# Optimum Biocide Concentration Required to Preserve the Highest Amount of Sucrose During Mud Filtration

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**Abstract**:

*This study focused on the determination of optimum biocide concentration required to minimize sugar loss during mud filtration as a way of keeping sugar loss to the lowest possible level. To this point, the study investigated cane juice purity at varying biocide concentrations and determined the minimum biocide concentration to inhibit microbial activities during sugar processing so that, at juice purification stage, the highest amount of sucrose is preserved during mud filtration. Accordingly, the study found the optimum biocide concentration required to preserve the highest amount of sucrose during mud filtration to be 5.0 ppm. Consequently, the study recommends the use of biocide for inhibition of microbial activities during sugar processing so that, at juice purification stage, the highest amount of sucrose is preserved during mud filtration. Further, the study recommends 5.0 ppm as the optimum biocide concentration to be used so that the highest amount of sucrose during mud filtration is preserved, especially when using the biocide known as dermacide-BD-2084.*

**Keywords:** *biocide concentration; sugar loss; microbial activity; juice purification; mud filtration; sucrose.*

## I. INTRODUCTION

The loss of sucrose at all stages from sugar cane in the field to crystal sugar in the bag is a serious economic problem to the sugar industry, making it necessary for the industry to cut the losses to the lowest possible level. This post-harvest deterioration in cane is principally the consequence of the action of the bacteria Leuconostoc mesenerioides, Lactobacillus which lead to inversion of sucrose and a large production of dextran, acids and ethanol affecting juice purification and kinetics of sucrose crystallization. In high enough concentration, dextran causes gummy solutions and difficulties in processing, along with sucrose loss. The presence of high concentrations of gums in sugarcane and subsequent processing streams adversely affect sugarcane processing, yields and the quality of the sugar produced. Not only does the formation of dextran result in expensive sucrose losses, but also the high-viscosity associated with this polysaccharide often interrupts normal processing operations and also interferes with the polarimetric analysis of sucrose because, having a positive rotation, it is interpreted as sucrose, resulting in false high estimates of the sugar content in the cane juice.

Usually, raw cane sugar is produced from sugarcane (Saccharum officinarum). The major unit operations involved in raw cane sugar processing are washing, size reduction, milling (where juice is extracted from harvested cane stalks), juice purification, vacuum evaporation and crystallization; as is graphically presented in the Figure 1 which follows.

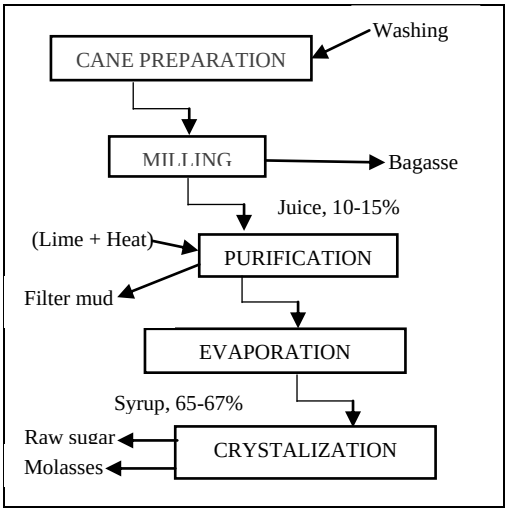


**Figure 1: Generalized Flow Diagram of Unit Steps in Raw Cane Sugar Processing, Adapted from [1]**

In this study, the unit of interest was cane juice preparation (refer to the Figure 1 above), given that cane juice contains sucrose and other nutrients in the right concentration for promoting microbial growth [2] and it was microbial activities that this study sought to inhibit.The first stage of purifying cane juice is called clarification or defection. Clarification employs lime and heat intended to remove maximum amount of impurities from the juice. It (clarification) affects juice filterability, evaporator scale composition, sucrose crystallization, and the quality of raw sugar produced [3].

Lime serves the dual purpose of raising the pH and stabilizing the juice against hydrolysis of the sucrose in the acid juice and forming a precipitate to remove impurities. The heat helps to disinfect the juice of harmful bacteria and improves coagulation and precipitation of the impurities. In simple lime defection, lime is added to the juice to raise the pH from 5.5–5.7 to 6.7–7.5 and to react with inorganic phosphate present in the cane juice to form a calcium phosphate floc which entraps insoluble impurities from the cane juice as it settles [2]. The lime is added either in the form of milk of lime, lime-juice mixtures or lime saccharate. Lime addition may be batch-wise or continuous: to cold juice (35–40 $^{o}$C), intermediate juice (72–76 $^{o}$C) or hot juice (100 $^{o}$C). Polyacrylamide flocculants are added to aid in the coagulation of the precipitate [3]. In cold liming, milk of lime is added to the cold juice, and the limed juice is then pumped through heaters in which the juice is heated to (90–115 $^{o}$C). Studies have shown the advantage of hot or intermediate liming over cold liming, resulting in less sucrose loss through inversion, less dextran formation, and better control of pH and turbidity[4], [5].

The combination of lime and heat forms a flocculent precipitate with various components in the juice, consisting mostly of insoluble lime salts, coagulated protein, entrapped colloidal and suspended matter. The precipitate is removed by sedimentation or settling in continuous closed-tray clarifiers. The juice leaving the clarifier is a clear brown liquid. The flocculent precipitates that settle on the clarifier trays are called "muds," [2]. Muds contain about five percent solid matter. In sugar manufacturing factory, entrained sugar is recovered from the mud by means of rotary vacuum filters equipped with a perforated metallic screen cloth. The turbid filtrate is returned to the clarification system and the press cake or filter mud is usually sent to the fields as fertilizer. Good clarification depends upon the formation of a stable flocculent precipitate that settles rapidly [6].

## II. THE PROBLEM AND STUDY OBJECTIVES

Several strategies involving the use of chemicals, particularly biocides, have been invoked to minimize bacterial inversion and these have been successful in preventing sugar losses. At very low dose, biocides cannot inhibit activities of microorganisms; at optimum concentration, biocides do substantially inhibit microbial activities. However, literature is generally silent on the minimum inhibitory biocide concentration required so that the highest amount of sucrose is preserved during mud filtration. Therefore, problem remained to find the optimum concentration of biocide required to preserve the highest amount of sucrose during mud filtration.

The main objective of the study was to determine the optimum biocide concentration required to minimize sugar loss during mud filtration, as a wayof cutting sugar loss to the lowest possible level during processing. Specifically, the study sought to investigate cane juice purity at varying biocide concentrations and to determine the optimum biocide concentration required to preserve the highest amount of sucrose during mud filtration.

## III. LITERATURE REVIEW

In the early 1960's, Chen [7] encountered some white gelatinous substance removed from juice heaters in a factory in Peru. It was identified as dextran. The name 'dextran' was introduced by Scheibler in 1874, when he discovered that the mysterious thickening of cane and beet sugar juices was caused by a carbohydrate of empirical formula ($C_6H_{10}O_6$) with a positive optical rotation [8]. Shortly thereafter it was suggested that Leuconostoc mesenerioideswas responsible for the slime production in sugar factories [9]. Today we know that dextran (and other gums) can be produced from sucrose by several bacterial species and that the structure of each type of gum depends on the microbial strain that produces it [10]-[15]. In sugar production, dextrans are undesirable compounds produced by contaminant microorganisms from sucrose [16]. The physical and chemical properties of sugarcane juice make it an excellent substrate for proliferation of a variety of microorganisms which ultimately cause sucrose degradation and the production of microbial metabolites such as acids (lactic and acetic acid), alcohols (mannitol and ethanol) and polysaccharides (levan and dextran) in the sugarcane juice [17]. The identification of dextran was the starting point of a long series of tests carried out in the following years confirming that physical cleaning can only achieve 50- 60 percent of mill sanitation and that dextran formation could not be avoided without the addition of biocide. Similar tests also conducted by Tilbury [18] showed that biocide addition had a significant benefit in sugar factories which could reduce the rate of sugar loss by saving of up to 60 percent sucrose.

### *A. Cane Juice Purification*

The first stage of purifying cane juice is called clarification or defection. Clarification employs lime and heat intended to remove maximum amount of impurities from juice [4],[5]. It, clarification, affects juice filterability, evaporator scale composition, sucrose crystallization and the quality of raw sugar produced [3]. According to [2], the cane juice obtained from milling (or diffusion) is acidic (pH around 5.5–5.7), turbid and dark brown in colour. By weight, it contains 12–18 percent sucrose (the more mature the cane is, the higher the concentration of sucrose) in addition to soluble and insoluble impurities such as soil, protein, waxes,

polysaccharides, starch, fine bagasse (bagacillo), organic and phenolic acids, soluble salts and pigments. Cane juice also contains 0.5-3 percent reducing sugars (glucose and fructose in approximately equal quantities).

The post-harvest deterioration in cane is principally the consequence of the action of the bacteria Leuconostoc, Lactobacillus saccharomyces, Rodotorula genera, which lead to inversion of sucrose and a large production of dextran, acids and ethanol affecting kinetics of sucrose crystallization [19], [20]. In sugar processing, not only does the formation of dextran results in expensive sucrose losses, but also the high-viscosity associated with this polysaccharide often interrupts normal processing operations [21] and also interferes with the polarimetric analysis of sucrose because, having a positive rotation, it is interpreted as sucrose, resulting in false high estimates of the sugar content in the cane juice [2].

Biocides or bactericides or microbial inhibitors are organic compounds designed to kill bacteria. These compounds are important for control in sugarcane processing, as the juice contains sucrose and other nutrients in the right concentration for promoting microbial growth [2]. In the sugar industry, there are several proprietary biocides available that are intentionally added to control *Leuconostoc* bacteria. Two classes of biocides important to the sugar industry are the carbamates and the quaternary ammonium compounds [22]. The biocides in most common use around the world are *dithiocarbamates*, *glutaraldehyde*, and *ammonium bisulfite*[23]. Although they differ in composition, they are all intended to kill or retard the growth of the *Leuconostoc* microorganism and prevent it from decomposing sucrose into dextran [7]. It is therefore evident that the application of biocide serves a two-fold purpose: (i) to reduce sucrose loss and (ii) to avoid or reduce the formation of dextran or gummy substances in cane juice.

### *B. Biocide Concentration Required for Sucrose Preservation*

According to [24], the chemical, physical, sensory and nutritional attributes of sugarcane juice are affected by several factors that can be physical (light, heat), chemical ($O_2$), biochemical (enzymes) and/or biological (microorganisms, insects). These properties of sugarcane juice make it an excellent substrate for the proliferation of a variety of microorganisms which ultimately cause sucrose degradation and the production of microbial metabolites such as acids (lactic and acetic acid), alcohols (mannitol and ethanol) and polysaccharides (levan and dextran) in the sugarcane juice [17]; depending on environmental conditions: varying qualitatively from place to place and season to season. Primary juice (PJ) contains about 106 to 109 colony forming units (cfu) of microorganisms per ml. Many of these metabolites are not eliminated by boiling juice for clarification and in fact some grow at clarifier and continue to grow during subsequent processes. Moreover, microbes produce various metabolites that interfere in the process and affect sugar recovery and sugar quality adversely. To make sucrose recovery even more difficult, microbes protect themselves by growing as biofilms or spores resistant to penetration by antimicrobials such as biocides [22].

The loss of sucrose at all stages from sugar cane in the field to crystal sugar in the bag, is a serious economic problem to the sugar industry, increasing costs and making it necessary for industry to cut the loses to the lowest possible level [24], [25]. According to [22], sugar losses are of four types, namely: (i) chemical, due to changes in pH and temperature – this can be reduced only by strict control on parameter (e.g. pH and temperature); (ii) microbial, due to direct consumption of sugars for growth; (iii) enzymatic – microbial and invertase present in sugarcane cells and (iv) indirect losses due to microbial metabolites.Microbial degradation of sugars occurs as follows [22]: (i) sucrose is first converted to glucose and fructose, which are then degraded or utilized by microbes to produce various metabolites; (ii) glucose is utilized to form dextran, acids, alcohol, gas and other polysaccharides; (iii) fructose is converted to glucose and also used to form complex polysaccharides and (iv) no rules apply for the conversion and all reactions can occur irrespective of pH and temperature.

Two major actions of contaminating microorganisms are (i) the removal of sucrose from the sugar production process, directly resulting in reduced sucrose availability for sugar production and, (ii) producing gums from the sucrose, resulting in a multitude of problems in sugarcane processing [26]. The main microorganisms of concern are those which are heat-resistant (thermophilic) such as Leuconostoc mesenerioides. Heat-resistant organisms can enter the process system with outside ingredients. Heat resistant molds, including Byssochlamys, Paecilomyces, Eupenicillium, Talaromyces and Eurotium have been found in juices even after heat treatment [27]. The minimum level of thermophilic bacteria found in cane entering the factory is between 600 and 800 per 10 grams. It seems that this level remains more or less constant during extraction and clarification. Declining cane quality, frequent breakdowns or process conditions increase the number of thermophiles and the use of a biocide then becomes necessary to inhibit their activities [28]. For example, the biocide di-methyl dithiocarbamate kills 90 percent of microbes in 45 minutes. However, to be effective in saving sucrose loss, a biocide must have the capacity to kill 90 percent of microbes within 10 minutes [22]. Microbial degradation of sugars at high temperatures involves the following [22]: (i) microbes capable of growing at higher temperature also gain entry via cane; they remain dormant at normal

temperature; (ii) these thermophiles grow in clarifier and during subsequent processes; (iii) major end product of their (thermophilic microbes) metabolism (80%) is lactic acid and (iv) thermal degradation of invert also produces acid.

The major microbiological losses in sugar processing are caused by the common soil bacterium Leuconostoc mesenerioides, which uses sucrose as a food source, producing a long-chain polysaccharide known as dextran as a waste product. 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 [16, 20]. In high enough concentration, dextran causes gummy solutions and difficulties in processing, along with sucrose loss. It is widely reported that the presence of high concentrations of gums in sugarcane and subsequent processing streams adversely affects sugarcane processing and yields and quality of the produced sugar [29], [30]; Gums are "carbohydrates of high molecular weight which are precipitated from aqueous solutions by acidified ethanol,"[29]. Not only does the formation of dextran results in expensive sucrose losses, but also the high-viscosity associated with this polysaccharide often interrupts normal processing operations [21]. It also interferes with the polarimetric analysis of sucrose because, having a positive rotation, it is interpreted as sucrose; resulting in false high estimates of the sugar content in the cane juice [2]. Dextrans in the sugar industry are predominantly linear, but [31] have shown that branching can be significant, particularly with the low molecular weight dextrans where five to eight percent branching is possible.

Measures adopted to combat the effects of dextran have included high-pressure washing with hot water and the use of biocides. Physical cleaning only achieves 50-60 percent of mill sanitation [7] and dextran formation could not be avoided without the use of biocide. While proper control on microbial activity is essential so as to minimize impurity development, at very low dose biocides cannot kill microorganisms[4], [22].

Though in raw sugar manufacturing, biocides such as carbamates, quaternary compounds, halogenated phenols and antibiotics are required to be added to sugar cane juice, not only are biocides costly, in large doses biocides have by-side effects on humans [32], [33]. Residual biocide has been reported as forming a white surface deposit after drying [34]. Indeed, excess biocide was suggested as the cause of this residue formation [35]. Hence, only minimum inhibitory concentration (MIC) of biocides should be required to recover sucrose from cane juice so as to avoid harm to human beings and the environment. Indeed, [37] studied and found optimization of concentration to be plausible. Evaluation of sucrose loss during sugar processing from cane juice is normally achieved through the following: purity drop from primary juice (PJ) to mixed juice (MJ) or analysis of dextran or analysis of alcohol or microbial count or rise in reducing sugars from PJ to MJ or rise in reducing sugars (RS) as well as acidity from PJ to MJ to clear juice till final molasses [22].

## IV. METHODOLOGY

This study focused on finding the optimum concentration of biocide required to preserve the highest amount of sucrose during mud filtration. The methods and materials that were moved to achieve the objectives of the study are presented as follow.

### *A. Apparatus and Materials*

The study monitored brix, polarization (pol) and purity change of the samples as biocide concentrations were varied from 0.0 to 7.0 ppm. To this end, samples were taken during the July to August 2016 sugar cane crop at the SCOUL. The following noteworthy apparatus and materials were put to use, namely: sampling cup and buckets; cold water for dilution; hot water as a water bath; beakers; measuring cylinders; filter paper; filter funnels; polarimeter for measuring pol value; refractometer for measuring brix and biocide, in particular Dermacide-BD-2084 (chemicals for controlling microorganisms in cane-sugar and beet-sugar mills) as detailed in the Table I which follows.

**Table I. Biocide (Dermacide-Bd-2084) Composition**

| Active ingredients | Parts per million |
|---|---|
| Disodium cyanodithioimidocarbonate | 2.5 |
| Ethylenediamine | 1.0 |
| Potassium N-methyldithiocarbamate | 3.5 |

(Source: US Drug and Food Administration. (2016). CFR - Code of Federal Regulations Title 21. Volume 3. CITE: 21CFR173.320) [18], [36]).

### *B. Methods*

The methods applied in order to achieve the objectives of the study involved treatment of muddy sugar cane juice samples with varying biocide concentrations, determination of brix, pol and purity.

#### *1) Treatment of Muddy Sugar Cane Juice with Biocide Concentration*

The procedure followed in the treatment of muddy sugar cane juice with varying biocide concentration was the following.

i) Samples were taken from the clarifier mud tray just as the muddy juice exited to the mud recirculation tank.
ii) The samples were then immediately transferred to the laboratory for analysis.

iii) In the laboratory, the samples were placed in a water bath of 100.0 $^{o}$C to maintain temperature.
iv) A portion of the sample (100.0 $cm^3$) was placed into a measuring cylinder and biocide added. The biocide concentrations were varied from 0.0 to 7.0 ppm.
v) The mud sample and biocide were allowed to react for a length of five (5) minutes, while still in the water bath.
vi) After five minutes, the sample was filtered and pol, brix and purity of the resultant filtrate, for the various concentrations determined.

*2) **Determination of Brix***

Brix was measured using an index instrument temperature controlled refractometer after the filtration of samples with kieselguhr through filter paper according to the standard method described as follows: (i) the prism surface and the presser were cleaned with a clean tissue soaked in distilled water and (ii) the muddy juice sample was introduced onto the prism surface using a dropper and the read key engaged. The Brix observation was then directly read off.

*3) **Pol Determination***

First, basic lead acetate was added to the samples, then filtered through filter paper according to the standard method described as follows.

i) A muddy juice sample (200 ml) was transferred into a beaker.
ii) Lead sub acetate (2g) was added to the sample and mixed well to coagulate the impurities and the sample filtered.
iii) The pol tube was cleaned thoroughly with distilled water to remove all traces of previous sample and then rinsed with the sample to be read.
iv) The filtrate of the clarified sample was poured into the pol tube which was then placed in the polarimeter to determine pol value.
v) The pol reading was taken when the readings stabilised.
vi) Pol% was obtained from Schmitz's table by multiplying the pol reading with the pol factor corresponding to brix, as follows,

Pol% = pol reading x Brix factor ... (3.1).

*4) **Purity Determination***

The purity of sugar product is the cane sugar present in percentage terms of the solid matter. Since the sugar may be expressed as pol, and the solid as brix, the apparent purity that is generally known as purity was derived as follows.

$$\text{Purity} = \frac{\text{Pol \%}}{\text{Brix}} \times 100\% \quad \ldots (3.2).$$

### *C. Limitations of the Study*

Limitations to the findings of this study would likely arise from the maturity of the sugar cane plant from which juice was extracted and the season of cane harvesting, since the amount of sucrose depends on the maturity of the sugar cane. The more mature the sugar cane, the higher the sucrose content [17] – certainly not for over mature sugar cane.

## V. RESULTS

The main objective of this study was to determine the optimum biocide concentration required to minimize sugar loss during mud filtration, so that sugar loss is cut to the lowest level possible. Specifically, the study set out to investigate sucrose purity against biocide concentration and to determine the minimum biocide concentration required to inhibit microbial activities so that the highest amount of sucrose is preserved during mud filtration. The Table II which follows presents brix, pol and purity at varying biocide concentration.

**Table Ii. Brix, Pol% And Purity Against Biocide Concentration**

| Biocide Concentra-tion [ppm] | Brix [1g of sucrose in 100g of solution] | Pol% | Sugar (Sucrose) % Purity |
|---|---|---|---|
| 0.0 | 10.65 | 8.29 | 77.84 |
| 1.0 | 9.76 | 7.74 | 79.30 |
| 2.0 | 8.77 | 7.06 | 80.50 |
| 3.0 | 10.81 | 8.73 | 80.76 |
| 4.0 | 11.24 | 9.10 | 80.96 |
| 5.0 | 10.10 | 8.25 | 81.68 |
| 6.0 | 11.12 | 9.06 | 81.50 |
| 7.0 | 8.34 | 6.79 | 81.40 |

Source: Author.

From the Table II at biocide concentration of 5.0 ppm, the highest amount of sucrose (82.59%) was recovered during mud filtration. The apparent slight drop in sucrose purity beyond biocide concentration 5.0 ppm appears to be a result of impurities, basing on the suggestion by [34], [35] that residual biocide forms a white surface deposit which are most likely impurities and not sucrose. The slight apparent drop may also be explained basing on the observation attributed to [22], that sugar loss is not only as a result of microorganisms' direct consumption of sugars for growth, but also result of changes in pH and temperature, microbial and invertase present in sugarcane cells and indirect losses due to microbial metabolites.

Plotting purity against biocide concentration and reading off the graph, for clarity, confirms that the optimum biocide concentration required to preserve the highest amount of sucrose during mud filtration is 5.0 ppm, as presented in the Figure 2 which follows.

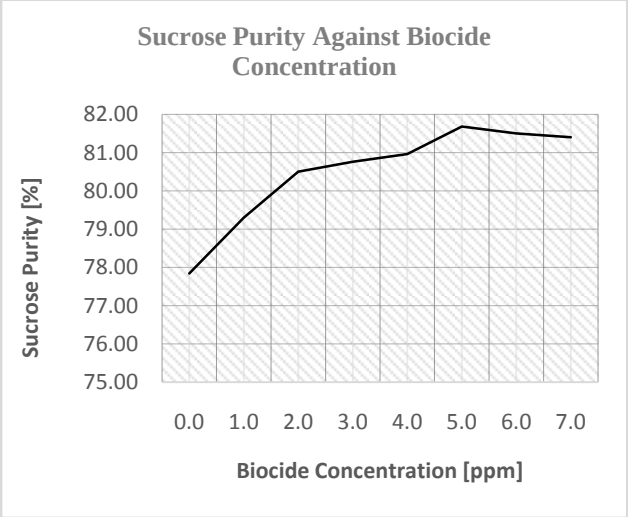


**Figure 2: Biocide Concentration Against Pol Percentage**

From the Figure 2, subsequent to biocide concentration 5.0 ppm, purity does not increase anymore. Therefore, 5.00 ppm is the optimum biocide concentration required to preserve the highest amount of sucrose during mud filtration.

## VI. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

### A. *Discussion*

The study found that the optimal biocide concentration during sugar processing so that the highest amount of sucrose is preserved during mud filtration was 5.0 ppm; thus demonstrating that biocides are successful in limiting sugar losses, in tandem with [22]. To attain this optimal concentration, the biocide concentration had to be increased gradually in consonance with the study by [33] who, using a similar procedure, attained minimum inhibitory concentration (MIC) to verify the effect of chlorine dioxide, a well-known biocide, for bacterial decontamination of water and equipment. With regards to sugar processing, from Table II recovery of sucrose (as reflected in the purity rise) increases with increase of concentration of biocide applied. Beyond biocide concentration 5.0 ppm, sucrose purity does not increase anymore. This then is the optimum biocide concentration required to preserve the highest amount of sucrose during mud filtration.

That at the beginning biocide concentration more-or-less linearly increase with sucrose purity, given that biocides inhibit activities of microorganisms is indicative that at low dose biocides do not sufficiently inhibit activities of microorganisms as noted by [4, 22]. Upon preserving the highest amount (82.59%) of sucrose during mud filtration, there was a slight drop in sucrose purity level apparently as a result of impurities, basing on the suggestion by [34], [35] that residual biocide forms a white surface deposit. It may also be noted that microbes protect themselves by growing as biofilms or spores resistant to penetration antimicrobials such as biocides [22], so biocides are seen to be effective only up to a certain concentration before microorganisms react to form biofilms and spores.

This study has demonstrated that optimum biocide concentration added to cane juice preserves sucrose during mud filtration in agreement with [7], [22]. According to this study, the optimum concentration of biocide required to preserve the highest amount of sucrose during mud filtration is 5.0 ppm.

### B. *Conclusion*

This study focused on the determination of the optimum biocide concentration required to minimize sugar loss during mud filtration, so that sugar loss is cut to the lowest possible level. To this point, the study investigated the brix, pol and purity of the resultant cane juice filtrate at varying biocide concentrations and determined the minimum biocide concentration required to inhibit dextran formation during sugar processing such that the highest amount of sucrose is preserved during mud filtration. The optimum biocide concentration required to preserve the highest amount of sucrose during mud filtration was found to be 5.0 ppm.

### C. *Recommendations*

Following the findings presented here, this study recommends the following: (i) since biocides are effective in inhibiting microbial activities, they may be used for inhibition of dextran formation during cane juice purification so that the highest amount of sucrose may be preserved during mud filtration and (ii) the optimum biocide (Dermacide-BD-2084) concentration to be used so that the highest amount of sucrose is preserved during mud filtration is 5.0 ppm.

## REFERENCES

[1] M. E.Clarke,"Cane sugar process and byproduct streams,"Paper presented at Iowa biotechnology consortium, San Diego, March 1991.

[2] A. J. Kent, Ed.,"Handbook of Industrial Chemistry and Biotechnology," Volume 1 and 2, 12th Edition,Springer, ISBN 978-1-4614-4258-5 ISBN 978-1-4614-4259-2 (eBook). DOI 10.1007/978-1-4614-4259-2 Springer New York Heidelberg Dordrecht London, Library of Congress Control Number: 2012952590, 2012.

[3] W. O. S.Doherty, J. Greenwood, D. Pilaski, P. G. Wright, "The effect of liming conditions in juice clarification," Proc Aust Soc Sugar Cane Technol 24:443–451,2002.

[4] G. Eggleston, A. Monge, A., Pepperman, "Preheating and incubation of cane juice prior to liming: a comparison of intermediate and cold lime clarification," J Agric Food Chem 50:484–490,2002.

[5] G. Eggleston, A. Monge, B. Ogier, "Further insights on the factory performance of cold, intermediate and hot lime clarification systems," Proceedings of the sugar processing

research conference, vol 348–365, New Orleans, Louisiana, USA,(2002b).

[6] R. J. Steindl, "The clarification of cane juice. Int Sugar J 101:213–215,1999.

[7] J. C. P.Chen,and J. S. Rauh, "Technical and Economic Justification for the use of Sugar Process Chemicals,"49th Annual Sugar Technologists Conf. (pp. 48-57), Hawaiian,1990.

[8] C. Scheibler, "Investigation on the nature of the gelatinous excretion (so-called frog's spawn) which is observed in production of beet-sugar juices,"Z Dtsch Zucker-Ind 24: 309-335,1874.

[9] P. Van Tieghem, "Sur la gomme de sucrerie (On sugar-mill gum),"Ann Sci Natur Bot 7: 180-203,1878.

[10] S. Kralj, G. H. van Geel-Schutten, M. M. G. Dondorff, S. Kirsanovs, M. J. van der Maarel, and L. Dijkhuizen, "Glucan synthesis in the genus Lactobacillus: isolation and characterisation of six glucansucrases genes, enzymes and glucan products from six different strains,"Microbiology 150(11): 3681-3690,2004.

[11] M. S.Bounaix, H. Robert, V. Gabriel, S. Morel, M. Remaud-Siméon, B. Gabriel, and C. Fontagné-Faucher, "Characterisation of dextran producing Weissella strains isolated from sourdoughs and evidence of constitutive dextransucrase expression,"FEMS Microbiol Lett 311(1): 18-26, 2010.

[12] A. Lakshmi-Bhavani, andJ. Nisha, J. "Dextran – The polysaccharide with versatile uses'"Int J Pharma Bio Sci 1(4): 569-573, 2010.

[13] S. Palomba, S. Cavella, E. Torrieri, A. Piccolo, P. Mazzei, G. Blaiotta, V. Ventorino, and O. Pepe, " Polyphasic screening, homopolysaccharide composition and viscoelastic behaviour of wheat sourdough from a Leuconostoc lactis and Lactobacillus curvatus exopolysaccharides producing starter culture,"Appl Environ Microbiol 78(8): 2732-2747, 2012.

[14] E. M.Paulo, M. P.Vasconcelos, I. S.Oliveira, H. M. J.Affe, R. Nascimento, I. S. de Melo,M. E. A.Roque., and S. A. de Assis, "An alternative method for screening lactic acid bacteria for the production of exopolysaccharides with rapid confirmation,"Ciênc Tecnol Aliment 32(4): 710-714,2012.

[15] M. H. P. B.Vettori, K. C. Blanco, M. Cortezi, M, C. J. B. de Lima, and J. Contiero, "Dextran: Effect of process parameters on production, purification and molecular weight and recent applications,"Diálogos Ciência 31: 171-186, 2012.

[16] E. R. Jimenez, "Biotecnologia Aplicada, 22(1), 20-27, 2005.

[17] G. Cerutti de Guglielmone, O. Diez, G. Cárdenas, and G. Oliver, "Sucrose utilisation and dextran production by *Leuconostoc mesenteroides* isolated from the sugar industry,"*Sug J* 62(10): 36-41, 2000.

[18] R. H.Tilbury, B. S.Hollingsworth, S. D. Graham, andP. Pottage,"Mill sanitation: A fresh approach to biocide evaluation," Proceeding of International Society of Sugar Cane Technologists, 16th Congress. pp. 2749-2767, 1977.

[19] P. Singh, N. Arya, P. Tiwari, A. Suman, R. K. Rai, A. K. Shrivastava, and S. Solomon, "Use of Glutaraldehyde and Benzalkonium Chloride for Minimizing Post-Harvest Physio-Chemical and Microbial Changes Responsible for Sucrose Losses in Sugar Cane," J. Agric. Food Chem,2008, 56, 7176–7183.

[20] P. G. Morel, S. du Boil and S. Wienese,"Enzymic reduction of dextran in process - laboratory evaluation of dextranasesproc s afrsugtechnol" ASS, 76, 2002.

[21] Z. G.Zhao, X. He, S. Yu, and H. Atsuya, "Optimizing conditions for dextranase application of sugarcane mixed juice using response surface methodology,"International Sugar Journal," 2014, Retrieved from: https://www.internationalsugarjournal.com/paper/2014/page/7 on 14th April 2017.

[22] V. M. Kulkarni, "importance of controlling microbes during sugar production from cane and its effect on sugar yield and losses," 6th International Sugar Conference, November 10 – 13, Aswan, Egypt, 2012.

[23] M. Fowers, and L. E. Lloyd, "Biocide synergy: reducing costs and losses through physical and chemical interactions," Presented at the 30th general meeting of the American Society of Sugar Beet Technologists, Orlando, FL, Feb., 1999.

[24] M. Kunitake, C. Ditchfield, C. Silva, and R. Petrus, "Effect of Pasteurization Temperature on Stability of an Acidified Sugarcane Juice Beverage" Ciênc. Agrotec, Lavras, v.38, n.6, p.554-561, nov./dez., 2014.

[25] M. M.Bukhari, S. El Khaseh, A. Osman, and S. E. F. Hegazi, "Investigations of the influence of dextran on sugar cane quality and sugar cane processing in Kenana sugar factory," Journal of Chemical and Pharmaceutical Research, 2015, 7(4):381-392.

[26] S. Nel, "Microbial Diversity Profiling In Sugarcane Processing: What, Why And How?" Proc S Afr Sug Technol Ass (2014) 87: 246 – 254.

[27] M., S., B.de Cássia, C. Muller, H. C. do Amparo, and G. M., de Aragão, "Survey of molds, yeast and Alicyclobacillus spp. from a concentrated apple juice productive process," Braz J Microbiol, 2014 May 19;45 (1):49-58, eCollection, 2014.

[28] P. Antier, "Microbiological Control in Acane Sugar Mill: Implications on Sugar Quality and on Losses,"Proc S Afr Sug Technol Ass, 1996, pp. 185-188.

[29] J. A.Cuddihy Jr, M. E. Porro, and J. S. Raiih, "The presence of total polysaccharides in sugar production and methods for reducing their negative effects,"J Am Soc Sug Cane Technol 21: 73-91, 2001

[30] A. B. Ravnö, and B. S. Purchase, "Dealing with dextran in the South African sugar industry,"Proc S Afr Sug Technol Ass 79: 28-47, 2005.

[31] L. A.Edye, M. A.Clarke, E. J. Roberts, and R. S. Blanco, "Dextran analysis by N.M.R," Publication of Technical Papers and Proceedings of the Sixty-Third Annual Meeting of Sugar Technologists, Vancouver, Canada, S. I. T,55-64, 1995.

[32] , F. A.Guardiola, A. Cuesta, J. Meseguer, and A. E. M. A.Maria,"Risks of Using Antifouling Biocides in Aquaculture," International Journal of Molecular Sciences; 2012, 13, 1541-1560; doi:10.3390/ijms13021541, ISSN 1422-0067 www.mdpi.com/journal/ijms.

[33] S. P.Meneghin, F. C.Fabricia Cristina-Reis, P. Garcia de Almeida, andS. R. Ceccato-Antonini, "Chlorine dioxide against bacteria and yeasts from the alcoholic Fermentation," Brazilian Journal of Microbiology (2008) 39:337-343, ISSN 1517-8382337, 2008.

[34] R. Payton, "The freeze drying of organic materials," unpublished report, Institute of Archaeology, 1980.

[35] A. M. Rosenquist, "The Stabilizing of wood found in the Viking ship Oseberg – Part IV,"Studies in Conservation. 4 (1959), 62-72, 1959.

[36] US Drug and Food Administration, 2016, CFR - Code of Federal Regulations Title 21, Volume 3. CITE: 21CFR173.320. Sec. 173.320 Chemicals for controlling microorganisms in cane-sugar and beet-sugar mills, 2016.

[37] S. J. Kulkarni, and J. P., Kaware, "Groundnut Shell Adsorbent in Packed Bed for Cadmium Removal- Modeling for Breakthrough Curve," SSRG International Journal of Chemical Engineering Research, ISSN: 2394 – 5370, – volume 2 Issue1 May to June 2014.