextranase efficiency. As seen in Table 3, when initial dextran levels entering the tank were low, hydrolysis across the tank was markedly less. This is further illustrated in Fig. 5, where the initial antibody dextran level is plotted against % dextran hydrolysis by the "concentrated" dextranase in all the trials at the factory. Below ~ 670 ppm/oBrix dextran, dextranase efficiency decreased (Fig. 5) and this became more dramatic below ~ 500 ppm/°Brix dextran. Thus, it is easier to hydrolyze high levels of dextran than low levels. Fulcher and Inkerman (1976) reported that the ability of dextranase to hydrolyze dextran increased with a rise in HMW dextran (>2000 KDa) although they did not discuss why. Earlier, Tilbury (1971) claimed that dextranase was not influenced by dextran level in cane juice. The explanation for the easier hydrolysis of high levels of dextran is illustrated in Fig. 6a. When a low level of dextran is initially present, contact between the dextranase and dextran is low, i.e., the enzyme/substrate ratio is high. Conversely, when a high level of dextran is present, contact is higher (Fig. 6a). To solve this, factory staff can apply working solutions of higher dilutions of the "concentrated" dextranase to improve contact (see Fig. 6b). Improving agitation in the factory by, for example, using a mechanical rotor in a tank, tank baffles, or even serpentine pipes, would also improve contact.

Figure 6b. Recommended dilutions for temporary factory working solutions of "concentrated" dextranases. The different dilutions represent the same ppm concentration of enzyme molecules (shown as squares), but larger volumes (shown as circles) to improve contact



Conclusions

Factory dextranase optimization trials were conducted in the 2004 LA processing season. As previous laboratory studies (Eggleston and Monge, 2005) had shown dextranase applications to syrup were relatively uneconomical, only juice applications were studied at two factories. Sugarcane factories need to uniformally use the simple titration method of Eggleston (2004) to measure the activities of dextranases to 1) compare the economically equivalent activities of different commercially available dextranases as activities and prices change regularly, 2) monitor changes on storage at the factory, and 3) check the activity of delivered batches. The preparation of a working solution of "concentrated" dextranases is required to improve the contact between dextranase and dextran,

and is more cost-effective than adding "non-concentrated" dextranases directly. Staff at factories are used to preparing working solutions of flocculant chemicals for the clarification process. Working solutions can be easily prepared at the factory with either 24 °Brix raw sugar solutions, distilled, or tap water. It is conservatively recommend that a working solution be prepared with tap water and stored for 12 to 24 h maximum. If factory staff prefer, they can store the working solution for longer if it is prepared with a 24 °Brix raw sugar solution treated with sodium dithiocarbamate biocide.

A dextranase optimization trial was conducted at a LA factory that had relatively high antibody dextran levels (>1000 ppm/°Brix) in juice, and the addition of 2-fold or 5-fold working solutions (the 6 ppm dosages were normalized to the original enzyme activity) of "concentrated"dextranase (52000 DU/ml) were successful in consistently hydrolyzing 70-94% antibody dextran. Furthermore, working solutions of "concentrated" dextranase were very effective in reducing severe dextran levels in an acute, emergency situations at the factory, although doses of up to 21 ppm were required. At another LA factory with relatively low antibody dextran levels (<300 ppm/°Brix), application of dextranase was more problematic, which is reported in Part II (Eggleston et al, 2006). It was observed that high levels of dextran are easier to hydrolyze with dextranase than low levels, due to higher contact between the dextran and dextranase (low enzyme/substrate ratio). Below ~ 670 ppm/°Brix antibody dextran, dextranase efficiency decreased, and this became more dramatic below ~ 500 ppm/°Brix antibody dextran. To solve this, factory staff can add working solutions of higher dilutions of "concentrated" dextranase to improve contact, as well as ensure there is adequate mechanical agitation. Serpentine pipes would provide turbulent flow without the added costs of mixers, occupy relatively little space, and increase retention time. A little experimentation in the factory is recommended to optimize dextranase applications under each factory's unique conditions.

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