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BIOGAS PRODUCTION AND GREENHOUSE GAS MITIGATION USING FISH WASTE FROM BRAGANÇA/BRAZIL

Article Highlights

- Fish waste from Bragança/Brazil and anaerobic sludge to the production of biogas
- Several inoculum/fish waste ratios were performed to determine the viability of the best condition
- Methane content ranged from 50 to 65%, and its yield varied from 80 to 140 mL·g_{vs}⁻¹
- Estimate of GHG reduction and electrical energy of 1.62x10³ tons of CO_{2e} and 370 MWh·year⁻¹
- Potential electrical energy can supply more than 100 local houses in the circular economy

Abstract

The potential of biogas production using fish waste (FW) and its effect on greenhouse gas (GHG) reduction and energy production were evaluated in this research. FW was co-digested with anaerobic sewage sludge (SS). The FW was collected in Bragança, northern Brazil, where the fish industry is the main activity with an FW production of approximately 9,000 kg·day⁻¹. The experimental part included five SS/FW ratios, and in two experiments, hydrogen was added. The experiments were carried out for 30 days, and the effect on the cumulative biogas and methane yields were analyzed. The GHG reduction was estimated using the amount of FW not discarded in the Bragança open dump, and the electricity generation was calculated using the methane yield. Besides, two kinetic models were performed. The results presented a GHG reduction of 1,619 tons of CO_{2e} and an electricity production of 372 MWh·year⁻¹ to 956 MWh·year⁻¹. Furthermore, the analysis of variance indicated that the methane production was highly dependent on the SS/FW ratios, which ranged from 76 mL·g_{vs}⁻¹ to 138 mL·g_{vs}⁻¹. Finally, this research showed the benefit of using FW to generate biogas and electricity while reducing GHG emissions in a city without energy.

Keywords: biogas, energy, fish waste, greenhouse gases, kinetic models, methane.

The fishing Industry is one of the leading market sectors worldwide, including traditional fishing on open systems and even inland aquaculture operations. The

world per capita fish consumption increased from 6.1 kg in 1950 to 20.3 kg in 2016 [1].

In 2014, global fish production was approximately 170 million tons in live weight [2]. Considering 45% of the live weight is waste, the environmental management of fish waste is a worldwide problem [3]. Usually, FW is discarded in landfills, open dumps, or incinerated. However, these methods cause environmental problems, such as groundwater pollution and the emission of toxic gases [4,5].

FW comprises many parts, like the viscera, head, skin, and bones. These sub-products can produce fish sauce, flour, oil and/or food. Besides, FW has great

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potential for energy production. The renewable energy market suggests that these feedstocks could play a part in the future of biofuels [1,6].

The anaerobic digestion process can be separated into four steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. During hydrolysis, the organic material is converted to oligomers by hydrolytic enzymes. Acidogenesis is the step whereby the products from hydrolysis are converted to volatile fatty acids and alcohols by primary fermentative bacteria. In acetogenesis, the different products from acidogenesis are converted by secondary fermentative bacteria to acetate, carbon dioxide, and hydrogen. Finally, in methanogenesis, the hydrogen and carbon dioxide and the syntrophic oxidized acetate are converted into methane [7]. Furthermore, hydrogen gas may be inserted into the anaerobic digestion reactor to enable the methanogenic communities to produce more methane through the biochemical reaction between H₂ and CO₂, augmenting the methane content in the biogas [8].

Anaerobic digestion is one of the alternatives that can be used for energy production. Biogas can be used as fuel in boilers, motor generators, gas turbines, and cogeneration units to produce heat or energy while reducing production costs and adding value to processes and products [9,10,53]. Besides, producing biogas using locally available substrates and renewable resources is an efficient and environmentally friendly technology, reducing GHG emissions and creating a circular economy [11–13]. Unfortunately, when improperly disposed at landfills or open dumps, many substrates, such as FW, may cause a natural emission of GHG (diffuse and unusable) at the landfill site, increasing the GHG emission. However, using this substrate, associated with anaerobic wastewater sludge abundant in Bragança, could be useful for three purposes: mitigating GHG emissions, generating clean energy using biogas as fuel, and producing electrical energy. These three options were evaluated in this paper.

The main objective of this paper was to evaluate biogas production using FW and anaerobic sewage sludge as co-substrates. Five SS/FW ratios were initially used, called experiments A (A₁ to A₅). Subsequently, among the five SS/FW ratios studied, the two best results regarding the methane yield and concentration were used in the experiments using hydrogen. These two new experiments were named experiments B (B₁ and B₂). First, the physical-chemical and microbiological characterization of the SS and FW were determined. Then, the cumulative biogas and methane yields were evaluated, and an analysis of variance was performed to study the process variables

to determine the influence of the SS/FW ratios and the digestion time. Next, a kinetic study was performed using the First-order and Gompertz models. Finally, the mitigation of GHG emissions and the production of electrical energy using the Bragança FW were determined.

MATERIALS AND METHODS

Fish waste and SS preparation

The FW was collected in a fish processing plant located in the city of Bragança, state of Pará, northern Brazil, which processes approximately 20,000 kg·day⁻¹ of fish, generating 9,000 kg·day⁻¹ of FW (45%) [3,14]. Generally, the FW is disposed of in an open dump in this location, emitting GHG as there is no biomass valorization program in this municipality.

The FW consists of viscera, head, skin, and bones from several types of fish species, such as *Epinephelus Marginatus* and *Cynoscion Acoupa* [14]. First, a sample of FW was separated and refrigerated at 5 °C. Then, it was crushed using Trapp TRF 80M crusher and a food processor, Philips-Walita RI7630. The processed waste was a finely divided suspension screened in a 40-mesh screen. Finally, the processed FW was stored in a closed container at -18 °C [15,16]. This reduction in the FW size (pretreatment) was necessary to improve the digestion process, especially the hydrolysis step [49].

It is important to emphasize that the pretreatment and freezing, i.e., the FW conditioning, were necessary to transport the substrate, by plane, from Bragança, in the state of Pará in the Amazon Region, to the Instrumental Chromatography Laboratory (LCI) at the UFPE-DEQ, in the state of Pernambuco, distant 1,530 km, to carry out the anaerobic digestion experiments and analysis. Furthermore, commercially, the FW will be treated on-site as feedstock in a biodigester to produce biogas locally.

The SS was collected from a sanitary sewage treatment plant in Recife, Pernambuco, in northeast Brazil, specifically from a biological digester decanter. The SS samples were collected in 2 L polyethylene flasks, sealed, and refrigerated at 5 °C [10].

Fish waste and SS characterization

The FW and SS were characterized at the LCI-UFPE, following different protocols, indicated in Table 1.

Experimental setup and analytical methods

The anaerobic digestion (AD) experiments were carried out using 50 mL flasks (bench scale biodigester)

Table 1. Main characterizations of fish waste and SS.

Fish Waste		SS	
Moisture	ISO 1442 [36]	Moisture	ISO 1442 [36]
pH	ISO 2917 [37]	pH	ISO 2917 [37]
Salt content	FAO Codex Stan 167 [42]	Conductivity	APHA/SM 2510 [44]
Phosphorus content	ISO 13730 [38]	COD	APHA/SM 5220 [46]
Fat content	ISO 1444 [39]	BOD ₅	APHA/SM 5210 [45]
Total solids	APHA/SM 2540 [43]	Total solids	APHA/SM 2540 [43]
Fixed solids	APHA/SM 2540 [43]	Fixed solids	APHA/SM 2540 [43]
Volatile solids	APHA/SM 2540 [43]	Volatile solids	APHA/SM 2540 [43]
Elemental Analysis	ASTM D3176-84 [46]	Elemental Analysis	ASTM D3176-74 [48]
Protein content	ISO 1871 [40]	Anaerobic and aerobic colony-forming units (CFU)	São Paulo [47]
Potassium content	ISO 5310 [41]		

FAO: Food and Agriculture Organization of the United Nations; ISO: International Organization for Standardization; ASTM: American Society for Testing and Materials - standard methods for the ultimate analysis of coal and coke; APHA/SM: American Public Health Association/standard methods; São Paulo Government - Secretary of agriculture - Normative Instruction n° 62.

with an adequate volume of 30 mL and a headspace of 20 mL, airtight closed by a rubber septum and aluminum seal. In each flask, the needle of a 60 mL syringe was inserted through the septum to enable the measurement of the biogas yield by the volume of the displaced plunger, as shown in Figure 1. The biogas yields were determined daily.

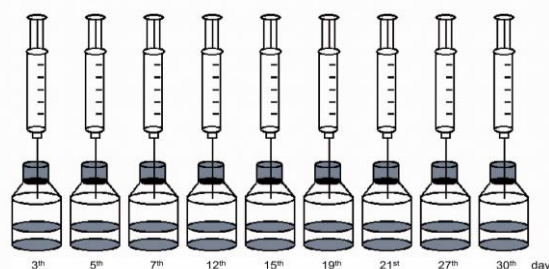


Figure 1. Anaerobic digestion experimental setup - 50 mL flasks and 60 mL syringe.

Five experiments (A1 to A5) were initially performed with five different SS/FW ratios. For example, experiment A₁ used 1.00 g of SS, 9.00 g of FW (SS/FW ratio of 0.11 g.g⁻¹), and 20.00 g of water. The same composition was used in 18 flasks (9 flasks in duplicate).

The biogas composition and the substrate pH of each flask pair were analyzed on days 3rd, 5th, 7th, 12th, 15th, 18th, 21st, 27th, and 30th. The methane, carbon dioxide, and hydrogen contents were determined by gas chromatography. To obtain the gas samples for each flask and to inject the biogas samples in the GC, 1000 µL gastight syringe was used. Finally, carefully, upon the internal pressure removal, the flask pair were opened to determine their pH. The other pairs of flasks (digesters), experiments A₂ to A₅, followed the same procedure, and they were analyzed on the predetermined days to conclude the AD experiments and pH analyses. The SS/FW ratios were:

$$A_2 = 2.15\text{g}/8.00\text{g} (0.27 \text{ g}\cdot\text{g}^{-1}),$$

$$A_3 = 3.00\text{g}/7.00\text{g} (0.43 \text{ g}\cdot\text{g}^{-1}),$$

$$A_4 = 8.25\text{g}/1.75\text{g} (4.71 \text{ g}\cdot\text{g}^{-1}) \text{ and}$$

$$A_5 = 9.00\text{g}/1.00\text{g} (9.00 \text{ g}\cdot\text{g}^{-1}).$$

These SS/FW ratios were based on the research found in the literature, such as 10.0 g.g⁻¹ and 6.7 g.g⁻¹, respectively [16,32]. As the best results were obtained on experiments A₄ and A₅, the same ratios were used in the same conditions. However, on the 7th and 18th days, 0.16 mmol of hydrogen was injected in the digesters, using a 5000 µL gastight syringe, through the rubber septum (with a very fine needle). These two new experiments were named B₁ and B₂. Hydrogen injection aimed to verify how hydrogen would affect the methane content in the biogas through the H₂ + CO₂ reaction in the methanogenic phase [8].

Determining the optimum SS/FW ratio is a very important parameter in anaerobic digestion studies, as it can maximize biogas yield with the methane concentration [17–19]. Therefore, the results of biogas yields and methane concentrations were expressed regarding volatile solids (VS), considering the FW and SS volatile solids. The volatile solids consist of the organic phase in the fermentable solid matter. Hence, the VS was standard to express the biogas and methane yields [50,51].

The cumulative biogas and methane yields from the SS control (blank experiments) were performed using the same conditions of experiments A₁ to B₂ without FW insert. In this case, the yields were evaluated only on the 12th, 21st, and 30th days. Blank (control) experiments are important to compare the biogas and methane yields without inserting both substrates.

All experiments are summarized in Table 2. These experiments were carried out for 30 days at a temperature of $30\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$, with manual agitation four times a day.

The methane and carbon dioxide content in each SS/FW ratio were analyzed by gas chromatography (GC) using a gas chromatograph HP 5890 with a thermal conductivity detector (TCD). The column used was a Porapak-N: 6.0 m x 2.5 mm i.d. The injector and detector temperatures were $100\text{ }^{\circ}\text{C}$. The GC oven temperature was $40.0\text{ }^{\circ}\text{C}$ (3.0 min), heated at

$20.0\text{ }^{\circ}\text{C}$ (1 min) to $100.0\text{ }^{\circ}\text{C}$ (2.0 min). Nitrogen was a carrier gas at a constant flow of $30.0\text{ mL}\cdot\text{min}^{-1}$. The volume of the biogas injected (splitless mode) was $100\text{ }\mu\text{L}$, using a $1000\text{ }\mu\text{L}$ GC syringe. The GC gas standard was a mixture of CH_4 (55%), CO_2 (40%), H_2 (1%), and N_2 (4% for balance), provided by White Martins Inc., with 99.9% purity. For experiments B₁ and B₂, the residual hydrogen content was measured by GC at the same temperatures and nitrogen flow rate. However, a 5A molecular sieve column, 3.0 m x 2.5 mm i.d, was used.

Table 2. Anaerobic digestion experimental resume.

Experiment	I (g)	FW (g)	SS/FW ($\text{g}\cdot\text{g}^{-1}$)	Water (g)	Control (blank)
A ₁	1.00	9.00	0.11	20.00	1.00 g (I) + 20 g (water)
A ₂	2.15	8.00	0.27	19.85	2.15 g (I) + 20 g (water)
A ₃	3.00	7.00	0.43	20.00	3.00 g (I) + 20 g (water)
A ₄	8.25	1.75	4.71	20.00	8.25 g (I) + 20 g (water)
A ₅	9.00	1.00	9.00	20.00	9.00 g (I) + 20 g (water)
B ₁	8.25*	1.75	4.71	20.00	8.25 g (I) + 20 g (water)
B ₂	9.00*	1.00	9.00	20.00	9.00 g (I) + 20 g (water)

T = $30\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$; *0.16 mmol of hydrogen at the days 7th and 18th.

Analysis of variance

The analysis of variance (ANOVA) was performed using the best results concerning the biogas and methane concentrations in the AD experiments with no hydrogen injected. The best results were obtained in experiments A₃, A₄, and A₅. In these analyses, the independent variables were the SS/FW ratios and the

digestion time (DT) [17–19]; and the dependent variable was the cumulative methane yield in terms of VS [50–51]. The pH and temperature were not evaluated in this statistical study, but they were quantified during the process, and the results were discussed [28,33,52]. All experiments were carried out according to Table 3.

Table 3. Experimental design for analysis of variance.

Experiment	SS (g)	FW (g)	SS/FW	DT (days)
A ₃	3.00	7.00	$0.43\text{ g}\cdot\text{g}^{-1}$ (-1)	12 (-1), 21 (0), 30 (+1)
A ₄	8.25	1.75	$4.71\text{ g}\cdot\text{g}^{-1}$ (0)	12 (-1), 21 (0), 30 (+1)
A ₅	9.00	1.00	$9.00\text{ g}\cdot\text{g}^{-1}$ (+1)	12 (-1), 21 (0), 30 (+1)

A 3^2 factorial design was developed, and the experimental conditions were: SS/FW ratios ($\text{g}\cdot\text{g}^{-1}$) 0.43 (-1), 4.71 (0) and 9.00 (+1); DT 12 days (-1), 21 days (0), and 30 days (+1), representing the experiments A₃, A₄, and A₅, respectively. In this case, ANOVA was justified to seek satisfactory methane yield in the function of the SS/FW ratios and DT within these process domains [20].

In this statistical study, a mathematical model was developed to synthesize this union of results, according to Eq. (1):

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_1x_2 \quad (1)$$

The domains of x_1 and x_2 were $\{x \in \mathbb{R} / -1 \leq x \leq 1\}$, attributed to SS/FW ratios and DT, respectively. Coefficients a_0 to a_3 are experimentally determined

model coefficients and image (y) represents the cumulative methane yield ($\text{mL}\cdot\text{g}_{\text{sv}}^{-1}$).

The coefficient of determination (R^2) was calculated according to the analysis of variance studies [20], and it represents the approach of the mathematical model to experimental data. This coefficient configures a relation between the regression sum of squares $(\hat{y} - \bar{y})^2$ and the total sum of squares $(y_{ij} - \bar{y})^2$. The percentage of the variation explained, or the coefficient of determination (R^2), was calculated according to Eq. (2):

$$R^2 = \frac{\sum_i^m \sum_j^n (\hat{y} - \bar{y})^2}{\sum_i^m \sum_j^n (y_{ij} - \bar{y})^2} = \frac{RSS}{RSS + rSS} = \frac{RSS}{TSS} \quad (2)$$

where y_{ij} are experimental values (i level and j repetition), \hat{y}_i represents the values calculated according to the model, Eq. (1), and \bar{y} represents the global mean. An F test was done to evaluate the model fit. This test was performed using the total sum of squares (TSS), regression sum of squares (RSS), residual sum of squares (rSS), and the corresponding mean squares [20]. The influence of process variables was presented using a Pareto chart, and the model visualization was done by response surface.

Kinetic modeling

Two kinetic models, First-order and Gompertz, were applied to simulate anaerobic biodegradation, using the best results obtained among the experiments described in Table 2, which were experiments A₄ and A₅.

Cumulative methane yield was fitted to the First-order kinetic model, described by Eq. (3), and the Gompertz model, described by Eq. (4) [10,21]:

$$y(t) = y_0 [1 - e^{-kt}] \quad (3)$$

$$y(t) = y_0 e^{-\left\{ -e \left[\left(\frac{2.72R}{y_0} \right) (y - t) \right] + 1 \right\}} \quad (4)$$

where y_0 is the methane production potential ($\text{mL} \cdot \text{g}_{\text{VS}}^{-1}$), k is the first-order hydrolysis constant (day^{-1}), R is maximum methane production rate [$\text{mL} \cdot (\text{g}_{\text{VS}} \cdot \text{day})^{-1}$], and γ is the lag phase (days).

The kinetic study is justified to fit a model of the methane yield as a function of the digestion time, founding a natural lag phase, maximum slope, and estimating methane potential. These parameters could be used to compare different systems in different conditions [10].

Estimation of the GHG mitigation

There are several standard methodologies to calculate GHG emissions [22,54,67] by the different biomass types of disposal. This research used the Excel GHG Protocol Brazil Spreadsheet [23], a framework created by World Resources Institute (WRI) in 1998, widely used in corporate and academic calculations.

The theoretical estimate of the GHG emissions reduction, in terms of carbon dioxide equivalent (CO_{2e}), was determined using these parameters: FW disposal of $3,240 \text{ tons} \cdot \text{year}^{-1}$ ($9,000 \text{ kg} \cdot \text{day}^{-1}$) [14], an average rainfall of $2,501 \text{ mm} \cdot \text{year}^{-1}$, an average temperature of $27 \text{ }^\circ\text{C}$, and shallow open dump ($< 5\text{m}$) [23]. The unit-denominated carbon dioxide equivalent represents the integration of emissions from various GHG based on their global warming potential [57]. The main idea is not

to discharge this biomass and use it as a substrate for biogas generation.

Estimation of the electrical energy production

According to the biogas yield, the electrical energy production (in terms of $\text{MWh} \cdot \text{year}^{-1}$) was determined using the methane lower heating value (LHV) of $35,500 \text{ kJ} \cdot \text{m}^{-3}$ [24]. The methane lower heating value can also be expressed, by mass, $50,000.0 \text{ kJ} \cdot \text{kg}^{-1}$ [55] (considering the methane density of $0.66 \text{ kg} \cdot \text{m}^{-3}$ [56]) or by mol, $890 \text{ kJ} \cdot \text{mol}^{-1}$ (considering the methane molar weight of $0,016 \text{ kg} \cdot \text{mol}^{-1}$) [56]. In this research, the methane potential production was measured regarding volatile solids in the batch experiments. For the estimation of the electrical energy production, the Bragança annual FW production and an efficiency of 35% for the motor-electricity generator set [25] were used, as seen in Eq. (5):

$$P = [CH_4 (\text{mL } g_{\text{VS}}^{-1})] [VS_{i+FW} (g_{\text{VS}})] \left[\frac{FW_{\text{annual}} (g)}{FW_{\text{batch}} (g)} \right] \left[\frac{1 \text{ m}^3}{10^6 \text{ ml}} \right] \left[\frac{35,500 \text{ kJ}}{\text{m}^3} \right] \left[\frac{1 \text{ MWh}}{3.6 \times 10^6 \text{ kJ}} \right] [0.35] \quad (5)$$

RESULTS AND DISCUSSION

Characterization of the fish waste and SS

The characterization of FW and SS is shown in Table 4.

As shown in Table 4, the FW sample presented a moisture content of 71.00%, which may favor the anaerobic digestion process due to the natural transport of nutrients and microorganisms [60]. Kaffle and Kim [21] and Cadavid-Rodríguez *et al.* [15] have found similar FW moisture values, 68.7 and 74.8, respectively. Besides, FW presented total solids and volatile solids contents of 29.0% and 24.9%, respectively, representing a good fermentable material content [50,51].

The C/N ratio from FW samples was 4.5. This result may be correlated to the high contents of nitrogen and protein, 11.22% and 18.09%, respectively, which may inhibit biogas formation [27]. Other researchers have found C/N ratios of 5.7, 10.7, and 6.5 in FW samples, and despite that, in their experiments, good biogas and methane yields were obtained [15,16,26]. These results may have occurred due to other parameters influencing the AD process, such as SS/FW ratios, DT, SS type, and so forth [61–63].

The COD/BOD₅ ratio was 2.9. It is recommended that the COD/BOD₅ ratio be lower than 4.0, as this indicates a good biodegradability of the substrate [28]. The SS presented a moisture content of 94.94%, favorable to the nutrients transport [60], total solids of

Table 4. Characterization of fish waste and SS.

Fish Waste		SS	
Moisture % (w/w)	71.00	Moisture % (w/w)	94.94
pH	7.19	pH	6.90
Salt content (g·100g ⁻¹)	0.30	Conductivity (mS·cm ⁻¹)	8,13
Phosphorus content (g·100g ⁻¹)	0.88	COD (gO ₂ ·L ⁻¹)	12.140
Fat content (g·100g ⁻¹)	7.00	BOD (gO ₂ ·L ⁻¹)	4.150
Protein content (g·100g ⁻¹)	18.09	Total solids (g·L ⁻¹)	50.6
Potassium content (mg·kg ⁻¹)	1.44	Fixed solids (g·L ⁻¹)	9.7
Total solids (g·L ⁻¹)	290.0	Volatile solids (g·L ⁻¹)	40.9
Fixed solids (g·L ⁻¹)	41.0	C % (w/w)	34.54
Volatile solids (g·L ⁻¹)	249.0	H % (w/w)	6.13
C % (w/w)	50.89	N % (w/w)	4.73
H % (w/w)	8.35	S % (w/w)	1.60
N % (w/w)	11.22	O % (w/w)	53.00
S % (w/w)	1.44	Anaerobic CFU	1.84 x 10 ⁹
O % (w/w)	28.10	Aerobic CFU	0.33 x 10 ⁹

5.06%, and volatile solids of 4.09%, comparable to others reported anaerobic sludges [10,15]. The SS C/N ratio was 7.3. In anaerobic digestion processes, the recommended values of the C/N ratio vary from 10 to 30. Values of a C/N ratio less than 10 may cause low biogas and methane yields [10,29,30]. The SS and FW C/N ratios were 7.3 and 4.5, respectively. Hence, they were lower than the recommended values in the literature [10,29,30]. However, other parameters can influence biogas production [61–63].

The anaerobic colony forming units (CFU) and the aerobic CFU presented values of 1.84×10^9 and 0.33×10^9 , respectively. So, the anaerobic CFU was 6 times greater than the aerobic CFU. These results suggested that the substrates (SS and FW) were good AD feedstocks. Furthermore, the SS anaerobic CFU value was similar to the results of 2.71×10^9 and 1.80×10^9 obtained by [65] and [66], respectively, in the same AD condition.

The elemental analysis of the FW and SS led to their chemical formulas, $C_5H_{10}O_3N$ and $C_8H_{18}O_{10}N$, respectively. These formulas were used to estimate the methane yield (M_{th}) through Eq. (6) ($C_aH_bO_cN_d$) [31]

$$M_{th} (mL \cdot g_{VS}^{-1}) = 1000 \left[\frac{(22.415/8)(4a + b - 2c - 3d)}{(12a + b + 16c + 14d)} \right] \quad (6)$$

Hence, the SS and FW theoretical methane yields were $213 \text{ mL} \cdot g_{VS}^{-1}$ and $446 \text{ mL} \cdot g_{VS}^{-1}$, respectively. The same equation was used to estimate the biogas and methane yields for experiments A₄ and A₅ which presented the best results concerning biogas yields and methane content in the biogas. Experiment A₄ used 8.25 g of SS and 1.75 g of fish waste. Then, its calculated theoretical methane yield was $268.7 \text{ mL} \cdot g_{VS}^{-1}$. For experiment A₅ (9.00 g of SS and

1.00 g of fish waste), the calculated theoretical methane yield was $190.3 \text{ mL} \cdot g_{VS}^{-1}$.

These results represent the maximum theoretical methane production based on the organic matter's elemental composition. Hence, these calculated results are likely higher than those obtained in the experiments due to the strong dependence on several operative parameters of the anaerobic process.

Cumulative biogas and methane yields and methane content

The cumulative biogas yields of experiments A₁ to B₂ are shown in Fig. 2. Table 5 illustrates the cumulative biogas yield ($\text{mL} \cdot g_{VS}^{-1}$), the cumulative methane yield ($\text{mL} \cdot g_{VS}^{-1}$), and the methane content (% v/v) data obtained on the 30th day for the experiments A₁ to B₂ and control.

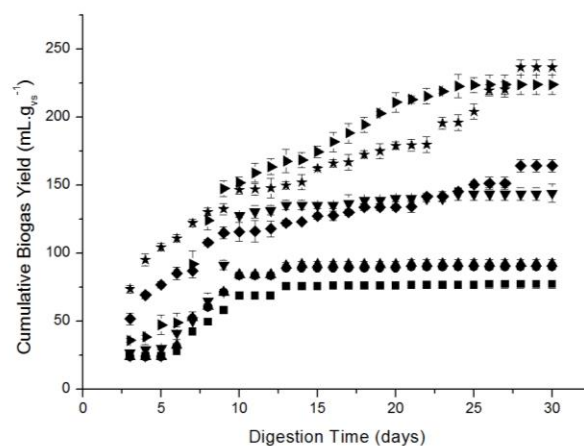


Figure 2. Cumulative biogas yield ($\text{mL} \cdot g_{VS}^{-1}$) - experiments: A₁ (■), A₂ (●), A₃ (▲), A₄ (▼), B₁ (◆), A₅ (▶), B₂ (★).

The low biogas yields, and methane content, after

30 days of fermentation, for experiments A₁, A₂, and A₃ were due to the small numbers of microorganisms capable of degrading the organic matter present in the SS /FW ratios completely, respectively, 0.11 g·g⁻¹ (A₁), 0.27 g·g⁻¹ (A₂), 0.43 g·g⁻¹ (A₃). The SS supplied the microorganisms to the AD process, acting as inoculum. However, 30 days (RT) seemed insufficient to form a colony capable of completely degrading the substrates due to the low SS to FW ratios. Hence, the SS/FW ratios for experiments A₁, A₂, and A₃ were inappropriate for the DT used [10,17,19]. For these reasons, the ratios used in experiments A₁, A₂, and A₃ were discontinued due to the low efficiency of the 30 days AD process.

On the other hand, the experiments A₄ (SS/FW of 4.71 g·g⁻¹), A₅ (SS/FW of 9.00 g·g⁻¹), B₁ (SS/FW of 4.71

g·g⁻¹, and the 0.16 mmol of hydrogen injected on the 7th and 18th days) and B₂ (SS/FW of 9.00 g·g⁻¹, and the 0.16 mmol of hydrogen injected on the 7th and 18th days), presented results showing that the biogas yields, methane yields, and methane contents increased. These results can be seen in Table 5 and Fig. 3. According to Table 5 and Fig. 3, the biogas yields ranged between 80 mL·g_{vs}⁻¹ and 140 mL·g_{vs}⁻¹ with the methane content exceeding 50%. These results showed an increase in the microbial population due to a higher SS content in AD reactors, enabling the substrate organic matter for SS and FW to degrade more efficiently. These results were similar to 50 mL·g_{vs}⁻¹–200 mL·g_{vs}⁻¹ of methane yields and 50%–75% of methane content obtained by other researchers [6,15,16,32].

Table 5. Summary of anaerobic digestion results on the 30th day.

Experiment	Cumulative Biogas Yield (mL·g _{vs} ⁻¹)	Biogas Yield from control (mL·g _{vs} ⁻¹)	Cumulative Methane Yield (mL·g _{vs} ⁻¹)	Methane Yield from control (mL·g _{vs} ⁻¹)	Methane Percentual in volume (% v/v)
A ₁	77.29 ± 2.95	10.05 ± 0.75	6.11 ± 0.70	0.77 ± 0.08	7.58 ± 1.48
A ₂	90.06 ± 2.31	16.85 ± 0.81	9.90 ± 1.05	1.85 ± 0.11	11.10 ± 2.50
A ₃	92.46 ± 2.92	18.30 ± 1.02	18.02 ± 5.50	3.48 ± 0.49	19.47 ± 5.35
A ₄	144.21 ± 6.81	30.91 ± 1.45	75.52 ± 5.47	6.48 ± 0.58	52.37 ± 1.32
A ₅	224.03 ± 7.11	40.30 ± 1.87	137.94 ± 9.29	9.92 ± 0.85	61.57 ± 2.19
B ₁	164.26 ± 5.45	56.91 ± 2.09	89.52 ± 5.40	10.85 ± 0.63	54.50 ± 2.78
B ₂	236.59 ± 5.69	81.02 ± 3.12	157.18 ± 8.94	17.81 ± 0.81	66.54 ± 5.38

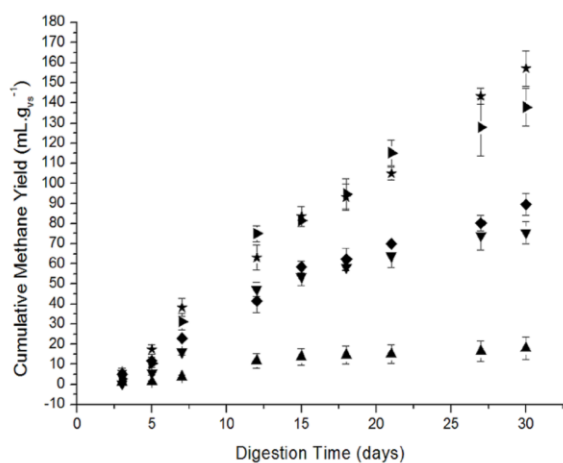


Figure 3. Cumulative methane yield (mL·g_{vs}⁻¹) - experiments: A₃ (▲), A₄ (▼), B₁ (◆), A₅ (▴), B₂ (★).

In the AD processes, several parameters affect the biogas yield and methane content. These parameters are, for instance, the substrate (S) type and composition, type of inoculum (I) used to start up the AD, the I/S ratio, moisture content, temperature, pH, agitation, and digestion time [15,17,19,21,28,33,60]. In this research, the variation of temperature and pH were not studied. However, in all experiments, the temperatures were kept at 30 °C ± 2 °C and the pH

varied between 6.2 and 7.4. When the pH ranges between 5.5 and 6.0, acidogenic bacteria increase, impairing the fermentative process. Conversely, methanogenic bacteria increase with pH values ranging from 6.8 to 7.2, favoring the fermentative process [28]. When the pH is below 8.0, the nitrogen remains in aqueous ammonium form NH₄⁺. However, when pH exceeds 8.0, the methanogens community is affected, inhibiting methane production [33].

After 30 days, the methane contents obtained in experiments A₄ and B₁ were 52.37% ± 1.32% and 54.50% ± 1.78%, originating at the same SS/FW ratio (4.71 g·g⁻¹). However, experiment B₁ used 0.16 mmol of hydrogen added on the 7th and 18th days. As a result, there was an increase in the methane content of 4.06% for B₁. Similarly, after 30 days, the methane contents from experiments A₅ and B₂ were 61.57% ± 2.19% and 66.44% ± 2.17%, originating at the same SS/FW ratio (9.00 g·g⁻¹). But, experiment B₂ used 0.16 mmol of hydrogen added on the 7th and 18th days. Hence, there was an increase of 7.9% in the methane content in the biogas. This augmentation of the methane content in the biogas in the experiments in which hydrogen was injected may suggest the researches were capable of

synthesizing more methane in the methanogenesis phase of the AD process, as there was more hydrogen available for the carbon dioxide and hydrogen reactions, as suggested by some researchers [8,61–62]. Further experiments injecting hydrogen in the AD reaction are necessary to obtain more detailed and confident data on the influence of hydrogen in the biogas methane content. The influence of the injected hydrogen on the biogas methane content will be investigated.

Despite the experiments using hydrogen, B₁ and B₂ produced interesting and promising results, more investigation will be needed to establish the effect of the injected hydrogen on the biogas methane content. This matter will be considered for future research. For this reason, experiments A₃, A₄, and A₅ were selected to continue the ANOVA, and only A₄ and A₅ for the kinetics, GHG emissions reduction, and electrical energy production studies.

Cumulative methane yield and analysis of variance (ANOVA)

The evolution of methane yield depended on the SS/FW ratio and DT (independent variables). An ANOVA was performed for experiments A₃, A₄, and A₅ to verify the effect of the SS/FW ratio and DT on the methane yield. The ANOVA results are shown in Table 6.

According to Table 6, the results indicated that SS/FW ratios and DT were significant to methane yield.

Among them, the influence of the SS/FW ratio was the most significant. The inoculum/substrate ratio can affect not only the biodegradability but also the methane production rate and/or hydrolysis rate [10,17]. SS provided the microbes for the organic matter degradation in the co-digestion experiments. At the same time, FW was the fresh substrate with insufficient microbes to decompose the organic matter faster and more efficiently.

The combined effect (x_1x_2) was significant. The diversity of combined effects and the dynamism of the process need a complex mathematical model. So, the mathematical model that described the methane yields in the domain of experimental design is shown in Eq. (7).

$$y(\text{mL g}_{\text{VS}}^{-1}) = 62.211 + 47.219x_1 + 16.224x_2 + 14.140x_1x_2 \quad (7)$$

The domains of x_1 and x_2 were $\{x \in \mathbb{R} / -1 \leq x \leq 1\}$, and they were attributed to SS/FW ratio and DT, respectively, as described in Table 3. Furthermore, an *F* test was performed, and this model was observed to be statistically significant because F_1 (RMS/rMS) was higher than $F_{3,14}$, $807.90 > 5.56$, respectively. Also, this model was predictive because F_2 (LFMS/PEMS) was less than $F_{5,9}$, $5.35 < 6.63$ [20]. Finally, the proportion of the explained variation relative or coefficient of determination was 0.99, which indicated a good approximation of the analytical model to the experimental data.

Table 6. Study of analysis of variance - experiments A₃ to A₅.

Experiment	SS/FW	DT (days)	Experimental	Designed	CH ₄ (mL.g _{VS} ⁻¹)
A ₃	0.43 (-1)	12 (-1)	-1	-1	11.73 ± 3.48
A ₃	0.43 (-1)	21 (0)	-1	0	15.18 ± 4.63
A ₃	0.43 (-1)	30 (+1)	-1	+1	18.02 ± 5.50
A ₄	4.71 (0)	12 (-1)	0	-1	47.31 ± 3.40
A ₄	4.71 (0)	21 (0)	0	0	63.89 ± 5.79
A ₄	4.71 (0)	30 (+1)	0	+1	75.52 ± 5.47
A ₅	9.00 (+1)	12 (-1)	+1	-1	75.09 ± 4.00
A ₅	9.00 (+1)	21 (0)	+1	0	115.21 ± 6.44
A ₅	9.00 (+1)	30 (+1)	+1	+1	137.94 ± 9.29
RSS	31,513.88	Df	3		RMS 10,504.63
rSS	182.03	Df	14		rMS 13.00
TSS	31,695.91	Df	17		F ₁ (RMS/rMS) 807.90
Lack of fit (LFSS)	136.19	Df	5		LFMS 27.24
Pure error (PESS)	45.84	Df	9		PEMS 5.09
R ² (RSS/RSS+rSS)	0.994				F ₂ (LFMS/PEMS) 5.35

Figures 4a and 4b illustrate the mathematical model, Eq. (7), and the Pareto chart, which shows the influence of the variables on the methane yield,

respectively. Experiments A₄ and A₅ will be considered for the kinetic study, energy production estimate, and GHG reduction.

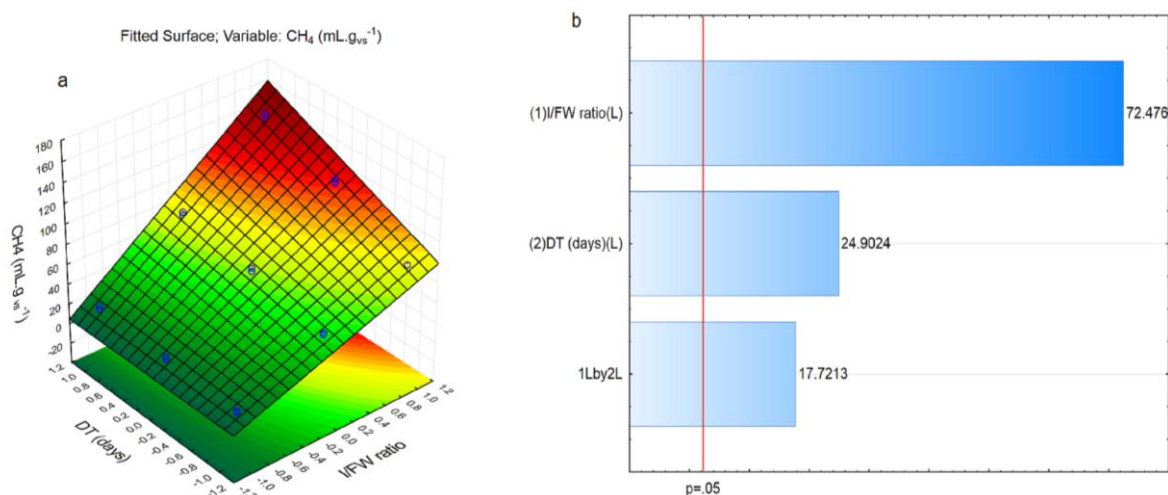


Figure 4. Fitted surface (a) and Pareto chart (b) - dependent variable: CH_4 yield ($mL \cdot g_{vs}^{-1}$).

Kinetic modeling

A kinetic study was performed for a DT of 30 days with SS/FW ratios equal to $4.71 \text{ g} \cdot \text{g}^{-1}$ (A_4) and $9.00 \text{ g} \cdot \text{g}^{-1}$ (A_5). Two kinetic models were fitted, the First-order kinetic model (Eq. 3) and the Gompertz model (Eq. 4). The results are shown in Figure 5. The model's parameters are shown in Table 7.

The first-order model considers continuous methane production, with a maximum production rate at the beginning of the AD process. Differently, the Gompertz model considers a lag phase before methane production starts and a maximum production rate during the process. And so, the results showed a better approximation of the Gompertz model ($R^2 > 0.97$) compared to the first-order model ($R^2 > 0.91$).

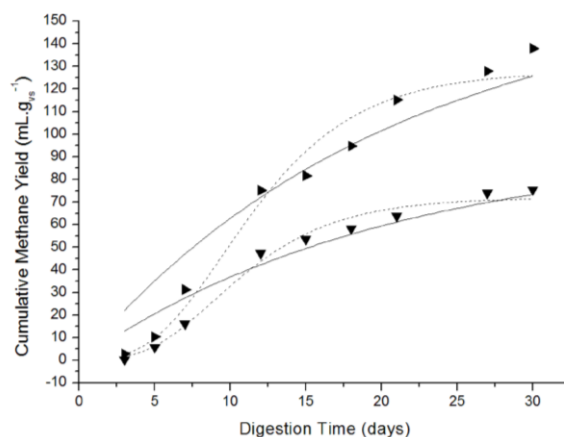


Figure 5. Kinetic study of the cumulative methane yield ($mL \cdot g_{vs}^{-1}$) - first-order model (solid line) and Gompertz model (dash line) - A_4 (\blacktriangledown), A_5 (\blacktriangle).

Table 7. Parameters of the kinetic models.

Experiment	First-order model			Gompertz model			
	y_0 ($mL \cdot g_{vs}^{-1}$)	k (day^{-1})	R^2	y_0 ($mL \cdot g_{vs}^{-1}$)	R ($mL \cdot g_{vs}^{-1} \cdot day^{-1}$)	γ (day)	R^2
A_4	95.661 ± 5.371	0.049 ± 0.005	0.912	71.996 ± 1.890	6.225 ± 0.398	4.502 ± 0.449	0.985
A_5	164.509 ± 8.882	0.048 ± 0.004	0.921	127.766 ± 4.984	10.415 ± 1.109	4.714 ± 0.676	0.970

The presence of lag phases and maximum derivatives of methane yields ($\partial y / \partial t$) were observed in other research [10,21], suggesting an initial period since the hydrolyses process to methanogenic reaction (obviously after acidogenic and acetogenic ways).

GHG emission reduction and estimate of energy production

In a real process involving biogas formation by biodigestion, it is necessary to perform a complete

study with several parameters (economic, operational, and logistic, among others).

At the end of this research, two scenarios were presented, performed by experiments A_4 and A_5 , both in a batch study. Moreover, the annual FW production from Bragança (Brazil) was $3,240 \text{ tons} \cdot \text{year}^{-1}$. This information was necessary to calculate the GHG emission reduction estimate and electrical energy production. Furthermore, a summarized study of the possible biogas plant implementation was conducted in

similar conditions.

GHG emission reduction

Using the Excel GHG Protocol Brazil Spreadsheet, with a waste amount of 3,240 tons·year⁻¹, and applying these conditions: average rainfall of 2,501 mm·year⁻¹, an average temperature of 27 °C, in a shallow open dump (< 5 m); the results showed an expected GHG reduction of 1,619 tons of CO_{2e} (cumulative reduction for 20 years). Regarding methane reduction, with a global warming potential of 1/25 compared to CO_{2e} [57], a reduction of 64.76 tons of CH₄ was observed (1,619/25 tons of CH₄). Or, in volume, 98,121 m³ (considering the methane density of 0.66 kg·m⁻³ [56]). Considering the biomass amount of 3,240 tons, the methane emission reduction would be 30.28 m³·ton⁻¹ (98,121/3,240 cubic meters per ton). This result can be compared with 26-62 m³CH₄·ton⁻¹, calculated from the LandGEM 3.02 model, using refractory organic compounds and/or easily degradable organic compounds for 20 years [54].

Estimate of the energy production

Conform was mentioned, and experiments A₄ and A₅ were considered to guide this research. To experiment A₄, the methane production potential was 75.52 mL·g_{vs}⁻¹, and the SS/FW proportion was 8.25 g per 1.75 g, respectively. So, the weight from volatile solids was 0.77 g (8.25x0.0409 + 1.75x0.249). To experiment A₅, the methane production potential was 137.94 mL·g_{vs}⁻¹, and the SS/FW ratio was 9.00 g per 1.00 g, respectively. So, the weight from volatile solids was 0.62 g (9.00x0.0409 + 1.00x0.249).

For experiments A₄ to A₅, the results of the estimation of the production of energy (P, MWh·year⁻¹) were calculated by Eq. (5), with respective values, which Eq. (5a) is correlated to experiment A₄, and Eq. (5b) is correlated to experiment A₅.

$$P = \left[75.52 \left(\text{mL g}_{\text{vs}}^{-1} \right) \right] \left[0.77 \left(\text{g}_{\text{vs}} \right) \right] \left[\frac{3.24 \times 10^9 \left(\text{g} \right)}{1.75 \left(\text{g} \right)} \right] \left[\frac{1 \text{ m}^3}{10^6 \text{ mL}} \right] \left[\frac{35,500 \text{ kJ}}{\text{m}^3} \right] \left[\frac{1 \text{ MWh}}{3.6 \times 10^6 \text{ kJ}} \right] [0.35] = 371.58 \quad (5a)$$

$$P = \left[137.94 \left(\text{mL g}_{\text{vs}}^{-1} \right) \right] \left[0.62 \left(\text{g}_{\text{vs}} \right) \right] \left[\frac{3.24 \times 10^9 \left(\text{g} \right)}{1.00 \left(\text{g} \right)} \right] \left[\frac{1 \text{ m}^3}{10^6 \text{ mL}} \right] \left[\frac{35,500 \text{ kJ}}{\text{m}^3} \right] \left[\frac{1 \text{ MWh}}{3.6 \times 10^6 \text{ kJ}} \right] [0.35] = 956.36 \quad (5b)$$

Finally, the estimated energy production varied between 372 MWh·year⁻¹ and 956 MWh·year⁻¹, which would be enough to power from 310 to 797 local houses approximately (e.g., fishermen community), which have an average consumption of 1.20 MWh·year⁻¹ (or 100 kWh·month⁻¹) [34,35].

Regarding electrical energy production per ton of biomass (3,240 tons·year⁻¹), the results showed a production of 0.11 MWh·ton⁻¹ to 0.30 MWh·ton⁻¹ (371 MWh or 956 MWh per 3,240 tons of FW). These results can be compared to 0.54 MWh·ton⁻¹, described by Ravanipour *et al.* [59], in a study of fish and shrimp waste disposed of in Bushehr, Iran [59].

Finally, the cost of energy in biogas plants was described by other researchers, such as 9.22 Indian Rupias per kWh (or approximately 0.11 USD·kWh⁻¹) [63]; or USD 1.54 kWh per 9.42 kWh, a cost of 0.16 USD·kWh⁻¹ [64]. So, it's now possible to establish a relationship between electrical energy production and the cost of a biogas plant (approximately).

CONCLUSION

Highlighting the SS/FW ratios of 4.71 g·g⁻¹ and 9.00 g·g⁻¹, the biogas production using anaerobic digestion of fish waste with domestic wastewater sludge was promising. The methane yields were between 76 mL·g_{vs}⁻¹ and 138 mL·g_{vs}⁻¹, and the methane content was superior to 50%. The study of analysis of variance indicated that the evolution of methane yield was dependent on the SS/FW ratio and digestion time. The First-order (R² > 0.91) and Gompertz kinetic models (R² > 0.97) fitted very satisfactorily. However, the Gompertz kinetic model presented the best adjustment. Using 9 tons·day⁻¹ (or 3,240 tons·year⁻¹) of FW, the estimate of GHG reduction was 1,619 tons of CO_{2e} for 20 years, according to the GHG Protocol Brazil Spreadsheet. Finally, the estimate of electrical energy production was between 372 MWh·year⁻¹ and 956 MWh·year⁻¹, which can be useful for generating energy for more than 300 local houses, increasing life quality, and developing a circular economy for the community.

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PROIZVODNJA BIOGASA I UBLAŽAVANJE GASOVA SA EFEKTOM STAKLENE BAŠTE KORIŠĆENJEM RIBLJEG OTPADA IZ BRAGANSE U BRAZILU

U ovom istraživanju je procenjen potencijal proizvodnje biogasa korišćenjem ribljev otpada (FW) i njegov uticaj na smanjenje gasova sa efektom staklene bašte i proizvodnju energije. Riblji otpad je ko-digestiran sa anaerobnim kanalizacionim muljem (SS). Riblji otpad je sakupljen u Braganci, u severnom Brazilu, gde je ribarska industrija glavna delatnost sa proizvodnjom ribljev otpada od približno 9.000 kg/dan. Eksperimentalni deo obuhvatao je pet odnosa SS/FV, a u dva eksperimenta je dodat vodonik. Eksperimenti su izvedeni 30 dana i analiziran je uticaj na kumulativne prinose biogasa i metana. Smanjenje gasova sa efektom staklene bašte je procenjeno korišćenjem količine ribljev otpada koja nije bačena na otvorenu deponiju Bragance, a proizvodnja električne energije je izračunata korišćenjem prinosa metana. Pored toga, urađena su dva kinetička modela. Rezultati su pokazali smanjenje gasova sa efektom staklene bašte za 1.619 tona CO_{2e} i proizvodnju električne energije od 372 MVh-godišnje do 956 MVh-godišnje. Štaviše, analiza varijanse je pokazala da je proizvodnja metana u velikoj meri zavisila od odnosa SS/FV, koji su se kretali od 76 ml/g do 138 ml/g. Konačno, ovo istraživanje je pokazalo korist od upotrebe ribljev otpada za proizvodnju biogasa i električne energije uz smanjenje emisija gasova sa efektom staklene bašte u gradu bez energije.

Ključne reči: biogas, energija, riblji otpad, gasovi staklene bašte, kinetički modeli, metan.

NAUČNI RAD