Research Square

Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

Anaerobic Digestion of Macrophytes (Lemna Minor, Spirodela intermedia, Spirodela Polyrhiza and Wolffia Columbiana) And Anaerobic Sludge From Uasb Reactor: Effect of I/S Ratio (Inoculum/substrate) and Temperature

Nestor Leonel Muñoz Hoyos (nestor.hoyos@ufpr.br)
Hydraulic Research Institute
Jose Carlos Alves Barroso Júnior
Universidade Federal do Ceará
Maria Cristina de Almeida Silva
Hydraulic Research Institute
Luiz Olinto Monteggia
Hydraulic Research Institute

Research Article

Keywords: Biogas, Biomass waste, Biochemical Methane Potential (BMP), Specific methane yield, Renewable energy

Posted Date: October 23rd, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3458098/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

The present study aimed to explore floating macrophytes biogas and methane production potential (*Lemna minor*, *Wolffia columbiana, Spirodela polyrhiza*, and *Spirodela intermedia*) through the digestion process using anaerobic sludge from a UASB reactor as inoculum. Substrate (macrophytes) and inoculum (anaerobic sludge) came from domestic wastewater treatment. Two exploratory trials were conducted at different operating conditions to determine their influence on biogas and methane production. The assays were carried out using biodigesters with a volume of 50 liters and operated in batches, using macrophyte *in nature*. The experiments were carried out in triplicates for blank (anaerobic sludge) and different I/S ratios (inoculum/substrate). In Test 1, who evaluated four I/S ratios (0.5, 1.0, 1.5, and 2.0), biodigesters operation occurred under winter temperature conditions (mean 18±2.4°C) for 66 days. Test 2 evaluated the effect of temperature on biogas and methane production in the biodigesters. This experiment maintained the same I/S ratios of Test 1, but at summer temperature (26.6±1.1°C), and the duration of the batches was 37 days. The results obtained in the operational conditions tested in this work indicated that the biomass of macrophytes contributed positively to the increase in biogas and methane production. The highest yields (Y_{CH4}) achieved in winter and summer conditions were for I/S=2.0 with 184 NmL CH4/gVS and for I/S=1.0 with 323.3 NmL CH4/gVS, respectively. It evidenced that biogas and methane production varied in function of the operational parameter I/S that determines the amount of substrate fed in the biodigesters and the test application temperature.

Highlights

- Anaerobic digestion of macrophytes (*Lemna minor, Spirodela intermedia, Spirodela polyrhiza, and Wolffia columbiana*) with UASB reactor sludge as inoculum increased biogas and methane production.
- Four I/S ratios (Inoculum/Substrate) were evaluated on biogas and methane production in winter and summer conditions.
- Potential of the aquatic plant of the *Lemnaceae* family as a raw material to produce biogas and methane. In the less favorable condition, increments in the methane production from the macrophyte in the winter of 285% (I/S=0.5), 472% (I/S=1.0), 693% (I/S=1.5) and 536% were determined. (I/S=2.0).
- Anaerobic digestion is a viable alternative for managing excess lignocellulosic plants (*Lemnaceae*) used in the bioremediation of domestic and industrial wastewater.
- The results of specific methane yield obtained in the Tests in winter (I/S:2.0 with 184 NmLCH4/gVS) and summer (I/S:1.0 with 323,2 NmLCH4/gVS) are comparable with other studies that used pre-treated substrate and heating of the reaction medium.

1. INTRODUCTION

Energy is a determining factor for social and economic development, and its consumption has intensified in recent years due to population growth, industrialization, and the rapid urbanization of cities. Currently, fossil fuels such as oil, natural gas, and coal are the primary sources that supply the world's energy demand. However, these energy sources are limited and will soon have restricted use due to their scarcity and the environmental problems related to atmospheric emissions (Nahar & Sunny, 2020).

The growing global concern with environmental issues, such as climate change and the urgency of abandoning dependence on fossil fuels, has promoted the search for new energy sources that transform the current world energy scenario. This is how the energy valuation of organic waste generated in the economic, productive, and basic sanitation sectors, using anaerobic digestion (AD) technology to produce biogas, has intensified in the world, positioning AD as a reliable technology for bioenergy production (Baena-Moreno et al., 2019).

The use of biogas to generate electricity, thermal energy, and biomethane has been gaining ground in Brazil and worldwide. The International Center for Renewable Energy (CIBiogás) in Brazil in 2021 estimated that the theoretical potential for biogas production was 82.5 billion Nm^3/year, and the amount of biogas used for the same year was 2.3 billion Nm^3/year, approximately 3% of its full potential (CIBiogás, 2022).

Factors such as availability and transportation cost are decisive in choosing a substrate. In addition to selecting the material with biodegradation potential, it is necessary to define the best operating conditions, such as the best mixing ratio of the substrate and inoculum that allows greater synergy between the food and the microorganisms, temperature, detention time, pH, and mixture (Chen et al., 2022; Elbeshbishy et al., 2012; Q. Li et al., 2017; Y. Li et al., 2019; McLeod et al., 2019).

In recent years, aquatic plants of the *Lemnaceae* family have aroused interest in energy use as a promising material for ethanol, methane, and hydrogen (Alzate et al., 2014; Chen et al., 2022; Cheng & Stomp, 2009; Ge et al., 2012; Shen et al., 2016; Xu & Deshusses, 2015). However, aquatic plants, including floating, emergent, and submerged macrophytes, have been over-cultivated and have caused several environmental problems in lakes, dams, and reservoirs worldwide (Abbasi et al., 1990; Haga & Ishikawa, 2011; Moorhead & Nordstedt, 1993; Muñoz Escobar et al., 2011; O'Sullivan et al., 2010).

Lemnaceae has been used in the remediation of domestic and industrial wastewater in recent decades. The use of ponds for cultivating macrophytes can combine the control of pollution of water resources through the treatment of effluents at a secondary and tertiary level, in addition to the generation of biomass that can be used as raw material for the generation of renewable energies, adding value to the biomass produced through effluent treatment (Femeena et al., 2022; Grobbelaar, 2009; Hadiyanto & Soetrisnanto, 2013; Oswald, 1988; Powell et al., 2011; Zhou et al., 2023).

Several studies show that the lagoon with macrophytes was efficient in treating different types of effluents, resulting in high removal of nutrients (phosphorus and nitrogen), suspended solids, heavy metals, micropollutants, and pathogens (Barroso Júnior et al., 2022; Ceschin et al., 2019; Gatidou et al., 2017; Kadir et al., 2020; Ozengin & Elmaci, 2007).

For the proper functioning of macrophyte phytoremediation ponds, plant density must be periodically controlled to maintain good efficiency in removing pollutants from wastewater. For Ceschin et al. (2019), there are limitations in the use of *Lemna* in the phytoremediation of polluted aquatic environments due to the excess of aquatic plants, some of the negative conditions identified were low temperature, formation of a hypoxic environment and the accumulation of necrotic biomass.

One option for managing this excess generated biomass in wastewater treatment could be anaerobic digestion or co-digestion (Gaur & Suthar, 2017; Koyama et al., 2014; Pena et al., 2017; Ren et al., 2018; Yadav et al., 2017). Thus, macrophytes are generally rich in lignocellulosic materials, proteins, lipids, fibers, and starch, making them a non-agricultural plant source with potential for sustainable biofuel production (Farrell & Gopal, 2008; Vassilev et al., 2015; Verma & Suthar, 2015).

Therefore, the objective of this work was to explore the potential of biogas production obtained in the anaerobic digestion of macrophytes of the *Lemnaceae* family with excess sludge from an upflow anaerobic sludge blanket reactor (UASB), generated in the treatment of domestic effluent. For this, tests were carried out using batch-operated digesters to evaluate the effect of the I/S ratio (inoculum/substrate) and temperature (winter and summer) on biogas and methane production.

2. MATERIALS AND METHODS

Inoculum and substrate samples were collected in an experimental pilot station in Wastewater Treatment Plants (WWTP) in Porto Alegre/RS, Brazil. Macrophytes (substrate) came from a system of high-rate, series-operated lagoons employed for post-treatment of effluent from an upflow anaerobic sludge blanket (UASB) reactor fed with sanitary sewage. The genera identified were *Spirodela, Lemna*, and *Wolffia*, with *Lemna minor, Spirodela intermedia, Spirodela polyrhiza*, and *Wolffia columbiana* being the predominant species.

The anaerobic sludge was collected from the experimental reactor type UASB used to treat sanitary sewage. It presented a flocculent physical characteristic due to the significant variability in the concentration of organic matter in the raw effluent (Hoyos et al., 2019).

The anaerobic sludge and macrophyte samples were collected on the same day and stored in a refrigerator at 3°C. To experiment setting up, the sludge and the macrophyte were removed from the fridge in advance to reach the operating temperature of each test.

Exploratory tests were carried out in 15 biodigesters with a volume of 50 L, using cylindrical PVC tubes with a diameter of 30 cm and a proper height of 70 cm. The construction of all biodigesters predicted a vertical axis manual stirring. The measure of produced biogas flow was daily, through the water displacement method using the modified *Mariotte* bottle (flask), using 20 L bottled mineral water coupled to each biodigester (Fig. 1).

2.1. Description of the Biochemical Methane Potential (BMP) test

The inoculum and substrate characterization analysis was carried out before each BMP test in terms of total solids (TS) and total volatile solids (TVS) and other physical and chemical parameters of interest. The biodigesters were manually mixed once a day for one minute to reduce heterogeneity between the inoculum and the substrate.

The medium reaction definition of the AD experiments was by the ratio (I/S) where I was the TVS mass of the inoculum (anaerobic sludge) and S was the TVS mass of the macrophytes substrate (*Lemnaceae*). The I/S ratio values were set to 0.5, 1.0, 1.5, and 2.0 to determine the best operating ratio, according to the performance of the anaerobic process in terms of biogas production, methane, and specific methane yield.

Twelve digesters were grouped into four sets, each consisting of three digesters (triplicates) to meet the I/S load ratios of 0.5, 1.0, 1.5, and 2.0. The three remaining digesters were used as a reference, corresponding to the inoculum used in the anaerobic process. The purpose of the reference reactors was to determine the volume of biogas produced by the anaerobic sludge and subtract it from the total biogas generated in the biodigesters containing the different I/S ratios. In this way, the net production of biogas and methane from the macrophyte was determined.

Two experimental tests were developed to evaluate the effect of the I/S ratio and temperature on the production of biogas, methane, and the specific yield of methane. Test 1 was carried out in the winter temperature conditions and Test 2 in the summer conditions, particularly seasons in the southern region of Brazil.

Test 1

The effect of the I/S ratio (0.5, 1.0, 1.5, and 2.0) on biogas production was studied by operating the digesters at usual winter temperatures. The substrate (macrophyte) was used *in nature*, and the average operating temperature was 18 ± 2.4°C for 66 days.

Test 2

The effect of temperature on biogas production, maintaining the four I/S ratios of Test 1, and the experiment was carried out at the usual temperatures of the summer season. The temperature of the experiment was in the range of 26.6 ± 1.2°C and the experimental time was 37 days.

All biodigesters were loaded with 10 L of anaerobic sludge to compose the different I/S sets and added an equivalent amount of fresh mass of macrophytes to obtain the four I/S ratios. The mass of macrophytes fed into the I/S = 0.5, 1.0, 1.5, and 2.0 biodigesters was 5.80, 2.90, 1.94 and 1.45 kg for Test 1 and 5.72, 2.82, 1.89 and 1.41 kg for Test 2, respectively.

The measurement of volumetric production of biogas was done until the daily production in the biodigesters was less than 1% of the accumulated value for 5 consecutive days. In Test 1, due to the variation in biogas production, the experiment was left an upper time to explore the behavior of the anaerobic process.

2.2. Analytical methods

The stability and performance of the AD process evaluation considered the monitoring of a set of physical and chemical parameters such as pH, TS, TVS, volatile fatty acids (VFA), Total Kjeldahl Nitrogen (TKN), and ammoniacal nitrogen performed according to the methodologies described in the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WEF, 2017) and Total Alkalinity (TA), Partial Alkalinity (PA) and Intermediate Alkalinity (IA), according to the method by Ripley et al. (1986).

The reaction medium characterization of the biodigesters was performed by collecting a volume of 300 mL after mixing for 1 minute. The sample was centrifuged for 10 minutes at 1,500 rpm to obtain the liquid phase of the reaction medium. Then the supernatant was vacuum filtered using a quantitative filter (JP-41) with a pore size of 28 μm and then a microfilter of particle retention size 0.45 μm. The reaction medium was collected for physical and chemical characterization at the experiment's beginning, during, and end. Samples were collected in one of the biodigesters of each triplicate of the I/S ratios and the reference.

Biogas quantity and quality were measured daily for each BMP test in the 12 I/S ratio digesters and the three reference digesters. The volume of water displaced from the *Mariotte´s* bottles by the biogas was weighed. A 1 mL biogas sample was collected and manually injected into the chromatography to determine its composition and to assess its quality. A DANI GC 1000 chromatograph was used with thermal conductivity detector (TCD) at 180°C, SUPELCO Carboxen-1006 PLOT capillary column. The oven temperature was 50°C, the S/SL injector operated in split mode with a flow rate of 3.0 mL/min at 40°C, the carrier gas used was helium.

The monitoring of VFA concentrations was performed using the DANI Instrument Master GC model gas chromatograph, equipped with a flame ionization detector (FID) at 250°C, GC Nukol 24107 capillary column with an oven temperature of 80°C. A 1 μm of sample was injected with the gas-tight *Hamilton syringe* in split mode with a flow rate of 1.0 mL/min and helium carrier gas. The total execution time of the method was 7 min, and the programmed temperature ramp (2 min – 40°C/min) started at 80°C and ended at 200°C.

The volume of biogas produced under the experimental conditions of the tests was converted to standard conditions of temperature and pressure with equations 1 and 2, using the standard references of 1,013 hPa (1 atm) and 0°C (273.15 K) of pressure and temperature, respectively.

$$V_N = V \bullet \frac{(P - P_W) \bullet T_N}{P_N \bullet T}$$

1

Where V_N is the normalized dry volume of biogas (NmL or STP mL), V is the volume of biogas produced (mL), P is the pressure of the biogas on the day of reading or measurement (hPa), P_W is the vapor pressure of the water as a function of ambient temperature (hPa), T_N is the normal temperature (273 K), P_N is normal pressure (1,013 hPa), and T is the experiment temperature (K).

The calculation of water vapor pressure was performed with the modified equation of Buck, (1996) (Eq. 2):

$$P_W = 6{,}1121 \bullet e^{\left(\left(18{,}678 - \frac{T_C}{234{,}5}\right) \bullet \frac{T_C}{257{,}14 + T_C}\right)}$$

2

Where P_W is the water vapor pressure as a function of ambient temperature in hPa (1 hPa = 0.1 kPa) and T_C is the ambient temperature of the experiment (°C).

3. RESULTS AND DISCUSSION

The macrophyte *in nature* presented 95% humidity and 86% TVS for Test 1. For Test 2, it was verified that 93.4 and 84% of humidity and TVS, respectively. Its chemical composition was 77.43% organic matter, 37.40%

crude protein, 15.14% crude fiber, 2.32% ethereal extract, and 22.57% ash.

The values found for protein, fiber, and moisture are in the range of values reported in the literature for different species of macrophytes, which vary from 16-44.7% of protein, from 8.8–15.6% of fiber, and 94–96% humidity (Appenroth et al., 2017; Gaur & Suthar, 2017; Verma & Suthar, 2016).

Table 1 presents the results of the physical and chemical characterization of the anaerobic sludge, highlighting that the pH was close to the neutral range. The fraction of active biomass in the sludge determined as TVS for Tests 1 and 2 was 42 and 48%, showing that the anaerobic sludge used as inoculum showed a low concentration of anaerobic microorganisms measured with TVS influence the efficiency of the AD process.

Table 1
Physical and chemical characteristics of the inoculum of Tests 1 and 2

Operating Parameters	**Test 1**	**Test 2**
	Anaerobic Sludge	**Anaerobic Sludge**
TS (mg/L)	30,446	32,562
TVS (mg/L)	12,704	15,734
Total Alkalinity (mg/L)	895	738
Partial Alkalinity (mg/L)	445	298
TKN (mg/L)	877	1,004
Ammoniacal Nitrogen (mg/L)	90	57
Temperature (°C)*	16.6	25.6
pH	7.2	6.8

* The temperature at the AD beginning.

3.1. Biochemical Methane Potential (BMP)

The experimental results of the different I/S ratios were plotted as daily production and accumulated production of biogas, the evolution of the methane content in the biogas, and specific methane yield.

The daily biogas production values as a function of the I/S ratio of Test 1 (Fig. 2. A1), show a period of zero production in the first 48 hours due to a possible adaptation of microorganisms to the environment and/or development of necessary enzymes for the hydrolysis of the new substrate. After this latency phase, the production of biogas was mainly due to the degradation of the residual organic matter contained in the anaerobic sludge. Then, a gradual increase in biogas production was observed after day 11, but with no defined trend about I/S values. It is believed that this is due to the high results variation of the daily biogas production, which remained until the experiment´s end.

The daily biogas production behaved differently from the winter season for the summer temperature. High biogas production was observed in the first week of Test 2 (Fig. 2. B1), with a tendency to decrease over time without depleting the biodegradable organic matter from the substrate. The results obtained in this test allow elucidating the positive effect of temperature on the anaerobic digestion of macrophytes, consequently adding the production of biogas in the process.

In the figure of accumulated biogas production of Test 1 (Fig. 2. A2), it is observed that the I/S = 1.5 ratio reached the highest value with 45 NL. However, the other I/S ratios (1.0, 0.5, and 2.0) showed similar results and a tendency to increase over time like the I/S = 1.5 ratio, thus making it challenging to identify the value of the I/S ratio recommended to produce biogas under these operating conditions.

The trend of accumulated biogas production for the I/S ratios (0.5, 1.0, 1.5, and 2.0) of Test 2 is shown in Fig. 2 (B2). Unlike the winter test, the curves show a different behavior, where it is possible to observe the effect of the organic loading parameter (I/S ratio) on biogas production. No latency phase was observed in this test, and biogas production started soon after the experiment. It shows that the microorganisms involved in the process could adapt more quickly to the new substrate and convert a more significant amount of biodegradable organic matter from the substrate to biogas. The 0.5 I/S ratio reached the maximum biogas volumetric production of approximately 145 NL, which meant an increase of 222% to that obtained in Test 1 with the 1.5 I/S ratio.

It is observed that the digesters of Test 1 reached the maximum accumulated production of biogas (80–90%) in the range of 58–62, 59–63, 55–59, and 40–55 days for I/S = 0.5, 1.0, 1.5 and 2.0, respectively. As for Test 2, the maximum accumulated production was reached in a shorter time, showing that the application temperature of the experiment accelerated the anaerobic process, reducing by at least 50% the digestion time of the different I/S ratios. The maximum accumulated production of biogas corresponding between 80 and 90% for the experiment in the summer temperature was in the range of 23–28, 19–27, 16–25, and 15–23 days for I/S = 0.5, 1.0, 1.5, and 2.0, respectively.

The composition of biogas at winter and summer temperatures showed different trends, as shown in Fig. 2. (A3 and B3). In Test 2, the methane fraction increased over time, notably in the first eight days, with values close to 50% for all I/S ratios. It is believed that the temperature (summer) allowed a more significant activity of acetogenic and methanogenic (*Archaea*), allowing the first group to transform the produced volatile fatty acids into acetate and later the methanogenic (*Archaea*) to convert the acetate to methane, principally.

With the specific methane yield curves from Test 1 (winter), it was possible to point out the effect of the I/S ratio on the AD process. As shown in Fig. 2 (A4), it appears that the highest I/S ratios were those that had the highest values of specific methane yield with 178 and 184 NmL CH_4/g VS for I/S = 1.5 and 2.0, respectively. The previous results show that in biodigesters with lower macrophyte mass, the microbial community better adapted to the substrate (macrophytes), which is reflected in higher methane production. The I/S ratio (0.5) with the most increased mass of macrophytes fed into the biodigester showed the worst yield in the anaerobic assay with 25 NmL CH_4/g VS. In these biodigesters with greater substrate mass, a more significant release of adverse intermediates to the microorganisms of the AD process may eventually occur.

In the summer condition (Test 2), the intermediate ratios I/S = 1.0 and 1.5 obtained the best results of specific methane yield, as shown in Fig. 2 (B4). The values reached were 323 and 306 NmL CH_4/g VS, respectively. The results obtained in this experiment are superior to those of Test 1, which shows the influence of temperature on the anaerobic conversion of lignocellulosic substrate to methane. When comparing the specific methane yield results of Test 1 with Test 2, it was found that there was a percentage increase of 867, 296, 72, and 11% for the I/S ratios = 0.5, 1.0, 1.5, and 2.0, respectively.

The specific methane yield results of Tests 1 and 2 are comparable with other studies that used macrophytes as substrate without and with pre-treatment in anaerobic digestion processes. For example, the results of the study by Toyama et al. (2018) are higher, as shown in Table 2, than those obtained by the 4 I/S ratios in the winter (Test 1) and summer (Test 2) temperature condition. The difference in the results obtained is probably due to the temperature applied to the experiments. The authors mentioned above operated the AD process at a constant temperature of 38°C.

The specific yield values of methane (Y_{CH4}) reported by Pena et al. (2017) and Ren et al. (2018) are lower than those obtained by the I/S ratios = 0.5, 1.0, 1.5, and 2.0 in the experiment applied in the summer condition (Test 2) of this study. Although the temperature of application of the experiments has been higher in the studies, it was found that the inoculum used in the test by Pena et al. (2017) showed much lower TVS content than what was used in the present work, which probably affected the performance of the AD process.

In another AD study of macrophytes (duckweed) carried out by Buragohain et al. (2021), the specific methane yields obtained in the mono- and co-digestion process were lower than those obtained in test 2. The authors reported a maximum yield of 217.1 ± 11.2 NmL/g VS for a concentration of 15% TS when the biological process was operated as monodigestion, lower than three I/S ratios (0.5, 1.0, and 1.5) of test 2. When manure was added to cattle in the 1:1 ratio to the monodigestion process, the maximum yield obtained was 271.7 ± 14.2 NmL/g VS, being even lower than the two I/S ratios (1.0 and 1.5) of Test 2.

It is noteworthy that the results of the macrophyte species studied in the present work are in the range of other correlated studies that are presented in Table 2. The variation of results between the different studies can be explained by variables such as the temperatures of the experiments, mixing, pre-treatment of the macrophyte, use of one or more co-substrates, the quality of the inoculum used, and the biochemical composition of the macrophyte.

Table 2
Comparison of the specific methane yield of Tests 1 and 2 with other studies.

Macrophyte	Pre-treatment	Protein (%)	I/S Ratio	Inoculum	Temp (°C)	Period (days)	Methane yield Y_{CH4} (NmL/gVS)	Reference
S. polyrhiza	Without	21.37	1.0	AS	37	70	340.7	(Cu et al., 2015)
L. minor	Without			SW	37	40	193	(Pena et al., 2017)
L. gibba	Thermal			AS	35	35	60–468	(Gaur et al., 2017)
L. minor	Without	19	1.0	AS	37	30	169	(Ren et al., 2018)
S. polyrhiza *L. minor* *L. gibba* *L. punctata*	Without			AS	38	28	340–413 334–375 334–370 343–408	(Toyama et al., 2018)
L. obscura	Without	18.3	0.5	AS	35	42	227–434	(Calicioglu et al., 2019)
E. crassipes *P. stratiotes* *S. molesta*	Ultrasound		2.0	AS	35	46	422 417 432	(Kist et al., 2020)
E. densa	Alkaline		2.0		35	20	231.8	(Dutra et al., 2021)
L. minor	Physical and thermal		0.5	SW	36 ± 1	54	347.2*	(Navarro et al., 2022)
L. minor, W. columbiana, S. polyrhiza, S. intermedia	Without	37.4	0.5, 1.0, 1.5, e 2.0	AS	18.1 ± 2.4	66	25-183.9	Present study
L. minor, W. columbiana, S. polyrhiza, S. intermedia	Without	37.4	0.5, 1.0, 1.5, e 2.0	AS	26.6 ± 1.2	37	204.9-323.2	Present study
AS: Anaerobic sludge, SW: Swine wastewater								

*Condition: IN:(FW + FD). IN: inoculum, FW: Food waste, FD: Fresh Duckweed.

According to the results presented in Table 2, it is observed that the application temperature of Test 1 (winter) and Test 2 (summer) are lower. However, the values obtained for specific methane yield were higher or close to other studies where the reaction medium was heating, indicating an implicit energy expenditure throughout the experiment in these studies. In the same way, the use *in natura* of the macrophyte (without pre-treatment) made it possible to apply the experiments, avoiding previous steps in the process and/or the use of equipment or chemical reagents that could make it energetically or economically unfeasible to reproduce on an expanded scale at the AD of macrophytes with anaerobic sludge. It stands out that the results obtained in Tests 1 and 2 are like those of other works (Table 2) that used substrate pretreatment, thus showing the potential of the macrophyte as a substrate in AD.

3.2. Monitoring the anaerobic digestion process of Tests 1 and 2

The physical and chemical characterization of the initial and final samples and the organic loading rate (OLR) of Tests 1 and 2 are presented in Table 3. According to the monitoring of the anaerobic process, it is observed that the reduction of TS and TVS was less than 20%, showing difficulty on the part of microorganisms in hydrolyzing the lignocellulosic components of the macrophyte structure. According to Nizami et al. (2009), the biodegradability of this material is limited by the cellulose crystallinity and the lignin content.

Table 3
Physical and chemical characterization of biodigesters - Tests 1 and 2

Parameters		**Test 1**				**Test 2**			
		I/S = 0.5	**I/S = 1.0**	**I/S = 1.5**	**I/S = 2.0**	**I/S = 0.5**	**I/S = 1.0**	**I/S = 1.5**	**I/S = 2.0**
TS (mg/L)	Initial	38,208	36,980	36,210	35,851	34,830	34,420	33,101	34,119
	Final	31,015	29,888	30,564	31,521	28,392	29,618	30,982	30,538
TVS (mg/L)	Initial	13,084	13,225	12,780	13,420	14,472	14,982	14,566	14,995
	Final	10,548	10,955	11,796	12,732	11,852	13,172	14,130	13,866
TKN (mg/L)	Initial	748	619	531	649	1,406	1,033	1,015	976
	Final	1,951	1,427	1,485	1,136	2,004	1,655	1,510	1,278
Ammoniacal Nitrogen (mg/L)	Initial	382	404	322	295	201	115	86	86
	Final	757	728	553	408	958	697	581	436
TA (mg/L)	Initial	1,300	1,770	1,823	1,481	1,115	1,056	1,198	1,027
	Final	4,649	3,725	3,314	3,002	4,776	3,818	3,226	2,904
PA (mg/L)	Initial	895	1,173	934	885	596	440	709	528
	Final	2,806	2,293	2,356	2,048	3,813	2,918	2,171	2,024
(IA/TA)	Initial	0.31	0.34	0.49	0.40	0.46	0.58	0.41	0.49
	Final	0.40	0.38	0.29	0.32	0.20	0.24	0.33	0.30
pH	Initial	7.10	7.15	7.11	7.14	7.02	6.80	6.83	6.73
	Final	7.20	7.22	7.25	7.19	7.42	7.24	7.11	6.99
OLR (g VS/L.d)	Initial	0.20	0.12	0.09	0.07	0.45	0.27	0.20	0.16

In both Tests (1 and 2), the TKN and ammoniacal nitrogen concentration increased throughout each experiment. The increase in nitrogen concentration in the biodigesters is probably related to the solubilization of nitrogen compounds present in the substrate (macrophyte). The values of ammoniacal nitrogen concentration in the biodigesters were within the concentration range 200-1,000 mg/L, a range that several authors have identified as having no adverse effect on anaerobic processes (Khanal, 2011; Lauterböck et al., 2012; Mata-Alvarez et al., 2000).

The TA and PA concentrations increased over time in Tests 1 and 2, and the highest concentrations were verified at the experiment end. Their increase occurred as a function of the amount of substrate added to the biodigesters. In general, the alkalinity values for tests 1 and 2 were within the normal range for anaerobic processes, which according to Metcalf et al. (2004), ranges from 1,000 to 5,000 mg/L.

This same observation can be made for the pH values, which, being in the range between 6.5 and 7.5, are considered optimal for methanogenic bacteria (Y. Li et al., 2019).

Figure 3 shows the evolution of VFAs in anaerobic sludge and I/S ratio digesters. The VFA concentrations in Test 1 (A5) showed accumulations over time without causing disturbances in the anaerobic process that could compromise biogas and methane production. For the summer condition (B5), VFA concentrations remained below 1,000 mg/L, showing that the temperature allowed a better dynamic balance between the production of fatty acids and their subsequent consumption and conversion into acetate, methane, and other gases.

Kumar et al. (2016) evaluated the effect of psychrophilic (15 ± 2°C), mesophilic (30 ± 2°C), and thermophilic (50 ± 2°C) temperature on food residue AD. The authors determined the temperature in the mesophilic range to be optimal for the biological process. At this temperature, a higher rate of growth and activity of microorganisms was associated, which led to achieving the highest yield of the experiment.

4. CONCLUSIONS

The use of natura macrophyte (*Lemnaceae*) as a substrate contributed to increasing the production of biogas, methane, and specific methane yield (Y_{CH4}) in the anaerobic digestion process for the two operating temperatures conditions, using excess sludge from the UASB reactor as inoculum.

The operational parameter´s importance and influence in the anaerobic process are highlighted, such as the ratio between inoculum and substrate I/S (0.5, 1.0, 1.5, and 2.0) and operating temperature.

In Test 1 at winter temperature (18 ± 2.4°C), the highest value of the specific methane yield was 184 NmL CH_4/gVS for the highest I/S ratio (2.0). The increase in methane production from the macrophyte in this experiment was 285, 472, 693, and 536% for the I/S = 0.5, 1.0, 1.5, and 2.0 ratio, respectively.

The summer temperature (26.6 ± 1.2°C) benefited the conversion of macrophyte biomass into biogas and methane. In Test 2 the biodigesters showed the highest values of volumetric production. The percentage increase in net methane production, when compared to Test 1 (winter temperature), was 1,157% for the I/S = 0.5 ratio, 400% for the I/S = 1.0 ratio, 116% for the I/S ratio = 1.5 and 40% for the I/S ratio = 2.0.

In the summer operation condition, the intermediate ratios (1.0 and 1.5) obtained the highest values of specific methane yield (Y_{CH4}) with 323.2 and 305.7 NmL CH_4/gVS, respectively.

The temperature effect Tests (1 and 2) for natural substrate present significantly different values of Y_{CH4}. However, an economic evaluation (benefit-cost relationship) of the viability of heating the digestion process is necessary, especially in winter. Likewise, the shorter time required in Test 2 (37 days) is also worth mentioning, which allows the processing of a more significant amount of biomass per unit volume of the biodigesters in the summer condition.

Declarations

Ethics approval and consent to participate

The authors declare that this is our original work, and the corresponding manuscript has not been published before and is only submitted to the Journal of Biotechnology for Biofuels and Bioproducts.

Consent for publication

Corresponding authors confirm that the submission of this manuscript has been approved by all the co-authors.

Availability of data and materials

The data generated or analyzed during this study are included in this published article and its additional files. Further datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

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

Funding

Not applicable.

Authors' contributions

Nestor Leonel Muñoz Hoyos: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resource, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization. **Jose Carlos Barroso Junior:** Resources, Writing - Review & Editing, Visualization. **Maria Cristina de Almeida Silva:** Resources, Writing - Review & Editing, Visualization. **Luiz Olinto Monteggia:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Project administration.

Acknowledgments

Eternal thanks to the great scientist and professor Luiz Olinto Monteggia (*in memoriam*). His legacy and his contributions to the sanitation of the World and Brazil have always remained in force. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) of Brazil for the PhD scholarship awarded to N. L M. Hoyos Finance Code 88882.345763/206-02.

References

1. Abbasi, S. A., Nipaney, P. C., & Schaumberg, G. D. (1990). Bioenergy potential of eight common aquatic weeds. *Biological Wastes*, *34*(4), 359–366. https://doi.org/10.1016/0269-7483(90)90036-R
2. Alzate, M. E., Muñoz, R., Rogalla, F., Fdz-Polanco, F., & Perez-Elvira, S. I. (2014). Biochemical methane potential of microalgae biomass after lipid extraction. *Chemical Engineering Journal*, *243*, 405–410.

https://doi.org/10.1016/j.cej.2013.07.076

3. APHA-AWWA-WEF. (2017). *The Standard Methods for the Examination of Water and Wastewater* (American Public Health Association -American Water Works Association -Water Environment Federation, Ed.; 21st ed.).
4. Appenroth, K., Sree, K. S., Böhm, V., Hammann, S., Vetter, W., Leiterer, M., & Jahreis, G. (2017). Nutritional value of duckweeds (Lemnaceae) as human food. *Food Chemistry*, *217*, 266–273. https://doi.org/10.1016/j.foodchem.2016.08.116
5. Baena-Moreno, F. M., Rodríguez-Galán, M., Reina, T. R., Zhang, Z., Vilches, L. F., & Navarrete, B. (2019). Understanding the effect of Ca and Mg ions from wastes in the solvent regeneration stage of a biogas upgrading unit. *Science of the Total Environment*, *691*, 93–100. https://doi.org/10.1016/j.scitotenv.2019.07.135
6. Barroso Júnior, J. C. A., Cristina De Almeida Silva, M., Leonel Muñoz Hoyos, N., & Monteggia, O. (2022). Evaluation of UASB effluent post-treatment in pilot-scale by microalgae HRP and macrophytes pond for nutrient recovery. *Journal of Cleaner Production*, *357*, 131951. https://doi.org/10.1016/J.JCLEPRO.2022.131951
7. Buck, A. L. (2012). *Model CR-1A Hygrometer With Autofill Operating Manual*. https://www.hygrometers.com/wp-content/uploads/CR-1A-users-manual-2009-12.pdf
8. Buragohain, S., Mahanta, P., & Mohanty, K. (2021). Biogas production from anaerobic mono- and co-digestion of lignocellulosic feedstock: Process optimization and its implementation at community level. *Environmental Technology and Innovation*, *24*, 101981. https://doi.org/10.1016/j.eti.2021.101981
9. Calicioglu, O., Richard, T. L., & Brennan, R. A. (2019). Anaerobic Bioprocessing of Wastewater-Derived Duckweed: Maximizing Product Yields in a Biorefinery Value Cascade. *Bioresource Technology*, *289*(May), 121716. https://doi.org/10.1016/j.biortech.2019.121716
10. Ceschin, S., Sgambato, V., Ellwood, N. T. W., & Zuccarello, V. (2019). Phytoremediation performance of Lemna communities in a constructed wetland system for wastewater treatment. *Environmental and Experimental Botany*, *162*, 67–71. https://doi.org/10.1016/J.ENVEXPBOT.2019.02.007
11. Chen, G., Zhao, K., Li, W., Yan, B., Yu, Y., Li, J., Zhang, Y., Xia, S., Cheng, Z., Lin, F., Li, L., Zhao, H., & Fang, Y. (2022). A review on bioenergy production from duckweed. *Biomass and Bioenergy*, *161*, 106468. https://doi.org/10.1016/J.BIOMBIOE.2022.106468
12. Cheng, J. J., & Stomp, A. M. (2009). Growing Duckweed to recover nutrients from wastewaters and for production of fuel ethanol and animal feed. *Clean - Soil, Air, Water*, *37*(1), 17–26. https://doi.org/10.1002/clen.200800210
13. CIBiogás, C. I. de E. R.-B. (2022). *Panorama do Biogás no Brasil 2021. CIBiogás (Brasil) Relatório Técnico nº 001/2022*.
14. Cu, T. T. T., Nguyen, T. X., Triolo, J. M., Pedersen, L., Le, V. D., Le, P. D., & Sommer, S. G. (2015). Biogas production from vietnamese animal manure, plant residues and organic waste: influence of biomass composition on methane yield. *Asian-Australasian Journal of Animal Sciences*, *28*(2), 280–289. https://doi.org/10.5713/ajas.14.0312
15. Dutra, J. da C. F., Passos, M. F., da Silva, T. F., & Mockaitis, G. (2021). Pyrolysis and anaerobic digestion of the Egeria Densa biomass in different pretreatment conditions for potential use as a substrate in the

generation of bioproducts. *Journal of Water Process Engineering*, *40*. https://doi.org/10.1016/j.jwpe.2020.101772

16. Elbeshbishy, E., Nakhla, G., & Hafez, H. (2012). Biochemical methane potential (BMP) of food waste and primary sludge: Influence of inoculum pre-incubation and inoculum source. *Bioresource Technology*, *110*, 18–25. https://doi.org/10.1016/J.BIORTECH.2012.01.025
17. Farrell, A. E., & Gopal, A. R. (2008). Bioenergy Research Needs for Heat, Electricity, and Liquid Fuels. *MRS Bulletin*, *33*(04), 373–380. https://doi.org/10.1557/mrs2008.76
18. Femeena, P. V., House, G. R., & Brennan, R. A. (2022). Creating a Circular Nitrogen Bioeconomy in Agricultural Systems through Nutrient Recovery and Upcycling by Microalgae and Duckweed: Past Efforts and Future Trends. *Journal of the ASABE*, *65*(2), 327–346. https://doi.org/10.13031/JA.14891
19. Gatidou, G., Oursouzidou, M., Stefanatou, A., & Stasinakis, A. S. (2017). Removal mechanisms of benzotriazoles in duckweed Lemna minor wastewater treatment systems. *Science of The Total Environment*, *596–597*, 12–17. https://doi.org/10.1016/J.SCITOTENV.2017.04.051
20. Gaur, R. Z., Khan, A. A., & Suthar, S. (2017). Effect of thermal pre-treatment on co-digestion of duckweed (Lemna gibba) and waste activated sludge on biogas production. *Chemosphere*, *174*, 754–763. https://doi.org/10.1016/j.chemosphere.2017.01.133
21. Gaur, R. Z., & Suthar, S. (2017). Nutrient scaling of duckweed (Spirodela polyrhiza) biomass in urban wastewater and its utility in anaerobic co-digestion. *Process Safety and Environmental Protection*, *107*, 138–146. https://doi.org/10.1016/j.psep.2017.02.005
22. Ge, X., Zhang, N., Phillips, G. C., & Xu, J. (2012). Growing Lemna minor in agricultural wastewater and converting the duckweed biomass to ethanol. *Bioresource Technology*, *124*, 485–488. https://doi.org/10.1016/J.BIORTECH.2012.08.050
23. Grobbelaar, J. U. (2009). Upper limits of photosynthetic productivity and problems of scaling. *Journal of Applied Phycology*, *21*(5), 519–522. https://doi.org/10.1007/s10811-008-9372-y
24. Hadiyanto, M., & Soetrisnanto, D. (2013). Phytoremediations of Palm Oil Mill Effluent (POME) by using aquatic plants and microalge for biomass production. *Journal of Environmental Science*. http://www.academia.edu/download/32836481/Publikasi_Marcel.pdf
25. Haga, H., & Ishikawa, K. (2011). Spatial distribution of submerged macrophytes in the southern basin of Lake Biwa in the summer of 2007, in comparison with that in 2002. *Japanese Journal of Limnology (Rikusuigaku Zasshi)*, *72*(1), 81–88. https://doi.org/10.3739/rikusui.72.81
26. Hoyos, N. L. M., Barroso_Junior, J. C., Leal, F. K., Barrantes, E. F. G., & Monteggia, L. O. (2019). Proposta de nova configuração de reator anaeróbio híbrido aplicado ao tratamento de esgoto sanitário. *Revista DAE*, *67*(219), 73–88. https://doi.org/10.4322/dae.2019.046
27. Kadir, A. A., Abdullah, S. R. S., Othman, B. A., Hasan, H. A., Othman, A. R., Imron, M. F., Ismail, N. 'Izzati, & Kurniawan, S. B. (2020). Dual function of Lemna minor and Azolla pinnata as phytoremediator for Palm Oil Mill Effluent and as feedstock. *Chemosphere*, *259*, 127468. https://doi.org/10.1016/J.CHEMOSPHERE.2020.127468
28. Khanal, S. K. (2011). *Anaerobic Biotechnology for Bioenergy Production: Principles and Applications* (S. K. Khanal, Ed.). John Wiley & Sons, Ltd. https://books.google.com.br/books?hl=pt-BR&lr=&id=1RbyLx-GkSMC&oi=fnd&pg=PT7&ots=oVO6oi9iOB&sig=pULJWLhHxUgZNdlHYGrikge5VsQ

29. Kist, D. L., Cano, R., Sapkaite, I., Pérez-Elvira, S. I., & Monteggia, L. O. (2020). Macrophytes as a Digestion Substrate. Assessment of a Sonication Pretreatment. *Waste and Biomass Valorization*, *11*(5), 1765–1775. https://doi.org/10.1007/s12649-018-0502-8
30. Koyama, M., Yamamoto, S., Ishikawa, K., Ban, S., & Toda, T. (2014). Anaerobic digestion of submerged macrophytes: Chemical composition and anaerobic digestibility. *Ecological Engineering*, *69*, 304–309. https://doi.org/10.1016/j.ecoleng.2014.05.013
31. Kumar, P., Hussain, A., & Dubey, S. K. (2016). Methane formation from food waste by anaerobic digestion. *Biomass Conversion and Biorefinery*, *6*(3), 271–280. https://doi.org/10.1007/s13399-015-0186-2
32. Lauterböck, B., Ortner, M., Haider, R., & Fuchs, W. (2012). Counteracting ammonia inhibition in anaerobic digestion by removal with a hollow fiber membrane contactor. *Water Research*, *46*(15), 4861–4869. https://doi.org/10.1016/j.watres.2012.05.022
33. Li, Q., Li, H., Wang, G., & Wang, X. (2017). Effects of loading rate and temperature on anaerobic co-digestion of food waste and waste activated sludge in a high frequency feeding system, looking in particular at stability and efficiency. *Bioresource Technology*, *237*, 231–239. https://doi.org/10.1016/j.biortech.2017.02.045
34. Li, Y., Chen, Y., & Wu, J. (2019). Enhancement of methane production in anaerobic digestion process: A review. *Applied Energy*, *240*(June 2018), 120–137. https://doi.org/10.1016/j.apenergy.2019.01.243
35. Mata-Alvarez, J., Macé, S., & Llabrés, P. (2000). Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. *Bioresource Technology*, *74*(1), 3–16. https://doi.org/10.1016/S0960-8524(00)00023-7
36. McLeod, J. D., Othman, M. Z., & Parthasarathy, R. (2019). Process intensification of anaerobic digestion: Influence on mixing and process performance. *Bioresource Technology*, *274*(December 2018), 533–540. https://doi.org/10.1016/j.biortech.2018.12.011
37. Metcalf, L., Eddy, H. P., & Tchobanoglous, G. (2004). Wastewater energy: treatment and reuse. In *New York [etc.]: McGraw-Hill* (4th ed.). McGraw-Hill.
38. Moorhead, K. K., & Nordstedt, R. A. (1993). Batch anaerobic digestion of water hyacinth: Effects of particle size, plant nitrogen content, and inoculum volume. *Bioresource Technology*, *44*(1), 71–76. https://doi.org/10.1016/0960-8524(93)90211-S
39. Muñoz Escobar, M., Voyevoda, M., Fühner, C., & Zehnsdorf, A. (2011). Potential uses of Elodea nuttallii-harvested biomass. *Energy, Sustainability and Society*, *1*(1), 4. https://doi.org/10.1186/2192-0567-1-4
40. Nahar, K., & Sunny, S. A. (2020). Duckweed-based clean energy production dynamics (ethanol and biogas) and phyto-remediation potential in Bangladesh. *Modeling Earth Systems and Environment*, *6*(1), 1–11. https://doi.org/10.1007/s40808-019-00659-y
41. Navarro, B. L., Challiol, A. Z., Bortoloti, M. A., Kuroda, E. K., & Fernandes, F. (2022). Methane production potential from anaerobic digestion of plant biomass residues and food waste as substrates. *Journal of Material Cycles and Waste Management*, *24*(6), 2470–2482. https://doi.org/10.1007/S10163-022-01496-5/TABLES/7
42. Nizami, A. S., Korres, N. E., & Murphy, J. D. (2009). Review of the integrated process for the production of grass biomethane. *Environmental Science and Technology*, *43*(22), 8496–8508. https://doi.org/10.1021/es901533j

43. O'Sullivan, C., Rounsefell, B., Grinham, A., Clarke, W., & Udy, J. (2010). Anaerobic digestion of harvested aquatic weeds: water hyacinth (Eichhornia crassipes), cabomba (Cabomba Caroliniana) and salvinia (Salvinia molesta). *Ecological Engineering*, *36*(10), 1459–1468. https://doi.org/10.1016/J.ECOLENG.2010.06.027
44. Oswald, W. J. (1988). Micro-algae and waste-water treatment. In L. J. B. (Eds.) M.A. Borowitzka (Ed.), *Micro-algae and waste-water treatment* (pp. 305–328). Micro-Algal Biotechnology, Cambridge University Press.
45. Ozengin, N., & Elmaci, A. (2007). Performance of Duckweed (Lemna minor L.) on different types of wastewater treatment. *Journal of Environmental Biology*.
46. Pena, L., Oliveira, M., Fragoso, R., & Duarte, E. (2017). Potential of duckweed for swine wastewater nutrient removal and biomass valorisation through anaerobic co-digestion. *Journal of Sustainable Development of Energy, Water and Environment Systems*, *5*(2), 127–138. https://doi.org/10.13044/j.sdewes.d5.0137
47. Powell, N., Shilton, A., Pratt, S., & Chisti, Y. (2011). Luxury uptake of phosphorus by microalgae in full-scale waste stabilisation ponds. *Water Science and Technology*, *63*(4). http://wst.iwaponline.com/content/63/4/704
48. Ren, H., Jiang, N., Wang, T., Omar, M. M., Ruan, W., & Ghafoor, A. (2018). Enhanced Biogas Production in the Duckweed Anaerobic Digestion Process. *Journal of Energy Resources Technology*, *140*(041805–1). https://doi.org/10.1115/1.4039782
49. Ripley, L. E., Boyle, W. C., & Converse, J. C. (1986). Improved alkalimetric monitoring for anaerobic digestion of high-strength wastes. *Water Pollution Contron Federation*, *58*(5), 406–411.
50. Shen, N., Wang, Q., Zhu, J., Qin, Y., Liao, S., Li, Y., Zhu, Q., Jin, Y., Du, L., & Huang, R. (2016). Succinic acid production from duckweed (Landoltia punctata) hydrolysate by batch fermentation of Actinobacillus succinogenes GXAS137. *Bioresource Technology*, *211*, 307–312. https://doi.org/10.1016/J.BIORTECH.2016.03.036
51. Toyama, T., Hanaoka, T., Tanaka, Y., Morikawa, M., & Mori, K. (2018). Comprehensive evaluation of nitrogen removal rate and biomass, ethanol, and methane production yields by combination of four major duckweeds and three types of wastewater effluent. *Bioresource Technology*, *250*(September 2017), 464–473. https://doi.org/10.1016/j.biortech.2017.11.054
52. Vassilev, S. v., Vassileva, C. G., & Vassilev, V. S. (2015). Advantages and disadvantages of composition and properties of biomass in comparison with coal: An overview. *Fuel*, *158*, 330–350. https://doi.org/10.1016/J.FUEL.2015.05.050
53. Verma, R., & Suthar, S. (2015). Utility of Duckweeds as Source of Biomass Energy: a Review. In *Bioenergy Research* (Vol. 8, Issue 4, pp. 1589–1597). Springer New York LLC. https://doi.org/10.1007/s12155-015-9639-5
54. Verma, R., & Suthar, S. (2016). Bioenergy potential of duckweed (Lemna gibba L.) biomass. *Energy Sources, Part A: Recovery, Utilization, and Environmental Effects*, *38*(15), 2231–2237. https://doi.org/10.1080/15567036.2015.1098750
55. Xu, J., & Deshusses, M. A. (2015). Fermentation of swine wastewater-derived duckweed for biohydrogen production. *International Journal of Hydrogen Energy*, *40*(22), 7028–7036. https://doi.org/10.1016/J.IJHYDENE.2015.03.166

56. Yadav, D., Barbora, L., Bora, D., Mitra, S., Rangan, L., & Mahanta, P. (2017). An assessment of duckweed as a potential lignocellulosic feedstock for biogas production. *International Biodeterioration and Biodegradation*, *119*, 253–259. https://doi.org/10.1016/j.ibiod.2016.09.007
57. Zhou, Y., Stepanenko, A., Kishchenko, O., Xu, J., & Borisjuk, N. (2023). Duckweeds for Phytoremediation of Polluted Water. *Plants*, *12*(3), 589. https://doi.org/10.3390/PLANTS12030589/S1

Figures

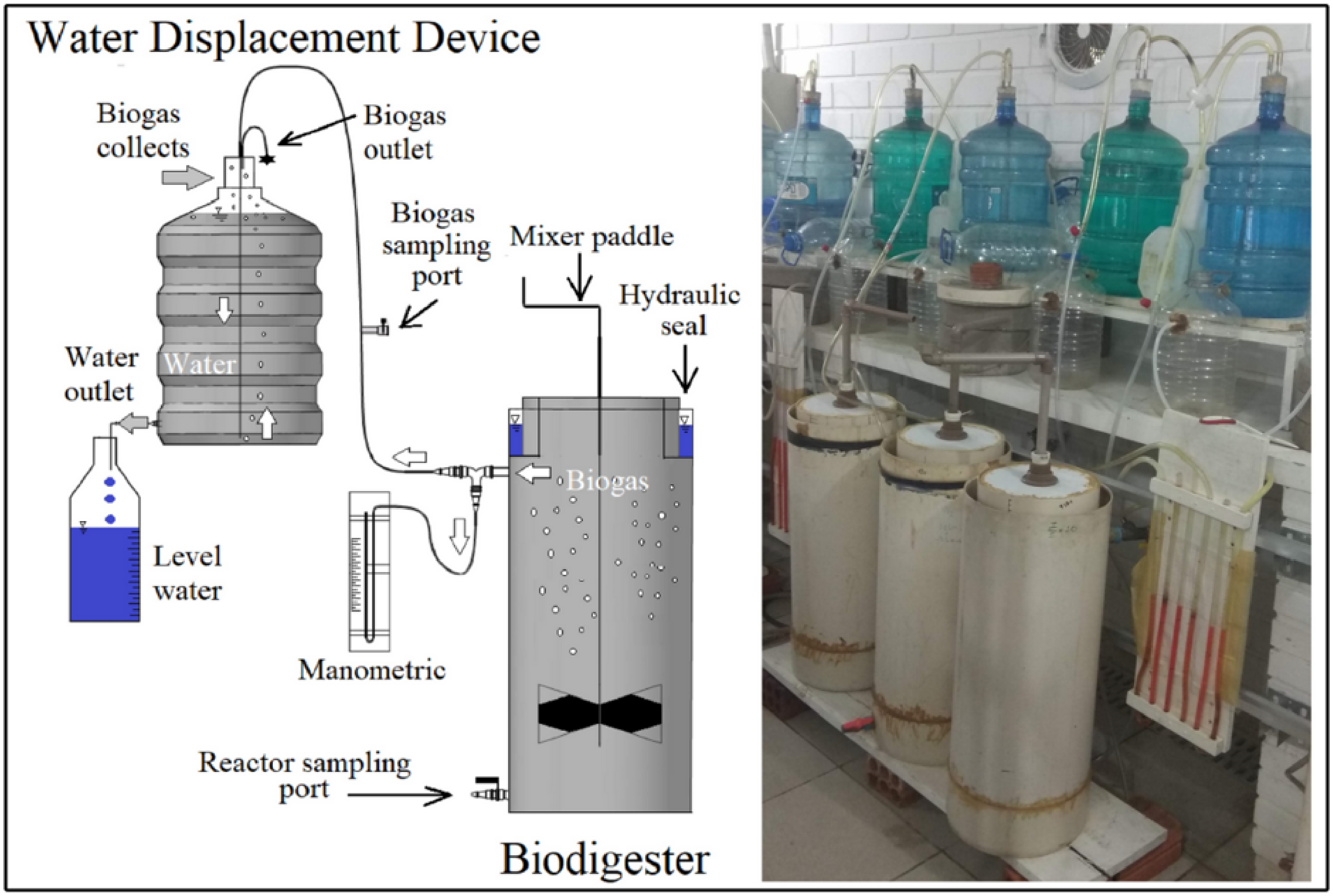

Figure 1

Biodigester and its components and biogas measurement system.

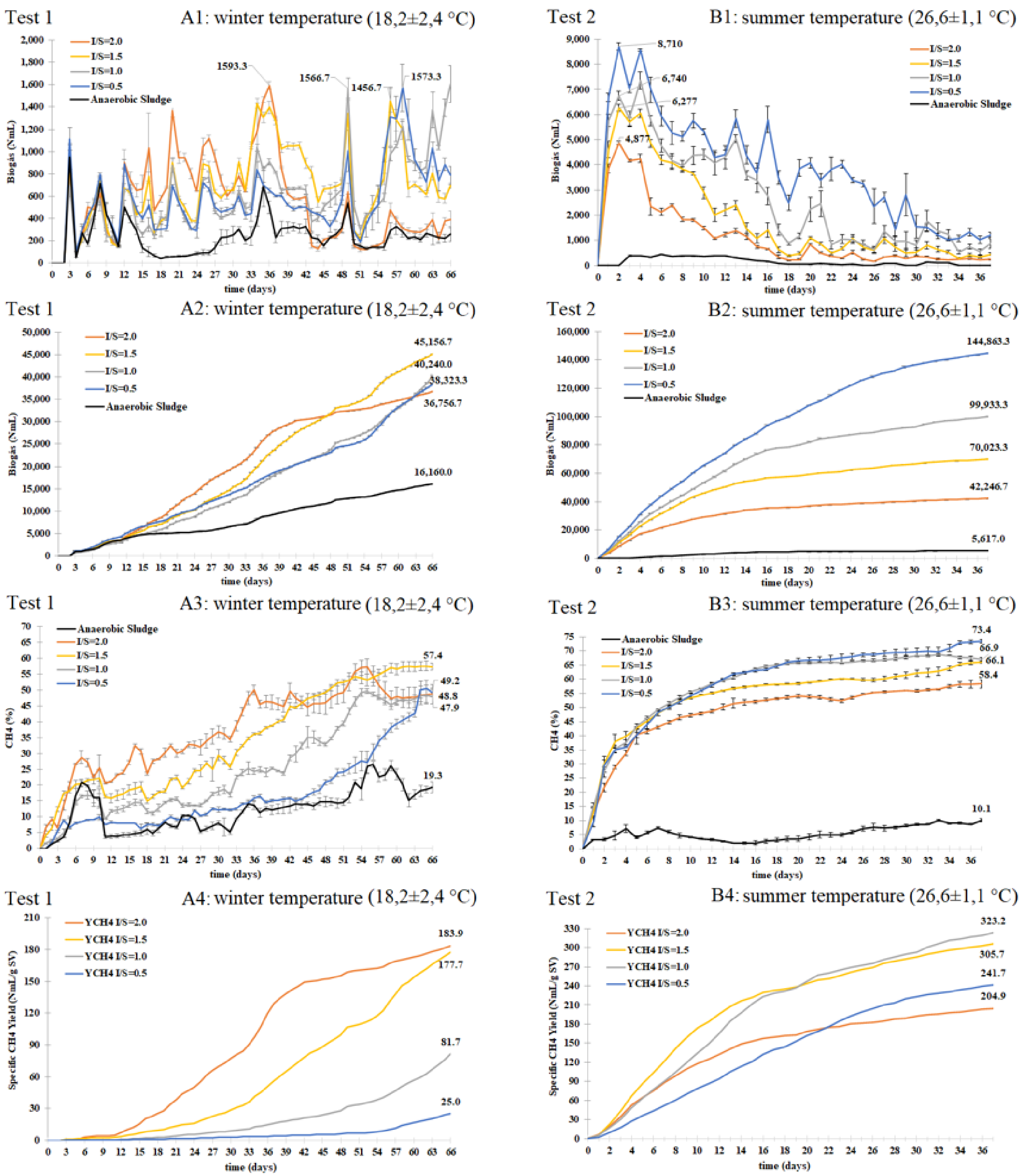

Figure 2

A - Test 1 (winter) - Daily biogas production (A1), accumulated biogas production (A2), methane content (A3) and specific methane yield (A4) and B - Test 2 (summer) - Daily biogas production (B1), accumulated biogas production (B2), methane content (B3) and specific methane yield (B4).

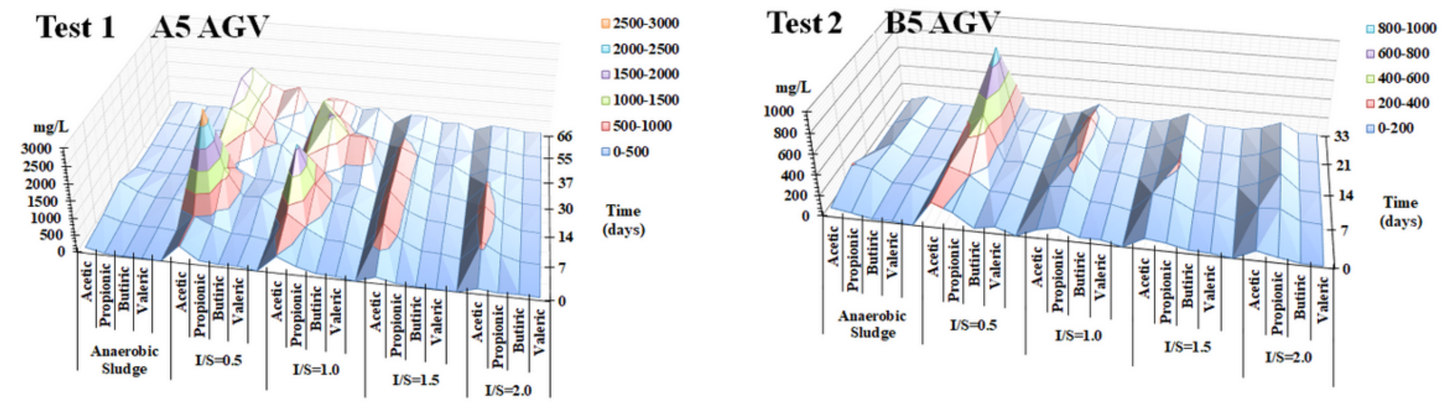

Figure 3

VFA production about time for the winter condition (A5) and the summer condition (B5).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.docx