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INTEGRATING INVASIVE MACROPHYTE BIOMASS CONTROL WITH BIOGAS PRODUCTION: A SUSTAINABLE APPROACH IN THE ITAIPU RESERVOIR

INTEGRANDO O CONTROLE DE BIOMASSA DE MACRÓFITAS INVASIVAS COM A PRODUÇÃO DE BIOGÁS: UMA ABORDAGEM SUSTENTÁVEL NO RESERVATÓRIO DE ITAIPU

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ABSTRACT: This study assessed the potential of *Hydrilla verticillata*, an invasive aquatic plant in the Itaipu Reservoir, as a source of biogas through anaerobic digestion, using swine slaughterhouse sludge as inoculum. The Biochemical Methane Potential (BMP) test was evaluated at three different Inoculum/Substrate Ratios (ISR): 3:1, 2:1, and 1:1. The biogas production in mL g^{-1} of volatile solids in these ISRs was 638,5 mL g^{-1} SV, 842,5 mL g^{-1} SV e 820,8 mL g^{-1} SV, respectively. Microcrystalline cellulose served as a positive control (ISR 2:1), and blanks (reactors without substrate containing only inoculum) had production (in mL g^{-1} VS) of 729,4 and 102,0 ± 10,9, respectively. The systems stabilized within 42 days, maintaining the appropriate pH. There was an 18.3% ± 3.7% reduction in partial alkalinity in the blanks, increases of 34.9% ± 4.0% in cellulose, and 138.5% ± 33.9% in the ISR. Intermediate alkalinity decreased in all tests. Despite the suboptimal C/N ratio, biogas production was satisfactory, with a gradual increase in methane content. On day 30, the ISR reached methane levels of 65.53% ± 3.87%. This study underscores the potential of *Hydrilla verticillata* as a biogas source, using swine slaughterhouse sludge as inoculum, providing an environmentally sustainable solution for waste utilization.
Keywords: bioenergy; anaerobic digestion; aquatic plants; sustainability.

RESUMO: *Este estudo avaliou o potencial da Hydrilla verticillata, uma planta aquática invasora no Reservatório de Itaipu, como fonte de biogás por meio da digestão anaeróbia, utilizando lodo de abatedouro de suínos como inóculo. O teste de Potencial Bioquímico de Metano (PBM) foi realizado em três diferentes Relações Inóculo/Substrato (RIS): 3:1, 2:1 e 1:1. A produção de biogás em mL g^{-1} de sólidos voláteis nessas RIS foi de 638,5 mL g^{-1} SV, 842,5 mL g^{-1} SV e 820,8 mL g^{-1} SV, respectivamente. A celulose microcristalina serviu como controle positivo (RIS 2:1), e os controles em branco (reatores sem substrato, contendo apenas inóculo) apresentaram produções (em mL g^{-1} VS) de 729,4 e 102,0 ± 10,9, respectivamente. Os sistemas se estabilizaram em 42 dias, mantendo o pH adequado. Houve uma redução de 18,3% ± 3,7% na alcalinidade parcial nos controles em branco, aumento de 34,9% ± 4,0% na celulose e de 138,5% ± 33,9% na RIS. A alcalinidade intermediária diminuiu em todos os testes. Apesar da relação C/N subótima, a produção de biogás foi satisfatória, com um aumento gradual no teor de metano. No dia 30, a RIS alcançou níveis de metano de 65,53% ± 3,87%. Este estudo destaca o potencial da Hydrilla verticillata como fonte de biogás, utilizando lodo de abatedouro de suínos como inóculo, proporcionando uma solução ambientalmente sustentável para a utilização de resíduos.*
Palavras-chave: bioenergia; digestão anaeróbia; plantas aquáticas; sustentabilidade.

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INTRODUCTION

The global population increase generates a growing need for food, water, and energy, putting pressure on natural resources. This pressure results in environmental challenges, including an increase in the production of waste, including organic waste, and the pollution of water bodies, exacerbating the proliferation of algae and aquatic plants.

In this context, bioenergy is a potential solution to face these challenges and promote sustainability. The production of biogas through anaerobic digestion of organic waste is a viable technology. This biological treatment occurs without oxygen, in which a complex microbial community degrades organic matter, producing biogas, a gaseous mixture rich in methane and carbon dioxide.

Although anaerobic digestion has traditionally been applied to various types of waste, aquatic plants are emerging as an alternative and innovative source of biomass for biogas production. Invasive aquatic plants, such as *Hydrilla verticillata*, have considerable potential due to their rapid growth and favorable physicochemical characteristics. However, these plants also pose an environmental challenge when uncontrolled, negatively affecting aquatic ecosystems.

Collecting this invasive biomass and using it for energy through biogas offers an environmentally sustainable approach to dealing with these challenges. In this way, it reduces aquatic weeds' environmental impact and generates renewable energy from non-agricultural sources. However, to make this approach viable, it is essential to overcome challenges such as variations in the chemical composition of biomass, the formation of inhibitors during anaerobic digestion, and the seasonality of biomass.

Therefore, this study addresses the challenge of invasive aquatic plants in Lake Itaipu and explores the opportunity to take advantage of digestate generated in pig slaughterhouses in the western region of Paraná. This integration seeks to develop an innovative and sustainable approach to biogas production, contributing to preserving aquatic ecosystems and promoting environmental sustainability.

MATERIALS AND METHODS

Collection of Inoculum and Plant Biomass

The inoculum was the digestate from anaerobically digested slaughterhouse sludge collected in a pig slaughterhouse in Medianeira-Paraná (Brazil). After collection, it was acclimatized for seven days to mesophilic temperature (Holliger *et al*., 2016).

Aquatic macrophytes of the species *Hydrilla verticillata* were collected on the banks of the Itaipu reservoir, in the geographic coordinates Lat. 25° 26' 49.4" S, Log. 54° 32' 58.9" W, close to the Bela Vista Biological Refuge. After collection, the macrophytes were washed, morphologically identified (Gettys & Enloe, 2016), dehydrated in the sun, and crushed before testing.

Physicochemical analysis

The substrates, inoculum, and digestates from the BMP tests were characterized using the solids content (total and volatile) of an oven-dry weight basis, according to method 2540 of the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Determining the pH of substrates and effluents followed the 4500 H^+ method (APHA, 2005). Partial and intermediate alkalinities were determined using the 2330B* potentiometric titration method (APHA, 2005).

Total nitrogen values were obtained from Kjeldahl analysis (Allen *et al.*, 1974). A methodology adapted by Meeker & Wagner (1933) was used for ammonia nitrogen. For protein content, a conversion factor of 6.25 was used (Boyd, 1970). The lipid content was determined by extraction with a Soxhlet apparatus, using hexane as solvent. The carbon content was estimated by dividing the volatile solids by 1.83 (Jain & Kalamdhad, 2019). The physicochemical analyses were carried out in triplicate to characterize the isolated substrates and throughout the tests.

Biochemical Methane Potential (BMP)

Penicillin flasks with a volume of 100 mL, with 60% useful volume, sealed with a rubber stopper and aluminum seal, were used as reactors in the biochemical methane potential tests. The samples were incubated in a BOD chamber at a mesophilic temperature, at 37ºC ± 1ºC.

Microcrystalline cellulose in the Inoculum: Substrate Ratio (ISR) 2:1 was used as a positive standard, and only the inoculum was used as a blank. Anaerobic digestion tests were carried out at ISR of 1:1, 2:1, and 3:1 based on volatile solids, as expressed in Table 01:

Table 01 - Composition of samples used in BMP tests.

Sample	Inoculum (g)	Macrophyte (g)	Microcrystalline cellulose (g)
ISR 3:1	49,6	0,4	-
ISR 2:1	49,4	0,6	-
ISR 1:1	48,8	1,2	-
Cellulose (ISR 2:1)	49,5	-	0,5
Blank 3	49,6	-	-
Blank 2	49,4	-	-
Blank 1	48,8	-	-

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Replicates were collected for physicochemical analyses on days 0 (initial), 20 and 42 (final). The daily biogas measurement was carried out by displacing the volume of a glass syringe by equalizing the system's internal pressure with atmospheric pressure using a digital manometer. The reading was terminated when the daily volume of biogas was below 1% of the total accumulated in the tests (Holliger *et al.*, 2016).

Biogas composition

The analysis of the biogas composition occurred on days 5, 15, and 30 of the anaerobic digestion tests using a Perkin Elmer – Clarus 68 chromatograph, with helium as a carrier gas. The chromatograph temperature ramp was programmed from 32 to 200ºC, with an analysis time of 2 minutes to determine the methane and carbon dioxide produced in the BMP test.

Statistical analysis

The Kruskal-Wallis test evaluated the effect of inoculum: substrate ratios on biogas production. Dunn's post-test was applied with a significance level of 5% after verifying the lack of normality in the data using the Assistat software version 7.7 (Silva & Azevedo, 2016).

RESULTS AND DISCUSSION

Preliminary analyses

Initial analyses indicated that the macrophyte composition agrees with the literature data (Chen *et al.*, 2016, Kainthola, Kalamdhad, Goud, 2019). The anaerobically digested slaughterhouse sludge used as inoculum had a lower solids content than the literature references (Blazy *et al.*, 2015; Guidoni *et al.*, 2021). All substrates demonstrated a high concentration of volatile organic matter, as shown in Table 02:

Table 02 - Analysis of solids (total and volatile) on a dry basis.

Sample	Total solid (TS) %	Volatile solid (VS) %	VS/TS
H. verticillata wet	11,8 ± 0,5	83,3 ± 2,3	7,1
H. verticillata sun-dried	90,3 ± 0,3	79,9 ± 0,4	0,9
Inoculum	2,3 ± 0,2	77,3 ± 0,2	33,6
Microcrystalline cellulose	95,2 ± 0,1	96,6 ± 0,3	1,0

Sun-drying macrophyte offers advantages over fresh biomass, allowing continuous availability of macrophyte for bioenergy production through anaerobic digestion (Priya *et al.*, 2018). The reduced volume of dehydrated macrophyte also facilitated the storage and handling of biomass for BMP tests. Other physicochemical analyses were carried out on the substrates to evaluate additional parameters, as seen in Table 03.

Table 03 - Physicochemical analyses of *H. verticillata* and inoculum.

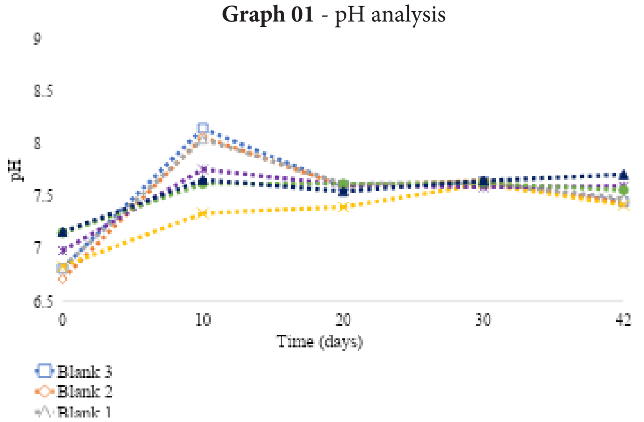
Sample	*H. verticillata*	Inoculum
Lipids (%)	3,44 ± 1,02	-
C (%)	43,88 ± 0,74	42,22 ± 0,10
NTK (%)	2,60 ± 0,26	4,08 ± 0,06
Ammoniacal N (%)	0,29 ± 0,04	0,82 ± 0,05
C/N	16,88	10,35
Protein content (%)	16,25 ± 1,60	25,48 ± 0,36

H. verticillata is highly degradable due to its high moisture, low C/N ratio, and low crude protein content. The findings accord with previous literature (Chen *et al.*, 2016; Shah, Sumbul & Andrabi, 2010; Lu *et al.*, 2015). Aquatic macrophytes, including this species, can potentially be converted into biofuels, such as biogas, due to their higher protein and lipid content than terrestrial plants (Kaur *et al.*, 2018). While slaughterhouse sludge is used as inoculum, its characteristics vary compared to the literature due to factors such as the animal species slaughtered, the production processes, and waste treatment techniques (Blazy *et al.*, 2015; Madeira *et al.*, 2023).

pH monitoring

Monitoring the pH during the anaerobic digestion tests revealed that, although there was no initial pH adjustment, the values remained adequate over time. Initially, they ranged from 6.70 to 7.16, within the recommended range (Kunz, Steinmetz & Amaral, 2019). In the first ten days, a significant increase indicated the release of ammonia nitrogen from protein degradation. Subsequently, between days 10 and 20, there was a general decrease in pH, except for the cellulose test, which continued to increase until the thirtieth day. From day 30 onward, fluctuations in pH values occurred, reaching a range of 7.42 to 7.71 on the 42nd day (Graph 01).

Graph 01 - pH analysis

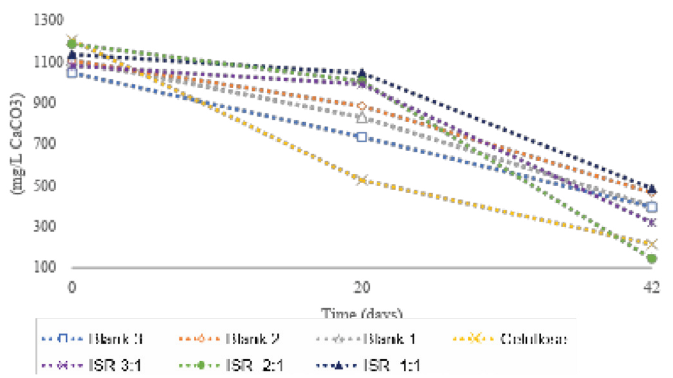


These variations reflect the complex interactions between organic compounds over time in different tests, but the proposed systems are in equilibrium, avoiding the need to add alkalizers for correction (Kunz, Steinmetz & Amaral, 2019; Potdukhe *et al.*, 2021).

Partial and intermediate alkalinity

Partial alkalinity was analyzed to evaluate the buffering power and consumption of organic acids during acidogenesis (Chernicharo, 2007). Initially, alkalinity values varied between 1080 and 1205 mg/L of $CaCO_3$. On the 20th day, we encouraged a reduction of 25.4% ± 3.9% in blanks and 11.9% ± 4.9% in RIS, while cellulose decreased 58.3% ± 2.6%, as seen in Graph 02.

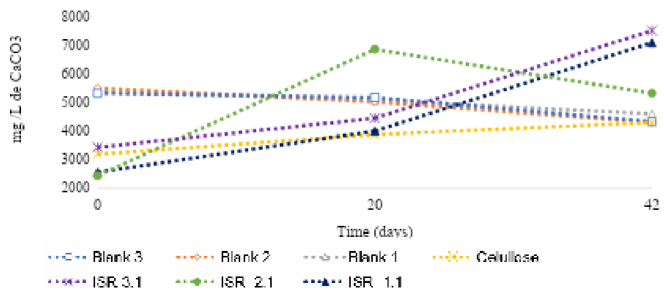
Graph 02 - Intermediate alkalinity



On the last day, intermediate alkalinity was between 145 and 485 mg/L of $CaCO_3$, with reductions of 66.5% ± 3.0% in blanks, 71.8% ± 15.3% in ISR, and 82.2% ± 1.8% in cellulose, indicating adequate buffering capacity in the experiments. The values obtained in the tests were higher than those of digestates treated anaerobically by co-digestion (Akhiar *et al.*, 2017).

Partial alkalinity, in turn, is directly related to the neutralization capacity of volatile acids generated during acidogenesis in the anaerobic digestion process (Chernicharo, 2007). During the analyses, it was found that the blanks presented an initial partial alkalinity in the range of 5310 to 5370 mg/L of $CaCO_3$, while the cellulose recorded an average value of 3180 ± 254.6 mg/L. The ISR in proportions 1:1, 2:1 and 3:1 presented, respectively, mean values of 2550 ± 212.1 mg/L, 2430 ± 42.4 mg/L and 3420 ± 84.9 mg/L, as seen in Graph 3.

Graph 03 - Partial alkalinity.

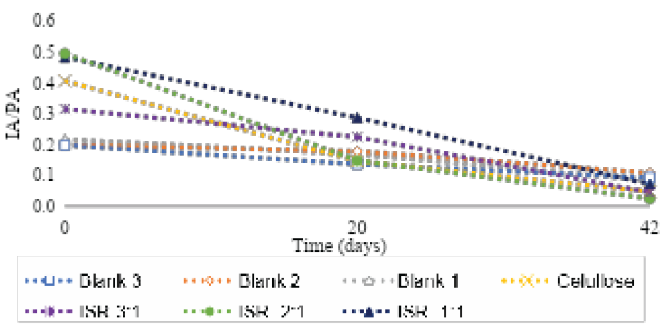


In the first 20 days, a 6.5% ± 3.1% reduction in partial alkalinity in blanks was observed. On the other hand, cellulose showed a 21.2% ± 0.7% increase in partial alkalinity, while ISR 1:1, 2:1, and 3:1 had increases of 58.2% ± 2.5%, 176.5% ± 7.0% and 31.6% ± 2.5%, respectively. These increases indicate the degradation of organic matter from substrates and conversion to organic acids during acidogenesis.

When comparing digestates with substrates, a reduction of 18.3% ± 3.7% in partial alkalinity in blanks was observed, indicating a decrease in organic acids throughout the process. Cellulose showed an increase of 34.9% ± 4.0% in the partial alkalinity of digestates, while ISR had an increase of 138.5% ± 33.9%. These results demonstrate the conversion of organic acids during anaerobic digestion, which agrees with the pH results, which remained within the neutral range at the end of the process.

The relationships between intermediate alkalinity and partial alkalinity (IA/PA) were calculated to evaluate the balance of the process, following the criteria of Ripley *et al.* (1986), which considers IA/PA values greater than 0.3 as indicative of instability in anaerobic digestion. The values are presented in Graph 04.

Graph 04 - IA/PA ratio



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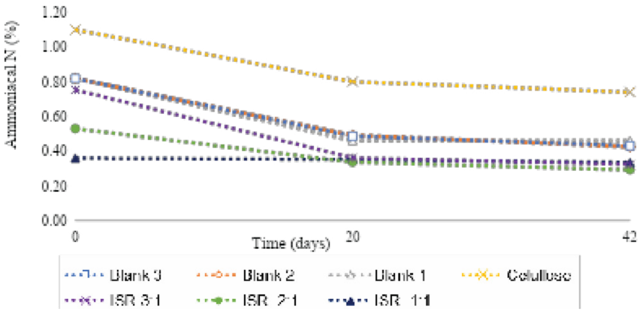
Initially, only the blanks presented values below 0.3, possibly because they had already been digested anaerobically. On day 40, the IA/PA values were below 0.3, indicating that the tests presented an IA/PA ratio suitable for anaerobic digestion, with a buffering effect that resulted in an ideal pH for biogas production.

Ammoniacal Nitrogen, NTK and Protein

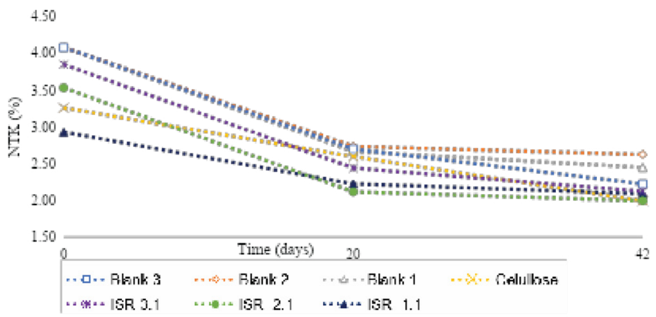
During the tests, there was continuous consumption of ammonia nitrogen, as shown in Graph 05.

Graph 05 - Ammoniacal N content on a dry basis.



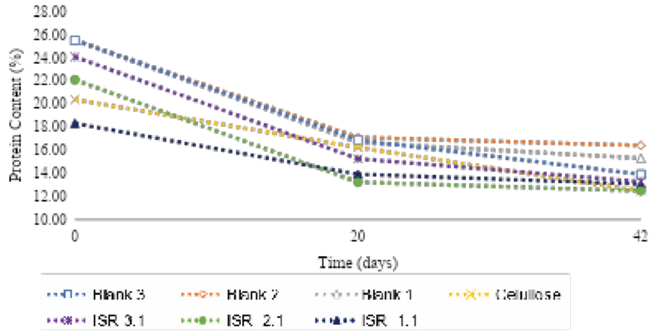
In the first 20 days, there was a reduction of 41.5% ± 2.1% in ammoniacal nitrogen in blanks, 27.3% ± 4.7% in cellulose and 2.1% ± 11.7%, 36.6% ± 8.4%, and 52.1% ± 5.5% at ISR 1:1, 2:1 and 3:1, respectively. Compared to substrates, digestates reduced a total of 46.5% ± 2.3% in blanks, 32.7% ± 9.2% in cellulose, and 36.4% ± 26.1% in RIS. NTK reductions can be seen in Graph 06.

Graph 06 - NTK content on a dry basis.



The NTK content had reductions of 33.8% ± 0.9% in blanks, 20.3% ± 2.5% in cellulose, and 33.5% ± 8.4% in ISR in the first 20 days. At the end of treatment, the reductions were 40.4% ± 4.9% in blanks, 38.8% ± 3.8% in cellulose, and 38.9% ± 9.0% in ISR. The identical decreases were observed for the crude protein content, as seen in Graph 07.

Graph 07 - Protein content on a dry basis

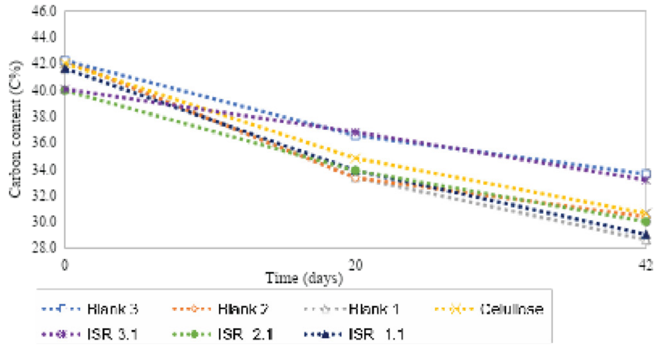


Digestates have the potential to be used in fertigation. However, it is important to highlight that, during the tests, reductions in nitrogen content were observed. Therefore, it is necessary to conduct further investigations to evaluate its viability as a biofertilizer. To be considered a good biofertilizer, it must contain nutrients such as nitrogen (N), phosphorus (P), and potassium (K) (Jurgutis *et al.*, 2021).

Carbon Content and C/N Ratio

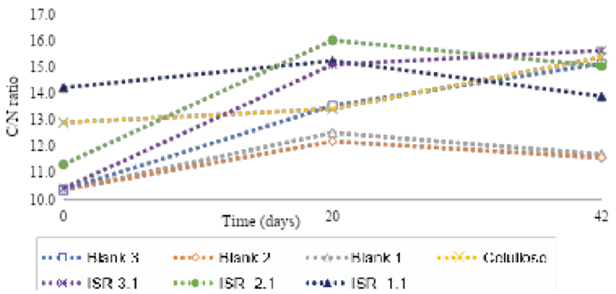
Anaerobic digestion tests demonstrated a continuous reduction in carbon content throughout the process due to the degradation of organic matter in the substrates, resulting in the conversion of carbon into simpler compounds and subsequent production of biogas, as represented in Graph 08.

Graph 08 - Carbon content on a dry basis.



After 20 days of treatment, there were significant reductions in carbon content in all tests, with variations of 18.5% ± 4.4% in blanks, 17.1% ± 1.5% in cellulose, and 14.0% ± 5.3% in RIS. At the end of the process, carbon contents reduced even further, with decreases of 26.9% ± 6.0% in blanks, 27.1% ± 7.5% in cellulose, and 24.1% ± 6.6% in ISR. The Carbon/Nitrogen ratio (C/N) was also evaluated and was below the ideal range recommended for biogas production, as seen in Graph 09.

Graph 09 - C/N ratio.

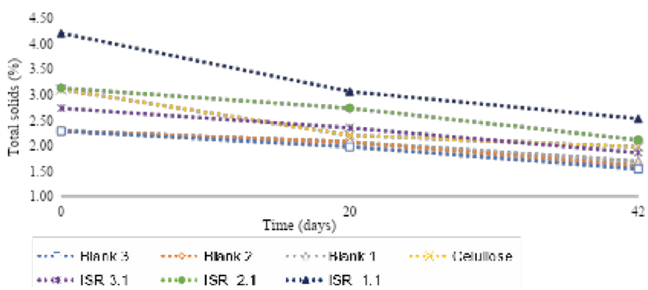


Despite the low C/N ratio, the results indicate that anaerobic digestion of the macrophyte *H. verticillata* is a viable and sustainable strategy for the treatment of organic waste, promoting the reduction of the amount of organic matter and the production of renewable energy in the form of biogas. Furthermore, this approach contributes to reducing environmental impact, preventing the release of greenhouse gases into aquatic environments, and improving water quality. However, monitoring and adjusting process conditions, especially the C/N ratio, is important to optimize biogas production and avoid problems related to ammonia toxicity and pH.

Total and Volatile Solids

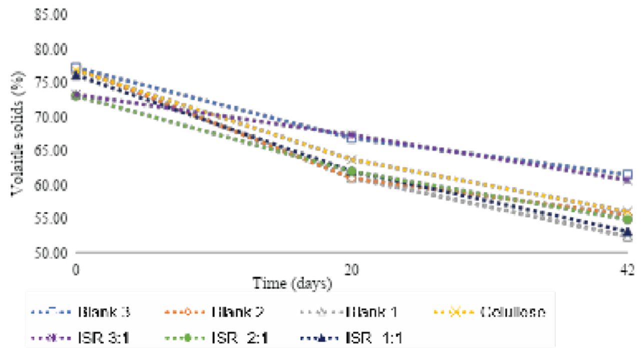
Substrates and digestates followed literature recommendations, presenting total solids values below 10%. This concentration range is essential to ensure efficient mass transfer and avoid possible system overload (Kunz, Steinmetz & Amaral, 2019). The analysis of total solids, illustrated in Graph 10, allows us to visualize the variation in these values over time:

Graph 10 - Total solids on a dry basis (%).



At the end of the process, blanks had a reduction of 29.6% ± 3.3%, cellulose decrease of 36.4% ± 1%, and ISR had a decrease of 34.8% ± 4.5%. As expected, over time, a gradual and continuous reduction in total solids was observed in all tests, which highlights the effectiveness of anaerobic digestion in treating aquatic macrophyte biomass. In Graph 11, it is possible to follow the reductions in volatile solids throughout the tests.

Graph 11 - Volatile solids on a dry basis (%).



Volatile Solids (VS) are also an important parameter in anaerobic digestion, representing the organic fraction that can be converted into biogas by the microorganisms present in the process. The tests revealed that all sample results showed a high content of volatile solids, greater than 70%, demonstrating the potential of these biomasses as a source of energy through anaerobic digestion (Kunz, Steinmetz & Amaral, 2019).

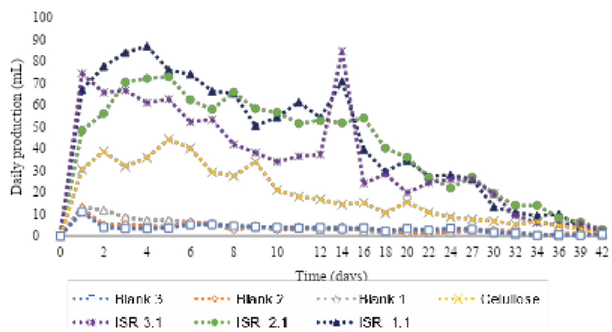
Over time, the systems showed a consistent and continued reduction in volatile solids. The total removal of volatile solids in the tests was 26.9% ± 6.0% in blanks, 27.1% ± 7.5% in cellulose, and reductions of 30.2% ± 2.3%, 24.9% ± 5.1%, and 17.2% ± 4.0% at ISR 1:1, 2:1 and 3:1, respectively.

This reduction in VS indicates the biogas production potential of the evaluated systems, highlighting the potential of anaerobic digestion as a sustainable way of using energy from the macrophyte *H. verticillata*, as observed by Chen *et al.* (2016).

BMP Test

Daily biogas production (Graph 12) during the tests revealed an initial increase followed by a slowdown after the 14th day, except for ISR ratios of 3:1 and 1:1, which peaked on the 14th day. Subsequently, all trials experienced a drop in biogas production. These results align with previous studies that obtained *H. verticillata*, indicating that most of the biodegradable components of the macrophyte are digested in the first two weeks, with more resistant compounds persisting after this period (Chen *et al.*, 2016).

Graph 12 - Daily biogas production.



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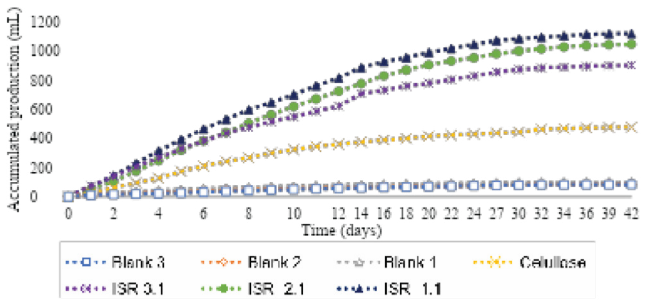
Accumulated biogas production (Graph 13) stabilized within 42 days, with daily production representing less than 1% of the accumulated total. The ISR 1:1, 2:1, and 3:1 assay significantly outperformed the controls, indicating that anaerobically digested slaughterhouse sludge is a suitable inoculum for the BMP test (Holliger *et al.*, 2016).

Graph 13 - Accumulated production of biogas (mL).



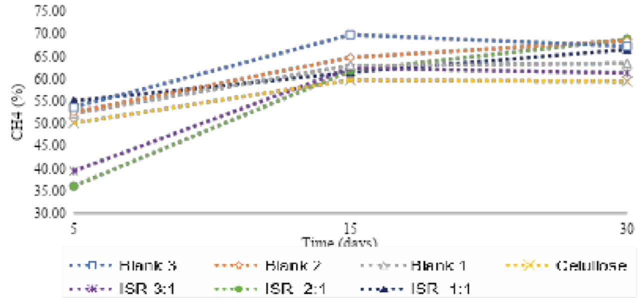
The Kruskal-Wallis test indicated a statistical difference in biogas production at a significance level of 5%. These differences were attributed to the effect of adding the inoculum, with a 95% confidence level. Dunn's test evaluated the statistical difference between biogas production averages. Accumulated production data is presented in Table 04:

Table 04 - Dunn's test applied to accumulated biogas production.

Sample	Accumulated production (mL)	Alpha-5% difference
ISR 1:1	1122,9 ± 42,4	c
ISR 2:1	1048,8 ± 32,7	c
ISR 3:1	905,2 ± 19,9	c
CELLULOSE (ISR 2:1)	478,3 ± 24,2	Bc
BLANK 1	99,3 ± 5,2	Ab
BLANK 2	84,9 ± 10,6	a
BLANK 3	83,5 ± 2,3	a

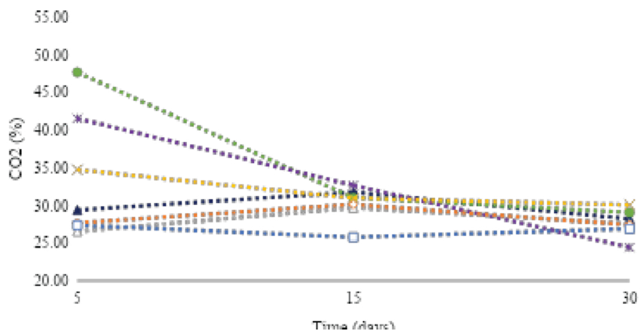
The total biogas production, discounting the inoculum production, was 729.4 mL g^{-1} VS for microcrystalline cellulose, reaching the expected production for the test (Holliger *et al.*, 2016). The production of blanks was 102.0 ± 10.9 mL g^{-1} VS, while for ISR 1:1, 2:1, and 3:1, it was 638.5 mL g^{-1} VS, 842.5 mL g^{-1} VS, and 820.8 mL g^{-1} VS, respectively. Although it presented a high biogas production, it was below the show found by Mahmood *et al.* (2015), who obtained 1000 mL g^{-1} VS of macrophytes when using digested sludge as inoculum.

Graph 14 - Methane content in biogas.



Carbon dioxide remained within acceptable standards, comprising 30% to 50% of biogas. Graph 15 shows the carbon dioxide content in biogas, characterized on days 5, 15, and 30.

Graph 15 - Carbon dioxide content in biogas.



These results demonstrate the potential for energy use of the macrophyte *H. verticillata* in anaerobic digestion, contributing to the production of biogas sustainably by taking advantage of the biomass of this aquatic weed.

CONCLUSIONS

Research has demonstrated that using the macrophyte *Hydrilla verticillata* in conjunction with anaerobically digested slaughterhouse sludge is a viable strategy for biogas production. Physicochemical analyses confirmed that all inoculum: substrate ratios had specific characteristics for anaerobic digestion. The test systems started biogas production immediately and reached stability in up to 42 days, even without prior pH adjustment, indicating the process's effectiveness.

The results of Biochemical Methane Potential (BMP) tests revealed that choosing the 1:1 ratio (ISR) is an economical option without compromising process efficiency. Anaerobically digested slaughterhouse sludge proved to be a suitable inoculum for this application. Despite the macrophyte's initial low C/N ratio, over time, there was a gradual increase in methane content, highlighting the potential of this resource for biogas production through anaerobic digestion.

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