AgriEngineering

Article

Sweet Sorghum Irrigated with Treated Domestic Wastewater in the Brazilian Semi-Arid: Agronomic Performance and High-Gravity Bioethanol Production

Leandro Candido Gordin [1], Amanda Alves da Silva dos Santos [2], Joyce Gueiros Wanderley Siqueira [2], Ariédenes Bandeira Rodrigues [3], Alex Luís Bernardo da Silva [3], Rafael Barros de Souza [4], Ênio Farias de França e Silva [1], Emmanuel Damilano Dutra [2] and Jorge Luiz Silveira Sonego [3,*]

1 Department of Agricultural Engineering, Federal Rural University of Pernambuco, Recife 52171-900, PE, Brazil; lcgengagricola@gmail.com (L.C.G.); enio.fsilva@ufrpe.br (Ê.F.d.F.e.S.)
2 Laboratory of Bioenergy and Environment, Department of Nuclear Energy, Federal University of Pernambuco, Recife 50740-545, PE, Brazil; amanda.alvess@ufpe.br (A.A.d.S.d.S.); joycegwsiqueira@gmail.com (J.G.W.S.); emmanuel.dutra@ufpe.br (E.D.D.)
3 Graduate Program in Biotechnology, Federal University of Pernambuco, Recife 50670-901, PE, Brazil; ariedenes.rodrigues@ufpe.br (A.B.R.); alex.luis@ufpe.br (A.L.B.d.S.)
4 Institute of Biological Sciences, University of Pernambuco, Recife 50100-130, PE, Brazil; rafael.souza@upe.br
* Correspondence: jorge.sonego@ufpe.br

Abstract

Sweet sorghum is a promising crop for bioethanol production in semi-arid regions, due to its tolerance to drought and salinity, where conventional energy crops face limitations. This study aimed to evaluate the agronomic performance of sweet sorghum irrigated with treated domestic wastewater (TDW) and its application as a substrate for bioethanol production under high-gravity (HG) and very-high-gravity (VHG) fermentation conditions. Field experiments were conducted in the Brazilian semi-arid using a 5 × 5 full factorial design consisting of five irrigation depths (40–160% crop evapotranspiration, ETc) combined with five potassium fertilization doses (0–80 kg·ha^{-1}), totaling 25 treatments. Agronomic performance, biomass production, and total reducing sugar accumulation were evaluated in both plant cane and ratoon crops. Sweet sorghum juice was subsequently combined with sugarcane molasses and fermented using *Saccharomyces cerevisiae* in batch and fed-batch processes. Irrigation with TDW associated with moderate potassium fertilization enhanced plant development, biomass yield, and sugar accumulation, particularly at irrigation depths between 100% and 130% of ETc, reaching up to 1908 kg·ha^{-1} of TRS. Bioethanol production achieved fermentation efficiencies of 91.83% and 84.80% and productivities of 4.63 and 4.21 g·L^{-1}·h^{-1} under HG and VHG conditions, respectively. These findings indicate that sweet sorghum irrigated with TDW is a promising feedstock for bioethanol production under high-gravity fermentation conditions while supporting the use of alternative water resources in semi-arid environments.

Keywords: sweet sorghum; drought resistant crops; microirrigation; VHG; drylands; TDW

Check for updates

Academic Editor: Pablo Martín-Ramos

Received: 24 March 2026
Revised: 27 June 2026
Accepted: 30 June 2026
Published: 4 July 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

The global transition toward sustainable energy systems has intensified the demand for renewable fuels capable of reducing greenhouse gas emissions and dependence on fossil resources [1]. Fuel ethanol stands out as one of the most consolidated biofuels worldwide. The United States currently leads global production, with approximately 61 billion liters

annually, while Brazil ranks second, producing between 33 and 37 billion liters per year [2]. In the United States, ethanol is produced mainly from corn, whereas in Brazil, the primary feedstock is sugarcane, a crop rich in fermentable mono and disaccharides such as sucrose, glucose, and fructose [3].

Despite this technological and productive consolidation, the expansion of ethanol production raises concerns related to land availability, freshwater consumption, and the long-term sustainability of conventional feedstocks, particularly in regions subject to climate variability and water scarcity [1]. Additionally, sugarcane seasonality restricts industrial operation to harvest periods, increasing the demand for complementary feedstocks. In this context, sweet sorghum (*Sorghum bicolor* L. Moench) has emerged as a promising alternative for bioethanol production due to its short growth cycle, adaptability to different environments, and tolerance to drought and salinity [4,5]. These characteristics make the crop particularly suitable for the Brazilian semi-arid region, which is characterized by low rainfall, prolonged drought periods, and saline soils [6].

Although adapted to adverse conditions, sweet sorghum cultivation in semi-arid conditions frequently requires irrigation to maintain yield stability. However, freshwater availability in these regions is limited and often competes with urban and industrial demands. In this scenario, treated domestic wastewater (TDW) has been proposed as a sustainable irrigation alternative because it simultaneously supplies water and nutrients while reducing dependence on conventional water resources [7]. Previous studies conducted in semi-arid regions of Saudi Arabia [8] and Brazil [9] demonstrated the feasibility of cultivating sweet sorghum using treated effluents.

In water-limited environments, irrigation management is a critical factor for maximizing crop productivity while improving water use efficiency. Although sweet sorghum exhibits tolerance to drought and saline conditions, irrigation strategies based on crop evapotranspiration (ETc) can significantly affect biomass production, sugar accumulation, and crop performance. Evaluating different irrigation depths becomes particularly relevant in semi-arid regions, where efficient water allocation is essential for sustainable agricultural production. Inadequate irrigation, whether deficit or excessive, may alter physiological responses, biomass formation, and sugar accumulation, directly affecting the potential of sweet sorghum as a bioethanol feedstock.

From both agronomic and industrial perspectives, sweet sorghum offers additional advantages. The crop can be cultivated during the sugarcane off-season and used for first-generation bioethanol production from stalk sugars as well as second-generation bioethanol production from lignocellulosic biomass, thereby increasing ethanol productivity per cultivated area [4,5]. Moreover, due to its structural similarity to sugarcane, sweet sorghum can be processed using existing industrial infrastructure [4]. Sweet sorghum also presents lower water requirements (approximately 8 ML·ha^{-1}) and a shorter cultivation cycle (~4 months) compared with sugarcane, which requires approximately 36 ML·ha^{-1} and 12–13 months to reach maturity [10]. Production costs are also estimated to be lower than those of sugarcane and corn-based ethanol systems [11].

Although the lower sugar concentration in sweet sorghum juice compared to sugarcane juice may limit ethanol productivity [3], strategies such as blending sweet sorghum juice with sugarcane molasses and applying high-gravity (HG; >200 and <250 g·L^{-1}) and very-high-gravity (VHG; >250 g·L^{-1}) fermentation processes can increase ethanol titers, improve productivity, and reduce water and energy consumption [12,13]. Also, strategies such as fed-batch operation and nitrogen supplementation improve the performance of those processes [12,14]. Yeast stress-response mechanisms can be activated under these conditions, enabling improved ethanol production. VHG fermentation has successfully

enhanced ethanol production from several substrates for both first [15,16] and second-generation [15,17] bioethanol, including sweet sorghum [18,19].

Despite the potential of treated wastewater irrigation and ETc-based irrigation management for sweet sorghum cultivation, studies integrating irrigation strategies, potassium fertilization, and subsequent application in ethanol production remain limited. Unlike studies focused solely on field productivity or fermentation, this work evaluates sweet sorghum grown under different irrigation levels and potassium doses, testing the resulting broth as a complementary substrate to sugarcane molasses in fermentations under high (HG) and very-high-gravity (VHG) conditions. Integrating sustainable water management with fermentation process intensification may therefore represent a strategic approach to improving agricultural and industrial system efficiency.

In this context, this study aimed to evaluate whether irrigation with treated domestic wastewater and potassium fertilization at different irrigation depths based on ETc in the Brazilian semi-arid region improve the performance of sweet sorghum cultivation and, subsequently, evaluate the overall biotechnological potential of sorghum juice in mixtures with sugarcane molasses as a medium for ethanol production under high and very-high-gravity fermentation conditions.

2. Materials and Methods

2.1. Experimental Field and Climate

The experiment was carried out at Pernambuco's semi-arid region, in the town of Ibimirim, 333 km from Recife. The experimental area is at 8° 32′ 05″ South and 37° 41′ 50″ West, with 408 m of altitude. According to the Köppen classification, the region exhibits a BSw'h' climate type, characterized as very hot semi-arid, with an average annual precipitation of 454 mm. The average annual temperature is around 24.9 °C, average maximum of 32.6 °C, and average minimum of 20.0 °C [20].

The experiment took place at the Pilot Unit of Hydro Agricultural Reuse of Domestic Wastewater, which belongs to the Agricultural Engineering Department (DEAGRI) of the Federal Rural University of Pernambuco (UFRPE), between 31 January 2020 and 30 July 2020 (180 days). During the experiments, temperature, humidity, and precipitation were monitored. The lowest, average, and highest temperatures were 23.10 °C, 27.73 °C, and 28.34 °C, respectively. The highest and lowest relative humidities were 81.54 and 69.33%, respectively. Finally, the accumulated precipitation was 559 mm.

The soil was classified as Neossolo Quartzarênico according to the Brazilian Soil Classification System (SiBCS; Embrapa 2025), corresponding to an Arenosol in the World Reference Base for Soil Resources (IUSS Working Group WRB 2022). It was collected from an area under hiperxerophilous Caatinga vegetation with predominantly flat terrain. Physical and chemical characterizations are shown in Table 1.

Table 1. Physical and chemical characteristics of the soil (B) basic infiltration velocity.

Depth	P	pH	Ca	Mg	Na	K	Al	H	S	CTC	V
(cm)	(mg dm^{-3})		(cmolc·dm^{-3})								%
0–20	74	6.5	2.50	0.65	0.03	0.26	0	0.24	3.4	3.7	93
20–40	31	5.5	1.65	0.60	0.06	0.28	0.05	0.69	2.6	3.3	78

Depth (cm)	Horizon	Sand	Silt	Clay	BIV$^{(B)}$	Texture Class
		g·kg^{-1}			mm·h^{-1}	
0–20	A	760	80	160	26	Sandy loam
20–40	C1	760	80	160		

2.2. Treatments and Experimental Design

The experimental design was carried out using a randomized block design (RBD), in a 5 × 5 factorial arrangement, resulting in 25 treatments. Each treatment was replicated 4 times, totaling 100 experimental plots. The treatments consisted of five irrigation levels using treated domestic wastewater (TDW), defined as L1 = 40%, L2 = 70%, L3 = 100%, L4 = 130%, and L5 = 160% of the estimated Localized Crop Evapotranspiration (LCE). LCE refers to crop evapotranspiration adjusted for localized irrigation systems, such as drip irrigation, where only a fraction of the soil surface is wetted, and water demand is estimated considering localized wetting conditions.

Irrigation levels above 100% LCE were included as an experimental strategy to evaluate sweet sorghum responses across a broad range of water availability scenarios and to establish response curves under both deficit and excess irrigation conditions. This approach enabled the identification of irrigation thresholds associated with maximum crop performance and contributed to determining optimal water management strategies under semi-arid conditions. These treatments were combined with five potassium fertilization doses: 0, 20, 40, 60, and 80 kg·ha^{-1}, corresponding to 0, 50, 100, 150, and 200% of the agronomic recommendation. Fresh and dry biomass production, productivity, and total reducing sugars per hectare were determined.

2.3. Collection and Treatment System for Domestic Wastewater

The raw sewage was obtained from a condominium sewage system designed to serve 500 households with around 3000 inhabitants, in the vicinity of Lajes, Ibimirim-PE. It was physically pretreated passing through a bar screen, a grit chamber, and a Parshall flume, followed by the primary treatment in an Upflow Anaerobic Sludge Blanket (UASB) reactor and finishing in polishing ponds.

The polishing pond in Ibimirim-PE had 7.0 m in length, 3.0 m in width and 1.0 m in depth, with a storage capacity of 2.0 m^3 of treated domestic sewage. Additionally, they were lined with plastic sheeting to prevent soil and groundwater contamination.

The domestic sewage treated for irrigation was collected monthly to evaluate its physical, chemical, and microbiological characteristics. Sampling was carried out right after the flow passed through the control head and the irrigation filter system. Analysis was performed according to the standard methodology established by the American Public Health Association [21]. Table 2 shows the characteristics of the treated domestic wastewater.

The treated domestic wastewater used in this study presented physicochemical characteristics that indicate both agronomic potential and environmental limitations for long-term agricultural reuse. The electrical conductivity value (2.10 dS·m^{-1}) indicates moderate salinity conditions, while sodium concentration (133.10 mg·L^{-1}) may contribute to gradual ion accumulation in soil under prolonged irrigation. According to the Brazilian Agricultural Research Corporation (EMBRAPA) [22], waters with up to 600 mg·L^{-1} of total salts, with less than 60% of those being sodium, are suitable for irrigation. Therefore, regarding salinity, the treated wastewater used in this work can be used to irrigate sweet sorghum. Salinity is the main challenge for wastewater use in irrigation systems, since excessive sodium accumulation may promote soil sodification, reducing soil hydraulic conductivity and negatively affecting water infiltration and nutrient dynamics. Also, the microirrigation system itself is susceptible to damage caused by the excess of salt, which can lead to clogging and corrosion [23].

Nevertheless, treated wastewater also supplied relevant nutrient concentrations, including nitrogen, potassium, and phosphorus, potentially reducing mineral fertilizer requirements and contributing positively to crop development. Additionally, sweet sorghum has proven its resilience to high salinity conditions [24].

Although the effluent used in the present study exhibited high values of biochemical oxygen demand (BOD), thermotolerant coliforms, and sodium adsorption ratio (SAR), the feasibility of the agricultural reuse of this water depends on the irrigation method adopted, crop type, soil characteristics, and management conditions employed in the experiment. Recent studies have highlighted that the agricultural reuse of wastewater represents an important strategy for water resource conservation and nutrient recycling, especially in semi-arid regions affected by increasing water scarcity.

The BOD value observed in the effluent can positively contribute to nutrient cycling, microbiological activity, and soil fertility. Recent studies have demonstrated that irrigation with treated wastewater can increase soil microbial activity and nutrient availability, particularly in agricultural systems subjected to continuous water reuse. Part of the applied organic load is mineralized in the soil and incorporated into biogeochemical cycles, partially reducing the dependence on mineral fertilizers [25].

Regarding microbiological quality, high concentrations of thermotolerant coliforms in wastewater do not constitute an absolute restriction for agricultural reuse when associated with appropriate irrigation methods. Localized irrigation substantially reduces contact between the effluent and the aerial parts of plants, minimizing pathogen dispersion and the risks of sanitary contamination. According to the World Health Organization [26], localized systems provide the highest level of health protection among irrigation application methods. Oron et al. [27] demonstrated that the risk of infection varies by several orders of magnitude depending on the irrigation method, with subsurface localized irrigation being one of the most effective strategies for mitigating sanitary risks, especially in crops not intended for human consumption. In the case of sweet sorghum, which is intended for bioenergy production, sanitary risks become substantially lower.

Table 2. Physical and chemical characterization of the treated domestic wastewater (TDW) used for sweet sorghum irrigation.

Parameters	Concentration	Unity
Conductivity	2.10	$dS \cdot m^{-1}$
pH	7.20	-
Hardness	273.40	mg de $CaCO_3 \cdot L^{-1}$
Calcium	74.90	$mg \cdot L^{-1}$
Magnesium	21.00	$mg \cdot L^{-1}$
Sodium	133.10	$mg \cdot L^{-1}$
Potassium	43.60	$mg \cdot L^{-1}$
Total nitrogen	126.00	$mg \cdot L^{-1}$
Total phosphorus	13.70	$mg \cdot L^{-1}$
Sulfur	5.30	$mg \cdot L^{-1}$
Manganese	1.30	$mg \cdot L^{-1}$
Iron	9.20	$mg \cdot L^{-1}$
SAR	13.10	$(mg \cdot L^{-1})^{-1/2}$
COD	154.00	mg de $O_2 \cdot L^{-1}$
BOD	39.00	mg de $O_2 \cdot L^{-1}$
Dissolved Oxygen	46.00	%
Total Coliforms	2.20×10^7	NMP/100 mL
Thermotolerant Coliforms	1.40×10^7	NMP/100 mL

2.4. Characterization and Management of the Irrigation System

The dripping irrigation (microirrigation) had lateral distribution pipelines consisting of 16 mm dripping tubes with pressure-compensation emitters (Dripper PCJ-LCN-Netafim) and a nominal flow rate of 2.3 $L \cdot h^{-1}$. Nominal pressure was defined as 12 mca.

The water demand of sweet sorghum was estimated using a climate-based technique. Firstly, the reference evapotranspiration (ET_0) was calculated using the Penman-Monteith standard method [28]. The crop evapotranspiration (ET_C) was measured to incorporate the crop and cultivation system characteristics. To calculate them, two coefficients were determined: the crop coefficient (Kc) and the mean localization coefficient (KL_{med}). Kc was proposed by Doorenbos and Kassam [29], and KL_{med} was calculated as the mean of five methods (Table 3).

Table 3. Crop coefficient (Kc) for sweet sorghum and methods to estimate the location coefficient (KL_{med}).

Crop Coefficient (Kc)		Location Coefficient (KL_{med})			
Development Stage	**Kc**		**Methods**	**DAE**	**KL_{med}**
I—Emergency—10% of CSS	0.40	1st	Aljibury, Marsh e Huntamer—(1974) [30]	5	0.208
II—10–80% of CSS	0.75	2nd	Hoare, Garzoli e Blackwell—(1974) [31]	10	0.386
III—80–100% of CSS	1.15	3rd	Decroix—(1978) [32]	15	0.561
IV—Physiological maturation	0.80	4th	Keller—(1983) [33]	20	0.722
V—Harvest	0.55	5th	Keller e Bliesner—(1990) [34]	25	0.905

The location coefficients were calculated according to the projection of the shaded area (S) by noon, every five days throughout the crop development. KL_{med} was determined as a function of the percentage of the shaded area, with values ranging from 0 to 1. When the value of KL_{med} reached 1.236, after 30 days of sorghum emergence (DAE), the value of 1.000 was used for irrigation management, as the crop grew. Thus, the evapotranspiration of sweet sorghum IPA–467 was estimated using Equation (1) [28].

$$ET_c = ET_0 \times Kc \times KL_{med} \quad (1)$$

where ET_c—crop evapotranspiration, $mm \cdot d^{-1}$; ET_0—reference evapotranspiration, $mm \cdot d^{-1}$; Kc—crop coefficient; KL_{med}—mean location coefficient.

Sweet sorghum IPA–467 irrigation was scheduled with a two-day irrigation interval, each one applied at 6:00 A.M. Irrigation time (Ti) for the different treatments was determined using Equation (2).

$$Ti = \frac{F \times ET_C \times (E_{LL} \times E_g)}{q_g \times E_a} \times 60 \quad (2)$$

where Ti—irrigation time, min; F—correction factor for irrigation depth (0.40, 0.70, 1.00, 1.30, and 1.60); ET_C—crop evapotranspiration, $mm \cdot d^{-1}$; E_{LL}—spacing between side lines, m; E_g—spacing between drippers in the lateral line, m; q_g—emitter flow rate, $L \cdot h^{-1}$; E_a—irrigation system efficiency, dec.

For the first cycle (plant sorghum), the irrigation depths using treated domestic sewage were 72.45 (40% ET_C), 126.74 (70% ET_C), 181.06 (100% ET_C), 235.38 (130% ET_C), and 289.7 mm (160% ET_C). Additionally, 213 mm was added for rainfall. In the second cycle (ratoon sorghum), the accumulated rainfall was 142 mm, and the irrigation depths were 84.81 mm (40% ET_C), 148.42 mm (70% ET_C), 212.03 mm (100% ET_C), 275.64 mm (130% ET_C), and 339.25 mm (160% ET_C).

To monitor climate-based irrigation management, tensiometers were installed at two different soil depths: 20 cm and 40 cm. The readings were carried out in a two-day interval, at 5:00 P.M, aiming to verify the effect of different irrigation depths on the soil matric potential.

Figures 1 and 2 show the variations in soil matric potential for 0–20 and 20–40 cm of irrigation depths, respectively, throughout the experiment as a function of the irrigation depths applied in the treatments corresponding to 40, 70, 100, 130, and 160% of ET_C.

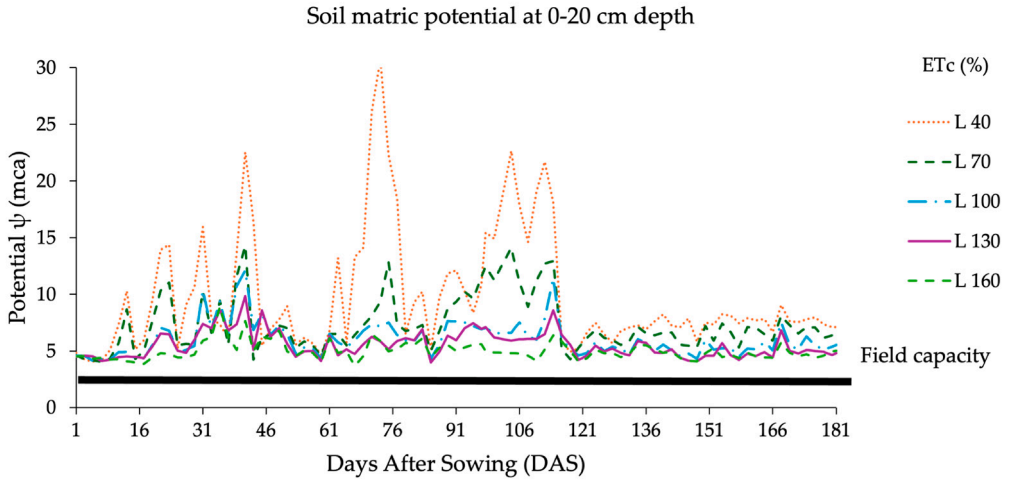


Figure 1. Soil matric potential (Ψ, mca) at 0–20 cm depth throughout the sweet sorghum cycle under different irrigation depths corresponding to 40, 70, 100, 130, and 160% of crop evapotranspiration (ETc). The black horizontal line represents the soil field capacity. DAS: days after sowing.

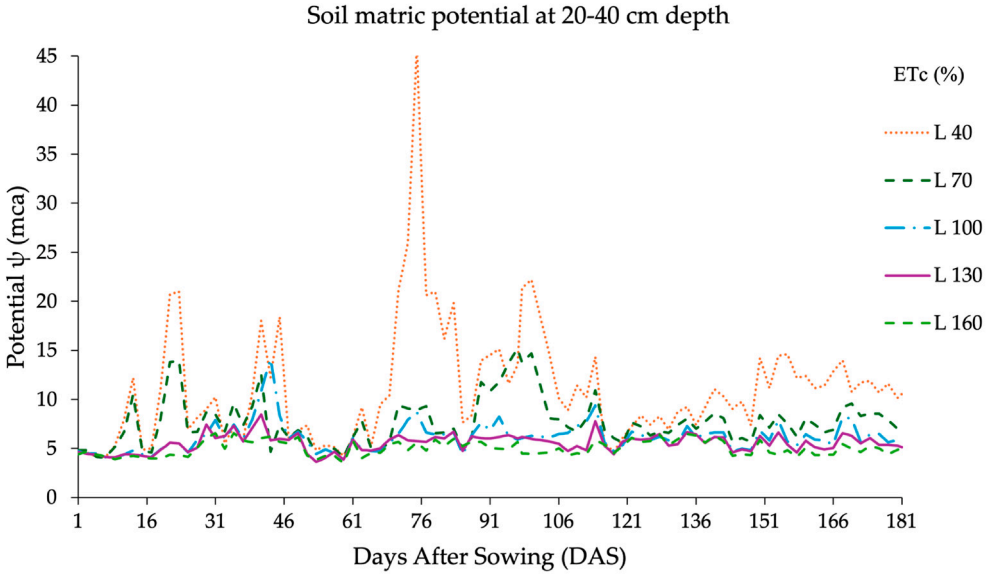


Figure 2. Soil matric potential (Ψ, mca) at 20–40 cm depth throughout the sweet sorghum cycle under different irrigation depths corresponding to 40, 70, 100, 130, and 60% of crop evapotranspiration (ETc).

2.5. Agronomic Management of Sweet Sorghum IPA–467

The experiment occupied an area of 1500 m^2, divided into 100 parcels, each with 15 m^2. The division had three lines with a length of 5 m and blank spaces of 1 m.

For sweet sorghum IPA–467 sowing, soil liming was carried out 30 days before planting, using dolomitic limestone with a Relative Neutralizing Power (RNP) of 70% at a rate of 300 $kg \cdot ha^{-1}$. The limestone was broadcast uniformly and incorporated into the soil. Then, the product was incorporated at a depth of 0.20 m with the aid of a leveling harrow, which made two cross passes across the entire area.

The basal mineral fertilization for sowing was carried out according to the recommendations of the Agronomic Institute of Pernambuco (IPA) [35], in furrows distant 10 cm from the planting lines, with 10 cm of depth. It was applied with a mass equivalent to 30 $kg \cdot ha^{-1}$

of nitrogen, using urea as nitrogen source (45% of nitrogen). Phosphorus fertilization was carried out at a rate of 30 kg·ha^{-1} using double superphosphate as a source (28% P_2O_5).

The sweet sorghum was sown on 31 January 2020. Prior to sowing, the experimental plots were irrigated for 3 h, until the soil reached its capacity of humidity. The sweet sorghum IPA-467 seeds were sown in furrows with 0.05 m of depth and spaced 1.0 m between lines, with a density of 25 seeds per linear meter. They germinated after 6 days of sowing (DAS). To ensure plant development, they all received 100% of their ET_C, with 10 irrigations corresponding to 41 mm. The application of different irrigation depths started at 20 DAS. Furthermore, the rainfall water was considered and subtracted for each irrigation. Thinning was performed at 15 DAS to obtain a density of 15 plants per linear meter.

Manual control of weeds was carried out twice (15 and 30 DAS) with the aid of hoes. Pest control of fall armyworm (*Spodoptera fugiperda*) was performed with pesticides recommended for the crop, such as Lannate BR–Du Pont, at 100 mL·100·L^{-1} of water and Decis–Bayer at 75 mL·100·L^{-1} of water, applied at 10,15, 30, and 40 DAS.

The first harvest (plant sorghum) was done at 97 DAS, when the plants were removed from the central row of each experimental parcel, with a length of 3 m and a distance of 1 m, with an area of 3 m^2. After that, the irrigation was continuous to ensure the development of tillers (ratoon sorghum).

Mineral fertilization for the second cycle was carried out five days after harvesting (102 DAS), using 60 kg·ha^{-1} of nitrogen, using urea as a source. Potassium fertilizing and pest control were consistent with those in the first cycle. The second harvest (sorghum ratoon) was conducted 83 days after the first, totaling 180 DAS for the entire experimental period. Harvesting procedures followed the same protocol as the initial harvest.

2.6. Analysis of Growth and Fresh and Dry Productivity

Harvest of plant sorghum and ratoon sorghum was done at 97 and 180 (83 DAH) DAS. A total of 500 g of sorghum fresh biomass (FB) was weighed and identified, then dried in a forced-air oven at 65 °C for 72 h and weighed again as dry biomass (DB). FB and DB were used to estimate fresh (FP) and dry (DP) productivities, expressed as Mg·ha^{-1}. Canopy heights (CH) were measured using a graduated ruler, from the canopy base to the tallest leaf, and culm diameters were measured using a caliper ruler [36]. For both variables, three measurements were performed throughout the crop cycle. In the first cycle (plant sorghum), evaluations were conducted at 20, 60, and 95 days after sowing (DAS). In the second cycle (ratoon sorghum), analyses were performed at 25, 55, and 75 days after cutting (DAC), corresponding to 120, 150, and 170 DAS, respectively.

For CH, the analyses were performed along the central row of all experimental parcels, each measuring 3 m in length and spaced 1 m apart, totaling an effective experimental area of 3 m^2. Measurements were taken at the beginning, middle, and end of the row to determine the average canopy height.

2.7. Efficiency in the Use of Treated Domestic Wastewater (WUE)

The efficiency of TDW use (WUE) was calculated considering the fresh productivity and the volume of TDW applied based on the different irrigation depths (mm), as defined by Equation (3), proposed by [37].

$$WUE = \frac{FB}{D_{TDW}} \quad (3)$$

where WUE—TDW efficiency (kg·ha^{-1}·mm^{-1}); FB—sweet sorghum fresh biomass (kg·ha^{-1}); D_{TDW}—irrigation depth with treated domestic wastewater (mm).

2.8. Total Reducing Sugars in the Juice of Planted Sweet Sorghum

The total reducing sugars (TRS) represent combinations of different sources of fermentable sugars (free and potential glucose and fructose from sucrose hydrolysis). To obtain the TRS values, samples from the different treatments were analyzed by HPLC [3].

2.9. Statistical Analysis

All data was analyzed using normality (Shapiro–Wilk) and homoscedasticity tests. Then, Mauchly's sphericity test was applied to assess the significance of the time, which, when identified, indicated the use of a split-plot-in-time model. The model was applied to SD and CH. Wilks' test was also applied with a significance of $p \leq 0.05$. The parameters were adjusted using multiple regression models (response surface), considering the time associated with the irrigation depths with TDW or with potassium doses.

The other variables were analyzed by analysis of variance (ANOVA) using the F test ($p \leq 0.05$). Regression models were selected by coefficient significance at a 5% probability level and the higher R^2 value.

2.10. Bioethanol Production from Stem Juice of Sweet Sorghum Irrigated with TDW

2.10.1. Microorganism, Sweet Sorghum Juice, and Sugarcane Molasses

The yeast used was a lyophilized *Saccharomyces cerevisiae* from Fleischmann, AB Brasil (Pederneiras, SP, Brazil), with an initial viability of 80.83 ± 7.63% and a moisture content of 5.18 ± 1.26%. The molasses was kindly provided by the distillery Impacto Bioenergia Alagoas S/A (Alagoas, Brazil) and stored in a plastic container, protected from sunlight, at ambient temperature (25 °C). The molasses was analyzed, presenting 756.02 ± 8.20 $g \cdot L^{-1}$ of total reducing sugars (TRS, obtained as the sum of glucose and fructose after the total inversion of sucrose by yeast invertase). The sweet sorghum juice (IPA 467 variety) used in the study was obtained by milling the sweet sorghum culms using a sugarcane juice mill.

Subsequently, juice obtained from different extractions was homogenized to obtain a single representative sample of sweet sorghum juice. This strategy aimed to reduce variability among extraction batches and standardize the substrate composition for process evaluation. The homogenized juice was stored in plastic bottles at −5 °C and presented a total reducing sugar (TRS) concentration of 58.24 ± 5.50 $g \cdot L^{-1}$. All fermentation media were prepared using the same homogenized sweet sorghum juice and sugarcane molasses batches.

2.10.2. Fermentative Capacity and Ethanol Tolerance

The fermentation test was carried out in duplicate, in 1 L shaking flasks with a working volume of 0.5 L, and incubated in a rotary shaker (New Brunswick Scientific C25KC, New Brunswick Scientific, Edison, NJ, USA) at 30 °C and 150 rpm for 44 h. The fermentation medium was prepared from a mixture of sweet sorghum juice and sugarcane molasses, with an initial TRS concentration of 205.02 $g \cdot L^{-1}$, supplemented with 5.32 $g \cdot L^{-1}$ of urea as a nitrogen source. The inoculum was added at an initial concentration (in dry mass) of 20 $g \cdot L^{-1}$ of yeast *S. cerevisiae*. During the fermentation process, 50 g of pure molasses was added at 13.5, 24.75, and 30 h, totaling 3 pulses. To analyze the process, a volume of 2 mL was collected for the analysis of cell, sugar, and ethanol concentrations. Also, cell viability was evaluated at the beginning of the fermentation, before each feed, and at the end of the assay.

2.10.3. Batch Ethanol Fermentations Using a Shaking Flask

The fermentation media were prepared using different proportions of sweet sorghum juice (SSJ) and sugarcane molasses. The use of mixed substrates was adopted because

sweet sorghum juice presents lower sugar concentrations than molasses but provides fermentable sugars and nutrients that may support yeast metabolism. In contrast, sugarcane molasses contains highly concentrated sugars but may contain inhibitory compounds and compositional limitations under high substrate concentrations. Therefore, combining both substrates was intended to improve nutritional balance and fermentation performance under high-gravity (HG) and very-high-gravity (VHG) conditions.

Four batch ethanol fermentation (F1, F2, F3, and F4) were performed in duplicates in 250 mL Erlenmeyer flasks with 150 mL work volume, using a cell concentration of around 20 $g \cdot L^{-1}$ (dry basis) and incubated in a rotary shaker (New Brunswick Scientific C25KC) at 30 °C and 150 rpm for 24 h. The fermentations were designated according to their compositions, as listed in Table 4. The fermentation medium was supplemented with urea at 5.32 $g \cdot L^{-1}$, adapted from [33]. Samples of 2 mL were withdrawn at 0, 20, and 24 h to monitor substrate consumption and ethanol production.

Table 4. Must composition of HG and VHG fermentations and substrate concentrations.

Fermentation	Composition	TRS ($g \cdot L^{-1}$)
F1	molasses (27.05%) + distilled water (72.95%)	204.50
F2	molasses (33.07%) + distilled water (66.93%)	239.44
F3	SSJ (28.00%) + molasses (72.00%)	238.54
F4	SSJ (32.00%) + molasses (68.00%)	251.00

SSJ = sweet sorghum juice; TRS = total reducing sugar.

2.10.4. Fed-Batch Ethanol Production in Bioreactor

The fermentation was carried out in a bioreactor (New Brunswick Scientific Bioflo 110, New Brunswick Scientific, Edison, NJ, USA) with a working volume of 1 L, in duplicate. As described by Sonego et al. [33], the fed-batch ethanol fermentation was performed considering a fed-batch (until the vat is filled), followed by the process continuing until the complete consumption of the substrate. An inoculum volume (0.3 L) corresponds to 30% ($v \cdot v^{-1}$) of the vat working volume, containing 80 g (dry weight) of lyophilized yeast (equivalent to C_{X0} = 20.0 $g \cdot L^{-1}$). The yeast was added to the vat along with the corresponding volume of water and maintained under agitation at 150 RPM in order to ensure complete dissolution of the yeast flake. A 0.7 L of culture medium, consisting of sugarcane molasses diluted in sorghum juice, was fed for 11 h until the vat was filled (fed-batch stage). The wort consisted of a mixture of 46.60% sweet sorghum juice and 53.40% molasses, supplemented with nitrogen in the form of urea at 8 $g \cdot L^{-1}$. The total concentration of reducing sugars in the medium was 301.55 $\pm$ 4.50 $g \cdot L^{-1}$. Subsequently, the fermentation proceeded until substrate exhaustion (batch stage). The temperature was maintained at 30.0 °C by the bioreactor's cooling system.

Fed-batch VHG fermentations in bioreactors were conducted in duplicate due to the operational complexity of the experiments, particularly their extended duration. Similar approaches are commonly employed in exploratory studies involving process optimization under VHG conditions. To minimize experimental variability, all operating conditions were strictly standardized, and analytical determinations were performed in replicate. Although the fermentation experiments were performed in duplicate, consistent technological trends were still identified. However, future research utilizing a higher number of replicates would provide greater statistical confidence in the evaluation of these fermentation parameters, overcoming the inherent limitations of duplicate assays.

2.10.5. Analytical Methods

The cell concentration (on a dry mass basis) was determined after sample centrifugation at 10,000 rpm and 4 °C for 10 min. The precipitate was washed twice with distilled

water and then dried at 100 °C for 24 h. The supernatant was stored at −4 °C for subsequent analysis of sugars and ethanol.

The concentrations of sucrose, glucose, fructose, and ethanol were determined by high-performance liquid chromatography (HPLC) using an Agilent Series 1100 system (Agilent Technologies, Santa Clara, CA, USA) equipped with a refractive index detector. The column used was Aminex HPX-87H (Bio-Rad Laboratories, Hercules, CA, USA) maintained at 35 °C, and the mobile phase consisted of a 0.005 mol/L H_2SO_4 solution, with a flow rate of 0.6 mL.min^{-1}, adapted from [38]. The total reducing sugars (TRS) represent combinations of different sources of fermentable sugars (free and potential glucose and fructose from sucrose hydrolysis).

Cell viability was determined by the methylene blue staining method [39], in which a Neubauer chamber was used under an optical microscope (Labomed Lx300, Los Angeles, CA, USA) at 40× magnification, where blue-stained cells were considered dead and unstained cells alive.

2.10.6. Fermentative Parameters

Parameters to evaluate fermentation performance in the process were ethanol yield coefficient ($Y_{E/S}$, in $g_E \cdot g_S^{-1}$) and productivity (P_E, $g \cdot L^{-1} \cdot h^{-1}$). The $Y_{E/S}$ was calculated using Equation (4) for batch fermentations and Equation (5) for fed-batch fermentations.

$$Y_{E/S} = \frac{C_{Ef} \times V_f - C_{E0} \times V_0}{C_{S0} \times V_0 - C_{Sf} \times V_f} \quad (4)$$

$$Y_{E/S} = \frac{(C_{Ef} \times V_f)}{(V_f - V_0) \times C_{SF} - C_{Sf} \times V_f} \quad (5)$$

The P_E was calculated using Equation (6):

$$P_E = \frac{C_{Ef}}{t_f} \quad (6)$$

where C_E is the ethanol concentration ($g \cdot L^{-1}$), C_{SF} is the substrate concentration in the must feed ($g \cdot L^{-1}$), V is the volume (L), t is the time of the fermentative process, and the subscripts "0" and "f" represent the initial and final times of the culture, respectively.

3. Results and Discussion

3.1. Vegetative Growth Under Treated Domestic Wastewater Irrigation

The multivariate analysis (Wilks' test) revealed significant effects ($p \leq 0.01$) of irrigation depth (ID), potassium dose (K), and evaluation time on canopy height (CH) and culm diameter (CD) in both plant and ratoon cycles. As shown in Table 5, significant ID × time and K × time interactions were observed, whereas the triple interaction (ID × K × time) was not significant, indicating independent contributions of irrigation and potassium supply to vegetative development.

Irrigation with treated sewage domestic wastewater, when combined with moderate potassium doses, improved sorghum development, especially at irrigation depths between 100% e 130% of ETc. Significant growth, high biomass production, and improved water use efficiency were observed under those conditions. Potassium fertilization was crucial to mitigate the negative effects of sewage salinity, allowing the plants' physiological development, particularly relating to photosynthesis and transpiration.

The tallest CH obtained from the mathematical model of the statistical analysis was 417 cm, with an ID of 183 mm (101.21% ETc) at 93 DAS, which represents an increase of 14.22 and 12.88% over the ID of 72 mm (40% ETc) and 289 mm (160% ETc), respectively.

Regarding the effect of K and time over CH, the highest value was 399.50 cm, with a K of 45.91 kg·ha^{-1}, at 93 DAS, with an increase of 2.25 and 1.25% when compared to 0 and 80 kg·ha^{-1}, respectively.

For the canopy height (CH) variable in the ratoon sorghum cycle under irrigation depths (ID), the highest estimated value, obtained through the partial derivative of the equation from the statistical analysis, was 402 cm, achieved with an irrigation depth of 169.92 mm (80.14% ETc) at 75 DAH. This showed increases of 21.80% and 32.24% when compared with irrigation depths of 72 and 289 mm (40 and 160% ETc), which resulted in canopy heights of 314 and 272 cm, respectively, at the same evaluation time. For the potassium (K) treatment, the highest estimated CH was 398.35 cm with an application of 45.12 kg·ha^{-1} K at 73 DAH, showing contributions similar to those observed in the plant sorghum cycle.

Table 5. Summary of multivariate analysis (Wilks' test) for canopy height and culm diameter under different irrigation depths and potassium doses.

Variables	F		Variables	F	
	Plant Sorghum			Raton-Sorghum	
	CH	CD		CH	CD
DAS	33,632.09 **	1960.66 **	DAH	9739.31 **	372.51 **
DAS:ID	7.31 **	8.46 **	DAH:ID	5.31 **	4.91 **
DAS:K	2.40 **	9.31 **	DAH:K	6.73 **	5.73 **
DAS:ID:K	0.75 ^{ns}	0.941 ^{ns}	DAH:ID:K	0.71 ^{ns}	1.48 ^{ns}

(^{ns}) No significance; (**) significant at 1%; (DAS) days after sowing; (DAH) days after harvesting; (ID) irrigation depths with treated sewage domestic wastewater; (K) potassium doses.

Considering the interaction between irrigation depth and time, the highest estimated CD value was 24.18 mm, obtained with an irrigation depth of 219 mm (121.43% ETc) at 95 DAS, representing an increase of 22.57% compared with the irrigation depth of 72 mm (40% ETc), for which the estimated CD value was 18.72 mm at the same evaluation time. Regarding potassium fertilization, the highest CD value obtained was 23.83 mm with the application of 47.51 kg·ha^{-1} K at 84 DAS, corresponding to increases of 9.89% and 4.33% compared with the treatments of 0 and 80 kg.

Based on the partial derivative of the statistical model, the highest CD value obtained was 21.98 mm with an irrigation depth of 195.94 mm (92.42% ETc) at 74 DAC, representing increases of 49.60% and 27.96% compared with irrigation depths of 72 and 289 mm (40 and 160% ETc), which resulted in CD values of 11.08 and 15.83 mm, respectively, at the same evaluation time. Under the interaction between potassium fertilization and time, the highest CD value was 21.23 mm with the application of 47.12 kg·ha^{-1} K at 74 DAC, showing contributions similar to those observed in the plant sorghum cycle.

Results from this work are aligned with those obtained by [40], who also irrigated sorghum with treated domestic sewage wastewater. The authors observed that the wastewater fulfilled the plant's hydric and nutritional needs. Both works show that this is an alternative to minimize the water scarcity in arid and semi-arid regions. Nevertheless, abiotic stress still imposes limits on the productivity of several crops worldwide. Thus, alternatives to mitigate salinity and drought are still needed [41].

The highest decrease in the absolute and relative growth rate was observed with the lowest and highest irrigation depths, and in the absence or super dosage of potassium, which induced the plant to change its morphology and, consequently, reduce its photosynthetic efficiency. The biggest growth was seen during the plant's initial stage of development, then the growth rate decreased. Growth rate is related to physiological characteristics and depends on the soil and climate conditions. In this work, irrigation

management, fertilizing, and salinity influenced sweet sorghum absolute and relative growth rate, which are also parameters associated with the liquid assimilation rate [42].

Despite sweet sorghum's resilience, under highly stressful conditions, it limits its metabolic processes, which include closing its stomata, inhibiting nutrient absorption, and reducing photosynthesis efficiency. This decreases the shoot system production. Also, high salinity can alter the assimilated partition between roots and shoot, leading to reduced shoot biomass accumulation [24]. The greatest reductions in absolute and relative growth were observed under potassium absence or excess, which induced morphological changes in the plants and consequently reduced photosynthetic efficiency [43,44].

Table 6 presents the summary of the analysis of variance (ANOVA) for fresh mass productivity (FP), dry mass productivity (DP), and water use efficiency (WUE) of sweet sorghum for both plant sorghum and ratoon sorghum cycles. FP and WUE in both cycles, as well as DP for ratoon sorghum, were significantly affected by irrigation depth with TDW and by potassium doses (K), as well as their interactions, while DP for plant sorghum was influenced only by the isolated treatments, all for $p < 0.01$.

Table 6. ANOVA table for fresh biomass productivity (FP), dry biomass productivity (DP), and water use efficiency (WUE) of sweet sorghum IPA-457 under the effect of different irrigation depths (ID) and potassium doses (K).

Source of Variation	GL	Medium Square					
		FP		DP		WUE	
		Cycle 1	Cycle 2	Cycle 1	Cycle 2	Cycle 1	Cycle 2
Irrigation depths (IB)	4	8262.50 **	1657.80 **	4561.02 **	9811.82 **	7294.90 **	3147.35 **
Linear regression	1	-	-	-	-	-	-
quadratic regression	1	-	-	-	-	-	-
Potassium doses (K)	4	6685.10 **	1840.00 **	6045.40 **	2843.20 **	2936.94 **	605.30 **
Linear regression	1	-	-	-	-	-	-
quadratic regression	1	-	-	-	-	-	-
IB:K	16	687.40 **	226.40 **	199.32 **	423.84 **	1429.08 **	605.30 **
Blocks	3	101.9 ^{ns}	308.21 ^{ns}	930.07 ^{ns}	111.03 ^{ns}	13.84 ^{ns}	7.40 ^{ns}
Residue	72	846.82	244.43	534.19	125.55	124.81	9.90
CV * (%)	-	15.49	11.49	17.23	13.49	17.89	13.49

(^{ns}) No significance; (**) significant at 1%; * CV = Coefficient of variation.

For the plant sorghum cycle (FP), based on the partial derivative of the multiple regression model, the maximum yield (74.26 Mg·ha^{-1}) was obtained with the combination of ID = 196 mm (108% ETc) and K = 38.53 kg·ha^{-1}. Regarding FP during the ratoon sorghum cycle, a similar response was observed, with a maximum FP of 35.59 Mg·ha^{-1}, with the association of ID of 255.24 mm (120% ETc) and K = 38.93 kg·ha^{-1}. Compared with irrigation depths of 72 and 289 mm (40 and 160% ETc), the increases were 21.70% and 11.96%, respectively, while the extreme K doses (0 and 80 kg·ha^{-1}) resulted in percentage reductions similar to those observed for ID. Regarding FP in the ratoon sorghum cycle, a response pattern similar to that of the initial cycle was observed, with a maximum FP of 35.59 Mg·ha^{-1} obtained with the combination of ID = 255.24 mm (120% ETc) and K = 38.93 kg·ha^{-1}. However, a significant increase was observed only under the treatment with 85 mm (40% ETc), which resulted in a 17.92% reduction in FP, while the response to K treatments was similar to that observed in the plant sorghum cycle.

Potassium (K) regulates essential processes related to crop growth and development. Under saline and water stress conditions associated with wastewater use, it affects multiple physiological mechanisms: improving the functioning of the photosynthetic system,

regulating stomatal opening and closure, maintaining cellular turgor, promoting osmoregulation, and reducing the excessive uptake of toxic ions such as Na^+ and Fe^{2+}. Potassium acts as a cofactor for more than 60 enzymes, including those involved in ATP, starch, and protein synthesis, in addition to stimulating lignin synthesis and cuticle thickening, thereby providing greater structural resistance to water loss [45]. Furthermore, K enhances K^+/Na^+ ionic selectivity in root channels, reducing sodium accumulation in plant tissues and preventing phytotoxicity [46]. As a consequence, K increases stomatal conductance, CO_2 assimilation, and photoassimilate transport, favoring sugar accumulation (especially sucrose and glucose) and improving fermentative efficiency. Plants adequately supplied with K exhibit greater water use efficiency (WUE), maintaining a higher ratio between accumulated biomass and transpired water under moderate water deficit conditions [47]. In addition, potassium plays a protective role in chloroplasts by increasing stromal pH and minimizing photo-oxidative damage during water deficit, thus contributing to the integrated mitigation of water and salt stresses through osmoregulation [48].

Based on the regression equations, the highest dry matter yield (DP) values were observed under the irrigation depth (ID) of 196 mm (109% ETc), combined with K doses of 40 or 60 kg·ha^{-1}, resulting in a maximum yield of 17 Mg·ha^{-1}. For DP, the extreme K doses (0 and 80 kg·ha^{-1}) caused deleterious effects compared with the intermediate treatments. In addition, the treatment without K_2O application (0 kg·ha^{-1}) showed an increment rate of 58.75% over the total irrigation range (72–289 mm). For the K interaction analysis, the highest DP value corresponded to irrigation depths of 181 and 235 mm (100 and 130% ETc) combined with the intermediate K dose of 40 kg·ha^{-1}. Furthermore, similarly to the ID treatments, the extreme K treatments caused detrimental effects on FP.

Fresh and dry biomass yields decreased with increasing salinity levels. These findings are consistent with those reported by Silva et al. [49], who evaluated five salinity levels (2.5, 5.0, 7.5, and 10.0 dS·m^{-1}) and four sweet sorghum cultivars (CSF 11, CSF 15, P 76, and P 298), concluding that plant dry matter decreased as salinity increased in all cultivars. However, Queiroz et al. [6] evaluated the sweet sorghum cultivar BRS 506 under different irrigation water electrical conductivity (EC) levels (1.5, 3.8, and 6.0 dS·m^{-1}) and irrigation depths (55, 83, and 110% ETc) and reported that biomass production was not significantly affected by the treatments.

The results of the present study are also consistent with those of Sheik et al. [50], who evaluated forage sorghum under five EC levels (0.30, 4, 6, 8, and 10 dS·m^{-1}) and observed that the highest salinity level reduced fresh and dry biomass by 16.21% and 7.86%, respectively, compared with the control treatment. Likewise, Punia et al. [51], in a study involving two sorghum varieties and different irrigation water salinity levels (60, 80, 100, 120, and 140 mM NaCl), reported reductions in fresh biomass of 87.60% and 64.90% for the cultivars PC-5 and SSG 59-3, respectively, at the concentration of 140 mM NaCl.

The highest water use efficiency (WUE) value (165.18 kg·mm^{-1}·ha^{-1}) was observed in the plant sorghum cycle under the irrigation depth (ID) treatment of 72 mm (40% ETc) combined with the application of 38.63 kg·ha^{-1}·K, according to the partial derivative of the multiple regression equation from the statistical analysis. In the ratoon sorghum cycle, the highest WUE value was 70.03 kg·mm^{-1}·ha^{-1}, obtained with an irrigation depth of 125.34 mm and potassium fertilization of 42.06 kg·ha^{-1}. In both cycles, the irrigation depths of 289 and 339 mm (160% ETc) resulted in significant reductions in WUE. In both cycles of sweet sorghum IPA-467, the maximum water use efficiency (WUE) values were observed under the lower irrigation depth treatments. These results demonstrate the adaptation of sorghum to water stress conditions and highlight the crop's potential to maintain productivity even under abiotic stress conditions.

3.2. Total Reducing Sugars (TRS) from Sweet Sorghum IPA-467

The harvested sweet sorghum culm was milled into juice, and the total reducing sugars were measured and used to determine the yield of reducing sugars per hectare. The results are shown in Table 7:

Table 7. Total reducing sugars per hectare of sweet sorghum planted.

Plant Sorghum			Ratoon Sorghum		
Irrigation Depth (mm)	**K (kg·ha^{-1})**	**TRS (kg·ha^{-1})**	**Irrigation Depth (mm)**	**K (kg·ha^{-1})**	**TRS (kg·ha^{-1})**
72.45	0	374.55 ± 134.23 cC	84.81	0	183.70 ± 27.60 cC
72.45	20	421.31 ± 63.01 cC	84.81	20	182.49 ± 29.55 cC
72.45	40	692.32 ± 112.48 aBC	84.81	40	275.35 ± 29.55 aC
72.45	60	615.20 ± 174.30 abBC	84.81	60	232.22 ± 46.91 bC
72.45	80	241.86 ± 74.73 cC	84.81	80	211.58 ± 42.31 bcC
126.74	0	381.97 ± 254.68 cC	148.42	0	181.79 ± 17.99 cC
126.74	20	727.59 ± 141.71 abBC	148.42	20	342.19 ± 98.29 bBC
126.74	40	856.23 ± 222.84 aB	148.42	40	632.53 ± 91.36 aB
126.74	60	861.95 ± 87.44 aB	148.42	60	301.05 ± 99.11 bcBC
126.74	80	350.97 ± 109.54 cC	148.42	80	291.88 ± 165.58 bcBC
181.06	0	968.81 ± 59.41 bA	212.03	0	381.51 ± 239.87 cB
181.06	20	1509.28 ± 81.38 abA	212.03	20	480.41 ± 50.42 bcAB
181.06	40	1466.97 ± 176.31 abA	212.03	40	648.61 ± 173.41 abAB
181.06	60	1908.47 ± 655.38 aA	212.03	60	822.53 ± 222.43 aA
181.06	80	760.13 ± 456.19 bA	212.03	80	439.64 ± 30.73 bcAB
235.38	0	625.43 ±183.45 bBC	275.64	0	413.51 ± 159.51 cB
235.38	20	813.12 ± 385.35 bBC	275.64	20	466.98 ± 19.89 cAB
235.38	40	1376.39 ± 395.04 aA	275.64	40	965.25 ± 134.99 aA
235.38	60	1398.78 ± 568.33 aA	275.64	60	936.09 ± 161.19 aA
235.38	80	727.23 ± 318.34 bBC	275.64	80	396.01 ± 107.45 cBC
289.70	0	460.28 ± 68.14 cBC	339.25	0	230.57 ± 93.41 cC
289.70	20	567.38 ± 116.79 cBC	339.25	20	518.24 ± 149.52 bAB
289.70	40	1116.66 ± 265.87 abAB	339.25	40	826.12 ± 103.52 aAB
289.70	60	1285.59 ± 304.61 aAB	339.25	60	630.41 ± 147.45 abAB
289.70	80	437.35 ± 141.61 cBC	339.25	80	439.73 ± 140.00 bBC

The letters represent the Tukey test for multiple comparisons among columns ($p < 0.05$). Lowercase letters compare the five K doses within each irrigation depth, and uppercase letters compare the five irrigation depths within each K dose.

For plant sorghum, the Tukey test at the 5% significance level indicated a significant interaction effect between irrigation depth and potassium dose on TRS production in sorghum. Overall, it was observed that increasing K doses up to 60 kg·ha^{-1} favored TRS increase, while the application of 80 kg·ha^{-1} resulted in a marked reduction in productive response. Among the irrigation levels evaluated, the 181.06 mm depth presented the best performance, standing out as the most favorable level for expressing the crop's productive potential.

The highest TRS value was obtained with the combination of 181.06 mm irrigation and 60 kg·ha^{-1} K, reaching 1908.47 kg·ha^{-1}. This treatment was assigned to the superior statistical group, evidencing significantly higher performance compared to most other combinations evaluated. At the same irrigation depth, K doses of 20 and 40 kg·ha^{-1} also presented elevated TRS values, albeit inferior to the maximum observed. On the other hand, at both lower and higher irrigation depths, performance was reduced, indicating that both water deficit and excess compromised TRS accumulation.

These results demonstrate that sorghum's response to potassium was not uniform across irrigation levels, characterizing a significant interaction between the factors. Thus,

maximum productive efficiency was achieved under intermediate water conditions associated with moderate potassium fertilization, suggesting that integrated water and potassium management is determinant for increasing TRS production in sorghum culture.

In the ratoon sorghum, the Tukey test at the 5% probability level indicated a significant interaction effect between irrigation depth and potassium dose on TRS production in sorghum. Overall, it was observed that increasing K doses up to 40 kg·ha^{-1} favored progressive TRS increase, while the application of higher doses (80 kg·ha^{-1}) resulted in a marked reduction in productive response, evidencing a possible toxicity effect or nutritional imbalance.

Among the irrigation depths evaluated, the 275.64 mm depth presented the best productive performance, standing out as the most favorable level for expressing the crop's productive potential. The highest TRS value was obtained with the combination of 275.64 mm irrigation and 40 kg·ha^{-1} K, reaching 965.25 kg·ha^{-1}. This treatment was assigned to the superior statistical group (letter aA), evidencing significantly higher performance compared to most other combinations evaluated.

At the same irrigation depth (275.64 mm), the K dose of 60 kg·ha^{-1} also presented elevated TRS values (936.09 kg·ha^{-1}), not differing statistically from the optimal treatment, while at lower depths (84.81 and 148.42 mm), performance was compromised even with adequate potassium fertilization. On the other hand, at the highest depth (339.25 mm), TRS reduction was observed compared to the optimal depth, indicating that water excess also compromised sugar accumulation.

These results demonstrate that sorghum's response to potassium was not uniform across irrigation levels, characterizing a significant interaction between the studied factors. Maximum productive efficiency was achieved under intermediate-high water conditions (275.64 mm), associated with moderate potassium fertilization (40 kg·ha^{-1}), suggesting that integrated water and potassium management is determinant for increasing TRS production in sorghum culture.

Based on the TRS results (Table 7) obtained during sweet sorghum cultivation, juice samples from the different irrigation depths and potassium doses were pooled before the fermentation assays. This strategy was adopted to evaluate the crop's overall biotechnological potential, but it obscured the specific contributions of each agronomic treatment. Consequently, future research should ferment each treatment separately to clarify the direct impact of field management practices on ethanol yield.

3.3. Ethanol Tolerance in Very-High-Gravity Fermentation Using Pulse-Fed Batch Strategy

To evaluate the ethanol tolerance of the commercial yeast strain, a fermentation process was performed using a high substrate concentration. To prevent substrate inhibition, the experiment was conducted under a pulse-feeding regime throughout the fermentation period. Figure 3 presents the profiles for substrate consumption, ethanol production, and cell viability over time. By distributing the substrate through pulse feeding, potential osmotic stress on the yeast was minimized. Substrate consumption continued until approximately 40 h of fermentation, reaching an accumulated ethanol concentration of about 122.21 g·L^{-1}. However, due to the high ethanol concentration in the fermented broth, a sharp decline in cell viability was observed throughout the process, resulting in approximately 25% viability at 40 h of the fermentative process.

The study conducted by Camargos [52], which used the flocculent yeast *S. cerevisiae* C2/00, sugarcane molasses diluted in water as culture medium, at 32 °C, also reported product inhibition around 40 h of fermentation, with a final ethanol concentration of 105.35 g·L^{-1}.

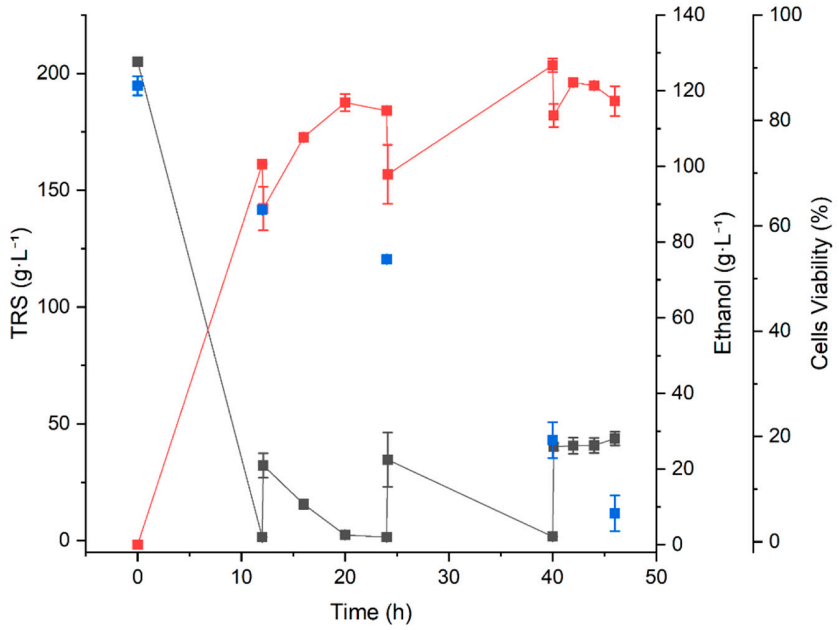


Figure 3. Cell viability (Blue), substrate –TRS (Black), and ethanol (Red) concentration profiles during pulse-fed batch ethanol fermentation.

In the study conducted by Cruz [53], maximum ethanol production by an industrial *Saccharomyces cerevisiae* strain (Y904) was investigated using a central composite design. The evaluated variables included fermentation temperature (24 to 34 °C), initial cell concentration (10 to 35 $g \cdot L^{-1}$), and initial total reducing sugar concentration (179.6 to 280.5 $g \cdot L^{-1}$). Optimal conditions for maximum ethanol (113.6 $g \cdot L^{-1}$) productivity (6 $g \cdot L^{-1} \cdot h^{-1}$) and yield (93%) were identified as 240 $g \cdot L^{-1}$ of total reducing sugars, 35 $g \cdot L^{-1}$ of inoculated cells, and a fermentation temperature of 26 °C.

Studies such as those by Cruz [53], Rivera [54], and Veloso [55] demonstrate that temperature influences ethanol production. Therefore, the higher ethanol yield observed in the present study may be attributed to the temperature difference between Camargo [52] (32 °C) and this work (30 °C). The use of sweet sorghum juice to dilute the molasses may explain the higher ethanol concentration obtained in this assay. Additionally, different *S. cerevisiae* strains were used in each study.

3.4. Batch Bioethanol Production in HG and VHG Fermentation

The fermentation performance tests using sweet sorghum juice mixed with molasses were carried out under four conditions described in Table 4, as shown in Table 8. Fermentations F1, F2, and F3 had their total reducing sugars almost completely depleted, with only fermentation F4 showing a residual concentration of 18.05 ± 4.50 $g \cdot L^{-1}$ of TRS. This value can be explained by the fact that fermentation F4 was conducted under VHG conditions.

Table 8. Comparison of fermentation parameters in HG and VHG systems (mean ± standard deviation).

Variable	Unit	Fermentation			
		F1	**F2**	**F3**	**F4**
Cs_0	$g \cdot L^{-1}$	204.50 ± 1.55	239.44 ± 2.37	238.54 ± 2.41	251.00 ± 1.28
Cs_f	$g \cdot L^{-1}$	2.17 ± 1.89	1.50 ± 1.68	1.64 ± 1.23	18.47 ± 4.50
C_{Ef}	$g \cdot L^{-1}$	87.08 ± 2.28	104.55 ± 2.89	111.16 ± 1.12	100.94 ± 3.89
$Y_{E/S}$	$g_E \cdot g_S^{-1}$	0.43 ± 0.01	0.44 ± 0.01	0.47 ± 0.01	0.43 ± 0.02
E_f	(%)	84.22 ± 2.21	85.99 ± 2.37	91.83 ± 0.92	84.8 ± 3.12
P_E	$g \cdot L^{-1} \cdot h^{-1}$	3.63 ± 0.10	4.36 ± 0.12	4.63 ± 0.05	4.21 ± 0.17

The highest ethanol concentration was achieved in fermentation F3 (111.16 $g \cdot L^{-1}$), a condition involving a mixture of sweet sorghum juice and molasses, which demonstrates that the combination improved the performance of this HG fermentation.

The fermentation F2 and F4 also yielded values above 100 $g \cdot L^{-1}$. The ethanol yields ranged from 0.43 to 0.47, which are considered acceptable to good by the industry [54,56]. The productivities were above 4.2 $g \cdot L^{-1} \cdot h^{-1}$, except for fermentation F1. This lower performance is attributed to its lower initial TRS concentration, which led to a lower final ethanol concentration.

Silva [3], using commercial yeast strains and fermentation media ranging from 100% sweet sorghum juice to 100% diluted molasses, with sugar concentrations between 172.7 and 187.5 $g \cdot L^{-1}$, conducted experiments at 34 °C for 9 h. They obtained similar ethanol yields, ranging from 0.42 to 0.44. The study was based on regular sugar concentrations, and in all tests using sweet sorghum juice, the total reducing sugars were nearly depleted—similar to the results of the present study, but under high-gravity (HG) conditions. This demonstrates the potential of sweet sorghum juice as a viable component for increasing ethanol production in industrial settings.

Camargos [52], using diluted molasses in water, conducted a fed-batch fermentation experiment in a bioreactor at a constant temperature of 32 °C, employing the flocculating yeast strain C2/00 under HG and very-high-gravity (VHG) conditions for up to 36 h. The best result was achieved at a concentration of 222.00 $g \cdot L^{-1}$ of total reducing sugars (TRS), yielding 104.4 $g \cdot L^{-1}$ of ethanol, with a yield of 85.95% and efficiency (E_f) of 89.45%. When compared to fermentation F3 in the present study, which was conducted under similar HG conditions and achieved better results, this suggests that the use of sweet sorghum could help the fermentation to reduce the inhibitory compounds commonly found in molasses-based fermentations.

3.5. Very-High-Gravity Fermentation Using a Mixture of Sorghum Juice and Sugarcane Molasses

To evaluate the use of sweet sorghum juice (IPA 467) as an alternative diluent for sugarcane molasses under very-high-gravity (VHG) conditions, fed-batch fermentation experiments were performed using a substrate composed of sweet sorghum juice and sugarcane molasses. The use of mixed substrates was adopted because sweet sorghum juice presents fermentable sugars in a good concentration and nutrients that may support yeast metabolism.

The must consisted of a mixture containing 46.60% sweet sorghum juice and 53.40% sugarcane molasses, resulting in an initial substrate concentration of Cs = 301.55 ± 4.50 $g \cdot L^{-1}$. This proportion was selected based on preliminary trials and substrate composition requirements to achieve VHG conditions while maintaining suitable operational conditions for yeast growth and fermentation performance.

The results showed that, at the end of the feeding phase, the concentration of total reducing sugars (TRS) in the wort reached 122.04 ± 3.60 $g \cdot L^{-1}$. After 30 h of fermentation, the residual substrate concentration was 2.30 ± 2.00 $g \cdot L^{-1}$, indicating an almost complete consumption of the available sugars. Ethanol production reached 122.35 ± 1.56 $g \cdot L^{-1}$, corresponding to a yield of 0.41 g_E/g_S. The process efficiency was estimated at 80.01%, with an ethanol volumetric productivity of 4.08 $g \cdot L^{-1} \cdot h^{-1}$ (Table 9). These results provide clear evidence that fed-batch fermentation can effectively minimize the substrate inhibition that occurs in the first hours of the process, allowing the fermentation to proceed until complete exhaustion of the substrate.

The final ethanol concentration achieved in the bioreactor fermentation was similar to that observed in the ethanol tolerance assay under very-high-gravity (VHG) conditions, both reaching values of 122 $g \cdot L^{-1}$. This result indicates that the process in the bioreactor

approached the maximum tolerance threshold for the yeast. The fermentative parameters obtained (Table 9) are within the ranges reported for fermentative processes [54,56].

Table 9. Performances of the very-high-gravity fed-batch ethanol fermentation.

Bioreactor Fermentation		
Variables	**Units**	**Values ***
C_{S11h}	$g{\cdot}L^{-1}$	122.04 ± 3.60
C_S final	$g{\cdot}L^{-1}$	2.30 ± 2.00
C_E	$g{\cdot}L^{-1}$	122.35 ± 1.56
$Y_{E/S}$	$g_E{\cdot}g_S{}^{-1}$	0.41 ± 0.01
P_E	$g{\cdot}L^{-1}{\cdot}h^{-1}$	4.08 ± 0.08
Initial Viability	%	87.72 ± 6.72
Final Viability	%	26.44 ± 3.85

* mean ± standard deviation.

In a study conducted by Thatiyamanee [18], the strain *Saccharomyces cerevisiae* NP01 was employed in VHG fermentations utilizing sweet sorghum juice diluted with distilled water. These experiments were carried out in Erlenmeyer flasks at 200 rpm for 72 h, with varying cell concentration, urea, and initial total reducing sugar (TRS) concentrations. In the four assays performed with 310 $g{\cdot}L^{-1}$ of TRS, ethanol production ranged from 97.20 to 117.52 $g{\cdot}L^{-1}$, sugar consumption ranged from 68 to 75% of the available TRS, and productivity ranged from 2.0 to 2.5 $g{\cdot}L^{-1}{\cdot}h^{-1}$. The substantial differences in substrate consumption and productivity, compared to the present work, can be attributed to the type of fermentation strategy applied. Whereas Thatiyamanee et al. operated in simple batch mode, this study utilized a fed-batch regime, demonstrating improved performance under VHG conditions.

The lower viability values (Table 9) obtained for fed-batch ethanol fermentation can be explained by the fact that at the end of VHG fermentation, a high ethanol concentration (C_E = 122.35 $g{\cdot}L^{-1}$) was measured in the broth. These findings confirmed that high ethanol concentrations had a negative effect on the yeast cells, hindering cell division and reducing viability, as described previously [38]. The satisfactory fermentative performance observed suggests that the use of sweet sorghum juice–molasses mixtures can provide adequate nutritional and fermentable conditions for VHG ethanol production.

The low cell viability observed at the end of fermentation makes it impossible to attempt to reuse the yeast cells. Because of this, future studies could adopt some strategies to mitigate that problem. Thatiaymanee [18] uses KCl/KOH for the protection of the cells against osmotic pressure. Pereira et al. [57] reported in their work that the CAT-1 and PE-2 yeast strains, which are industrial strains, were able to ferment under VHG conditions, demonstrating their robustness in producing ethanol in an unfavorable environment while maintaining their viability.

The results of this study were obtained under specific field and experimental conditions in the semi-arid region of Brazil and should be validated under different cultivation and processing scenarios. Therefore, future studies should evaluate additional sweet sorghum cultivars, investigate the long-term effects of irrigation with treated domestic wastewater on soil and plant health, and assess scale-up strategies for high-gravity fermentation systems. In addition, further research is needed to investigate the fermentative behavior of juice obtained from specific irrigation depths and potassium doses, enabling the identification of agronomic strategies that simultaneously maximize crop productivity and fermentation efficiency. Finally, although the integrated system appears promising, its environmental sustainability and economic viability must be confirmed through com-

prehensive life-cycle assessments (LCA) and economic evaluations to support the broader application of this approach.

4. Conclusions

This study demonstrates that treated domestic wastewater (TDW) can be effectively integrated into sweet sorghum production systems under semi-arid conditions, improving both agronomic performance and supporting bioethanol production within a circular resource-use framework. Irrigation depths between 100 and 130% of crop evapotranspiration (ETc), combined with moderate potassium fertilization (approximately 40–60 kg ha^{-1}), optimized vegetative growth, fresh biomass yield, and total reducing sugar (TRS) accumulation.

The plant crop cycle achieved a maximum fresh biomass yield of 74.26 $Mg{\cdot}ha^{-1}$ and TRS production of 1908.47 $kg{\cdot}ha^{-1}$ under optimized water–nutrient management. Both water deficit (40% ETc) and excessive irrigation (160% ETc) negatively affected growth and sugar accumulation, confirming the importance of balanced irrigation strategies when using wastewater with moderate salinity. Although ratoon productivity declined, its response pattern remained consistent, reinforcing the robustness of the system across cropping cycles.

Fermentation trials further validated the industrial potential of sweet sorghum juice supplemented with molasses. High-gravity (HG) systems achieved ethanol concentrations above 110 $g{\cdot}L^{-1}$ with fermentation efficiencies of up to 91.83%. Under very-high-gravity conditions, ethanol concentration reached 122.35 $g{\cdot}L^{-1}$, demonstrating a high substrate conversion capacity, although ethanol toxicity became the primary limiting factor.

From an agricultural engineering perspective, the integration of treated wastewater irrigation, optimized potassium fertilization, and intensified fermentation strategies constitutes a technically feasible and potentially promising bioethanol production system. This approach aims to enhance water reuse, nutrient recycling, and process intensification, offering a strategic pathway for decentralized biofuel production in water-scarce regions.

Overall, the results support the use of sweet sorghum irrigated with treated domestic wastewater as a resilient feedstock with potential for bioethanol production, bridging agronomic management and bioprocess engineering within an integrated semi-arid production model. However, its long-term environmental sustainability remains to be fully validated. Future research incorporating economic evaluation and long-term soil monitoring is essential to confirm the sustainability and environmental risks of this integrated production chain.

Author Contributions: Conceptualization, J.L.S.S., E.D.D., R.B.d.S. and Ê.F.d.F.e.S.; methodology, L.C.G. and A.A.d.S.d.S.; software, L.C.G., J.G.W.S. and A.L.B.d.S.; validation, A.A.d.S.d.S. and L.C.G.; formal analysis, E.D.D., A.B.R., A.L.B.d.S. and J.G.W.S.; investigation, L.C.G., A.A.d.S.d.S., A.B.R., A.L.B.d.S. and J.G.W.S.; resources, J.L.S.S., E.D.D., R.B.d.S. and Ê.F.d.F.e.S.; data curation, J.L.S.S., E.D.D. and Ê.F.d.F.e.S.; writing—original draft preparation, A.B.R., A.L.B.d.S. and J.G.W.S.; writing—review and editing, J.L.S.S., E.D.D. and R.B.d.S.; visualization, J.L.S.S., E.D.D., R.B.d.S. and Ê.F.d.F.e.S.; supervision, J.L.S.S. and E.D.D.; project administration, J.L.S.S., E.D.D. and R.B.d.S.; funding acquisition, J.L.S.S. and E.D.D. All authors have read and agreed to the published version of the manuscript.

Funding: The authors are grateful for the financial support provided by Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES, Finance Code 001), National council for Scientific and Technological Development (CNPq), Pernambuco State Research Foundation (FACEPE grant APQ-2157-2.01/24 and APQ-1601-4.03/25).

Data Availability Statement: The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: The authors acknowledge the NBiotec for the use of their infrastructure.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

TDW	Treated Domestic Wastewater
HG	High Gravity
VHG	Very High Gravity
ETc	Crop Evapotranspiration
TRS	Total Reducing Sugars
SSJ	Sweet Sorghum Juice
FP	Fresh Productivity
DP	Dry Productivity

References

1. Thanapornsin, T.; Phongsri, R.; Laopaiboon, L.; Laopaiboon, P. Use of spent yeasts from bioethanol production plant as low-cost nitrogen sources for ethanol fermentation from sweet sorghum stem juice in low-cost bioreactors. *Carbon Resour. Convers.* **2025**, *8*, 100269. [CrossRef]
2. Renewable Fuels Association. Annual Ethanol Production [Internet]. 2025. Available online: https://ethanolrfa.org/markets-and-statistics/annual-ethanol-production (accessed on 6 August 2025).
3. Silva, E.H.; Lemos, D.A.; Cruz, A.J.G.; Badino, A.C.; Silva, R.G.; Sonego, J.L.S. Bioethanol Production Using Mixtures of Sorghum Juice and Sugarcane Molasses: Experimental Data and Kinetic Modeling. *Sugar Tech* **2024**, *26*, 799–808. [CrossRef]
4. Vieira, L.F.; da Silva, J.W.; Nascimento, M.R.; Santos, P.R.; Baroni, D.F. Path analysis in the evaluation of ethanol and culm yield components in sweet sorghum. *Pesqui. Agropecu. Trop.* **2022**, *52*, e72850. [CrossRef]
5. Ameen, M.; Mahmood, A.; Shahzad, A.N.; Zia, M.A.; Javaid, M.M. *Sorghum's Potential Unleashed: A Comprehensive Exploration of Bio-Energy Production Strategies and Innovations*; Bioresource Technology Reports; Elsevier Ltd.: Amsterdam, The Netherlands, 2024; Volume 27.
6. de Queiroz, G.C.M.; de Medeiros, J.F.; da Silva, R.R.; da Silva Morais, F.M.; de Sousa, L.V.; de Souza, M.V.P.; da Nóbrega Santos, E.; Ferreira, F.N.; da Silva, J.M.C.; Clemente, M.I.B.; et al. Growth, Solute Accumulation, and Ion Distribution in Sweet Sorghum under Salt and Drought Stresses in a Brazilian Potiguar Semiarid Area. *Agriculture* **2023**, *13*, 803. [CrossRef]
7. Lima, B.L.d.C.; Araújo, J.S.; Souza, J.T.A.; Lira, E.C.d.; Tabosa, J.N.; Alencar, E.L.d.N.; Moraes, J.E.F.d.; Almeida, C.D.G.C.d.; Silva, A.O.d.; Rolim, M.M.; et al. Domestic Reclaimed Water for Circular Agriculture: Improving Agronomic Performance of Sweet Sorghum in a Semiarid Tropical Climate. *Sustainability* **2025**, *17*, 5765. [CrossRef]
8. Seleiman, M.F.; Al-Suhaibani, N.; El-Hendawy, S.; Abdella, K.; Alotaibi, M.; Alderfasi, A. Impacts of long-and short-term of irrigation with treated wastewater and synthetic fertilizers on the growth, biomass, heavy metal content, and energy traits of three potential bioenergy crops in arid regions. *Energies* **2021**, *14*, 3037.
9. de Lira, R.B.; Ferreira-Neto, M.; da Silva Dias, N.; de Medeiros, J.F.; de Brito, R.F.; de Lemos, M.; dos Santos Fernandes, C.; da Silva Sá, F.V. Biomass, grain yield, ethanol production, and energy cogeneration of sweet sorghum irrigated with domestic sewage effluent. *Biomass Convers. Biorefin.* **2023**, *13*, 9131–9140.
10. Somadder, P.D.; Trzcinski, A.; Chen, G.; Chow, Y.; Manan, M.A. *Fermentation of Sorghum with Aspergillus Strains: A Promising and Sustainable Pathway to Enzyme Production-Comprehensive Review*; Renewable and Sustainable Energy Reviews; Elsevier Ltd.: Amsterdam, The Netherlands, 2025; Volume 213.
11. Oktem, A.; Oktem, A.G. Bioethanol obtained from soluble sugars in sweet sorghum, influencing properties and their interrelationships. *Ind. Crops Prod.* **2024**, *221*, 119351. [CrossRef]
12. Gomes, D.; Cruz, M.; de Resende, M.; Ribeiro, E.; Teixeira, J.; Domingues, L. Very high gravity bioethanol revisited: Main challenges and advances. *Fermentation* **2021**, *7*, 38. [CrossRef]
13. Deesuth, O.; Laopaiboon, P.; Klanrit, P.; Laopaiboon, L. Improvement of ethanol production from sweet sorghum juice under high gravity and very high gravity conditions: Effects of nutrient supplementation and aeration. *Ind. Crops Prod.* **2015**, *74*, 95–102. [CrossRef]
14. Yang, H.; Coldea, T.E.; Zeng, Y.; Zhao, H. Wheat gluten hydrolysates promotes fermentation performance of brewer's yeast in very high gravity worts. *Bioresour. Bioprocess* **2021**, *8*, 5. [CrossRef] [PubMed]
15. Iwuozor, K.O.; Emmanuel, S.S.; Bello-Hassan, M.T.; Emenike, E.C.; Adeniyi, A.G. Advancements in High Gravity Fermentation Strategies for Optimizing Ethanol Production from Sugarcane-Based Substrates. *Sugar Tech* **2024**, *26*, 1016–1032. [CrossRef]

16. Moreno Alzate, J.L.; Tran, T.; Ceballos, H.; Nguyen, C.N.; Nguyen, T.C.; Zhang, X.; Newby, J.; Dufour, D.; Chu-Ky, S. Physico-chemical characterization of special cassava starches and their application for bio-ethanol production through no-cook technology at very high gravity. *Ind. Crops Prod.* **2024**, *219*, 119095.
17. Ajayo, P.C.; Ajayo, B.S.; Zhao, L.; Huang, M.; Zou, J.; Tian, D.; Guo, J.; Hu, J.; Shen, F. Ethanol production from the wood and fruit juice of paper mulberry via simultaneous saccharification and juice co-fermentation under normal and very high gravity conditions. *Renew. Energy* **2024**, *229*, 120759. [CrossRef]
18. Thatiyamanee, P.; Laopaiboon, P.; Laopaiboon, L. Optimizing bioethanol production from sweet sorghum stem juice under very high gravity fermentation and temperature stress conditions. *Carbon Resour. Convers.* **2024**, *8*, 100274.
19. Sriputorn, B.; Laopaiboon, P.; Phukoetphim, N.; Uppatcha, N.; Phuphalai, W.; Laopaiboon, L. Very high gravity ethanol fermentation from sweet sorghum stem juice using a stirred tank bioreactor coupled with a column bioreactor. *J. Biotechnol.* **2021**, *332*, 1–10. [CrossRef] [PubMed]
20. Cavalcanti, E.P.; Silva, V.P.; Sousa, F.A. Programa computacional para a estimativa da temperatura do ar para a Região Nordeste do Brasil. *Rev. Bras. Eng. Agríc. Ambient.* **2006**, *10*, 140–147. [CrossRef]
21. American Public Health Association—APHA. *Standard Methods for the Examination of Water and Wastewater–SMEWW*, 22nd ed.; American Public Health Association—APHA: Washington, DC, USA, 2012.
22. Cordeiro, G.G. *Qualidade de Água para Fins de Irrigação: Conceitos Básicos e Práticos*; Documentos 167; Embrapa Semi-Árido: Petrolina, Brazil, 2001; p. 20. Available online: https://www.infoteca.cnptia.embrapa.br/bitstream/doc/151757/1/SDC167.pdf (accessed on 29 June 2026).
23. Anyango, G.W.; Bhowmick, G.D.; Bhattacharya, N.S. A critical review of irrigation water quality index and water quality management practices in micro-irrigation for efficient policy making. *Desalin. Water Treat.* **2024**, *318*, 100304. [CrossRef]
24. Santos, G.C.L.; Garcia, P.H.M.; Viana, T.B.L.; Borges, P.F.; Araujo, L.S.; Gonzaga, S. Crescimento e eficiência do uso da água do sorgo sob distintos regimes hídricos contínuos. *Arch. Zootec.* **2020**, *69*, 266. [CrossRef]
25. Carpanez, T.G.; Moreira, V.R.; Assis, I.R.; Amaral, M.C.S. Potential for nutrients reuse, carbon sequestration, and CO_2 emissions reduction in the practice of domestic and industrial wastewater recycling into agricultural soils: A review. *J. Environ. Manag.* **2024**, *370*, 122443. [CrossRef]
26. WHO (World Health Organization). *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture*; Technical Report Series, 778; WHO: Geneva, Switzerland, 1989.
27. Oron, G.; Gillerman, L.; Lael, A.; Manor, Y.; Braude, E.; Bick, A. Minimizing health risks during secondary effluent application via subsurface drip irrigation. *Water Sci. Technol.* **2010**, *62*, 2330–2337. [CrossRef] [PubMed]
28. Allen, R.G.; Pereira, L.S.; Raes, D.; Smith, M. *Crop Evapotranspiration: Guidelines for Computing Crop Water Requirements*; FAO: Rome, Italy, 1998.
29. Doorenbos, J.; Kassam, A.H. Yield response to water. In *FAO Irrigation and Drainage Paper*; FAO: Rome, Italy, 1979; p. 193.
30. Aljibury, F.K.; Marsh, A.W.; Huntamer, J. Water use with drip irrigation. In Proceedings of the Second International Drip Irrigation Congress, San Diego, CA, USA, 7–14 July 1974.
31. Hoare, E.R.; Garzoli, K.V.; Blackwell, J. Plant water requirements as related to trickle irrigation. In Proceedings of the Second International Drip Irrigation Congress, San Diego, CA, USA, 7–14 July 1974.
32. Decroix, M. *Conception et Calcul d'un Projet de Micro-Irrigation a la Parcell*; Publicação do Centre International de Hautes Etudes Agronomiques Mediterraneennes; Institut de Bari: Bari, Italy, 1978.
33. Keller, J. Trickle irrigation. In *En Soil Conservation Service National Engineering Handbook*; United States Department of Agriculture: Washington, DC, USA, 1978; p. 129.
34. Keller, J.; Bliesner, R.D. *Sprinkle and Trickle Irrigation*; Van Nostrand Reinhold: New York, NY, USA, 1990; p. 652.
35. Cavalcanti, F.J.d.A. *Recomendações de Adubação para o Estado de Pernambuco: Segunda Aproximação*; Instituto Agronômico de Pernambuco, IPA: Recife, Brazil, 2008.
36. Farinelli, R.; Lemos, L.B. Produtividade e Eficiência Agronômica do Milho em Função da Adubação Nitrogenada E Manejos de Solo. *Int. J. Maize Sorghum* **2011**, *9*, 135–146.
37. Oliveira, E.C.A.; José Freire, F.; Campelo de Oliveira, A.; Euzébio Simões Neto, D.; Tavares da Rocha, A.; Alves de Carvalho, L. Produtividade, eficiência de uso da água e qualidade tecnológica de cana-de-açúcar submetida a diferentes regimes hídricos. *Pesq. Agropec. Bras.* **2011**, *46*, 617–625.
38. Sonego, J.L.S.; Lemos, D.A.; Pinto, C.E.M.; Cruz, A.J.G.; Badino, A.C. Extractive Fed-Batch Ethanol Fermentation with CO_2 Stripping in a Bubble Column Bioreactor: Experiment and Modeling. *Energy Fuels* **2016**, *30*, 748–757. [CrossRef]
39. Lee, S.S.; Robinson, F.M.; Wang, H.Y. Rapid determination of yeast viability. In *Biotechnol Bioeng Symp; (United States)*; University of Michigan: Ann Arbor, MI, USA, 1981.
40. Carvalho, A.A.; Montenegro, A.A.D.A.; Silva, L.; Costa, R.S.; Almeida, G.C. Análise do crescimento fisiológico do sorgo irrigado com água residuária tratada associado com cobertura morta. *Rev. Bras. Agric. Irrig.* **2021**, *14*, 4258–4270.

41. Abd El-Mageed, T.A.; Rady, M.O.; Abd El-Wahed, M.H.; Abd El-Mageed, S.A.; Omran, W.M.; Aljuaid, B.S.; El-Shehawi, A.M.; El-Tahan, A.M.; El-Saadony, M.T.; Abdou, N.M. Consecutive Seasonal Effect on Yield and Water Productivity of Drip Deficit Irrigated Sorghum in Saline Soils. *Saudi J. Biol. Sci.* **2022**, *29*, 2683–2690. [PubMed]
42. Schneider, J.R.; Caron, B.O.; Elli, E.F.; Schwerz, F.; Engroff, T.D. Cutting Management Modifies the Phyllochron, the Growth and the Production of Forage Sorghum under the Cool Environment. *Arch. Zootec.* **2019**, *68*, 552–560.
43. Souza Filho, E.J.; Barros, K.K.; Bezerra Neto, E.; Gavazza, S.; Florencio, L.; Kato, M.T. Effect of reclaimed water and dehydrated sludge on the morpho-physiology and yield of sorghum. *Environ. Technol.* **2024**, *45*, 3479–3495. [CrossRef] [PubMed]
44. Calone, R.; Sanoubar, R.; Lambertini, C.; Speranza, M.; Vittori Antisari, L.; Vianello, G.; Barbanti, L. Salt Tolerance and Na Allocation in Sorghum bicolor under Variable Soil and Water Salinity. *Plants* **2020**, *9*, 561. [CrossRef] [PubMed]
45. Rohbakhsh, H. Alleviating adverse effects of water stress on growth and yield of forage sorghum by potassium application. *Adv. Environ. Biol.* **2013**, *7*, 40–46.
46. Ashraf, M.A.; Abid, M.; Teixeira Da Silva, J.A.; Shahzad, S.M.-H.; Hussain, A.; Imtiaz, M. Potassium and salt stress: Role of potassium in alleviating salt stress in plants. *Crit. Rev. Plant Sci.* **2015**, *34*, 348–370.
47. Zörb, C.; Senbayram, M.; Peiter, E. Potassium in agriculture: Status and perspectives. *J. Plant Physiol.* **2014**, *171*, 656–669. [CrossRef] [PubMed]
48. Feitosa, D.R.; Rigo, M.M.; Lima, B.L.D.C.; Ferraz, E.X.; Leal, M.H.D.S.; Oliveira Filho, R.A.D.; Tabosa, J.N.; Ferreira, M.; Silva, A.O.D.; Silva, Ê.F.D.F.E. Potassium nutrition improves sweet sorghum yield under brackish water use in a semi-arid tropical climate. *Rev. Bras. Eng. Agrícola Ambient.* **2026**, *30*, e294203. [CrossRef]
49. Silva, M.L.D.S.; Sousa, H.G.D.; Silva, M.L.D.S.; Lacerda, C.F.D.; Gomes-Filho, E. Growth and photosynthetic parameters of saccharine sorghum plants subjected to salinity. *Acta Sci. Agron.* **2019**, *41*, e42607.
50. Sheikh, A.A.; Ahmed, K.; Akhter, B.; Qadir, G.; Nawaz, M.Q.; Rafa, H.; Wakeel, A.; Saeed, A.M. Evaluation of Quantitative and Qualitative Attributes of Forage Sorghum Irrigated with Saline Water. *Pak. J. Agric. Res.* **2021**, *34*, 905.
51. Punia, H.; Tokas, J.; Malik, A.; Singh, S.; Phogat, D.S.; Bhuker, A.; Mor, V.S.; Rani, A.; Sheokand, R.N. Discerning morpho-physiological and quality traits contributing to salinity tolerance acquisition in sorghum [*Sorghum bicolor* (L.) Moench]. *S. Afr. J. Bot.* **2021**, *140*, 409–418. [CrossRef]
52. Camargos, C.V.; Moraes, V.D.; de Oliveira, L.M.; Guidini, C.Z.; Ribeiro, E.J.; Santos, L.D. High Gravity and Very High Gravity Fermentation of Sugarcane Molasses by Flocculating Saccharomyces cerevisiae: Experimental Investigation and Kinetic Modeling. *Appl. Biochem. Biotechnol.* **2021**, *193*, 807–821. [CrossRef] [PubMed]
53. Cruz, M.L.; Resende, M.M.; Ribeiro, E.J. Evaluation of process conditions in the performance of yeast on alcoholic fermentation. *Chem. Eng. Commun.* **2018**, *205*, 846–855. [CrossRef]
54. Rivera, E.C.; Yamakawa, C.K.; Saad, M.B.W.; Atala, D.I.P.; Ambrosio, W.B.; Bonomi, A.; Junior, J.N.; Rossell, C.E. Effect of temperature on sugarcane ethanol fermentation: Kinetic modeling and validation under very-high-gravity fermentation conditions. *Biochem. Eng. J.* **2017**, *119*, 42–51. [CrossRef]
55. Veloso, I.I.K.; Rodrigues, K.C.S.; Sonego, J.L.S.; Cruz, A.J.G.; Badino, A.C. Fed-batch ethanol fermentation at low temperature as a way to obtain highly concentrated alcoholic wines: Modeling and optimization. *Biochem. Eng. J.* **2019**, *141*, 60–70. [CrossRef]
56. Basso, L.C.; Basso, T.O.; Rocha, S.N. Ethanol Production in Brazil: The Industrial Process and Its Impact on Yeast Fermentation. In *Biofuel Production-Recent Developments and Prospects*; InTech: Rijeka, Croatia, 2011; pp. 85–100. [CrossRef] [PubMed]
57. Pereira, F.B.; Guimarães, P.M.R.; Teixeira, J.A.; Domingues, L. Robust industrial Saccharomyces cerevisiae strains for very high gravity bio-ethanol fermentations. *J. Biosci. Bioeng.* **2011**, *112*, 130–136. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.