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Scaling-Up the Anaerobic Digestion of Pretreated Microalgal Biomass within a Water Resource Recovery Facility

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Abstract: Microalgae-based wastewater treatment plants are low-cost alternatives for recovering nutrients from contaminated effluents through microalgal biomass, which may be subsequently processed into valuable bioproducts and bioenergy. Anaerobic digestion for biogas and biomethane production is the most straightforward and applicable technology for bioenergy recovery. However, pretreatment techniques may be needed to enhance the anaerobic biodegradability of microalgae. To date, very few full-scale systems have been put through, due to acknowledged bottlenecks such as low biomass concentration after conventional harvesting and inefficient processing into valuable products. The aim of this study was to evaluate the anaerobic digestion of pretreated microalgal biomass in a demonstration-scale microalgae biorefinery, and to compare the results obtained with previous research conducted at lab-scale, in order to assess the scalability of this bioprocess. In the lab-scale experiments, real municipal wastewater was treated in high rate algal ponds (2×0.47 m^3), and harvested microalgal biomass was thickened and digested to produce biogas. It was observed how the methane yield increased by 67% after implementing a thermal pretreatment step (at 75 °C for 10 h), and therefore the very same pretreatment was applied in the demonstration-scale study. In this case, agricultural runoff was treated in semi-closed tubular photobioreactors (3×11.7 m^3), and harvested microalgal biomass was thickened and thermally pretreated before undergoing the anaerobic digestion to produce biogas. The results showed a VS removal of 70% in the reactor and a methane yield up to 0.24 L CH_4/g VS, which were similar to the lab-scale results. Furthermore, photosynthetic biogas upgrading led to the production of biomethane, while the digestate was treated in a constructed wetland to obtain a biofertilizer. In this way, the demonstration-scale plant evidenced the feasibility of recovering resources (biomethane and biofertilizer) from agricultural runoff using microalgae-based systems coupled with anaerobic digestion of the microalgal biomass.

Keywords: agricultural runoff; anaerobic digestion; biogas; biomethane; biorefinery; microalgae; photobioreactor; pretreatment; wastewater

1. Introduction

The treatment of wastewater is fundamental for ensuring public health and environmental quality. European regulations such as the Urban Waste Water Treatment Directive (91/271/EEC) [1] and the

Water Framework Directive (2000/60/EC) [2] aim at protecting surface waters from the adverse effects of wastewater discharges, such as organic pollution and oxygen depletion, which degrade aquatic life. This has been partially achieved through the collection and treatment of wastewater in urban settlements. In most of these cases, wastewater is subject to biological treatment (secondary treatment) for the removal of organic matter and suspended solids, but in cases where the receiving water bodies are considered sensitive to eutrophication, more stringent tertiary treatment may be required to reduce nitrogen and phosphorus pollution. In 2015, the percentage of population connected to wastewater treatment facilities ranged from 75% in Eastern Europe to 97% in central Europe, while the percentage connected to wastewater treatment plants that implement tertiary treatment ranged from 21% in south Eastern Europe to 80% in central Europe [3]. The percentage not connected to wastewater treatment facilities mostly corresponds to population living in scattered communities outside agglomerations, usually in rural areas.

Nature-based sanitation systems, such as constructed wetlands and microalgae-based systems, may be the most feasible solution for rural areas, since they have lower costs and less sophisticated operation and maintenance requirements [4,5]. Moreover, these systems can provide treatment efficiencies similar to those of activated sludge wastewater treatment plants (WWTPs) including tertiary treatment. The main disadvantages of natural systems are that they are susceptible to seasonality and require larger land areas compared to conventional treatment systems [6]. The effects of seasonality can be lessened by a proper design under the most adverse conditions. Regarding land availability, it may not be an issue in rural areas as compared to urban agglomerations. In addition, these systems are suitable for the treatment of agricultural runoff.

In particular, microalgae-based treatment systems have much lower energy input compared to conventional activated sludge units, since oxygen for biological treatment is supplied through microalgae photosynthesis. Moreover, these microorganisms are responsible for nutrient assimilation, allowing nitrogen and phosphorus removal [7,8]. Experimental and demonstration-scale facilities of microalgae-based systems treating municipal wastewater have shown removal efficiencies of 90% for COD, 75–95% for N-NH_4 and 37% of P-PO_4 [9–11]. On the other hand, WWTPs are shifting from being just a sanitation technology towards a bioproduct recovery industry, as biorefineries or water resources recovery facilities (WRRFs). Microalgae-based systems fit in this approach, since the treatment of wastewater is associated with the production of microalgal biomass that could be recovered or reused for further purposes. Thus, microalgae have gained research interest due not only to their great potential and impact applications on wastewater treatment, but also for resource recovery and societal development [12,13]. Harvested microalgal biomass can be processed into protein for animal feed, agricultural fertilizer, pigments and biopolymers, while biogas can be produced by means of anaerobic digestion of the total or residual biomass [14–20]. Biogas production from microalgae is suitable and of special interest for small agglomerations and rural areas, since a positive energy balance can be achieved, producing more energy from the biogas than the energy required for the operation of the whole plant, if environmental conditions (solar radiation, temperature) are appropriate [11,21].

For microalgae-based wastewater treatment, open ponds are normally justified as more economical than closed photobioreactors, which seem to be only recommended for high-value by-products. Nonetheless, closed tubular photobioreactors have interesting advantages, as more independency on weather conditions, lower risk of microbial contamination and lower CO_2 losses [22]. Systems combining open and closed compartments aim at taking advantage of the features and avoiding the main drawbacks of both types of systems, which may encourage the use of semi-closed photobioreactors in microalgae-based WRRFs [23].

Regarding bioenergy production, anaerobic digestion is the most straightforward and applicable technology to date. According to the literature, results on microalgal biomass methane yield at lab-scale range from 0.07 to 0.56 L CH_4 g VS^{-1}, depending on microalgae species, substrate characteristics and operating conditions, among other factors [15]. In any case, for improving biomass biodegradability, pretreatment methods have been tested in order to disrupt the cell wall and enhance the hydrolysis

step. Pretreatment techniques that have so far been applied to microalgae include physical, chemical and biological methods, as well as their combinations [24]. Even if they all seem effective in terms of methane production increase, thermal pretreatments at low temperature (<100 °C) seem more feasible to scale-up, since they have led to 70% methane yield increase and positive energy balances in lab scale reactors [25–27]. However, full-scale experience on anaerobic digestion of pretreated microalgal biomass is limited, despite its implementation is increasing according to the number of research projects worldwide [28].

In this context, a demonstration-scale plant including anaerobic digestion of pretreated microalgal biomass was implemented and operated in the framework of the projects INCOVER and AL4BIO. The projects aimed at changing the current wastewater treatment concept towards a bioproduct recovery industry and a reclaimed water supplier. One of the main outcomes of the projects was the evaluation at demonstration-scale processes and technologies that were previously tested only at the lab or pilot-scale. In particular, agricultural runoff and domestic wastewater were treated in demonstration-scale semi-closed photobioreactors, assessing the feasibility of selection of cyanobacteria and accumulation of polyhydroxybutyrate (PHB) and carbohydrates [29,30]. The biomass was harvested in a lamella settling tank and thickened in gravity settlers. Subsequently, the biomass was digested anaerobically for the production of biogas, after undergoing thermal pretreatment. The biogas was upgraded to biomethane in a photosynthetic absorption column [17], while the digestate was further stabilised and dewatered in a sludge treatment wetland for the production of biofertilizer.

This study compiles the data from the anaerobic digestion of pretreated microalgal biomass, with the objective of evaluating the results and comparing the production of biogas with previous research conducted at lab-scale. The discussion regarding the performances obtained at both scales aims at assessing the scalability of this bioprocess.

2. Materials and Methods

2.1. Demonstration-Scale Set-Up

The microalgae-based WRRF was located outdoors in the Agròpolis Campus of the Universitat Politècnica de Catalunya (UPC) in Viladecans (Barcelona, Spain, Figure 1). It treated a mixture of agricultural runoff (90% *v*/*v*) and domestic wastewater from a septic tank (10% *v*/*v*). The agricultural runoff was obtained from a drainage collection channel beside the campus. The system comprised three horizontal tubular semi-closed photobioreactors, a lamellar settler with polymer addition for biomass harvesting, two gravity thickeners, an anaerobic digestion unit for biogas production and upgrading to biomethane, and a constructed wetland for digestate stabilisation and dewatering. The clarified effluent was post-treated in a solar-driven ultrafiltration-disinfection unit and in three adsorption columns for nutrients recovery, and eventually reused for irrigation of rapeseed and sunflower crops by means of a smart irrigation system. Further details on the start-up of the plant may be found in [31].

2.1.1. Microalgal Biomass Production and Harvesting

Agricultural runoff was pumped from the collection channel to a homogenisation tank (10 m^3), where it was mixed with the partially treated domestic wastewater pumped from a septic tank. The influent was conveyed to the three semi-closed tubular photobioreactors. Each photobioreactor (11.7 m^3) was composed by two lateral open tanks (2.5 m^3) equipped with paddle-wheels, connected by sixteen horizontal transparent tubes (9.2 m^3). The paddle-wheels in the lateral open tanks provided a difference in the water level between the two tanks, causing the mix liquor to flow from one tank to the other through eight tubes and returning to the first open tank through the other eight tubes. The liquid velocity inside the tubes was 0.25 m/s, ensuring a turbulent flow and homogeneous mixing. Moreover, the open tanks provided dissolved oxygen release and preserved temperature increase.

The system was operated in a semi-batch mode, with a discharge of 2.3 m^3 of mixed liquor from each photobioreactor, followed by feeding the same volume of influent wastewater, each and

every day at 5 a.m. and 7 a.m., respectively. During the experimental period, the operation of the photobioreactors was changed according to the research and innovation objectives and the goals to be attained, e.g., wastewater treatment and biomass production optimisation or PHB accumulation by cyanobacteria. On the whole the plant was operated for 20 months; during the first 12 months the photobioreactors were operated in parallel with a HRT of 5 days, while during the following 8 months they were connected in series with a total HRT of 15 days [29,30].

Figure 1. Global view of the demonstration-scale plant.

Microalgal biomass was harvested in a lamellar settler (700 L), which comprised a flocculation chamber (50 L), which received the influent mixed liquor and an addition of coagulant; a stilling zone (180 L) after the flocculation chamber; a lamellar zone (350 L) which was the main settling volume; an effluent weir and collection channel over the lamella zone; and a sludge hopper at the bottom for collecting the settled biomass (120 L). The total daily volume of mixed liquor discharged from the photobioreactors was pumped to the settling tank at a surface loading rate of 0.135 m/h (including the lamellae's surface), with a HRT of 1.75 h. Biomass coagulation and flocculation was enhanced by dosing aluminium polychloride. The dose of coagulant was modified according to the influent mixed liquor characteristics. The sludge was drawn off from the bottom of the settling tank by means of an electro valve and a timer several times every day, until no more sludge remained in the hopper. Following, harvested microalgal biomass was further thickened in two gravity settlers (200 L each) working in series.

2.1.2. Thermal Pretreatment and Anaerobic Digestion

A diagram and an image of the thermal pretreatment and the anaerobic digestion unit are shown in Figure 2. Thickened microalgal biomass was conveyed to a homogenisation tank (100 L) under constant stirring. The biomass was then fed to the thermal pretreatment unit at a flow rate between 15 and 30 L per day. In order to distribute the load during the day, the microalgal biomass was pumped at a constant flow of 0.5 L/min during one minute every 25–45 min (OEM 520FAM/R2 peristaltic pump, Watson-Marlow®, United Kingdom). The time interval between each consecutive pumping event was adjusted in order to feed the desired total volume of biomass. The thermal pretreatment was carried out in a stainless steel tank (25 L), with constant stirring and an electrical resistance (1.5 kW, Electricfor SA, Barcelona, Spain) for maintaining the temperature at 75 °C. The pretreatment temperature was selected according to previous studies on the increase of microalgae anaerobic biodegradability after evaluating several pretreatment methods and validating the thermal pretreatment in continuous lab-scale reactors [26,27,32]. The pretreatment tank was equipped with an electronic temperature sensor (TD2517, IFM electronic LTD, Essen, Germany), and temperature data were collected and

recorded in a datalogger every 20 min. The tank also included an electronic liquid level sensor (PI2789, IFM) to control filling and emptying operations.

Pretreated biomass was pumped to the anaerobic digester (Watson-Marlow OEM 520FAM/R2 peristaltic pump, United Kingdom). The anaerobic digester (1 m^3) was maintained under constant stirring by means of liquid recirculation at 2 m^3/h (BN 2–6 L rotating positive-displacement pump, Seepex, TD2517, IFM electronic LTD, Essen, Germany) and at mesophilic temperature (35 °C) by means of an electrical resistance (CR212II0030 M77 LIR 589, Electricfor SA, Barcelona, Spain). Furthermore, the digester was equipped with electronic liquid level sensors, pressure, temperature and redox (PI2798, PI008A and TD2517—IFM Electronic LTD.—Essen, Germany) and pH (K100, Seko—Santa Rufina, Italy). Data of these parameters were measured online and recorded in a datalogger every 20 min. Biogas production was quantified using a mechanical flowmeter (TG0.5-PVC-PVC, Ritter® Bochum, Germany) and stored in a gasometer. The volume of biogas was recorded manually from the mechanical flowmeter every working day. Therefore, results are expressed as weekly average values of biogas production (L biogas/$L_{reactor}$·day) and methane yield (L CH_4/g VS).

Over an experimental period of 14 months (420 days), the digester was operated with two different HRT: 20 days (Period 1, until day 271) and 32 days (Period 2, days 272 to 420). Previous research had shown how increasing the HRT to 28–30 days could improve the biogas production from microalgae [27,33], and here we wanted to evaluate if it was also the case with pretreated microalgal biomass.

2.2. Analytical Methods

The performance of the photobioreactors and harvesting unit was monitored as described elsewhere [29]. In brief, grab samples from the influent wastewater (homogenization tank), the effluent of each photobioreactor, and the effluent of the lamella settling tank were collected and analysed weekly. The main operational parameters were analysed, among them the nutrients orthophosphate (PO_4^{3-}-P) and ammonium (NH_4^+-N) in the influent and in the photobioreactors, and turbidity, total suspended solids (TSS) and volatile suspended solids (VSS) in the photobioreactors and the harvesting unit. Turbidity was measured using a HI-93703 turbidimeter (Hanna instrumental, Limena, Italy). TSS and VSS were analyzed following Standard Methods for the Examination of Water and Wastewater [34]. NH_4^+-N was analyzed according to the methods described in Solórzano (1969) [35] and PO_4^{3-}-P was measured by means of a DIONEX ICS1000 ion chromatography system (Thermo-Scientific®, Waltham, MA, USA).

Samples of biomass were observed under a bright light microscope (Motic, Kowloon, Hong Kong) equipped with a camera (Fi2, Nikon, Tokyo, Japan) and a fluorescence microscope (Eclipse E200, Nikon, Tokyo, Japan) using the NIS-Element viewer® software, in order to observe the composition of microorganisms during the experimental period. The identification of microalgae and cyanobacteria was based on taxonomic books and databases [36,37].

The performance of the anaerobic digester was monitored as follows. The pH, redox potential, temperature and volume of the digester were continuously monitored on-site and recorded every 20 min, as well as the temperature and volume of the pretreatment unit. The volume of produced biogas was recorded every working day. The CH_4 and CO_2 content were periodically analysed from biogas samples using a GC equipped with a thermal conductivity detector (Trace GC with Hayesep packed column, Thermo Finnigan—Thermo-Scientific®, Waltham, MA, USA), as described by Marín et al. [17]. Samples of the influent biomass, pretreated biomass and digestate were analysed on a weekly basis. The concentration of Total Solids (TS), Volatile Solids (VS), total and soluble Chemical Oxygen Demand (COD and CODs) were determined according to the Standard Methods for the Examination of Water and Wastewater [34]. Total organic carbon (TOC) and total nitrogen (TN) were measured using an automatic analyser (multi N/C® 2100S analyser, Analytik Jena—Jena, Germany). TOC was analysed with an infrared detector (NDIR) according to the combustion-infrared method of the Standard Methods for the Examination of Water and Wastewater [34], by means of catalytic

oxidation at 800 °C using CeO_2 as catalyst. Following, a solid-state chemical detector (ChD) was used to quantify TN as NOx.

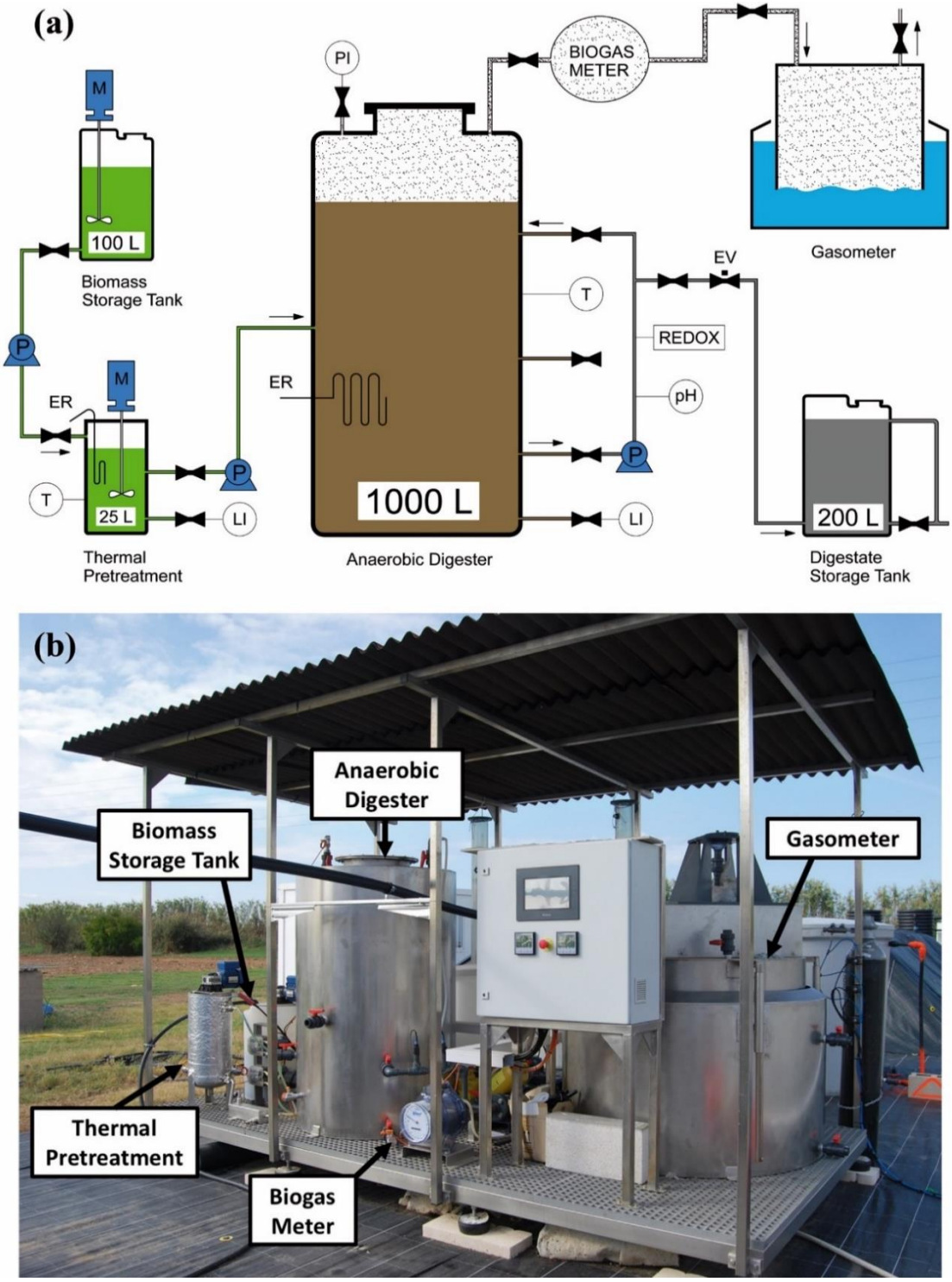

Figure 2. (**a**) Diagram of the anaerobic digestion plant. Mixers (M), pumps (P), electrical resistances (ER) and electrovalves (EV) are indicated in the figure, as well as the temperature (T), liquid level (LI), pressure (PI), redox and pH sensors. (**b**) Image of the anaerobic digestion plant.

2.3. Determination of Parameters

The performances of the thermal pretreatment, anaerobic digestion and biogas production were evaluated by calculating the following parameters.

The degree of solubilisation of microalgal biomass in the thermal pretreatment was calculated according to Equations (1) (S, percentage of solubilisation of the influent particulate COD) and (2) (SR, solubilisation ratio), where COD_{sp} is the soluble COD after pretreatment, COD_{so} is the soluble COD of the influent microalgal biomass, and COD_o is the total COD of the influent microalgal biomass.

$$S = \frac{COD_{sp} - COD_{so}}{COD_o - COD_{so}} \cdot 100 \quad (1)$$

$$SR = \frac{COD_{sp}}{COD_{so}} \quad (2)$$

The removal of VS ($VS_{removed}$, %) in the anaerobic digester was calculated as the difference between the VS concentration in the influent and effluent, with respect to the VS concentration in the influent, according to Equation (3), where VS_{inf} and VS_{eff} are the influent and effluent concentration of VS. VS_{inf} has been estimated as the mobile average of the influent VS concentration during the previous HRT period:

$$VS_{removed} = \frac{VS_{inf} - VS_{eff}}{VS_{inf}} \cdot 100 \quad (3)$$

The organic loading rate (OLR, kg VS/m^3·d) was determined as the amount of organic matter fed to the anaerobic digester per day, referred to the reactor working volume ($V_{reactor}$), according to Equation (4). At this aim, the organic matter concentration in the influent was expressed as the concentration of VS (VS_{fed}):

$$OLR = \frac{Q \cdot VS_{fed}}{V_{reactor}} \quad (4)$$

The methane production rate ($P_{methane}$, L CH_4/L·d) was calculated as the volume of methane produced per day, referred to the reactor working volume, according to Equation (5), where $\%CH_4$ is the methane content in the biogas:

$$P_{methane} = \frac{\text{L of methane per day}}{V_{reactor}} = \frac{\text{L of biogas per day} \cdot \%CH_4}{V_{reactor}} \quad (5)$$

Finally, the methane yield (Y_{CH4}, L CH_4/g VS) or specific methane production, was calculated by referring the methane production rate to the organic loading rate, according to Equation (6):

$$Y_{CH4} = \frac{P_{methane}}{OLR} \quad (6)$$

3. Results

3.1. Microalgal Biomass Production and Harvesting

Microalgal biomass produced in the semi-closed photobioreactors varied throughout the experimental period, as a result of the mode of operation and performance of the photobioreactors and harvesting unit, the weather conditions of the season and the variability of influent wastewater characteristics. Indeed, the biomass (expressed as VSS) concentration fluctuated with the solar radiation and water temperature, attaining low microalgae production during winter (7 g/m^3·day) and early spring, and increasing in summer and early autumn (up to 43 g/m^3·day) [29].

The operational conditions and performance of the harvesting unit also varied during the experiment. The turbidity of the influent mixed liquor to the lamella settling tank ranged between 20 and 500 NTU, and the doses of coagulant ranged between 1 to 12 mg Al/L for achieving an effluent

turbidity < 5 NTU. Harvested biomass was further thickened by gravity, reaching a concentration of VS between 2 and 18 g VS/L (Figure 3).

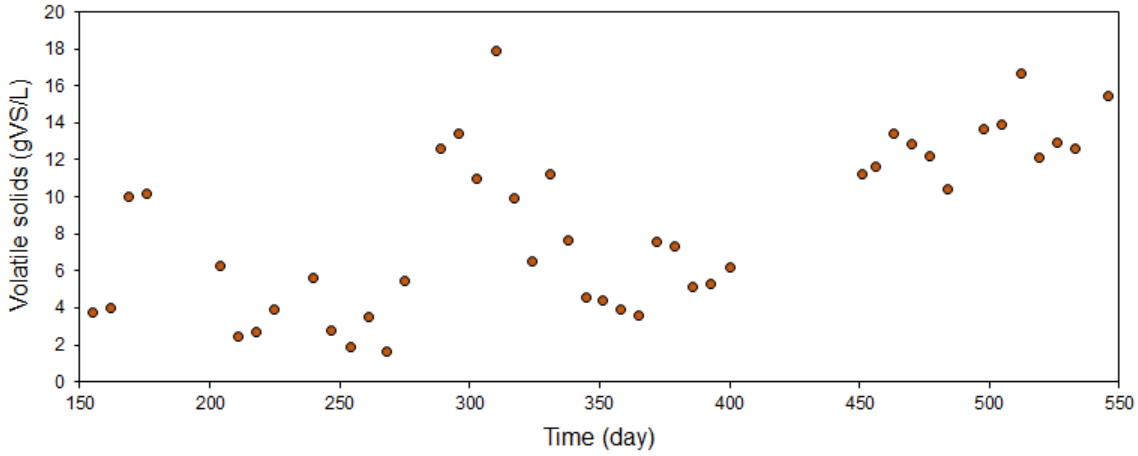

Figure 3. Concentration of volatile solids (VS) of thickened microalgal biomass.

The production of microalgal biomass seemed to be limited by the concentration of nutrients in the influent agricultural runoff, with average seasonal concentrations of N-NH_4 ranging between 1.2 and 3.6 mg/L and of P-PO_4 between 0.32 and 1.84 mg/L [38]. These values are quite low when compared to primary treated domestic wastewater (24–53 mg N-NH_4/L and 8–25 mg P-PO_4/L) [39]. In addition, the modification of the photobioreactors operation mode on day 330, from operation in parallel (5 days of HRT) to operation in series (15 days of HRT), also had an influence on the biomass production. Indeed, in spite of the favourable environmental conditions of springtime, after the modification the biomass production decreased, which was attributed to the lower influent flowrate and nutrients loading during the operation of the photobioreactors in series, with a total HRT of 15 days.

In general, the mixed culture was dominated throughout the whole period by cyanobacteria belonging to a coccoid species resembling *Synechococcus* sp. (especially during the operation in series), along with some filamentous cyanobacteria like *Pseudanabaena* sp. and green microalgae [29,30,38] (Figure 4a–d).

3.2. Thermal Pretreatment of Microalgal Biomass

The anaerobic digestion system was operated for 18 months. For the purposes of this study, only periods of stable operation were considered, in order to compare the anaerobic digestion of thermally pretreated microalgal biomass under lab-scale controlled conditions [26,27] and pilot-scale real conditions, and assess the scalability of the process. Thus, results from steady-state operation (days 204 to 455) are shown in Table 1. The temperature of the thermal pretreatment was steadily maintained at about 75 °C during the whole period, and the exposure time was around 20 h.

Table 1. Average values and standard deviation (SD) of thermal pretreatment parameters.

	Average	**SD**
HRT (h)	20.0	4.5
Influent		
Soluble COD before (mg/L)	456	265
VS/TS	0.47	0.08
Effluent		
Soluble COD after (mg/L)	2625	1262
VS/TS	0.45	0.08

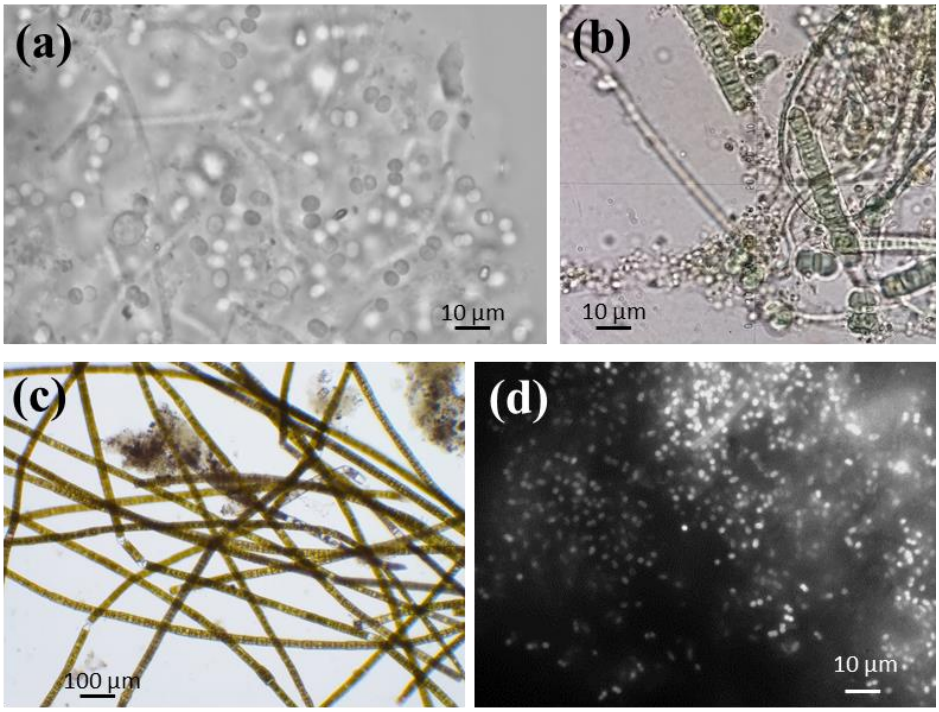

Figure 4. Microscopic images of the mixed liquor of the photobioreactors: (**a**) Coccal Cyanobacteria resembling *Synechococcus* sp. and small filamentous Cyanobacteria, surrounded by green microalgae, (**b**) filamentous Cyanobacteria resembling to *Oscillatoria* sp. and *Leptolyngbya* sp. and coccoid Cyanobacteria resembling to *Chroococcus* sp., *Synechococcus* sp. and *Synechocystis* sp., and (**c**) filamentous green microalgae, observed under bright light microscopy during the operation of the photobioreactors in parallel; and (**d**) higher dominance of *Synechococcus* sp. with some presence of *Pseudanabaena* sp., observed under fluorescence microscopy during the operation of the photobioreactors in series.

One of the most important parameters for evaluating the pretreatment effectiveness is the solubilisation of organic matter. Since microalgae cells are complex and resistant, in particular those grown in wastewater, organic compounds may be retained inside the cell wall, hindering the anaerobic biodegradability. Pretreatment methods aim at disrupting the cell wall and releasing intracellular compounds, enhancing the bioavailability of these compounds for anaerobic bacteria, and ultimately enhancing the anaerobic digestion rate and extent. This is commonly measured by the degree of solubilisation achieved after applying the pretreatment. In this study, the solubilisation degree (calculated from Equation (1)) was on average 45.7%, which means that almost half of the influent particulate COD was converted into soluble COD. When comparing the soluble COD before and after the pretreatment, it was increased from 456 to 2625 mg/L, representing a 5.8-fold solubilisation (calculated from Equation (2)).

These results fall within the range reported in the literature under laboratory conditions. For instance, the thermal pretreatment of mixed microalgal biomass at 75 °C for 10 h reached a 10.6-fold solubilisation [32], while the pretreatment of *Scenedesmus* biomass at 90 °C for 3 h increased soluble organic matter by 4.4-fold [40]. Indeed, the pretreatment effectiveness may vary depending on the microalgae species and growth characteristics, which depend on the culture medium composition [24]. For instance, in this study microalgal biomass was mainly composed of cyanobacteria in the demonstrative-scale plant treating agricultural runoff (with nutrients limitation), while in our lab-scale studies treating municipal wastewater the predominant species were green microalgae such as *Stigeoclonium* sp., *Monorraphidium* sp., or the diatoms *Nitzchia* sp. and *Amphora* sp.; the latter ones with an extremely resistant cell wall composed of silica [26].

According to results obtained, it seems that there was no organic matter loss during the pretreatment at 75 °C for 20 h, as the VS/TS ratio was maintained (Table 1), reproducing what was already observed in the lab-scale [26,27]. This is a matter of concern, since organic matter should not be lost prior to its conversion into biogas in the anaerobic digester.

3.3. Anaerobic Digestion Performance and Biogas Production

The anaerobic digestion performance is shown in Figures 5–7, where two experimental periods are differentiated: Period 1, when the anaerobic digester was operated with a HRT of 20 days (until day 271); and Period 2, when HRT was 32 days (days 272 to 420). Both periods operated under mesophilic conditions (35.8 ± 0.3 °C). The OLR ranged from 0.2 to 0.5 g VS/L·day in Period 1 and from 0.2 to 1.0 g VS/L·day in Period 2 (Figure 5). Indeed, it was more stable during the first period than during the second one, as a result of the VS concentration in thickened microalgal biomass, which follows a similar trend (Figure 3). Despite the variability, the average OLR was higher during the second period (0.5 vs. 0.28 g VS/L·day), even if the HRT was increased from 20 to 32 days. The reason for this is the increase in microalgal biomass production during summer time (Period 2), when microalgae growth was the highest (around 40 g/m^3·day). The correlation between the photosynthetic activity and the weather conditions is widely reported in the literature. In a pilot-scale study carried out at the same location, microalgae growth and biomass production followed the same trend as the solar radiation, reaching the highest values in spring and summer [11].

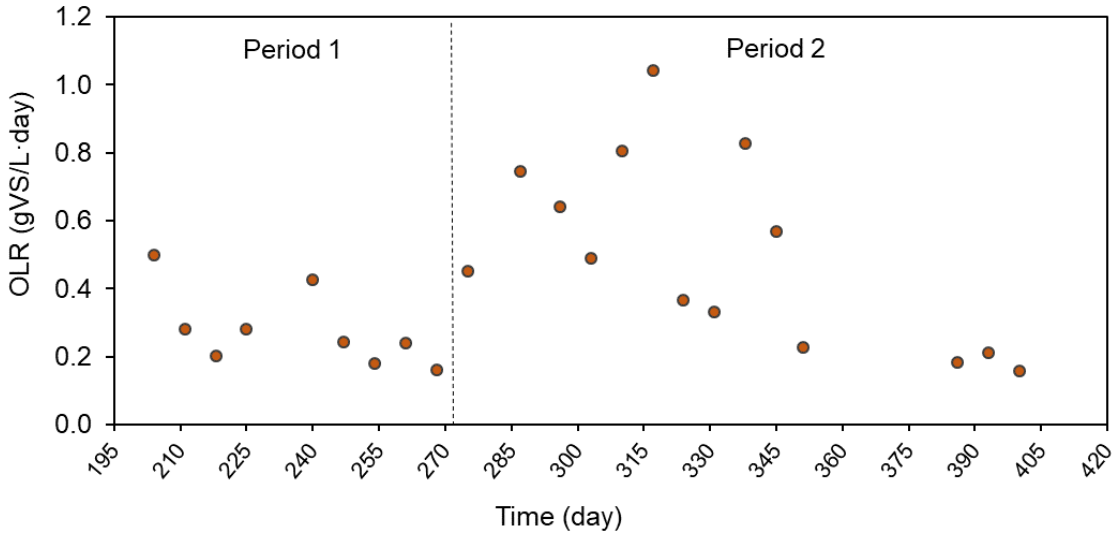

Figure 5. Organic loading rate in the anaerobic digester over the experimental periods 1 (HRT of 20 days) and 2 (HRT of 32 days).

The biogas production rate showed a similar trend as the OLR, with the highest values during summer (days 280–320) (Figure 6a). Indeed, the OLR was fairly low, and therefore increasing the OLR also increased the biogas production resulting from higher organic matter biodegradation. The methane content in biogas was around 76% in both periods, which is considered high upon the anaerobic digestion of particulate organic matter, suggesting an appropriate methanogenic activity. In terms of methane yield (Figure 6b), it ranged between 0.11 and 0.38 L CH_4/g VS during the first period and between 0.07 and 0.28 L CH_4/g VS during the second one, with average values of 0.24 and 0.16 L CH_4/g VS, respectively. It could be speculated that increasing the HRT (from 20 to 32 days) would concomitantly increase the anaerobic biodegradability and methane yield, as previously reported [27,33] and especially upon the anaerobic digestion of particulate organic matter, characterised by a slow hydrolysis step. However, microalgal biomass had already been pretreated with the aim of accelerating the hydrolysis, and in this case no further improvement was observed by increasing the HRT from

20 to 32 days. Most probably, all the soluble organic matter attained after the thermal pretreatment was already digested at 20 days of HRT and no further intracellular, hardly digestible or recalcitrant components were converted into biogas at 32 days of HRT. This indicates that the lower HRT of 20 days was already enough for operating the anaerobic reactor under the conditions of this study.

Another strategy for improving the anaerobic digestion performance would be the co-digestion with carbon-rich substrates, as agricultural biomass, to counter-balance the low C/N ratio of microalgae [41]. Indeed, the C/N ratio of pretreated microalgal biomass was fairly low, ranging from 4 to 10 (Figure 7), as a result of the high protein concentration in cells. This may jeopardize anaerobic digestion when ammonium concentrations arrive at inhibitory or toxic levels. According to the literature, optimal values for microbial growth are around 25–30 [42], which may lead to faster and higher methane production, while promoting the stability of the anaerobic digestion process. Besides, it is a way of increasing the OLR and biogas production potential.

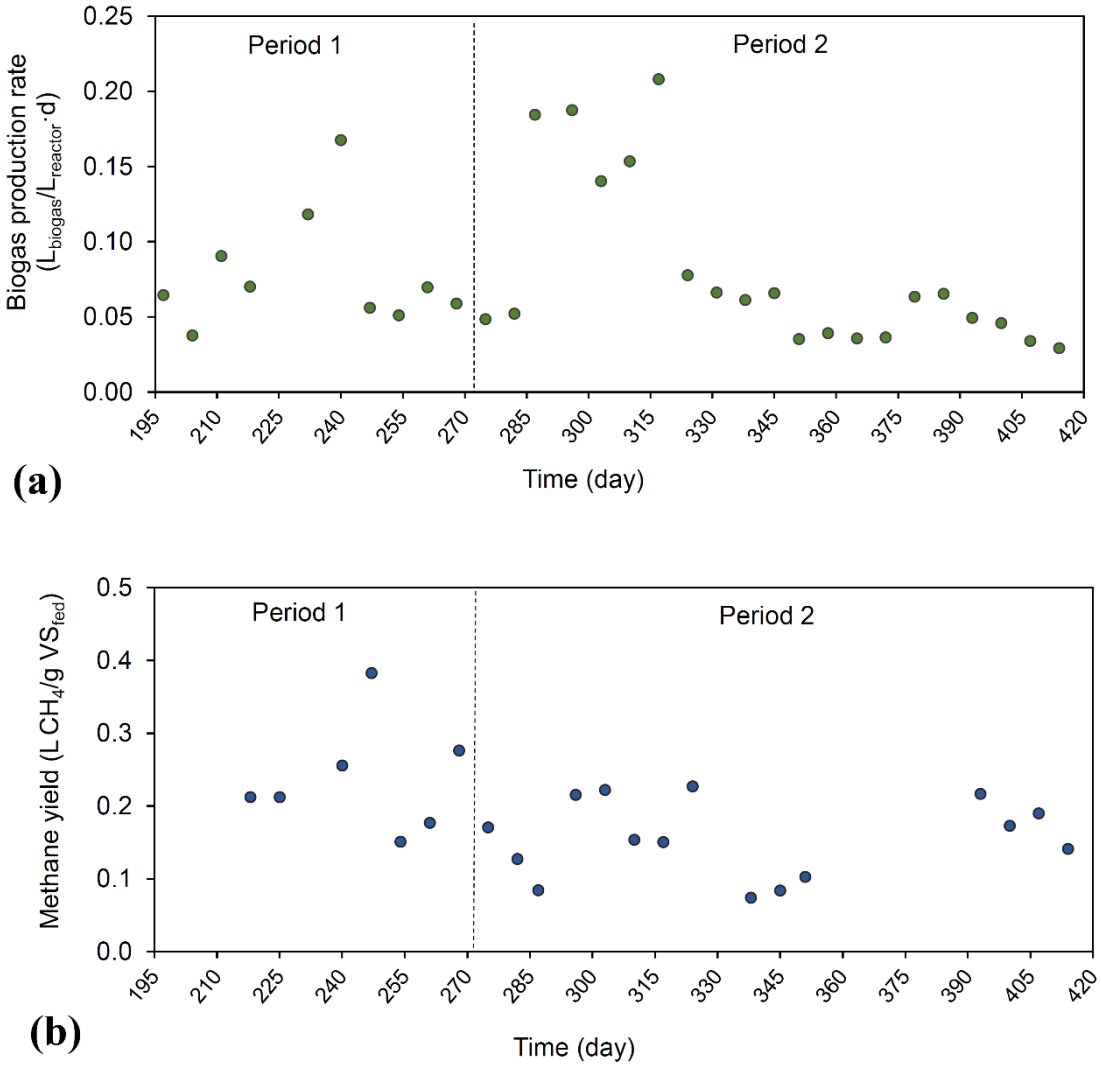

Figure 6. Biogas production rate (**a**) and methane yield (**b**) over the experimental periods 1 (HRT of 20 days) and 2 (HRT of 32 days).

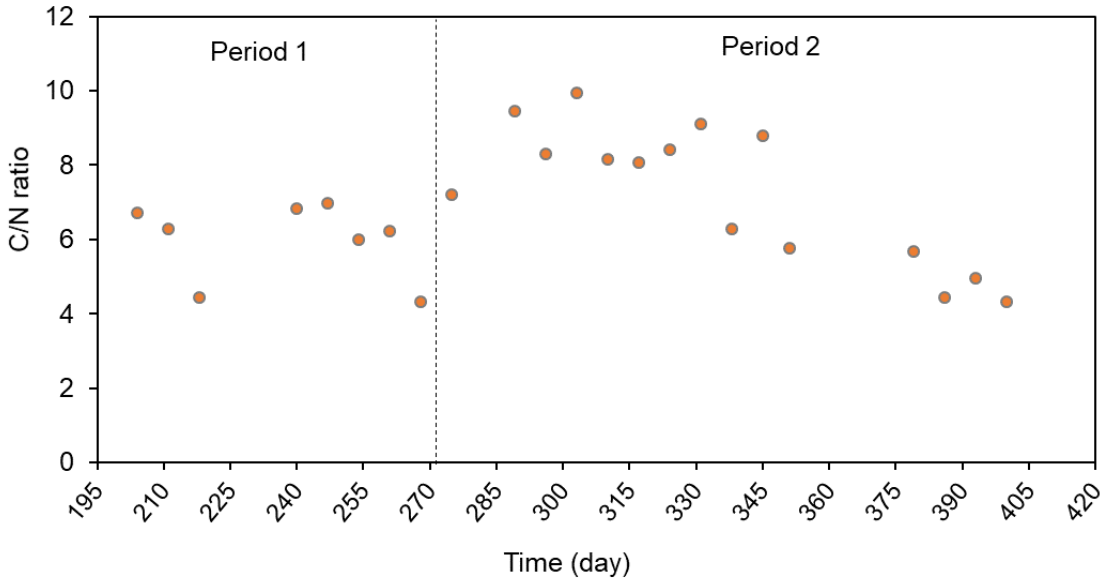

Figure 7. C/N ratio of the anaerobic digester influent (pretreated microalgal biomass) over the experimental periods 1 (HRT of 20 days) and 2 (HRT of 32 days).

4. Discussion

This study was intended to evaluate the anaerobic digestion of thermally pretreated microalgal biomass within a microalgae-based WRRF at demonstration-scale in outdoors conditions. Microalgae-based systems and biomass valorisation technologies have mostly been investigated in lab-scale facilities under controlled conditions. Such experiments are useful to quantify and compare operating conditions, yet do not provide information on the scalability under real conditions, with a strong seasonality and variations in influent wastewater characteristics, which are known to affect the wastewater treatment effectiveness, microalgal biomass production and biomass characteristics (predominant microalgae species and macromolecular composition). In fact, a recent study comparing microalgae-based systems at a lab-scale (5 m^2), pilot-scale (330 m^2) and full-scale (1 ha) revealed that full-scale units showed the lowest values in nutrient removal and microalgal biomass production [43]. The mentioned work indicated that the use of lab-scale data for designing and optimising full-scale plants is still uncertain. On the other hand, literature also suggests that there is an urgent need for more pilot and full-scale studies, since that represents a more realistic approach of the technology in comparison with lab-scale results [44].

In our previous studies, the thermal pretreatment conditions were optimised by comparing the effect of different temperatures (55, 75 and 95 °C) and exposure times (5, 10 and 15 h) on microalgal biomass solubilisation and biochemical methane potential (BMP) [32]. Subsequently, semi-continuous lab-scale reactors (1.5 L) were operated with microalgal biomass pretreated under the optimal conditions (75 °C for 10 h) [26,27]. Both studies were carried out under mesophilic conditions (35 °C) with a HRT of 20 days [26] and 30 days [27]; and in both cases two reactors were run in parallel, the first one receiving pretreated microalgal biomass and the second one raw microalgal biomass (control). In the present study, the same bioprocess was scaled-up in a microalgae-based WRRF, where the biogas produced was upgraded to biomethane and the digestate was post-treated in a constructed wetland to produce a biofertilizer. Thermal pretreatment has been described in the literature as the method to give the best result in microalgae pretreatment [15], however still with very few results in pilot and full-scale systems [7].

The main results obtained in the lab-scale reactors [26,27] and pilot set-up (Periods 1 and 2) are summarised in Table 2. In all cases the anaerobic digesters operated under mesophilic conditions (35–37 °C) with a HRT of 20 or 30–32 days. Microalgal biomass pretreatment was always conducted at

75 °C, with an exposure time of 10 h in the lab-scale experiments and 20 h in the pilot set-up. The OLR was considerably higher in the lab-scale reactors (around 0.7–0.8 g VS/L·day) than in the pilot ones (around 0.3 g VS/L·day in Period 1 and 0.5 g VS/L·day in Period 2), which is attributed to different influent wastewater characteristics, hence biomass production. In the lab-scale experiments, microalgae were grown in high rate algal ponds (HRAPs) treating urban wastewater (without limitation of N and P), manually harvested and thickened reaching higher concentration of VS than in the automated demonstration-scale facility, where microalgae were grown in photobioreactors treating agricultural runoff with some nutrients limitation [38]. In fact, a previous study using microalgae for treating agricultural stormwater showed nutrient limitation, which hampered biomass production, mainly in months with low rainfall events [45].

Table 2. Anaerobic digestion performance for thermally pretreated microalgal biomass in laboratory-scale and pilot-scale reactors. Mean values (standard deviation).

Parameter	Laboratory Scale *	Demonstration-Scale (Period 1)	Laboratory Scale **	Demonstration-Scale (Period 2)
Operational conditions				
Thermal pre-treatment HRT (h)	10	21.3 (0.0)	10	21.7 (5.6)
Anaerobic digester HRT (days)	20	20 (0)	30	32 (10)
OLR (g VS/L·day)	0.68 (0.10)	0.28 (0.11)	0.81 (0.02)	0.50 (0.28)
Influent composition				
VS (g/L)	11.2 (1.40)	6.4 (0.7)	23.7 (1.00)	18.1 (7.2)
TS (g/L)	21.1 (3.10)	16.6 (1.7)	34.2 (2.80)	36.1 (15.4)
COD (g/L)	11.84 (0.71)	9.04 (0.98)	25.2 (1.8)	20.92 (11.98)
N-NH_4 (mg/L)	218 (9.54)	156 (120)	260 (6.00)	312 (300)
Effluent composition				
VS (g/L)	9.50 (1.0)	1.8 (1.2)	14.5 (1.10)	9.9 (5.5)
TS (g/L)	19.80 (2.70)	4.6 (3.1)	26.7 (2.70)	23.4 (13.2)
COD (g/L)	10.6 (0.5)	11.3 (8.9)	25.2 (2.1)	14.7 (10.1)
N-NH_4 (mg/L)	323 (17.15)	458 (250)	8.0 (1.0)	456 (310)
Anaerobic digester pH	7.6 (0.4)	7.0 (0.2)	7.55 (0.08)	7.4 (0.1)
VFA (mg COD/L)	150 (58.6)	-	130 (<596 [1])	-
Anaerobic digestion performance				
VS removal (%)	52.3 (3.8)	70.0 (23.6)	39.5 (3.7)	45.7 (18.0)
Methane production rate (L CH_4/L·day)	0.20 (0.10)	0.072 (0.035)	0.19 (0.07)	0.064 (0.053)
Methane yield (L CH_4/g VS)	0.30 (0.09)	0.24 (0.08)	0.24 (0.07)	0.16 (0.05)
Methane content in biogas (%)	68.1 (0.6)	76.7 (0.0)	69.5 (1.7)	76.8 (2.0)

Note: * Data published by Passos and Ferrer, 2014 [26]; ** Data published by Solé-Bundó et al., 2018 [27]; [1] Maximum value achieved.

Consequently, in our study, the methane production rate was much higher in the lab-scale experiments (Table 2), yet the methane yield was not so different. With a HRT of 20 days, the methane yield was 25% higher in the lab-scale experiment (0.30 vs. 0.24 L CH_4/g VS) but with a HRT of 30–32 days it was 50% higher (0.24 vs. 0.16 L CH_4/g VS). When comparing the results, we should bear in mind that the anaerobic biodegradability depends on the microalgae species, which in systems treating real wastewater keep changing over time, depending on the weather conditions and influent characteristics [28,44]. Furthermore, these experiments were conducted with spontaneous mixed cultures dominated by green microalgae in the HRAPs treating urban wastewater (lab-scale experiments), and by cyanobacteria in the photobioreactors treating agricultural runoff (demonstration-scale facility). In addition, lab-scale experiments were conducted under controlled conditions, and manual microalgae harvesting and digester feeding ensured a constant flow rate of

thickened microalgal biomass with a fairly stable OLR. Conversely, the demonstration-scale facility was fully automated, meaning that the operation of a process depended on the success of the previous one and, despite the complexity of operating a microalgae biorefinery like this, with operational issues occurring regularly, the anaerobic digestion stage showed to be quite robust and reproduced reasonably well lab-scale results under real conditions resembling full-scale operation. This was reinforced by the results of stable pH, high methane content in biogas and the similar methane yield when compared to lab-scale results. On the whole, the results suggests that even with a variable microalgal biomass production and composition, and a lower OLR, the anaerobic digestion was a quite robust and straight forward downstream option for microalgal biomass valorisation at demonstration-scale.

In the context of microalgae-based biorefinery or WRRF, the bioproducts obtained in the anaerobic digester were further processed. The produced biogas was subsequently sparged into a 45 L absorption column, fed with mixed liquor from the photobioreactors. The photosynthetic biogas upgrading process was validated at demonstration-scale under outdoors conditions. The continuous operation of the system resulted in the production of biomethane, reducing the content of CO_2 and H_2S and obtaining a concentration of CH_4 between 94.1% and 98.8%, complying with most international regulations for methane injection into natural gas grids [17]. Moreover, the digestate was further stabilised in a sludge treatment wetland with an effective surface area of 6 m^2 and height of 1.5 m. The wetland was planted with common reed (*Phragmites australis*) and the digestate was daily pumped and fed to the wetland through a sludge distribution system consisting in a net of pipes with risers. The digestate was mineralised and dewatered in the wetland, producing a soil like structure with 12.5–12.8% dry matter content. According to the nutrient and heavy metals content (below the limits for reuse of sludge in arable land), the material could be used as soil amendment or biofertilizer.

5. Conclusions

This study assessed the scalability of the anaerobic digestion of pretreated microalgal biomass by comparing the results from a demonstration-scale microalgae biorefinery with those previously obtained at lab-scale. With the thermal pretreatment of microalgal biomass, the degree of solubilisation was on average 45.7%, which means that almost half of the influent particulate COD was converted into soluble COD. When comparing the soluble COD before and after the pretreatment, it was increased from 456 to 2625 mg/L, representing a 5.8-fold solubilisation. In the anaerobic digester, the average VS removal was 70% and the methane yield up to 0.24 L CH_4/g VS, which were similar to the lab-scale results. Overall, the anaerobic digestion step of the microalgae biorefinery showed to be quite robust and reproduced reasonably well lab-scale results under real conditions resembling full-scale operation.

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