Waste Biomass Valor
DOI 10.1007/s12649-016-9800-1

ORIGINAL PAPER

Pre treatment of Duckweed Biomass, Obtained from Wastewater Treatment Ponds, for Biogas Production

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Received: 1 June 2016 / Accepted: 5 December 2016

Abstract Considering the capacity of duckweed to treat wastewater and to produce valuable biomass, the present study aimed to highlight the potential of duckweed biomass harvested from wastewater treatment plant for biogas (methane) production. In this way a pilot system, comprising an anaerobic pretreatment and two duckweed ponds designed in series (10 m^2 each), was operated with real domestic sewage. The treatment efficiency was evaluated through the monitoring of conventional physical–chemical water quality variables sach as Temperature, pH, total phosphorus (TP), phosphate (PO_4), total nitrogen (TN), ammoniacal nitrogen (NH_4^+–N) and chemical oxygen demand (COD). Simultaneously the excess of biomass produced during the treatment was submitted to Biochemical Methane Potential test (BMP) carried out in a multi-batch reactor system. Three pretreatment approaches (fermentative, drying and alkaline) were performed in triplicate to evaluate their influence on methane production. Findings showed that the duckweed ponds removed the organic matter and nutrients from the wastewater (TN = 94%, TP = 92% and COD = 91%). Moreover, the biomass submitted to a fermentative pretreatment returned higher gas production (0.39 $Nm^3_{biogas}/kgVS_{fed}$) compared with the anaerobic digestion (AD) of unpretreated biomass (0.25 $Nm^3_{biogas}/kgVS_{fed}$). These results highlight the potential of duckweed ponds technologies to treat wastewater and produce clean energy simultaneously.

Keywords Duckweed ponds · Wastewater treatment · Nutrient uptake · Anaerobic digestion · Biogas

Introduction

Considering the global worry about environmental impacts and the stringency of protection laws, there is a need to explore some “non-conventional” methods for energy production which are not only efficient and economically viable but are also eco-friendly. Bio-renewable energy sources, such as biomethane are seen as one of key factors to fight this problem. According to Holm-Nielsen et al. [1], the EU policies concerning renewable energy systems (RES) have set forward a fixed goal of supplying 20% of the European energy demands from RES by year 2020. At least 25% of all bioenergy in the future can originate from biogas, produced from wet organic materials such as: animal manure, whole crop silages, wet food and feed wastes [1]. Backed by “The Blue Economy” approach, the production and use of biomathene could increase the local economy once it could be obtained from many types of organic waste, through anaerobic fermentation [2].

The biomethane is a compound of biogas (50–85% of CH_4, 20–35% of CO_2 and H_2, N_2, H_2S in lower content) [3], with high calorific power (about 20–22 MJ Kg^{-1}) and can be used as an energy source in industries, vehicles, electricity generation and others [4]. Recently, researchers have highlighted the potential of duckweed biomass yielded during wastewater treatment for energy generation, i.e. bioethanol and biogas [5–7]. Duckweed is a small floating macrophyte, belonging to the Araceae family, that have been assessed for decades due their notable capacity to uptake nutrients from water and for their biomass production [5, 8]. Due to their fast growth, great tolerance to high

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nutrient levels, and extraordinary nutrient uptake ability, duckweeds of various species have been intensively studied for the treatment of nutrient-rich wastewaters [5]. As a by-product of wastewater treatment, a high amount of valuable biomass is available to produce energy and food [9, 10].

The potential of duckweed biomass for biogas production is evident due to their high growth rate and composition of low cellulose and lignin content, but high leaf starch content [5, 11, 12]. Also some studies have pointed out the advantages of the codigestion combining duckweed biomass with other substrates like swine manure and dairy wastes, mainly due the nutrient supplied [13, 14]. For example, Henderson et al. [13] using batch anaerobic digesters at 35 °C with dairy manure showed a significant increase in methane production by adding 2–3% of duckweed (reaching 2.5 times higher than manure without duckweed). This authors attribute this effect to macro and micronutrients in duckweed tissues, as well the changes in C:N ratio. Also, Weidong et al. [15] assessed the codigestion of swine manure and duckweed (1:1) and the results showed that the biogas yield and the COD conversion rate were higher when compared with swine manure only (0.31 L gVS^{-1}, 63.2% and 0.28 L gVS^{-1} 57.1%, respectively).

In order to break up the structure of duckweed cell wall to improve microbial access several pretreatments could be performed (acid, alkaline, thermal) [6]. On the other hand, the pretreatment often leads to costs increase mainly in full scale plants. Xu and Deushes [6] evaluated different pretreatment before anaerobic digestion of duckweed biomass to produce hydrogen and biogas and concluded that acidification with H_2SO_4 (1%) in higher temperatures (85 °C) result in higher yield. However, high sodium concentrations resulting from pH adjustment after biomass pretreatment were inhibitory to fermentation. Moreover, substantially higher biogas and hydrogen production can be expected if a carbohydrate (starch) enrichment step is included in duckweed cultivation [5]. The leaf starch is easier and faster degraded by microorganisms than complex sugars as cellulose and hemicelluloses setting duckweed biomass in advantage over others plants [5, 6]. In spite of the cited potential, studies with focus on pretreatment for biodegradability and methane yield are scarce in scientific literature. In this context, the present works aimed to understand the biodegradability and biogas production kinetic of duckweed biomass, obtained from a pilot wastewater treatment plant. Also, the tests comprised different pretreatment methods to evaluate an alternative in order to optimize biogas yield through low costs techniques.

Materials and Methods

Duckweed Pond System Description and Operation

The experiment was developed in a pilot-system installed in Florianópolis, South Brazil (27°35′46.74″S, 48° 30′58.64″W), under a sub-temperate climate. The system is composed of an equalization tank (ET) of 5000 L and two fiber glass duckweed ponds (DP1 and DP2) covered by duckweeds from species *Landoltia punctata*. Both ponds have dimension of 4.2 m × 2.4 m × 1 m (10 m^2) and 0.42 m depth. Municipal wastewater from residential condominium was applied through peristaltic pump providing a continuous flow rate of 200 L d^{-1} and resulting in a hydraulic retention time (HRT) of 17 days in each pond. The sewage was stored in ET for 25 days HRT prior to being fed into in the DP system. The applied organic load rate was about 25.1 kg COD ha^{-1} day^{-1} and 10.5 kg NH_4^+–N ha^{-1} day^{-1}.

During 10 months (from Oct 2014–Jul 2015) the efficiency of the pond system was assessed through samples collected weekly in the inlet and outlet of each stage (Fig. 1). These samples were submitted for laboratorial analysis to determine the concentration of the following parameters: TP, TN, NH_4^+–N and COD, according to

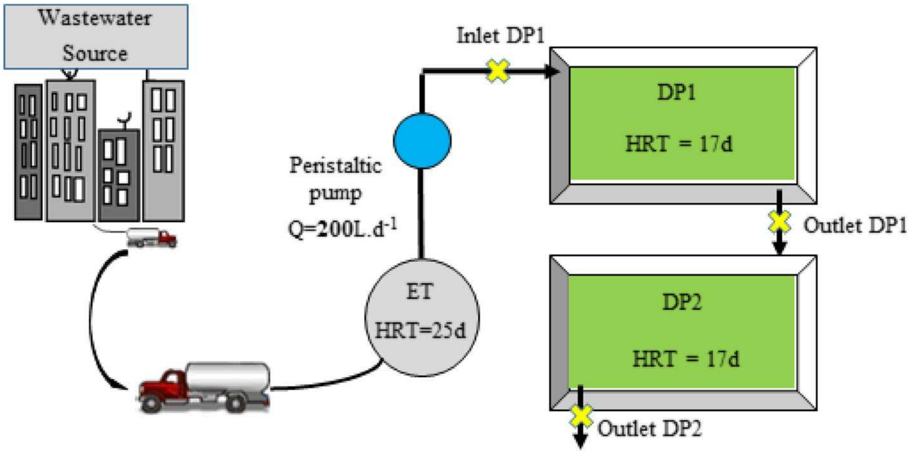

Fig. 1 Pilot system and collection sampling (*yellow markers*). (Color figure online)

Standard Methods [16]. In addition, PO_4^- was determined by liquid chromatography using Dionex® chromatographer while temperature and pH were determined using pHmeter Hanna HI8314.

Biomass Productivity

According to Landesman et al. [17] the evaluation of duckweed biomass productivity is determined by specific growth rate (g g^{-1} day^{-1}) and relative growth rate (g m^{-2} day^{-1}). Determine specific growth rate is necessary to find the produced biomass density [8]. According to Mohedano et al. [8], the biomass density was sampled through a floating plastic square with 0.25 m^2 internal area, which was released randomly over the pond surface whereas the imprisoned biomass was collected, dried and weighed providing biomass weight per area. The specific growth rate (SGR) and relative growth rate (RGR) were calculated from the relation between the average density (g m^{-2}) and total biomass harvested as shown in Equations 1 and 2, respectively (1, 2).

$$SGR = (Bt\ /N)/(A \times D) \tag{1}$$

$$RGR = (Bt/N)/A \tag{2}$$

where SGR is the specific growth rate (kg kg^{-1} day^{-1}), RGR is the relative growth rate (g m^{-2} day^{-1}), Bt is the total biomass removed in the period (kg), N represents the number of days in the period, D is the medium density (kg m^{-2}) and A is the surface area (m^2)

Biochemical Methane Potential (BMP)

The BMP tests were carried out in a multi-batch reactor system (AMPTS II, Lund, Sweden), which was specially designed for the determination of biochemical methane potential (BMP). Batches were performed in 500 mL glass bottles with a headspace volume of 100 mL continuously agitated by mechanical stirring and placed in a thermostatic water bath at 35 °C. Ramaraj et al. [12] showed that thermophilic digestion of duckweed (50 °C) produced less biogas and less methane than in mesophilic (35 °C) temperature. The produced biogas was passed through a 3 N NaOH solution to capture CO_2 and the remaining volume (methane) was automatically converted to standard temperature and pressure (0 °C and 1 bar). Digested sewage sludge from a municipal wastewater treatment plant in Florianópolis (Brazil) was used as inoculum (5.8 gVS kg^{-1}) and duckweed as a substrate (70.4 gVS kg^{-1}) in the BMP tests.

In order to break up the structure of the duckweed cell wall to improve microbial access, drying, alkaline and fermentative pretreatment were performed, and the effects on biogas and methane production were investigated. Thus, triplicates were performed for all BMP batches with an inoculum substrate ratio (I/S ratio) of 1.4 for the untreated, drying and alkaline pretreated duckweed batches while it was 2.5 for the batches fed with fermented duckweed.

Because duckweed is lacking a recalcitrant cell wall structure as cellulose and lignin, the temperature applied in the pretreatment was lower than those reported in typical pretreatment of lignocellulosic biomass [6, 18, 19]. Thus, during the drying pretreatment, the duckweed biomass was heated at 35 °C for 24 h to simulate a natural drying bed in a full-scale plant [20] In addition, the drying of biomass may be used as a tool since it may lead to less rapid decomposition and prevent losses of dry matter, available energy and greenhouse gas emissions during storage thereafter [21]. During alkaline pretreatment of lignocellulosic biomass could occur solvation and saponification. This causes a swollen state of the biomass and makes more accessible for bacteria [18, 22]. Hence, alkaline solution at 1% concentration NaOH was added in the substrate at a solid:liquid ratio of 1:5 for 24 h of contact to perform the alkaline pretreatment [22]. Fermentation was developed using the two-phase anaerobic digestion concept which can enhance the acidogenic phase regarding the fermentation of organic solid substrates by anaerobic bacteria into volatile fatty acids (VFA). The fermentative condition can be reached using high substrate loads in order to boost up the hydrolysis and fermentation first order kinetics of enzymatic hydrolysis of duckweed biomass [5, 9, 20]. In this way, the fermentative pretreatment was performed in 500 mL glass bottles (triplicates) set using low I/S ratio (0.2), incubated at 35 °C for 3 days using the same inoculum used in the batches. All analytical methods to determine total and volatile solids, COD, TP, TKN, ($N{-}NH_4^+$) were performed in accordance with Standard Methods [16].

The methane production was calculated by the amount of accumulated methane production per unit volatile solid (VS) that was added to each reactor. In this study, the inoculum was firstly degassed (25 days at 35 °C), in order to prevent biogas formation from the endogenous organic content [23]. Thus, the specific methane produced from the duckweed biomass was calculated as follows (3):

$$\mathrm{SMP} = VCH4\ \mathrm{accumulated}/mVS\ \mathrm{substrate} \tag{3}$$

where SMP is the specific methane production (Nm^3 $kgSV^{-1}$), VCH_4 represents accumulated cumulative methane volume (Nm^3) and mVS is the substrate mass.

Substrate biodegradability is related to the first-order kinetics of carbohydrate, lipid and protein degradation (K_h) and therefore the hydrolysis kinetics can be estimated from the BMP results. From the accumulated methane production curve, it was possible to calculate K_h ($days^{-1}$) for each pretreatment used in this study according to the following Eq. (4) [23]:

$$dS/dt = -K_h S \tag{4}$$

where S is the substrate biodegradability, t is the time (d), *Kh* is the hydrolysis constant. After the integration, the value of the variable K_h can be obtained by the following Eq. (5):

$$\ln(B_\infty - B)/B_\infty = -K_h t \tag{5}$$

where B_∞ is the final cumulative methane production, B is the cumulative methane produced in a given time, t.

Results and Discussion

Wastewater Treatment in Duckweed Pilot System

The average characteristics of the wastewater used treated in the pilot-system are shown in (Table 1). Concentrations of chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) in the DP1 influent were approximately 100.6, 57.3, and 6.4 mg L^{-1}, respectively. Considering the DP2 effluent, efficiencies of the duckweed system in terms of COD, TN and TP removals were 70.6, 92.1 and 93.6%. These results are in accordance with other similar studies using duckweed ponds for wastewater treatment purposes [24–26]. The low C:N:P ratio (usually found in tertiary effluents) does not affect the high efficiency since the main nutrient removal mechanism is based on autotrophic metabolism such as nitrification and plant absorption. Earlier studies have shown that nitrogen assimilation by duckweed arises as the main removal way in these systems [8, 10, 26]. The low organic loading rate of 25.2 kgCOD ha^{-1} day^{-1} and high HRT could contribute to this high performance, however, recent studies have also presented COD removal efficiencies ranging from 76 to 80% [24, 27, 28].

Results from Table 1 depict that the first duckweed pond (DP1) could fit the effluent on Brazilian standard law by itself (CONAMA 430), since the TP and NH_4^+-N remained below 4.0 and 20.0 mg L^{-1} respectively. Also, the average concentration of TN found in the final effluent was 4.5 mg L^{-1}, even well fitted for more restrictive environmental control standards, for example the UE legislation 91/271/EEC of 15 May 1991 [29]. Other peculiarity of the presented system was the excellent phosphorus removal efficiencies which provide a final effluent with less than 1.0 mgTP L^{-1}. According to Farrel [30] duckweeds have more phosphorus in their biomass than other aquatic macrophytes. As with for nitrogen removal, the phosphorus is also assimilated by the plant (especially in the form PO_4^{3-}) and removed from the system exclusively by harvesting. According to Öbek and Hasar [31] the daily harvesting resulted in phosphorus efficiency removal of 50% and it greatly increases to 96.7% whenever harvesting is performed every 2 days.

Biomass Productivity

The literature highlights that biomass management is a sensitive and important step for maintaining the treatment yields, because the removed biomass amount should agree on the biomass growth in order to keep a constant density [17]. Also, duckweed growth rates depend on climate conditions, used species and effluent composition, therefore a wide range is noted in scientific literature. In the present study, the relative average growth rates were 5.72 and 3.27 g m^{-2} day^{-1} (dry weight) and the specific growth rate obtained were 0.096 and 0.054 g g^{-1} day^{-1} for DP1 and DP2, respectively.

These data are in accordance with most findings in specific literature which present a large range of values. Iatrou et al. [32] investigated the *Lemna minor* growth using human urine and treated domestic wastewater under different dilutions, temperatures, initial mass of duckweed and presence of different microelements in laboratorial scale. The obtained maximum values of 0.097 g g^{-1} day^{-1} to SGR and 3.7 g m^{-2} day^{-1} to RGR. In contrast Mohedano et al. [11] studying a full-scale duckweed pond for swine waste treatment found high growth rates of 0.24 g g^{-1} day^{-1} to SGR and 18 g m^{-2} day^{-1} to RGR.

Table 1 Loading rate, mean values, standard deviation and removal efficiencies of DP1 and DP2

Parameters N = 37	Loading rate (kg ha^{-1} day^{-1})	DP1 inlet	DP1 outlet	DP2 outlet	Efficiency (%)
T (°C)		21.8 ± 2.8	21.8 ± 2.8	22.1 ± 2.9	
pH		7.1 ± 0.2	7.1 ± 0.2	6.8 ± 0.2	
TN (mg L^{-1})	14.4	57.3 ± 14	22.2 ± 10	4.5 ± 3.6	92.1
NH_4^+–N (mg L^{-1})	11.4	45.7 ± 9.8	16.3 ± 5.4	1.5 ± 1.5	96.6
TP (mg L^{-1})	1.6	6.4 ± 1.5	2.2 ± 1.1	0.4 ± 0.6	93.6
P–PO_4 (mg L^{-1})	1.0	4.1 ± 2.1	1.3 ± 1.0	0.2 ± 0.4	96.1
COD (mg L^{-1})	25.2	100.6 ± 54.1	39.0 ± 10.7	29.4 ± 8.6	70.6

N = samples number (campaigns)

Table 2 Reactors contents before startup of biochemical methane potential (BMP) test

Variable	Pretreatment			
	Untreated	Drying	Fermentative	Alkaline
COD ($gO_2\ L^{-1}$)	17.22	13.51	14.24	12.95
SCOD ($gO_2\ L^{-1}$)	3.14	2.31	11.18	2.12
TS ($g\ kg^{-1}$)	11.58	11.63	8.87	9.26
VS ($g\ kg^{-1}$)	9.21	9.54	7.48	7.00
TP ($g\ kg^{-1}$)	13.22	14.52	13.64	10.87
TKN ($g\ kg^{-1}$)	25.20	30.24	24.08	21.98
$N–NH_4^+$ ($g\ L^{-1}$)	0.19	0.23	0.18	0.18

Table 3 Methane productions from untreated and pretreated duckweed biomass

Pretreatment	Kinetic constant (day^{-1})	SGP (Nm^3_{biogas}/$kgVS_{fed}$)
Untreated	0.027 (0.758)	0.25
Drying pretreatment	0.076 (0.898)	0.32
Alkaline pretreatment	0.072 (0.991)	0.32
Fermentation pretreatment	0.137 (0.958)	0.39

Values in parenthesis are correlation factors (R^2)

BMP Assay

Reactors were setup with each one using a different pretreatment procedure. Their characteristics are shown in Table 2. Regarding the total solids content (TS), the highest TS concentration (11.63 g kg^{-1}) was obtained after the drying pretreatment while the lower TS concentration was presented in the fermented batch reactor. However, the latter reactor presented the greatest VS content (85%) suitable for bacteria metabolism. Nutrients were quite similar in all pretreated batches, except for the alkaline reactor whose concentrations were quite a bit lower.

The cumulative biogas and methane production during anaerobic digestion of untreated and pretreated duckweed biomass are shown in Fig. 2. Biogas and methane production clearly presented a different behavior when the process was followed for longer than 14 days. The untreated duckweed batches leveled off methane and biogas productions after 12 days of incubation. However, a slight increase in this curve was observed thereafter, reaching a new horizontal asymptote around the 25 days. The maximum gas production from the untreated biomass was 407 NmL_{biogas} (Fig. 2) and 245 NmL_{CH4}.

Biogas and methane productions from drying and alkaline pretreatments were quite similar ($p > 0.05$) and higher than values obtained from the anaerobic digestion of untreated biomass (Table 3). Besides, both batches levelled off gas production as observed in the untreated biomass. The fermented biomass yielded 336 NmL of biogas and 201 NmL of methane after 23 days of incubation. However, the curve showed in Fig. 2 does not consider the biogas yield during the fermentation step prior to the start of the methanogenic phase (234 NmL of CH_4).

The untreated duckweed biomass seemed to present a diauxic degradation pattern in which two exponential phases were observed: one fast phase during the first 10 days and a slower phase after the twentieth day (Fig. 2). According to Hamilton et al. [33] this behavior is often observed in substrates that have either a readily degradable fraction of small molecules that require little hydrolysis or a large molecular weight fraction that requires a longer hydrolysis period. In fact, the untreated duckweed presented the lowest k_h value (0.027 day^{-1}) while all pretreated biomasses presented greater k_h

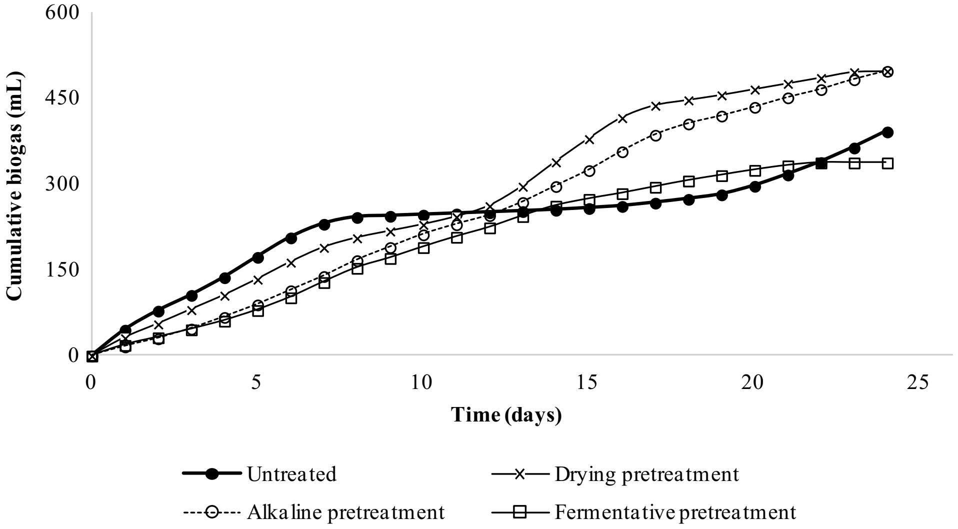

Fig. 2 Biogas productions of untreated and pretreated duckweed biomass (the data were obtained continuously however some markers were added for better viewing)

values, indicating an improvement in microbial digestibility and biomass biodegradability. Pretreatments often break down the complex organic structure into simpler molecules which are then more susceptible to microbial degradation [6, 18].

Conclusively, the specific biogas production obtained from the pretreated biomass was almost 36% higher than SGP of untreated biomass. In fact, the SGP ranged between 0.32 and 0.39 Nm³/kgVS$_{fed}$ (0.19–0.23 Nm³CH$_4$/kgVS$_{fed}$) for the pretreated biomass (Table 3). Since the duckweed biomass digestibility increased after the pretreatment, the obtained specific yields can be very promising for sustainable biofuel production. These yields confirm some advantages of duckweed pretreatments regarding the biogas productivity. Besides, among all the pretreatment approaches, it is important to highlight the best one should comprise not only the greater SGP but also the simpler implementation considering a full scale sewage plant treatment (5, 33). Since the alkaline and the drying pretreatments presented similar SGP values (0.32 m^3/kgVS$_{fed}$), the drying pretreatment would be the more attractive option especially due to the lower usage of alkaline and acids and less need for high capacity employers to manage the pretreatment stage in a full scale plant. Besides the high costs of chemical inputs, many inhibitory substances could be formed after acid or alkaline pretreatment [6, 34].

Recently, the interest of using the waste macrophyta biomass harvested from wastewater treatment ponds for gas production has significantly increased. Keesano [35] evaluated the biogas production of the anaerobic digestion of waste duckweed harvested in sewage treatment. The study reported an average production of 0.39 and 0.36 m^3 kgSSV^{-1}, and the methane composition ranged from 67.1 and 62.5% for the AD of fresh biomass and AD of dry biomass, respectively. Triscari et al. [14] added five different concentrations of duckweed biomass in a digester set to treat rural waste. Results showed that using 0.5–2% of duckweed biomass as a co-substrate increased the methane production.

Weidong et al. [15] studied the addition of duckweed biomass in a digester designed for pig manure treatment. The specific biogas production, COD efficiency conversion and gas production rate were 0.31 m^3 kgCOD^{-1}, 63.2% and 1.0 m^3 m^{-3} day^{-1}, respectively. Previously, yields from the single substrate AD trial had been 0.28 m^3 kgCOD^{-1}, 57.1% and 0.71 m^3 m^{-3} day^{-1}, respectively. In this way, the addition of duckweed biomass in the digester strongly benefited all digester productivities.

These outcomes highlight the possibility of using anaerobic digestion as an alternative to enhance and integrate the duckweed biomass management. This would lead to biogas recovering as a renewable energy.

Conclusions

During the experimental period the duckweed based treatment system had delivered a high efficiencies mainly for nitrogen (92%) and phosphorus (93%) but also for COD removal (70%). Also, this process generates a surplus biomass which could be used for renewable energy generation. Between the three pretreatments tested the highest specific biogas production was obtained with fermentative pretreatment (0.39 Nm³$_{biogas}$/kgVS), followed by drying and alkaline pretreatment (both 0.32 Nm³$_{biogas}$/kgVS). The highest hydrolysis constant in the process was obtained after the fermentative pretreatment (0.137 day^{-1}) and the lowest one regarded the untreated duckweed (0.027 day^{-1}). Hence, aiming the energy generation integrated with wastewater treatment in full scale duckweed ponds, this findings point out for two promising procedures, that is solar drying (35 °C) and the use of two-stage reactors (fermentative pretreatment). Thus, applying these two low-cost techniques, without chemical inputs or high temperature process, the biomethane yield could be improved and biogas production by anaerobic digestion could be a suitable alternative to duckweed biomass harvested during wastewater treatment.

Acknowledgements The authors would like to thank the team from the Laboratory of Effluents, the Federal University of Santa Catarina, CNPq, CAPES and the TSGA project.

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