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Review

Electron Beam on Fermentation Medium as an Alternative Disinfection Method for Ethanol Distilleries: A Comprehensive Review

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Abstract: Corn and sugarcane are the primary feedstocks for ethanol production, but microbial contamination hinders yeast fermentation efficiency. Current control methods include antibiotics and sulfuric acid, but they have limitations, resulting in dependence on external inputs and the risk of antibiotic-resistant bacteria. This review examines electron beam technology as an industrial-scale disinfection solution for both corn- and sugarcane-based ethanol production, highlighting its advantages, limitations and opportunities for adoption in Brazil. A critical evaluation highlights the importance of optimal operating conditions for scalability, cost effectiveness and sustainable implementation. Through a practical example, we demonstrate the effectiveness of electron beam treatment in improving fermentation efficiency and reducing contamination-related losses. Notably, the ionizing radiation from this process does not affect wort sugar content or generate radioactive residues. While acknowledging the potentially high energy input requirements, cogeneration in sugarcane mills can address this, making it a viable option; however, further technical and economic evaluation should be made. In addition, electron beam technology is a promising approach for the production of high-value products such as neutral alcohol, amino acids, animal feed and pharmaceuticals. Therefore, this comprehensive review provides valuable insights for researchers, industry stakeholders and policymakers to promote sustainable and efficient ethanol production practices.

Keywords: electron beam; biorefinery; bioprocess; bioproduct; distillery; contaminant; sterilization

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1. Introduction

Brazil is the second largest producer of ethanol, accounting for 27% of world production [1]. The ethanol is mainly produced from sugarcane, and the residual biomass from the bagasse is used for cogeneration energy in the boilers. The bagasse meets the energy needs of industrial plants, and the extra energy produced is commercialized in the grid as bioelectricity, representing a significant part of the Brazilian energy matrix [2,3].

The United States established an ethanol production chain to reduce its energy dependence on imported oil [1], emphasizing ethanol as a major alternative energy source to fossil

fuels [1], and improve air quality in major cities [4]. In the 2000s, the U.S. produced about 6.2 billion liters of corn-based ethanol, and the scale increased to about 49.3 billion liters in the next decade [1]. U.S. technology is often considered the standard for corn-based ethanol production. However, Brazilian corn-based ethanol has a lower carbon footprint, reducing greenhouse gas emissions by 83% compared to its fossil fuel equivalent. This reduction in its carbon footprint is mainly achieved by substituting natural gas with alternative biomasses such as wood chips [1,4,5].

Until 2014, Brazilian ethanol was exclusively produced from sugarcane. However, the emergence of local corn surpluses in the central-west region and the limited infrastructure for transporting the cereal to ports, which are approximately 2000 km away, made it an interesting feedstock for ethanol production [6,7]. Thus, in the last decade, corn ethanol has witnessed a significant increase and has contributed more than 14% of ethanol production [8]. However, one of the main limitations of ethanol production from corn [9] and sugarcane [10] is the presence of contaminants, which can reduce the fermentation efficiency and produce undesirable by-products [11]. Currently, antibiotics are used to control microorganisms, but, in addition to increasing the risk of producing antibiotic-resistant microorganisms, antibiotics also remain in the yeast, which can be used as an ingredient in animal feed [12–14].

Recent studies have proposed the use of an electron beam as a disinfection treatment for sugarcane, corn and mixed worts [11,15,16]. However, to the best of our knowledge, there is no comprehensive literature review that evaluates the advantages and disadvantages of adopting this technology on an industrial scale for ethanol production. Therefore, in this review, we highlight the limitations of the electron beam, but also show the opportunities for the adoption of this technology in the context of ethanol production in Brazil. We also demonstrate the efficiency of this process through a practical example.

2. Contaminating Microorganisms

Even in the current ethanol production systems, the vulnerability associated with microbial contamination is present and has a significant impact [17]. Contaminating microorganisms cause significant losses to the processes in terms of efficiency and productivity [18,19]. Losses are even higher when the population of contaminating bacteria in wort for fermentation reaches levels higher than 10^7 cells per mL [20], and it can even reach between 10^8 and 10^9 bacterial cells per milliliter [21].

Contaminants in ethanol production are primarily derived from mineral impurities carried with the raw material from the field to the industry [22–24]. Several studies have shown that ethanol distilleries, whether sugarcane- or corn-based, have a higher bacteria diversity. Contaminating bacteria can generate substances that retard the fermentation process or compete with the yeast for sugars and nutrients in the wort [23]. Wild yeast is another microorganism that can significantly affect the fermentation process in addition to bacterial contamination [17,25].

Effects of Contaminants on Wort

The presence of contaminants in the wort can affect fermentation by causing reduced cell viability, increased yeast flocculation and acid formation, resulting in lower process efficiency and productivity [18,26–28]. If the population of contaminating bacteria in the wort exceeds 10^7 cells per mL, it can cause a significant reduction in alcohol yield, up to 55% of the expected theoretical value [20]. Contamination levels ranging from 10^8 to 10^9 bacterial cells per mL can result in a reduction of up to 90% in alcohol yield [21]. It represents a production loss of between 10 and 30 thousand liters of ethanol per day for a distillery with a daily production capacity of one million liters [29].

Wild yeasts can dominate the fermentation process since they have an aggressive nature and higher tolerance to acid treatment, antibiotics and high concentrations of sugars and ethanol in the medium [30]. To mitigate these issues, it is important to carefully monitor and control the fermentation process, as the presence of wild yeasts can cause problems

such as yeast cell flocculation, excessive foam formation and reduced yeast cell viability, leading to decreased process productivity and efficiency indicators [31]. These effects result in several losses during the yeast recovery process (Melle-Boinot) [32,33], including:

i. Yeast cream with a lower yeast cell concentration;
ii. A larger volume of wine undesirably directed to acid treatment;
iii. A higher buffering capacity of the yeast cream due to dilution in a higher proportion of wine;
iv. A higher dose of acid required for acid treatment of the yeast cream;
v. Reduced yeast cell viability during acid treatment;
vi. Increased cost of performing this acid treatment process.

In addition, flocculating yeast can cause yeast cells to settle to the bottom of fermentation tanks, resulting in reduced productivity and efficiency during the fermentation process [29]. Contamination also prevents the production of wine with consistent microbiological characteristics throughout the season, which, in turn, hinders the production of specialty alcohols such as neutral alcohol. Another negative effect of contamination is the lack of stability in the non-aseptic production process, which makes potentially applicable techniques and technologies not feasible [25], including practices such as recycling and recovery of the same standard yeast strain [32,34,35].

3. Current Control Methods

Ethanol distilleries apply several methods to reduce or control contamination [36,37]. Measures such as using sulfuric acid for yeast acidification or antibiotics for infection control can control bacterial contamination levels to reduce fermentation losses but do not completely eliminate the population of contaminating microorganisms [38]. In fact, these methods can lead to the selection of tolerant bacteria and wild yeast strains, which end up dominating the process [17,39].

It is important to note that ethanol plants have not achieved optimal conditions yet. This means that complete inactivation of microorganisms in the wort is not practiced, and contaminants and their metabolites are present throughout the system, resulting in suboptimal fermentation performance [10,17,38,40]. Contamination hinders process enhancements, reduces fermentation efficiency and decreases industry revenue.

The use of certain antibiotics may promote the development of bacterial antibiotic resistance [41]. These antibiotics do not prevent reinfection by *Lactobacillus* spp., which can form biofilms on the inner linings of industrial pipes, thereby increasing their tolerance to high concentrations of antibiotics [42,43].

For the selected yeast strain introduced into the process as a fermentation agent, acid treatment can be detrimental, causing osmotic stress and reducing fermentation efficiency [31]. Furthermore, there may be potential hazards associated with residual antibiotics present in the yeast retrieved at the end of the process [44]; these are particularly relevant in systems using this material for animal feed [44,45]. Certain antibiotics, such as virginiamycin, persist in both yeast and distiller's dried grains (DDGs), resulting in undesired residual effects in high-quality animal feed products [45]. This applies to commercialized yeasts, WDG or DDG [32].

Brazilian ethanol plants have been exploring alternatives to antibiotics for wort disinfection. Existing methods for microorganism inactivation require further adjustments to ensure sustainability and overcome limitations for immediate large-scale implementation in distillery wort treatment. The use of steam is both time and energy intensive [46], while gamma radiation demands a prolonged treatment duration due to the low dose rate of cobalt-60 [47]. Biological control methods, such as bacteriophages, have been investigated as natural bacterial antagonists [48]. Furthermore, chemical compounds with bacteriostatic and bactericidal activities have been tested [49]. Recent studies have proposed the use of an electron beam as a disinfection treatment for sugarcane, corn and mixed worts [11,15,16]

4. Electron Beam

Based on the experience reported in production systems, ionizing radiation can be an effective alternative for the elimination of pathogenic and spoilage microorganisms [32,50,51]. This technology has been successfully tested by various researchers as a means of controlling microorganisms in sugary solutions [47,52,53] while preserving the nutritional and sensory qualities of the material [54]. Gamma radiation is known as an alternative for the decontamination of sugarcane juice [55], though it has a low dose rate and high time of residence [47], while recent studies have also assessed the irradiation of mixed worts [11,56].

Radiation is a form of energy in transit that propagates from an emitting source [57]. When this energy is capable of displacing electrons from atoms, it is classified as ionizing radiation. This results in the production of ions [58,59], which are electrically charged due to the gain or loss of electrons [60]. The displaced electrons dissipate their energy through interactions with electrons and nuclei of other atoms, potentially leading to the successive formation of new ions [60–62].

When an atom loses electrons, the entire molecular structure can be compromised due to the instantaneous rearrangement of electrons in search of a more stable configuration. This search can result in the loss of the chemical identity of the molecule involved and the formation of molecules that are foreign to the environment [60,62]. These interactions between ionizing radiation and matter can be classified as either direct or indirect. Direct action occurs when ionizing radiation directly hits a portion of DNA or RNA or other vital components of a cell and causes a loss of the physical integrity of the genetic material, leading to cell failure.

Indirect action occurs when vital components of cells are affected by highly reactive chemical compounds formed after the interaction of ionizing radiation and water [61,63,64]. This interaction, known as water radiolysis [58,62,65], leads to the formation of free radicals [66], capable of interacting with other molecules and causing their oxidation, reduction, dissociation or degradation [61,65,67]. Water molecules are the most abundant in a biological organism or in an aqueous solution such as wort, making this interaction a critical phenomenon for indirect inactivation of microorganisms [58,60,68,69].

The major radiation-induced damages to intracellular DNA are related to chemical changes in purines, pyrimidines and deoxyribose, as well as physicochemical damage that can result in single- or double-strand breaks in the phosphodiester structure [70]. The variation in the sensitivity of microorganisms to radiation is attributed to variability of their physical and chemical structures and their capability to recover after radiation-induced damage [54,58].

For this reason, the energy required to manage microorganisms varies depending on the resistance of the species and the microbial load [54]. Additional factors affect the susceptibility of vegetative cells to irradiation, such as the composition of the medium, moisture content, irradiation temperature and the presence or absence of oxygen, and whether the product is fresh or frozen can also have an impact [51,71,72]. Spores or endospores are resistant structures and tend to be more resilient than vegetative cells, often requiring higher doses of ionizing radiation for their inactivation [16]. In any case, the radiation dose applied must strike a balance between what can be tolerated in the product without undesirable changes and what is necessary to achieve the desired effects [50,65,72]. Establishing minimum doses is of paramount importance when the goal is to eliminate pathogenic organisms [54,73,74].

Gamma rays, X-rays and electrons differ only in the primary radiation that interacts with the material. After the initial interaction, the energy transport mechanism inside the product is the same for all three types of radiation, with secondary electrons (electrons ejected from their orbits) predominating and producing the majority of excitations and ionizations [54,58,65,68].

The electron beam is generated in electron accelerators, which can be defined as systems in which a voltage difference is established between a cathode and an anode

inside a vacuum tube. Electron beams are released from the cathode, also called cathode rays. The accelerated electrons are characterized by low penetration and high dose rate (kGy s^{-1}) [32], which significantly reduces the treatment time of the material [65]. This characteristic makes it possible to use the electron beam in production lines with high throughput [32,54].

However, unlike that of photons, the range of an accelerated electron in a medium is finite [75]. In other words, the thickness of the material to be treated and its density are determining factors in the application of accelerated electrons [15]. On the other hand, accelerated electron processing is much faster than gamma (60Co) processing due to the dose rates [47,52]. This is a decisive factor from a technical and operational point of view [65]. Irradiation of sugars leads to the production of acids, carbonyl compounds and other compounds that are highly dependent on specific medium conditions [76]. The use of ionizing radiation has been successfully tested for microbial control in sugar solutions [54].

The inactivation of microorganisms in production lines is of interest to prevent infection and process inefficiency [32]. The use of ionizing radiation is an alternative treatment with advanced oxidative processes that allow the control of the microbial load [65]. However, the operational conditions require a radiation source that allows the composition of a dynamic method that meets the scale and production flow, as well as economic aspects [47,71].

The amount of energy from ionizing radiation is called the radiation dose and can be measured in gray (Gy = J kg^{-1}) [50]. The amount of absorbed dose is called the D10 dose, and it helps to numerically evaluate the radiosensitivity of a microorganism to radiation. It represents the dose capable of reducing the population of microorganisms in the irradiated product by one logarithmic cycle, corresponding to 90% of the initial number [77]. Equation (1) is used to calculate the *D*10 value.

$$D10 = \frac{Di}{Log\ N0 - Log\ Nf} \quad (1)$$

where *Di* = irradiation dosage (kGy); *N*0 = the initial contaminant's colony-forming units (UFC mL^{-1}); and Nf = ln(*n*/*r*), where *n* = total amount of material irradiated *Di*, and *r* = the amount of material that did not have microbial growth.

4.1. Electron Beam Application

An electron beam as ionizing radiation [55,78] is a potential option for wort treatment [11] for different types of fermentation [32,79]. Electron accelerators were originally designed for scientific research. The first facilities used for industrial purposes were installed in the 1950s. They are now used in various industries to improve the physical, chemical and microbiological properties of materials [80]. These devices are of electrical origin, which allows them to be switched on or off instantly. In order for the entire material to be exposed to the electron beam and receive the minimum dose necessary to achieve the desired effect [72], the solution to be treated is presented to the beam in an upflow stream. The solution is pumped vertically inside the channel, undergoes treatment and flows out the sides to be collected [65].

Electron accelerators are DC (direct current) machines, like industrial equipment. They are known for their robustness and high reliability, allowing an operational availability of at least 8000 h per year [65].

They have advantages such as the following:

- The possibility of applying a high dose rate, which allows a larger volume of wort to be treated per unit of time [54];
- Investment equivalent with costs to achieve the same treatment capacity as other techniques [81];
- Greater ease of licensing and acceptance by the community since radiation emission stops as soon as the electrical power supply is interrupted [15];
- Ease of operation and control of the electron beam [65];

- In the case of ethanol production in Brazil, the high energy demand can be met by the cogeneration.

Easy operation and control of the activity of the electron accelerator as an on–off system makes electron beam application technology even more interesting for use in agro-industry [11,52,54,65]. Moreover, its limitation in terms of low penetration capacity in materials [69] is easily overcome by the countercurrent operating condition [65], which exposes the solution in a thin layer under controlled flow [47]. It is worth noting that the radiation doses used for material treatment do not generate radioisotopes or radioactive waste [32].

4.2. Effects of Electron Beam on Wort Treatment

Podadera (2007) and Lima et al. (2016) emphasized that irradiation of a sugary solution may result in the possible disruption of glycosidic linkages [54,82]. However, this effect would only result in the formation of reducing sugars (monosaccharides) at the expense of the disaccharides present in the substrate, without any loss of total sugars. Other products, such as hydrogen peroxide, carbon dioxide and formic acid, may be formed on the treated substrate by secondary reactions of irradiation [61,71].

Calegari et al. (2023) [16] did not find the formation of flavonoids and aldehydes, such as furfural and 5-HMF, which are generally produced from sugar degradation, using doses of up to 40 kGy of electron beam [83]. The breakdown of carbohydrates, especially D-xylose, D-glucose and L-arabinose, may generate compounds such as phenolics [84], which are known to inhibit the biocatalyst [82,83].

It is also important to highlight that the moment of wort treatment in the distillery plant is immediately before the yeast inoculation and start of fermentation. The electron beam does not have any radioactive waste in wort; it is an immediate treatment and does not damage the yeast [32]. On the other hand, authors report improved fermentation efficiency in worts that have been irradiated [47,85].

Douradinho et al. (2024) did not observe any yeast inhibition in the fermentative process using a 20 kGy dosage [32]. Moreover, the authors reported a fermentation improvement when the process was conducted in wort irradiated with an electron beam. They observed the inactivation of wort contaminants, which led to an increase in the yield, productivity and efficiency of fermentation by 2.6%, 0.21 g L^{-1} h^{-1} and 4.7%, respectively [32].

5. A Practical Example of Wort Irradiation

5.1. Materials and Irradiation

In Table 1, there is a description of the worts used in this study and the treatments they were subjected to. Following the tendency of feedstock integration in corn-based ethanol plants in Brazil [22,86], the worts had different compositions to be evaluated under contaminants and irradiation conditions. They were obtained from sugarcane juice (S) and from 39% of the solids of corn hydrolyzed in cane juice (CS) and in water (C).

Table 1. Composition of worts obtained from corn, cane and feedstock integration.

Abbreviation	C0	C20	CS0	CS20	S0	S20
Description	Corn hydrolyzed in water with no radiation	Corn hydrolyzed in water irradiated with 20 kGy	Corn hydrolyzed in cane juice with no radiation	Corn hydrolyzed in cane juice irradiated with 20 kGy	Sugarcane juice with no radiation	Sugarcane juice irradiated with 20 kGy
Corn (g)	639		639		0	
Water (mL)	1000		0		0	
Juice (mL)	0		1000		1000	
Fermentable sugars from juice (g L^{-1})	0		58		304	
Fermentable sugars in wort (g L^{-1})	302		342		304	

The corn used in this study was obtained from an agricultural supply store located in the municipality of Piracicaba, near ESALQ. To obtain fragments with a particle size below 2 mm, the corn grains were subjected to hammer milling using an automated sieve. The syrup was obtained from concentrated sugarcane juice at a sugarcane mill in the municipality of Piracicaba, São Paulo, Brazil. The syrup had a soluble solids content of 69° BRIX. It was then clarified with lime and monobasic sodium phosphate, following the procedure outlined by Sica et al. (2021) [22], after dilution.

The cane syrup was diluted to a concentration of 300 g of fermentable sugars per liter (L^{-1}), representing the treatment using only sugarcane-derived substrate. For the corn wort, 639 g of corn was added to one liter of distilled water. In the case of the mixed wort (CS), 639 g of corn was combined with one liter of diluted syrup, which contained 58 g of fermentable sugars per liter (L) [32].

Distilled water and diluted syrup were preheated to 55 °C. An 80 mg amount of Liquozyme® α-amylase enzyme (Novozymes, Copenhagen, Denmark) was added, followed by corn particulates. The system was heated for approximately 40 min until the temperature reached stability at 88 °C. An additional 80 mg of the same α-amylase enzyme was introduced once the temperature hit 88 °C. The mixture was consistently stirred at 80 rpm for 150 min [15].

Once the liquefaction of corn starch was complete, the system was gradually cooled until the temperature stabilized at 65 °C. At this juncture, the mixture had a pH of 5.0, requiring no further adjustments. Under these established conditions, 224 mg of Spirizyme® glucoamylase enzyme (Novozymes, Copenhagen, Denmark) was introduced, and the system was consistently stirred at 80 rpm for the saccharification process [15]. Following the completion of saccharification, the amalgam of corn particles and sugar solution underwent centrifugation using a Thermo Scientific® (Waltham, MA, USA) horizontal centrifuge, model Sorvall ST40R, with a rotational speed of 10,000 rpm (3924× *g*) at a temperature of 5 °C for a duration of 10 min. The sugar solutions obtained as supernatant from centrifugation were subsequently filtered through a 210 µm sieve and utilized in the preparation of worts.

To simulate the microbiological contamination commonly found in industrial processes [24,40], we intentionally added 10 g L^{-1} of the mineral impurities present in the raw materials to the wort. This mixture was agitated for 12 h at 30 °C, creating a representative microbial contaminant similar to those typically observed in industrial settings [23,87,88]. The contaminated wort was then divided into six equal portions. Three of them (S20, CS20 and C20) were irradiated with an electron beam at a 20 kGy dose. The other three portions (S0, CS0 and C0) were not irradiated (0 kGy).

The wort was irradiated using the industrial electron accelerator Dynamitron DC1500/25/4—JOB188 (Radiation Dynamics Inc., Edgewood, NY, USA) located at the Technological Radiation Center (CTR) of the Institute of Energy and Nuclear Research—IPEN/USP. The accelerator operates at a power of 150 kW, a beam energy of 1.5 MeV, an electric current of 5.61 mA and a beam width of 1.12 m. An electron beam is emitted with a dose rate of 19.99 kGy s^{-1} (19.99 kJ kg^{-1} s^{-1}) [65]. The worts were arranged in glass containers with a layer thickness of 4 mm to facilitate penetration by the electron beam [32]. Dosimeters were used to monitor and confirm the dose uniformity and consistency, validating the irradiation process [89].

Bacterial and total mesophilic counts were determined using the serial dilution and pour plate technique on Plate Count Agar (PCA) medium, following the protocols outlined by Oliveira et al. (1996) [90]. Serial dilutions were prepared in test tubes containing 9 mL of deionized water supplemented with 0.1% (*w*/*v*) peptone and sterilized. The Petri dishes were incubated in a Marconi® oven (model MA415) at 30 ± 0.5 °C for 48 h to facilitate microbial growth. Afterward, colonies were enumerated. To inhibit yeast growth in bacterial colony counts, cyclohexamide (Actidione®, Sigma-Aldrich; Darmstadt, Germany) was added to the culture media at a concentration of 10 mg L^{-1}. The results were analyzed using analysis of variance (ANOVA) to determine significant differences among treatments.

The Tukey test was used to compare means between treatments ($p < 0.05$). These statistical analyses were performed using the SAS 9.4v software package.

5.2. Main Findings

The diluted cane syrup replaced the pure water conventionally added in the hydrolysis step without compromising hydrolysis efficiency (CS). In addition to saving about 1 L of water per 639 g of corn, it also provided 58 g of sugar and essential nutrients to the wort. On an industrial scale, this amount is significant and corresponds to approximately 1.56 m^3 of water that is no longer captured for every 1 ton of corn processed for ethanol production. Considering average agro-industrial yields [91], approximately 3.9 L of water would be saved for every 1 L of ethanol produced.

Treatments before irradiation (treatments C20, CS20 and S20) had no differences in the counting bacteria. Bacteria were the majority of the mesophilic microorganisms in the worts, which ranged from 8.5 to 8.6 Log CFU+1 mL^{-1}. The electron beam radiation was efficient in removing mesophilic and total bacterial contamination in treatments C0, CS0 and S0 (Table 2).

Table 2. Assessment of microbiological and chemical effects of different physicochemically composed worts irradiated by electron beam.

Treatment		Bacteria	Mesophilic	Fermentable Sugars
		Log (UFC+1) mL^{-1}		g L^{-1}
T1	C0	8.5 ± 0.10 a	8.4 ± 0.13 a	298.7 ± 1.56 b
T2	C20	0.0 ± 0.00 b	0.0 ± 0.00 b	298.8 ± 7.53 b
T3	CS0	8.8 ± 0.09 a	8.8 ± 0.05 a	335.6 ± 1.04 a
T4	CS20	0.0 ± 0.00 b	0.0 ± 0.00 b	335.3 ± 9.10 a
T5	S0	8.7 ± 0.06 a	8.7 ± 0.07 a	298.1 ± 0.49 b
T6	S20	0.0 ± 0.00 b	0.0 ± 0.00 b	298.3 ± 0.99 b

Standard errors are indicated after the mean. Significant differences between treatments within the same cycle are denoted by distinct uppercase letters (Tukey < 0.05), while significant differences for the same treatment in different cycles are indicated by varying lowercase letters.

Considering the complete inactivation of microorganisms observed in the worts of different physicochemical compositions, the dose of 20 kGy was then sufficient to reduce the microbial load by about 8 log units. The decimal reduction dose (D10 value) was estimated from this electron beam dose validated for contaminant inactivation. The mean D10 values for the C20, CS20 and S20 treatments were 2.27, 2.35 and 2.30 kGy, respectively.

Contamination Control

The population level of contaminating microorganisms was designed to represent a recurrent condition in industrial fermentation processes with infection by contaminants—A population of bacteria in the order of 10^8 CFU mL^{-1} [25], which is considered by [20,21,92] as detrimental to the fermentation efficiency and cellular viability of yeast. Due to the wide variety of microorganisms introduced into the wort by the impurities present in the raw materials, bacteria and fungi are found in both sporulated and vegetative forms in agro-industrial processes. The population intensity of 10^8 CFU mL^{-1} is a realistic representation of the industrial ethanol production scenario. To validate the effectiveness of the electron beam dose in inactivating the microorganisms present in the worts, the minimum dose required for spore inactivation is 20 kGy [93].

The microbiological contamination of the wort is one of the major factors negatively affecting industrial alcoholic fermentation [47,55,94,95]. Nobre et al. (2007) also observed complete inactivation of contaminating microorganisms by 20 kGy of ionizing radiation using a Co60 source [55].

After irradiation, the presence of microorganisms was observed only in the musts not treated with electron beam (dose 0 kGy), i.e., treatments C0, CS0 and S0. While

the absence of microbial growth of any nature in culture media inoculated with musts irradiated at a dose of 20 kGy (C20, CS20 and S20) demonstrated the effect of complete inactivation of bacteria and total mesophiles, the treatments at a dose of 0 kGy showed a number of bacteria and total mesophiles equal to 8.5 and 8.4 units log (CFU+1) mL^{-1} in C0, 8.8 and 8.8 units log (CFU+1) mL^{-1} in CS0 and 8.7 and 8.7 units log (CFU+1) mL^{-1} in S0, respectively. These results indicate a predominantly bacterial composition, i.e., the presence of non-significant fungi.

The electron beam treatments of the wort at a dose of 20 kGy did not show significant effects on the fermentable sugars content in the wort ($p < 0.05$). The total fermentable contents in worts subjected to C20, CS20 and S20 were 298.8, 335.3 and 298.3 g L^{-1}, respectively. Preservation of sugars is essential in substrate decontamination procedures [32]. Any reduction in the sugars present in the wort would be undesirable because it would result in a lower volume of ethanol formed per volume of wort. These observed results also confirm those of [96,97], who reported the preservation of sugars ($p < 0.05$) in sugar solutions and molasses treated with ionizing radiation at doses up to 30 and 40 kGy, respectively. The authors of [98] also found no degradation of total sugars in foods treated with up to 30 kGy of gamma radiation ($p < 0.05$).

Air bubbles can be observed in worts immediately after receiving a dose of 20 kGy of ionizing radiation and may be related to the release of CO_2 formed by the interaction with the radiation, or even to the release of oxygen and other gases released by the moderate temperature change of the material [99]. Possible undesirable side effects of irradiating the wort with 20 kGy must be clarified by evaluating the alcoholic fermentation processes of these materials [50,65,72].

Doses higher than 20 kGy would not bring additional benefits to the microbiological quality of the wort in terms of contaminant control or, consequently, to the performance of alcoholic fermentation. This is because it would increase the chances of forming secondary compounds. The presence of these compounds in the wort would be undesirable if detrimental to the fermentation process [61]. Also, the operation of the electron accelerator at a dose higher than sufficient for the complete inactivation of the contaminating microorganisms could generate an unnecessary demand for electricity [72].

The mean D10 doses did not have considerable differences (Figure 1). The values are in agreement with the studies of [71], who observed that samples containing different species of microorganisms (including sporulated forms) had an average *D*10 value of 2.48 kGy. The results are also consistent with the observations of [53], who evaluated several substrates in a specific culture of *B. subtilis* and obtained a D10 value of up to 2.60 kGy. From the D10 value, it is still possible to estimate the effects of lower electron beam doses on microbial load reduction using the equation provided by [100]. According to [71], the estimation of the D10 value supports the prediction of the effectiveness of irradiation processes.

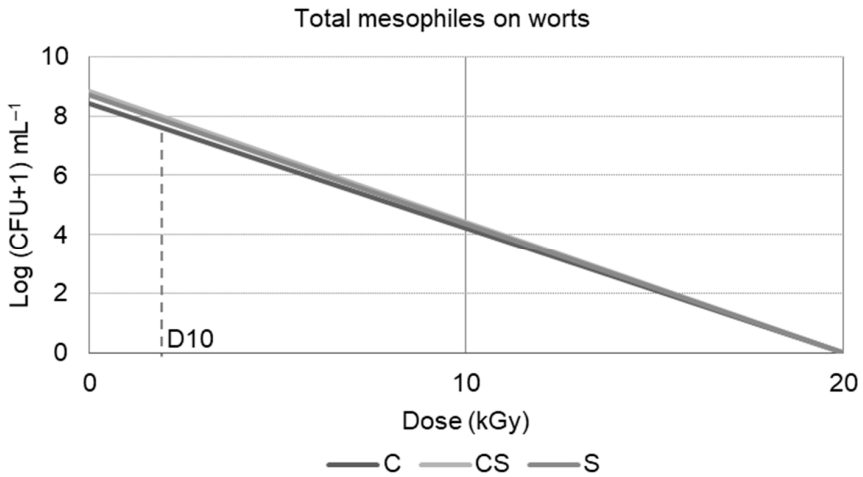

Figure 1. Population of total mesophiles and the D10 dose of electron beam.

6. Opportunities and Future Perspectives

This review highlights the possibilities of applying electron beam technology in the fermentation process. Firstly, it would be necessary to disseminate didactically the correct concepts about ionizing radiation, how the electron beam works and its advantages and to clarify that there is no radiological risk or radioactive waste. There is a real opportunity to use this technology to sterilize musts in other industrial fermentation processes, such as the production of hydrogen, acids, amino acids, biomass and pharmaceuticals.

Distilleries can use excess electricity from ethanol plants to power the electron accelerator. Ethanol distilleries can use their excess energy to trigger an electron beam as ionizing radiation to inactivate microorganisms in the wort and treat impurities. It is estimated that, with approximately 350 ethanol plants in Brazil, a 5% increase in yield [32] can be achieved, considering 5% losses in yield fermentation and fermentations with completely inactivated contaminants. According to this calculation, the national 31 million m^3 [8] could produce an additional 1.5 million m3 of ethanol per year by minimizing losses during the process.

Also, the cost to implement a 20 kGy electron accelerator in a production line of 220 thousand cubic meters per year of ethanol is about USD 5 million [101]. It would represent less than 2.5% of the total capital expenditure required to build an ethanol plant of this size, which would also be an affordable investment into a plant that can produce about 5% more ethanol every year [32].

Further studies to make an in-depth evaluation of an electron accelerator installed on the ethanol plant production line to sterilize the wort are also necessary to ensure the conditions are adequate and properly scale up the technology, even if it is already applied in industrial scale. However, the scientific benefits of wort sterilization and a contamination-free process are clear.

In the alcoholic fermentation process, the benefits of this approach include reduced sugar degradation, reduced osmotic stress on yeast, reduced competition between yeast and contaminating microorganisms and elimination of the need for antibiotics and other inputs to control contaminants. Indirect benefits include a fermentation process with fewer metabolites produced by bacteria and yeast, more stable process conditions and the ability to produce higher-value-added products. In addition, fermentation by-products are free of antibiotic residues, including yeast and DDGs, and also produce an environment free of antibiotic residues, an environment and industrial processes free of antibiotic-resistant bacteria and a fermentation process free of recontamination by biofilms formed in the pipes.

All this is reflected in increased efficiency, productivity and yield of alcoholic fermentation, and the possibility of running the process while maintaining the yeast strain selected and introduced at the beginning of the harvest as a high-performance inoculum throughout the harvest (Melle-Boinot) is being considered. Maintaining a single yeast strain throughout the process allows the isolation of a specific strain that can be marketed as a high-performance fermentation agent or protein biomass with desired nutritional or nutraceutical properties.

Figure 2 displays a flowchart depicting the process integration proposed by Douradinho (2023) [56]. Figures 3 and 4 display a flowchart of the conventional process of ethanol production from sugarcane or corn. The contaminants carried into the system by the raw materials may be inactivated by electron beam just before the fermentation phase, regardless of whether the wort comes from corn, cane or a mixed wort [15,32]. After the irradiation stage, the entire clean system and piping are kept closed to receive irradiated must and prevent any reinfection or recontamination.

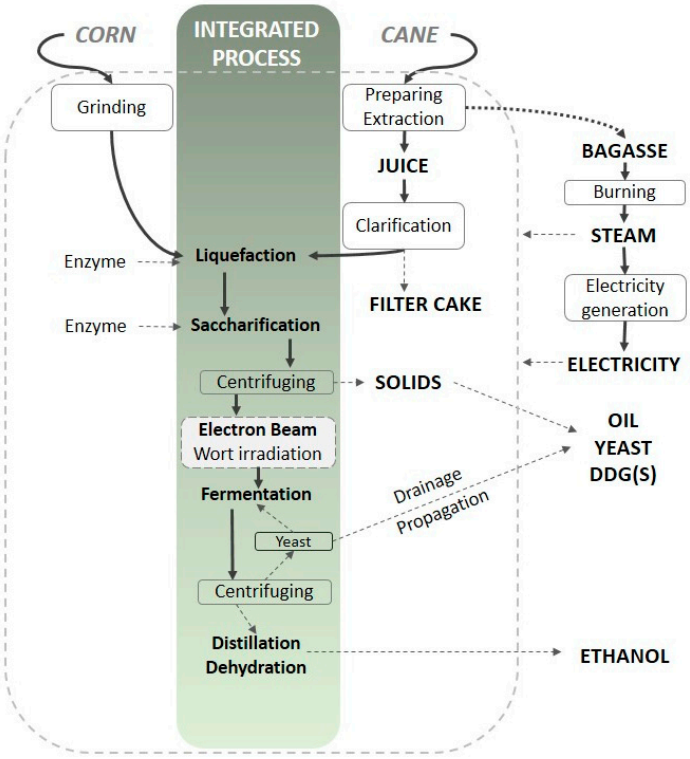

Figure 2. Diagram of the integrated corn- and cane-based ethanol process, indicating the possibility of adding the electron accelerator into the production line.

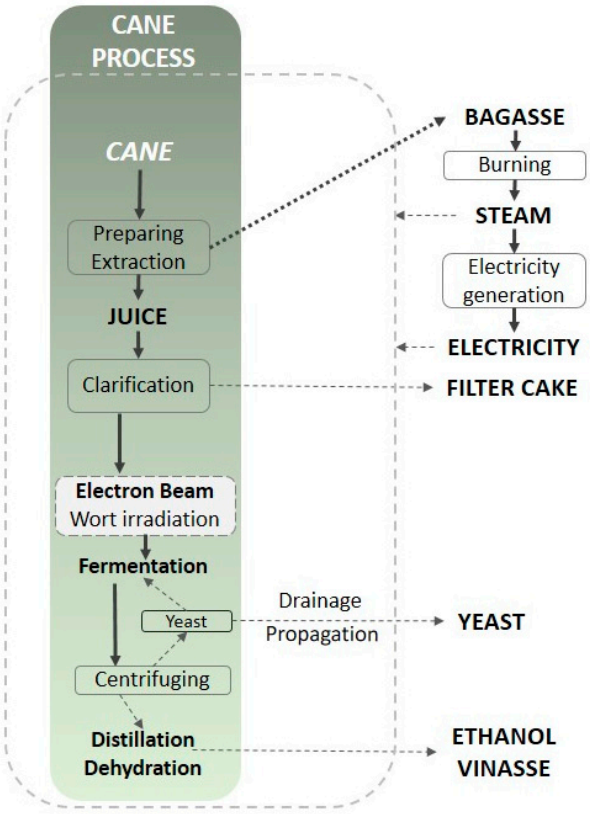

Figure 3. Diagram of the cane-based ethanol process, indicating the possibility of adding the electron accelerator into the production line.

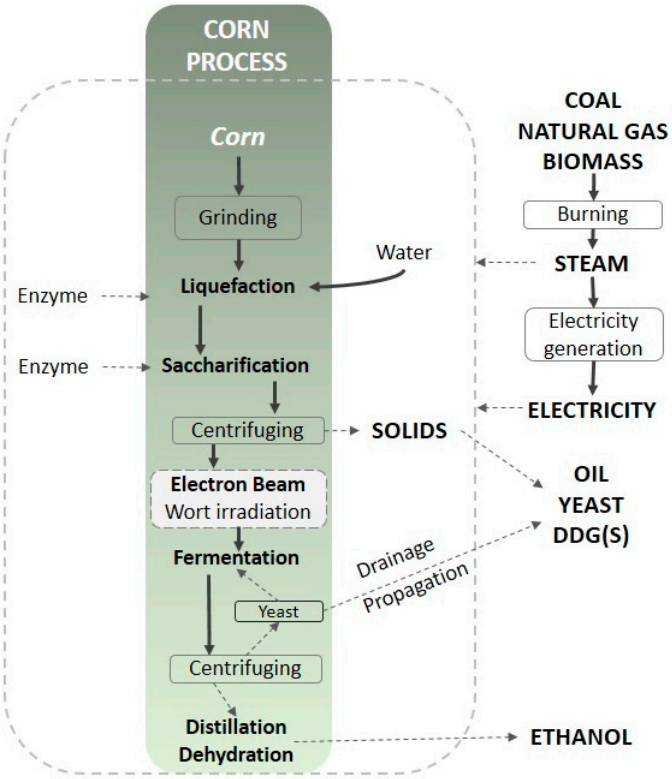

Figure 4. Diagram of the corn-based ethanol process, indicating the possibility of adding the electron accelerator into the production line.

7. Conclusions

Therefore, the implementation of ionizing radiation technology can bring several benefits to the ethanol industry, reducing the dependence on antibiotics, improving fermentation efficiency, reducing the synthesis of by-products and allowing the production of high-value products, such as neutral alcohol, amino acids and pharmaceuticals. This review showed that the context of ethanol production in Brazil provides opportunities for the adoption of this process and for making the ethanol production cleaner, meeting the demands of human and animal safety and sustainability.

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