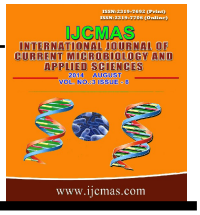
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 9 (2014) pp. 1147-1160**
http://www.ijcmas.com



Original Research Article

Application of Immobilized *P. aculeatum* Dextranase in Sugarcane Processing

A.Y. Gibriel[2], Azza A. Amin[1], Nessrien[2] Yassien N. M., Hanna A. El Banna[1] and F.M.Khaled[1*]

[1]Food Science and Technology Dept., National Research Center, Dokki, Giza, Egypt
[2]Food Science Dept., Fac. of Agric., Ain Shams Univ., Shoubra El-Kheima, Cairo, Egypt
**Corresponding author email:*

Keywords

Immobilized *P. aculeatum* Dextranase in Sugarcane Processing

A B S T R A C T

The application of *P. aculeatum* dextranases in Abu Kerkas sugarcane juice and final evaporate syrup indicated that dextranase activities in final evaporate syrup temperature (50 °C, °Brix 57.3) were dramatically reduced after 30-50 °Brix. Overall, juice applications were more efficient and economical than adding then to evaporate syrup. However, immobilized dextranase can be applied to syrup at levels as low as 10 ppm/solids to remove up to 70.65 % dextran which is useful to consider when several dextran problems occur. Heating juice to 55 °C in the presence of all dextranases, dramatically removed more dextran from a juice than at the current ambient temperature of application 32 °C and was much more economical. Free dextranase removed 47.89 % dextran after 5 min and at 55 °C compared to 8.03 % at 32 °C. While, 56.26 % of dextran was removed after 5 min, at 55 °C compared to 9.77 % at 32 °C for the immobilized dextranase. Storage effect of immobilized dextranase at 25–30 °C proved that the activity had decreased slightly from 97518.32 to 79982.91 units, while storage under 4 °C also decreased the activity from 81592.37 to 75297.37 units/100ml. Repeated batch processing in juice sugarcane as a simulation to factories conditions proved that immobilized dextranase can be used for over 16 cycles with 49.8 % activity.

Introduction

One of the major industrial applications of dextranases is the reduction of sliming in sugar production processes. The growth of *Leuconostoc* and *Lactobacillus* spp. is the most important factor in contributing to the postharvest deterioration of cane sugar and frost-damaged beet sugar (Brown and Inkerman, 1992). Problems caused by dextran in raw sugar include sucrose loss, increased viscosity of process syrups, and poor recovery of sucrose due to inhibition of crystallization.

The application of immobilized enzyme technology has great potential for improving the industrial uses of enzymes. The overall cost of an enzyme-catalyzed industrial process can be greatly reduced if the enzymes can be easily recovered and reused, usually by simple filtration or centrifugation (Ohba *et al.*, 1978).

Organic and inorganic supports have been employed in the immobilization of dextranase by several workers

(Laurinavichyus and Kulis, 1978). Bentonite was used to bind dextranase as a stable clarifying agent for cane juices. Several inorganic clarifying agents, such as Kieselguhr, charcoal, bentonite, Hyflo-Supercel and super-phosphate, are used in the sugar industry for the clarification of cane juices (Meade and Chen, 1977).

The action pattern of endo-dextranases from *P. funiculosam* and *P. lilacinum* are altered when covalently bound to silanized and glutaraldehyde-treated silica (Smiley *et al.,* 1982). The active immobilized enzyme produced a substantial amount of reducing sugar but its action had little effect on the viscosity of a high molecular weight dextran (2000 kDa) at pH 5.1 and 40 °C.

By contrast soluble dextranase slightly increased the level of reducing sugars and produced a rapid decrease in substrate viscosity. The immobilized dextranase can hydrolyse relatively few internal bounds of the high molecular weight dextrans, and, as a result, the bound dextranase converts from an endo- to an exo-type of action and produces high levels of reducing sugar. The thermal stability of immobilized dextranase from *P. aculeatum* maintained high operational activity following six cycles of repeated use, and 60% of dextran was hydrolyzed at 50 °C in 1 h (Prabhu and Prabhu, 1985).

Bentonite was selected as the support for dextranase immobilization because of its frequent use in the clarification of juices, its inert microporous structure with a large surface area and its high stability. No work seems to have been done on the application of immobilized dextranase in sugar-cane juices. Application of this bentonite matrix-bound enzyme in acetone powder form or suspension in acetate buffer or sucrose solution will help greatly in the sugar industry, with the possibility of continuous removal of dextran from juices. The operational stability of immobilized dextranase is the most important factor affecting dextran biodegradation in many industrial applications such as food, beverage, and sugar cane industries and other undesirable effects of dextran (Madhu and Prabhu, 1985_b).

The immobilized *P. funiculosum* 258 dextranase operational stability was evaluated in a repeated batch process, there was a rapid drop in the concentration of reducing sugars released (17%) after the second cycle. This rapid drop in activity was possibly due to the release of un-tightly bound enzyme from the carrier (Sakai *et al.,* 1991) and (Kusano *et al.,* 1989).

The immobilized dextranase was used after washing in a repeated batch process and the percent of residual dextranase activity was determined for 6 cycles. The immobilized enzyme was able to maintain a good yield of reducing sugars, while drop in activity was possible after many cycles due to the release of bound enzyme from the carrier. The immobilized dextranase retains 76% of its original activity after 5 cycles (Abdel-Naby *et al.,* 1999).

Immobilization of isolated *Streptomyces anultus* dextranase on hydroxyapatite in micro reactor led to production of 0.7mg isomaltotriose/ml/min. compared with macro reactor 0.45 mg isomaltotriose /ml/min. This rapid mass transfer is the key advantage of micro reaction technology. In this respect this direction of research may be of interest in the innovative application of cold-active dextranase in synthesis of pure isomaltotriose and overcoming dextran problems at lower temperature especially in the crystallization process. Doaa and wafaa (2009).

This study aimed to remove dextran from juice and final evaporate sugarcane by applying the produced immobilized *P. aculeatum* dextranase as well as reduce the viscosity and increase the sugarcane production.

Materials and Methods

3,5-dinitrosalysilic acid reagent, ammonium sulphate, dextran (M.W. 40,000 Da) , 3-Amino-propyl-triethoxy-silane (3-APTES), glutaraldehyde, cross-linking monomer (*N*,*N*, methylene-*bis*-acrylamide), cyanuric chloride ($CNCL_2$), thionyl chloride ($SOCL_2$), cyanogen bromide and carbodiimide were purchased from Sigma Company, England.
Sugarcane juice and final evaporate syrup (FES) were obtained from Abu kerkas factory (Menya Governorate – Egypt).

Dextranase activity

Dextranase activity was assayed by a modification method described by Webb and Spencer-Martin (1983).

Protein determination

Protein was determined according to the method described by Lowry *et al.* (1951) as follows:

°Brix measurement

The mean °Brix of triplicate samples was measured using an Index Instrument TCR 15–30 °C controlled refractometer accurate to 0.01 °Brix (Eggleston and Monge, 2005).

Determination of dextran in Sugarcane (juice or syrups)

Dextran in sugarcane syrups was determined by an alcohol method ICUMSA GS 1-15 (Anon, 1994) with the modifications of Gillian and Adrian (2004). Standard curve of dextran (MW 40,000 Da) (Fig. 1) was precipitated with 100% absolute ethanol to form haze dextran. Juice (35ml) was pipetted into a conical flask and 0.1 ml of α-amylase was added at 55°C for 15 min to degrade starch, 10 ml was then pipetted into a 25 ml plastic syringe with a filter holder attached, containing a coarse glass filter (25 min).

Two ml of 10 % Tri-Coloro Acitic Acid (TCA) was then pipetted into the syringe body and 0.5 g high-grade celite filter aid, and 5 ml filtrate was added to 5 ml absolute ethanol for 2 min, then the absorbance was immediately read in spectro-photometer at 720 nm. The amount of dextran was calculated using the same standard curve as for the ICUMSA haze dextran method mentioned above.

Viscosity

The viscosity of syrup was measured on a Brookfield DV-II$^+$ rotational viscometer at 25 °C. Spindle no. 18, and the temperature was maintained via a jacketed sample cell (8 ml) which was connected to water-bath accurate to 25±0.1 °C. The rpm rate applied was 150, which is equivalent to a shear rate of 3.96. Viscosity in centipoise (cP) was determined with Brookfield Wingather software and was calculated as percentage torque multiplied by the spindle factor. Preliminary experiments were undertaken to ensure repeatability of the method applied (Eggleston and Monge, 2005).

Effect of free and immobilized dextranase on the removal of dextran in juice and final evaporate sugarcane syrup

The effect of free and immobilized dextranase on the removal of dextran in juice and final evaporate syrup containing dextran 3.18 g/100 ml juice and 7.23 g/100

ml syrup, respectively were carried out at 50 °C for 30 min, and every 5 min the % dextran remaining was determined (Gillian and Adrian, 2004).

Effect of free and immobilized dextranase on the reduction of viscosity in final evaporate sugarcane syrup

The effect of the free and immobilized dextranase on viscosity reduction in the syrup was carried out at 50 °C for 30 min, then after 5 min the viscosity was determined (Gillian and Adrian, 2004).

Effect of biocide on reactivity of dextranase

The method described by Gillian and Adrian, (2004) was used to measure the effect of adding 20 ppm biocide (dithiocarbamate) to juice (14.1°Brix) in the presence of free dextranase (132.56 units) or immobilized dextranase (1.5 g). Biocide (dithiocarbamate) was added (20 ppm/juice) to prevent further dextran formation reactions at 55 °C. Free dextranase was added to 250 m1 of juice and mixed thoroughly for 30 min. The juice/dextranase mix was then immediately placed in a shaking water-bath at 55 °C and 90 rpm. Aliquots (40 ml) were removed after 0, 5, 10, 15, 20, 25 and 30 min, and boiled immediately for 2.5 min. After cooling, the samples were analyzed, in duplicate, for haze dextran. For the immobilized dextranase and the reaction conditions were the same.

Storage Characteristics of free and immobilized dextranases

Free and immobilized dextranases were stored in dark brown bottles in a cool and dark laboratory corner (ambient temperatures ranged from 23-27 °C). Dextranases activity was measured at time intervals (10 days) over a period of 90 days, then dextranase sample was stored in a refrigerator at 4°C for 150 days and periodically analyzed as well (Gillian and Adrian, 2004).

Results and Discussion

Applications of dextranases in laboratory

Immobilized *P. aculeatum* dextranase was directly applied on juice and syrup sugarcane in the laboratory. To understand how free and immobilized dextranases react during industrial processing and how they can be manipulated, a laboratory study of their effects on sugarcane juices and syrup was initially under-taken.

The composition of juice and final evaporate sugarcane syrup (FES) from Abu Kerkas factory as a model (Table 1), indicated that the final evaporate syrup had higher °Brix (% dissolved solids) and dextran values 57.3 % and 7.23 gm/100 ml than the juice 14.1 % and 3.18 gm/100 ml, respectively.

Effect of ˚Brix on free and immobilized dextranase activity

The efficiency of dextranases in the factory depends on the pH, °Brix, temperature, retention time, activity and dosage of the dextranase applied. The effect of °Brix on the activity of free (215.37 units) and immobilized (132.56 units) dextranase at 50 °C for 25 min was illustrated at Fig. (2).

Dextranase activity was stable up to 10 – 30 °Brix, but afterwards decreased rapidly because of the low concentration of water reactant, while immobilized dextranase activity was stable up to 50 °Brix (97.31 – 84.19 % activity) and decreased to 67.14 % at 70 °Brix.

This result indicated that the immobilization process increased the ability of dextranase to carry high concentrations of sucrose compared to the free enzyme, which shows a reduction in its activity to 1.97 % at 70 °Brix.

Overall, the pH, temperature and °Brix conditions in factory last evaporators are sub-optimal for dextranase reaction. This was confirmed and shown to be uneconomical when used free dextranase to final evaporate syrup in the laboratory. But there was a significant improvement for the use of the immobilized dextranase either to final evaporate syrup or juice.

The dramatic loss of dextranase activity is because more water is available to de-activate and denature the enzyme protein structure and increase its conformational mobility. Furthermore, transport conditions can affect of dextranases activity, e.g. they have arrived at the factory with no activity (Eggleston *et al.,* 2006). Therefore, the activity delivered batched to factory should be monitored.

Effect of free and immobilized dextranase on removal of dextran in final evaporate sugarcane syrup

The effect of free and immobilized dextranase to remove dextran from Abu Kerkas final evaporate syrup (7.23 g dextran /100 ml syrup) at 50 °C (Fig. 3), showed very little effect on dextran degradation by using free dextranase, with only 25.92 % of dextran was removed after 30 min. while, the immobilized dextranase was able to remove a considerable amount of dextran 70.65 % after 30 min. Dextran remaining in syrup after 10 min from adding free or immobilized dextranase was 87.67 and 61.93 %, respectively. These results indicated that it is not economically to add free dextranase to sugarcane syrup.

Although results are reliable, there must be underestimations of % dextran removal, to ensure that processing efficiency was definitely improved upon by degrading dextran in syrup with free or immobilized dextranase.

Effect of free and immobilized dextranase on reduction of viscosity in final evaporate sugarcane syrup

The effect of adding free or immobilized dextranase to reduce the viscosity in syrup at 65 °C for 30 min (Fig. 4), indicated that the addition of free dextranase (215.37 units) to the evaporate syrup slightly reduced the viscosity over 30 min, while the immobilized dextranase (132.56 units) reduced only 12.49 % from total viscosity of syrup after 5 min, and reached to 40.66 % after 30 min. These results indicated that the higher level of free dextranase applied, the lower viscosity achieved after 30 min and vise versa for the immobilized dextranase and more economics. Small viscosity reductions of syrup in the presence of free dextranase were found by Hidi and Staker (1975), as the viscosity of syrups is mostly caused by sucrose.

Effect of free and immobilized dextranase on removal of dextran from sugarcane juice at 32°C

The effect of free (215.37 units) and immobilized (132.56 units) dextranase on the removal of dextran from sugarcane juice at 32 °C (Fig. 5) showed that after 5 min addition of free dextranase, 8.03 % of dextran was removed compared to only 9.77 % at 32.0 °C for the immobilized dextranase. The removal of dextran from juice reached after 30 min incubation to 37.21% and 84.17 % for free and immobilized dextranase, respectively.

Tucker (1995) tried to understand how commercial dextranases react during industrial processing and how they can be manipulated. Eggleston and Monge, (2004, 2005) initially undertaken a comprehensive laboratory study of the effect of dextranases on sugarcane juices and syrups.

Effect of free and immobilized dextranase on removal of dextran from sugarcane juice at 55°C

The effect of adding free (215.37 units) and immobilized dextranase (132.56 units) on the removal of dextran from sugarcane juice at 55 °C (Fig. 6) indicated that heating juice to 55 °C increased dextran hydrolysis to 47.89 % after 5 min addition of free dextranase compared to 8.03 % at 32 °C after the same time. As for the immobilized dextranase heating the juice to 55 °C improved the hydrolysis of dextran to 56.26 % compared to 9.77 % at 32 °C after 5 min. However, in comparison with immobilized dextranase at 55 °C and after 30 min, 90.82 % of dextran was hydrolyzed than 68.74 % for the free enzyme at the same condition.

These results indicated that heating the juice to 55 °C with immobilized dextranase markedly improves the efficiency of the application and to some extent, overcomes insufficient retention time and were much economical.

If industrial processing conditions are ideal, an applied hydrolyze enzyme such as dextranase will repeat the hydrolysis reaction many times during the process. However, the addition of hydrolyases to an industrial process is often non-ideal, problematic, and difficult to optimize because conditions are frequently harsh.

Also, extrapolation from laboratory and pilot scales to the industrial scale is not always linear. Although, the initial laboratory results are reliable and gave a useful indication of the necessary dosage of immobilized dextranase to either juice or syrup, they are obtained under ideal conditions compared to those at factories, where dextranase is added to much larger volumes of juice in tanks and pipes with fluctuating of flow rates and agitations.

Consequently, this study suggested that, a factory trials must be conducted to any Egyptian sugarcane factory across the processing season to verily if heating juice under industrial conditions could improved immobilized dextranase application.

It would be expect to increase factory energy inputs and costs. These will be negligible as existing heated juices could be recirculated into the juice tank or juice pipes. Moreover, because heating the juice will reduce the immobilized dextranase dosage, any costs from increased energy than costs for the relatively expensive dextranase.

Eggleston *et al.,* (2006) showed that laboratory result gave a solid foundation from which to start the factory trials, and allowed decision not to study dextranase applications to last evaporate syrup, as they are not cost-effective.

Most factory application of dextranases to juice occurred at ambient temperature (26 – 32 °C). Recently Eggleston and Monge, (2005) showed that maximum activity of dextranase in many U.S. sugarcane juice was viable at 50 °C.

Addition of free or immobilized dextranase to sugarcane juice in the presence of biocide (dithiocarbamate)

Although, heating juice with immobilized

dextranase at 55 °C may lead to more optimum temperature for *Leuconostoc* growth and dextran formation, but the addition of biocide will inhibit this. Fig. (7) Illustrated the effect dithiocarbamate (biocide) on the activity of free and immobilized dextranase in sugarcane juice at 55 °C and pH 5.5.

Results showed that the immobilized dextranase still worked in the presence of the biocide, and the addition of 10 ppm of biocide may be slightly more favorable than 20 ppm of addition. Eggleston and Monge (2005) showed that when severe dextran problems occur, an option in the factory could be to add 10 ppm/juice to the mill tandem and 10 ppm to mixed juice.

Effect of storage on free and immobilized dextranase under simulated factory conditions

Free or immobilized dextranase were stored in the coolest and shadiest area of the factory in order to prevent loss of activity at higher temperatures. To simulate such factory storage conditions, the *P. aculeatum* free and immobilized dextranase were stored in a cool and dark corner of a laboratory at ambient temperatures range from 25–30 °C, and under refrigeration at 4 °C. The effect of storage time on the free or immobilized dextranase activity across the approximate length of a grinding season (90 days) was studied.

Storage stability of dextranases at room temperature

After 90 days of storage under room temperature at 25–30 °C, the activity of the immobilized dextranase decreased slightly from 97518.32 to 79982.91 units (Fig. 8). In dramatic contrast, the activity of the free dextranase decreased across the storage time under these simulated factory conditions from 97518.32 to 8212.78 units. This result indicated that storage at 25–30 °C for 90 days significantly decrease the activity of the free dextranase.

Storage stability of dextranases under refrigeration (4°C)

After 150 days of storage under refrigeration at 4 °C, the activity of the immobilized dextranase decreased slightly from 81592.37 to 75297.37 units (Fig. 9). While, there was a dramatic decrease in the activity of the free dextranase from 81592.37 to 36945.04 units after 150 days.

This result indicated that there is no ideal or easy storage conditions for free dextranase in the factory which led them not to purchase all the dextranase needed early in the season.

Furthermore, this problem highlights the need for the factory staff to be able to monitor the activity of their dextranase across the season.

The reuse of immobilized dextranase by batch processing

The operational stability of the immobilized *Penicillium aculeatum NRRL-896* dextranase was evaluated in a repeated batch process (Fig. 10). Results indicated that after the eight cycle, there was a drop in the concentration of reducing sugars released (16.5 %). This drop in activity was possible due to the release of untigthly bound enzyme from the carrier.

A little decrease in dextran hydrolysis (by only 8.4 % in relation to eight cycles) was recorded from the 11^{th} up to 8^{th} cycle. Later the hydrolysis efficiency declined distinctly and in the twentieth cycle it achieved a value of about 13.4 %. The immobilized

dextranase was able to maintain a good yield of reducing sugars (50.2 %) as high as 49.8 % of the initial activity after the sixthten cycles.

These results indicated that the bound enzyme could be used repeatedly for 16 cycles without any appreciable loss in sugar yield which reached average value of 50.2 %.

The operational stability of *P. aculeatum* dextranase immobilized on oyster stem modified with glutaraldehyde by cross-linking technique appears to be more stable than *P. aculeatum* dextranase immobilized on other carriers.

Economic costs of different dextranase applications

The relative equivalent cost for the immobilized dextranase versus the free one was shown in Table (2).

Results indicated that the complete hydrolysis of dextran by using free dextranase costs 0.5 L.E. while it was 1 L.E. for the immobilized one. In case of syrup dextranase it should be added at 50 °C that cause hydrolysis of dextran to 70.65 % and 25.92 % for the immobilized and free dextranase, respectively. Although, the cost of free dextranase at such level was half that of the immobilized one. However, the immobilized dextranase could be used for 16 times.

Cost calculation of dextranases juice addition after 5 min were undertaken Table (2).

The relative cost of free dextranase in juice at either 32 or 55 °C was almost similar 1.23 and 1.27 L.E. /100 ml juice, while it was one L.E. expensive for the immobilized one at the same two temperature used.

It could be concluded that the application of the immobilized *P. aculeatum* dextranase on oyster mushroom stem by physical adsorption with cross-linking was more economical in juice at 55 °C as it could be used for 16 times and gave high hydrolysis of dextran.

Fig.1 Standard curve of haze dextran (MW 40,000 Da)

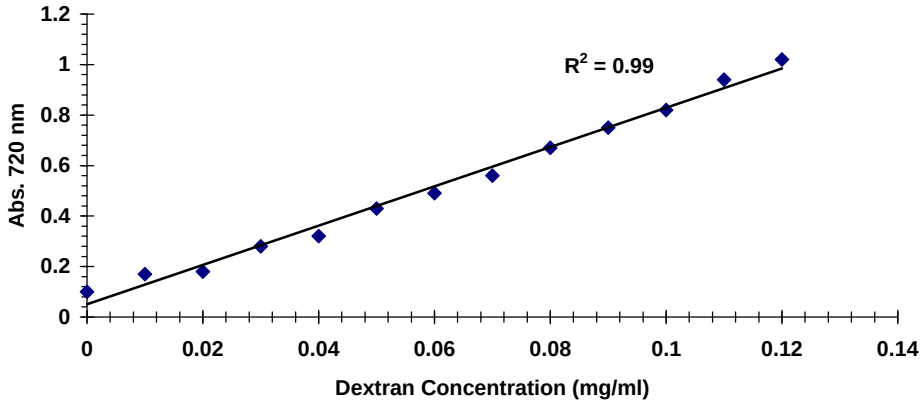


Table.1 Composition of Juice and final evaporate sugarcane syrup from an Abu Kerkas factory

Samples	°Brix	pH	Dextran g/100ml
Sugarcane juice	14.1	5.4	3.18
Final evaporate syrup (FES)	57.3	6.2	7.23

Fig.2 Effect of °Brix on free and immobilized dextranase activity under conditions pH 5.0; 25 min at 50 °C

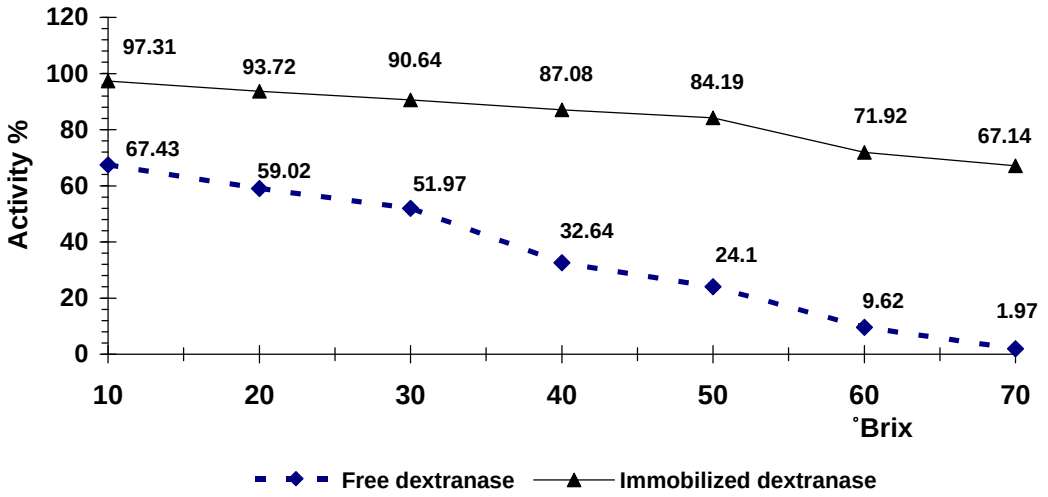


Fig.3 Effect of free and immobilized dextranase on removal of dextran in final evaporate sugarcane syrup:

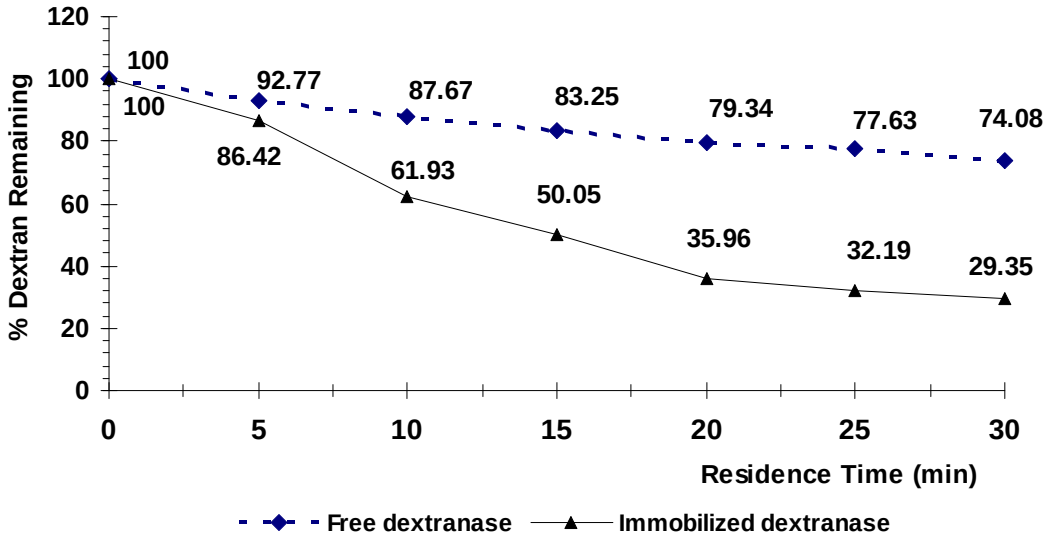


Fig.4 Effect of free and immobilized dextranase on reducing of viscosity in final evaporate sugarcane syrup

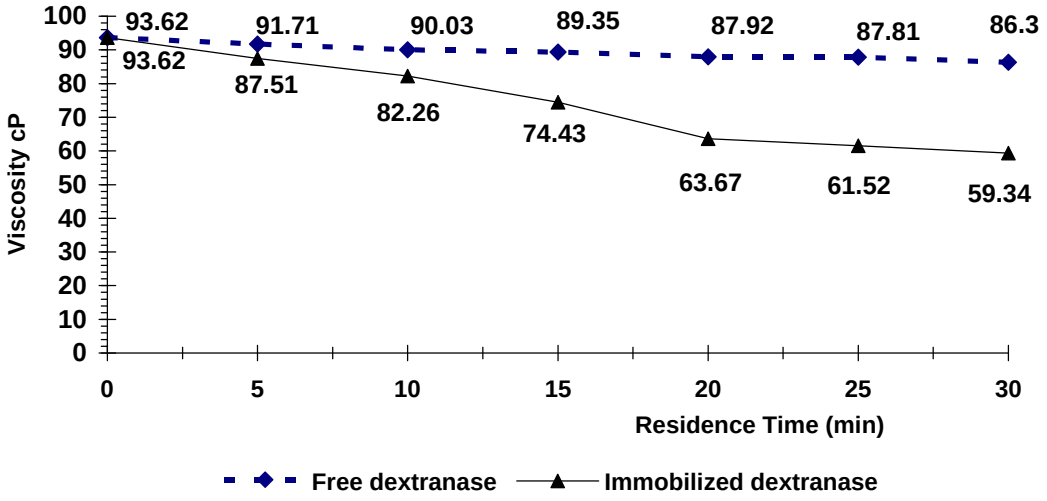


Fig.5 Effect of free and immobilized dextranase on removal of dextran from sugarcane juice at 32°C

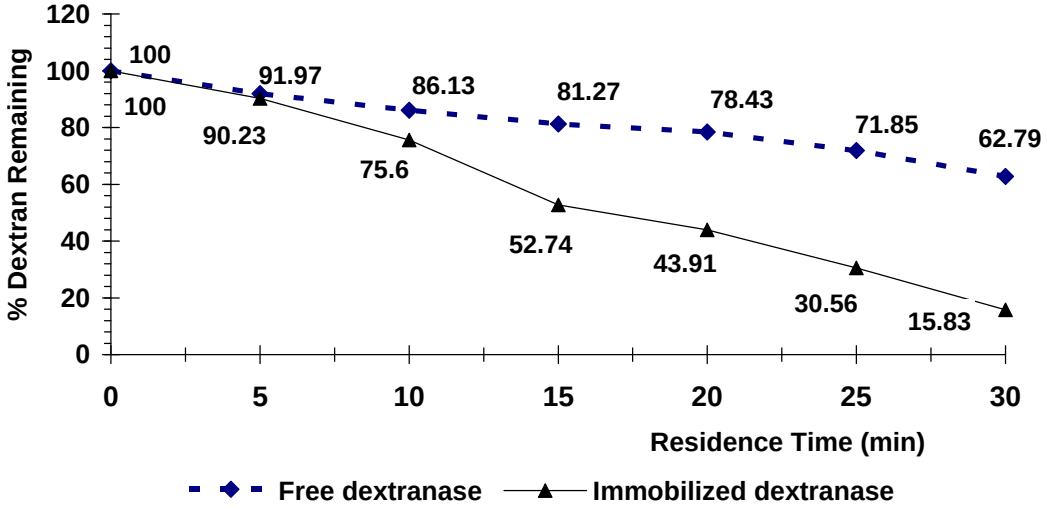


Fig.6 Effect of free and immobilized dextranase on removal of dextran from sugarcane juice at 55 °C

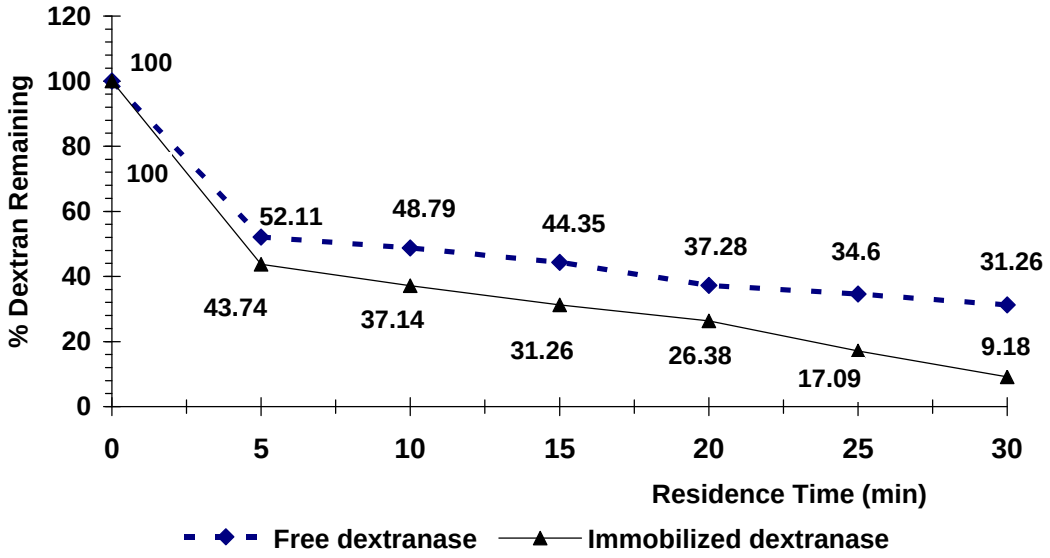


Fig.7 Effect of biocide (dithiocarbamate) on free and immobilized dextranase activity on sugarcane juice at 55 °C and pH 5.5

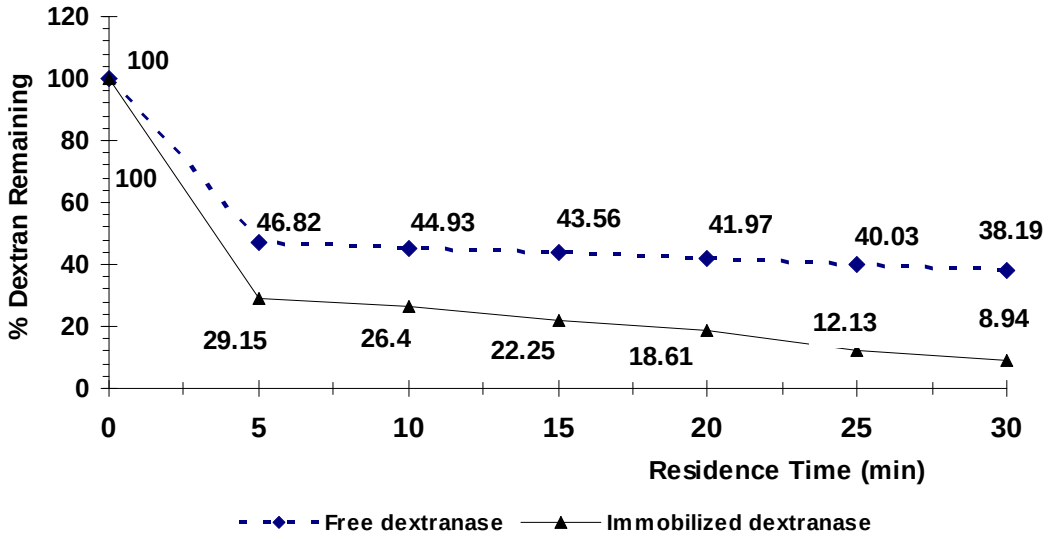


Fig.8 Changes in activity of free and immobilized dextranase under simulated factory storage conditions, stored at room temperature 25-30 °C in shaded place over a 90-day.

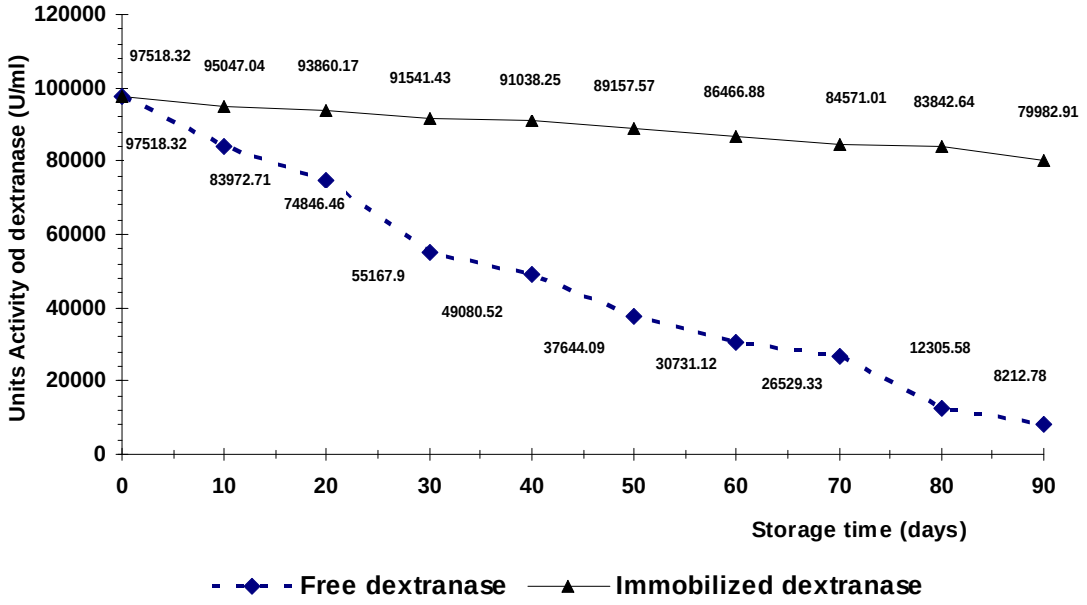


Fig.9 Changes in activity of free and immobilized dextranase stored under refrigeration°C over 150-day

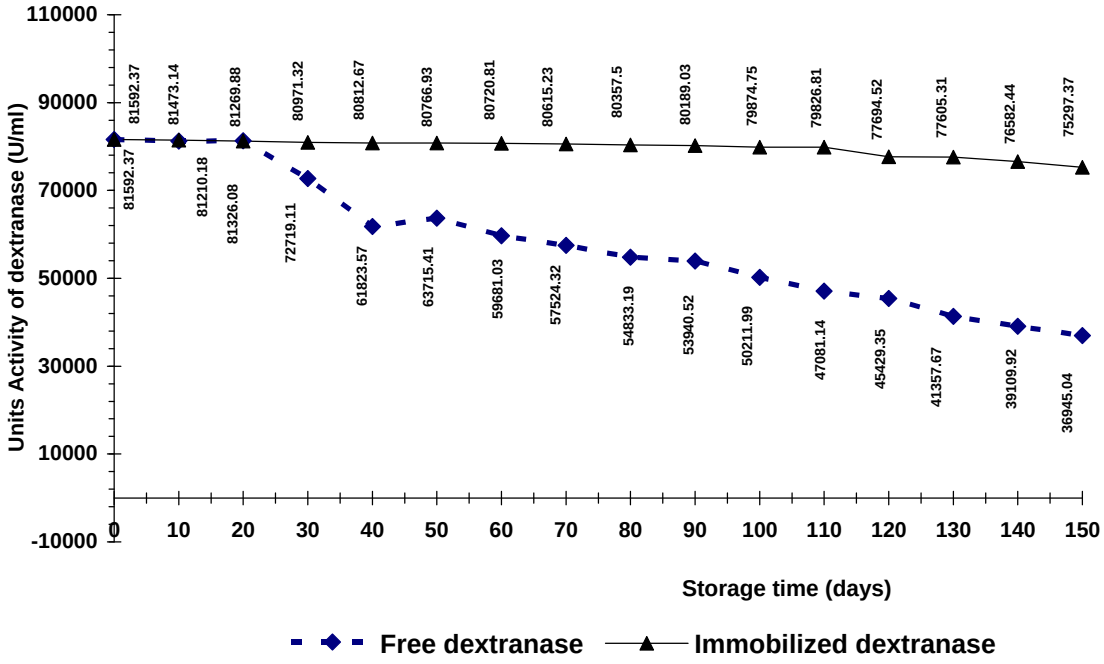


Fig.10 Operational stability of immobilized *Penicillium aculeatum NRRL-896* dextranase

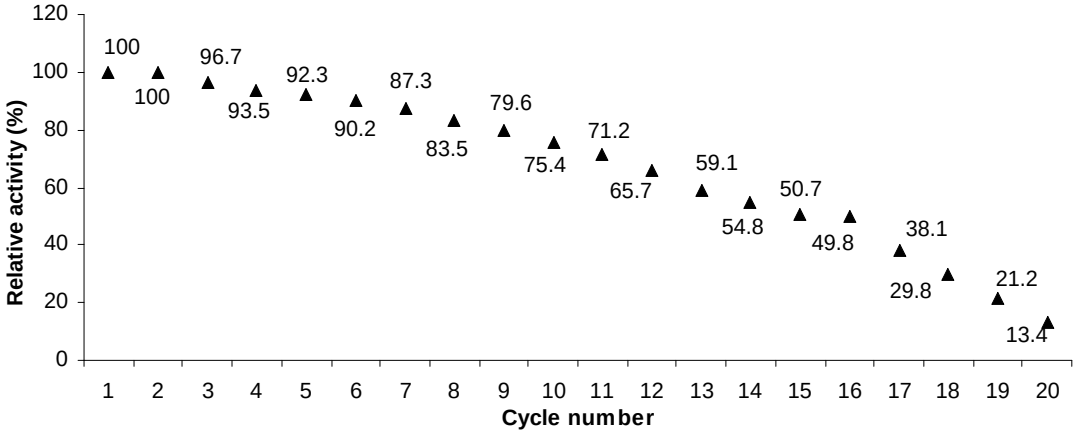


Table.2 Cost-effective calculations for different dextranase applications

Form of dextranase	Enzyme added U/ml	Dextran breakdown %	Breakdown ration %	Cost L.E. for 100 % hydrolysis	Relative equivalent cost L.E.
Syrup sugarcane (7.23 g dextran/57.3 °Brix) application at 50 °C and for 30 R^+					
Immobilized	132.56	70.65	1	4.2	1
Free	215.37	25.92	2.72	7.7	0.5
Juice sugarcane (3.18 g dextran/14.1 °Brix) application at 32 °C and for 5 R^+					
Immobilized	132.56	9.77	1	30.7	1
Free	215.37	8.03	1.2	24.9	1.23
Juice sugarcane (3.18 g dextran/14.1 °Brix) application at 55 °C and for 5 R^+					
Immobilized	132.56	56.26	1	5.3	1
Free	215.37	47.89	1.17	4.17	1.27

R^+ is Residence time

References

Anon S. (1994). The determination of dextran in raw sugar by a modified alcohol haze method (Method GSI-15)-ICUMSA Methods Book. ICUMSA Publication Department, Norwich, England.

Cheetham N. W. H., M. E. Slodki and G. J. Walker (1991). Structure of linear, low molecular weight dextran synthesized by a D-glycosyltransferase (GTF-S3) of *Streptococcus sobrinus*. Carbohydr. Polymers, 16: 341–353.

Cuddihy J. A. and D. F. Day (1999). The process and financial impact of dextran on a sugar factory. Sugar Journal Mar: 27–30.

De Bruijn J. M. (2000). Processing of frost damaged beets at CSM and the use

of dextranase. Zuckerindustrie, 125(11): 892–902.

Eggleston G. and A. Monge (2004). Optimization of factory applications of dextranases in the U.S. Proc. Sugar Proc. Res. Conf. 371–394.

Eggleston G. and A. Monge (2005). Optimization of sugarcane factory application of commercial dextranases. Process Biochemistry, 40: 1881–1894.

Eggleston G., A. Monge, B. Montes and D. Stewart (2006). Factory trials to optimize the industrial application of dextranase in raw sugar manufacture: Part I. Intern. Sugar Journal, 108(1293): 528–537.

Gillian E. and M. Adrian (2004). Optimization of sugarcane factory application of commercial dextranases in the U.S. Conference on Sugar Processing Research, SPRI, 371–394.

Gudohnikov S. (2007). World sugar market-Back to sulplus. World perspectives for sugar Beet and cane as a Food and Energy crop, Sharm El Sheikh GL 1.1/1–11.

Hidi P. and R. Staker (1975). Enzymic hydrolysis of dextran in mills, part 1. Proc. 42nd Conf. of Queensland Society Sugar Cane Technol. [QSSCT], 331–344.

Inkerman P. A. and L. Riddell (1977). Dextranase III. Refinements to the enzyme process for the treatment of deteriorated cane. Proc. Queensl. Soc. Sugar Cane Technol., 44: 215–233.

Jimênez E. R. (2005). The dextanase along sugar-making industry. Biotec. Apll., 22: 20–27.

Khalifah M. A. (2001). Effect of dextran on sugarcane quality and raw sugar manufacture. Dissertation, Assiut University, Egypt.

Khalikova E., P. Susi and T. Korpela (2005). Microbial dextran hydrolyzing enzymes: Fundamentals and applications. Microbiol. Mol. Biol. Rev., 69(2): 306–324.

Lowry O. H.; N. J. Rosebrough; L. Farr and R. J. Randall (1951). Protein measurement with folin phenol reagent. Journal Biol. Chem., 193: 265–275.

Madhu G. L. S. and K. A. Prabhu (1984). Application of dextranase in the removal of dextran from cane juice. Int. Sug. Journal, 86: 136–138.

Matheson R. R. (1980). "Viscosity of solutions of rigid rodlike macromolecules". Macromolecules, 13: 643–648.

Tilbury R. H. and S. M. French (1974). Further studies on enzymic hydrolysis of dextran mill juices by dextranase and fungal α-amylase. Proc. Int. Soc. Sug. Cane Technol., 15: 1277–1287.

Tucker G. A. (1995). Fundamentals of enzyme activity. In Enzymes in Food Processing. Tucker G.A., Woods LFJ, Eds. Blackie, p 1-25.

Webb E. and I. Spencer-Martin (1983). Extracellular endodextranase from the yeast *Lipomyces starkeyi*. Can. Journal Microbiol., 29: 1092–1095.