

Application of Enzydex During Sugar Process for Improving Sugar Yield

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Abstract

Dextrans are undesirable compounds, synthesized by *Leuconostoc mesenteroide* from sucrose, increasing the viscosity of the sugar flow and reducing industrial recovery, resulting significant losses to the sugar industries. The use of dextranase enzyme is the most efficient method for hydrolyzing the dextrans at sugar mills. A preparation of dextranase enzyme namely "Enzydex" developed by Catalysts Biotechnologies Pvt. Ltd which was shown significant reduction at very low concentration 3-5ppm during six plant trials in India and in Philippines. During plant trials, the 38-78% dextran reductions were observed at various stages of sugar process.

1. Introduction

Dextrans are bacterial originated glucose polymer formed by the bacterium *Leuconostoc mesenteroide* after harvest or during the damage of sugar cane rind. Dextrans are almost zero or very low before harvest of sugarcane. *Leuconostoc mesenteroide* is lactic acid a bacterium comes under genus of *Leuconostoc*, able to produce enzyme dextranase. Under favorable temperature and humidity conditions the dextranase hydrolyses the sucrose for the formation of dextrans.

These dextrans are extracted in the sugar 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.

Dextrans are a high molecular weight glucose polysaccharide comprising mainly α -(1—6) linkages, but also contains a small amount of α -(1—4), α -(1—3) and some α -(1—2) linkages. Most dextrans in the sugar industry are linear but some branching may occur. During sugar production, the formation of dextran in sugarcane not only causes expensive sucrose losses but also the high viscosity associated with this polysaccharide often slows evaporator and crystallization rates, raises losses of sucrose to molasses and distorts factory pols readings.

The harmful effect of dextrans begins at their formation due to the irreversible sucrose consumption. The formation of one gram dextran molecule required 4 gram of sucrose molecule. 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. Recent studies showed that a *Leuconostoc mesenteroide* strain isolated in a sugar mill in Argentina during the first 6 h of culture at 30°C consumed sucrose at a rate of 8.46 g/L/h.

In sugar production, the use of enzyme dextranase is the most efficient method for hydrolyzing the dextrans at sugar mills. Some bacterial strains, filamentous fungi and a small number of yeasts have also 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. The concentration of dextranase enzyme application to reduce the dextran level in sugar juices and syrup is known to 5ppm to 10ppm. In some cases, the dextranase applications were observed at high concentration. Resulting these applications of dextranase at this concentration is not economic viable to the sugar industries.

Keeping all these views in mind Catalysts Biotechnologies Pvt. Ltd developed a unique product named as ENZYDEX which consists of enzyme dextranase, hydrolyze insoluble and viscosity enhancer molecules dextrans in to simple soluble oligosaccharides and short chain molecules at very low concentration 3ppm to 5ppm. ENZYDEX enhances clarification, filtration & crystallization process efficiency of sugar process. Ultimately increase the quality of

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recovered sucrose crystals in term of uniform crystallization & color reduction of sucrose crystals.

2. Material and Methods

Application of ENZYDEX was used to hydrolyze dextran at 3 - 5 ppm concentration in split manner at national and international sugar mills. Dosing points selected in Indian sugar mills were given as below. Dextranase activity was observed before and after dosing of product ENZYDEX at dosing points.

1. Mill station (0.5 – 1.0 ppm)
2. Mixed juice tank (1.0 – 2.0 ppm)
3. Clarifier (0.5 – 1.0 ppm)
4. Evaporator (0.5 – 1.0 ppm)

2.1 Dextranase Activity: Dextranase activity was carried out by 3, 5 dinitrosalicylic acid (DNS) method.

2.2 Chemicals and Reagent Preparation: 1M Acetic Acid solution; 0.05M Sodium Acetate buffer –working solution pH 5.0; 3, 5 Dinitrosalicylic acid (DNS); 2.5% Dextran:

3. Procedure

Pre-warmed the test tubes with enzyme dilutions in a water bath maintained at 50°C. Pipette 0.1 ml of the enzyme dilution and added 0.4 ml of buffer to each tube. Inoculated 0.5 ml of the substrate solution to the tubes at regular intervals, shaken the tubes and incubated for 10 minutes. After incubation added 1.0ml of DNS with tartrate to the sample tubes. Then added 1.0ml of DNS to the blank tubes and immediately added 0.5ml of the enzyme to the respective tubes. Added 1.0ml of DNS to the Reagent Blank and the Substrate blank. Boiled the tubes for 10 minutes in a boiling water bath. Cooled the tubes in water to room temperature and added 2 ml of water. Vortexed the tubes and took the O.D. at 540nm in Shimadzu UV visible Spectrophotometer.

3.1 Calculations

Activity of sample = ((Net O.D + constant)/slope)*1000*D.F.) / (180*10*0.1) units/gram

Net O.D.: (OD1+OD2)/2-Blank OD

3.2 Unit Definition

One Dextranase unit is the amount of enzyme which liberates 1.0 micromoles of reducing sugar (expressed as glucose equivalent) in one minute under the assay conditions specified.

4. Results

To observe the performance of ENZYDEX at national and international level, many plant trials were carried out and observed significant reduction in dextran level at various stages of sugarcane juice lines in sugar industries. Enzydex applied on various points like milling station, mix juice, syrup, massecuites and final molasses etc., depending on dextran level at various stages. The effectiveness of product was observed through plant trials conducted at various location of India and Philippines.

The plant trial 1 showed the 77% dextran reduction at A heavy position while 48% dextran reduction was observed in sulphured syrup at 3ppm of Enzydex. Plant trial 2 showed highest 78% dextran reduction at B heavy level while lowest 38% dextran reduction was observed in B messecuite at 4ppm. Plant trial 3 showed 76% dextran reduction in B messecuite while 58% dextran reduction was

observed in clear juice at 3ppm. In Plant trial 4, the 71% dextran reduction was observed at A masseuite and lowest reduction was observed 58% in final molasses at 4ppm of Enzydex. Plant trial 5, the maximum dextran reduction was observed 75% while lowest reduction was observed at 47% in final molasses at 3ppm.

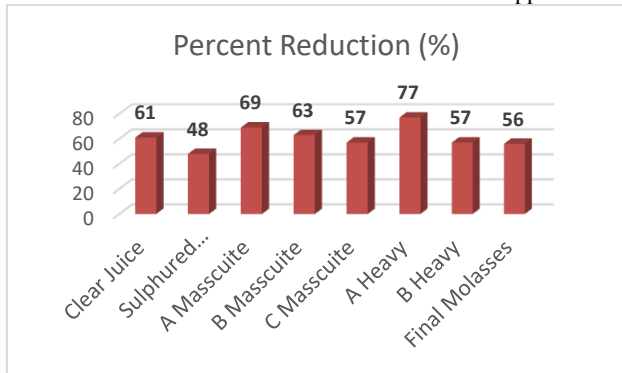


Fig.1. Plant Trial 1 - Dose at 3ppm (1.0ppm in mixed juice, 0.5ppm in filtrate juice, 1.5ppm in syrup juice) at Ghatwa, MP, India

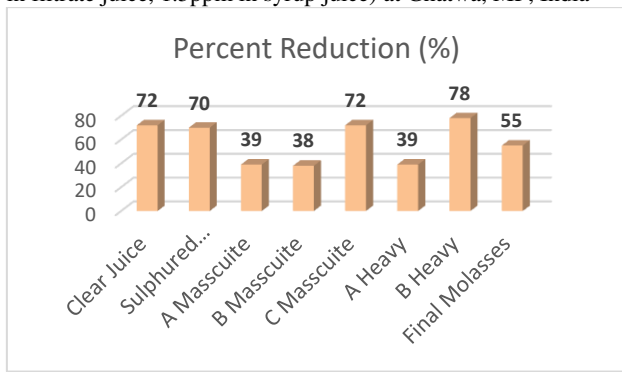


Fig. 2: Plant Trial 2 - Dose at 4ppm (1.5ppm in mixed juice, 1.5ppm in syrup, 1.0ppm in B heavy) at Latur, MP, India

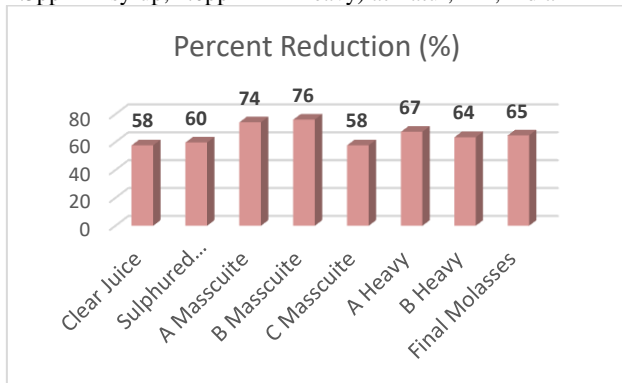


Fig. 3. Plant Trial 3 - Dose at 3ppm (1ppm in mixed juice, 1ppm in filtrate juice & Raw Melter, 1ppm in Syrup) at Kolhapur, MH, India

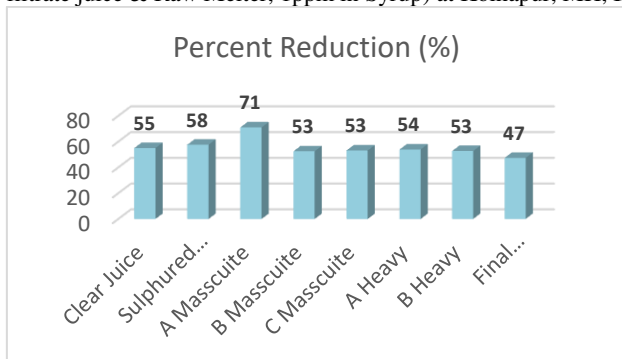


Fig. 4. Plant Trial 4 -Dose at 4ppm (2.5ppm (In mixed juice & Syrup Tank and 1.5ppm (In mixed juice & Syrup from 6th Day) at Nellikuppam, Tamilnadu, India

5. Discussion

Based on the five plant trials it was observed that the average 60.0% dextran reduction was observed during sugar process at 3-5ppm of

Enzydex. Plant trial 3 which was conducted at Kolhapur, Maharashtra India, showed maximum dextran reduction average 65.9% at the concentration 3ppm (Figure 6). Basis this significant dextran reduction at low ppm of Enzydex another single Plant Trial 6 was conducted at Bacolod, Philippines for three days. These 3 days observations indicated that the application of Enzydex on Raw Syrup, the dextran reduction was observed is 43.52 % and Raw Syrup purity enhancement observed by ~ 3.6 unit. While another example of dextran reduction through literature it was reported that the application of "non-concentrated" dextranase to evaporator syrup was uneconomical. However, "concentrated" dextranase can be applied to syrup levels as low as 10 ppm/solids to remove up to 37% dextran which is useful to consider when severe dextran problems occur.

Basis all these observations it was concluded that the concentration of Enzydex 3-5ppm is suitable for application which can control dextran at significant level at various stages in sugar flow which is economic viable for application at various level of sugar process.

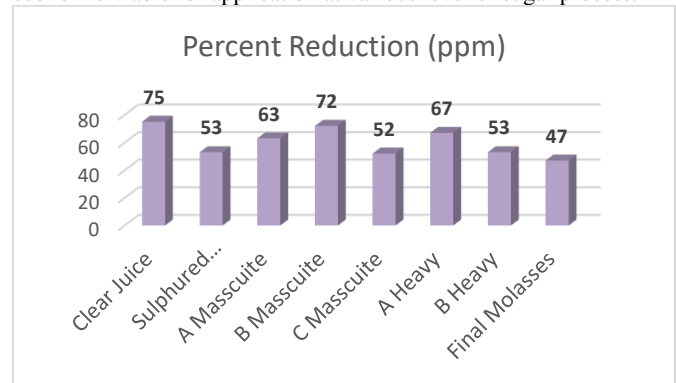


Fig. 5. Plant Trial 5 - Dose at 3ppm at Ramgarh, Uttar Pradesh, India

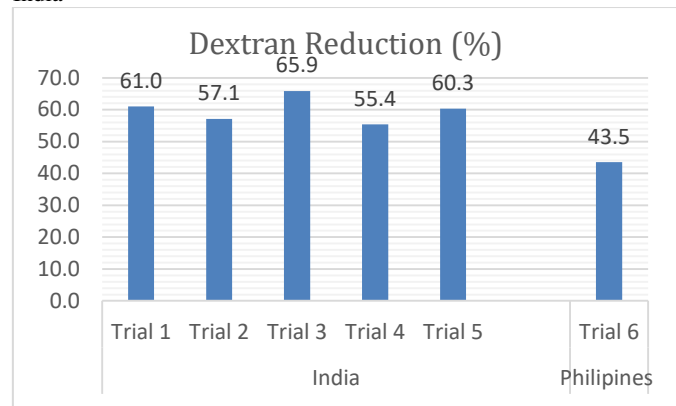


Fig.6. Comparative dextran reduction in various plant trials (1-6).

6. Conclusions

Enzydex is a preparation of enzyme dextranase which hydrolyze insoluble, viscosity enhancer dextran molecules into simple, soluble oligosaccharides and short chain molecules. Enzydex majorly act on α -1.6 along with α -1.2, α -1.3 & α -1.4 glycosidic linkages of dextran molecule to hydrolyze them. Enzydex application is suitable to apply on various sugar process points like milling station, mix juice, syrup, masseuites, etc. where dextran level occurred at various concentrations. Enzydex offered significant benefits in term reduce process viscosity, prevent excess scaling, enhances clarification, filtration & crystallization process efficiency.

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