The dextranase along sugar-making industry

Efraín Rodríguez Jiménez

División Química Física, Centro de Ingeniería Genética y Biotecnología, CIGB
Ave. 31 e/ 158 y 190, Cubanacán, Playa, AP 6162, CP 10600, Ciudad de La Habana, Cuba
Fax: (53–7) 271 47 64; E–mail: efrain.rodriguez@cigb.edu.cu

REVIEW

ABSTRACT

In sugar production, dextrans are undesirable compounds synthesized by contaminant microorganisms from sucrose, increasing the viscosity of the flow and reducing industrial recovery, bringing about significant losses. The use of the dextranase enzyme is the most efficient method for hydrolyzing the dextrans at sugar mills. Some bacterial strains, filamentous fungi and a small number of yeasts have been shown to produce dextranase. The fungal dextranases showed the highest reaction rate at low Brix, with pH and temperature close to 5.0 and 50 °C, respectively, that is, conditions existing in juice extraction. Some of these dextranases formulated in enzymatic preparations have been efficiently used for hydrolyzing dextrans in sugar mill juices. In more advanced points of the process, where the dextrans have already caused losses, the conditions of temperature and Brix are high. However, although the volumes are smaller, the treatment with these enzymes in syrup showed the need to increase the dose, equaling dextranase consumption. Some thermo tolerant bacterial dextranases identified up to now showed a much reduced specific activity that makes their industrial use unfeasible. The fungal dextranases from *Chaetomium sp.* have shown the best results on dextrans treatment both in juices and syrups. Any attempt to obtain a new natural or recombinant dextranase enzyme, must be comparable with to the *Chaetomium* enzyme.

Keywords: Dextranase, industrial enzymes, industrial application, dextran

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RESUMEN

La dextranasa a lo largo de la industria azucarera. En la producción de azúcar, las dextranas son compuestos indeseables sintetizados por microorganismos contaminantes a partir de la sacarosa, que conllevan a pérdidas significativas al incrementar la viscosidad en los flujos y reducir el recobrado industrial. El empleo de la enzima dextranasa es el método más eficiente para la hidrólisis de las dextranas en el central azucarero. Se han identificado cepas de bacterias, hongos filamentosos y menor número de levaduras que producen dextranasa. Las dextranasas fúngicas manifestaron la mayor velocidad de reacción y encontraron las condiciones más favorables de reacción a bajo Brix, con valores de pH y temperatura próximos a 5.0 y 50 °C, respectivamente, es decir, condiciones existentes en el procesamiento de jugos. Algunas de estas dextranasas formuladas en preparados enzimáticos se han empleado para hidrolizar eficientemente las dextranas en los jugos de los centrales. En puntos más avanzados del proceso donde ya las dextranas han provocado pérdidas, las condiciones de temperatura y Brix son elevadas. Sin embargo, aunque los volúmenes a tratar son inferiores, el tratamiento con estas enzimas en meladura mostró la necesidad de incrementar la dosis llegando a igualar el consumo de dextranasa. Algunas dextranasas termo tolerantes de bacterias identificadas hasta el momento manifestaron la actividad específica muy reducida que hace impracticable su uso industrial. Las dextranasas fúngicas de *Chaetomium sp.* han mostrado los mejores resultados en el tratamiento de las dextranas tanto en jugos como en meladura. La búsqueda de nuevas dextranasas, ya sean naturales o recombinantes debe dirigirse a la obtención de una dextranasa comparable con la enzima de *Chaetomium sp*.

Palabras claves: Dextranasa, enzimas industriales, aplicación industrial, dextrana

Introduction

Dextrans are high molecular weight polysaccharides formed, of at least 50%, by 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 [1]. Branches are significant in low molecular weight dextrans which in can raise up to 8% [2]. Solubility of dextrans decreases as the proportion of other α linkages increases with respect to the α-1.6 linkages [3].

Dextrans are not normal compounds of sugar cane. The contents of these polysaccharides in sugar cane is very low or almost zero. They are formed by the action of the dextransucrose enzyme from contaminant microorganisms that home to the plant sap [1] or that attack it when its cortex is damaged. The infestation of sugar cane by the insect "borer" *Diatraea saccharalis*, and the attack of rodents favor microbial contamination of the cane in the field [4]. *Leuconostoc mesenteroides* is the lactic acid bacterium that mainly attacks sugarcane. The level of exposure of the internal tissues of sugar cane increases with mechanical cutting, chopping or burning, provoking the inactivation of the phenol oxidase enzymes which have a protective or anti bacterial action in the plant [1]. Under favorable temperature and humidity conditions, the dextransucrose hydrolyses the sucrose, forming dextrans. These dextrans are extracted in the mills along with the juices and contaminate the sugar mill flow, reaching levels in the juice exceeding 10 000 ppm (1%) in very extreme cases [5].

Statistics of 5 years in Louisiana, showed that in 60% of the harvest time the contents of dextrans did not exceed 250 ppm in the juice of whole stalk sugar cane while the chopped sugar cane exhibited a faster dextran formation due to a greater exposed stalk area

1. Cuddihy JA, Porro ME, Rauh JS. The presence of total polysaccharides in sugar production and methods for reducing their negative effects. Midland Research Laboratories, Inc. Available from URL: http://www.midlandresearchlabsinc.com/doclib/polysach.pdf.

2. Morel du Boil PG, Wienese S. Enzymatic reduction of dextran in process- Laboratory evaluation of dextranases. Proc S Afr Sug Technol Ass 2000;76:435-43.

3. Imrie FKE, Tilbury RH. Polysaccharides in sugar cane and its products. Sugar Technology Reviews 1972;1:291-361.

4. Rampaud M. Manejo de los roedores en la caña de azúcar. Sugar y azúcar 1993:46-7.

and hence, a higher degree of bacterial infection [6]. Additionally, a massive infection with *Leuconostoc* and other bacteria of up to six inches from the extremes of the sugar cane was reported in chopped sugar cane two hours after harvesting [7, 8]. In burnt sugar cane a rapid increase in the level of dextrans of almost ten times was observed from 12 to 48 hours, reaching 3 200 ppm [7]. It was also reported that dextran content in sugar cane that was burnt and left standing in the field increased rapidly from 280 ppm on the third day after burning to 2 900 ppm, after one week [1].

Deleterious effects of dextrans in sugar production

Once dextrans are present in the sugar production process the viscosity of the solution increases depending on the concentration and the molecular weight or the polymers formed which may range from 10^5 to 10^7 or more [3]. The dextrans of a very high molecular weight are insoluble, while those of low molecular weight and soluble, are the ones that cause more difficulties in the production process [6, 9].

The control of dextrans in the sugar industry is carried out by the rigorous adjustment between the burning, when performed, the cutting, mechanical or manual, and the delivery of the fresh sugar cane to the mill. Sanitation techniques of the technological equipment with steam every 8 hours during the sugar mill operation and the use of biocides on the sugar-cane in tandem are also executed [1]. Any delay of over 14 hours in the arrival of the cut sugar cane to the mill under warm and humid conditions, favors the formation of dextrans [3], which will reach the mills and enter with the juice into the industrial flow. Dextran content increases progressively along the process from the dilute juice to the final molasses [1].

The harmful effect of dextrans begins at their formation due to the irreversible sucrose consumption they produce. A study to evaluate these sucrose losses showed that the presence of 0.05% dextrans in raw sugar consumed 0.2 kg/t of sugar or 0.02 kg/t of processed sugar cane [1].

Recent studies showed that a *L. mesenteroides* strain isolated in a sugar mill in Argentina during the first 6 hours of culture at 30 ºC consumed sucrose at a rate of 8.46 g/L/h [1]. The sucrose consumption reduced with an increase in temperature.

The economic losses caused by dextrans are continuous throughout the process, since its early content in the juices falsely increases the amount of sugar calculated for them and alters the production indicators of the factory. This is due to the dextrorotatory characteristic of dextrans that polarize approximately three times more than sucrose producing a high false Pol value [1]. A study performed by adding standard dextrans to pure sucrose solutions showed that for each 180 ppm of the polysaccharide there was a mean increase of polarization of 0.05 ºS [10].

The high viscosity of the juices and the presence of high molecular weight dextrans in them along with other insoluble matter cause a blockage of the filters provoking juice losses due to spills that are generally underestimated.

The viscosity of the solution during clarification reduces the precipitation speed of impurities, forming scale deposits and decreasing the heating efficiency of the flow, thereby generally impoverishing the process. The corresponding juice derived from the deteriorated sugar cane with more acid pH values, consumed larger amounts of lime for its neutralization, hence providing higher turbidity, generating a larger volume of sticky mud that causes prees filter blockage [3].

The presence of dextrans during evaporation provokes an increase of scale deposits in the heating surface and hence a greater energy loss. The massecuites cooking time is also increased and their exhaustion is reduced.

In the crystallizers, the increase of sugar crystallization time causes the massecuites to become cooler than normal which increases the already abnormally high viscosity of the fluid. An increase in washing time of the centrifugals is needed to get the required quality of sugar, increasing total centrifuging and purging time [9]. The raw sugar derived from these massecuites is sticky and difficult to handle, dry and pack [1]. Studies confirmed that the quality of the massecuites significantly affected the yield of the sugar crystals reducing it to 86% in the case of 755 ppm of dextrans in massecuites with 84.3% purity [9].

The reduction of the sugar crystallization speed is specifically expressed in the decrease of crystal growth speed in the *a* and *b* axes. That is, the sugar crystals are deformed in the *c* axis, acquiring an elongated form with the dextrans occluded in them [3]. This so-called needle-shaped crystal reduces the purging efficiency of the massecuites in the centrifugals resulting in a poor separation of crystal and molasses, hence reducing the refined quality of the sugar [1].

Studies showed that at low dextran concentrations, close to 10% of them were included in the sugar crystals while the inclusion reached 30% when the syrup concentration surpassed 5 000 ppm [6, 9].

Raw sugar with dextran concentrations above 250 ppm [5] is subject to payment penalties of the magnitude of 0.007% of the price multiplied by the amount of tons sold. The fine value increases gradually in 0.002% with the increase in dextran concentration every 160 ppm, until reaching 0.013% for the contents equal or higher than 1 010 ppm [6].

Studies in Louisiana sugar mills showed that the sugar contents in the final molasses increased in 0.6 points per 1 000 ppm of dextran in molasses, which is equivalent to 250 ppm in mixed juices, generating the loss of 0.6 pounds (0.272 kg) of sugar per ton of sugar cane [9].

To this amount of sugar loss we must include the amount consumed for the formation of the 250 ppm of dextran: in the mixed juice, which according to prior data corresponded to 0.022 pounds (0.01 kg) per ton of sugar cane. That is, in the presence of 250 ppm of dextran in the mixed juice a total of 0.282 kg of sugar per ton of processed sugar cane was lost, which linearly extrapolated to the presence of 1 000 ppm of dextran in mixed juice generating a loss of 1.128 kg of sugar /t cane.

Another study using conservative numbers from different studies around the world showed that each 0.1% increase of dextran in the juice (1 000 ppm), resulted in the loss of 8.8 pounds (4 kg) of sugar per ton of sugar produced without considering the indus-

5. Cuddihy JA, Rauh JS, Porro ME. Improving sugar recovery with sugar process chemicals. Midland Research Laboratories, Inc. 1998. Available from URL: http://www. midlandresearchlabsinc.com/doclib/sugrcvry.pdf.

6. Cuddihy JA, Day DF. The process and financial impact of dextran on sugar factory. Midland Research Laboratories, Inc. 1999. Available from URL: http://www. midlandresearchlabsinc.com/doclib/dexfinan.pdf.

7. Cuddihy JA, Mendez F, Bernhard C. Dextranase in sugar production: factory experience. Midland Research Laboratories, Inc. 1999. Available from URL: http://www.midlandresearchlabsinc.com/doclib/dexexper.pdf.

8. Midland Research Laboratories, Inc. Recovery of additional sucrose with an integrated program using biocide and dextranasa to reduce undetermined losses. 1998. Available from URL: http://www.midlandresearchlabsinc.com doclib/biodxtrn.pdf.

9. Rauh JS, Cuddihy JA, Falgout RN. Analyzing Dextran in the Sugar Industry: A Review of Dextran in the Factory and a New Analytical Technique. Midland Research Laboratories, Inc. 1999. Available from URL: http://www. midlandresearchlabsinc.com/doclib/dexrevew.pdf.

trial recovery [5]. This implies the loss of an additional 0.77 pounds (0.35 kg) of sugar per ton of ground sugar cane assuming a recovery of 88%.

Another analysis for determining the losses of sugar caused by dextran showed a loss between 7.4 and 8 kg per ton of ground sugar cane [5].

Other studies at more advanced stages of the process showed that for each 300 ppm of dextran in the syrup the purity of the molasses increased in 1% [11]. Additionally it was shown that a 1 point rise in final molasses purity was equivalent to the loss of 0.454 kg of sugar per ton of sugar cane processed [8].

The exact data on sugar losses caused by dextran are very difficult to determine since there are many influencing factors, starting from the distorted values of the initial sucrose contents, the variability between the methods used to determine dextran content and even the variation of the criteria assumed when analyzing this [9].

In general, the results of all these studies show that the losses generated by dextran went from 0.35 kg of sugar per ton of ground sugar cane caused by the presence of each 1 000 ppm of dextran in the mixed juice and reached 8 kg. Whatever the accuracy of this result, it shows the need to eliminate dextran from the sugar manufacturing process.

Physical methods such as ultrafiltration, dialysis and reverse osmosis are very useful for this but are not yet technologically developed for their economical application in the sugarcane process [2]. Some have been introduced in the sugar industry in the United States, but with a significant capital cost and the return on investment has not been demonstrated [5].

The only method applicable today in the sugar industry is the enzymatic hydrolysis of dextrans. Dextranase EC 3.2.1.11 (α-D-1,6-glucan-6-glucanohydrolase), is the enzyme that hydrolyses the α-1.6 linkages mainly present in the dextran polysaccharides, breaking these bonds to form smaller olygosaccharide molecules.

A study at Midland Research Laboratories, Inc., of Kansas, United States showed that the use of dextranase has an economic value, with the use of the enzyme one can expect at least a minimum improvement of the sugar production cost of about 3.00 USD per ton of sugar cane [6].

Chronology of the isolation of dextranase producing microorganisms

From the beginning, fungi and the bacteria were identified as the main enzymatic sources capable of hydrolyzing dextrans. In the early 50's Japanese researchers identified *Penicillium lilacinum* and *Penicillium funiculosum* fungi that produced dextranase in the presence of dextrans and later in the 60's others strains from *Chaetomium gracile* and *Gibellela funiculosum* fungi [12] were identified. The dextranase from *P. lilacinum* showed maximum activity in the pH range of 5.0 to 5.5 and its between 53 and 60 ºC [13].

After an extensive search that continued in Japan during the 70's the *Aspergillius carneus* fungus strain that accumulates the enzyme when cultured in dextrans and another from the *Penicillium luteum* were identified [12]. Both were characterized in detail. The pure enzyme of the *P. luteum* showed its maximum activity in pH of 4.0 to 6.0 and 50 ºC. The dextranase from *P. funiculosum* also purified and characterized showed a maximum activity at pH 6.0 and it was inactivated rapidly when submitted to temperatures above 40 ºC [14].

Until then there were few dextranase producing bacteria identified among which were strains of *Lactobacillus bifidus*, two species of *Bacillus* and intestinal bacteria identified during the 50's. The dextranase of the *Brevibacterium fuscum* var. *dextranlyticum*, published in 1974 by Japanese researchers presented totally different characteristics from those already studied with maximum activity in the range of pH from 7.0 to 7.5 and stable from pH 5.0 to 11.0 [15]. Around this period, in isolations performed in dental plates, the dextranase producing bacterial strains *Fusobacterium fusiforme, Actinomyces israeli, Bacteroides ochraceus* and *Streptococcus mutants* [16] were found. The dextranase from *Streptococcus mutants* presented the optimum pH at 5.5 and 37 ºC.

The next reports of new dextranase producers induced by dextran were made in 1975 by American researchers in the fungal strains *Fusarium miniliforme, Fusarium oxysporum, Fusarium roseum* and *Penicillium roqueforii* [17]. The dextranase from *Fusarium miniliforme* showed optimum pH of 5.5, and 55 ºC and became totally inactive after 15 minutes of exposure to 80 ºC and pH 6.0 [18].

In 1978 the identification of a dextranase producing strain of *Flavobacterium* sp. M-73 was published and was later characterized [19]. The enzyme was stable in the pH range of 6.5 to 12.0 with the optimum activity at pH 7.0 and 35 ºC.

In 1981 Japanese researchers at the Sankyo company published the characterization of the dextranases produced by the fungus strain *Chaetomium gracile* isolated in the 60's [20]. The two enzymes identified were stable in a wide pH range from 5.5 to 11.0, with the optimum activity at pH 5.5, and 65 ºC and very stable at temperatures below this.

Among the dextranases identified and characterized from bacteria and fungi in these early days, the one from *C. gracile* tolerated the highest temperature and had the capacity to function in a very wide pH profile.

The first report of a dextranase produced by yeast, specifically by a strain of *Lipomyces starkeyi* in the presence of dextran, was performed in 1983 and afterwards researchers from the University of Louisiana, obtained a mutant of this strain that produced dextranase in the presence of glucose as a cheaper substrate [21]. The enzyme produced was stable in a pH ranging from 2.5 to 7.0 and reached maximum activity at pH 5.0 and 55 ºC.

In 1984, Indian researchers published the dextranase secreted by the fungus strain of *Penicillium aculeatum* [22]. For the first time, the significance of the application of the dextranase in the sugarcane industry was pointed out. The enzyme was very stable when raw with maximum activity in the pH range from 4.5 to 5.6 and 50 ºC.

In 1984 Soviet researchers, published studies performed with new dextranases from strains of *Penicillium piscarium*, *Aspergillus insuetus* and *Aspergillius ustus* [23].

10. De la Rosa RD. Las dextranas: su efecto sobre la polarización de la sacarosa y la economía azucarera. II. International Sugar Journal 1998;100(1192):198-203.

11. Clarke MA, Edye L, Cole F, Kitchar J. Sugarcane factory trials with dextranase enzyme. Sugar Journal 1997:20-2.

12. Fukumoto J, Tsuji H. Studies on dextranases. I. *Penicillium luteum* dextranasa: Its production and some enzymatic properties. J Biochem 1971;69:1113-21.

13. Novo Nordisk A/S. Dextranase Novo 25L. A dextran decomposing enzyme for the sugar industry. Product data in-formation 112-GB, Novo Enzyme Division, Dagsvaerd, Denmark; 1977.

14. Sugiura M, Ito A, Ogiso T, Kato K, Asano H. Studies on dextranase. Purification of dextranase from *Penicillium funiculosum* and its enzymatic properties. Biochimica et Biophysica Acta 1973;309:357-62.

15. Sugiura M, Ito A, Yamaguchi T. Studies on dextranase. II. New exo-dextranase from *Brevibacterium fuscum* var. dextranlyticum. Biochimica et Biophysica Acta 1974;350:61-70.

16. Ellis DW, Miller CH. Extracellular dextran hydrolase from *Streptococcus mutans* strain 6715. J Dent Res 1977; 56(1):57-69.

17. Simonson LG, Liberta AE. New sources of fungal dextranase. Mycologia 1975; 67:845-51.

18. Simonson LG, Liberta AE, Richardson A. Characterization of an extracellular dextranase from *Fusarium miniliforme*. Applied Microbiology 1975;30(5):855-61.

19. Kobayashi M, Takagi S, Shiota M, Mitsuishi Y, Matsuda K. An isomaltotriose-producing dextranasa from *Flavobacterium sp.* M-73: Purification and properties. Agric Biol Chem 1983;47(11): 2585-93.

20. Hattori A, Ishibashi K, Minato S. The purification and characterization of the dextranase of *Chaetomium gracile*. Agric Biol Chem 1981;45(11):2409-16.

21. Koenig DW, Day DF. Induction of *Lipomyces starkeyi* dextranasa. Appl Environ Microbiol 1989;55(8):2079-81.

22. Madhu GLS, Prabhu KA. Studies on dextranasa from *Penicillium aculeatum*. Enzyme Microb Technol 1984;6:217-20.

From then on publications of the isolation and characterization of dextranases from diverse sources of fungi and bacteria and their evaluation in the hydrolysis of the polysaccharides both in the oral cavity and in the sugarcane process continued.

In 1986, American researchers reported the isolation from the oral cavity of the dextranase producing strain of *Streptococcus sobrinus*, with the objective, of obtaining the purified enzyme as an immunogen against the oral *Streptococci* that causes dental caries.

In 1987 Nigerian researchers published the production of dextranase by a toxigenic fungus strain of *Aspergillus clavatus*, found in poultry feed [24].

Studies in Cuba isolated dextranase producing strains identified as *Penicillium funiculosum* and *Penicillium purpurogenum* [25 and 26]. The later characterization of the strain of *P. funiculosum* provided its reidentification as *Penicillium minioluteum* [27]. The enzyme produced by this fungus in the presence of dextranase had maximum activity at pH 4.5 to 5.0 and 35 ºC [28].

In 1988 Japanese researchers reported the production of dextranase by a strain of Arthrobacter *globiformis* that presented mild thermo tolerance maintaining 20% of the enzymatic activity at 70 ºC [29]. This was the first report made of a bacterial dextranase that tolerated temperatures above 40 ºC.

The techniques of genetic recombination already available in the decade of the 80´s were applied in this field. The first report of a recombinant dextranase was performed in 1991 by researchers of the Medical School of the University of Florida in reference to the expression of the gene of *Streptococcus salivaris* in *Escherichia coli* [30].

Following this is in 1993, Japanese researchers published the expression of a dextranase of the strain CB-8 of *Arthrobacter sp.* in the bacteria of the oral environment, *Streptococcus gordinii*, with the objective of using it for the preventive therapy of caries [31].

Rapid development of molecular biology in Cuba lead researchers from the Center for Genetic Engineering and Biotechnology in 1993 to obtain a strain of the methylotrophic yeast *Pichia pastoris* that expressed the gene of the fungus *Penicillium miniuloteum* and secreted high levels of the active recombinant dextranase to the culture media [32]. This constituted the first yeast producing recombinant dextranase. The enzyme increased the value of the optimum temperature to 57 ºC with respect to the natural dextranase from the same fungus but similarly in both dextranases, at temperatures above the optimum the enzymatic activity falls drastically. The pH range of maximum activity remained between 4.0 and 5.0.

In 1994 Japanese researchers published the intracellular expression in *E. coli* of the dextranase of *Arthrobacter globiformis* [33] and American researchers the expression in the same host of the dextranase from *Streptococcus sobrinus* [34]. The recombinant enzyme of S. *sobrinus* presented a specific activity of 4 000 U/mg of protein and the maximum activity was reached at pH 5.3 and 39 ºC.

In spite of the appearance of recombinant dextranases, the identification of dextran producing strains continued. In 1994, Polish researchers published the isolation of a strain of the fungus *Penicillium notatum* as a new source of the enzyme [35]. The dextranase produced by it showed to be relatively stable in the raw stage and reached a maximum activity at pH 5.0 and 50 ºC.

The work on bacterial gene expression coding for dextranase continued in Japan during 1995 and the intracellular expression in *E. coli* of the *Streptoccoccus mutans* enzyme was published [36].

Between the years 1995 and 1997 ingenious work performed in Australia on the selection of dextranase enzyme sources tolerating higher temperatures [37] were reported. Four strains were isolated that produced dextranase with optimum temperatures above 60 ºC and optimum pH in the range of 5.0 to 5.5, but unlike the dextranases known until then they presented very low specific activity, below 0.7 U/mg of proteins, that is, three orders lower with respect to that of *Chaetomium gracile*. The characterization of one of the isolated strains included it in the *Thermoanaerobacter genus* as the closest phylogenetic relation, with 98.8% homology at the RNAr level with the *Thermoanaerobacter wiegelii* [38]. The dextranase of this strain reached maximum activity at pH 5.5 and 70 ºC. Another sample isolated was included in the same genus of the *Thermoanaerobacter* and produced dextranase that exhibited maximum activity at the pH range of 4.5 to 5.5 and 80 ºC, while in the crude stage the temperature was 85 ºC [39]. The dextranases produced by these *Thermoanaerobacter* are until today those with the greatest thermal tolerance compared to both the natural ones derived from fungi, yeast and bacteria and the recombinant ones.

As a consequence of the continued recombination studies, in 1995 researchers from the University of Osaka published the expression of dextranase in the bacteria of the oral environment *Streptoccoccus salivarius* [40]. In 1997 Canadian researchers published, the construction of the recombinant library of the gene codifying for the dextranase of the *Streptococcus suis* performed in phage [41].

In 1998 Japanese researchers showed the production of dextranase by the Gram negative bacteria of the oral environment *Capnocytophaga ochracea, Capnocytophaga sputigena, Prevotella loescheii, Prevotella melaninogenica* and *Prevotella oralis* [42], and Canadian researchers published the observation of the synthesis of dextranase in the dimorphus and pathogenic fungus *Sporothrix schenkii*, during the yeast type phase of the cells [43]. This last enzyme showed pH 5.0 as the optimum value for maximum activity.

In 1999 Japanese researchers published the expression in *E. coli* of the dextranases of another strain of *Arthrobacter globiformis* [44] and of *Brevibacterium fuscum* var. *dextranlyticum* [45].

The work of Danish researchers concerning the cloning of the DNA fragment of the fungus *Paecilomyces lilacinum* coding for the dextranase enzyme in different species of filamentous fungi belonging to the genus *Aspergillus*, *Fusarium* and *Gribberella* was published in the year 2000 [46]. The optimum temperature of the recombinant enzyme was 60 ºC, not different from the natural enzyme produced by the *P. lilacinum.*

From here on the search continued for new dextranase sources that would provide enzymes with characteristics for new uses. Thus in the year 2001 Ger-

23. Zinchenko ON, Lobanok AG, Shishlo VI. Hydrolysis of streptococcal polysaccharides by micromycete dextranases. Prikl Biokhim Mikrobiol 1984;20:369-72.

24. Ogundero VW, Adebajo LO. Polysaccharide degrading enzymes of a toxigenic strain of *Aspergillus clavatus* from Nigerian poultry feeds. Nahrung 1987; 31:993-1000.

25. Guilarte B, Cuervo R, Rodríguez J, Pacheco N. Aislamiento y caracterización de microorganismos productores de dextranasa. Cuba Azúcar 1988:13-9.

26. Guilarte B, Cuervo R, Rodríguez J, Puente L. Biosíntesis de dextranasa por el *Penicillium funiculosum*. Cuba Azúcar 1985:9-12.

27. Guilarte B, Cuervo R, inventors; ICIDCA, assigneé. Método para la producción de dextranasa. CU 22546 A1. 1999.

28. Raíces M, Moleiro MC, Li IM, Roca H, Delgado J, Cremata J, Herrera L. Purificación y caracterización parcial de una enzima dextranasa de una cepa de hongo del género *Penicillium*. Biotecnología Aplicada 1991;8(2):248-55.

29. Okada G, Takayanagi T, Miyahara S, Sawai T. An Isomalto-Dextranase accompanied by isopullulanase activity from *Arthrobacter globiformis* T6. Agric Biol Chem 1988;52:829-36.

30. Lawman P, Bleiweis AS. Molecular cloning of the extracellular endodextranase of *Streptococcus salivarius*. J Bacterial 1991;173:7423-8.

31. Kubo S, Kubota H, Ohnishi Y, Morita T, Matsuya T, Matsushiro A. Expression and secretion of an *Arthrobacter* dextranase in the oral bacterium *Streptococcus gordonii*. Infect Immun 1993;61:4375-81.

32. Roca H, García B, Margóllez E, Curbelo D, Delgado J, Herrera L, *et al*., inventors; CIGB, assigneé. Dextranase enzyme, method for its production and DNA encoding the enzyme. European patent 0 663 443 B1. 1998.

33. Iwai A, Ito H, Mizuno T, Mori H, Matsui H, Honma M, *et al*. Molecular cloning and expression of an isomalto-dextranase gene from *Arthrobacter globi-formis* T6. J Bacteriol 1994;176:7730-4.

34. Wanda SY, Curtiss R. Purification and characterization of *Streptococcus sobrinus* dextranase produced in recombinant *Escherichia coli* and sequence analysis of the dextranase gene. J Bacteriol 1994;176: 3839-50.

35. Szczodrak J, Pleszczynska M, Fiedurek J. *Penicillium notatum* 1 a new source of dextranasa. J of Ind Microb 1994;13: 315-20.

36. Igarashi T, Yamamoto A, Goto N. Characterization of the dextranase gene (dex) of *Streptococcus mutans* and its recombinant product in an *Escherichia coli* host. Microbiol Immunol 1995;39:387-91.

37. Wynter CVA, Galea CF, Cox LM, Dawson MW, Patel BKC, Hamilton S, *et al*. Thermostable dextranasa: screening, detection and preliminary characterization. J Appl Bacteriol 1995;79:203-12.

man researchers published the isolation of a strain of the thermophylic, Gram positive and strictly anaerobic dextranase producing bacteria *Thermoanaerobacterium thermosaccharolyticum* [47]. The enzyme showed maximum activity at pH 5.5 and between 65 and 70 ºC.

In 2003 American researchers published the isolation of the strain of *Streptomyces anulatus* that produced two alkaline tolerant dextranases during growth in dextrans [48]. The enzymes produced had a pH tolerance range between 5.0 and 9.5, with an optimum temperature of 40 ºC for one and of 50 ºC for the other, while the optimum pH was 7.0 for both. These characteristics focused the enzymes to be use in the formulation of detergents.

Table 1 summarizes the main characteristics of some dextranases. The difference in the optimum pH range and temperature among them with respect to the source of origin is evident. In the case of the ones derived from fungi, the optimum pH values in general are slightly acid and in case of the ones derived from bacteria, the optimum pH values are closer to the neutral range. As exceptions the enzymes of the fungus *Chaetomium gracile* maintain their optimum activity at a wide range of pH from 5.5 to 11.0 and those of the bacteria of the genus *Thermoanaerobacter,* have an optimum value at a pH close to 5.5, similar to fungi derived enzymes.

The optimum temperature in general in dextranases from fungi range from 50-60 ºC, slightly higher than those from bacteria that average 40 ºC, again the exceptions are those derived from thermal tolerant bacterial strain of the genus *Thermoanaerobacter*, that surpass 65 ºC and one of the enzymes of the fungus *Chaetomium gracile*, at 65 ºC.

With respect to recombinant enzymes, the behavior of the optimum temperature and pH values were reported to be similar to the natural enzymes in the case of the dextranases derived from *Paecilomyces lilacinum* expressed in different fungi hosts but the enzyme from the *Penicillium minioloteum* expressed in *Pichia pastoris* yeast, as mentioned before, increased in optimum temperature from 35 to 57 ºC and extended its pH range from to 4.0 to 5.0.

Table 1. Optimum pH and temperature values of dextranases synthesized by different microorganisms

Dextranases	Source		Optimum Value	
			pH	Temperature
Natural	Fungi	*Penicillium lilacinum* [13]	5.0-5.5	53-60 °C
		Penicillium luteum [12]	4.0-6.0	50 °C
		Penicillium funiculosum [14]	6.0	NR
		Penicillium aculeatum [22]	4.5-5.6	50 °C
		Penicillium minioluteum [28]	4.5-5.0	35 °C
		Penicillium notatum [35]	5.0	50 °C
		Chaetomium gracile (two enzymes) [20]	5.5-11.0	55 y 65 °C
		Fusarium miniliforme [18]	5.5	55 °C
		Sporothrix schenkii [43]	5.0	NR
	Bacteria	*Brevibacterium fuscum var. Dextranlyticum* [15]	7.0-7.5	NR
		Streptococcus mutans [16]	5.5	37 °C
		Streptomyces anulatus (two enzymes) [48]	7.0	40 y 50 °C
		Flavobacterium sp. M-73 [19]	7.0	35 °C
		Thermoanaerobacter wiegelii [38]	5.5	70 °C
		Strain of *Thermoanaerobacter* [39]	4.5-5.5	80 °C
		Thermoanaerobacterium thermosaccharolyticum [47]	5.5	65-70 °C
	Yeast	*Lipomyces starkeyi* [21]	5.0	55 °C
Recombinant	Fungi	*Paecilomyces lilacinum* in *Aspergillus*, *Fusarium* and *Gribberella* [46]	NR	60 °C
		Penicillium miniuloteum in *Pichia pastoris* [32]	4.0-5.0	57 °C
	Bacteria	*Streptococcus sobrinus* in *Escherichia coli* [34]	5.3	39 °C

NR: Not reported

Dextranase enzymatic preparations for the sugar industry

The use of dextranases in the sugar industry was suggested by Tilbury more than 30 years ago, when the enzyme was only studied for the preparations of medicinal dextrans used as substitutes for blood plasma and more recently in tooth paste formulations to hydrolyze the dextrans present in the dental surfaces.

Since 1972 when Inkerman and James presented their results of the use of dextranase to improve the deteriorated sugar cane process in sugar mills of Queensland [49], several enzymatic preparation of dextranase had been available in the market, different companies had produced them and few had been the microbial source used. In these initial studies of its industrial application the enzymatic preparation of Glucanase D-1, produced by the Phizer Chemicals company was used for hydrolyses in mixed juices with a high concentration of dextrans.

During the 80's the companies producing industrial enzymes started to produce preparations of dextranases to be used in the sugar manufacturing process.

In 1986 the enzymatic preparation Dextranex, produced by the American company Miles Laboratories appeared in the market. It was derived from a strain of *Chaetomium sp*. [32]. This preparation was proposed for its use in the sugar industry however there is no published information available about its use.

Among the first commercially launched dextranase enzymatic preparations was also the DN 25 L and later the DN 50 L, both from the Danish company Novo Nordisk A/S, produced from a strain of *Penicillium lilacinum* [13], renamed as *Paecilomyces lilacinum* in the enzymatic preparation Dextranase 50 L.

None of the enzymatic preparations from Novo Nordisk A/S obtained the approval for safe use (GRAS) by the regulatory agency of the United States, the food and drug administration (FDA) and could not be commercialized in that country, loosing its markets.

In the specifications of the enzymatic preparations DN 25 L and DN 50 L from Novo Nordisk A/S the advantages of the dosage in juices or syrups were not differentiated suggesting they could be used in any case while always keeping the temperature between 50-60 ºC and the pH between 5 and 6 [13]. The enzymatic preparation Dextranase 50 L, accor-

38. Wynter CVA, Patel BKC, Bain P, de Jersey J, Hamilton S, Inkerman PA. A novel thermostable dextranase from a *Thermoanaerobacter* species cultured from the geothermal waters of the Great Artesian Basin of Australia. FEMS Microbiology Letters 1996;140(2-3):271-6.

39. Wynter CVA, Chang M, De Jersey J, Patel BKC, Inkerman PA, Hamilton S. Isolation and characterization of a thermostable dextranase. Enzyme and Microbial Technology 1997;20:242-7.

40. Onishi Y, Kubo S, Ono Y, Nozaki M, Gonda Y, Kano H, *et al*. Cloning and sequencing of the gene coding for dextranase from *Streptococcus salivarius*. Gene 1995;156:93-6.

41. Serhir B, Dugourd D, Jacques M, Higgins R, Harel J. Cloning and characterization of a dextranase gene (dexS) from *Streptococcus suis*. Gene 1997; 190(2):257-61.

42. Igarashi T, Yamamoto A, Goto N. Detection of dextranase-producing gram-negative oral bacteria. Oral Microbiol Immunol 1998;13:382-6.

43. Arnold WN, Nguyen TBP, Mann LC. Purification and characterization of a dextranase from *Sporothrix schenckii*. Arch Microbiol 1998;170:91-8.

ding to the data available suggested it should be used specifically to hydrolyze the dextrans in the juices.

The isolation of the strain of *Penicillium minioluteum* as a source of active dextranase under industrial conditions of temperature and pH performed in Cuba, allowed the researchers to obtain an enzymatic preparation in 1988 that was used for several years in different sugar mills of the country [27].

The development of the production process in *Pichia pastoris* of the recombinant dextranase of *P. minioluteum* performed by Cuban researchers concluded in the formulation of the enzymatic preparation Hebertec-Dextranase, which was used for industrial application studies in different sugar mills in the country between 1995-1999.

In laboratory studies at 50 ºC and pH 5.0, with an initial concentration of dextrans equal to 1 500 ppm in the presence of 15% sucrose showed that the dose of 16 ppm of Hebertec-Dextranase hydrolyzed 90% of the polysaccharide in 10 minutes. The treatment of higher concentrations of dextrans under the same conditions had a lower level of hydrolysis. It was also observed that the reduction of the reaction temperature to 35 ºC caused a decrease of the hydrolysis level reaching 90% for an initial concentration of dextrans of 500 ppm. The industrial studies performed in juice at a dose of 16 ppm of this enzymatic preparation with a reaction time of 10 minutes provided a hydrolysis of 85% of the 1 600 ppm of dextrans present in juices.

With the advanced techniques of molecular biology several research groups worked to improve the enzymatic preparations of dextranase available from the companies manufacturing industrial enzymes continued but there are no reports demonstrating that another recombinant dextranase preparation has been developed.

The work at the Novo Nordisk A/S laboratories of the expression of the gene coding for the dextranase of the fungus *Paecilomyces lilacinum* in different filamentous fungi did not end in the development of a new enzymatic preparation applicable to the sugar industry, although it did produce a patent for its use in tooth paste preparations [46].

The enzyme produced by the fungus *Chaetomium gracile* characterized by the Sankyo company, was used in industrial studies of the hydrolysis of dextrans performed in sugar mills in Australia and was formulated in an enzymatic preparation produced by the American company Genencor with the brand name Dextranex™. It was evaluated in studies in Louisiana during the harvests of 1996 and 1997 [11].

In 1999, the Sankyo company obtained GRAS approval issued by the FDA for the dextranase enzyme produced by the *Chaetomium gracile* [50]. In spite of the scarce information available of the enzymatic preparation Dextranase® produced with this fungus, Sankyo began its exportation to refiners of Europe and the United States in the fiscal years 2000 and 2001 [51, 52].

During the year 2002 a new enzymatic preparation appeared in the market, Dextranase Plus L from Novo Nordisk A/S, with improved characteristics having a thermal stability at up to 85 ºC and a wider pH range between 3 and 7, produced from the fungus *Chaetomium erraticum* [53].

The characteristics of the enzyme and the source microorganism that were reported for the enzymatic preparation Dextranase Plus L, were similar to the Dextranase L offered in the market since 1998 by the Japanese company Amano [54]. This preparation Dextranase L was declared for use both in the juice and syrup at a dose ten times higher 50 and 100 mL per ton of syrup treated with the reaction time in the range of 30 to 60 minutes, three times longer than the time proposed for the treatment of the juice.

The enzymatic preparation Dextranase Plus L from Novo Nordisk A/S did define its specific use for the juice or syrup, extending the possibility of its use in the presence of higher temperatures due to the thermo tolerance.

Talozyme D is an enzymatic preparation that appeared in Internet in 2002. It was recommended for use in juices with pH in the range of 5 to 7, and 50 to 60 ºC, but the enzymatic source was not reported [55].

Since the use of dextranases in the sugar industry was identified, the manufacturing companies the enzymatic preparations made available with prices that for approximately 20 years, were of about 30 000 USD per ton. This high price was based on the benefit generated by its use need to avoid on excess level of dextrans in the sugar crystals above the refiners admissible value.

Although the enzymatic preparations were available their high price did not allow their generalization in the sugar industry. Unfortunately, there are not many industrial application results published and many important judgments that could have been provided by sugar experts were silenced, and the only criteria were those of the will of the manufacturers of the enzymatic preparations.

Enzymatic treatment of juices

From the economic point of view it was considered sufficient to hydrolyze two thirds of the dextrans present in the process and it was argued that this was able to provide a formidable economic improvement of sugar production [49]. It was assumed that the consumption of a larger amount of enzyme would only generate unrecoverable expenses.

The pioneer studies performed with the enzymatic preparation Glucanase D-1 in Australia, which did not specify the dose used, reported the hydrolysis of 70% of the dextrans present in the juice with the lowest dose [49]. It was concluded that the complete elimination of dextrans was not essential for high economic advantages in the application of the enzyme.

The preliminary work with the dextranase from *P. aculeatum* in juices extracted from different parts of the sugar cane harvested under diverse conditions showed that the enzyme was especially useful in the case of critical state of the production process and when the sour cane was supplied [56]. They also indicated that prolonged reaction times and low Brix were favorable conditions for enzymatic hydrolysis and suggested the enzyme should be added as soon as possible in the process in the mills or during evaporation.

The criteria of calculating the dextranase dose for a needed hydrolysis level was first published in the manuals of the enzymatic preparations DN 25 L and DN 50 L from Novo Nordisk A/S [13].

44. Oguma T, Kurokawa T, Tobe K, Kitao S, Kobayashi M. Cloning and sequence analysis of the gene for glucodextranase from *Arthrobacter globiformis* T-3044 and expression in *Esche-richia coli* cells. Biosci Biotechnol Biochem 1999;63:2174-82.

45. Mizuno T, Mori H, Ito H, Hirokazu M, Kimura A, Chiba S. Molecular cloning of isomaltotrio-dextranase gene from *Brevi bacterium fuscum* var. dextranlyticum strain 0407 and its expression in *Es cherichia coli*. Biosci Biotechnol Biochem 1999;63(9):1582-88.

46. Christensen T, Fuglsang CC, Halkier T, Johansen D, inventors; Novo Nordisk A/S, assigneé. Recombinant enzyme with dextranase activity. US 6,156,553. 1998.

47. Hoster F, Daniel R, Gottschalk G. Isolation of a new *Thermoanaerobacte-rium thermosaccharolyticum* strain (FH1) producing a thermostable dextranase. J Gen Appl Microbiol 2001;47:187-92.

48. Decker SR, Adney WS, Vinzant TB, Himmel ME, inventors; Midwest Research Institute, assigneé. Alkaline tolerant dextranase from *Streptomyces anulatus*. US 6,509,184. 2003.

49. Inkerman PA, James GP. Dextranase II, Practical application of the enzyme to sugar mills. Proc Queensland Soc Sugar Cane Technologists. 43rd Conf, Watson Ferguson and Co; Brisbane, Queensland, Australia 1976. p 307-15.

50. CFSAN/Office of Food Additive Safety. Summary of All GRAS Notices. 2004. Available from URL: http://www.cfsan. fda.gov/~rdb/opa–gras.html.

51. Sankyo Co. Sankyo Review of Opperations. Special Merchandise. P 20. Sankyo Co., Ltd., Japan. 2000. Available from URL: http://www.sankyo.co.jp/english/ir/information/annual/2000/pdf/010.pdf.

52. Sankyo Co. Sankyo Review of Opperations. Health Care Department. P 21. Sankyo Co., Ltd., Japan. Available from URL: http://www.sankyo.co.jp/english/ir/information/annual/2001/pdf/19-21.pdf. 2001.

53. Novo Nordisk A/S. Ficha técnica Dextranase Plus L®. 2002. 2002-19760-01 10 14 2002 © Novozymes A/S, Krog-shoejvej 36, 2880 Bagsvaerd, Denmark. 2002.

54. Amano. Dextranase L "Amano". Amano Pharmaceutical Co., Ltd., Nagoya, Japan. 1998.

55. CYTEC. Talozyme D. Liquid dextranasa concentrate for the sugar industry. WTT-1216. CYTEC Industries Inc., 1.5k 9/02 HOR. 2002. Available from URL: http://www.cytec.com. 2002.

56. Madhu GLS, Prabhu KA. Application of dextranase in the removal of dextran from cane juice, International Sugar Journal 1984;86(1025):136-8.

A very important factor considered to favor the hydrolysis was the establishment of the residence time of the enzyme that in the case of juice ranged between 10-15 minutes [11].

During the harvests of 1996 and 1997 in Louisiana studies of the industrial application of the Dextranex™ preparation, dosed in mixed juice at a rate of 6 g/t of juice, with a reaction time of 12-15 minutes were done [11]. A reduction of the dextran levels between 50 and 85% resulted, also a reduction in viscosity in the pans, a decrease in purity of the molasses to normal values and the decrease of the dextran contents in sugar crystals from 3 200 to 630-780 ppm.

Recently, the prices of the enzymatic preparations available decreased almost eight times [7]. The criteria of only hydrolyzing two thirds of the dextrans changed and the objective of achieving a larger amount of hydrolysis was retaken. Nevertheless, the conclusion about the use of the enzyme only of high dextran levels in the process did remain valid [56].

The Audubon Sugar Institute reported that during the year 2002 dextranase was used in all the sugar mills of Louisiana when it was required [57].

Enzymatic treatment of syrups

The experimental results showed that with the increase of the sugar concentration in the solution to 65 ºBrix, the dextranase enzymes produced by the fungi *Chaetomium gracile* and *C. erraticum*, increased their thermal stability up to 85 ºC, but as a negative aspect, the increase in Brix reduced the hydrolysis rate of the dextrans [53, 54]. In the presence of a sugar concentration of 60 ºBrix the enzymatic activity was reduced between 30-40% [1].

The result obtained from an increase in thermal tolerance of dextranase stimulated the interest of researchers to reevaluate its use in syrup and molasses with the main objective of reducing the enzyme consumption when applying it in the lower volume processing sites.

Treatments in Australia showed that the viscosity in the B molasses with high dextran contents was reduced 20% as a result of enzymatic hydrolysis in the advanced stages of the process [1].

A trial in Louisiana, the supply of Dextranex™ preparation in the 4th (last) evaporator at 85 ºC and 65 ºBrix [11], although the dose used was not reported, the dextran levels in the syrup was reduced between 70-75% and in A molasses by 20-60% while the contents of dextrans in sugar fell from 2 450 to 780 ppm. It was also reported that studies on the industrial application in the sugar mill of Alma Plantation provided similar results [7].

To reach a satisfactory level of dextran hydrolysis, with this enzyme that showed increased thermal tolerance, in sites of the process after that the evaporators, it was necessary to correct the dose due to the loss of activity caused by the high Brix. As a result, the dose per unit volume increased up to six times, equaled the daily consumption of the enzyme and thus did not decrease the cost of the enzymatic treatment [7].

This eliminated the possibility of economizing the enzymatic preparation used the process where the reduction of the volume of the material treated combines higher concentrations of sugar with an increase in temperature.

Studies in Cuba with the enzymatic preparation of natural dextranase from the fungus *Penicillium minioluteum* indicated the need of increasing the dose 6 and 50 times to treat the syrup and B molasses respectively as well as doubling the reaction time of the juice [58].

It was also determined that the recombinant dextranase enzymatic preparation Hebertec-Dextranase increased its thermal tolerance with the increase in Brix in the solution treated. The residual activity of the enzyme was of 85%, after being submitted in the presence of 65 ºBrix, to 65 ºC, for 30 minutes. The evaluation of the recombinant enzyme in sucrose solutions with higher Brix required a higher consumption of the enzyme, the same as was obtained for the natural enzyme. Laboratory studies showed that 160 ppm of the Hebetec-Dextranase hydrolyzed 80% of the 1500 ppm of dextrans in the presence of 60% sucrose, in 15 minutes of reaction at 65 ºC at pH 6.0. For higher initial dextran concentration, the hydrolysis was lower.

Other attempt at moving the use of the dextranase to the later stages of the process are in the works promoting the use of biocide products [59]. The important function carried out by the enzyme in the production process does not contradict the use of biocides, but complements it, to achieve the recovery of all the sugar present. The earlier the viscosity is reduced in the process, the larger the amount of sugar that will be recovered and thus less economic losses will be generated. Even with the use of biocides in tandem and thus the absence of bacterial contamination in the mills, the dextrans can be formed in the sugar cane during its transportation to the sugar mill due to unavoidable climatic factors, for example, and thus cause the disastrous effect on the process that is only eliminated by the action of dextranase at the beginning of the process. The situation in dextrans has worsened in recent years in South Africa compared to historical data [2].

Differing from the immediate application of the enzymatic preparation in the juices, when the dose is applied later in the process, the previous stages have already had perturbations generated by the elevated viscosity. Thus, the economic losses, as well as the initial losses suffered by the sucrose hydrolyzed to dextrans have to be added to the total cost of the enzymatic treatment. Thus, enzymatic treatment in advanced stages of the process makes it more expensive and is only justified in the case when the dextrans unavoidably reaches the pans. In this case, the effect of its direct addition at that point is obtained earlier than if the dose is applied to the juice.

This aspect of the ideal application point of the enzyme is hardly discussed in literature. These only mention the site used without a comparative analysis. The most explicative information on this aspect was given by the Audubón Sugar Institute in a recent report in which they affirmed that the most effective site for adding the enzyme was in the mills [57]. It referred that at that point the conditions of temperature, pH and hydration were such that allowed a rapid action of the enzyme. The treatment of syrup and molasses was referred as a secondary point for the application, which is very useful in case of recycling sugar C with high dextran contents.

57. Audubon Sugar Institute. Annual Report 2002-2003; p. 15. 2003. Available from URL: http://www.lsuagcenter.com/Inst/Research/Departments/audubonsugar/PDF/Audubon%20annual%2003.pdf.

58. Guilarte B, Cuervo R. Aplicación de dextranasa. Quimización de la industria azucarera ICINAZ–MINAZ. 1990. p. 43-60.

59. Cuddihy JA, Day DF. Biocide usage at Louisiana factories. Midland Research Laboratories, Inc. 1998. Available from URL: http://www.midlandresearchlabsinc.com/ doclib/biousage.pdf.

Recent reports of laboratory studies to establish the favorable reaction conditions for three different dextranases: the preparations from Novo Nordisk A/S Dextranase 50 L (*P. lilacinum*) and Dextranase Plus L (*C. erraticum*) and from Genencor Dextranex L-4 000 (*C. gracile*) [2], indicated the possibility of the use in the syrup at 60 ºC of both enzymes deriving from *Chaetomium sp*., with the readjustment of the dose due to the increase in Brix and the temperature suggested by the manufacturers. However, at 70 ºC, the dose has to be doubled and it is not considered feasible under those conditions.

In general there is insufficient data published on the economic effect of the use of dextranases in the treatment of juices at the early stages in the mills, as well as in the syrups or molasses in the later stages of the process. What is evident is that the irreversible losses of the sugar converted to dextrans and the losses in the sites prior to enzymatic treatment, wherever it was performed, are unrecoverable and must be considered in the strict estimations.

As a result of their analysis performed years ago, Cuban researchers proposed the possibility of using dextranase for the recovery of 0.8 kg of sugar per ton of ground sugar cane by decreasing the purity in the final molasses in 8% during the 30 most rainy days of the harvest [60]. According to all the above analysis, for the exact calculation of cost effectiveness in the use of dextranase it is necessary to subtract from the recovered sugar, the cost of the enzymatic preparation consumed, the amount of sugar losses incurred due to the conversion to dextrans and the spills, as well as, a correction of the initial contents of sugar in the juice by usingthe readjusting factor of the false Pol.

The approximate cost of the use of the enzymatic preparation in the present conditions, that is, with the price of the ton of the enzymatic preparation being approximately 3 500 USD, the dose used in juice of 16 ppm that hydrolyzes 90% of up to 1 600 ppm of dextrans present and that achieves a recovery of 0.8 kg sugar per ton of grinded cane affected by dextrans, shows more attractive results. Additionally considering that the amount of juice generated per ton of sugar cane is approximately of 1.075 m_3, this means that for each ton of sugar cane treated, 14.88 ppm of the enzymatic preparation will be applied and assuming that it will have a density of 1 g/mL, its costs corresponds to 0.052 USD. Moreover, the value of the amount of sugar recovered, assuming the price per pound in the market at 0.05 USD, is of 0.088 USD. Thus the economic effect of the use of the enzyme in the juice from the initial stage of the process, given as the correlation of the money recovered in sugar with the expense in the enzymatic preparation, is approximately 1.7 times. Added to this is the savings by not paying the penalties. In addition, it is also very important to consider the blockages and shutdowns avoided which converge in the stability of sugar mill productivity.

According to the data presented in this study, it is not the same if the doses of the enzymatic preparation are applied in advanced points of the process, where other losses already mentioned unavoidably occur due to the action of the dextrans, besides needing a dose ten times higher for the treatment.

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60. Namer I, Pérez JR, Dávila H. Análisis de los beneficios económicos del empleo de las enzimas en la producción del azúcar de exportación cubana. Atac 1988; 3:34-8.

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