Heliyon 9 (2023) e12537

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Biogas from aquatic plants: A bioenergetics incentive for constructed wetlands usage

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ARTICLE INFO

Keywords:
BMP
Biomethane
Aquatic plants
Kinetic analysis

ABSTRACT

Our study demonstrated the energy gains when using biomass from three macrophyte, used commonly in constructed wetlands for wastewater treatment, the water hyacinth, cattail, and dwarf papyrus, as a substrate for biogas generation. The biochemical methane potential for the three biomass was evaluated in batch and at bench at 37 °C. A kinetic analysis of anaerobic digestion was also conducted for these substrates, evaluating the biogas composition and energy potential. Anaerobic digestion resulted in 94.27, and 25 $mL_{CH4}/gVS_{substrate}$ of dry mass; and 19,569.65, 5617.88, and 6068.45 kJ/t of cattail, water hyacinth, and dwarf papyrus, respectively. Biomass from water hyacinth did sustain the fastest degradation, indicating that models considering the lag phase are more adequate to evaluate the anaerobic digestion of this type of substrate. Higher digestion speed resulted in the generation of 2901.88 kJ/t more energy with biomass from water hyacinth versus cattail, highlighting its value for use in constructed wetlands.

1. Introduction

Constructed wetlands (CW) reproduce flooded ecosystems [1,2] where aquatic plants – the macrophytes – grow, such as cattail, water hyacinth, water lily, and water lettuce. In CWs, when wastewater flows through the roots, it is treated by the action of plants and microorganisms in the root area and the support medium [3–8]. Macrophytes store pollutants in their plant tissue [3,4,9,10], returning them to the medium at senescence, requiring regular cutting [1,2,4,7,10,11] to maintain the quality of the CW effluent [5,8] and is need a friendly environmental disposal for the residues.

Macrophyte productivity ranges from 5 to 60 kg.m-2 [5,11,12]. As a result, cutting generates a large volume of residues for disposal [13], which can be a disadvantage and discourage the use of CW [2,14,15].

Nonetheless, the residues from the cuts of macrophytes can be treated by anaerobic digestion (AD), a suitable process that converts organic matter into biogas ([19] [1,16–18]; and the digested solids can be used as a biofertilizer. Mainly constituted of methane, biogas can replace natural gas and generate heat and electricity [1,2,19]; [16]; [20,21].

Lignin is a compound that inhibits AD [22]; [43]) and macrophytes, as they have low level of this compound [1,23,24], represent an alternative substrate for biogas production [25,26]. The literature mainly shows water hyacinth (Eicchornia crassipes) as a

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https://doi.org/10.1016/j.heliyon.2022.e12537
Received 8 July 2022; Received in revised form 28 October 2022; Accepted 14 December 2022

substrate, reporting up to 400 L_{biogas}. kg^{-1} made of plant [16,18,23–30]. [31] estimated that one ton of macrophyte and seaweed mixes could generate up to 3900 kWh. The organic matter composition as sugar concentrations varies between the different plants that can be used in CW and thus the production of biogas and methane levels are distinct.

Therefore, it is possible to use residual plant biomass from CW to produce biogas and biofertilizer, including CW in the context of the circular economy [25,26,28,32]. Residues of macrophytes can serve as a substrate for local methane production and this used in heating systems, for example, on a rural property or generate electricity. This proposal would encourage the use of CW and, consequently, effluent treatment, generation and use of renewable energy, uptake of atmospheric carbon by macrophytes, mitigation of the greenhouse effect, aesthetic improvement of wastewater treatment plants due to vegetation, etc. Considering the above, this study analyzed three macrophytes in terms of potential bioenergy production by anaerobic digestion.

2. Materials and methods

2.1. Substrate: biomass from macrophytes

The biochemical methane potential (BMP) of biomass from macrophytes cattail (Typha dominguensis), water hyacinth (Eicchornia crassipes), and dwarf papyrus (Cyperus papyrus Nanus) was assessed. Cattail grew in wetlands, at the Educational Unit for the Production of Cattle Dairy from the Federal Institute of Education, Science and Technology of the South of Minas Gerais, Campus Inconfidentes, Brazil – only the leaves were used. Water hyacinth was harvested at the Salto Grande Reservoir, in Americana, São Paulo, Brazil, and its root and leaves were used. Dwarf papyrus was grown in a CW performing tertiary treatment of sewage from the School of Agricultural Engineering at UNICAMP, and only its leaves were used in this study.

Biomass were first dehydrated in the sun and then in an oven with air circulation (65 ± 2 °C) until constant weight; then they were stored at room temperature. They were ground in (i) a grinding mill and (ii) a grinding mill followed by domestic food processor, resulting in coarse (C) and fine (F) granulometry, respectively. The granulometric composition was determined by sieving [33].

Biomass were characterized (Table 1), with determination of volatile solids (VS) in g.kg^{-1} [34]; cellulose, hemicellulose, and lignin in percentage [35,36]; and nitrogen in percentage [37].

2.2. Inoculum: anaerobic reactor sludge

The inoculum was obtained from sludge of a compartmented reactor used in dairy cattle effluent treatment [38]. Before the tests, sludge was adapted with fine biomass for each macrophyte, at 37 °C, substrate/inoculum ratio 0.1 gVSsubs/gVSinoc, and supplemented with nutrient medium [39]. After adaptation, inoculum were characterized in terms of total and volatile solids and showed the concentrations 43.08 and 30.36 g.L-1, 48.67 and 32.63 g.L-1, and 56.80 and 35.69 g.L-1 TS and VS in the experiments with cattail, water hyacinth, and dwarf papyrus, respectively. The amount of biogas generated by the inoculum (control reactors, without substrate) was subtracted from the amount of biogas produced from biomass [40]; [41]).

2.3. Biochemical methane potential (BMP) tests

The BMP tests were conducted according to the VDI 4630 test standard [40,41], observing the influence of (i) granulometry and (ii) inoculum to substrate (based on VS) ratio (φ, in gVS_{subs}/gVS_{inoc}). Microcrystalline cellulose was the positive control substrate [41] with φ 0.5 gVS_{subs}/gVS_{inoc}, considering 5% humidity.

Schott DURAN borosilicate glass bottles capacity 1 L were used as batch reactors, with reactional volume of 400 mL Batch fermentation tests were conducted as double determinations inside a water batch provided of an electric resistance, controlled at

Table 1
Characterization of several macrophytes as substrate of anaerobic digestion.

Species	Popular name	VS	Lignin	Cellulose	Hemicellulose	Nitrogen	Author
		(g/kg)	(%)				
Typha dominguensis	Cattail	**758 ± 38**[a]	**12.4 ± 2.1**	**42.9 ± 1.7**	**9.5 ± 0.5**	**2.23**	**Our study.**
Cyperus papyrus Nanus	Dwarf papyrus	**843.521 ± 0.004**[a]	**16.7 ± 0.4**	**41.8 ± 1.5**	**11.6 ± 0.3**	**2.19**	**Our study.**
Eichhornia crassipes	Water hyacinth	**751 ± 6***	**7.5 ± 0.9**	**43.01 ± 0.1**	**12.3 ± 0.5**	**1.79**	**Our study.**
		*	12.12	21.63	25.94	1.16	[28]
		700–850	23.3	38.1	30.2	3.15 (N_{org})	[26]
		795	7.72	35.0	24.8	2.13	[25]
		64.1	18.9	*	*	*	[16]
Azolla microphylla	Water fern	720	10.13	27.4	15.67	2.8	[25]
Egeria densa	–	800	6.33	39.54	7.81	*	[47]
Lemna minor	Water lentils	770	4.1	28.8	22.3	2.53	[25]
Oxycarium cubense	–	*	25.20	35.6	14.8	8.56	[28]
Pistia stratiotes	Water lettuce	*	15.70	16.47	16.91	1.48	[28]
Salvinia	–	*	13.70	29.16	10.16	1.05	[28]

[a] Dry matter; *Not available.

37 ± 1 °C [41]. The reactors were manually shaken at least once a day [41] and prior to daily measurements of biogas volume produced. The volume was measured by liquid displacement in eudiometers filled with water acidified to a pH between 2.5 and 3.5 with 0.036 mol L^{-1} H_2SO_4, and 0.5 g L^{-1} methyl orange to prevent CO_2 absorption. The tests ended when the daily production was less than 1% of the total accumulated volume [41]. Silicone hoses connected reactors and eudiometers, and silicone stoppers were used to seal the reactors.

Biogas yield (Y, in $mL_{biogas}/gVS_{substrate}$) was evaluated by dividing the produced volume (mL) and using normal temperature and pressure (NTP) correction (273 K and 1.013 hPa) by dry mass (g) of added VS. To evaluate VS reduction [34] multi-factor analysis of variance (ANOVA) with 95% confidence was used in Statgraphics Centurion software. Factors included particle size – coarse (C) and fine (F) – and substrate/inoculum ratio (0.25; 0.5; 1.0 gVS_{subs}/gVS_{inoc}). According to the standard indicated by the authors (VDI 4630).

For the kinetic analysis, two first-order models were compared, and the resulting difference is in lag phase weighting (1st order w/ Lp – [42]; [17]) or considering it as zero (1st order w/o Lp – [17,43], illustrated in Equations (1) and (2), respectively. Modified Gompertz model was also evaluated (Equation (3)) ([22]; [17,44,45].

$$Y(t) = Y_{max}\left(1 - exp^{-k\left(t - L_p\right)}\right) \quad 1$$

$$Y(t) = Y_{max}\left(1 - exp^{-kt}\right) \quad 2$$

$$Y(t) = Y_{max}\ exp^{-exp\left[\left(\frac{R_m\ \ exp}{Y_{max}}\right)\left(L_p - t\right)\right]+1} \quad 3$$

In equations (1)–(1)–(3), Y(t) is the biogas yield (mL/gVS); $Ymax^{\#}_{biogas}$ is the maximum biogas yield ($mL_{biogas}/gVS_{inoculum}$); $k^{\#}$ is the hydrolysis constant (day^{-1}); t: time (day); $Lp^{\#}$: lag phase (day); $Rm^{\#}$: max. rate of biogas production ($mL.day^{-1}$). The parameters marked with $^{\#}$ were estimated by nonlinear regression in Statgraphics Centurion.

To monitor the concentrations of CH_4 and CO_2 in biogas, Construmaq UL-13 gas chromatograph was used, equipped with a thermal conductivity detector; stainless steel column 3 m long, 1/8" diameter, equipped with Hayesep D 80–100 mesh for CO_2 separation; and hydrogen gas at 40 ± 2 mL/min as the mobile phase. Biogas was analyzed at room temperature (25 ± 2 °C); the amount of 2–5 mL was injected, and 4 to 6 injections were performed. Data were the arithmetic means between the different injections, excluding outliers with 95% confidence.

Chromatography generated values in mmolCH4/Lbiogas and we considered 22.413 L/mol at NTP as the molar volume to obtain the methane percentage in biogas and the BMP in mLCH4/gVSsubstrate. For the energy potential, we used 50.0 MJ.kg-1 as the lower heating value of methane ([46] [21]; and 0.72 kg.m-3 density [46].

3. Results and discussion

Here, φ refers to the substrate/inoculum ratio, and F and C refers to the granulometric composition: fine and coarse, respectively. The value of φ was followed by F or C. For example, 0.25-F and 1.00-C indicate 0.25 gVS_{subs}/gVS_{inoc} and fine granulometry, and 1.00 gVS_{subs}/gVS_{inoc} and coarse granulometry, respectively.

3.1. *Characterization of macrophyte biomass*

After being submitted to two grinding methods, cattail presented the most expressive differences in granulometry. More than 60% of the particles were larger than 2.38 mm (coarse granulometry) and more than 60% of the particles were smaller than 0.6 mm (fine granulometry), and about 30% of them were 1.19 mm. For water hyacinth, around 70% of the particles were smaller than 0.6 mm and 80% were smaller than 0.6 mm in coarse and fine granulometry, respectively. For dwarf papyrus, about 70% of the particles were smaller than 0.6 mm and 24% of the particles were retained in a 1.19 mm sieve in coarse granulometry; in fine granulometry, almost 85% of the particles were smaller than 1.19 mm and 12% of the particles were retained in the sieve.

Biomass from cattail, water hyacinth, and dwarf papyrus presented 758, 751, and 843 gVS.kg-1 of dry matter; and 2.23%, 1.79%, and 2.19% nitrogen, respectively and these values are consistent with the literature, as shown in Table 1. These low levels of nitrogen combined with the organic content demonstrate the possibility of converting these substrates into biogas [25].

The content of structural carbohydrates also indicates residues from macrophytes as an interesting raw material to produce biofuels [25]: cellulose 42–43%, lignin 7–17%, and hemicellulose 9–13% (Table 1). Low levels of lignin are beneficial to AD because it resists breakdown by enzymatic hydrolysis, inhibits microbial access to other carbohydrates, and delays the production of biogas [16,28]. Water hyacinth and *Egeria densa* [47] contain the lowest level of lignin, possibly due to its small aerial part, which does not require support. On the other hand, cattail and dwarf papyrus have long aerial parts, requiring a more rigid and resistant structure.

According to dates shown in Table 1 the origin and growth medium of the macrophyte influence its composition [25]; [16]), highlighting the importance of investigating previously the potential for biogas production from macrophytes residues in order to ensure safe real-scale application.

Table 2

Reduction of volatile solids and kinetic parameters of anaerobic digestion of substrates.

Sample[a]	Reduction of VS (%)	$Ymax_{biogas}$b(mL/gVS)	1st order w/o Lp			1st order w/Lp				Modified Gompertz model			
			Ymax (mL/gVS)	k ($days^{-1}$)	R^2 (%)	Ymax (mL/gVS)	k ($days^{-1}$)	Lp (days)	R^2 (%)	Ymax (mL/gVS)	Rm (mL/day)	Lp (days)	R^2 (%)
CATTAILrowhead													
0.25-F	21.23 ± 5.13	189 ± 2	181 ± 2	0.200 ± 0.010	95.90	186 ± 2	0.155 ± 0.007	- 0.90 ± 0.10	98.23	183 ± 2	15.1 ± 0.8	- 2.2 ± 0.3	97.14
0.25-C	16.82 ± 0.19	137 ± 40	131 ± 1	0.226 ± 0.009	97.09	133 ± 1	0.186 ± 0.007	- 0.64 ± 0.09	98.71	131 ± 1	13.5 ± 0.6	- 1.6 ± 0.2	97.88
0.50-F	21.77 ± 1.94	207 ± 30	200 ± 2	0.162 ± 0.007	97.17	206 ± 2	0.128 ± 0.004	- 0.90 ± 0.10	99.00	201 ± 2	14.3 ± 0.6	- 2.4 ± 0.3	97.52
0.50-C	31.13 ± 1.12	179 ± 8	174 ± 2	0.166 ± 0.007	96.77	179 ± 2	0.129 ± 0.004	- 1.00 ± 0.10	99.04	175 ± 2	12.4 ± 0.5	- 2.4 ± 0.3	97.96
1.00-F	6.44 ± 0.01	190 ± 10	187 ± 2	0.130 ± 0.004	98.32	192 ± 2	0.109 ± 0.003	- 0.77 ± 0.09	99.31	185 ± 2	11.8 ± 0.5	- 2.1 ± 0.3	97.89
1.00-C	26.94 ± 2.52	199 ± 2	194 ± 2	0.136 ± 0.005	98.34	199 ± 2	0.114 ± 0.003	- 0.77 ± 0.08	99.40	193 ± 2	12.8 ± 0.5	- 2.0 ± 0.3	98.24
WATER HYACINTHrowhead													
0.25-F	14.63 ± 1.10	117.0 ± 20	120 ± 3	0.33 ± 0.02	97.97	119 ± 3	0.35 ± 0.03	0.12 ± 0.07	98.20	114.0 ± 1.0	30 ± 1	0.28 ± 0.07	99.55
0.25-C	16.65 ± 10.39	93.0 ± 0.7	90 ± 3	0.38 ± 0.04	94.81	90 ± 3	0.41 ± 0.05	0.16 ± 0.09	95.33	87.0 ± 1.0	27 ± 2	0.50 ± 0.10	98.36
0.50-F	35.64 ± 1.12	94.0 ± 8.0	96 ± 2	0.49 ± 0.04	97.38	95 ± 2	0.53 ± 0.04	0.13 ± 0.05	97.92	93.1 ± 0.5	38 ± 1	0.35 ± 0.04	99.79
0.50-C	27.47 ± 0.47	108.0 ± 3.0	109 ± 2	0.38 ± 0.02	98.38	108 ± 2	0.41 ± 0.03	0.12 ± 0.05	98.67	104.2 ± 0.7	32 ± 1	0.28 ± 0.05	99.69
1.00-F	24.13 ± 11.48	102.3 ± 0.4	99 ± 2	0.40 ± 0.02	98.61	98 ± 1	0.44 ± 0.02	0.12 ± 0.04	98.94	94.7 ± 1.0	30 ± 2	0.22 ± 0.09	99.00
1.00-C	30.19 ± 0.58	102.0 ± 6.0	105 ± 2	0.40 ± 0.03	97.79	104 ± 2	0.44 ± 0.03	0.16 ± 0.05	98.34	100.6 ± 0.7	34 ± 1	0.36 ± 0.05	99.69
DWARF PAPYRUSrowhead													
0.25-F	12.13 ± 9.07	107.0 ± 40	103 ± 6	0.13 ± 0.02	85.97	110 ± 8	0.090 ± 0.020	- 1.60 ± 0.60	91.15	105 ± 5	6.0 ± 0.7	- 2.0 ± 1.0	92.03
0.25-C	27.42 ± 1.40	122.2 ± 0.6	126 ± 9	0.13 ± 0.02	81.63	142 ± 13	0.070 ± 0.020	- 2.50 ± 0.80	91.02	131 ± 7	6.4 ± 0.8	- 4.0 ± 1.0	91.84
0.50-F	28.67 ± 6.12	100 ± 14	92 ± 3	0.22 ± 0.02	93.37	96 ± 3	0.160 ± 0.020	- 0.90 ± 0.30	96.29	94 ± 3	8.3 ± 0.8	- 2.0 ± 0.5	95.44
0.50-C	20.52 ± 9.43	199 ± 60	208 ± 8	0.13 ± 0.01	96.60	207 ± 8	0.130 ± 0.010	0.20 ± 0.20	96.67	195 ± 4	20.0 ± 1.0	0.6 ± 0.3	98.42
1.00-F	35.56 ± 3.19	196 ± 96	185 ± 3	0.18 ± 0.08	98.97	187 ± 3	0.165 ± 0.008	- 0.30 ± 0.10	99.22	180 ± 3	18.0 ± 1.0	- 0.8 ± 0.3	98.15
1.00-C	32.96 ± 2.51	131 ± 10	126 ± 2	0.15 ± 0.05	99.38	127 ± 2	0.140 ± 0.006	- 0.17 ± 0.08	99.47	121 ± 2	11.2 ± 0.6	- 0.6 ± 0.3	98.28
Cellulose	41.64 ± 19.63	260.0 ± 10	272 ± 10	0.15 ± 0.02	96.06	267 ± 8	0.170 ± 0.020	0.50 ± 0.10	97.15	253 ± 3	36.3 ± 2.0	1.3 ± 0.1	99.45

Substrate/inoculum ratio (gVS_{subs}/gVS_{inoc})-granulometry (fine or coarse); [b]value measured experimentally ($mL_{biogas}/gVS_{inoculum}$).

3.2. Removal of volatile solids

In the experiment with cattail, granulometry had a more significant effect (p-value 0.0074) than φ (p-value 0.0577) in VS removal (95% confidence). The highest degradation (31.1%) of cattail was observed at φ 0.50 gVS_{subs}/gVS_{inoc} and with fine granulometry (Table 2). Lower consumption of organic matter at 1.00-C may have occurred due to difficult homogenization and contact between inoculum and substrate caused by excess of larger pieces.

AD of biomass from water hyacinth reached 35.64% VS reduction to 0.50-C. However, none of the factors had a more pronounced effect on the result (p-value 0.7792 and 0.0517, respectively, for granulometry and φ). Like cattail, a more balanced ratio between substrate and inoculum favored the removal of VS.

This result is consistent with the rate of 36.8% reported by Ref. [18] obtained with *E. crassipes* digested at 1.5 substrate/inoculum ratio. However, higher values have been reported: 81% and 65% [16,26]; respectively). This difference in results shows that the macrophyte origin and the AD methodology influence the VS removal.

Digestion of dwarf papyrus reached 35.56% VS reduction to 1.00-C. It was the most suitable treatment condition for this residue. Like water hyacinth, no significant influence was observed from one of the factors (p-value 0.6902 for granulometry and 0.2036 for φ).

“Digestion of microcrystalline cellulose (Table 2) consumed 41.64% of VS, lower than the value of 75% reported by Ref. [16]. Nevertheless, was demonstrated the inoculum was viable for AD and the difference between the consume of VS may be due to the distinct origins of the macrophytes used by the authors.”

For water hyacinth and dwarf papyrus, the highest VS removal was observed with coarse granulometry. Smaller particles tend to

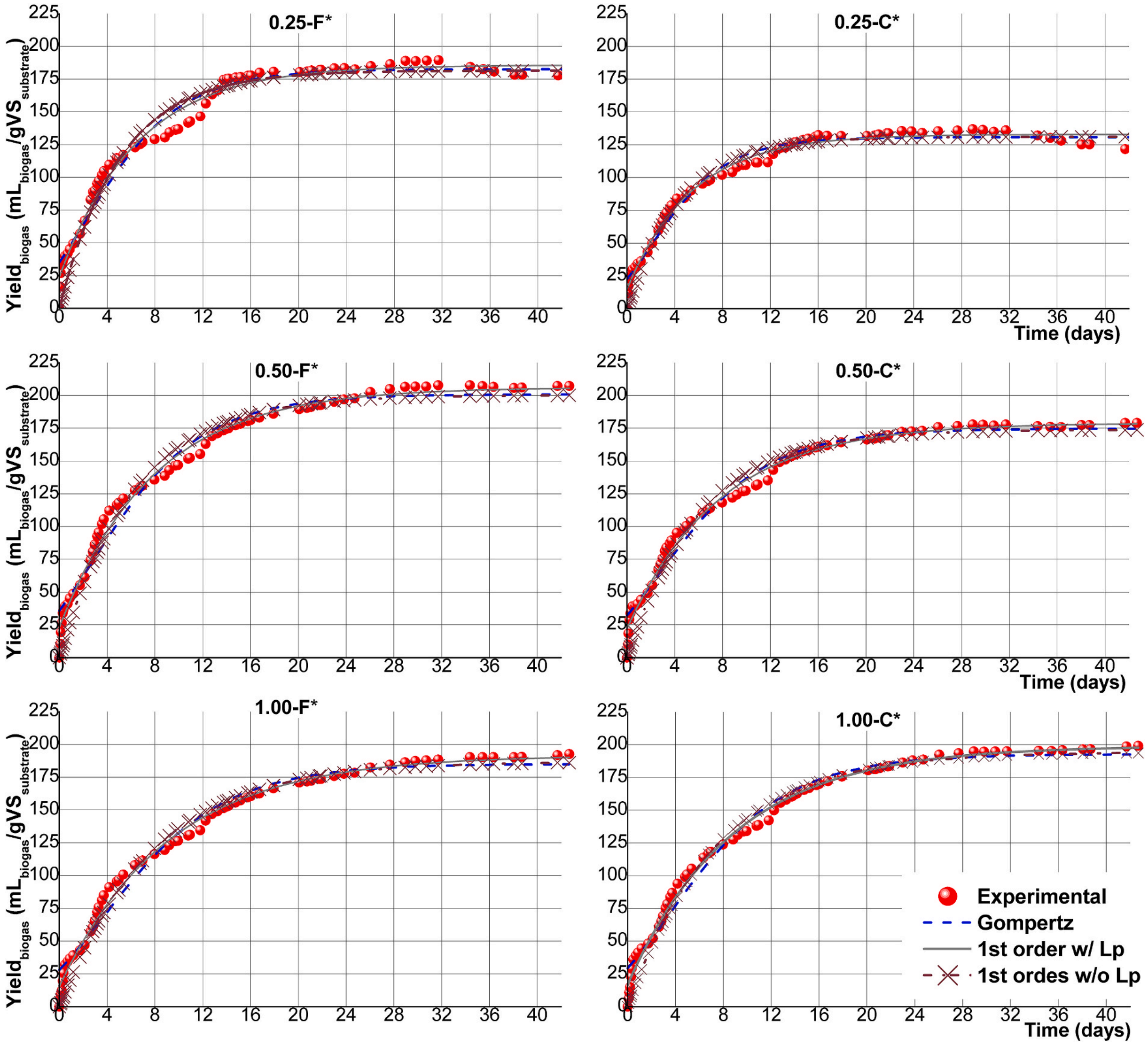

Fig. 1. Biogas yield (experimental and kinetic models) from cattail anaerobic digestion.

benefit AD by increasing the surface area for hydrolysis. However, this process can accelerate the production and accumulation of inhibitory intermediate compounds, justifying a lower performance. Then, from the perspective of VS reduction, there is no justification for incurring expenses with additional grinding process.

3.3. Kinetic analysis

The absence of lag phase (Figs. 1–3) indicates the microbiota was in an environment that favored degradation [20] and adapted to the substrate, so null Lp considered is coherent. However, the “1st order w/Lp” model presented the best fit (R^2) and the discussion will be based on it.

The literature [22,43–45,48] does not mention negative Lp in most cases (Table 2); it means the instantaneously consumable (limiting) substrate is not sufficient to support the excessive amount of biomass at the initial period of digestion, besides confirming inoculum adaptation.

For water hyacinth and dwarf papyrus 0.50-C, Lp was less than 4 h, shorter compared to 16 and 32 days of testing, respectively. It emphasizes the inoculum activity, especially when compared to 3 out of 35 total days of AD of water hyacinth, as reported by Ref. [18]. The positive control showed Lp of 0.5 and 1.3 days for the first order and modified Gompertz, respectively. Although shorter than 5–7 days [17], such longer time is reasonable because the inoculum was not adapted to microcrystalline cellulose.

Each model presented the best fit to a substrate, but both properly estimated the maximum biogas yield (Y_{max}). For water hyacinth and microcrystalline cellulose, Gompertz showed R^2 of 98.4%–99.8% (Table 2). In turn, “first order w/Lp” better modeled AD of cattail, with R^2 of 98.2%–99.4%. The advantage of modified Gompertz, as it is based on a sigmoidal curve, was that it followed the

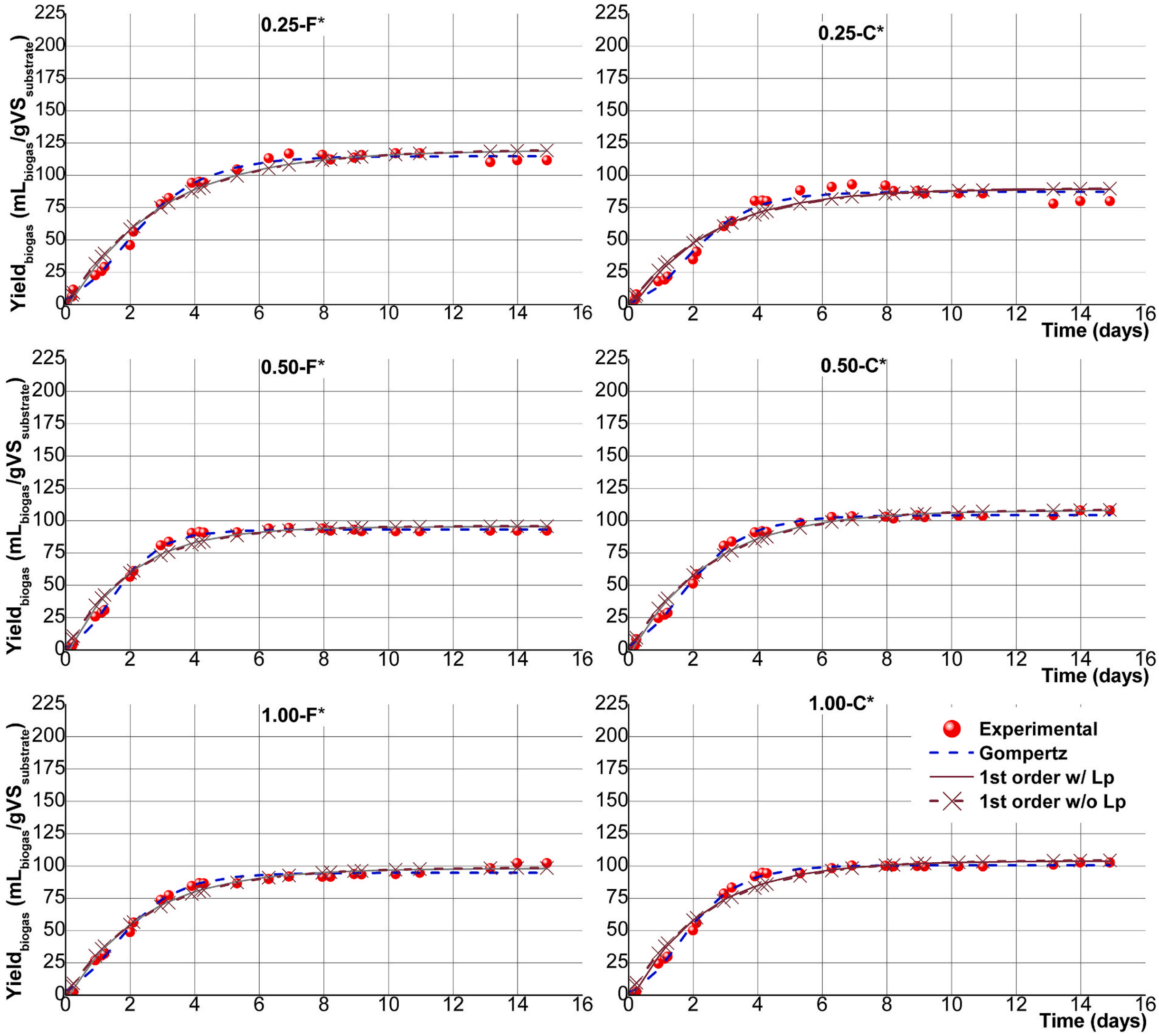

Fig. 2. Biogas yield (experimental and kinetic models) from water hyacinth anaerobic digestion.

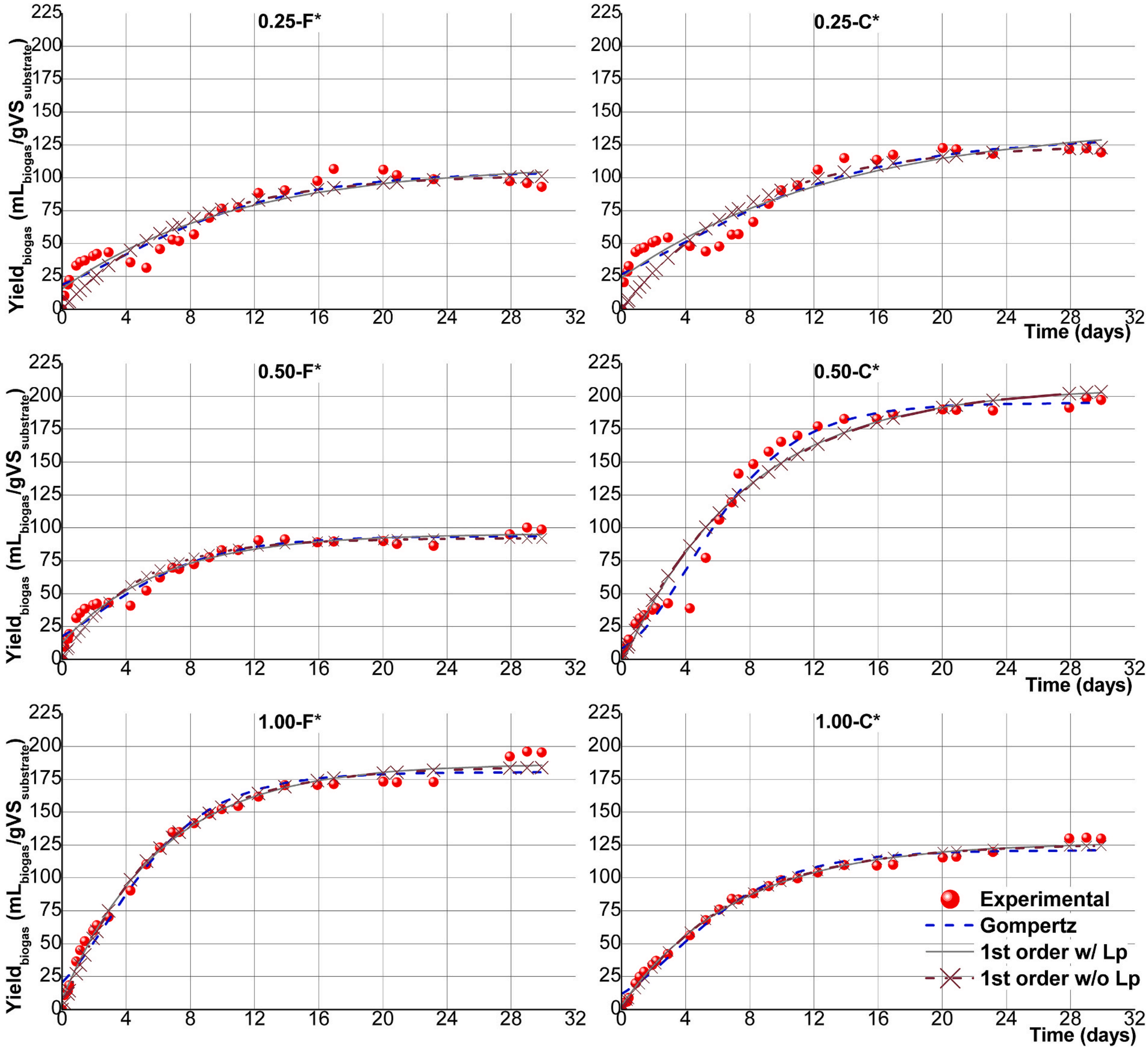

Fig. 3. Biogas yield (experimental and kinetic models) from dwarf papyrus anaerobic digestion.

decline in biogas production at day 5 (Fig. 3) of dwarf papyrus AD. These observations show the model must consider the lag phase and that the most appropriate model is conditioned to the substrate.

Water hyacinth showed the highest degradation rate, k between 0.35 and 0.53 day^{-1}, which was expected due to its lower level of lignin () when compared to cattail and dwarf papyrus: 7.5, 12, and 17%, respectively. This degradation rate contributed to the lowest total AD time.

The lowest value of k was observed in dwarf papyrus AD at 0.25 gVS$_{subs}$/gVS$_{inoc}$, 0.07 and 0.09 d^{-1}, while for the other cases, it was 0.109–0.186 d^{-1} for cattail, and 0.130 to 0.165 day^{-1} for dwarf papyrus. Both cattail and dwarf papyrus presented values of k close to microcrystalline cellulose: 0.170 d^{-1}.

Floating macrophyte *Egeria densa* presented k of 0.27 d–1 [49]; an intermediate value when compared to the plants analyzed in this study, but similar to the value obtained for water hyacinth. Its low lignin content (6.33%, Table 1) reinforces its influence on the hydrolysis rate. In summary, biomass from cattail was the substrate with the most difficult degradation, followed by dwarf papyrus and water hyacinth, which had an impact on the total AD time.

For water hyacinth, Rm was 27 ± 2 to 38 ± 1 mL d^{-1}, which highlights its easy degradation, but does not cause higher gross biogas production, probably due to the VS content. For cattail, Rm was between 11.8 ± 0.5 and 15.1 ± 0.8 mL.day^{-1}. Dwarf papyrus was less homogeneous, with 0.25-F, 0.25-C and 0.50-F presenting Rm values between 6 and 8 mL.day^{-1}; while 0.50-C, 1.00-F and 1.00-C presented Rm values of 11–20 mL.day^{-1}.

Considering the above, both models were appropriate, as they predicted different and equally important parameters. The parameters were influenced by the substrate, but not with the substrate/inoculum ratio or particle size.

3.4. Biogas yield

The substrate-inoculum balance, 0.50 gVSsubs/gVSinoc ratio, presented highest amounts of biogas (Table 2) and it is recommended for better buffer and prevention of biodegradation inhibition [20]; [41]). In general, smaller particles favored the maximum yield of biogas (Ymax).

In AD of cattail biomass, fine particles benefited φ 0.25 and 0.50 gVS_{subs}/gVS_{inoc}, presenting 189 versus 137 mL_{biogas}/gVS_{subs} and 207 versus 179 $mL_{biogás}/gVS_{subs}$ in fine and coarse granulometry, respectively (Table 2). With similar results at 1.00-F and 1.00-C, 190 ± 10 versus 199 ± 2 mL_{biogas}/gVS_{subs}, respectively, the higher energy consumption in fine grinding did not result in higher Y_{max}.

Water hyacinth 0.25-F showed the most promising yield, 117 mLbiogas/gVSsubs (Table 2). However, this condition changed for 0.50-F and 0.50-C: 94 and 108 mLbiogas/gVSsubs, respectively; and 102 mLbiogas/gVSsubs for 1.00 gVSsubs/gVSinoc in fine and coarse granulometry. The literature reports higher values for water hyacinth, ranging from 150 [18] to 398 mLbiogas/gVSsubs [26]. It shows variation according to the macrophyte origin and AD methodology, requiring more information about this topic. In addition to a higher degradation rate, the speed of AD stabilization (Fig. 2) and lower biogas yield demonstrated an influence of its composition, with lower lignin and VS contents.

Dwarf papyrus-inoculum balance also benefited AD, but C yielded more at 0.25 and 0.50 gVSsubs/gVSinoc: 122 and 199 mLbiogas/gVSsubs, respectively. A hypothesis to be tested refers to the accumulation of acids and system intoxication [43], considering the decline at day 4 (Fig. 3). At 1.00-F, 196 mLbiogas/gVSsubs was obtained versus 131 mLbiogas/gVSsubs at 1.00-C (Table 2). Dwarf papyrus and cattail generated more biogas than 66 and 144 mLbiogas/gVS obtained from *Phragmites australis*, another emergent aquatic plant with long stems [1].

3.5. Biogas composition and biochemical methane potential

The quality of biogas is discussed in terms of the percentage of methane (Table 3). In general, higher amounts of substrate resulted in more CH_4 in the biogas. Granulometry affected 1.00 gVS_{subs}/gVS_{inoc} ratio, with fine grinding favoring methane generation. However, energy balance must be studied to assess the practical feasibility of such milling.

Biogas from *Typha dominguensis* 1.00-C had the highest proportion of CH_4, with 49.66% and 94.36 $mL_{CH4}/gVS_{substract}$ (Table 3). The ratio of 0.5 gVS_{subs}/gVS_{inoc} showed 39.34 and 38.38% CH_4, but 179 and 207 $mL_{biogas}/gVS_{substract}$ for coarse and fine granulometry, respectively. A better biogas yield did not result in CH_4 release, although the value is high when compared to other substrates.

Lin [1] reported 66 to 144 mLCH4/gVS for *Phragmites australis* (reed). Cattail resulted in yields in the range mentioned above (Table 2). The author's estimates powering eight buses to run 8000 km per year when collecting 100 ha of planted wetlands and it can be extrapolated for *T. dominguensis*.

The greater amount of *E. crassipes* (**water hyacinth)** at 1.00-F had an impact on biogas quality: 25.95% CH_4 and 26.54 $mL_{CH4}/gVS_{substracte}$ (Table 3). On the other hand, in coarse grinding, only 17.27% CH_4 was obtained, although $Ymax_{biogas}$ was similar. The same was observed for the reference substrate, which generated 53 $mL_{biogas}/gVS_{substrate}$ more than cattail 0.50-F (the second highest

Table 3
Reduction of volatile solids, concentration and yield of methane in biogas, and energy potential of macrophytes.

Sample[a]	Methane concentration in biogas	Methane yield	Energy potential		
	(%)	($mL_{CH4}/gVS_{substrate}$)	(kJ/kgVS)	(kJ/kgST)	(kJ/kg_{plant})
CATTAIL					
0.25-C	17.34 ± 0.00	23.75 ± 0.69	854.99 ± 24.96	648.08 ± 18.92	4.93 ± 0.14
0.25-F	26.19 ± 6.15	49.50 ± 0.05	1.781.94 ± 1.89	1.350.71 ± 1.43	10.27 ± 0.01
0.50-C	39.34 ± 5.71	70.42 ± 0.31	2.535.01 ± 11.33	1.921.54 ± 8.59	14.60 ± 0.07
0.50-F	38.38 ± 1.76	79.46 ± 1.15	2.860.44 ± 41.46	2.168.21 ± 31.42	16.48 ± 0.24
1.00-C	28.42 ± 2.22	56.55 ± 0.06	2.035.87 ± 2.05	1.543.19 ± 1.55	11.73 ± 0.01
1.00-F	49.66 ± 0.57	94.36 ± 0.50	3.397.04 ± 17.88	2.574.95 ± 13.55	19.57 ± 0.10
WATER HYACINTH					
0.25-C	9.32 ± 1.74	8.67 ± 0.01	312.09 ± 0.23	234.38 ± 0.18	1.84 ± 0.00
0.25-F	8.52 ± 0.69	9.97 ± 0.17	359.04 ± 6.14	269.64 ± 4.61	2.11 ± 0.04
0.50-C	16.10 ± 1.01	17.39 ± 0.05	626.09 ± 1.74	470.19 ± 1.31	3.68 ± 0.01
0.50-F	14.55 ± 3.23	13.67 ± 0.12	492.25 ± 4.19	369.68 ± 3.15	2.89 ± 0.02
1.00-C	17.27 ± 7.51	17.61 ± 0.10	633.98 ± 3.73	476.12 ± 2.80	3.73 ± 0.02
1.00-F	25.94 ± 3.21	26.54 ± 0.01	955.37 ± 0.3	717.48 ± 0.28	5.62 ± 0.00
DWARF PAPYRUS					
0.25-C	6.09 ± 0.14	7.44 ± 0.00	267.93 ± 0.13	226.01 ± 0.11	1.74 ± 0.00
0.25-F	6.28 ± 0.39	6.72 ± 0.25	241.97 ± 9.05	204.10 ± 7.63	1.57 ± 0.06
0.50-C	8.63 ± 0.95	17.18 ± 0.52	618.60 ± 18.65	521.80 ± 15.73	4.03 ± 0.12
0.50-F	8.38 ± 1.66	8.38 ± 0.12	301.54 ± 4.22	254.35 ± 3.56	1.96 ± 0.03
1.00-C	10.13 ± 1.02	13.27 ± 0.10	477.72 ± 3.65	402.97 ± 3.08	3.11 ± 0.02
1.00-F	13.22 ± 0.59	25.90 ± 1.27	932.49 ± 45.67	786.58 ± 38.53	6.07 ± 0.30

[a] Substrate/inoculum ratio (gVS_{subs}/gVS_{inoc})-granulometry (fine or coarse).

value), but only 15.63% CH_4.

Biogas from cattail and water hyacinth contained CH_4 in levels of 56%, 46%, 43%, and 30% reported for the water lettuce, O. cubense, water hyacinth, and Salvinia, respectively [28].

Romero De León et al. [16] reported 62 and 58% CH_4 in biogas from water hyacinth at the substrate:inoculum ratio of 1:1 and 2:1, respectively. The temperature of 55 °C used in the experiment favored AD, but it consumes more energy and requires equipment operation, which is not consistent with our proposal of simplified operation. The highest value, 66% in the ratio of 2:1, was observed at day 20 (out of total 50), demonstrating the periods of this study are supported by the literature.

Dwarf papyrus had the highest organic content (Table 1), but showed max. CH_4 of 13.22% in biogas. Its 16.7% lignin content may explain its difficult degradation and lower quality of biogas, although *Brachiaria* generated biogas with 60% CH_4, even with 27.39% lignin [28].

Data provided above confirm the importance of monitoring biogas quality. They also demonstrate that estimates of CH_4 generation from substrate characterizations may lead unrealistic results, highlighting the relevance of BMP testing for AD viability studies.

In general, φ 0.50 gVS_{subs}/gVS_{inoc} presented higher removals of VS than 1.00 gVS_{subs}/gVS_{inoc}. For the treatment of CW residue and production of methane, we propose the use of two reactors in the first relationship, maintaining the stability of food/microorganism balance, increasing the overall yield of methane and maintaining the amount of residues to be treated.

3.6. *Energy generation from macrophytes biomass*

The energy potential validates the previous discussions: in general terms, cattail has the greatest potential, followed by water hyacinth and dwarf papyrus (Table 3). Condition 1.00-F favored such potential, resulting in 19569.65 kJ t^{-1}; 5617.88 kJ t^{-1} and 6068.45 kJ t^{-1} for cattail, water hyacinth and dwarf papyrus, respectively. However, energy balance should be considered, including expenses to obtain smaller particles.

A comparison of 0.50-F and 1.00-F for cattail is also relevant. The same amount of substrate can be treated in two reactors operating at 0.50 gVS_{subs}/gVS_{inoc} and still obtain more energy: 13,387 kJ per ton of cattail biomass.

Cattail and water hyacinth showed stabilization times of 25 and 6 days, respectively. At 1.00-F, and considering that a batch of cattail AD is equivalent to 4 of water hyacinth AD, it would allow the generation of 2901.88 kJ more with one ton of water hyacinth. In this perspective, and given its easy harvesting for being a floating plant, this macrophyte should be considered as a substrate.

In addition to AD time, biomass productivity, growth time, and easy handling of these macrophytes should be considered. *Typha latifólia L.*, the same genus as cattail, produces around 3.22 kg m^{-2} of dry matter in the aerial portion every year [5], the same portion used in our study. Considering this productivity, cattail could yield 63,014 kJ m^{-2} per year.

Cyperus papyrus, same genus as dwarf papyrus, produces 5 kg m^{-2} [11]. Then, dwarf papyrus AD could generate 30,342 kJ m^{-2} annually; while *Eicchornia crassipes*, which produces 60 kg m^{-2} [12], could generate 337,073 kJ m^{-2}.

Based on the information above, water hyacinth was the macrophyte with the highest energy potential, followed by cattail and dwarf papyrus.

However, the efficiency of each macrophyte for the wastewater treatment in CW should be considered. Future studies should conduct comprehensive assessments of energy demand for the maintenance of constructed wetlands, anaerobic digester and milling, efficiency in wastewater treatment, biomass productivity, and efficiency and stability in energy availability. This way, sewage treatment and energy generation could be combined.

4. Conclusions

The ratio substrate:inoculum 0.50 gVS_{subs}/gVS_{inoc} was the better condition for AD of macrophytes biomass evaluated in this work and the kinetic models with lag phase showed better fit to experimental data. Water hyacinth was the most interesting substrate, followed by cattail. Dwarf papyrus generated 6068 ± 297 kJ t^{-1} of plant and hydrolysis constant 0.165. day^{-1}. Cattail had the best energy recovery but slower degradation: 19,570 ± 103 kJ/t and k 0.109. day^{-1}. Water hyacinth provided only 5618 ± 2.20 kJ t^{-1} but rapid digestion: k 0.53. day^{-1}, obtaining 2.902 kJ t^{-1} more in the same period. However, the efficiency of macrophytes in CWs must be observed to combine sewage treatment and energy generation.

Acknowledgements

The authors thank Espaço da Escrita – Pró-Reitoria de Pesquisa - UNICAMP - for the language services provided.

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