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The Influence of Oxidizing and Non-Oxidizing Biocides on Enzymatic and Microbial Activity in Sugarcane Processing

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Abstract: Processing aids are utilized during raw sugar manufacturing at sugarcane processing facilities to mitigate unwanted contamination from microorganisms and their associated exopolysaccharides (EPS). Microorganisms in processing facilities contribute to sugar losses through sucrose inversion and consumption, with many bacteria strains subsequently producing dextran and fructan EPS that can cause downstream issues related to viscosity and crystallization. Similar issues also result from the presence of unwanted starches from plant material in cane juices. Processing aids include biocides for bacterial inhibition, and enzymes (e.g., dextranase, amylase) to break down polysaccharides in juices. However, oxidizing biocide processing aids (e.g., sodium hypochlorite) may inhibit enzymatic processing aid activity. In this study, biocides (sodium hypochlorite, carbamate, and hop extract) and enzymes (dextranase and amylase) were simultaneously added to sugarcane juice to measure residual enzymatic activity for dextranase and amylase. The same biocides were also tested to estimate minimum inhibitory concentrations against bacterial strains isolated from Louisiana sugarcane processing facilities. These experiments provide evidence to suggest that sodium hypochlorite may interfere with enzymatic processing aid activity, with lesser/limited enzymatic inhibition from carbamates and hop extracts. Biocide susceptibility assays suggest that sodium hypochlorite has limited effectiveness against tested bacterial strains. Hop extract biocide was only effective against Gram-positive *Leuconostoc* while carbamate biocide showed more broad-spectrum activity against all tested strains.

Keywords: sugarcane; biocides; dextran; dextranase; starch; amylase; raw sugar

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1. Introduction

Sugarcane processing facilities use enzymes and biocides as processing aids to alleviate microbial and polysaccharide contamination issues that arise during the production of raw sugar. Microbes contribute to sucrose losses and subsequently generate exopolysaccharides (EPS; e.g., dextrans and fructans), which introduce additional processing challenges related to viscosity and crystallization [1–5]. Sugarcane plant matter can also introduce unwanted quantities of starch in juice after milling, which contributes to further challenges during processing [6]. Typical concentrations of starch and dextran found in sugarcane juice are given in Table 1 [7,8]. There are temporal variations in the quantities of polysaccharides present in sugarcane juices during processing throughout a given season/year. For example, a higher content of green leaves and tops in delivered cane contributes to higher resulting starch content in juices [9]. Conversely, freezes may result in lower starch content, but greatly increased dextran content [8]. Microbial loads can also vary during a given processing year/season due to changes in environmental conditions, harvesting practices, and factory sanitation practices [10]. Because microbial loading is generally correlated with EPS content in juices, dextran content can also fluctuate correspondingly [11–13]. For these reasons, it is unlikely that a "one-size-fits-all" approach exists for the application of biocidal and enzymatic processing aids to manage unwanted polysaccharides during raw sugar manufacturing.

Table 1. Reported starch concentrations and dextran concentrations for sugarcane juice across different varieties from Brazil and Louisiana.

Juice Starch Content (ppm/Brix) [1]	Cane Variety for Starch Measured in Juice (Brazil)	Juice Dextran Content (ppm/Brix) [2]	Cane Variety for Dextran Measured in Juice (Louisiana)
2628	RB 86-7515	112	CP 70-321
1679	SO 83-2847	135	CP 79-318
1896	RB 72-454	133	HoCP 85-845
1740	SP 80-3280	126	HoCP 91-555
1798	RB 85-5536	195	HoCP 96-540

[1] Average values from data reported across several measurements taken over seven months for Brazilian sugarcane varieties grown in Brazil [7]. [2] The ASI (Audubon Sugar Institute) II method uses dextranase enzyme for dextran quantification [14]. Data for USDA ("CP" and "HoCP") varieties grown in Louisiana [8].

Enzymatic processing aids for polysaccharides include dextranase and amylases, while oxidizing (e.g., sodium hypochlorite (in bleach) and sodium permanganate) and non-oxidizing (e.g., carbamates and hop extracts) biocides are applied to manage microorganisms. A summary of some industrially relevant, bacterially-derived amylase and dextranase enzymes that are reported in literature is given in Table 2 [15–30]. The enzymes used for research in this study are from bacteria, as per information available from their supplier. Fungal-derived enzymes are also common; for example, a major source of dextranases in the U.S. is from *Chaetomium* spp. fungi [31,32]. Although many of these enzymes have optimal pH and temperature ranges that are suitable for application during raw sugar production, there is the risk of inhibition by sugars, salts, oxidizers, metal ions, and chelators (like ethylenediaminetetraacetic acid, EDTA) [15,33].

Table 2. Selected bacterial amylase and dextranases reported in published literature.

Enzyme	Source	Opt. pH	Opt. Temp. (°C)	Enzyme mol. wt. (kDa)	Ref.
Amylase	*Chromohalobacter* sp. TVSP 101	7.0–9.0	65	72	[17]
Amylase	*Haloarcula hispanica*	6.5	50	43	[18]
Amylase	*Bacillus* sp. PS-7	6.5	60	71	[19]
Amylase	*Bacillus* sp. *Ferdowsicous*	4.5	70	53	[20]
Amylase	*Bacillus* sp. KR-8104	4.0–6.0	70–75	59	[21]
Amylase	*Bacillus dipsosauri* DD1	6.1	60	80	[22]
Amylase	*Lactobacillus manihotivorans* LMG 18010T	5.5	55	135	[23]
Dextranase	*Brevibacterium fuscum*	7.0–7.5	-- [1]	-- [1]	[24]
Dextranase	*Streptococcus mutans*	5.5	37	-- [1]	[25]
Dextranase	*Streptomyces anulatus* (two strains)	5.0–9.5	40 and 50	63 and 82	[26]
Dextranase	*Flavobacterium* sp. M-73	7.0	35	114	[27]
Dextranase	*Thermoanaerobacter wiegelii*	5.5	70	-- [1]	[28]
Dextranase	*Thermoanaerobacter* strain	4.5–5.5	80	150	[29]
Dextranase	*Thermoanaerobacterium thermosaccharolyticum*	5.5	65–70	200	[30]

[1] Entries with dashes (--) represent values that were not reported/available in the cited reference.

Oxidizing biocides, like bleach, affect microbial activity through oxidizing reactions involving the free chlorine content from sodium hypochlorite (NaClO). Sodium permanganate reacts through similar oxidation mechanisms involving permanganate anions, although recent work has suggested that it may be less efficacious and more cost-prohibitive

for widespread industry adoption as a biocide [34]. Additionally, biocide trials for sugarcane juices have typically used raw juices, which vary in the loading and composition of microbes. This has led to poor reproducibility and understanding of which microbes are susceptible to the tested biocide [12].

Carbamate biocides rely on the inactivation of enzymes involved in cellular respiration and other bacterial metabolic pathways [35]. Recent work in South Africa showed the effectiveness of two commercial carbamate biocides against several gum-producing bacterial isolates from a sugarcane factory; carbamates also apparently may not inhibit the effectiveness of dextranases [31,36]. Other non-oxidizing biocides, like hop extracts, use the effect of hop compounds to act as ionophores, ultimately interfering with essential enzyme reactions of hop-sensitive bacteria for microbial inhibition [37]. Biocide application levels are typically on the order of magnitude of 10 ppm [12,38]. Notably, yeasts and Gram-negative bacteria are not inhibited by hop compounds [39,40], although Gram-positive *Leuconostoc* is typically reported as the most abundant problematic bacteria in sugarcane processing [41]. It is also possible that oxidizers, like hypochlorite, may react with dextrans and starch polysaccharides. This may elicit modified enzymatic affinity for the degradation of these polysaccharides, as shown in previous work for corn starch [42]. Non-oxidizing biocides are not expected to participate in structure-modifying oxidation reactions with polysaccharides.

The simultaneous application of these processing aids (i.e., biocides and enzymes) into sugarcane juices, however, may cause unwanted interactions. It may be possible for the non-specific reactive nature of oxidizing biocides to interfere with or eliminate the effectiveness of enzymatic processing aids. For a sugarcane processing facility, this would result in the compounded negative effect of greater costs associated with processing aids, that would ultimately perform less effectively for the management of microbes and polysaccharides. The aim of this work is to explore the effects of (1) biocides on microbial growth activity for relevant sugarcane bacterial isolates, and (2) assess any potential impacts that biocides and processing aid enzymes may have on one another during sugarcane juice processing for raw sugar manufacturing.

2. Materials and Methods

2.1. Sugarcane Juice, Enzymes, and Biocides

Sugarcane juice was collected from factories in south Louisiana and transported on ice for less than 2 h before being stored frozen (at −20 °C) until use. All enzymatic activity experimental samples for this work utilized factory-mixed juice collected in the Fall season of 2020. Prior to further analyses, juice samples (2 L volume) were allowed to thaw under refrigeration for 48–72 h. Juice pH, Brix, and polarization (pol) were measured to verify relative consistency among juice samples used for processing aid experiments. The pH was measured with a HI991300 pH/EC/TDS meter (Hanna Instruments; Woonsocket, RI, USA). Brix was measured with a DR6000-T digital refractometer (A.Kruss Optronic; Hamburg, Germany), and pol was measured with an Autopol 880 automatic saccharimeter (Rudolph Research Analytical; Hackettstown, NJ, USA). In the context of sugar technology, Brix is defined as the percentage of dry substance (or dissolved solids) in a sugar solution, and pol is defined as the percentage of sucrose as measured using a polarimeter [43]. Sodium hypochlorite (5% free chlorine) was purchased from Acros Organics (now ThermoFisher Scientific; Waltham, MA, USA). Carbamate biocide (Magnacide D; dithiocarbamate compounds), hop extract biocide (Beta Stab 10A), bacterial amylase enzyme (Magnazyme S), and bacterial dextranase enzyme (Magnazyme DEX) were generously provided by a commercial supplier (PRO TECH International; Thibodaux, LA, USA).

2.2. Bacterial Isolations

Bacteria used in this study were isolated from sugarcane processing facilities in south Louisiana, as previously described [44,45]. The strains were isolated from sugarcane crusher juices and sugarcane mixed juices and were collected during the 2020 and 2021

processing seasons (approximately September to January). Bacteria utilized in this study are *Leuconostoc suionicum* strain LASM7; *Gluconobacter japonicus* strain LASM12; and *Pantoea dispersa* strain LASM22.

2.3. Susceptibility Testing of Bacterial Isolates

The susceptibility of bacterial isolates described previously [44] from sugarcane factories was determined based on the microdilution technique [46,47]. Precultures were grown for approximately 24 h in Tryptone-sucrose-yeast extract (TSY; modified from TGY [48]) medium containing 50 g/L sucrose at 28 °C with 250 rpm agitation using a MaxQ 6000 shaker (ThermoFisher Scientific; Waltham, MA, USA). Optical density (OD_{600}) was measured in a spectrophotometer (Evolution 201 UV-Vis.; ThermoFisher Scientific; Waltham, MA, USA) and precultures were used as inoculum for susceptibility testing in sterile 96-well plates at a starting OD_{600} of 0.05 in triplicate. Stock solutions of each biocide were used to make working solutions in TSY broth at 2000 ppm. Each biocide was tested using 2-fold serial dilutions in TSY broth in the microwell plate with final working concentrations ranging from 1000 ppm down to 1.95 ppm. Microwell plates were sealed with sterile breathable plate sealers (Aeraseal, Excel Scientific; Victorville, CA, USA) to prevent splashing between wells. Susceptibility assays were incubated at 28 °C, at 250 rpm for 18 h. Readings for OD_{600} of the microwell plate were measured on a BioTEK Synergy Neo2 plate reader with Gen5 3.03 software (BioTEK; Winoosky, VT, USA).

2.4. Enzyme Activity Measurement with Biocides

To assess residual enzyme activity after biocide addition, enzymes (5 ppm for Magnazyme S; 100 ppm for Magnazyme DEX), and biocides (at prescribed concentrations up to 500 ppm) were added to 100 mL of juice and allowed to react for 10 min (based on approximate factory residence times [6,31]) at room temperature before subsequent activity measurement. Although enzyme and biocide concentrations may exceed those in industrial application, the levels applied herein were chosen for laboratory procedural and reproducibility reasons. To avoid any potential thermal deactivating effects, all analyses were conducted at room temperature. Suitable enzyme concentrations were determined based on (approximate) minimum values needed for sufficient optical density readings in their respective assays. Residual amylase activity was measured using Phadebas® Amylase tablets (Phadebas AB; Kristianstad, Sweden), and residual dextranase activity was measured using a tablet assay (Dextrazyme) from Megazyme Ltd. (Neogen Corp.; Lansing, MI, USA) [49,50]. These assays rely on spectrophotometric measurements (accounting for blank samples) at 620 nm and 590 nm for amylase and dextranase activity, respectively. Further details for these protocols are available from test manufacturers [49,50].

For amylase activity measurements, prescribed quantities of amylase enzyme and biocide were concurrently added to sugarcane juice (as above) and stirred briefly. Sample volumes of 200 µL were pipetted into 10 mL centrifuge tubes followed by 4 mL of deionized water. Tubes were pre-incubated for 5 min at 37 °C in a water bath (Branson Ultrasonics Corporation; Brookfield, CT USA). Next, a Phadebas® tablet was added to each sample and vortexed (VWR analog vortex mixer; VWR International; Radnor, PA, USA) for 10 s. Mixed samples were then incubated for 15 min at 37 °C, and the reaction was stopped by the addition of sodium hydroxide (1.0 mL, 0.5 M) and subsequent vortexing for approximately 10 s. Samples were then centrifuged (Accuspin Micro17 centrifuge; ThermoFisher Scientific; Waltham, MA, USA) at 10,000× *g* for 5 min and transferred to cuvettes through a 0.45 µm polyvinylidene difluoride (PVDF) syringe filter (MilliporeSigma/Sigma-Aldrich; St. Louis, MO, USA). Amylase activity was determined using spectrophotometer (Evolution 201 UV-Vis.; ThermoFisher Scientific; Waltham, MA, USA) measurement of the absorbance at 620 nm.

For dextranase activity measurements, prescribed quantities of dextranase enzyme and biocide were added to sugarcane juice (as above) and stirred briefly. A 0.5 mL aliquot was transferred to a 15 mL test tube, which was then pre-incubated at 40 °C for 10 min in a water

bath (Branson Ultrasonics Corporation; Brookfield, CT, USA) without stirring. Tris buffer (10 mL, 2% w/v) was added and mixed by vortexing for approximately 10 s to terminate the reaction. Finally, a 1.5 mL aliquot was centrifuged (Accuspin Micro17 centrifuge; ThermoFisher Scientific; Waltham, MA, USA) at 10,000$\times$ g for 10 min and filtered through a PVDF syringe filter (MilliporeSigma/Sigma-Aldrich; St. Louis, MO, USA). Dextranase activity was determined using spectrophotometer measurement (Evolution 201 UV-Vis.; ThermoFisher Scientific; Waltham, MA, USA) of absorbance at 590 nm.

All experiments were run in at least duplicate, and experimental error is reported in subsequent figures as the standard error of the mean. Fisher's least significant difference procedure was used to assess for statistical significance, where Levene's test and Shapiro-Wilk test were employed to verify equality of variances and normality, respectively. Analyses and data visualization were performed in Microsoft Excel and Python (Jupyter notebook environment).

3. Results

3.1. Biocide Susceptibility Results

The results from biocide susceptibility testing are depicted for three biocides (carbamate, hop extract, and sodium hypochlorite) against relevant isolates. Figures 1–3 show the susceptibility of *Leuconostoc suionicum* isolate LASM7, *Gluconobacter japonicus* isolate LASM12, and *Pantoea dispersa* isolate LASM22, respectively. These strains were isolated from Louisiana sugarcane processing facilities and are representative of microbes that contribute to sucrose loss and EPS production [44]. Statistical analyses were not conducted for comparison among these data, but error bars are included as a quantification of experimental error/reproducibility.

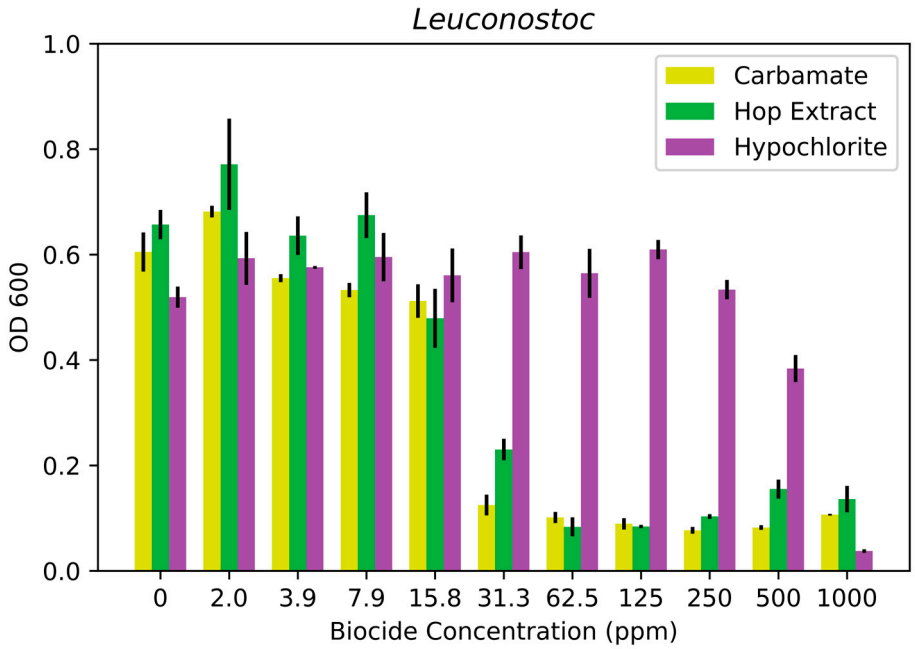

Figure 1. Testing of *Leuconostoc suionicum* strain LASM7 susceptibility to sodium hypochlorite, carbamate, and hop extract biocide concentrations ranging from 0 to 1000 ppm.

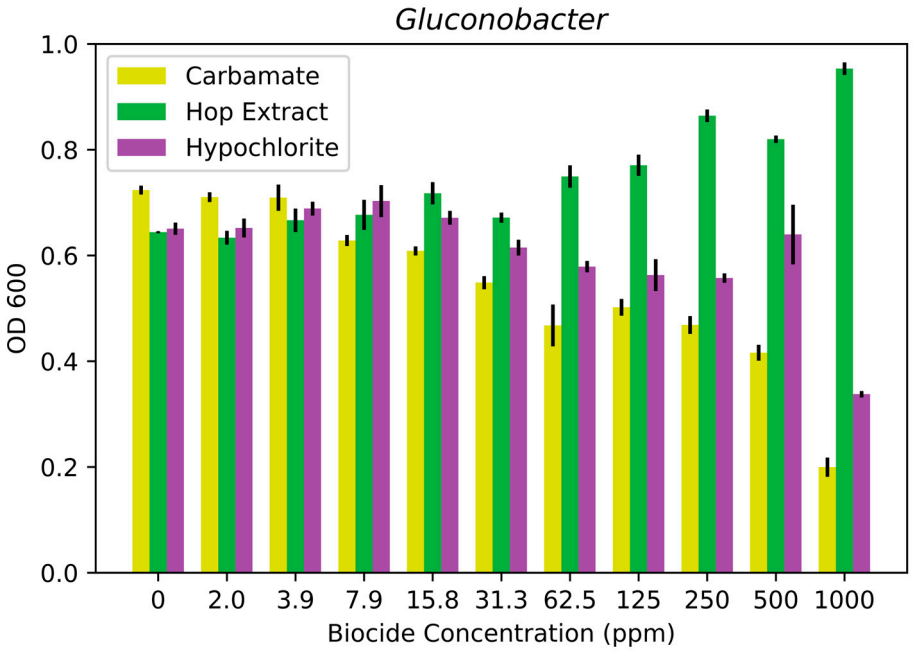

Figure 2. Testing of *Gluconobacter japonicus* strain LASM12 susceptibility to sodium hypochlorite, carbamate, and hop extract biocide concentrations ranging from 0 to 1000 ppm.

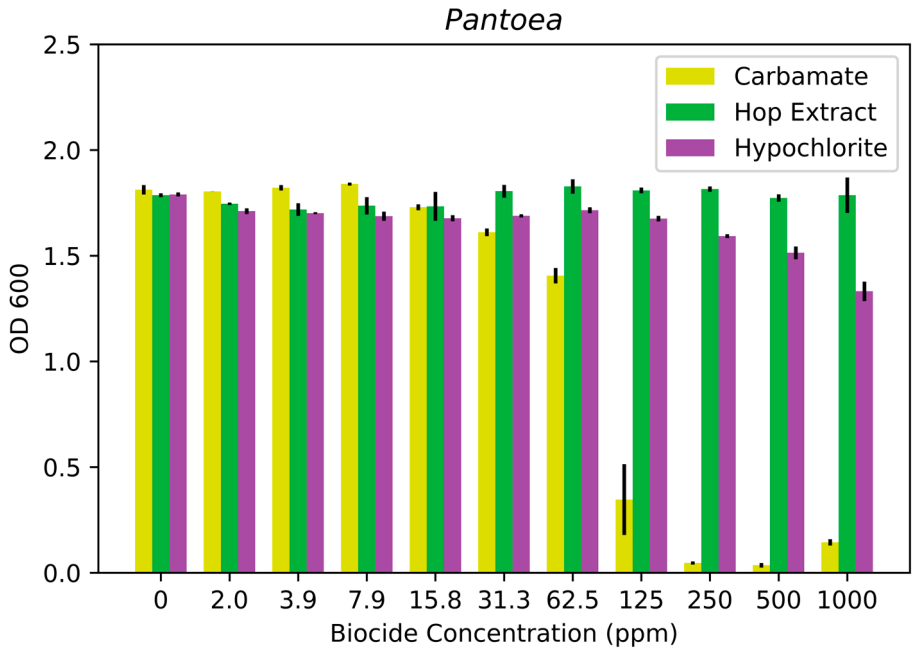

Figure 3. Testing of *Pantoea dispersa* strain LASM22 susceptibility to sodium hypochlorite, carbamate, and hop extract biocide concentrations ranging from 0 to 1000 ppm.

For *Leuconostoc suionicum* strain LASM7, the minimal inhibitory concentration (MIC) was approximately 60 ppm for both the carbamate and hop extract biocides. Modest inhibition was observed at 500 ppm sodium hypochlorite. Previous work suggests that the actual MIC for this strain is close to 600 ppm for sodium hypochlorite [44]. For *Gluconobacter japonicus* strain LASM12, hop extract had no observable biocidal effect, consistent with literature reports concerning Gram-negative bacteria [39,40]. The same is also true for Gram-negative *Pantoea dispersa* strain LASM22. Sodium hypochlorite and carbamate biocides show OD_{600} reductions of roughly 50% and 70% for *Gluconobacter japonicus* strain LASM12, respectively, at 1000 ppm. Sodium hypochlorite showed a very limited effect on

Pantoea dispersa strain LASM22, while the carbamate MIC level for this strain is between approximately 125 and 250 ppm. A summary of these results is given in Table 3.

Table 3. Observed approximate levels for minimum inhibitory concentrations (MIC) of three tested biocides against three tested bacterial isolates in this study.

Isolate	NaClO MIC	Carbamate MIC	Hop Extract MIC
Leuconostoc suionicum strain LASM7	>500 ppm	60 ppm	60 ppm
Gluconobacter japonicus strain LASM12	>1000 ppm	>1000 ppm	No effect
Pantoea dispersa strain LASM22	>1000 ppm	125–250 ppm	No effect

3.2. Enzyme Activity Results

Analyses of sugarcane juices used for experiments are given in Table 4. Measurements include pH, refractometer Brix, pol (from saccharimeter), and calculated purity (quotient of pol and Brix). Averages are given, with uncertainty reported as the standard error of the mean from four juice batches used in enzymatic activity experiments. In addition, the same data is given for the average of the first six weeks of mixed juice from a sugarcane processing facility in Louisiana (2022–2023 season). There is a general correspondence between measured parameters for (stored frozen) mixed juice samples used in this work and recent, early-season factory data from a Louisiana location.

Table 4. Mixed juice parameters for samples used in this work, in comparison with recent factory data from a Louisiana processing facility.

Parameter	Juice in This Work (Samples Stored Frozen, from Fall 2020)	Juice Data from 2022 to 2023 Season (Louisiana Factory, First Six Weeks)
pH	5.38 ± 0.15	5.37 ± 0.03
Brix	15.4 ± 0.8	15.6 ± 0.1
Pol	13.1 ± 0.9	13.1 ± 1.0
Purity (%)	84.6 ± 2.0	84.3 ± 0.3

Residual enzymatic activity was measured to quantify the remaining effectiveness of processing aid enzymes following concurrent addition with biocides. The results from these experiments are given in Figure 4 for amylase and Figure 5 for dextranase enzymes. In each case, statistical analyses suggest sufficiently normal distribution and equality of variances. For amylase experiments comparing residual enzyme activity with biocide addition, the one-way ANOVA result was statistically significant ($p < 0.05$). Dextranase ANOVA results were not statistically significant ($p > 0.05$). Post-hoc testing of amylase (5 ppm initial loading) experimental results was used to assess for statistical significance among biocide concentrations. Although this is an apparent dosage-dependent response in average residual amylase activity (reported as optical density (OD) at 620 nm for this assay), only the 500 ppm sodium hypochlorite sample was significantly different from the control with no biocide addition.

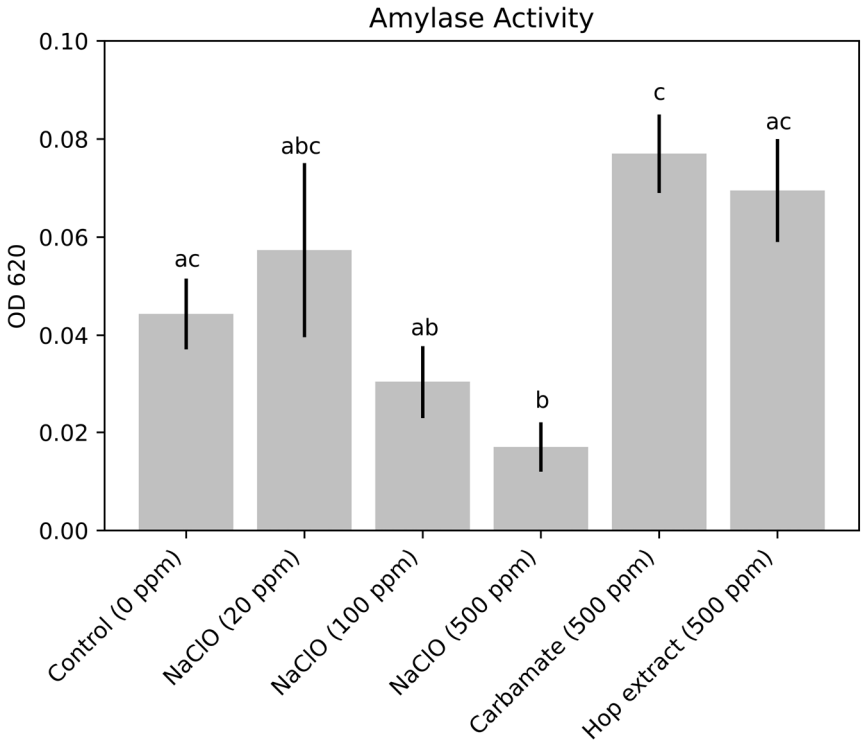

Figure 4. Residual amylase activity (reported as assay OD reading) for sodium hypochlorite (NaClO), carbamate, and hop extract biocide treatments (letters denote statistical significance).

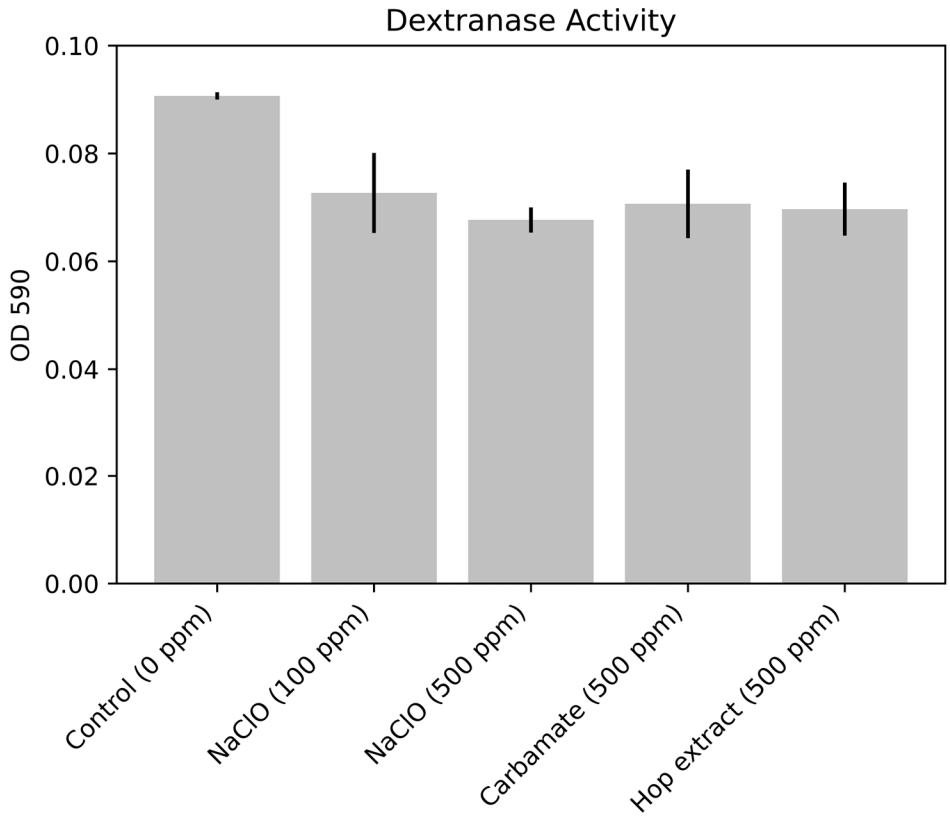

Figure 5. Residual dextranase activity (reported as assay OD reading) for sodium hypochlorite (NaClO), carbamate, and hop extract biocide treatments.

Post-hoc testing was not conducted for dextranase results (ANOVA not significant). A sodium hypochlorite concentration of 20 ppm was not analyzed in conjunction with dextranase due to higher dextranase loadings of 100 ppm necessary for adequate OD readings in the assay. In general, there is a drop-off in residual amylase activity following biocide addition, but further work is needed for more conclusive results. Conducting analysis in sugarcane juice may also contribute to larger uncertainties when taking spectrophotometric readings. As mentioned previously, although enzyme and biocide concentrations may exceed those in industrial application, the levels applied herein were chosen for laboratory procedural and reproducibility reasons.

4. Discussion

The results from the presented analyses provide evidence to suggest that there may be concerns with residual enzyme activity when an oxidizer, like sodium hypochlorite, is deployed in conjunction with dextranases and amylases. These observations have been similarly reported in relevant literature, briefly summarized in Table 5 [33,51–54]. Because sugarcane processing facilities may have greatly differing engineering practices, ultimately, decisions about how much, when, and where to apply various processing aids are left up to relevant operational personnel. It is certainly probable that the early addition of an oxidizer (like bleach applied during cane milling) may have no impact on amylases applied in a later, downstream unit operation. It is also probable that the spatially concurrent addition of enzymes and biocides can result in locally high processing aid concentrations, especially if there is an absence of well-mixed conditions. These considerations can be broadly extended to any biomass processing and/or agricultural industry, beyond sugarcane, that may employ both biocidal and enzymatic processing aids (for example, pulp and paper [55–57]; dairy processing [58,59]).

Table 5. Summary of previously reported results on residual enzyme activity in conjunction with various treatments (e.g., surfactants and oxidizers).

Enzyme	Summary	Ref.
Amylase from thermophilic *Bacillus* sp.	Residual activity is reported for amylase with the following inhibitors: sodium dodecylsulfate (SDS), EDTA, NaClO, H_2O_2 at concentrations of 5 mM. For EDTA and NaClO specifically, a time of 20 min showed between 40 and 50% activity, and a time of 60 min showed between 10 and 20% activity.	[33]
Amylase from *Bacillus* sp. PN5	Residual activity is reported for amylase with the following inhibitors: sodium dodecylsulfate (SDS), EDTA, NaClO, H_2O_2 at concentrations of 5 mM. For EDTA and NaClO specifically, a time of 20 min showed between 40 and 50% activity, and a time of 60 min showed between 10 and 20% activity.	[51]
Metagenome-derived Amylase P109	Residual activity was tested for oxidizing agents H_2O_2 and NaClO at concentrations of 5, 10, 15, 20, and 25 mM and the reducing agent β-mercaptoethanol (BME). All three reagents showed dosage-dependent increasing levels of inhibition, with BME having the greatest residual activities and NaClO having the least (between 50 and 60%).	[52]
Amylase from marine *Streptomyces* sp. D1	Residual activity of approximately 80% is reported for both NaClO and H_2O_2 at concentrations of 1% w/v. A gradual, dosage-depended decline in residual activity is shown for both oxidizers, although concentrations of 0.2% show nearly 100% residual activity.	[53]

Table 5. *Cont.*

Enzyme	Summary	Ref.
Glucan 1,6-α-isomaltosidase from *Arthrobacter globiformis*	Enzyme with reported substrate specificity for several native NRRL dextrans. Stable at pH between 3.0 and 8.0, with 90% residual activity following heating to 60 °C. Reported inhibitors include some metal ions (Ag^{2+}, Hg^{2+}, Fe^{3+}) and potassium permanganate oxidizer.	[54]

The most abundant literature on this subject is related to starch oxidation and amylase; it was considerably more difficult to find any comparable studies involving dextran and dextranases. Previous work has also examined the effects of sodium hypochlorite on starch oxidation reactions, which form carboxyl and carbonyls in a modified starch matrix [42,60,61]. In general, these studies report starch oxidation to occur at sodium hypochlorite loadings between 0.1 and 1% (1000 and 10,000 ppm), which are larger than concentrations for the inhibition of enzymatic activity and susceptibility for bacteria studied herein. Other work has reported that oxidation reactions in starch can occur at glucose moiety C2 and C3 positions, creating steric hindrance that may limit subsequent amylase activity [62,63]. An example of potential oxidation pathways of starch with hypochlorite is given in Figure 6 [64].

Furthermore, concentrations of sodium hypochlorite that show appreciable biocidal effects, based on the results of the work herein, exceed those necessary which potentially inhibit enzymatic activity. These concentrations are higher than what is typically found in factories, raising questions about the efficacy of sodium hypochlorite at current application levels in industry. These results warrant further research, especially at an industrial scale, if possible. Conversely, observations in this study suggest that non-oxidizing biocides (i.e., carbamates and hop extracts) may have biocidal utility without subsequently causing dextranase and amylase inhibition (with the caveat of overall higher costs). Additionally, while hop extract effectively inhibited the Gram-positive *Leuconostoc suionicum* isolate LASM7, Gram-negative bacteria that are also present in juice and/or biofilms (e.g., *Gluconobacter japonicus* isolate LASM12 and *Pantoea dispersa* isolate LASM22) can continue to consume sucrose and produce EPS in the presence of hop extracts.

Future work can assess in greater depth the enzymatic activity of amylase and dextranases toward breaking down oxidized starches and dextrans in solution over a range of relevant concentrations, as well as synergistic effects with combinations of biocidal treatments. Potential steric hindrance from oxidized glucose moieties presents interesting challenges/considerations for the degradation of (oxidized) polysaccharides. Emphasis must also be given to conducting these kinds of experiments under industrially relevant sugar-processing conditions, with acidic, sugar-containing media (as in Table 4). Finally, in comparison to starch/amylase studies, there is a relative lack of similar studies on dextran/dextranase (and fructan analogs). This could also be an emphasis or goal for future work.

There are also opportunities for advancement regarding the ways in which biocide effectiveness is studied in the context of sugar crop processing and raw sugar manufacturing. As mentioned previously, bacterial loadings and/or populations are dynamic across sugar mills and during processing seasons. This may present issues with conducting reproducible analyses using juice samples alone. The research presented herein seeks to circumvent this challenge with a more fundamental approach that relies on cultured bacterial isolations. Biocides can then be tested against relevant, individual isolates. Future work can also explore novel biocides in greater depth, as presented in a recent sugar beet study using plant extracts and/or essential oils [65], which tend to be generally recognized as safe for food/agriculture processing applications.

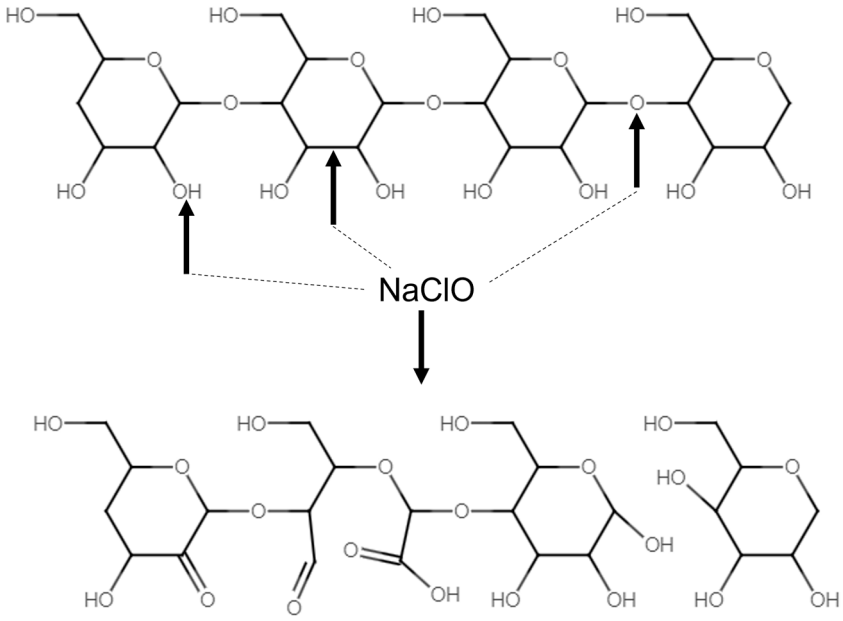

Figure 6. Potential mechanisms for sodium hypochlorite oxidation (not accounting for stereochemistry; molecular shapes and/or bond angles are presented for illustration only) of glucose moieties in starch polysaccharides. Adapted from Vanier NL, et al., Copyright (2017), with permission from Elsevier [64].

5. Conclusions

In this study, the effects of oxidizing (sodium hypochlorite) and commercial non-oxidizing biocide (carbamate and hop extract) treatments on residual enzymatic processing aid (amylase and dextranase) activity were assessed. In addition, the susceptibility of industrially relevant bacteria strains (*Leuconostoc*, *Gluconobacter*, and *Pantoea*) was measured with the same biocides. Results for residual amylase activity experiments suggest that at a sufficiently high loading (approximately 500 ppm), sodium hypochlorite may inhibit enzyme activity. Non-oxidizing biocides did not show the same level of inhibition. Dextranase results were less statistically conclusive in comparison to amylase, although some level of enzyme activity loss was observed from biocide treatments. Susceptibility testing showed that higher sodium hypochlorite concentrations (greater than 500 ppm) were necessary for bacteria inhibition in all strains. Hop extract biocide showed inhibition of Gram-positive *Leuconostoc* at roughly 60 ppm, but it did not inhibit the Gram-negative *Gluconobacter* and *Pantoea* strains. Carbamate biocide showed more broad-spectrum activity, with minimal inhibitory concentrations around 60 ppm for the *Leuconostoc* strain and 125 to 250 ppm for the *Pantoea* strain, although inhibition of *Gluconobacter* was more limited. Future work will continue to explore the influence of combinations of biocides and enzymes in sugarcane processing for raw sugar production, with potentially greater emphasis given to the assessment of managing bacterially derived polysaccharides like dextrans and levan fructans.

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