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Research Article

Wort disinfection treatment with electron beam for bioethanol production

Rubens Perez Calegari[1]*, Eric Alberto da Silva[2], Ana Paula Maria da Silva[3], Marcelo Pego Gomes[3], Layna Amorim Mota[1], Valter Arthur[1], Antonio Sampaio Baptista[3]

[1]Universidade de São Paulo/CENA, Av. Centenário, 303 – 13416-000 – Piracicaba, SP – Brasil.
[2]Universidade de São Paulo/IPEN, Av. Lineu Prestes, 2242 – 05508-000 – São Paulo, SP – Brasil.
[3]Universidade de São Paulo/ESALQ – Depto. de Agroindústria, Alimentos e Nutrição, Av. Pádua Dias, 11 – 13418-900 – Piracicaba, SP – Brasil.
*Corresponding author <rubenscalegari@live.com>

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ABSTRACT: Microbial contamination of the wort during the fermentation process causes significant losses in ethanol production worldwide and creates a dependence of the industry on chemicals and antibiotics to control contamination. Therefore, this study used electron beam (e-beam) to disinfect wort from sugarcane (*Saccharum officinarum* L.) molasses and investigate the bioethanol fermentation. Four treatments (T0 – T3) were carried out using ionizing doses of radiation through the electron accelerator: 0 (control), 10, 20, and 40 kGy. Total mesophiles, total bacteria, sucrose, glucose, fructose, phenolics, flavonoids, hydroxymethylfurfural (5-HMF), and Furfural were measured. An alcoholic fermentation assay was performed after the irradiation process. The irradiated treatments showed no inversion of sugars and formation of the inhibitory by-products flavonoids, furfural and 5-HMF, except for the phenolic compounds. The lower dose tested (10 kGy) reduced more than 99.9 % of the total mesophiles and more than 99.99 % of the total bacteria in the substrate. In the fermentation, the irradiated worts presented similar ($p > 0.05$) yields (92, 93, and 94 %) and ethanol productivity levels (0.89, 0.88, and 0.87 g L^{-1} h^{-1}, for T1, T2, and T3 respectively). However, all treatments presented higher yields and productivity ($p < 0.05$) when compared to the control (88 % and 0.85 g L^{-1} h^{-1}), highlighting the possible use of e-beam in wort fermentation at a lower dose (10 kGy). This allows reduction in losses caused by microbial contamination, besides increasing fermentation yield and productivity with lower energy consumption.

Keywords: alcoholic fermentation, biofuel, electron accelerator, microbial contamination, reduction of contamination

Introduction

The efficiency of yeast to turn sugar from sugarcane (*Saccharum officinarum* L.) juice or molasses into alcohol depends on the wort quality. In industrial production, wort is not usually sterilized prior to the fermentation process, which allows the entry of many microbial contaminants into the process, affecting efficiency and productivity (Amorim et al., 2011; Lopes et al., 2016). The formation of acids, increased flocculation, and reduction of yeast viability are among the significant losses caused by microbial contamination. The population of contaminating bacteria in fermentation can reach levels higher than 10^8 cells mL^{-1}, significantly reducing ethanol production (Ceccato-Antonini, 2018).

In Brazil, sugarcane mills usually perform the yeast cream treatment with acid (Melle-Boinot process), which requires a considerable volume of sulfuric acid (8 to 10 g per liter of ethanol generated) to reduce bacterial contamination (Basso et al., 2008; Costa et al., 2018; Silva-Neto et al., 2020). Although repeated countless times throughout the year crop (Brown et al., 2013), this process is not entirely efficient (Ceccato-Antonini, 2018), generating resistant bacteria and promoting osmotic stress of yeasts. Thus, reducing fermentation yield (Basso et al., 2011). Furthermore, wild yeast strains rapidly contaminate the fermentation and, after a few cycles, only wild strains survive the acid treatment (Brown et al., 2013). Another way to control bacterial contamination in distilleries is the use of antibiotics; nevertheless, resistance to drugs is a limiting factor in the contamination control efficiency due to bacterial resistance (Muthaiyan et al., 2011).

Electron beam (e-beam) is ionizing radiation (IR) and is somewhat effective for microorganisms inactivation. E-beam generally depends on the radiation dose applied, where the logarithmic number of microorganisms decreases linearly with increasing doses (Sampa et al., 2007). Furthermore, e-beam is a very safe and cold method requiring a a short exposure time. In e-beam, the dose applied is the control parameter of the method. Moreover, e-beam is an on-off technology that operates with electric power and has a higher dosing rate than other radiation technologies, such as gamma (γ) and X-rays (Silindir and Özer, 2009). Therefore, the use of e-beam shows potential for controlling wort contamination, because it can be installed in the production line and use part of the energy surplus produced by the industrial plant, providing more efficiency to alcoholic fermentation processes.

In this study, we assessed the application of e-beam to control wort contamination in sugarcane molasses for alcoholic fermentation. We investigated the efficacy and yield of alcoholic fermentation from wort treated with e-beam ionizing radiation and evaluated the electric energy consumption of e-beam.

Materials and Methods

Materials

The sugarcane molasses used for wort preparation in alcoholic fermentation were obtained from a sugarcane mill in the municipality of Charqueada, São Paulo State, Brazil (22°31′05″ S, 47°42′49″ W, altitude 571 m) and stored in a freezer (–20 °C) shortly after collection.

Wort clarification

The molasses underwent the clarification process by adding of 2.5 g L^{-1} of NaH_2PO_4 to the boiling molasses. Afterward, the molasses were autoclaved and kept for 48 h to separate the supernatant from the sedimented material (Sica et al., 2021).

At the end of the clarification step, the molasses with an initial concentration of 70 °Brix, 628.74 g L^{-1} of total reducing sugars were diluted with distilled water to obtain the final concentration of 16.7 °Brix (150 g L^{-1} of total reducing sugars).

Preparation of contaminating inoculum and inoculation

To prepare the contaminant inoculum, we collected a sample of 10 g of soil from several points in a cane field to simulate the groups of contaminating microorganisms usually found in alcoholic fermentation at sugarcane mills. This sample was mixed with 90 mL of the clarified molasses, filtered with a quantitative filter paper N.640, 125 mm and, placed in a 250 mL Erlenmeyer flask. This Erlenmeyer was kept at 30 °C under stirring at 100 RPM for 24 h, using an orbital shaker incubator.

After 24 h, the inoculum suspension reached 2.01×10^{12} CFU mL^{-1} (Colony Forming Unit) of total mesophiles and 1.32×10^{12} CFU mL^{-1} of total bacteria. Then, the inoculum was used to contaminate the wort. The final concentration in the wort was 1×10^{7} CFU mL^{-1} of total mesophiles.

Treatments

The wort used in the investigation was subject to four treatments. The control treatment – without elimination of contaminants microorganisms (T0) – and three treatments using different ionization radiation doses from e-beam source: 10 kGy (T1), 20 kGy (T2), and 40 kGy (T3). An e-beam accelerator irradiated the samples.

We used the batch irradiation process because of the small volume of wort processed. The wort was added to borosilicate rectangular glass vessels and packed with polyvinyl chloride (PVC) plastic film 0.1 mm to avoid contamination after the irradiation process. Each vessel received 300 mL of wort from clarified molasses, corresponding to 4 mm of a sample height. Four vessels were irradiated for each batch.

The electron accelerator was set to the energy source, the width and current of the e-beam to 2.4×10^{-13} J (1.5 MeV), 0.112 m and 5.61×10^{-3} A, respectively. The tray speed was 0.112 m s^{-1}, proportional dose of 5 kGy per run.

Evaluation of contamination control

The growth of total bacteria and total mesophiles were measured by the logarithmic variation based on the number of CFU: Log (CFU mL^{-1} + 1). It was added 1 to the treatments that presented 0 CFU, as the result of Log 0 is an undefined value.

The D_{10} (required dose to destroy 90 % of the population or 1 log) for total bacteria and total mesophiles were calculated in kGy, according to Eq. (1), where N_0 is the initial CFU mL^{-1} and N_{final} is the CFU mL^{-1} after irradiation.

$$D_{10} = \frac{applyed\ dose}{Log_{10}N_0 - Log_{10}N_{final}} \quad (1)$$

The efficiency control of the microorganisms was calculated according to Eq. (2).

$$Efficiency\ of\ control\ (\%) = \left(\frac{N_0 - N_{final}}{N_0}\right) \times 100 \quad (2)$$

Chemical and microbiological analyses

After the treatments, the materials underwent chemical and microbiological analyses, as described below.

Total mesophiles and total bacteria

For the enumeration of the microbial contamination levels, the total mesophiles and total bacteria were measured using an aseptic sample of 1 mL of the wort and serially diluted with 9 mL of saline solution (0.9 % NaCl in distilled water).

After the serial dilution, the samples were poured plated in Plate Count Agar (PCA) to determine the total mesophiles and in PCA with 10 mg L^{-1} of cicloheximide to determine the total bacteria. All platings were performed in triplicate with incubation at 30 °C for 48 h.

Sugars, glycerol, and mannitol

Sucrose, glucose, fructose, glycerol, and mannitol were measured by ion chromatography, following the method described by Eith et al. (2006). The equipment used was an ion chromatograph equipped with an amperometric detector and with a Metrosep Carb 1 –150/4.0 column. The eluent was NaOH 200mM solution with 1.0 mL min^{-1} flow under 35 °C for 9 min.

The wort samples were diluted 200 times, and the wine samples were diluted 50 times with ultrapure water. Afterward, the samples were filtered with 0.45 µm cellulose acetate filter. All samples were measured in triplicate and the volume of sample injected was 20 µL.

Furfural and 5-HMF

The furfural and 5-HMF compounds were measured by gas chromatography with Flame Ionization Detector (FID), following the method 72 described by the United States Department of Labor Occupational Administration Safety and Health (1988). The wort samples were filtered with 0.45 µm cellulose acetate membrane and the injection volume of the sample was 2.0 µL.

Total phenolics

The total phenolics concentration of the wort samples were determined according to the Folin-Ciocalteu method described by Julkunentiitto (1985). The samples were diluted with ultrapure water 25 times and measured in triplicate.

Total flavonoids

The total flavonoids concentration of the wort samples were measured according to Mabry et al. (2012) with modifications described by Braga et al. (2021). The samples were diluted with ethanol (70 % v v^{-1}) 50 times and measured in triplicate.

Fermentation

The worts of all treatments were subjected to the fermentation process, conducted in 500 mL Erlenmeyer flasks containing 200 mL of wort with 16.7 °Brix, 150 g L^{-1} of total reducing sugars, and 3 % of dry yeast *Saccharomyces cerevisiae* "Fleischmann".

The fermentation process was conducted with five replicates (reactors) per treatment at 30 °C under 100 RPM stirring using an orbital shaker incubator. The process was monitored through CO_2 losses from the reactors during the fermentation. At the end of the fermentation process, yeast cell viability and total bacteria were measured and the fermented wort was centrifuged at 3738.8 *g* at 10 °C for 10 min. Then, the wine was immediately frozen (–20 °C) for further chemical analyses.

The chemical and microbiological analyses of the wines were: total bacteria, sucrose, glucose, fructose, glycerol, and mannitol, as previously described, as well as yeast cell viability, yeast cell biomass, and alcohol content, as described below.

Yeast cell viability

The yeast cell viability was determined according to Pierce (1970) by the differential staining of living and dead cells using 0.1 % methylene blue solution and observation in a Neubauer chamber (0.0025 mm^2) using an optical microscope (400x). This analysis was performed at the beginning and the end of the fermentation process.

Yeast cell biomass

The wet weight determined the yeast cell biomass. The fermented wort was centrifuged at 3738.8 g, at 10 °C for 10 min. Then, the pellet mass was measured on a semi-analytical scale and compared to the initial mass of the sample.

Alcohol content

The wine alcohol content was measured by the distillation of 25 mL of sample in a micro-distiller followed by measuring the density with a Digital Densimeter at 20 ± 0.05 °C (Basso et al., 2008; Sica et al., 2021). The density value of the solution was used to calculate the alcohol concentration of the sample by converting the density read into %w w^{-1} using a conversion table at 20 °C / 20 °C.

Fermentation yield and productivity

The practical yield (*Yp*) was calculated based on the volume of ethanol obtained from 100 g of sugars supplied in the substrate according to Eq. (3).

$$Pratical\ yield\ (Yp) = \left(\frac{mL\ of\ final\ ethanol}{g\ of\ sugars\ supplied}\right) \times 100 \quad (3)$$

The theoretical yield (*Yt*) was calculated based on Gay-Lussac optimal yield, which defines that 1.00 g of total reducing sugars (TRS) generates 0.5111 g of ethanol. Therefore, the fermentation efficiency (ηP) was calculated based on Eq. (4).

$$\eta P(\%) = \left(\frac{Yp}{Yt}\right) \times 100 \quad (4)$$

The productivity was calculated according to Eq. (5) based on the alcohol content at the end of the fermentation and the fermentation time. The yield was expressed in grams of ethanol per hour (g L^{-1} h^{-1}).

$$Productivy = \frac{ethanol\ concentration\ in\ the\ wine\ g\ L^{-1}}{fermentation\ time\ (h)} \quad (5)$$

Electrical consumption estimation

To estimate the energy cost to operate the electron accelerator used in this study, initially we calculated the energy consumption in KWh of the e-beam operating with a voltage of 1.5×10^{6} V and electric current of 5.61×10^{-3} A for 1 h, which is the power required to irradiate a sample with a dose of 5 kGy approximately. The total energy consumed by the accelerator peripherals, such as the cooling system, vacuum system, and compressed air, was determined through the nominal values of the power supplied by the equipment manufacturers.

The sucroenergetic industry in Brazil is self-sufficient regarding electricity, and it is also an exporter of the energy surplus produced. Thus, the energy consumption needed for the electron accelerator would no longer be commercialized.

Therefore, the MWh value was equivalent to the average amount that would be paid to the sugarcane mill, according to the electric energy commercialization contracts of the Brazilian Electricity Regulatory Agency (ANEEL). For this calculation, it was considered that the amount paid for 1 MWh of the energy surplus produced by burning biomass (sugarcane bagasse and wood chips) by sugarcane mills in Brazil through public bidding processes held by ANEEL for energy distributors (CCEE, 2021).

According to the bidding processes held in Mar 2016 and Apr 2017 (energy supplied respectively in 2020 and 2021), the average amount paid for each MWh of energy was US$ 42.50 or US$ 0.0425 kWh^{-1} (ANEEL, 2021). The dollar rate (US$ 1.00 = R$ 5.53) was consulted on 21 Mar 2021 on the website of the Brazil Central Bank (BCB, 2021).

Experimental design and statistical analysis

The experimental design was entirely randomized with four treatments and five replicates per treatment. The results were subjected to analysis of variance (ANOVA) by the F test, and the averages were compared in the Tukey test at the significance level of 5 % ($p \leq 0.05$). The statistical analyses were performed using SISVAR 5.6 software.

Results and Discussion

Wort irradiation

The demand for renewable biofuels has increased to reach the targets for GHG emission reduction. Therefore, industrial yield needs to be improved and better control of the microbiological contamination is necessary.

During the season, the reuse of yeast cells influences the wort contamination level by bacteria and wild yeasts (Brexó and Sant'Ana, 2017; Lopes et al., 2016). The development and predominance of wild strains of yeasts are undesirable for the process due to the lower yield, flocculation, foaming, and biofilm formation produced by these microorganisms (Beckner et al., 2011; Della-Bianca et al., 2013; Della-Bianca and Gombert, 2013) These drawbacks increase the use of antifoams, acids, and antibiotics in ethanol production at sugarcane mills (Brexó and Sant'Ana, 2017).

The wort samples subjected to treatments T0, T1, T2, and T3 presented 7.00, 3.72, 3.3, and 2.41 Log CFU mL^{-1} ($p < 0.05$) of total mesophiles, respectively (Figure 1). These results correspond to an efficiency of total mesophiles control of 99.95 %, 99.98 %, and > 99.99 %, ($p < 0.05$) for T1, T2, and T3, respectively.

For total bacteria, T0, T1, T2, and T3 presented 5.00, 2.92, 3.22, and 2.33 Log CFU mL^{-1} of total bacteria, respectively (Figure 2). These results correspond to an efficiency of bacterial control of 99.99 %, 99.98 %, and > 99.99 % for T1, T2, and T3, respectively.

Therefore, wort microbial contamination decreased as the irradiation dose increased, agreeing with Sampa et al. (2007). The authors describe that when the irradiation dose increases, the logarithm of the number of microorganisms per volume decreases linearly.

Nobre et al. (2007) treated sugarcane juice with ionizing radiation (γ – Co^{60}) and reported that the dose of 15 kGy was not enough to inactivate the *Bacillus subtillis* culture entirely, but there was a reduction of more than 99.9 % of these bacteria. In our study, the dose of 10 kGy provided inactivation of total bacteria higher than 99.9 % and similar results were observed for total mesophiles. Furthermore, Nobre et al. (2007) used pure bacteria cultures while we used the total microbiota from a sugarcane field soil in our study.

Most studies on microbial radioresistance are based on reports of experiments typically involving pure cultures grown under near-optimal conditions (Shuryak, 2019). Our study used microbiota from the soil of a sugarcane field, as it presents many microorganisms groups at different levels. In addition, other authors

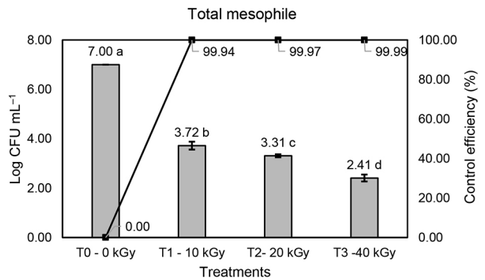

Figure 1 – Total mesophiles in sugarcane molasses wort after electron beam irradiation treatment at different doses. The error bars represent the standard deviation. CFU = Colony Forming Unit.

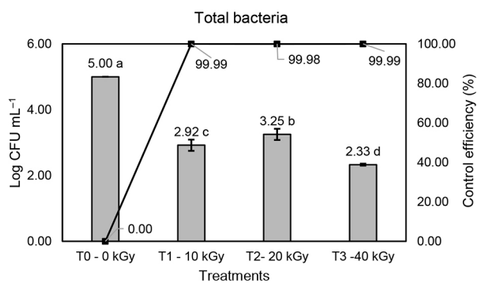

Figure 2 – Total bacteria in sugarcane molasses wort after electron beam irradiation treatment at different doses. The error bars represent the standard deviation. CFU = Colony Forming Unit.

have reported that bacterial contamination in alcoholic fermentation is primarily from the sugarcane field soils (Costa et al., 2015).

Costa et al. (2015) assessed microbial diversity at different stages of sugarcane ethanol production and identified 22 archaeal groups, 203 fungi groups, and 355 bacterial groups. The authors also mentioned that the microbial contamination increased through the sugarcane mill processes primarily from feedstock and soil impurities.

Many soil microorganisms are organic matter decomposers and opportunistic plant/animal pathogens (Diezmann and Dietrich, 2009; Sykes et al., 2014). This requires tolerating and possibly exploring the oxidizing compounds used as a defense mechanism by their hosts (Heller and Tudzynski, 2011), justifying the high radiotolerance of soil microorganisms. In addition, some microorganisms can synthesize antioxidant compounds and pigments that aid in radioprotection (Kim et al., 2007), such as vitamin C (Mao et al., 2006), carotenoids (Jain et al., 2015), and flavonoids (Molins, 2001; Shuryak, 2019; Shuryak et al., 2017). These compounds are commonly found in sugarcane juice (Abbas et al., 2014) and occur in the sugarcane molasses, the raw material used in our study (Table 1).

The D_{10} (required dose to destroy 90 % of the population) for total mesophiles was 3.06 kGy, whereas D_{10} was 4.81 kGy for total bacteria. Bacteria (prokaryotic) were described as more radioresistant than other microorganisms, such as fungi and viruses. Two cellular mechanisms of prokaryotes explain their radioresistance: the DNA and proteome protection induced by IR, and extensive and very complex DNA repair systems (Pavlopoulou et al., 2016), justifying that the total bacteria D_{10} is higher than the total mesophiles. In addition, the values found to follow the literature, which states that fungi and bacterial spores present D_{10} values between 1 and 10 kGy (Jung et al., 2017; Shuryak et al., 2017).

Other studies report on radioresistant microorganisms, namely fungi with chronic and acute radioresistance (D_{10} from 0.1 to 6.5 kGy) (Shuryak et al., 2017) and bacteria, such as *Deinococcus radiodurans*, which can withstand high radiation doses (D_{10} of 16 kGy) (Omelchenko et al., 2005) and can reconstruct the functional genome (Lim et al., 2019), also archaea, such as *Thermococcus gammatolerans* sp. nov., which was isolated after exposure to 30 kGy (γ- radiation) (Jolivet et al., 2003). Moreover, *Lactobacillus plantarum*, one of the major contaminants of alcoholic fermentation (Dellias et al., 2018; Dong et al., 2015), is described as a chronic and acute radioresistant microorganism (Daly et al., 2004; Shuryak et al., 2017). This shows that even the higher dose of 40 kGy was insufficient to eliminate all the contaminant microorganisms.

Most bacterial contaminants are found in the *Lactobacillus* genera (Bonatelli et al., 2017), especially lactic acid bacteria (LAB), such as *L. plantarum*, which are responsible for reducing yeast cell viability, due to the competition for nutrients and the production of toxic compounds, such as lactic and acetic acids during the fermentation process (Costa et al., 2008; Narendranath et al., 1997). These acids decrease sugar consumption, inhibit yeast growth, and decrease ethanol production (Seo et al., 2020).

In general, sugarcane mills use antibiotics to control bacterial contamination. However, in some cases, antibiotics do not prevent *Lactobacilli* infection recurrence, since these microorganisms can form a biofilm, which is tolerant to the high concentrations of antibiotics and cleaning (Dellias et al., 2018; Saunders et al., 2019).

The large-scale use of antibiotics can induce bacterial resistance (Carvalho et al., 2020). Besides, antibiotic residues, such as virginiamycin, can be found in distiller's dried grain (DDG) from bioethanol fermentation of corn, which is used as animal feed (Bischoff et al., 2016). Regarding sugarcane bioethanol, there is a concern about antibiotic resistance in microorganisms that may be discharged into the environment through the fertigation use of vinasse, the liquid waste from wine distillation (Mendonça et al., 2016). Furthermore, antibiotics in the vinasse can negatively affect its anaerobic digestion for biogas production by inhibiting acetogenic bacteria and methanogenic archaea (Sanz et al., 1996) and reducing the potential to use vinasse to produce other products.

Therefore, a more efficient disinfection process is needed, such as ionizing radiation (IR). However, IR may promote the formation of inhibiting by-products from sugar degradation (Molins, 2001). In our study, formation and alteration did not occur in the concentration of the inhibiting flavonoids, furfural, and 5-HMF ($p > 0.05$) in any condition of treatment

Table 1 – Chemical determinations of inhibitory by-products in wort from sugarcane molasses after treatments.

Treatment	Flavonoids	Phenolics	Furfural	5-HMF
		μg mL^{-1}		
T0 - Control	26.74 ± 1.57[a]	925.27 ± 13.72[c]	0.61 ± 0.00[a]	0.44 ± 0.00[a]
T1 - 10 kGy	23.47 ± 5.22[a]	980.68 ± 11.74[b]	0.64 ± 0.02[a]	0.44 ± 0.02[a]
T2 - 20 kGy	25.18 ± 7.92[a]	1012.64 ± 16.20[b]	0.63 ± 0.02[a]	0.43 ± 0.02[a]
T3 - 40 kGy	25.44 ± 1.11[a]	1089.38 ± 9.94[a]	0.62 ± 0.01[a]	0.43 ± 0.01[a]
CV	19.19	1.31	2.30	3.95

CV = Coefficient of Variation. Averages of n = 5 ± standard deviation. Superscript equal letters in the same column do not differ statistically by the Tukey test at 1 % level of significance.

evaluated (Table 1). These compounds are generally produced from sugar degradation, especially in thermal conditions (Chi et al., 2019; Eggleston and Amorim, 2006; Molins, 2001); however, they did not occur in our study. Therefore, we recommend doses between 10 and 40 kGy to reduce contamination and avoid the formation of inhibitors.

Aldehydes, such as furfural and 5-HMF, may inhibit key enzymes that affect the rate of protein synthesis of the central metabolism of yeasts, hindering growth and fermentation (Cabañas et al., 2019). Therefore, the presence of these compounds is highly unwanted in the fermentation substrate. On the other hand, there was a gradual increase in phenolic compound levels (6 %, 9.4 %, and 17.8 % for T1, T2, and T3, respectively), according to the radiation dose applied (Table 1).

The degradation of carbohydrates, especially D-glucose, D-xylose and L-arabinose can be related to the production of compounds, such as phenolics (Rasmussen et al., 2014). These compounds are considered biocatalyst inhibitors (Chi et al., 2019), and Lima et al. (2016) reported gradual production according to e-beam dose increase. However, in our study, the presence of phenolics did not inhibit yeast cell viability and biomass production ($p > 0.05$) in any treatment during the fermentation process (Table 3).

Martín et al. (2007) investigated sugarcane bagasse hydrolysate and reported that 2100 µg mL^{-1} of phenolic compounds concentration was responsible for yeast (*S. cerevisiae*) inhibition and consequently, poor fermentability. The authors adapted a strain of the same yeast and observed a higher ethanol yield on total sugar after 24 h (0.38 g g^{-1}) than the non-adapted yeast (0.18 g g^{-1}) in a wort with 1,400 µg mL^{-1} of phenolic compounds. In our study, although the yeast was not adapted to inhibitory toxins, the concentration of phenolics was below 1089.38 µg mL^{-1} (Table 1) in all fermented treatments, which probably reflected the *S. cerevisiae* tolerance to these compounds.

In addition to the low formation of inhibitors, there was no significant inversion of Total Reducing Sugars (TRS) in all treatments ($p > 0.05$) nor a decrease in sucrose concentration in irradiated treatments ($p > 0.05$) (Table 2). On the other hand, Lima et al. (2016) observed significant ($p < 0.05$) TRS inversion in sugarcane juice irradiated with 20 kGy e-beam dose.

There was no reduction in TRS concentration, which is interesting because low sugar degradation is essential in a decontamination method to avoid a decrease in ethanol efficiency due to the sugar degradation (Alcarde et al., 2003, 2001).

Fermentation process

In the fermentation process, sucrose was not detected in any treatment wine. In addition, glucose and fructose presented low concentrations (< 0.1 %) in the residual sugars in all treatments ($p > 0.05$), evidencing the efficient consumption of sugars by yeasts or other microorganisms during the fermentation process (Table 3).

The glycerol concentration was similar in wine from all treatments, approximately 15 grams per liter ($p > 0.05$). Bai et al. (2008) indicate that a level of about 1 % (w v^{-1}; 10 g L^{-1}) of glycerol is commonly produced during the fermentation process. The high glycerol concentration in wine can indicate a yeast response to adversity. High sugar values lead to high glycerol concentrations in the wort due to the increase in osmotic pressure (Ponce et al., 2016), and the presence of bacterial contamination (Li et al., 2009).

There was bacterial contamination in the wine of all treatments; however, the control treatment presented a higher value ($p < 0.05$) of 5.55 log CFU mL^{-1}. The presence of bacteria in all the treatments may be due to contamination during the experiment sampling and poor asepsis.

Like the high bacterial contamination, the control treatment (T0) presented a higher concentration of mannitol (0.41g L^{-1}) when compared to other treatments ($p < 0.05$). Mannitol is a sensitive contamination indicator, and its presence indicates the enzymatic dehydrogenation of fructose by bacteria (Eggleston et al., 2007). According to Eggleston et al. (2007), high mannitol concentrations may promote yeast flocculation and reduce the efficiency and yield of fermentation. The authors also described that a concentration around 6 g L^{-1} of mannitol could decrease ethanol yield by 4 %.

Fermentation of the control treatment (T0) showed the lowest efficiency (88 %) of the treatments ($p < 0.05$) due to high bacterial contamination in wine (5.55 log CFU mL^{-1}) and conversion of sugars into metabolites,

Table 2 – Behavior of sugars, glycerol, and mannitol in wort from sugarcane molasses after treatments.

Treatment	Sucrose	Fructose	Glucose	Glycerol	Mannitol	TRS
	g L^{-1}					
T0 - Control	102.03 ± 0.09^{a}	22.47 ± 0.31^{a}	22.55 ± 0.14^{a}	<LoQ	0.28 ± 0.01^{a}	152.15 ± 0.52^{a}
T1 - 10 kGy	102.77 ± 0.47^{a}	21.04 ± 0.89^{ab}	22.24 ± 1.43^{a}	<LoQ	0.28 ± 0.01^{a}	151.19 ± 2.44^{a}
T2 - 20 kGy	103.30 ± 3.79^{a}	20.04 ± 1.00^{b}	20.88 ± 1.55^{a}	<LoQ	0.30 ± 0.06^{a}	149.38 ± 4.33^{a}
T3 - 40 kGy	99.65 ± 0.09^{a}	20.35 ± 0.54^{b}	21.00 ± 0.65^{a}	<LoQ	0.27 ± 0.01^{a}	145.99 ± 1.19^{a}
CV	1.96	3.19	4.6	0	9.88	1.72

TRS = Total Reducing Sugars; < LoQ = lower than Limit of Quantification; CV = Coefficient of Variation. Averages of n = 5 ± standard deviation. Superscript equal letters in the same column do not differ statistically by the Tukey test at 5 % level of significance.

Table 3 – Microbiological and biochemical parameters after fermentation.

Treatment	Total bacteria	pH	Produced during the fermentation: Glycerol	Mannitol	Sucrose	Glucose	Fructose	Alcohol Content	Yeast biomass	Productivity	Yield	Yeast viability
	Log CFU mL^{-1}		g L^{-1}					w w^{-1}	g mL^{-1}	g L^{-1} h^{-1}	%	
T0 - Control	5.55 ± 0.37^{a}	4.38 ± 0.06^{bc}	15.51 ± 0.67^{a}	0.41 ± 0.02^{a}	<LoQ	0.25 ± 0.02^{bc}	0.10 ± 0.02^{b}	6.82 ± 0.19^{b}	0.14 ± 0.01^{a}	0.85 ± 0.02^{b}	88 ± 2^{b}	81 ± 2^{a}
T1 - 10 kGy	2.68 ± 0.40^{b}	4.44 ± 0.11^{b}	15.56 ± 0.25^{a}	0.39 ± 0.01^{ab}	<LoQ	0.23 ± 0.07^{c}	0.13 ± 0.02^{ab}	7.09 ± 0.13^{a}	0.15 ± 0.00^{a}	0.89 ± 0.02^{a}	92 ± 2^{a}	80 ± 2^{a}
T2 - 20 kGy	2.25 ± 0.05^{b}	4.27 ± 0.02^{c}	15.81 ± 0.52^{a}	0.38 ± 0.02^{b}	<LoQ	0.37 ± 0.28^{a}	0.14 ± 0.04^{a}	7.09 ± 0.04^{a}	0.15 ± 0.00^{a}	0.88 ± 0.01^{a}	93 ± 1^{a}	79 ± 2^{a}
T3 - 40 kGy	2.60 ± 0.49^{b}	4.58 ± 0.05^{a}	15.97 ± 0.31^{a}	0.32 ± 0.03^{c}	<LoQ	0.31 ± 0.44^{ab}	0.12 ± 0.01^{ab}	6.97 ± 0.13^{ab}	0.16 ± 0.02^{a}	0.87 ± 0.02^{ab}	94 ± 2^{a}	81 ± 2^{a}
CV	11.17	1.51	2.72	3.51	0	13.59	16.43	1.94	5.85	1.72	1.99	2.79

CFU = Colony Forming Unit; <LoQ = Lower than Limit of Quantification; CV = coefficient of variation. Averages of n = 5 ± standard deviation. Superscript equal letters in the same column do not differ statistically by the Tukey test at 5 % level of significance.

such as glycerol and mannitol (Table 3). Fermentation yields of T1 (92 %), T2 (93 %), and T3 (94 %) were significantly similar ($p > 0.05$) and higher than usually fed-batch industrial fermentations with 87 % average using molasses as raw material (Andrietta and Maugeri, 1994; Viegas et al., 2002).

The dose of 10 kGy (T1) also showed a greater fermentative yield than the efficiency described by Alcarde et al. (2001), who achieved 90.56 % in the fermentation of sugarcane juice treated with 10 kGy (γ radiation).

There is great importance and interest in increasing the yield of industrial fermentation. A yield of 92 % could mean a significant increase in ethanol production and thus in the revenue of sugarcane mills.

Ethanol productivity decreased with contamination, whereas fermentation of the control treatment showed the lowest value ($p < 0.05$) of 0.85 g L^{-1} h^{-1} (Table 3). The highest yields were achieved in T1 (0.89 g L^{-1} h^{-1}) and T2 (0.88 g L^{-1} h^{-1}) ($p > 0.05$).

Treatment T1 (10 kGy) is the most recommended since it presented similar ($p > 0.05$) efficiency and yield to T2 (20 kGy) and required less energy consumption to reduce the microbial contaminants.

Main changes in the fermentative behavior of the irradiated wort could have been observed if consecutive fermentative recycles and acid yeast treatment of the control treatment were carried out. Since the microbial contamination tended to increase throughout the fermentative recycles during the harvest season (Ceccato-Antonini, 2018), the differences between irradiated and non-irradiated wort could possibly have been more evident. This technology needs further studies and an increase in scale and economic viability.

Estimation of electrical energy consumption

In our study, electrical energy consumption to operate the electron accelerator at full power for one hour was approximately 150 kWh, and the e-beam alone accounted for 25 % (37.5 kWh) of this total. The cooling, vacuum, compressed air, and other devices consumed the remaining 75 % (112.5 kWh). In this case, the cost was US$ 6.43 per hour of use of the electron accelerator.

In our study, the e-beam was not used at its maximum power. Thus, for each hour of accelerator use, the electrical energy consumption was 122 kWh and the devices of each system mentioned above consumed 113.58 kWh. The e-beam consumed only 8.42 kWh. The cost was about US$ 5.23 per hour of use of the electron accelerator.

The operating cost considering only energy consumption of the electron accelerator for each treatment is presented in Table 4. Δt is the processing time (or sterilization) of the samples by e-beam. Their values were obtained considering the conveyor speed of 0.112 m s^{-1} and the linear length of two aligned trays equal to 0.40 m.

Table 4 – Operating cost of electron beam for different radiation doses applied to sugarcane molasses wort.

Treatment	Radiation dose	Δt	Cost
	kGy	s	US$
T0	0	0	0
T1	10	11.6	0.0644
T2	20	23.2	0.128
T3	40	46.4	0.258

Therefore, operating at a dose of 10 kGy, energy consumption by the electron accelerator is estimated at 129.34 kWh (16.34 kWh consumed by e-beam and the rest by the peripheral equipment). The cost of each hour of operation of the accelerator is estimated at US$ 5.54. Sugarcane mills in Brazil produce an average of 450 m^3 of wort per hour; thus, the estimated cost of processing 1 m^3 of sugar cane is US$ 0.012.

Sugarcane mills can process 1 m^3 of the wort in a short time. Furthermore, the e-beam technology is fast and the desired result in the microbial control can be achieved in a few seconds, which allows the treatment of large wort volumes in a short time, facilitating the process of implementation at large enterprises.

Recommendations

The disinfection of wort is required in the ethanol industry, and effective control of contamination needs an adequate system to clean fermenters, pipelines, centrifuges, valves, and other compartments to transport or store wine, yeast cream, and wort.

Therefore, the use of e-beam to sterilize the substrate could ensure productive yeast strains in the fermentation process, such as the thermotolerant strains of *S. cerevisiae*, described by Pattanakittivorakul et al. (2019), which show highly ethanol production at 40 °C as well as tolerance to high gravity fermentation and high concentrations of furfural, 5-HMF, and acetic acid.

Conclusions

The lower dose of 10 kGy reduced more than 99.9 % of the total mesophiles and more than 99.99 % of the total bacteria in the substrate. In addition, there was no production of the inhibiting compounds furfural, 5-HMF, and flavonoids in all doses tested.

All the irradiation treatments (10, 20, and 40 kGy) presented similar fermentation efficiency and ethanol yield. However, all showed significantly higher fermentation efficiency and ethanol yield when compared to the control.

The energy cost estimation by the electron accelerator to operate at a dose of 10 kGy was estimated at US$ 0.012 per m^3 of processed wort. This evidenced the possibility of using e-beam in wort treatment with a lower dose of 10 kGy, which may reduce losses caused by microbial contamination, promoting fermentation efficiency and yield gain with lower energy consumption.

Authors' Contributions

Conceptualization: Calegari, R.P.; Arthur, V.; Baptista, A.S. **Data acquisition**: Calegari, R.P.; da Silva, E.A.; Gomes, M.P. **Data analysis**: Calegari, R.P.; da Silva, E.A.; da Silva, A.P.M.; Gomes, M.P.; Mota, L.A. **Design of methodology**: Calegari, R.P.; da Silva, E.A.; Arthur, V.; Baptista, A.S. **Writing and editing**: Calegari, R.P.; Baptista, A.S.

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