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# Sustainable bioprocess combining subcritical water pretreatment followed by anaerobic digestion for the valorization of jabuticaba (*Myrciaria cauliflora*) agro-industrial by-product in bioenergy and biofertilizer

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ABSTRACT

The management of agri-food by-products has received worldwide attention due to concerns about the environmental impacts caused by incorrect deposition. This study presented a sustainable bioprocess combining subcritical water pretreatment (SWP) followed by semi-continuous anaerobic digestion (AD) for the valorization of jabuticaba (*Myrciaria cauliflora*) agro-industrial by-product in bioenergy and agricultural fertilizer. The SWP was conducted at 180 °C, 15 MPa, water flow rate of 10 mL $min^{-1}$, solvent to feed of 22.5 g $g^{-1}$, and a kinetic time of 45 min. The AD process was operated in semi-continuous mode under mesophilic and methanogenic conditions. The results demonstrated that the hydrolysate presented glucose (5.78 g $L^{-1}$), fructose (3.63 g $L^{-1}$), arabinose (1.82 g $L^{-1}$), and cellobiose (1.28 g $L^{-1}$) as major compounds. The use of pretreated jabuticaca by-product increased the methane yield (239.04 L $CH_4$ $kg^{-1}$ TVS) in the designed bioprocess combining SWP and AD when compared to the AD without pretreatment (42.31 L $CH_4$ $kg^{-1}$ TVS). The methane produced in the bioprocess with SWP followed by AD can generate 543 kWh $t^{-1}$ of electricity and 2,243.17 MJ $t^{-1}$ of heat, avoiding a total of 177.54 kg $CO_{2\text{-eq}}$ $t^{-1}$. The digestate generated after AD can be used as a biofertilizer up to a concentration of 0.3 g $L^{-1}$, without toxic effects on the germination of *Lactuca sativa*. Finally, the sustainable bioprocess designed could be an alternative to the management of jabuticaba by-product within a circular economy framework, producing bioenergy and agricultural fertilizer that can reduce greenhouse gas emissions and environmental pollution in the food industry.

## 1. Introduction

The combustion of petroleum-based fuels for energy purposes results in significant air pollution and greenhouse gas (GHG) emissions, which are the primary cause of global warming and climate change [1]. In 2021, 36.3 Gt $CO_2$ were released into the atmosphere because of fossil energy use. The massive worldwide tax and monetary incentives from postpandemic governments have pushed annual levels to an all-time high, with an increase of 2.1 Gt when compared to 2020 [2]. The strategy stimulates interest in renewable fuels from biomass that can be used to replace traditional energy [3].

The agri-food industry is responsible for the generation of high amounts of lignocellulosic by-products during processing. In underdeveloped countries, most of the residues generated are not disposed of in an environmentally friendly manner [4]. However, agri-food by-products can be considered promising raw materials for producing clean energy, and their worldwide abundance facilitates their application in technological routes [5]. For instance, it is possible to use lignocellulosic sugars from switchgrass to produce acetone-butanol-ethanol [6,7]. Hydrogen and biobutanol can be recovered from food waste

*Abbreviations:* AD, anaerobic digestion; sCOD, soluble chemical oxygen demand; tCOD, total chemical oxygen demand; FID, flame ionization detector; GC, gas chromatograph; GHG, greenhouse gas; HPLC, high-performance liquid chromatography; HRT, hydraulic retention time; OLR, same organic load rate; RID, refractive index detector; SWP, subcritical water pretreatment; SEM, scanning electron microscopy; TFS, total fixed solids; TS, total solids; TVS, total volatile solids; UASB, upflow anaerobic sludge blanket reactor; VFA, volatile fatty acids; VSR, volatile solids loading rate.

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fermentation with *Clostridium* [8,9]. Hence, the valorization of agri-food by-products with aerobic or anaerobic microorganisms can be an eco-friendly solution to produce biofuels, electrical energy, biosurfactants, bioplastics, and biofertilizers [10]. Notwithstanding, there are social, political, and sanitary concerns about the proper disposal of agri-food waste and achieving sustainable development based on the bio-economy [11]. Industries and governments began seeking novel and cleaner processes to generate energy, driven by the global demand for bioenergy increasing as people become more aware of climate change [12].

In the food industry, the jabuticaba (*Myrciaria cauliflora*) agro-industrial by-product is an example of lignocellulosic biomass that can be exploited for energy generation. During the industrial processing of jabuticaba, several marketable products are produced in the production chain, including sweets, jellies, extracts, and liqueurs [13]. However, the peel and seeds correspond to approximately 50 % of the weight of the jabuticaba fruit [14]. A recent bibliometric analysis elucidated that the jabuticaba by-product could be used as feedstock to recover bio-based products and bioenergy. However, for bioenergy recovery, a pretreatment step should be required to hydrolyze the lignocellulosic biomass and release monosaccharides [15].

Anaerobic digestion (AD) technology can be used to recover bio-energy from biomass. After AD, it is possible to produce a digestate rich in mineral and organic components that can be used as a soil biofertilizer to replace fossil minerals [3,16]. The AD process entails the conversion of organic matter into biogas via the phases of hydrolysis, acidogenesis, acetogenesis, and methanogenesis, which are carried out by various microorganisms [17]. The methane-rich biogas produced by AD can be used as a renewable fuel for automobiles and cooking and can be burned to generate power and heat [18].

However, hydrolysis can be a limiting stage in the AD of lignocellulosic biomass since the polymeric structure of the material may hinder or prevent the conversion to more biodegradable sugars [19]. Therefore, for the management of lignocellulosic biomass (e.g., jabuticaba by-product) with AD, a previous pretreatment may be necessary to facilitate the metabolism of bacteria, providing more fermentable sugars with a shorter chain [20]. Several pretreatment methods have been suggested in the literature, including alkalis, acids, and enzymes [21]. However, these methods present some environmental and economic drawbacks for industrial applications [22].

Hydrothermal pretreatment of biomass has gained attention due to the high yield of biogas produced from lignocellulosic waste, especially when compared to conventional processes without pretreatment [23]. Hydrothermal pretreatment allows for less generation of contaminants without chemical inputs and reduced inhibitory products and residues [24]. Subcritical water has been proposed as a promising hydrothermal pretreatment for depolymerizing different components from lignocellulosic biomass [25]. To reach the subcritical state of water, the temperature and pressure conditions should be higher than the boiling point (100 °C, 1 MPa) and lower than the critical point (374 °C, 22 MPa) [26]. Therefore, subcritical water pretreatment (SWP) can be an alternative to the hydrolysis of the lignocellulosic structure of the jabuticaba by-product for a further AD process for bioenergy recovery.

Based on the above, the aim of this study was to evaluate the effect of a combined bioprocess based on SWP followed by semi-continuous AD of jabuticaba by-product to recover bioenergy and agricultural fertilizer. The performance of methanogenic reactors was evaluated by operational parameters, biogas production, methane composition, and volatile fatty acids. This study focused on the recovery of bioenergy from biogas, avoided GHG emissions, and the application of digestate after AD in the germination of lettuce. Hence, this study provides scientific information for the application of SWP and AD as alternatives for waste management in a circular economy framework, recovering bioenergy from biogas and biofertilizer from digestate.

**Table 1**
Initial characterization of the raw materials.

| Parameters | Jabuticaba by-product | Hydrolysate | Inoculum | Feed | Unit |
|---|---|---|---|---|---|
| pH | 3.25 ± 0.01 | 4.17 ± 0.03 | 7.03 ± 0.13 | 3.45 ± 0.05 | – |
| Moisture | 65.75 ± 0.08 | 98.98 ± 0.02 | 94.66 ± 0.09 | 89.10 ± 0.76 | % |
| Total solids | 34.25 ± 0.08 | 1.02 ± 0.02 | 5.34 ± 0.09 | 10.90 ± 0.76 | % |
| Total fixed solid | 0.70 ± 0.04 | 0.03 ± 0.01 | 0.71 ± 0.01 | 0.17 ± 0.01 | % |
| Total volatile solid | 33.55 ± 0.04 | 0.99 ± 0.00 | 4.63 ± 0.08 | 10.73 ± 0.77 | % |
| Alkalinity | n.d. | n.d. | 147.25 ± 3.27 | n.d. | mg $CaCO_3$ $L^{-1}$ |
| Ammonium nitrogen | 69.16 ± 0.03 | 29.26 ± 7.98 | 15.96 ± 0.04 | 26.60 ± 5.32 | mg N-$NH_3$ $L^{-1}$ |
| Soluble chemical oxygen demand | 9.69 ± 0.25 | 1.08 ± 0.01 | 0.23 ± 0.04 | 1.65 ± 0.01 | g $O_2$ $L^{-1}$ |
| Total chemical oxygen demand | 13.34 ± 0.32 | 1.44 ± 0.02 | 0.44 ± 0.02 | 2.75 ± 0.09 | g $O_2$ $L^{-1}$ |
| Soluble proteins | 0.013 ± 0.001 | 0.07 ± 0.002 | 0.036 ± 0.001 | 0.034 ± 0.002 | g $L^{-1}$ |
| Total phosphorus | 0.012 ± 0.004 | 0.04 ± 0.003 | 0.03 ± 0.02 | 0.02 ± 0.003 | g $L^{-1}$ |

The results are expressed as the mean ± standard deviation. Analysis conducted in triplicate (n = 3).

## 2. Materials and methods

### 2.1. Raw materials and inoculum

The jabuticaba agro-industrial by-product (wet basis) was provided by the company *Maria Preta* (Campinas, SP, Brazil). The mesophilic inoculum was obtained from an upflow anaerobic sludge blanket (UASB) reactor of a poultry slaughterhouse (Dacar Company, Tietê, SP, Brazil). Table 1 presents the characterization of the raw materials used for SWP and AD.

### 2.2. Subcritical water pretreatment of jabuticaba by-product

The SWP was carried out in a semi-continuous flow-through process (Fig. 1). A hydrolysis reactor with an internal volume of 110 mL was used in the subcritical system. A high-pressure water pump was installed in the system. A preheater and an electric jacket-type heat exchanger (1500 W) insulated by ceramic fiber heated the water flowing into the reactor. The hydrolysis temperature was measured by thermocouples (type K), and the pressure was measured by monometers (0 – 7.500 psi, 0.1 % accuracy). After hydrolysis, the hydrolysate was cooled in a heat exchanger connected to a thermostatic bath. The pressure was adjusted with a micrometer valve.

The operational conditions of SWP were adopted based on previous research [27]. The reactor was fed with jabuticaba by-product (20 g, wet basis) and operated with a hydrolysis temperature of 180 °C, pressure of 15 MPa, and water flow rate of 10 mL $min^{-1}$. The solvent-to-feed ratio was 22.5 g water $g^{-1}$ jabuticaba by-product. The SWP was conducted for 45 min, and every 5 min, an aliquot of the hydrolysate was collected to perform the hydrolysis kinetics.

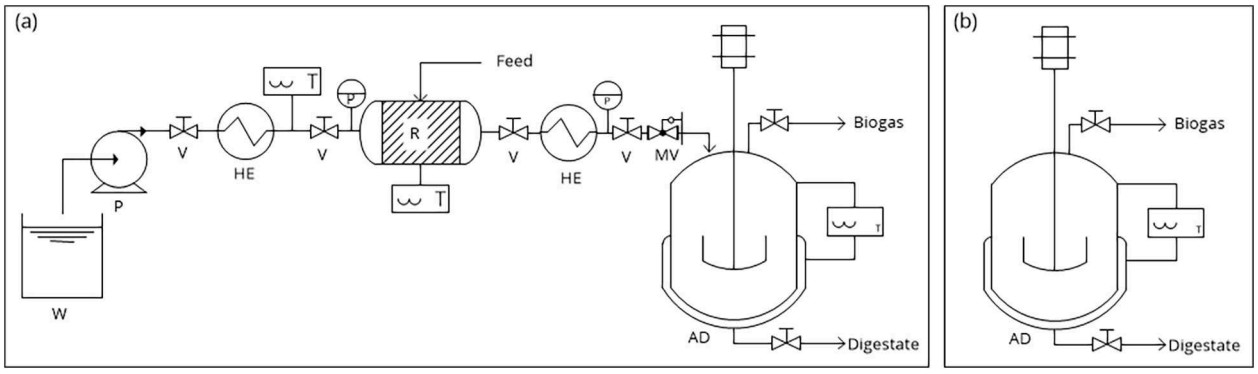


**Fig. 1.** Designed bioprocess for subcritical water pretreatment and anaerobic digestion. (a) SWP + AD reactor and (b) AD reactor. Label: W, water tank; P, high-pressure pump; V, block valves; P, manometer; T, thermocouples; R, subcritical reactor; HE, heat exchanger; MV, micrometric valve; AD, anaerobic digestion reactor.

### 2.3. Characterization of hydrolysates and solid residues after SWP

#### 2.3.1. pH

A digital pH meter (IonLab, model THS-3E, New York, NY, USA) was used to determine the pH of the hydrolysate during the hydrolysis kinetics. The pH meter was calibrated with buffer solutions before the readings, and the measurements were taken at 25 °C.

#### 2.3.2. Monosaccharides, organic acids, and inhibitors

High-performance liquid chromatography (HPLC) with a refractive index detector (RID) was used to quantify the sugar monomers (monosaccharides), organic acids, and inhibitors. Separation was performed with a Rezex™ column (Phenomenex, model ROA-Organic Acid H+ (8 %), 8 μm, 300 × 7.8 mm, Torrance, CA, USA) with an isocratic flow rate of 0.6 mL $min^{-1}$ of $H_2SO_4$ (5 mmol $L^{-1}$) at 60 °C. The RID was maintained at 40 °C. For the HPLC analysis, the hydrolysates were centrifuged (10,000 × *g*) and filtered (nylon 0.22 μm). 10 μL of hydrolysate was injected, and the run time was set to 48 min. The concentrations of cellobiose, glucose, fructose, arabinose, formic acid, acetic acid, furfural, and 5-hydroxymethylfurfural (5-HMF) were calculated from the calibration curves of each standard. The analysis was conducted in triplicate, and the results were expressed as g $L^{-1}$.

#### 2.3.3. Characterization of the solid residue

The solid residue remained in the reactor after SWP was collected and dried (105 °C, 24 h). The remaining solids were quantified by the difference considering the initial mass of the jabuticaba by-product used in the experiments. The raw biomass and solid residue were analyzed by scanning electron microscopy (SEM) (Tescan Vega 3 microscope) equipped with an energy dispersive X-ray microsound (Penta FET Precision, Oxford Instruments). For SEM analysis, the samples were dried (105 °C, 24) and then fixed to the surface of double-face adhesive tape and coated with a thin gold layer. The visualization occurred at an excitation voltage of 10 kV.

### 2.4. Semi-continuous anaerobic digestion of jabuticaba by-product

The AD process was started-up in a 4.3 L stirred tank reactor and operated in semi-continuous mode for 50 days. The following bioprocesses were started-up:

i) AD reactor (control, without pretreatment): substrate composed of 46.3 % wet jabuticaba by-product (1.54 L or 500 g, density of 0.324 g $mL^{-1}$), 31.87 % inoculum (1.07 L or 874 g, density of 0.812 g $mL^{-1}$) and 21.83 % water (0.7095 L). The substrate accounted for 3.32 L (77.2 % of the reactor's total volume), with the remaining 0.98 L for headspace (22.8 %). The AD reactor was fed daily with 14.58 g of jabuticaba by-product and 55 mL water.

ii) SWP + AD reactor (process with pretreatment): substrate composed of 52.5 % hydrolysate (1.354 L), 17.5 % wet jabuticaba by-product (0.451 L or 146 g, density of 0.324 g $mL^{-1}$), and 30 % inoculum (0.774 L or 953.7 g, density of 0.8115 g $mL^{-1}$). The substrate accounted for 2.58 L (60 % of the reactor's total volume), with the remaining 1.72 L for headspace (40 %). The SWP + AD reactor was fed daily with 14.58 g of jabuticaba by-product and 55 mL water.

The AD and SWP + AD reactors were operated under hydraulic retention times (HRT) of 33.2 and 25.8 days, respectively. The organic load rate (OLR) was 4.32 and 5.57 g $O_2$ $L^{-1}$ $d^{-1}$ for the AD and SWP + AD reactors, respectively. The volatile solids loading rate (VSR) was 1.47 and 1.89 g TVS $L^{-1}$ $d^{-1}$, respectively, for the AD and SWP + AD reactors.

The reactors were kept at a mesophilic temperature (36 °C) with a thermostatic bath (Marconi Equipment, model MA184, Piracicaba, SP, Brazil). The pH was maintained between 7 and 8.5 to enable methanogenic processes by adding NaOH (6 mol $L^{-1}$) to the feed. Mechanical stirrers (Fisatom®, model 715, São Paulo, SP, Brazil) were used for 5 min before sample collection and 5 min after feed to keep the reactors homogenized. The biogas produced in the reactor was collected daily in a Tedlar bag connected to the system (Supelco Analytical, Darmstadt, Germany). The digestate was collected to determine the operational performance.

### 2.5. Operational performance of the digestate from anaerobic digestion

#### 2.5.1. Physicochemical parameters

The operational performance of the reactors was assessed over 50 days by measuring pH, alkalinity, total nitrogen, ammonia nitrogen, soluble protein, soluble chemical oxygen demand (sCOD), total chemical oxygen demand (tCOD), total solids (TS), total fixed solids (TFS), and total volatile solids (TVS) using the Standard Methods for the Examination of Water and Wastewater [28]. The content of soluble protein was determined according to the method of Bradford [29]. All analyses were performed in triplicate ($n = 3$). The phosphorus content in the digestate was determined according to [30], with modifications. For this, 2.5 g of digestate was solubilized in 25 mL of Mehlich solution (HCl 0.05 mol/L and $H_2SO_4$ 0.0125 mol/L) to extract the phosphorous. The solution was stirred for 10 min (125 rpm, 25 °C) and then rested for 24 h before being filtered. The filtered solution (or water as a control) was reacted with 2 mL ammonium molybdate solution containing 300 mg ascorbic acid as a reducing agent. The absorbance was measured in a spectrophotometer at 660 nm after 1 h. The calibration curve was conducted with monopotassium phosphate ($KH_2PO_4$), and the results were expressed as g phosphorus $L^{-1}$ (g $L^{-1}$).

#### 2.5.2. Volatile fatty acids (VFA)

The VFA were extracted from the digestate using 5 g of sample in 50

mL of water. The solution was homogenized for 1.0 h (150 rpm, 25 °C) and filtered to remove non-soluble particles. The filtered solution was centrifuged (10.000 × g), and the supernatant was filtered (nylon 0.22 μm). The quantification and separation of VFA were conducted by HPLC-RID according to the method described in Section 2.3.2. Concentrations of acetic acid, propionic acid, isobutyric acid, and valeric acid were measured. The VFA was calculated from the calibration curves of each standard. The analysis was conducted in triplicate, and the results were expressed as g $L^{-1}$.

### 2.5.3. X-ray fluorescence (XRF)

The ashes from the TFS analysis of raw jabuticaba by-product and the digestate from AD and SWP + AD reactors (initial and final day) were used to quantify the minerals profile by XRF. Approximately 50 mg of sample was weighed, WAX binder was added, and then the samples were homogenized in a mortar. Another 50 mg of WAX binder was sieved in a plastic cup, after which the mortar containing the sample and the homogenized binder, together with the plastic cup containing the binder, were taken to the hydraulic press (AMEF, model AP-25T) with the tablet of aluminum inserted into the cavity of the press. First, the binder was added, and on top of the binder, the contents of the mortar were added, after which the material was pressed. The tablet with the pressed material was placed in X-ray fluorescence equipment (Panalytical, model Axios 1KW), and then the sample was read. The results are presented as a mass percentage of the elements obtained in the analyses.

## 2.6. Biogas volume, composition, and methane yield

The biogas produced from the AD and SWP + AD reactors was collected from the Tedlar bag, and the volume was measured daily using a syringe. The volume of biogas was adjusted for standard temperature and pressure conditions (1 atm and 298.15 K). The accumulated biogas volume was calculated by the daily biogas produced.

For the quantification of biogas composition, a gas chromatograph (GC) with a thermal conductivity detector (TCD) (Shimadzu®, model GC 2014, Kyoto, Japan) was used. Approximately 0.5 mL of biogas was collected from the reactor's headspace and injected into the GC-TDC. A micropacked column (length of 6 m and internal diameter of 3 mm) (ShinCarbon, ST 50/80 mesh) was used to determine the composition of oxygen ($O_2$), hydrogen ($H_2$), methane ($CH_4$), and carbon dioxide ($CO_2$). The following chromatographic conditions were employed: injection port and detector temperatures were set to 200 °C; the GC column temperature was initially set to 50 °C (held for 3 min) and then increased by 5 °C $min^{-1}$ to 180 °C (held for 5 min); and $N_2$ was used as the carrier gas (35 mL $min^{-1}$, 5 bar). The quantification was determined by the relative area of each compound.

The experimental methane yield was determined according to **Eq. (1)**.

$$Methane\ yield\left(\frac{L\ CH_4}{kg\ TVS_{added}}\right) = \frac{V_{biogas} \times CH_4}{TVS} \quad (1)$$

*where* $V_{biogas}$ is the accumulated volume of biogas (L), $CH_4$ is the percentage of methane in the biogas (%), and TVS is the content of volatile solids added in the reactor.

## 2.7. Potential for bioenergy recovery and avoided GHG emissions

The potential for electrical and thermal energy recovery from the methane-rich biogas was assessed assuming that the biogas is burning in a cogenerator, according to **Eqs. (2) and (3)**.

$$Electricity\left(\frac{MWh}{ton}\right) = \frac{Q_{biogas} \times LCV_{CH_4} \times C_m \times \eta_e \times CF}{M_{Jabuticaba}} \quad (2)$$

$$Heat\left(\frac{MJ}{ton}\right) = \frac{Q_{biogas} \times LCV_{CH_4} \times C_m \times \eta_e}{M_{Jabuticaba}} \quad (3)$$

*where* $M_{Jabuticaba}$ is the mass of jabuticaba by-product used during the 50 days of the experiment; $Q_{biogas}$ is the volume of biogas produced during the 50 days of AD ($m^3$ of biogas); $LCV_{CH_4}$ is the lower calorific value of methane (35.59 MJ $m^{-3}$); $C_m$ is the percentage of methane in biogas (%); $\eta_e$ is the engine efficiency (%), assumed to be 40 % for electric energy and 50 % for thermal energy; and CF is the conversion factor from MJ to MWh (1 MWh = 3600 MJ).

GHG emissions are avoided when electricity from the national grid is replaced with electricity from a local renewable source (e.g., from methane). A similar approach can quantify the avoided GHG from heat, where the biogas produced can replace natural gas (non-renewable fuel) in boilers. The quantification of avoided GHG emissions was calculated according to **Eqs. (4) and (5)**.

$$Avoided\ GHG_{electricity} = EF_{CO_2-Electricity} \times Electricity \quad (4)$$

$$Avoided\ GHG_{heat} = EF_{CO_2-Heat} \times Heat \quad (5)$$

*where* $EF_{CO_2-Electricity}$ is the emission factor of $CO_{2eq}$ for the 2019 Brazilian national electric energy generation, assumed to be 0.075 $tCO_{2\text{-eq}}$ $MWh^{-1}$ [31], and $EF_{CO_2-Heat}$ is the emission factor of heat energy (0.056 $tCO_{2\text{-eq}}$ $GJ^{-1}$), assuming a replacement of natural gas for biogas in the boiler [32].

## 2.8. Global energy balance of the bioprocesses

The global energy balance of the AD and SWP + AD processes was evaluated based on the experimental results obtained in this study. The energy balance was utilized to determine and quantify the amount of energy consumed, accumulated, transformed into another form, and lost during the process [33]. For the SWP, the first rule of thermodynamics was used to achieve energy balance, considering a steady state with constant pressure and no shaft work [34]. The variations in kinetic and potential energy were not considered. During SWP, mass (M) is constant in the process, and the heat required in the subcritical reactor (Q) is the difference in enthalpy (H) (**Eq. (6)**). Furthermore, the enthalpy can be determined using the mixture's specific heat in the reactor. It was able to use the specific heat of water (Cp*), which is 4.178 kJ $kg^{-1}$ $K^{-1}$ at 25 °C, after considering the mass of water greater mass of jabuticaba by-product [33]. A pressure pump was employed to regulate the pressure during hydrolysis, and it was assumed to be constant in the current operation. In addition, the subcritical reactor keeps the pressure constant over time while raising the water temperature from 25 to 180 °C. Thus, enthalpy was calculated based on **Eq. (7)**.

$$\frac{Q}{M} = H_2 - H_1 \quad (6)$$

$$H\left(\frac{kJ}{kg}\right) = C_p^*\left(\frac{kJ}{kg \bullet K}\right) \times T(K) \quad (7)$$

## 2.9. Application of digestate for the germination of Lactuca sativa

A germination experiment was carried out with the digestate obtained from the AD and SWP + AD reactors after 50 days. The phytotoxicity test identifies the agronomic quality of digestate for use as an agricultural substrate [35,36]. For the germination test, the digestate was diluted to different concentrations in deonized water (0.1, 0.5, and 1 g $L^{-1}$) followed by homogenization (150 rpm, 30 min, 25 °C). The homogenized solution was vacuum filtered using qualitative filter paper, and the fileted solution was used for the germination experiment. Briefly, 10 seeds of iceberg lettuce (*Lactuca sativa*) were placed in 11 cm Petri dishes containing 5 mL of the different digestate solutions soaked

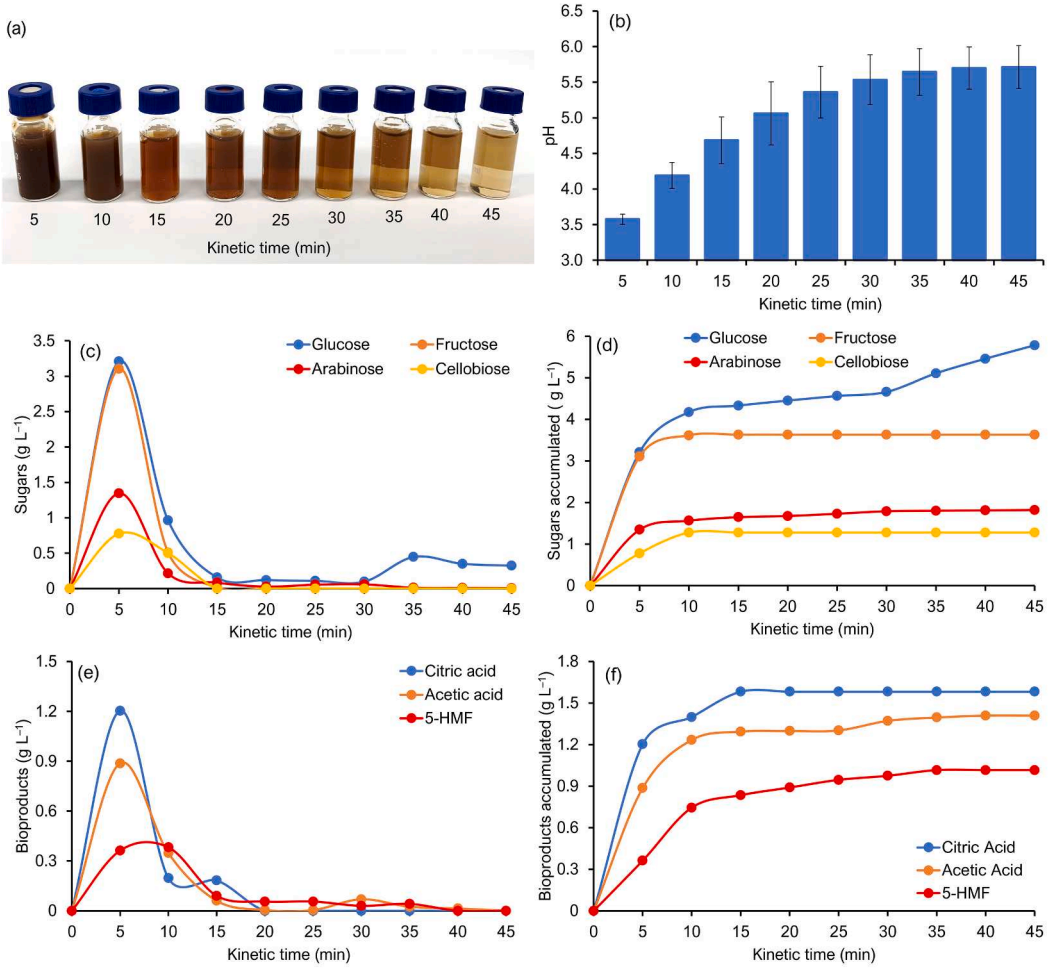


**Fig. 2.** Kinetic profile during the SWP of jabuticaba by-product. (a) Visual appearance of the hydrolysate. (b) pH. (c) Sugars (non-accumulated). (d) Sugar (accumulated). (e); bioproducts (non-accumulated). (f) bioproducts (accumulated).

in filter paper. The control was conducted with deionized water. The seeds were incubated in the dark for 7 days at 20 °C, which is the optimal germination temperature for Iceberg lettuce [37]. Tests were conducted in five repetitions.

The number of germinated seeds and the length of the roots were measured to determine the germination index (**Eq. (8)**) [38] and the inhibition percentage (**Eq. (9)**).

$$Germination\ Index\ (\%) = \frac{NGS_{Digestate} \times ARL_{Digestate}}{NGS_{Control} \times ARL_{Control}} \times 100 \quad (8)$$

$$Germination\ Inhibition\ (\%) = \frac{NGS_{Control} - NGS_{Digestate}}{NGS_{Control}} \times 100 \quad (9)$$

*where* $NGS_{Digestate}$ is the number of germinated seeds in the experiment with the digestate; $NGS_{Control}$ is the number of germinated seeds in the control experiment (with water); $ARL_{Digestate}$ is the average length of roots in the experiment with the digestate; and $ARL_{Control}$ is the average length of roots in the control experiment (with water).

### 2.10. Statistical analysis

All the data were evaluated in triplicate ($n = 3$), and the results are expressed as the mean ± standard deviation. The data were statistically analyzed using one-way analysis of variance (ANOVA), and the difference between the averages was validated using Tukey's test ($p \leq 0.05$) (Statistica® version 10.0, StatSoft Inc., Tulsa, OK, USA).

## 3. Results and discussion

### 3.1. Subcritical water pretreatment of jabuticaba by-product

Fig. 2 shows the kinetic profile of pH, sugar, and bioproducts evolution during the SWP of the jabuticaba by-product. From the visual appearance of the hydrolysate (Fig. 2**a**), it was observed that the first points of the hydrolysis kinetics resulted in a hydrolysate with a more concentrated color. The pH of the hydrolysate was acidic, ranging between 4.69 and 5.71 **(**Fig. 2**b)**. The pH values are within those found in the literature where it undergoes a slight increase during the hydrolysis kinetics [39], with similar behavior to a previous study on the hydrolysis of jabuticaba peels at different temperatures [27].

The release of sugars is the expected phenomenon from the SWP, since high temperature and pressure can promote the degradation of lignocellulose into monosaccharides. The hydrolysate obtained had a high amount of monosaccharides released during the kinetics (Fig. 2**c and 2d**). At the end of the pretreatment, the hydrolysate was composed of glucose (5.78 g $L^{-1}$), fructose (3.63 g $L^{-1}$), arabinose (1.82 g $L^{-1}$), and cellobiose (1.28 g $L^{-1}$). Glucose was the primary monosaccharide, as it comes from the hydrolysis of hydrolytically accessible cellulose and hemicellulose [40,41]. The sugar composition obtained in this study was similar to a previous study on the hydrothermal pretreatment of jabuticaba by-product, where glucose, fructose, arabinose, and cellobiose were the monosaccharides obtained [27]. Moreover, using subcritical water technology, some studies obtained glucose as the major monosaccharide using orange peel [42] and sugarcane bagasse [43] as

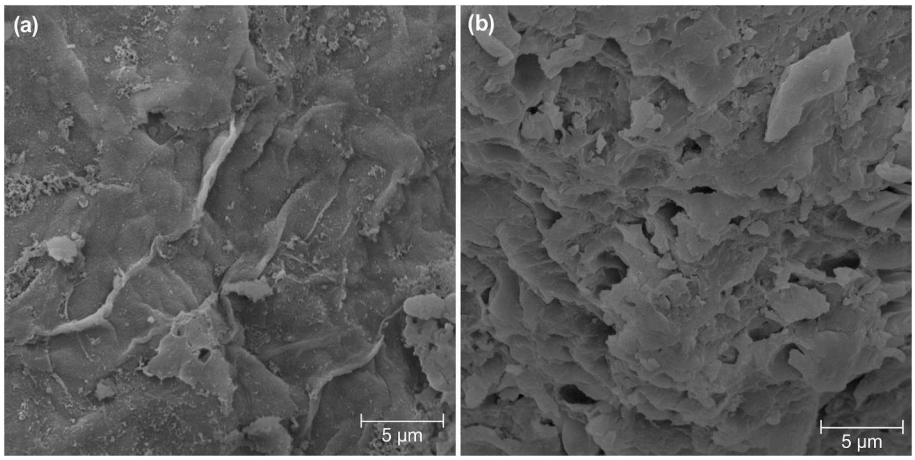


**Fig. 3.** Scanning electron microscopy of the (a) raw jabuticaba by-product and (b) solid residue after SWP.

feedstocks.

The formation of bioproducts (organic acids and inhibitors) can occur during the SWP of lignocellulosic biomass (Fig. 2**e and f**). In this study, citric acid (1.58 g $L^{-1}$) and acetic acid (1.99 g $L^{-1}$) were the organic acids obtained. The formation of organic acids occurs due to hydrolysis being carried out at high temperatures, which causes the decomposition of glucose and fructose into organic acids [44]. In addition, the jabuticaba by-product presents a high concentration of organic acids (1.38–1.48 g $g^{-1}$) in its composition [45], and during SWP, these compounds can be released into the hydrolysate. Additionally, a low concentration of 5-HMF (1.02 g $L^{-1}$) was obtained during SWP of the jabuticaba by-product. 5-HMF is an inhibitor of fermentation processes [46]. The formation of 5-HMF occurs due to the high temperature of hydrolysis, and it is usually formed by the dehydration of the six-carbon sugars formed by the degradation of cellulose [47–49].

The residual solids that remained in the reactor after pretreatment were measured and characterized by SEM. In this study, the initial biomass loaded in the reactor was reduced to 83.83 ± 2.05 %. That is, the initial dry mass of 6.7 g (equivalent to 20 g on a wet basis) of jabuticaba by-product was reduced to 1.08 ± 0.11 g (dry mass). This fact demonstrates that SWP acts in the biomass and converts cellulose, hemicellulose, and lignin into a hydrolysate. From the SEM analysis of the raw biomass (Fig. 3**a**) and the solid residue after SWP (Fig. 3**b**), it was not possible to observe that the raw biomass presented a uniform structure with a flat surface. After the SWP, the remaining biomass presented some pores, which can be indicative of changes in the physical structure of the biomass, suggesting that the SWP breakdowns the lignocellulosic structure.

Finally, the pretreatment of jabuticaba with subcritical water technology can be an alternative to produce a hydrolysate containing high concentrations of sugars, especially glucose and fructose. The hydrolysate showed a low concentration of organic acids and 5-HMF, demonstrating that the hydrolysate can be used for anaerobic fermentative processes.

### 3.2. Characterization of raw materials

The initial characterization of the raw materials is summarized in Table 1. The pH value deserves special attention, as it plays an essential role in AD. The pH of the jabuticaba by-product (3.25 ± 0.01) and the hydrolysate obtained from SWP (4.17 ± 0.03) were acids. When applying these feedstocks in AD, the pH should be adjusted to the ideal range to promote methanogenic reactions [50]. Concerning TVS, the raw jabuticaba by-product presented 33.55 ± 0.04 %, and after SWP, the hydrolysate presented a removal yield of 97 % of TVS. In addition, no alkalinity was detected in the raw material, hydrolysate, and feed.

**Table 2**
General parameters recorded during the semi-continuous AD and SWP + AD of jabuticaba by-product.

| Parameters | AD reactor | | SWP + AD reactor | | Unit |
|---|---|---|---|---|---|
| | Day 0 | Day 50 | Day 0 | Day 50 | |
| pH | 3.75 ± 0.09$^{b}$ | 8.47 ± 0.12$^{a}$ | 4.43 ± 0.05$^{B}$ | 8.48 ± 0.21$^{A}$ | – |
| Total solids | 11.08 ± 1.30$^{a}$ | 7.5 ± 0.06$^{b}$ | 3.46 ± 0.35$^{B}$ | 7.69 ± 0.03$^{A}$ | % |
| Total fixed solid | 0.45 ± 0.03$^{b}$ | 3.6 ± 0.03$^{a}$ | 0.21 ± 0.06$^{B}$ | 3.58 ± 0.03$^{A}$ | % |
| Total volatile solid | 10.63 ± 1.33$^{a}$ | 3.9 ± 0.09$^{b}$ | 3.25 ± 0.40$^{B}$ | 4.11 ± 0.00$^{A}$ | % |
| Alkalinity | n.d. | 992.75 ± 19.00 | n.d. | 1011.75 ± 23.75 | mg $CaCO_3$ $L^{-1}$ |
| Ammonium nitrogen | 26.6 ± 1.28$^{b}$ | 58.52 ± 5.32$^{a}$ | 7.98 ± 2.66$^{B}$ | 77.14 ± 10.64$^{A}$ | mg $NH_3$ $L^{-1}$ |
| Soluble chemical oxygen demand | 1.64 ± 0.47$^{a}$ | 4.55 ± 0.00$^{b}$ | 0.89 ± 0.13$^{B}$ | 4.26 ± 0.12$^{A}$ | g $O_2$ $L^{-1}$ |
| Total chemical oxygen demand | 3.16 ± 0.04$^{a}$ | 6.97 ± 0.01$^{b}$ | 1.64 ± 0.07$^{B}$ | 7.21 ± 0.09$^{A}$ | g $O_2$ $L^{-1}$ |
| Soluble proteins | 66.03 ± 0.26$^{a}$ | 28.78 ± 0.09$^{b}$ | 23.93 ± 0.45$^{B}$ | 27.35 ± 0.54$^{A}$ | g $L^{-1}$ |
| Total phosphorus | 0.05 ± 0.01$^{a}$ | 0.02 ± 0.01$^{a}$ | 0.04 ± 0.01$^{B}$ | 0.01 ± 0.01$^{A}$ | g $L^{-1}$ |

The results are expressed as the mean ± standard deviation. Analysis conducted in triplicate (n = 3). Different letters in each line (lowercase for the AD reactor and uppercase for the SWP + AD reactor) indicate significant differences by Tukey's test at $p \leq 0.05$. Label: n.d., not detected.

The SWP reduced the ammonia nitrogen, soluble proteins, sCOD, tCOD, and phosphorus of the jabuticaba by-product. The high sCOD (9.69 g $O_2$ $L^{-1}$) and tCOD (13.34 g $O_2$ $L^{-1}$) of the jabuticaba by-product demonstrate that this feedstock may be suitable for AD since methanogenic microorganisms consume organic matter and produce methane. From the characterization of raw materials, it can be observed that the pretreatment effectively converted the jabuticaba by-product into smaller organic molecules, making it possible to apply this hydrolysate into biotechnological processes for bioenergy recovery.

### 3.3. Operational performance of AD reactors

The effectiveness of the jabuticaba by-product with and without pretreatment on operating parameters, biogas generation, and bioenergy recovery was evaluated by characterization of AD and SWP + AD

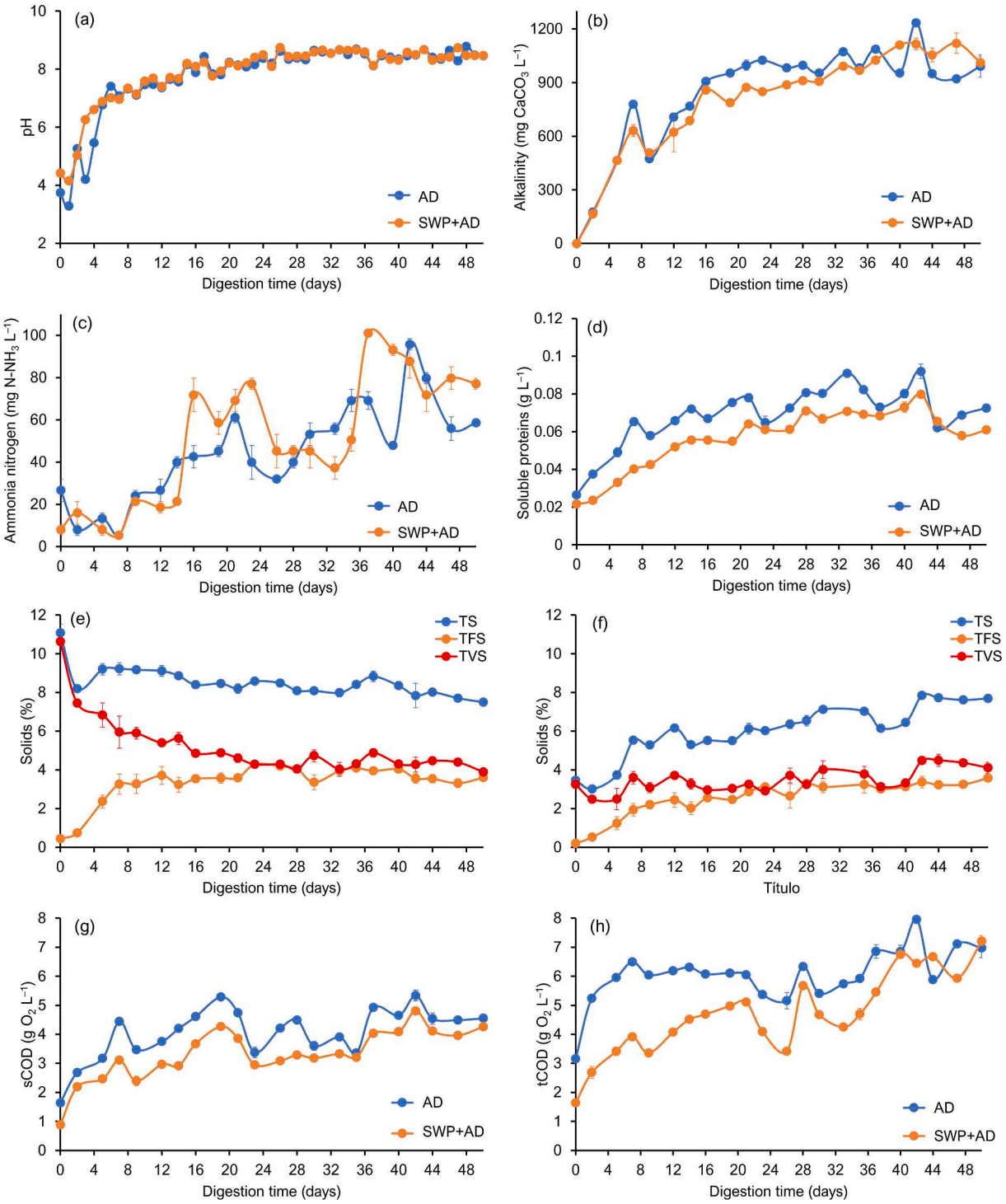


**Fig. 4.** Operational parameters during the semi-continuous AD and SWP + AD of jabuticaba by-product. (a) pH. (b) Alkalinity (mg $CaCO_3$ $L^{-1}$). (c) Ammonia nitrogen (mg N-$NH_3$ $L^{-1}$). (d) Soluble proteins (g $L^{-1}$). (e) Solids (%) for the AD reactor. (f) Solids (%) for the SWP + AD reactor. (g) Soluble chemical oxygen demand (g $O_2$ $L^{-1}$). (h) Total chemical oxygen demand (g $O_2$ $L^{-1}$).

reactors. Table 2 shows the reactor characterization on the initial and final days of AD, enabling an overview of the impact of SWP on AD performance. Fig. 4 shows the changes in pH, alkalinity, ammonia nitrogen, solids, and COD during the semi-continuous AD. A deep discussion was conducted of each operational parameter to observe the effect on biogas production and digestate quality.

### 3.3.1. *pH and alkalinity*

The pH in a solution demonstrates the concentration of protons ($H^+$) in the rector. For biotechnological processes, most microorganisms prefer a neutral pH range [51]. pH is one of the most important parameters influencing organic hydrolysis and acidogenesis [52], affecting many aspects of AD, such as the microbial community and metabolic pathways [17]. Fig. 4**a** shows the pH values of the AD and SWP + AD

reactors during 50 days of digestion. The two reactors had very similar behavior. Both oscillated in the first days of AD due to the predominance of hydrolysis and acidogenesis phases. During the hydrolysis phase, the enzymes of hydrolytic bacteria convert carbohydrates, proteins, and lipids into sugars, amino acids, and fatty acids, respectively. These compounds are transformed into VFA during the acidogenic phase, where there is formation and accumulation of organic acids, resulting in a drop in pH [53].

From day 0 until day 14, the pH ranged between 3.75 and 7.57 in the AD reactor. In contrast, for the SWP + AD reactor, the pH ranged between 4.43 and 7.68, which favors the hydrolysis of lignocellulosic compounds from the jabuticaba by-product [54]. From 15 days of AD, the methanogenesis phase was predominant in both reactors, as the pH ranged from 7.5 to 8.7. To maintain the pH values around the optimal range for methane production, 369 mL of NaOH (6 mol/L) was needed for the AD reactor, and 328 mL of NaOH (6 mol $L^{-1}$) was needed for the SWP + AD reactor over 50 days of the experiment in each reactor.

Alkalinity demonstrates the ability of a system to neutralize weak acids, and in AD, this is associated with the buffering capacity in this system. Alkalinity is necessary to maintain a stable pH in the digester to achieve optimal biological activity [55]. Fig. 4**b** demonstrates that alkalinity increased for both reactors (AD and SWP + AD). At the beginning of AD, no alkalinity was detected in the reactors. In the subsequent days of the experiment, there was an almost equal increase in both reactors. Nevertheless, from 12 days on, the alkalinity of the AD reactor was slightly higher than the alkalinity of the SWP + AD reactor, and from 40 days on, the SWP + AD reactor showed higher alkalinity. On the last day of digestion, the alkalinity was 992.75 mg $CaCO_3$ $L^{-1}$ in the AD reactor and 1011.75 mg $CaCO_3$ $L^{-1}$ in the SWP + AD reactor.

The increase in alkalinity can be explained by the formation of carbonates, bicarbonates, methane, and carbon dioxide [56]. The considerably favorable alkalinity for AD varies from 1000 to 5000 mg $CaCO_3$ $L^{-1}$, where in this range, the alkalinity has a positive effect on methane production, as the pollutant removal process is accelerated and the buffering capacity increases without inhibiting methanogenesis reactions [55]. In this experiment, the ideal alkalinity range was reached by the AD reactor on day 33 with 1073.5 mg $CaCO_3$ $L^{-1}$, while the SWP + AD reactor only reached the range on day 37 with 1026 mg $CaCO_3$ $L^{-1}$. This delay can be explained by the acidic pH of the raw material, which made it difficult to increase the alkalinity.

### 3.3.2. Ammonia nitrogen and soluble proteins

Fig. 4**c** presents the results for ammonia nitrogen. The end product of anaerobic fermentation of proteins, urea, and nucleic acids is ammonia, which can be present in the free ammonia form ($NH_3$) or ammonium ($NH_4^+$) [50,57]. Ammonia is essential in AD, as it influences microbial growth. Although necessary for AD, excess ammonia can inhibit methanogenesis [58].

In the experiments, the reactors showed regular ammonia nitrogen contents and did not show inhibitory concentrations. This increase is associated with the degradation of nitrogen compounds present in the jabuticaba by-product during hydrolysis. Ammonia nitrogen at the beginning and end of the AD reactor ranged from 26.6 to 58.52 mg N-$NH_3$ $L^{-1}$, while SWP + AD ranged from 7.98 to 77.14 mg N-$NH_3$ $L^{-1}$. Concentrations below 500 mg N-$NH_3$ $L^{-1}$ can lead to loss of biomass and, consequently, a reduction in biogas production due to a lack of nitrogenous nutrients [59]. In both reactors, the ammonia nitrogen values were below and an essential factor in biogas production in both systems.

Proteins in organic matter are converted into soluble forms during AD. From the results of soluble proteins (Fig. 4**d),** it was possible to observe an increase in the concentration of soluble proteins in the digestate. The results showed that the total proteins were converted into soluble proteins. Several groups of microorganisms participate in the degradation of different types of proteins. The proteins in AD are important because there is a high correlation between biogas production and protein degradation [60].

### 3.3.3. Phosphorus

Phosphorus plays a vital role in ecosystems and is an irreplaceable element for agriculture. The major problem is that phosphorus is a finite and scarce resource [61,62]. The phosphorus obtained in the digestate

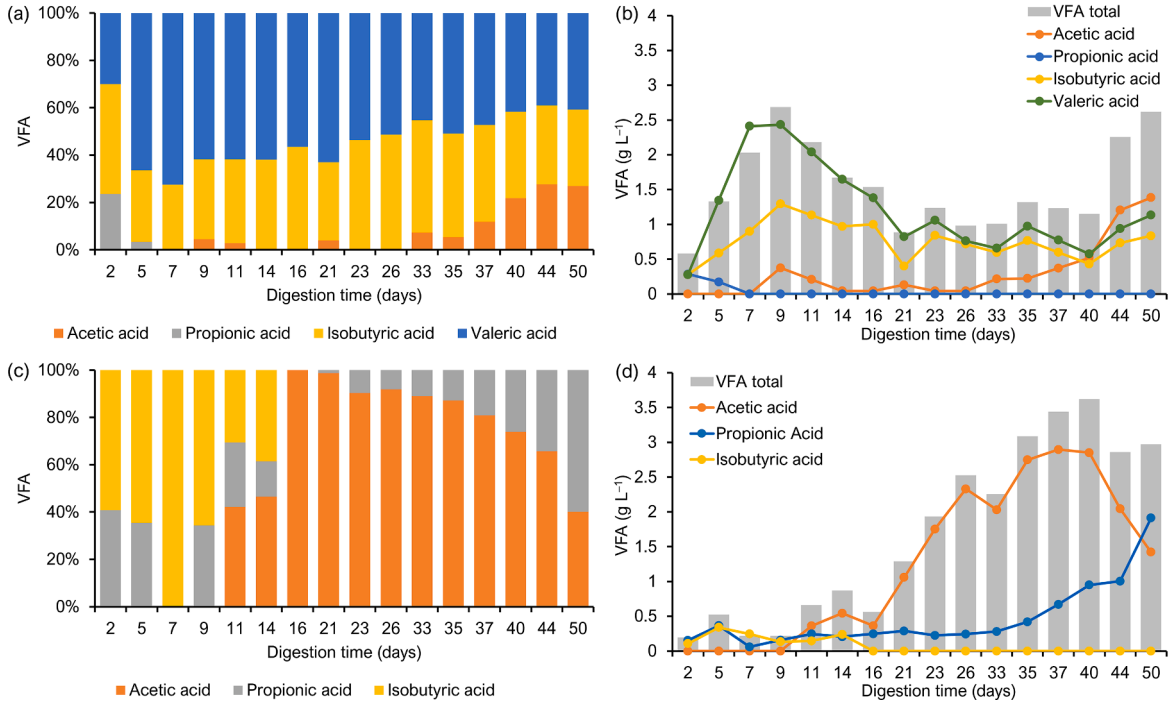


**Fig. 5.** Production of volatile fatty acids during the semi-continuous AD and SWP + AD of jabuticaba by-product. (a) The percentage of VFA in the AD reactor (%). (b) The concentration of VFA in the AD reactor (g $L^{-1}$). (c) The percentage of VFA in the SWP + AD reactor (%). (d) The concentration of VFA in the SWP + AD reactor (g $L^{-1}$).

can be used in agriculture as a fertilizer. Phosphorus is a very important nutrient for the physiological and biochemical processes of plants and is one of the most important nutrients for carrying out the photosynthesis process [63].

In this study, both reactors showed a decrease in phosphorus content during AD. The AD reactor had a phosphorus content that ranged from 0.054 g $L^{-1}$ on day 0 to 0.018 g $L^{-1}$ on day 50, while the SWP + AD reactor ranged from 0.04 g $L^{-1}$ (day 0) to 0.12 g $L^{-1}$ (day 50). The decrease in phosphorus values can be explained by the fact that the microbiota used the amount of available phosphorus, since phosphorus is an essential nutrient for living cells and is important for energy metabolism by adenosine triphosphate and for the constitution of deoxyribonucleic and ribonucleic acids [64]. Finally, the digestate obtained is rich in bioavailable nutrients, one of which is phosphorus, which makes digestate an excellent alternative for agricultural use as a fertilizer for plants [65].

### 3.3.4. Solids

The TS, TVS, and TFS evolution during AD can be seen in Fig. 4**e** (AD reactor) and Fig. 4**f** (SWP + AD reactor). Initially, the AD reactor presented a TVS of 10.63 %. The TVS decreased significantly in the initial days of digestion, reaching 5.95 % on the 7th day of AD. This decrease can be associated with the high microbial activity in the early stages of the process, where bacteria hydrolyze complex materials, reducing organic matter in the system [57]. The TVS was practically constant from day 16 until the end of the experiment, with an average of 4.4 %. The AD reactor removed 63.4 % of TVS, showing that the AD reactor is advantageous in removing organic matter with untreated jabuticaba by-product and can be an alternative for the adequate management of this agro-industrial by-product.

The SWP + AD reactor was started with a TVS content of 3.25 %. Unlike the AD reactor, which showed a decrease in the TVS value, the SWP + AD reactor showed a small increase in the value. At the end of digestion, the SWP + AD reactor had a content of 4.11 % TVS. This increase was due to the solid feed used in the system, in which a VSR of 1.95 g TVS $L^{-1}$ $d^{-1}$ was used. This feed was used for nutrient supplementation for the methanogenic microbiota.

### 3.3.5. Chemical oxygen demand

The chemical oxygen demand is one of the most important parameters to verify the efficiency of the AD process regarding the biodegradation of organic matter and methane production [1]. COD also provides the amount of oxygen needed to completely oxidize the organic content of the digestate [50]. In this study, the reactors were evaluated for sCOD (Fig. 4**g**) and tCOD (Fig. 4**h**). The sCOD and tCOD values increased during digestion, which can be associated with the OLR (3.71 g $O_2$ $L^{-1}$ $d^{-1}$ for the AD reactor and 4.93 g $O_2$ $L^{-1}$ $d^{-1}$ for the SWP + AD reactor) applied to the feed. The AD reactor started with a sCOD value of 1.64 g $O_2$ $L^{-1}$ and a tCOD of 3.15 g $O_2$ $L^{-1}$, and at the end of the digestion, the values were 4.55 and 6.97 g $O_2$ $L^{-1}$, respectively. For the SWP + AD reactor on day 0, the sCOD value was 0.88 g $O_2$ $L^{-1}$, and tCOD was 1.64 g $O_2$ $L^{-1}$, a lower value than the AD reactor. The results showed lower COD in the SWP + AD reactor on day 50 of digestion. The sCOD and tCOD were 4.25 and 7.20 g $O_2$ $L^{-1}$, respectively. There was an increase of 2.8- (AD reactor) and 4.8-fold higher (SWP + AD reactor) in the sCOD, while for tCOD the increase was 2.2- (AD reactor) and 4.4-fold higher (SWP + AD reactor).

### 3.3.6. Volatile fatty acids

Fig. 5 shows the production of VFA (acetic, propionic, isobutyric, and valeric acids) during the AD of jabuticaba by-products. VFA are the main intermediate metabolites of the anaerobic process. VFA play an important role in methane production, which is formed due to the conversion of VFA by bacteria during the methanogenic phase [66]. VFA are influenced by some environmental conditions, including pH, organic loading rate, and retention time. Nevertheless, it is worth mentioning that the production of specific VFA depends not only on pH but also on the type of substrate [67]. Although the production of VFA is not solely dependent on pH, it is a key factor in controlling the production of VFA in the acidification process [52].

**Table 3**
Chemical composition recorded during the semi-continuous AD and SWP + AD of jabuticaba by-product.

| Parameters | Jabuticaba by-product | AD reactor | | SWP + AD reactor | | Unit |
|---|---|---|---|---|---|---|
| | | Day 0 | Day 50 | Day 0 | Day 50 | |
| CaO | 4.04 | 7.72 | 1.45 | 7.27 | 1.17 | % |
| Cl | 0.24 | 0.52 | 0.13 | 0.12 | 0.16 | % |
| $CO_3O_4$ | n.d. | 0.02 | n.d. | n.d. | 0.01 | % |
| $Cr_2O_3$ | n.d. | 0.04 | 0.02 | 0.04 | n.d. | % |
| CuO | 0.06 | 0.41 | 0.05 | 0.36 | 0.05 | % |
| $Fe_2O_3$ | 0.31 | 11.93 | 1.74 | 11.77 | 1.41 | % |
| $K_2O$ | 60.44 | 22.90 | 7.63 | 9.97 | 7.46 | % |
| MgO | 6.75 | 4.79 | 1.29 | 3.29 | 1.24 | % |
| MnO | 0.03 | 0.11 | 0.01 | 0.09 | 0.02 | % |
| $MoO_3$ | n.d. | 0.02 | n.d. | 0.36 | n.d. | % |
| $Na_2O$ | 3.59 | n.d. | 80.32 | 12.54 | 81.53 | % |
| $Nd_2O_3$ | n.d. | n.d. | n.d. | n.d. | 0.02 | % |
| NiO | n.d. | 0.02 | 0.01 | 0.03 | n.d. | % |
| $P_2O_5$ | 13.36 | 13.56 | 2.38 | 10.14 | 2.21 | % |
| $Rb_2O$ | 0.10 | 0.03 | 0.02 | n.d. | 0.01 | % |
| $SO_3$ | 7.02 | 14.09 | 2.12 | 14.90 | 1.82 | % |
| $SiO_2$ | 0.24 | 14.41 | 1.41 | 18.95 | 1.53 | % |
| SrO | n.d. | 0.01 | n.d. | n.d. | n.d. | % |
| $TiO_2$ | n.d. | 0.40 | 0.04 | 0.41 | 0.04 | % |
| $Yb_2O_3$ | 0.06 | n.d. | n.d. | n.d. | n.d. | % |
| ZnO | 3.51 | 7.61 | 1.13 | 8.45 | 1.21 | % |
| $ZrO_2$ | 0.19 | 1.32 | 0.18 | 1.32 | 0.13 | % |

Label: n.d., not detected.

In the AD reactor **(**Fig. 5 **a-c),** valeric acid was the most produced VFA, with an average production of 0.7 g $L^{-1}$, followed by isobutyric acid, with an average of 0.5 g $L^{-1}$. Although acetic acid is one of the significant VFA in AD processes [52], in this reactor, it started to be produced mainly from the 40th day of digestion. The jabuticaba by-product may have hindered the microbial community from forming acetic acid until the 40th starting day. For the SWP + AD reactor **(**Fig. 5 **b-d),** acetic acid was the most produced VFA, with an average of 1.27 g $L^{-1}$, followed by propionic acid (0.37 g $L^{-1}$). The production of acetic acid tends to increase with increasing pH [52]. In this study, the pH was controlled between 7 and 8.5, which favored a better production of acetic acid during digestion. Acetic and propionic acids were the main products found in alkaline conditions with mixed culture fermentation [68], corroborating the results of the present study.

Traditionally, VFA are produced from petroleum-based sources. Although high-yield and fast-producing, the production of VFA from non-renewable sources and technologies will end up being hampered due to overexploitation and the depletion of fossil resources [69,70]. In a biorefinery concept, some studies evaluated the possibility of using AD technology for the recovery of VFA, being an additional product when compared with the strandad process that generates only biogas [71]. For the recovery of VFA, nanofiltration, reverse osmosis, pervaporation, membrane contactors, and membrane distillation are the available technologies for purification and isolation from the digestate [70].

### 3.3.7. X-ray fluorescence

Table 3 presents the results of the mineral composition of the jabuticaba by-product and the digestate of the AD and SWP + AD reactors. The jabuticaba by-product presented $K_2O$ as the major component, with 60.47 %, followed by $P_2O_5$ (13.36 %), $SO_3$ (7.02 %), MgO (6.75 %), and CaO (4.04 %). These compounds represent more than 90 % of the chemical composition of the jabuticaba by-product.

In the beginning of AD, the digestate presented in its chemical composition the $K_2O$ as the major component with 22.90 %, which was

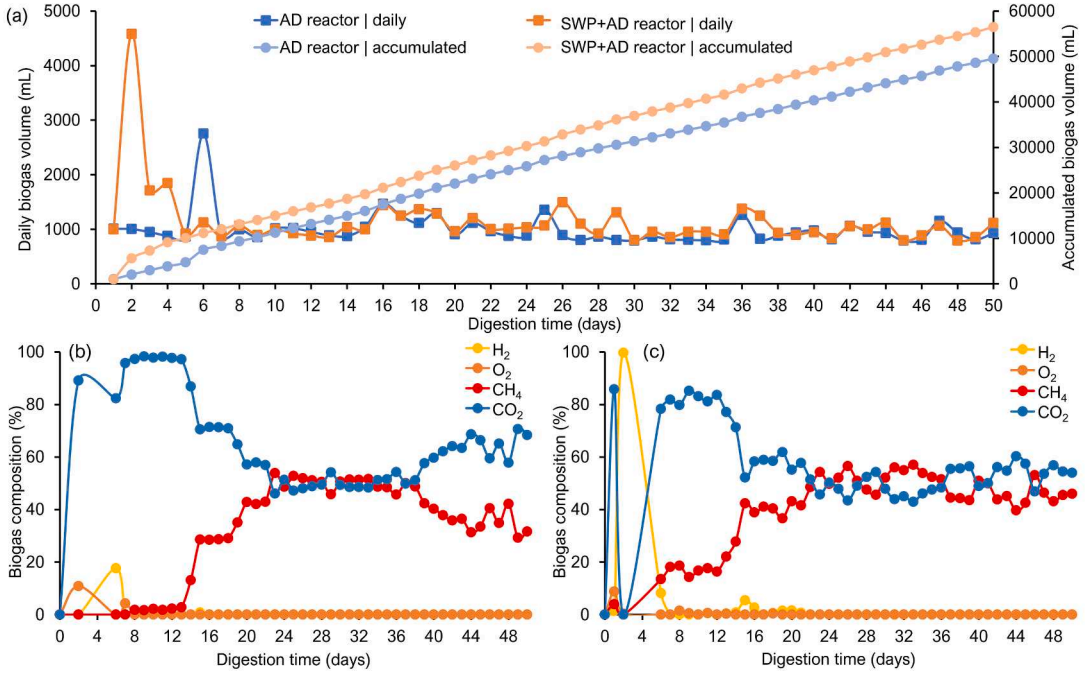


**Fig. 6.** Production of methane-rich biogas during the semi-continuous AD and SWP + AD of jabuticaba by-product. (a) Volume of biogas produced (daily and accumulated). (b) Biogas composition (AD reactor). (c) Biogas composition (SWP + AD reactor).

already expected since a large concentration of jabuticaba by-products was placed in the reactor. $SiO_2$ (14.41 %) was the second most abundant compound, followed by $SO_3$ (14.09 %), $P_2O_5$ (13.56 %), and $Fe_2O_3$ (11.93 %), and these compounds correspond to more than 75 % of the chemical composition of the digestate from the AD reactor. For the SWP + AD reactor, the digestate composition on day 0 had $SiO_2$ (18.95 %). Unlike the AD reactor, the SWP + AD reactor had a small amount of the jabuticaba by-product, so the digestate did not have a large amount of $K_2O$. The other components were $SO_3$ (14.90 %), $Na_2O$ (12.54 %), $Fe_2O_3$ (11.77 %), $K_2O$ (9.97 %) and CaO (7.27 %). These elements correspond to more than 75 % of the composition of the initial digestate of the SWP + AD reactor.

On the last day of AD (day 50), the digestate from both reactors showed a very similar chemical composition, indicating that the microorganisms acted very similarly in the two reactors at the end of the experiment due to the solid feed. The majority composition of the digestate on day 50 was $Na_2O$, with 80.32 % for the AD reactor and 81.53 % for the SWP + AD reactor. The second compound with the highest amount was $K_2O$ with 7.63 % and 7.46 %, respectively, for the AD and SWP + AD reactors. The change in the chemical composition of the digestate during AD can be explained by the fact that during digestion, a series of biochemical reactions occur, where microorganisms breakdown organic matter from the substrate [51]. In addition, because it is a biological process with several stages and involves different microorganisms, the use of different trace elements by the microorganisms is expected so that the reactor operates stably [72].

### 3.4. Production of methane in the anaerobic digestion process

In Fig. 6**,** it is possible to observe the daily and accumulated volume and the biogas composition in the AD and SWP + AD reactors. The daily production of biogas and its composition suffered some oscillations during the AD process. The oscillations of methane production are expected until the stabilization of the reactor because each digestion period is carried out by a different group of microorganisms [73].

The accumulated biogas production **(**Fig. 6**a)** was higher in the SWP + AD reactor, with a total production of 56.5 L, while the AD reactor produced 49.6 L. This result demonstrates that the reactor with the pretreatment was more efficient than the reactor without pretreatment for biogas production. The biogas production in the SWP + AD reactor was 13.9 % higher than that in the AD reactor. Regarding the biogas composition, the AD reactor **(**Fig. 6**b)** had an initial composition of $O_2$ (10.82 %) and $CO_2$ (89.18 %), whereas the SWP + AD reactor **(**Fig. 6**c)** had a composition of $H_2$ (1.43 %), $O_2$ (8.75 %), $CH_4$ (3.98 %) and $CO_2$ (85.84 %). Methane production started first in the SWP + AD reactor, with production on day 1. In contrast, methane production in the AD reactor started only on day 7, showing that pretreatment accelerated the start-up of methane production. The highest $CH_4$ peak for the AD reactor (53.91 %) occurred on day 23, while for the SWP + AD reactor, the highest $CH_4$ peak was on day 33 (57.01 %). The SWP + AD reactor presented a more stable methane content in the biogas than the AD reactor, demonstrating that the SWP is positive for the solubilization of the biomass components and increasing the production of methane-rich biogas. Some factors that affect the methane composition in biogas, such as OLR, HRT, temperature, pH, substrate composition, particle size, and feed material consistency, are parameters that deserve special attention and must be monitored to produce a stable content of methane in biogas [74–76].

The methane yield for the AD reactor was 42.31 L $CH_4$ $kg^{-1}$ TVS, while the AD reactor had a much higher yield of 239.04 L $CH_4$ $kg^{-1}$ TVS. The methane yield increased 5.64-fold higher for the SWP + AD reactor. In the literature, this is the first study on the AD of jabuticaba by-product. Comparing with other feedstocks, macaúba peel (590 L $CH_4$ $kg^{-1}$ TVS) and açaí processing residue (791.81 L $CH_4$ $kg^{-1}$ TVS) that received SWP also obtained high methane yields when compared to reactors without pretreatment [77,78]. Finally, the bioprocess designed by combining SWP and AD can be considered an excellent and promising technology for biomass treatment to produce methane-rich biogas.

### 3.5. Bioenergy potential and avoided GHG emissions

AD is a promising technology to produce methane-rich biogas in the context of energy demand and the circular economy. Compared to other technologies, such as incineration, gasification, and pyrolysis, AD causes

**Table 4**
Methane yield, potential of electric energy, heat, and avoided GHG emissions for the semi-continuous AD and SWP + AD of jabuticaba by-product.

| Parameters | AD reactor | SWP + AD reactor | Unit |
|---|---|---|---|
| Methane yield | 42.31 | 239.04 | L $CH_4$ $kg^{-1}$ $TVS_{added}$ |
| Electricity | 118.1 | 543.0 | kWh $t^{-1}$ |
| Heat | 531.38 | 2,443.17 | MJ $t^{-1}$ |
| Avoided $GHG_{electricity}$ | 8.85 | 40.72 | kg $CO_{2\text{-eq}}$ $t^{-1}$ |
| Avoided $GHG_{heat}$ | 29.76 | 136.81 | kg $CO_{2\text{-eq}}$ $t^{-1}$ |
| Avoided $GHG_{total}$ | 38.61 | 177.54 | kg $CO_{2\text{-eq}}$ $t^{-1}$ |

less air and solid waste pollution [79]. AD can be used to reduce the consumption of fossil fuels by generating energy through methane, resulting in a decrease in GHG emissions [80]. The biogas produced can occur in different ways, such as fuels for vehicular use from the use of biogas, as well as in the generation of electricity and heat from the combustion of biogas [81]. Global energy generation from biogas reached 1,331,949 TJ in 2017, an increase of 57.8 % from 2010 and 367.35 % from 2000 [82].

In this study, considering the biogas produced, methane composition, and jabuticaba by-products mass used during AD, the estimated electrical and thermal energy were evaluated (Table 4). Each ton of jabuticaba by-products submitted to the AD reactor could produce 118.1 kWh of electricity and 531.38 MJ of heat. Furthermore, this value increased to 543 kWh $t^{-1}$ of electricity and 2,443.17 MJ $t^{-1}$ of heat for the SWP + AD reactor, considering only the mass of jabuticaba by-products initially added in both reactors. With the accomplishment of this work, it was possible to obtain an increase in bioenergy production of 4.6-fold higher for electricity and heat, demonstrating that pretreatment is a great option to increase bioenergy production and that this process can be profitable for industrial implementation, reducing energy costs.

The energy and heat generated can be used by industries and even maintain the reactor's temperature. Since excess electricity can be sold to public networks, the use of bioenergy contributes to the mitigation of GHG. [79]. In this study, the methane-rich biogas produced in the AD reactor could mitigate a total of 38.61 kg $CO_{2\text{-eq}}$ $t^{-1}$ (8.85 and 29.76 kg $CO_{2\text{-eq}}$ $t^{-1}$, respectively, for electricity and heat) (Table 4). SWP increased methane production and avoided GHG emissions, reaching 177.54 kg $CO_{2\text{-eq}}$ $t^{-1}$ (40.72 and 136.81 kg $CO_{2\text{-eq}}$ $t^{-1}$, respectively, for electricity and heat).

Finally, the use of biogas generated in AD and SWP + AD reactors can be used for energy recovery in the jabuticaba processing industry, resulting in financial savings and generating an economic and

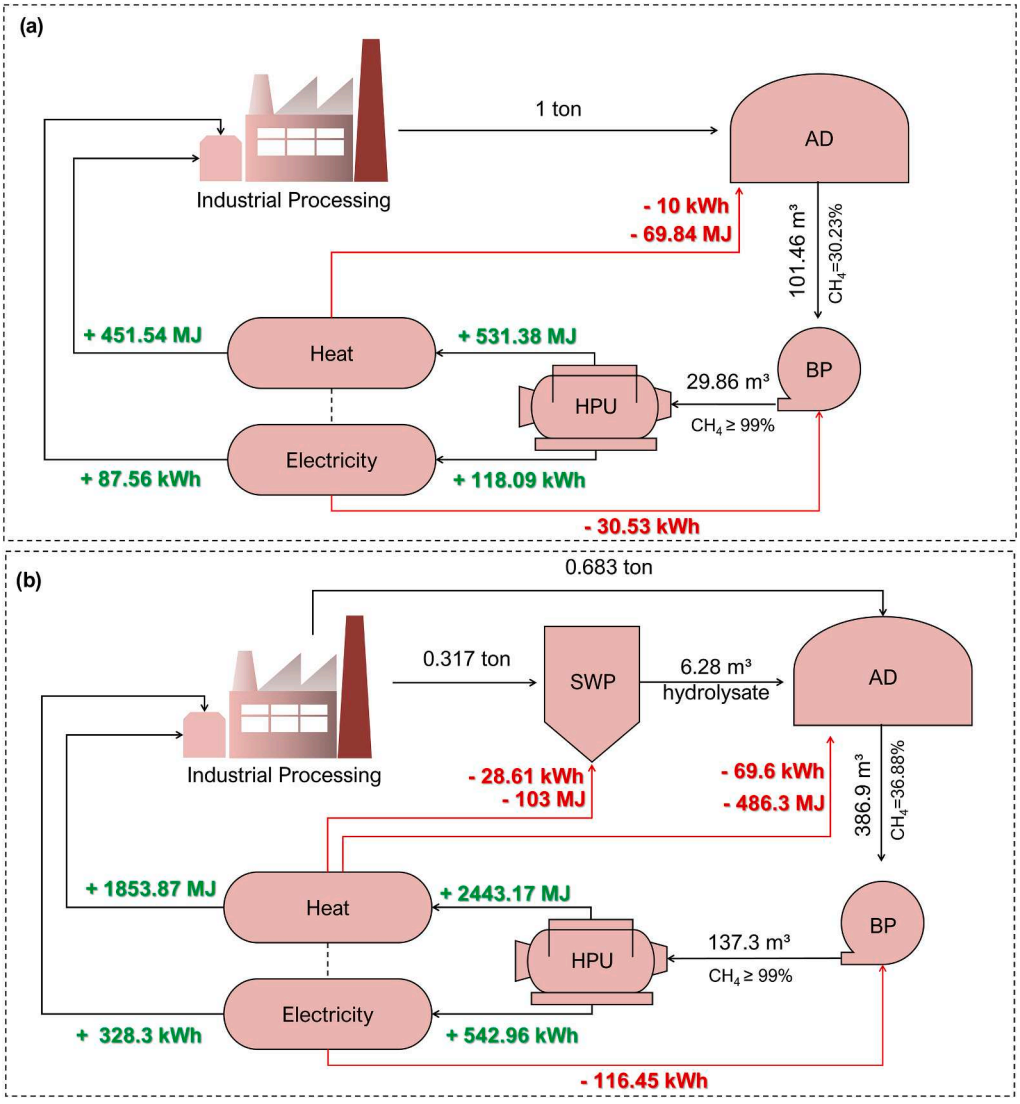


**Fig. 7.** Industrial mass and energy balance for the AD of jabuticaba by-product with and without SWP. (a) Process with the adoption of the AD reactor (without pretreatment). (b) Process with the adoption of the SWP + AD reactor (with pretreatment).

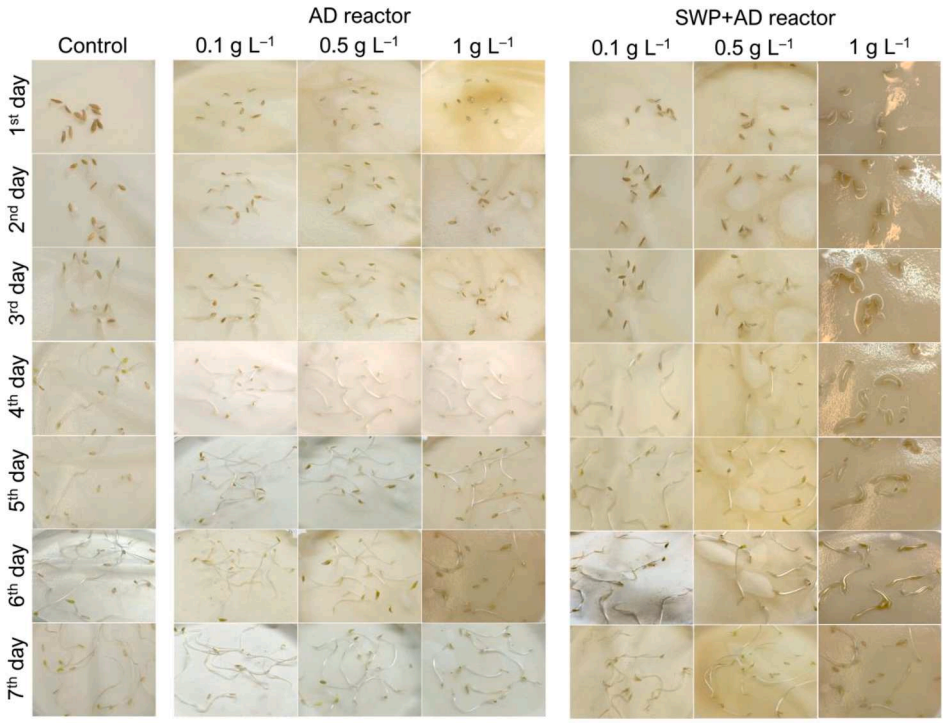


**Fig. 8.** Germination of lettuce with different concentrations of digestate obtained from the semi-continuous AD and SWP + AD of jabuticaba by-product.

environmental return. The AD is an alternative for the decentralized production of electric energy, diversifying the energy matrix and reducing GHG emissions.

### 3.6. Energy balance

The energy balance was performed considering the input of 1 ton of jabuticaba by-product (Fig. 7). The energy consumption in the process was determined to verify the surplus of the electricity and heat generated from the combustion of the methane generated from AD and SWP + AD. The AD of jabuticaba by-product without pretreatment could produce 101.46 $m^3$ biogas with an average of 30.23 % methane, considering the operational performance described in the AD reactor (Fig. 7**a**). For the application of biogas in a heat and power unit, purification is necessary to remove $CO_2$, hydrogen sulfide ($H_2S$), water vapor, and other contaminants that can be obtained in the industrial process of AD.

After purification, a cogenerator can transform methane into electrical and thermal energy. The electric energy generated can be used to supply the energy demand for purification, which was calculated for the AD process at 30.53 kWh, considering an electrical consumption of 0.301 kWh $m^{-3}$ [83]. In addition, a standard anaerobic reactor with a capacity of 1 ton, 10 kWh of electricity, and 69.84 MJ of heat is required for the mesophilic treatment [84]. In the simulated process operated with 1 ton of jabuticaba by-product, the energy required can be supplied from the self-energy produced. Finally, net electricity (87.56 kWh) and heat (451.54 MJ) can be used in the jabuticaba processing industry to replace the acquisition of national grid energy and natural gas to supply the heat in boilers. In this case, the total avoided GHG emissions are 6.56 kg $CO_{2\text{-eq}}$ for electricity and 25.29 kg $CO_{2\text{-eq}}$ for heat.

For the bioprocess with the adoption of SWP, the energy balance was estimated in the laboratory-scale hydrolysis reactor [34]. The heat required for the pretreatment was estimated at 647.6 kJ $kg^1$. In the process described in Fig. 7**b, a** total of 1 ton of jabuticaba by-products can be used for the pretreatment (0.317 ton) and AD (0.683 ton), considering that it is necessary to feed the process. From the SWP of 0.317 tons, it is possible to produce 6.28 $m^3$ hydrolysate that will be used in the start-up of the SWP + AD reactor. For the energy balance, 50 % of the energy for SWP was supplied by thermal energy (103 MJ), and the other was provided by electricity (28.61 kWh), both generated in the heat and power unit. In addition, the anaerobic reactor demands 69.6 kWh and 486.3 MJ, respectively, for electricity and heat. In this scenario, using SWP, the energy surplus was estimated at 328.3 kWh electricity and 1853.87 MJ heat. In this process, the avoided GHG emissions are 24.62 kg $CO_{2\text{-eq}}$ for electricity and 103.87 kg $CO_{2\text{-eq}}$ for heat.

Finally, the SWP of jabuticaba by-product followed by AD had a surplus of electricity (3.75-fold higher) and heat (4.1-fold higher) compared with the AD of jabuticaba by-product without pretreatment. The processes studied can contribute to the reduction of the carbon footprint of the agri-food sector since the energy generated can be used in the processing of jabuticaba. Therefore, the waste management

**Table 5**
Germination index and percentage of inhibition of the digestate obtained at the end of the semi-continuous AD and SWP + AD of jabuticaba by-product.

| Parameters | Day | AD reactor | | | SWP + AD reactor | | |
|---|---|---|---|---|---|---|---|
| | | 0.1 g $L^{-1}$ | 0.5 g $L^{-1}$ | 1 g $L^{-1}$ | 0.1 g $L^{-1}$ | 0.5 g $L^{-1}$ | 1 g $L^{-1}$ |
| Germination index (%) | 4 | $77.79 \pm 5.28^{aA}$ | $25.48 \pm 1.63^{cA}$ | $18.83 \pm 2.80^{dA}$ | $67.46 \pm 0.92^{bB}$ | $22.76 \pm 0.30^{cA}$ | $23.77 \pm 0.40^{cA}$ |
| | 7 | $63.73 \pm 0.42^{bB}$ | $19.36 \pm 2.64^{dB}$ | $13.75 \pm 1.02^{eB}$ | $75.32 \pm 3.55^{aA}$ | $23.29 \pm 1.45^{cA}$ | $17.63 \pm 2.73^{dB}$ |
| Inhibition (%) | 4 | $22.21 \pm 5.28^{cB}$ | $74.52 \pm 1.63^{aB}$ | $81.17 \pm 2.80^{aB}$ | $32.54 \pm 0.92^{bA}$ | $77.24 \pm 0.30^{aA}$ | $76.23 \pm 0.40^{aB}$ |
| | 7 | $36.27 \pm 0.42^{cA}$ | $80.64 \pm 2.64^{aA}$ | $86.25 \pm 1.02^{aA}$ | $24.68 \pm 3.55^{dB}$ | $76.71 \pm 1.45^{bA}$ | $82.37 \pm 2.73^{aA}$ |

Different letters (lowercase for lines and uppercase for the columns) indicate significant differences by Tukey's test at $p \leq 0.05$.

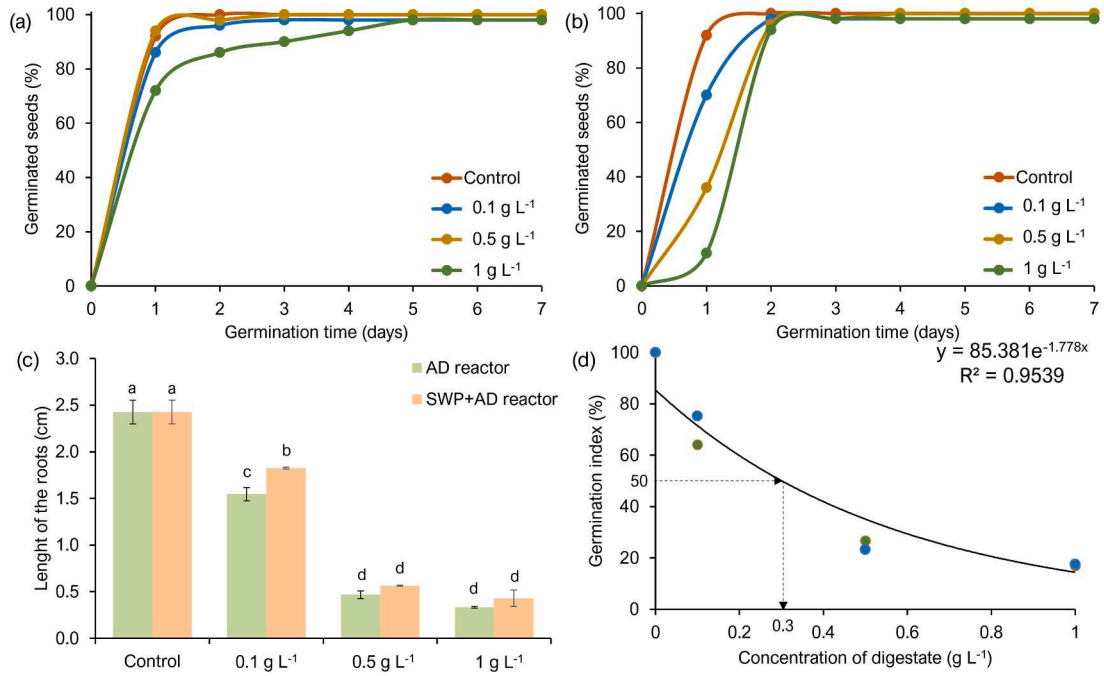


**Fig. 9.** Variation in germination seeds, length of the germinated roots, and germination index of the seeds of lettuce as a function of dilution levels. (a) Germinated seeds (%) treated with digestate from the AD reactor. (b) Germinated seeds (%) treated with digestate from the SWP + AD reactor. (c) Length of the roots (cm). (d) Regression analysis to predict the germination index as a function of the concentration of digestate.

process studied can operate in a circular economy, reducing energy costs, replacing the grid energy and natural gas with biogas, and reducing GHG emissions.

### 3.7. Application of digestate for the germination of Lactuca sativa

The effect of the digestate on lettuce germination was evaluated. The visual appearance of the germinated lettuce with digestate and with water (control) is presented in Fig. 8**.** The application of digestate decreased the germination index with the increase in the amount of digestate applied (Table 5).

The germination test using digestate from the AD reactor (Fig. 9**a**) and the SWP + AD reactor (Fig. 9**b)** showed that the time for seed germination was associated with the concentration of digestate. The digestate from the SWP + AD reactor had a greater inhibitory effect on the first day when compared with the AD reactor, as the germination index was approximately 10 % (SWP + AD reactor) and 70 % (AD reactor). Even though the digestate from the SWP + AD reactor had a higher inhibitory power, the root lengths of the germinated seeds (Fig. 9**c**) were longer than the roots that used the digestate from the AD reactor. The germination index of digestate from SWP + AD had a slight increase on day 7 compared to day 4, probably due to the lower concentration of digestate, where the presence of inhibitory compounds did not cause growth deceleration over time.

Notwithstanding, a germination index lower than 50 % indicates high toxicity [85]. In this study, the use of 0.5 and 1 g/L digestate had high toxicity and was not suitable for agricultural application in the germination of lettuce. The inhibition percentage increased for the digestate from the AD reactor, with concentrations higher than 0.5 g $L^{-1}$, and for the SWP + AD reactor, the same fact was observed for concentrations higher than 1 g $L^{-1}$. This fact can be explained by the presence of inhibitory compounds that affect lettuce germination, corroborating the literature [35]. The digestate submitted to ultrafiltration reduced its toxicity, increasing the germination rate of watercress (*Lepidium sativum*) [38]. Further technologies should be developed to reduce the toxicity of the digestate and increase the concentration for agricultural application.

The germination index of lettuce was predicted as a function of the concentration of digestate applied (**Eq. (10)**).

$$y = 85.381e^{-1.778x}\left(R^2 = 0.953\right) \quad (10)$$

The regression analysis demonstrated that to achieve a germination index of 50 % (limit to indicate toxicity), a digestate concentration of 0.3 g $L^{-1}$ can be applied. Therefore, the digestate obtained after AD and SWP + AD can be used up to 0.3 g $L^{-1}$, without toxic effects.

## 4. Conclusion

The subcritical water pretreatment of jabuticaba by-product proved to be effective in producing sugars. The hydrolysate showed high concentrations of glucose (5.78 g $L^{-1}$), fructose (3.63 g $L^{-1}$), arabinose (1.82 g $L^{-1}$), and cellobiose (1.28 g $L^{-1}$). The use of pretreated jabuticaba by-product was excellent for methane generation. The methane production in the SWP + AD reactor (239.04 L $CH_4$ $kg^{-1}$ TVS) was 5.64-fold higher than that of the AD reactor (42.31 L $CH_4$ $kg^{-1}$ TVS) without pretreatment. The methane-rich biogas from the AD reactor could produce 451.54 MJ of heat and 87.56 kWh of electricity per ton of jabuticaba by-product, while the SWP + AD reactor could generate 1853.87 MJ of heat and 328.3 kWh of electricity. Furthermore, the avoided GHG emissions were estimated at 177.54 kg $CO_{2\text{-eq}}$ $t^{-1}$ and 38.61 kg $CO_{2\text{-eq}}$ $t^{-1}$ for the SWP + AD reactor and AD reactor, respectively. The digestate generated after the anaerobic process can be applied as a sustainable fertilizer with a concentration up to 0.3 g $L^{-1}$, without toxic effects on the germination of *Lactuca sativa*. In conclusion, the designed bioprocess combining subcritical water pretreatment followed by anaerobic digestion can be a promising alternative for sustainable waste management and the recovery of bioenergy and fertilizer, advocating a circular economy transition of the agri-food industry.

### CRediT authorship contribution statement

**Rafael Gabriel da Rosa:** Methodology, Investigation, Validation,

Writing – original draft. **William Gustavo Sganzerla:** Conceptualization, Methodology, Investigation, Validation, Writing – review & editing. **Tiago Linhares Cruz Tabosa Barroso:** Methodology, Investigation, Writing – original draft. **Luiz Eduardo Nochi Castro:** Methodology, Investigation, Writing – original draft. **Mauro Donizetti Berni:** Supervision, Resources, Writing – review & editing, Funding acquisition. **Tânia Forster-Carneiro:** Supervision, Resources, Writing – review & editing, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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