Integrating microalgae tertiary treatment into activated sludge systems for energy and nutrients recovery from wastewater

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Abstract

In this study, microalgae digestate and secondary effluent were used to grow microalgae in a tertiary wastewater treatment, and then, the biomass was co-digested for biogas generation. A 30L closed-photobioreactor was used for microalgae cultivation. The biomass, mainly composed by *Scenedesmus* sp., reached and maintained a concentration of 1.1 gTSS/L during 30 days. A complete removal of N-NH$_4^+$ and P-PO$_4^{3-}$ and high nitrates and organic matter removals were achieved (58 % N-NO$_3^-$ and 70 % COD) with 8d of HRT. The potential biogas production of the cultivated microalgae was determined in batch tests. To improve their biodegradability, a novel method combining their co-digestion with activated sludge after a simultaneous autohydrolysis co-pretreatment was evaluated. After the co-pretreatment, the methane yield increased by 130 %. Thus, integrating microalgae tertiary treatment into activated sludge systems is a promising and feasible solution to recover energy and nutrients from waste, improving wastewater treatment plants sustainability.

Keywords: anaerobic digestion, autohydrolysis pretreatment, bioenergy, biogas, centrate, microalgal biomass
1. Introduction

Until now, wastewater treatment plants (WWTPs) were mainly conceived for removing contaminants and organic matter, and were designed and managed to protect human and environmental health (Muga and Mihelcic, 2008). However, the increasing water scarcity forces the need for new technological solutions with low cost and low energy demand (Chisti, 2008). To transform a conventional wastewater treatment system into a self-sustainable process it is necessary to shift from the current model towards a new one in which wastewater treatment systems will become a low energy processing industry, able to generate marketable products rather than wastes. For this reason, special efforts have been made recently to increase energy and resource recovery from wastewater by producing valuable byproducts (e.g. biofuels) from WWTPs.

Under this scenario, nature-based treatment solutions, such as microalgae-based systems, are conceived as a breakthrough to a new model for wastewater treatment (Pittman et al., 2011). Indeed, such systems are able to reuse nutrients from wastewater and other wastes (i.e. digestate from anaerobic digestion) in order to grow microalgae biomass which can be used as bioenergy feedstock (Uggetti et al., 2014a). However, the alternative of recycling microalgae digestate has been poorly explored. The main concern in the use of digestate as nutrient for microalgae growth is the elevated ammonium content. Though, this inconvenience may be solved by diluting it with another low strength waste effluent (i.e. secondary effluent from wastewater treatment).

Considering small-medium conventional WWTPs based on the activated sludge process with anaerobic digestion for waste activated sludge (WAS) treatment, a microalgae
photobioreactor (PBR) could be introduced as a tertiary treatment in order to improve the treated water quality and increase the biogas production (Figure 1). Indeed, the microalgal biomass produced in the PBR could be co-digested with waste activated sludge from the conventional plant. In such a case, their co-digestion could improve the methane productivity and the hydrolysis efficiency compared to each substrate mono-digestion, increasing the bioenergy recovery efficiency of the plant (Zhen et al., 2016). In fact, recent investigation has reported higher methane yield and/or rate when microalgae and WAS are co-digested (Beltran et al., 2016; Neumann et al., 2015). Besides, WAS has inherent enzymes inside its extracellular polymeric substances (EPS) which are released after a thermal pretreatment at 55ºC resulting in autohydrolysis of WAS (Carvajal et al., 2013). Hence, the co-pretreatment and subsequent co-digestion of microalgae and WAS may improve the hydrolysis. Moreover, the digestate from the anaerobic digestion could be reused as a source of nutrients for microalgae biomass growth together with the secondary effluent. In this way, the quality of treated wastewater would be improved, as compared to conventional biological systems, and the digestate would be treated while increasing the concentration of nutrients for microalgae growth.

Following the scheme proposed in Figure 1, this article addresses a novel approach in the field of wastewater treatment. Previous studies focused on microalgae production for biogas production (i.e., Passos et al., 2015, 2013; Passos and Ferrer, 2014), were addressed to treat urban wastewater by means of high rate algal ponds as a secondary treatment. Differently, this study proposes an integrated system of activated sludge and microalgae tertiary treatment for nutrients and bioenergy recovery from wastewater. Thus, the objectives of this research were: 1) to study the microalgal biomass production treating the secondary wastewater
effluent and digestate; and 2) to quantify the methane yield of harvested microalgae biomass co-digested with waste activated sludge after an autohydrolysis pretreatment.

2. Methodology

2.1 Experimental set-up

Experiments were carried out at the laboratory of the GEMMA Research Group (Barcelona, Spain). Microalgae were grown in a closed cylindrical photobioreactor (30L). The PBR was fed with microalgae uncentrifuged digestate diluted in secondary effluent from a pilot high rate algal pond (HRAP) treating municipal wastewater. The latter came from a pilot system treating municipal wastewater which comprised a primary settler, a high rate algal pond (HRAP) and a secondary settler (Gutiérrez et al., 2016). The digestate was obtained from lab-scale anaerobic digesters (1.5 L) that produced biogas from microalgae biomass harvested from the HRAP. A detailed description of the anaerobic digesters and HRAP may be found in Passos et al. (2015).

2.2 Photobioreactor operation

A mixed microalgae culture obtained from a pilot high rate algal pond was utilized as inoculum to start-up the photobioreactor. This inoculum consisted of a community of microalgae, bacteria, protozoa and small metazoan, specifically dominated by the microalgae genus *Chlorella* sp., *Scenedesmus* sp. and *Stigeoclonium* sp. The closed photobioreactor was located indoors and consisted of a cylindrical vessel made of polymethyl methacrylate with a working volume of 30 L. The mixed liquor was stirred by means of an air sparger placed at the bottom of the photobioreactor, at a flow of 10 L/min and a pressure of 0.034 MPa using a 105 W air compressor (model ACQ-012, JAD, China). The photobioreactor design and
operation characteristics may be found elsewhere (Arias et al., 2017). The culture in the photobioreactor was in continuous operation alternating light:dark periods of 12 h. During the illuminance period, light was supplied by an external lamp (600W, Sunmaster, USA) placed at 80 cm in front of the photobioreactor, providing 19,000 lux (289 µmol/m²s). The temperature of the culture along the experimental period ranged from 25 to 29 ºC.

The photobioreactor was fed once a day (semi-continuously) with microalgae digestate diluted in secondary effluent at a ratio of 1:50, and operated at 8 days of hydraulic retention time (HRT) and solids retention time (SRT). The dilution ratio of 1:50 was performed in order to decrease the ammonium (N–NH₄⁺) content to concentrations below 10 mg/L in the photobioreactor influent. The physico-chemical characterization of the digestate and secondary effluent used as influent for microalgae growth in the photobioreactor is shown in Table 1.

2.3 Biochemical methane potential assay

2.3.1. Substrates and inoculum

The microalgae biomass used in the biochemical methane potential (BMP) assays was collected from the photobioreactor effluent after stable operation. At the time, the microalgae biomass was clearly dominated by Scenedesmus sp. Harvested biomass was settled for 1 day, and then thickened for 3h to reach the target total solids (TS) concentration of 2.8 %. This procedure was performed at 5ºC to preserve microalgae properties.
WAS was used as co-substrate for *Scenedesmus* sp digestion. It was obtained from a secondary settler of a conventional WWTP (Barcelona, Spain). WAS had a TS and VS content of 1.8% and 1.3%, respectively. It was stored at 5 °C until use.

Mesophilic digested sludge from the same WWTP (Barcelona, Spain) was used as inoculum for BMP assays and was stored at 5 °C until use.

### 2.3.2. Autohydrolysis pretreatment: preliminary solubilisation assay

A preliminary solubilisation assay was carried out in order to determine the optimal contact time for the autohydrolysis pretreatment. The assay was performed at 55 °C in order to activate WAS enzymes (Carvajal et al., 2013).

The autohydrolysis pretreatment was carried out in four glass bottles with a total volume of 250 mL and liquid volume of 200 ml each. Bottles were placed in a heater under mild continuous mixing using multi magnetic stirrers at a constant temperature of 55 °C. Trials were prepared with microalgae and WAS alone (controls) and with mixtures of microalgae and WAS at different proportions: 50% microalgae + 50% WAS and 80% microalgae + 20% WAS (on a VS basis).

Time course of biomass solubilisation was analysed from the solubilisation curves defined by the solubilisation ratio (S) obtained at increasing exposure times. The solubilisation ratio was defined as follows:

\[ S = \frac{VS_s}{VS} \cdot 100 \]  (1)
where $S$ is the solubilisation ratio expressed as a percentage, $VS_s$ is the soluble volatile solids concentration and $VS$ refers to the total volatile solids concentration.

In order to compare the experimental data of the microalgae and WAS mixtures with the expected solubilisation ratio without substrates interaction, the theoretical solubilisation ratio was calculated using the following equation:

$$S_{\text{calc}} = f_A \cdot S_A + f_{WAS} \cdot S_{WAS}$$

where $S_{\text{calc}}$ is the calculated solubilisation ratio expressed as a percentage, $f_A$ and $f_{WAS}$ refer to the proportion of microalgae and WAS content in each solubilisation trial, respectively, and $S_A$ and $S_{WAS}$ are the experimental solubilisation ratio of microalgae and WAS tested alone, respectively.

### 2.3.3. Microalgae and WAS co-digestion BMP assays

BMP tests were carried out in order to determine the methane yield and rate ($k$) of co-digestion trials with microalgae and WAS, after an autohydrolysis pretreatment. The pretreatment was applied simultaneously to both substrates, taking into account the results of the preliminary solubilisation assay in terms of exposure time (Section 2.2.2). Three conditions were tested: i) 20% of microalgae and 80% of WAS, ii) 50% microalgae and 50% of WAS and iii) 80% of microalgae and 20% of WAS (on a VS basis). The mono-digestion of each substrate (with and without pretreatment) was also performed as control.
All experimental trials were prepared in triplicate with a substrate to inoculum (S/I) ratio of 0.5 g COD/VS/g VS according to Passos et al. (2013). A blank trial without substrate was used to quantify the amount of methane produced by the inoculum. After adding the proper amount of both substrates and the inoculum, serum bottles (160 mL) were filled with distilled water up to 100 mL, flushed with Helium gas, sealed with butyl rubber stoppers and incubated at 35 ºC until biogas production ceased.

A first-order kinetic model (Equation (3)) was applied to assess the performance and the kinetics of (co-)digestion assays.

\[ B = B_0 \cdot [1 - \exp(-k \cdot t)] \]  

where \( B \) represents the cumulative methane production (mL CH\(_4\)/gVS), \( B_0 \) is the final methane production (mL CH\(_4\)/gVS), \( k \) refers to the first-order kinetic constant (days\(^{-1}\)) and \( t \) is time (days).

The pair of experimental data \((B, t)\) was adjusted by the least square method using the SOLVE function from Excel. This allowed the determination of parameters \( k \) and \( B_0 \) of each co-digestion assay.

Furthermore, experimental data obtained by each co-digestion mixture was compared to theoretical values calculated from microalgae and WAS specific methane productions (Equation (4)):

\[ BMP_{calc} = f_A \cdot BMP_A + f_{WAS} \cdot BMP_{WAS} \]
where $BMP_{calc}$ is the calculated BMP, $f_A$ and $f_{WAS}$ refer to the percentage of microalgae and WAS content in each trial, respectively, and $BMP_A$ and $BMP_{WAS}$ are the experimental methane yield of microalgae and WAS mono-digestions, respectively.

### 2.4 Analytical procedures

#### 2.4.1 Tertiary wastewater treatment

Nutrients removal (nitrogen and phosphorous) was monitored taking samples twice per week at the end of the light phase in the photobioreactor influent (1/50 digestate/secondary effluent) and in the mixed liquor of the photobioreactor. Orthophosphate ($P-\text{PO}_4^{3-}$), nitrite ($\text{N}^-\text{NO}_2^-$) and nitrate ($\text{N}^-\text{NO}_3^-$) were determined using ion chromatograph DIONEX ICS1000 (Thermo-scientific, USA), operated in isocratic mode with $\text{Na}_2\text{CO}_3$ and $\text{NaHCO}_3$ as eluents at a temperature of 30 ºC and a flow of 1 ml/min. Values lower than 0.9 mg/L of $\text{N}^-\text{NO}_2^-$, 1.12 of $\text{N}^-\text{NO}_3^-$, and 0.8 mg/L of $\text{P-PO}_4^{3-}$ were considered below the limit of detection (LOD). On the other hand, ammonium ($\text{N}^-\text{NH}_4^+$) was measured by the colorimetric method indicated in Solorzano (1969). Total inorganic nitrogen (TIN) was calculated as the sum of $\text{N}^-\text{NH}_4^+$, $\text{N}^-\text{NO}_2^-$ and $\text{N}^-\text{NO}_3^-$. Samples were analyzed in triplicate. Soluble chemical oxygen demand (CODs) was determined according to Standard Methods (APHA-AWWA-WPCF, 2001).

Culture conditions as water temperature and pH were continuously measured by probes placed in situ and monitored by a pH-meter with a temperature sensor (Mettler Toledo, USA). Data was collected in periods of 2–3 min in a computer with the software LabVIEW®.
2.4.2 Microalgae biomass production

In order to evaluate the microalgae biomass production, turbidity was measured by means of a turbidimeter (HI 93703, HANNA Instruments, Italy) 3-5 days per week sampling at the end of the light phase. Then, total suspended solids (TSS) were determined from the correlation shown in Eq. (5) ($R^2 = 0.9951$) between turbidity and the dry weight of algal biomass determined gravimetrically as total suspended solids according to the standard method 2540-D (APHA-AWWA-WPCF, 2001).

\[
TSS \left(\frac{g}{l}\right) = 0.0026 \cdot Turbidity + 0.2046
\]  (5)

Microalgaeevolution was monitored once a week using an optic microscope (Motic, China) equipped with a camera (Fi2, Nikon, Japan), connected to a computer with the software NIS-Element viewer®. Microalgae species were identified in vivo using conventional taxonomic books (Bourrelly, 1985; Palmer, 1962).

2.4.3 Biogas production

The total volatile solids (VS) and soluble volatile solids (VSs) were analysed according to Standard Methods (APHA AWWA-WPCF, 2001). The soluble fraction was obtained after biomass centrifugation (UNICEN20, 4200 rpm, 8min, 20 °C) followed by filtration via glass-fiber filters (0.45 µm).

The cumulative biogas production was determined from the pressure increase in the headspace volume of the bottles measured with a manometer (GMH 3161 Greisinger,
Germany). The methane content in biogas was periodically analysed by gas chromatography, using a chromatograph with a thermal conductivity detector (Trace GC Thermo Finnigan with Hayesep packed column) and injector/detector/oven temperatures were 150, 250, 35 °C, respectively, using helium gas as carrier.

3. Results and discussion

3.1 Wastewater treatment performance

The closed photobioreactor was operated as a tertiary wastewater treatment to remove nutrients (N and P) from the secondary effluent (treated wastewater). Additionally, it treated the digestate, which in turn increased the concentration of nutrients for microalgae growth. Although the concentration of nutrients was not constant over the experimental period, N–NH$_4^+$ was almost completely removed and P-PO$_4^{3-}$ was never detected in the photobioreactor effluent (Figure 2). The pH was not regulated and values ranged from 9.4 to 11.5 in dark and light periods, respectively, due to the photosynthetic activity.

As shown in Figure 2, initial N-NO$_3^-$ showed a decreasing pattern over time. This is due to the variations on nitrification processes in the secondary effluent caused by seasonal changes in the HRAP performance (Arias et al., 2017 and Garcia et al., 2000), leading to changes in N-NO$_3^-$ concentrations in the influent. In any way, the average removal during the period of the experiment was 58 %. Indeed, the lack of N–NH$_4^+$ could enhance nitrates consumption as nitrogen source by microalgae since it has been shown that microalgae tend to prefer N–NH$_4^+$ over N-NO$_3^-$, and nitrate consumption does not occur until N–NH$_4^+$ is almost completely consumed (Garcia et al., 2000).
Regarding the CODs, the average concentration in the influent was 141±4 mg/L, which was reduced by 50.6 % over the first 3 weeks of operation and 70 % during the last 2 weeks (Figure 2). This increase in the COD removal efficiency during the last 2 weeks might be caused by an increment in the proportion of biodegradable organic matter in the influent. Notwithstanding, the CODs of the photobioreactor effluent was always below the discharge limit of 125 mg O₂/L (Directive 98/15/EC, 1998).

The biomass was clearly dominated by *Scenedesmus* sp. In general, the performance of this culture as a tertiary treatment for the digestate diluted in secondary effluent is comparable to other studies using different microalgae species that typically grow on wastewater. Olguín et al. (2003) treated anaerobically digested pig slurries diluted in seawater, and achieved removals around 90, 87 and 50 % for N--NH₄⁺, P-PO₄³⁻ and COD, respectively. Similar results were obtained by Cañizares et al. (1994), achieving removals above 90 % in both N--NH₄⁺ and P-PO₄³⁻ during the treatment of the pretreated pig slurries with *Spirulina maxima*.

In previous studies most of the removal efficiencies achieved with different microalgae consortia range between 60 % and 99 % (Olguín et al., 2003; Ruiz-Marín et al., 2010; Van Den Hende et al., 2016; Viruela et al., 2016). Such removals demonstrate that in general, algae-based wastewater treatment systems are a feasible alternative for nutrients and organic matter removal regardless of the type of culture. Remarkably, the results of this study reached higher removals of NH₄⁺ and P-PO₄³⁻ in comparison to the study of Viruela et al. (2016) and Wang et al. (2010) treating only anaerobic effluents (centrate), and the study of Arias et al. (2017), treating microalgal digestate diluted with secondary effluents. This fact could be
directly influenced by an efficient uptake of nutrients by microalgae, which can be considered by means of the high biomass concentration reached in this study. Additionally, the HRT of 8 d might be also contributing to the high removals obtained in both nutrients and COD in this study. Indeed long retention times are recommended to improve removal efficiencies in cases of low nutrients availability (Munoz and Guieysse, 2006).

3.2 Microalgae growth

As shown in Table 1, the secondary effluent had low N–NH$_4^+$ concentration (0.5 mg/L) and the digestate provided an additional N–NH$_4^+$ and phosphorous source to the photobioreactor which enhanced microalgae growth. During the experiment, the biomass showed an exponential growth during the first 5 days, increasing the initial concentration of 0.5 gTSS/L by 57.0 %. After that, a constant concentration of 1.1±0.1 gTSS/L was achieved and maintained throughout the experiment. The high biomass obtained in this study suggests the utilization of all the influent dissolved inorganic N and P available in form of N–NH$_4^+$, N–NO$_3^-$ and P–PO$_4^{3-}$, but also of other organic forms of N and P as shown by (García et al., 2002).

At the beginning the mixed culture was mainly dominated by Stigeoclonium sp. However, after the 10th day, the culture was clearly dominated by Scenedesmus sp. This could be influenced by the N/P ratio (12:1) in the photobioreactor. Indeed, Viruela et al., (2016) and Xin et al., (2010) reported ratios from 5:1 to 12:1 to be the optimal for the dominance of Scenedesmus sp. over other species. This specie in particular is known to have high growth rate in spite of low nutrients availability, specially to P limitation (Cai et al., 2013; Xin et al., 2010). In addition to nutrients availability in the culture, high adaptability of this genus to
several factors could facilitate their dominance over other green microalgae and cyanobacteria. These factors include high tolerance to light limitation (Liu et al., 2017) as well as high light intensities (Huisman et al., 1999), efficient adaptation to wide ranges of pH from 7.1 (Zhang et al., 2014) to 10.5 (da Fontoura et al., 2015). Indeed, their adaptability to grow in the digestate of different biomass feedstocks has already been demonstrated (Marcilhac et al., 2014; Uggetti et al., 2014a). These studies highlighted their capacity to grow under high N–NH$_4^+$ content, phosphorous limitation and high pH. Furthermore, this species is among the fastest growing green microalgae in wastewater and produce high yields in terms of carbohydrates or lipids (Komolafe et al., 2014; Rodolfi et al., 2009), which represents an advantage in terms of their conversion to biogas or biofuels.

In addition to *Scenedesmus* sp., a variety of microalgae and cyanobacteria have shown the capacity to grow on diluted and undiluted digestates from various sources. For instance, the digestate from swine slurry (Cheng et al., 2015), sewage sludge (Uggetti et al., 2014b), abattoir digestate (Bchir et al., 2011), swine manure (Hu et al., 2012) and poultry manure (Iyovo et al., 2010) are adequate for microalgae biomass production. Regarding the studies focused on recycling microalgae digestate for biomass production, Prajapati et al., (2014) used the digestate from anaerobic digestion of *Chroococcus* sp. diluted in tap water as nutrient supplement for microalgal growth. In that case, the microalgae concentration was 0.8±0.1 g TSS/L in a batch process. Likewise, in the study of Arias et al. (2017), digestate diluted with secondary effluent was employed to grow and select cyanobacteria, achieving a biomass production between 0.4 and 1.05 g TSS/L. In our research, higher concentrations (1.1±0.1 g TSS/L) were reached by utilizing digestate diluted with secondary effluent under semi-continuous mode.
3.3 Autohydrolysis pretreatment effect on biomass solubilisation

The effect of the autohydrolysis pretreatment was initially evaluated by the biomass solubilisation increase (Figure 3). WAS reached the highest solubilisation ratio (25.7 %) and microalgae the lowest (11.4 %). In view of the results, microalgae showed to be less biodegradable than WAS due to the resistant structure of their cell wall. Case in particular, *Scenedesmus* has been reported to have a complex multilayer cell wall (Tukaj and Bohdanowicz, 1995).

The results obtained in this study are in accordance with those obtained by Mahdy et al., (2015), who observed higher solubilisation rates with WAS than microalgae after a thermal pretreatment at 120 °C for 40 min. Besides, similar solubilisation rates for WAS were obtained by Carvajal et al. (2013) (25 % for proteins and 21 % for carbohydrates), who studied how inherent enzymes of WAS were released by applying a thermal pretreatment at 55 °C.

Considering the mixed substrates, at the end of the assay the solubilisation ratios were 21 % and 15 % for the mixtures with 50 % and 80 % of microalgae, respectively. Indeed, the solubilisation ratio decreased proportionally to the concentration of WAS decrease (R²=0.95). This proportionality was confirmed by comparing experimental data with theoretical solubilisation ratios, calculated from Equation (2). This means that there was no co-pretreatment effect, since microalgae solubilisation was not improved by pretreating it together with WAS. Therefore, inherent enzymes of WAS released during the autohydrolysis pretreatment were not effective at disrupting microalgae cell wall.
Finally, Figure 3 shows that all assays reached an asymptote by the end of the assay, meaning that solubilisation ratio increase was stabilised by that time. An increase on the contact time would not entail a significant increase of substrate solubilisation, whereas it would increase the amount of energy needed for the pretreatment. Therefore, 7.5 hours was selected as the optimum contact time for the autohydrolysis pretreatment prior to biochemical methane potential assays. This is in accordance with our previous studies which showed that a contact time of 8 hours was the optimum when pretreating microalgae at low temperature (Passos et al., 2013).

### 3.4 Biochemical methane potential of pretreated microalgae and WAS co-digestion

The anaerobic co-digestion BMP assays lasted 41 days (Figure 4). Regarding the pure substrates, WAS showed the highest methane yield (139 mL CH₄/g VS) while microalgae presented the lowest (82 mL CH₄/g VS) (Table 2). Nonetheless, after the pretreatment, microalgae presented a higher increase with respect to WAS. Indeed, the pretreatment applied to microalgae increased the methane yield by 64 %, achieving a value of 134 mL CH₄/g VS. On the other hand, pretreated WAS showed a production of 204 mL CH₄/g VS, which represents an increase of 47 %. These results are in accordance with the literature highlighting the importance of microalgae pretreatment, since their resistant cell wall hampers microalgae hydrolysis and anaerobic fermentation (Passos et al., 2014). Particularly, Scenedesmus sp. has a complex rigid cell wall which makes even more difficult the accessibility of enzymes to the substrate during the digestion process (González-Fernández et al., 2012).
The cumulative methane yield of the co-digestion trials were 187 mL CH$_4$/g VS, 162 mL CH$_4$/g VS and 132 mL CH$_4$/g VS for the mixtures of WAS with 20 %, 50 % and 80 % of microalgae, respectively. In order to detect potential co-digestion synergies, the theoretical methane yields were calculated according to Equation (4). The results showed neither positive nor negative synergies between substrates, meaning that the co-digestion did not improve microalgae anaerobic biodegradability. The lack of WAS enzymes effect on *Scenedesmus* sp. cell wall disruption, or the low C/N ratio might be responsible for the lack of synergies. These results are in agreement with Costa et al. (2012), who studied the co-digestion of macroalgae species (*Ulva* and *Gracilaria*) with WAS without any pretreatment. Additionally, Neumann et al. (2015) studied the co-digestion of *Botryococcus braunii* and WAS and synergies were neither identified. On the contrary, Wang et al. (2013) observed 23 % increase in biogas production when co-digesting *Chorella* sp. and WAS, with 41 % of microalgae. Despite *Chorella* sp. has a rigid cell wall due to its high content of cellulose, the co-digestion with WAS enhanced the hydrolysis.

The methane content in biogas of each co-digestion assay was periodically measured (Table 2). Results showed no differences among trials. Thus, the methane content was independent of the ratio between co-digestion substrates (Caporgno et al., 2015) and it was neither affected by the autohydrolysis pretreatment nor by the co-digestion.

Moreover, the methane production rate was also analysed through the apparent kinetic constant ($k$) of the first-order experimental model, as defined in Equation (3). Table 2 shows that substrates without pretreatment had the lowest values of $k$ (0.16 days$^{-1}$ and 0.17 days$^{-1}$ for microalgae and WAS, respectively), whereas pretreated substrates increased their kinetic
constants up to 0.27 days\(^{-1}\) and 0.25 day\(^{-1}\) for microalgae and WAS, respectively. Thus, a significant increase of the production rate (69% for microalgae and 47% for WAS) was observed by applying the pretreatment. Moreover, the co-digestion trials showed higher kinetic constants (0.29 days\(^{-1}\), 0.32 days\(^{-1}\) and 0.30 days\(^{-1}\) for 20%, 50% and 80% of microalgae content co-digestions) as compared to the mono-digestions. This evidenced how the co-digestion of microalgae and WAS can improve the mono-digestion of both substrates. Costa et al. (2012), Neumann et al. (2015) and Wang et al. (2013) agreed that co-digestion of microalgae and WAS improved the kinetic constant despite having different conclusion in terms of the final methane yield. This result was considered the main advantage of the studied microalgae and WAS co-digestion, as it may reduce the time needed for reaching the highest biogas production. This means that lower hydraulic retention times, hence smaller digesters could be used, reducing the costs.

3.5 The approach of recycling nutrients in a bioenergy producing system

This study highlights the viability of integrating an algae-based tertiary wastewater treatment system in a conventional WWTP that includes both processes: activated sludge and anaerobic digestion. This short term study also offers an alternative to the recycling use of digestate. Although the reuse of digestate as biofertilizer can promote a sustainable biogas production (Solé-Bundó et al., 2017), this substrate can be combined with secondary effluents as an alternative substrate to produce microalgal biomass. Additionally, this process could improve the treatment of remaining nutrients from secondary effluents and taking advantage of the
nutrients contained in the digestate. Considering the promising results here included, further studies based in long term conditions are recommended. This approach would involve a promising opportunity to close the biorefinery loop, accomplishing a sustainable and self-supporting use of resources and reducing disposal costs and environmental impacts.

4. Conclusions

Microalgal anaerobic digestate diluted with secondary wastewater was an effective source of nitrogen and phosphorus for microalgae growth in a photobioreactor. A complete uptake of N-NH$_4^+$ and P-PO$_4^{3-}$ was observed, while a constant production of 1.1 gTSS/L of algal biomass was achieved. This biomass, mainly composed by *Scenedesmus* sp., supported a low methane yield (82 mlCH$_4$/gVS) that was improved by 130% after an autohydrolysis co-pretreatment and co-digestion with waste activated sludge. Thus, integrating microalgae tertiary treatment into activated sludge systems is a promising and feasible solution to recover energy and nutrients from waste, improving wastewater treatment plants sustainability.

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Table 1. Composition of the wastewater used as photobioreactor feedstock.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Digestate</th>
<th>Secondary effluent</th>
<th>Photobioreactor influent&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>-</td>
<td>-</td>
<td>7.9 ± 0.3</td>
</tr>
<tr>
<td>TSS (g/L)</td>
<td>13.4 ± 8.5</td>
<td>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.26 ± 0.17</td>
</tr>
<tr>
<td>VSS (g/L)</td>
<td>12.3 ± 6.5</td>
<td>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24 ± 0.13</td>
</tr>
<tr>
<td>Alkalinity (mg CaCO&lt;sub&gt;3&lt;/sub&gt;/L)</td>
<td>-</td>
<td>-</td>
<td>153 ± 38.4</td>
</tr>
<tr>
<td>CODs (mg O&lt;sub&gt;2&lt;/sub&gt;/L)</td>
<td>122.8 ± 25.9</td>
<td>18.3 ± 5.5</td>
<td>141.1 ± 36.1</td>
</tr>
<tr>
<td>N–NH&lt;sub&gt;4&lt;/sub&gt; + (mg/L)</td>
<td>459 ± 166.5</td>
<td>0.21 ± 0.84</td>
<td>9.17 ± 3.33</td>
</tr>
<tr>
<td>N–NO&lt;sub&gt;2&lt;/sub&gt; + (mg/L)</td>
<td>&lt;LOD&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.44 ± 0.69</td>
<td>1.53 ± 0.91</td>
</tr>
<tr>
<td>N–NO&lt;sub&gt;3&lt;/sub&gt; – (mg/L)</td>
<td>&lt;LOD&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.94 ± 4.94</td>
<td>15.94 ± 4.94</td>
</tr>
<tr>
<td>TIN</td>
<td>-</td>
<td>-</td>
<td>26.64 ± 3.06</td>
</tr>
<tr>
<td>P–PO&lt;sub&gt;4&lt;/sub&gt; 3– (mg/L)</td>
<td>&lt;LOD&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.18 ± 0.87</td>
<td>2.18 ± 0.87</td>
</tr>
</tbody>
</table>

<sup>TIN</sup>: Total Inorganic Nitrogen

<sup>a</sup>Photobioreactor influent prepared by diluting the digestate in secondary effluent (1:50 ratio).

<sup>b</sup>TSS and VSS in the secondary effluent presented values <0.03 g L<sup>−1</sup>.

<sup>c</sup>LOD: Limit of Detection.
Table 2. Experimental results and data analysis at the end of the biochemical methane potential assays.

<table>
<thead>
<tr>
<th>Methane yield</th>
<th>% CH₄</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg CH₄/g VS</td>
<td>%</td>
</tr>
<tr>
<td>Microalgae (M)</td>
<td>82 ± 10</td>
<td>63.3 ± 0.1</td>
</tr>
<tr>
<td>WAS</td>
<td>139 ± 3</td>
<td>63.9 ± 0.8</td>
</tr>
<tr>
<td>(M)p</td>
<td>134 ± 6</td>
<td>64.0 ± 0.1</td>
</tr>
<tr>
<td>(WAS)p</td>
<td>204 ± 3</td>
<td>63.5 ± 0.3</td>
</tr>
<tr>
<td>(20 %M+80 %WAS)p</td>
<td>187 ± 9</td>
<td>64.0 ± 0.4</td>
</tr>
<tr>
<td>(50 %M+50 %WAS)p</td>
<td>162 ± 6</td>
<td>64.3 ± 0.9</td>
</tr>
<tr>
<td>(80 %M+20 %WAS)p</td>
<td>132 ± 2</td>
<td>64.6 ± 0.7</td>
</tr>
</tbody>
</table>

p = pretreated
Figure 1. General scheme of the system proposed in this study.
Figure 2. Influent and photobioreactor concentrations of ammonium (N−NH₄⁺), orthophosphates (P−PO₄³⁻), nitrates (N−NO₃⁻) and soluble chemical oxygen demand (CODs).
Figure 3. Solubilisation ratio over the solubilisation assay (10 h).

Note: M = microalgae; WAS = waste activated sludge.
Figure 4. Cumulative methane yield (mg CH\textsubscript{4}/g VS) over the biochemical methane potential assays with \textit{Scenesdesmus} sp. and WAS (co-digestion and mono-digestion). Symbols represent the mean value and standard deviation.

Note: M= microalgae; WAS= waste activated sludge; p = pretreated