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PRESERVATIVES FOR MOLASSES AND INFLUENCE OF DEXTRAN IN SUGAR INDUSTRY

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ABSTRACT (11 Pts Times New Roman, Bold, Capital Letters, Center Aligned)

Sugar industry process is very typical and multilevel operational process having various parameters to control simultaneously. Sugar loss is a critical part of this industry, which involves physical, microbiological and chemical aspect. Sugar loss can be recovered by enhancing the process efficiency by analyzing gaps. Physical part can be handle by strict operation & supervision while microbiological and chemical part is critical to handle without any efficient additives. The common additives are; Mill sanitizer for prevention of sugar & purity drop by bacterial (specially leuconostoc) contamination. Enzymes for starch & dextran removal resulting reduction in all related parameters like viscosity, color, flocculation, scaling etc. Other chemical aids for viscosity, color, flocculation, scaling, etc. which could be replaced by non-toxic ecofriendly enzymatic solutions.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. In this article a laboratory investigation have been done on the concentration of dextran in sugar factory deteriorated cane and sugar industry products, as well as their effects on the sugar factory operation. In addition, the effective concentration of biocides (Busan and formaldehyde) as inhibitor to bacteria responsible for formation of dextran has been done.. This sector is among the countries leading economic enterprises. Sugar is mainly extracted from sugarcane and sugar beet. Studies have indicated that nearly 20-30% of total sucrose synthesized by sugarcane plant is lost during various stages of raw material handling and sugar mill processing. The post-harvest sugar loss is one of the most alarming problems of sugar industry and has attracted widespread attention in the recent years..

I. INTRODUCTION

The formation of dextran as a result of microbiological activity is well documented. Dextran is currently recognized as having significant financial impact in cane sugar more than in beet sugar as penalties are often levied by cane sugar refiners. The presence of dextran in the factory is known to cause multiple processing problems, each having financial impact beyond merely sucrose loss. The processing and financial impact of dextran on the total factory operation is significant. However, the ability to measure the presence of dextran may be a limiting factor in recognizing its true impact on sugar beet operations. It is known that losses in sucrose may occur in beets in storage even under favourable conditions. The adaptation of dextran testing in beet sugar processing can identify at any stage of the process where sucrose losses are occurring and to what extent. A recently developed test method using a monoclonal antibody procedure has just been introduced into the cane industry. The new test method eliminates the time consuming and labour intensive methods currently practiced for measuring haze formation. It also has the advantage of being able to test dextran in juice and syrup as well as on the final sugar, while the currently used methods test only the final sugar.

Dextran is an extracellular bacterial homopolysaccharide of D-glucose composed predominantly of α-1,6-glucopyranosidic linkages within the main chain. A polysaccharide usually referred to as dextran compound widely occurs in deteriorated sugar cane and beet. These molecules are derived from the metabolic activities of microorganisms growing during plant cultivation or at some stage in the subsequent processing (James and Day, 2000). Dextrans can be responsible for problems in sugar processing which reduce both the recovery of sucrose during sugar production and the final quality of the sugar. Dextrans can be formed by many microorganisms and are

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not well-defined substances with specific properties. Dextran is the name given to a large class of extra-cellular bacterial polysaccharides composed almost exclusively of glucose units linked predominantly by 1:6 bonds, but also containing 1:4, 1:3 and some 1:2 glucosyl linkages. Dextrans in the sugar industry are predominantly linear, but (Edyeet al. 1995) have shown that branching can be significant, particularly with the low molecular weight dextrans where 5 to 8% branching was indicated. Dextrans in sugar processing occur as a result of post-harvest delay and, infrequently, as a result of poor factory hygiene. (Morel2002). In sugar production, dextrans are undesirable compounds produced .Analytical methods for determination of dextran in the sugar industry, several different methods are in use for the determination of dextran. These methods are Haze, Robert's copper and Polarimetric method.

Robert method Dextran was determined according to Roberts, (1983) and AOAC, (1990). Roberts copper method determines dextran after polysaccharide precipitation from sugar solutions by 80 %ethanol. Quantification is made calorimetrically using Phenol- H2SO4, after a second precipitation with alkaline copper reagent.

Haze method The determination of dextran in sugar solutions by a modified alcohol Haze method is conducted according to the ICUMSA method (1994). The test sample is dissolved in water.On contrast, dextrans are used in the msoluble starch is destroyed by incubation with a suitable enzyme (Novo Termamyl 120L, Novo Industri A/S, Bagsvaerd, Denmark). Protein is removed by precipitation with trichloroacetic acid (TCA) followed by filtration with acid-washed kieselguhr. The dextran haze is produced by diluting an aliquot of the treated, filtered solution to twice the aliquot volume by the addition of ethanol. The turbidity of the dextran haze is measured by reading the absorbance in a spectrophotometer at a wavelength of 720 nm. The method is standarized against a commercially available dextran. This method measure the haze formed by dextran, like polysaccharides, when alcohols added to a solution of raw sugar. The test sample was dissolved in water; soluble starch was destroyed by incubation with a suitable enzyme. Protein was removed by precipitation with trichloroacetic acid followed by filtration with acid – washed kiesselghur. The dextran haze was produced by diluting an aliquot of the treated, filtered solution, to twice the aliquot volume by the addition of ethanol. The turbidity of dextran haze was measured by reading in spectrophotometer at a wave length of 720nm.The method was calibrated against a commercially available dextran. The haze method is accepted commercially for dextran analysis. Therefore it was used in this study to determine the dextran concentration in raw sugar. Dextran and starch, like other non sugars, may be incorporated into the sugar crystal through adsorption-occlusion at the surface, through liquid inclusions, and/or co-crystallization (Sang-wal., 2007) during the crystal growth process. The degree of dextran and starch incorporation is indicated by a partition coef-ficient (Keff) between the mother liquor and the sucrose crystal (Promraska et al., 2009; Eggleston et al., 2012; Cole et al., 2013).

Based on this knowledge, a set of experiments was developed in order to determine the influence of these variables (dextran and starch content in syrup) on the sugar quality. The content of these non sugars in the crystallization trials varied between 0 and 2000 mg/kg.

PARAMETERS AFFECTING SUGAR PROCESS

Complex polysaccharides
Dextran: A complex polysaccharide, produced by leuconostoc, create hassle in process in term of viscosity enhancement, Flocc formation, color formation, filtration efficiency reduction and recovery loss

Starch: A complex polysaccharide, produced by natural process in cane, depend on cane variety, create hassle in process in term of viscosity enhancement, Floc formation, color formation, filtration efficiency reduction and recovery loss

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CANE VARIETY

Pre-aged cane: High in starch, leads to loss in cane yield, sugar recovery, poor juice quality and problems in milling, filtration, evaporation, crystallization and centrifugation due to extraneous matter.

Matured cane: Good for cane yield, sugar recovery, good juice quality but highly probable for microbial contamination due to maximum soluble sugar concentration.

Over-aged cane: High in starch, leads to loss in cane yield, sugar recovery, poor juice quality and problems in milling, filtration, evaporation, crystallization and centrifugation due to extraneous matter.

Cut to crush delay: Increase chance of contamination & dextran formation within the cane juice leads sucrose yield loss.

CONTAMINATIONS

Leuconostoc: Consume sucrose leads purity drop and form dextran leads process efficiency drop due to increased viscosity, scum formation and lower filterability, etc.

Other microbial contaminants: Consume sugar leads purity drop, scum formation, etc.

Processing parameters
Clarification: Improper clarification resulting process efficiency & yield loss
Mud separation: Improper mud separation leads contamination retention, resulting process efficiency & yield loss
Filtrate recycling: Recycling increase contamination concentration, leads fastidious growth of leuconostoc resulting sucrose loss

Effect of Starch in sugar process
What is Starch?
It is a carbohydrate consisting of a large number of glucose units joined by glycosidic bonds. This polysaccharide is produced by most green plants as an energy store. Starch consists of amylose and amylopectin. Amylose is polymer of glucose units linked together with α-(1→4) linkage. -Amylopectin are highly branched polymer in which braches occur at α-(1→6) linkages. In immature and maturing cane the starch concentration is high than in matured cane. Raw sugar produced in early in the crop season can be high in starch.

IMPACT OF STARCH ON PROCESS

Higher viscosity and starch content adversely affect the rate of settling and filtrations. i.e. reduce the capacity of plant. Starch content in syrup will adversely affect the rate of crystallization and increase the pan boiling time. Due to Higher Starch content ,more quantity of wash water is required during centrifugation i.e. reduce the capacity of centrifugal and significantly increase power consumption at centrifugal.Poor Clarification and Lower quality Sugar Causes turbidity/Floc in the solution hence limits uses in acid or alcoholic beverages.

Effect of Dextran in sugar process

- **What is Dextran?**
  - Dextran is a complex; polysaccharide made of many glucose molecules, In the formation of 1.0 g. dextran 4.0 g sucrose is lost.
  - Dextran is a collective name for high-molecular-weight polymers composed of D-glucose units connected with α-(1→6) linkages and various amounts of side branches linked with α-(1→2), α-(1→3) or α-(1→4) to the main chains.

- Produced by the enzyme dextran sucrase secreted by bacteria species Leuconostoc mesenteroides.
- Enzyme hydrolyses sucrose to fructose and glucose. Fructose is used by the organism and the remaining glucose fragment is converted to various polymers.

Impact of Dextran on process

Directly representing the loss in terms of sucrose.
Delaying the process due to excess viscosity.

Due to higher dextran content , more quantity of wash water is required during centrifugation i.e. reduce the capacity of centrifugal and significantly increase power consumption at centrifugal.
Higher dextran level in syrup/ molasses is leads to the formation of irregularly shaped crystals and decreases the exhaustion of molasses, i.e. higher molasses purity and Molasses % cane.

Apparent Pol and purity of juice and molasses is increased with increase in dextran level because the dextran is a dextrorotatory substance which gives positive polarization.

Preservative for BH-Molasses, C Molasses

C-Molasses/Final Molasses has been traditionally used for producing Ethanol in India.

1. With recent changes in Ethanol policy, alternative feed stocks such as BH- Molasses and Cane Juice have become viable for Ethanol Production.
2. However, Sugar season in India is limited to 120 - 150 days and distilleries can be operated for more than 300 days. Therefore Molasses need to be stored for 3-6 months.
3. Storage of molasses is a challenge due to deterioration of sugars during storage.
4. Sometimes, this deterioration is accelerated and instances of foaming with or without a rise in temperature, which changes the color and smell of molasses.
5. Molasses storage generally results in accumulation of byproducts and thus affecting the quality of molasses to be used in distillery.

This ultimately results in decreased fermentation efficiency and revenue losses

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Deterioration Causes

Cause # 01 – Growth of high sucrose tolerance bacterial consortium

- Due to challenges related to environment conditions differ from region to region
- Cleaning/sterilization challenges on regular basis of storage/transportation vessels like; pipelines, transfer pumps, road tankers, decantation pits, storage tanks & circulation pumps.
- These challenges promoting the growth of undesirable microbes, resulting in rapid deterioration in sugars and a rise in acidity.
- Microscopic observation and detailed microbial evaluation confirmed the growth of two bacteria growing in consortium as a single bacterium, which required a minimum sucrose content of 30% when grown under anaerobic conditions.
- When this consortium was broken in aerobic conditions, none of the isolates could tolerate >5% sucrose.

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Cause # 02 - Maillard reaction due to rise in temperature

- Spontaneous exothermic chemical change with evolution of carbon dioxide and the formation of volatile acids, mainly acetic.
- The decomposition is essentially a reaction between amino acids and reducing sugars (Maillard, 1912a, 1912b).

Honig (1965) summarized the mechanism of the Maillard reaction as follows:

1. Amino acid + hexose = amino acid-hexose compound (colourless)
2. Amino acid +amino acid - hexose compound polymerized product (I) with dehydration (yellow)
3. Polymerized product (I) undergoes decarboxylation and internal combustion

$C0_2$ + dehydration product (II) (brown melanoidin)
Polymerization product (II) humic acid + insoluble non-sugars (dark reddish-brown)

Probable Losses Due to Deterioration

- Reduction in sugar content
- Accelerate foaming
- Probability of rise in temperature
- Changes the color (Increase caramel value)
- Increase unpleasant smell in molasses
- Increase robust taste
- Accumulation of byproducts
- Increase volatile acidity
- Affects quality of molasses to be used in distillery
- Decreased fermentation efficiency on molasses
- Revenue losses
- Microbial growth
- **General Storage Period**
- C-Molasses : 3 – 6 Months
- BH-Molasses : 2 – 4 Months
- Sec. Juice Syrup : Few Hours – Few Days

Deterioration Trend
Base: 1000 Quintal Molasses
TRS/FS loss (in %)
In 1 month storage : 600 – 1000 Kg
In 3 month storage : 2000 – 3000 Kg
In 6 month storage : 4000 – 6000 Kg

Fermentable Sugar loss (in Kgs)
In 1 month storage : 0.6 – 1.0 %
In 3 month storage : 2.0 – 3.0 %
In 6 month storage : 4.0 – 6.0
Therefor the **total loss in ethanol yield** by using deteriorated molasses during entire storage period will be as follows (**ENA/RS loss)**
In 1 month storage : 360 – 600 litres
In 3 month storage : 1200 – 1800 litres
In 6 month storage : 2400 – 3600 litres
(ENA/RS loss in liters: Sugar quantity saved in kg. X 0.644 X 0.9 X 0.985/0.95)

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PREVENTIVE MEASURES

Check out the level of caramelization

1. Check out the level of Acids (Volatile & Non-Volatile Both)
2. Check out the level of contamination
3. Identify the type of Contamination
4. Check out the point of contamination origin
5. Check out the mixing feasibility of suitable product solution
6. Identify the average storage period for dosing pattern establishment
7. Time-course repetition of above check points as preventive measure and suitable solution identification
8. Cool molasses <38 degree Celsius using cooler before transfer to storage tank.
9. Execute appropriate aeration by proper circulation to prevent internal combustion.

Use suitable Preservative to prevent microbial deterioration
Use of Enzymol Protect will help to maintain TRS/FS as such till the entire storage period
Therefore the total saving of TRS & ENA/RS will be as follows
In 1 month storage : 0.6 – 1.0 % : 360 – 600 litres
In 3 month storage : 2.0 – 3.0 % : 1200 – 1800 litres
In 6 month storage : 4.0 – 6.0 % : 2400 – 3600 litres

Therefor the total gain in revenue will be as follows (ENA/RS @ 40 INR per litres)
In 1 month storage : 14400 – 24000 INR
In 3 month storage : 48000 – 72000 INR
In 6 month storage : 96000 – 144000 INR

Total enzyme input cost to maintain TRS during time-course storage will be as follows
In 1 month storage 7000 INR
In 3 month storage : 21000 INR
In 6 month storage : 42000 INR

Where; Enzymol Protect A will be required 2 Kg/month @ 1500 INR/Kg; once in 30 day & Enzymol Protect B will be required 4 Kg/month @ 1000 INR/Kg; once in 15 day

So, the average ROI will be ~ 3 times or 300 %
* This ROI is based on average minimum estimated values and can be increased with actual outputs.

- Application Protocol: Select two storage tanks with same molasses and storage condition.
- Use one storage tank as control and analyze the difference in characteristic of stored molasses by doing analysis of TRS, UFS, FS & TVA in every 15 days as mentioned in observation sheet.
- Use other storage tank as test to observe the effect of enzyme in storage period.
- Dose suitable product in test storage tank and mix properly by circulation using installed circulation pumps.
- Allow proper cooling of storage tank by continuous spraying of water.
- Note down the date and time of enzyme dosing and collect sample for pre-analysis.
- Proper routine analysis of TRS, UFS, FS & TVA is necessary and these analysis should be strictly done in every 15 days interval.

(Note: Maximum circulation will be more beneficial to proper mixing of enzyme resulting better results).
For maximum output of this product, four circulation pumps is required at every 90 degree angle; which will collect the molasses from center of the tank bottom and discharge on top near wall of the tank at same side. Alternate of this circulation system; Minimum 2 circulation pumps are necessary at every 180 degree angle; which will collect the molasses from center and discharge on top of the tank with 3 output line. One will be discharge on top near wall of

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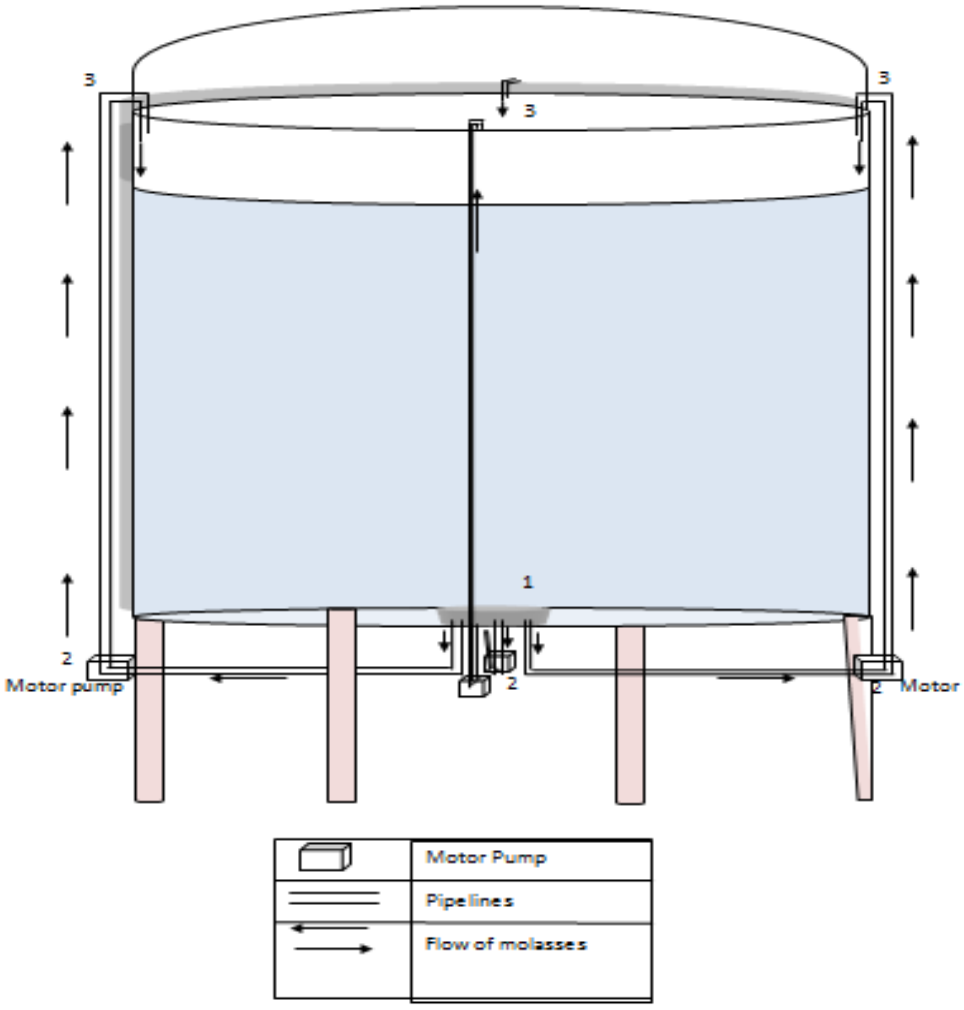
tank at same side and other two will be discharge at 90 degree of first discharge at different wall side. **See the diagram**



Circulation process details for Molasses Preservation

- Collection of molasses from bottom of the tank. The collection must be from centre.
- Electric motors used to circulate the molasses.
- Discharge of the molasses at the walls of tank.
- Four electric motors to be installed such that each motor collects molasses from the bottom of the tank & discharge it into the tank from upside in such that the molasses falls into the tank through the same side walls respectively.

The motors must be capable to circulate the whole tank in 24 – 36hrs.

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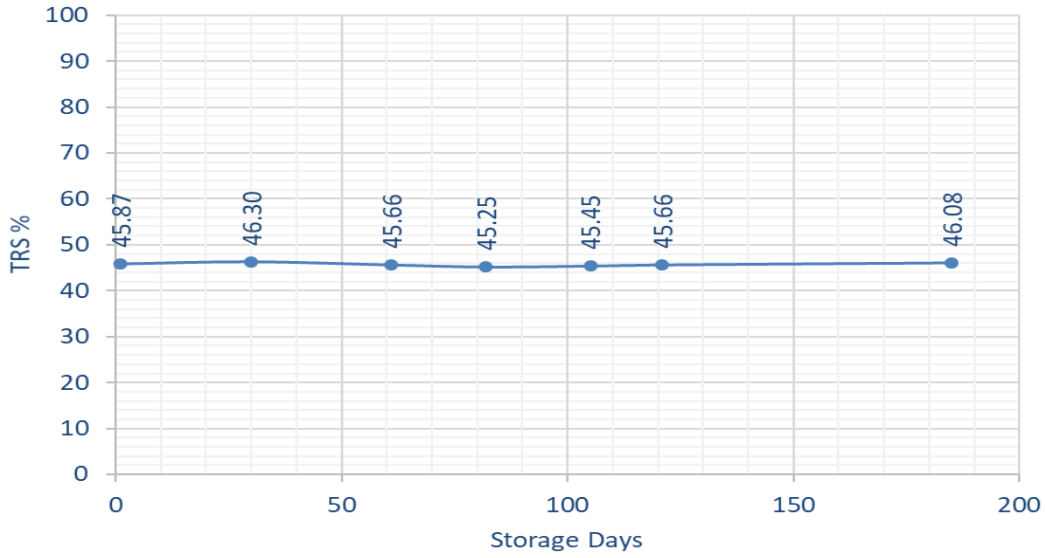

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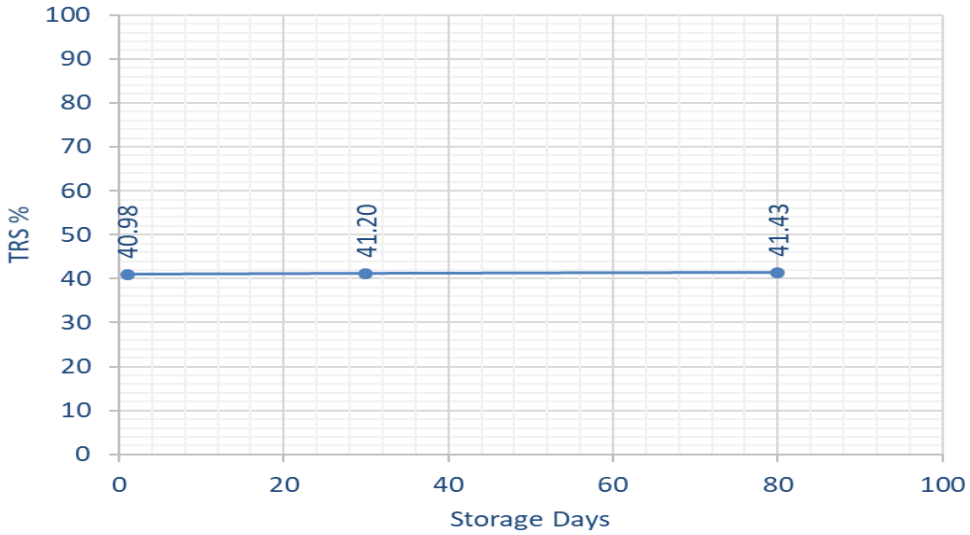
Case Studies on C-Molasses: North Region



Case Studies on C-Molasses: North Region
Case Study on BH-Molasses

Enzymol Protect Advance trial running in South Region

- Molasses Characteristics

TRS	: 57.23%
VA	: 4050 ppm
pH	: 5.35
UFS	: 2.52
Specific Gravity	: 1.412

- After one month storage period, No deterioration has been observed

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