Water resource recovery coupling microalgae wastewater treatment and sludge co-digestion for bio-wastes valorisation at industrial pilot-scale

Romina Avila[a], Álvaro Justo[ab], Elvira Carrero[b], Eudald Crivillés[b], Teresa Vicent[a], Paqui Blánquez[a*]

[a] Chemical, Biological and Environmental Engineering Department, Escola d'Enginyeria, Universitat Autònoma de Barcelona, E-08193, Bellaterra, Barcelona, Spain.

[b] Miguel Torres S.A., Miquel Torres i Carbó 6, 08720, Villafranca del Penedès, Barcelona, Spain.

* Corresponding author: Paqui Blánquez

E-mail address: paqui.blanquez@uab.cat

Full postal address: Chemical, Biological and Environmental Engineering Department, Escola d'Enginyeria, Universitat Autònoma de Barcelona, E-08193, Bellaterra, Barcelona, Spain.

Abstract

This case study is part of a circular bioeconomy project for a winery company aiming to integrate a microalgae-based system within the existing facilities of the winery WWTP, promoting nutrient recovery and transformation into valuable products and bioenergy. Microalgae were used for wastewater treatment, removing $N\text{-}NH_4^+$ (97%) and $P\text{-}PO_4^{-3}$ (93%). A pilot anaerobic reactor was used for batch anaerobic mono-digestion of secondary sludge (WAS) and for co-digestion of WAS and algal biomass. The methane yield using WAS from two different wine production seasons was 155.4 and 132.9 NL CH_4 $kgVS^{-1}$. Co-digestion led to the highest methane yield (225.8 NL CH_4 $kgVS^{-1}$). The application of the bio-wastes for fertilization was assessed through plant growth bioassays: mono- and co-digestion digestates and dry algal biomass enhanced plant biomass accumulation (growth indexes of 163%, 155% and 121% relative to those of the control - commercial amendment, respectively), demonstrating a lack of phytotoxicity.

Keywords: Algae - Waste activated sludge - Biogas - Biofertilizer - Circular bioeconomy

1. Introduction

A transition to Water Resource Recovery Facilities (WRRFs) is crucial to addressing the current challenges of conventional wastewater treatment plants (WWTPs) associated with secondary pollution generated by sludge, energy demand, greenhouse gas (GHG) emissions, and scarcity of mineral resources. WRRFs are focused on recovering water, energy, and nutrients from waste with self-energetic sufficiency (energy neutral) or even generating a positive net energy balance (Sarpong et al., 2020). Microalgae-based systems are a nature-based solution offering a breakthrough towards a new paradigm integrating wastewater treatment with resource and energy recovery (Wollmann et al., 2019). Wastewater is an abundant energy source that can be recovered and reused in a circular economic approach (Nagarajan et al., 2020). By coupling a microalgal photobioreactor (PBR) to conventional biological wastewater systems, the quality of the treated water can be improved, obtaining biomass that can be processed downstream and valorised as a bioenergy feedstock as well as an organic fertilizer (Solé-Bundó et al., 2019).

The European Commission has considered anaerobic digestion of organic wastes among the most energy-efficient technologies to harness the energy potential of biological wastes (EC, 2009a). Coupling microalgae production to wastewater treatment for biofuel production through anaerobic digestion or co-digestion with other substrates is technically and economically feasible (Rajesh Banu et al., 2020). Moreover, the use of microalgae for energy production increases system viability, enhances the efficiency of the process and diminishes WWTP costs (Barroso Soares et al., 2019). Co-digestion of microalgae with a carbon-rich substrate, such as waste activated sludge (WAS), leads to an appropriate C/N ratio of the mixture and prevents inhibition by ammonia (due to the high protein content in microalgal biomass) and volatile fatty acids (VFAs) (Milledge et al., 2019). Other advantages of co-digestion are that it balances macro- and micronutrients, enhances alkalinity and process stability, increases the load of biodegradable organic matter, improves hydrolysis efficiency while reducing the risk of inhibition, and increases methane productivity (Hagos et al., 2017).

The European Commission set a goal of a 30% reduction in non-renewable sources used in fertilizer production, with the aim of substituting recycled bio-wastes or other sources (EC, 2016). In microalgal anaerobic digestion-based biorefineries, nutrients can be recycled from wastewater, and the harvested microalgae can be used as a bio-based fertilizer, reducing or avoiding dependency on synthetic fertilizers and their associated GHG emissions (Coppens et al., 2016). In addition, the anaerobic can be applied for agronomic use since it is a biologically stable organic matrix and a source of nutrients, thereby increasing nitrogen, phosphorous, and microbial biomass in the soil (Guilayn et al., 2019).

This work includes a case study of an industrial winery WWTP (“winery WWTP” hereafter) aiming to identify a circular bioeconomic model for nutrients and bioenergy recovery from wastewater and sludge. Currently, the disposal of the WAS generated by the WWTP is delivered for external management and disposal, having an impact on the costs of running the WWTP. Efficient resource recovery is the major driving force for shifting from the current industrial WWTP to a WRRF. To this end, this study evaluates integrating a microalgae-based system for tertiary wastewater treatment into an industrial WWTP, bioenergy production from sludge and microalgae co-digestion, and the application of digestate and dry algal biomass as biofertilizers. This case study at the pilot scale will contribute to identifying challenges to and potentialities for establishing baselines for full-scale implementation.

2. Materials and methods

2.1. Photobioreactor

One litre of microalgal culture (TS = 0.8 g L^{-1}) obtained from a 3 L stock photobioreactor fed with Mann and Myers media (Mann and Myers, 1968) was used as inoculum to start-up the wastewater column PBR (“PBR” hereafter). Wastewater treatment was performed in a 50 L PBR (working volume of 45 L) made of transparent methacrylate and fed with 44 L of the secondary effluent from the winery WWTP. An air sparger (at a flow of 3.9 L min^{-1}) placed at the bottom of the PBR was used for microalgal stirring and aeration. The PBR was located inside a greenhouse in the facilities of the winery (Barcelona, Spain). The mean temperature during the experimental period was 19.5 °C, and the net sunlight irradiation was 26.3 MJ m^{-2} d^{-1}.

Microalgae in the PBR grew autotrophically in batch mode for 7 days until reaching the exponential growth phase and were harvested by natural sedimentation. An optical microscope and conventional taxonomic literature were used for morphological identification of algal biomass as previously reported (Avila et al., 2021). A mixed microalgal culture of *Scenedesmus* sp. and *Chlorella* sp. as the predominant species and bacteria and protozoa at a minor abundance was identified.

2.2. Pilot anaerobic reactor

A cylindrical stainless-steel digester (AISI 316 L) with a total volume of 70 L (working volume of 50 L) was designed and employed as the pilot anaerobic reactor. The reactor contained three outlet pipes and a safety valve (1 bar). The two upper outlet pipes of the reactor were connected to a temperature sensor (Waft, Barcelona) and a Mariotte column to measure volumetric biogas production by water displacement. The content inside the reactor was kept homogenized by continuous agitation (S.S.C. 9-2G, Agitaser, Barcelona). The reactor had a thermal jacket (Elementos calentadores, Barcelona, Spain) to maintain mesophilic conditions (35 – 37 °C).

2.3. Anaerobic digestion process

Mono- and co-digestion were performed in the anaerobic reactor under mesophilic conditions using WAS and WAS-microalgae mixtures, respectively. WAS was obtained from the secondary clarifier of the winery WWTP after the biological treatment, while algal biomass was collected from the PBR effluent. A mesophilic digestate from the anaerobic digester of a municipal WWTP (Vilafranca del Penedès, Barcelona) was used as inoculum. The pilot anaerobic reactor was operated in sequencing batch reactor (SBR) mode. Each cycle included the following steps: feeding, reaction, settling, and discharge. First, the reactor was fed with the inoculum and the substrate; second, the anaerobic digestion was carried out for 30 days; third, the reactor content was settled; and fourth, 1/3 of the reactor content was discharged, with the rest kept as inoculum for the next cycle. Before the beginning of a new cycle, the reactor content was left for 5 days for degasification to guarantee the depletion of the residual organic matter. Two cycles were performed for WAS mono-digestion, while two cycles were carried out for WAS-microalgae co-digestion. In the co-digestion cycles, two microalgal doses were employed: 0.2% and 1.8% (on a VS basis). These percentages were set according to the annual generation of both substrates by the winery WWTP.

Anaerobic digestion process performance was evaluated by monitoring the following parameters: total solids (TS), volatile solids (VS), VFAs, pH, and alkalinity (total alkalinity (TA), partial alkalinity (PA), and intermediate alkalinity (TA-PA)). TS, VS, and alkalinity were determined according to the procedures defined in Standard Methods (APHA, 2008) while VFAs were analysed as previously defined in Avila et al. (2021). The alkalinity index (IA/PA ratio) was employed to evaluate reactor stability, indicating to what extent the concentration of acids (estimated by the IA) exceeded the buffer capacity of the system provided by HCO_3^- (estimated by the PA) (Martín-González et al., 2010). Samples were taken from the reactor three times per week, in addition to the samples taken at the beginning and end of each cycle. Biogas production was quantified in the Mariotte columns. Periodically, samples of the generated biogas were taken in sealed gas sampling bags to analyse the CH_4 and CO_2 contents.

2.4. Plant growth bioassay

The reuse of liquid and solid bio-wastes from the anaerobic reactor and the PBR as a soil amendment or biofertilizer was assessed through a modified phytotoxicity assay employing plant growth (Solé-Bundó et al., 2017). Bioassays are phytotoxicity tests used to evaluate the influence of bio-wastes on biomass accumulation in plants (Alburquerque et al., 2012) and were carried out at the end of the winter season and the beginning of the spring season (mean temperature of 23 °C and daily photoperiod of 12.6 h). They were performed in plastic pots (⌀ 8.5 cm and height 8.5 cm, with drainage holes in the bottom) placed inside holder vessels in a greenhouse located at the facilities of the winery company. The studied bio-wastes included digestate from the 1st cycle (WAS mono-digestion) (F1), digestate from the 3rd cycle (WAS-microalgae co-digestion) (F2), algal biomass harvested by sedimentation and solar drying (F3), and sedimented PBR effluent (without biomass) (F4). Moreover, an organic commercial amendment (Fervo-64, Fervosa, Barcelona) used in conventional agriculture was employed as the reference control (FC). A total of 24 pots were used to test each bio-waste (n = 120 pots). First, the pots were filled with 281.0 ± 8.9 g of compost and commercial perlite and placed inside the holder vessels. Then, the holder vessels were filled with water to reach saturation of the substrate. After 24 h, surplus water was withdrawn from the holder vessels, and after 1 h, the pots were weighed. After 6 days, the pots were weighed to quantify water evaporation and determine the amount of water to be added. Then, lettuce seedlings (*Lactuca sativa)* were sown in each pot (day 0) and maintained under the same moisture conditions. On day 1, the bio-wastes were applied to the pots according to their mode of application as an irrigation liquid (in case of F1, F2, and F4) or by soil drench (in case of solid F3 and FC). The dose of bio-waste added to each pot was determined based on the nitrogen content in the target bio-waste, similar to the total nitrogen (TN) dose applied by other authors (Mulbry et al., 2005; Sigurnjak et al., 2017). The bioassay was performed for 40 days. On days 20, 27, and 40, a defined quantity of seedlings (on day 20, n = 7 pots; on day 27, n = 8 pots; and on day 40, n = 9 pots) were harvested from the pots, and the following parameters were measured to monitor plant growth: plant height (cm), shoot fresh weight (g), and root fresh weight (g). Plant height was measured from the point where roots started to grow to the top of the highest fully expanded leaf. At the end of the experiment, the total dry mass (TS) of the plants (shoots + roots) was determined after drying overnight at 105 °C. The growth index (GI) was used to assess the influence of the four bio-wastes and the control on plant growth and was expressed as a percentage of the total plant weight with respect to the reference control.

2.5. Analytical methods

TS and VS were determined according to the procedure defined in Standard Methods (APHA, 2008). COD, TN, ammonium (N-NH_4^+) and orthophosphate (P-PO_4^{-3}) were measured using test kits (Merck, Germany). TA and PA were determined by a titration method at pH 4.3 and pH 5.75, respectively, and IA was calculated as the difference between TA and PA, following procedure 2320B in Standard Methods (APHA, 2008).

Biogas production was normalised and expressed as the volume of biogas or methane generated per mass of VS of added substrate (NL biogas or CH_4 kg VS^{-1}) under standard conditions (273.15 K and 1.0133 bar). The CO_2 and CH_4 contents in the biogas were analysed by gas chromatography, and the VFA concentrations were analysed by high-performance liquid chromatography. Both procedures were previously described in Avila et al. (2020).

Macro- and micronutrients and heavy metals in the plant bioassays were analysed by an external laboratory (Eurofins Agroambiental, Lleida, Spain).

2.6. Data analysis

The experimental data from the bioassays were statistically analysed using ANOVA for repeated measures in the R environment (version 3.6.3). Differences were considered significant when $p < 0.05$, and post hoc comparisons were performed when the null hypothesis was rejected.

Biogas production was modelled by the modified Gompertz equation, and kinetic parameters for anaerobic degradation were calculated according to Eq. 1 (Nielfa et al., 2015):

$$\mathrm{P_{net}(t) = P_{max}.\exp\left\{-\exp\left[\frac{R_{max}.e}{P_{max}}(\lambda - t) + 1\right]\right\}} \qquad \text{Eq.1}$$

where $P_{net}(t)$ is the net cumulative biogas yield (NL biogas kg VS^{-1}) at time t, P_{max} is the biogas yield potential (NL biogas kg VS^{-1}), R_{max} is the maximum biogas production rate (NL biogas kg VS^{-1} d^{-1}), t is the digestion time (d), and λ is the lag phase (d). The experimental data were adjusted using a first-order kinetic model to assess the performance of the digestion according to Eq. 2 (Angelidaki et al., 2009):

$$\mathrm{B\ (t) = B_0\ (1 - \exp^{-K_H.t})} \qquad \text{Eq.2}$$

where B(t) is the cumulative biogas yield at time t (NL biogas kg VS^{-1}) obtained experimentally, B_0 is the ultimate biogas yield (NL biogas kg VS^{-1}), K_H is the hydrolysis rate constant (d^{-1}), and t is the digestion time (d). These parameters were estimated using MATLAB R2015a (MathWorks Inc. Natick, MA, USA).

3. Results and discussion

3.1. Microalgal tertiary wastewater treatment

The PBR used for tertiary wastewater treatment was fed secondary effluent from the winery WWTP using wastewater as a nutrient source for microalgal cultivation. The average values for the parameters of the PBR influent and the settled effluent during the experimental period (from September 2018 to March 2019) are shown in Table 1. High removal efficiencies were achieved for N-NH_4^+ (97%) and P-PO_4^{-3} (93%). Although the mean COD value in the effluent increased by 18%, this concentration was lower than the threshold value that was authorized for the company to discharge (160 mg O_2 L^{-1}). This surplus could be associated with the accumulation of extracellular organic matter produced during algal wastewater treatment (Higgins et al., 2018; Wang et al., 2015). Moreover, the N-NH_4^+ and P-PO_4^{-3} concentrations in the settled effluent were in compliance with threshold values for reutilization or discharge into receiving waters (0.4 and 0.6 mg L^{-1} for N-NH_4^+ and P-PO_4^{-3}, respectively). Overall, the quality of the effluent was improved. The results obtained were similar to those reported for algae-based tertiary wastewater treatment (Arbib et al., 2013; Arias et al., 2018). As stated in other studies, it is shown that the symbiotic interactions between microorganisms in microalgae-based systems contribute to nutrients´ removal (Qu et al., 2021).

Table 1

3.2. Anaerobic co-digestion of waste activated sludge and algal biomass

This work is part of a circular bioeconomy proposal that aims for the integration of a microalgae-based system for tertiary wastewater treatment, with the ultimate goal of transitioning from a WWTP to a WRRF, as represented in Fig. 1. A closed-loop use of resources includes algal biomass valorisation for energy recovery through anaerobic co-digestion of WAS and algal biomass, using the biogas for tractor biofuel and the digestate as soil amendment or biofertilizer in the company vineyards. This system is also coupled to algal biomass valorisation for nutrient recovery from wastewater through the application of dry algal biomass as biofertilizer in the vineyards and the use of the PBR effluent for irrigation. The assessment of the current winery WWTP and the use of the generated biogas for tractor biofuel are beyond the scope of the present study.

The performance of a pilot-scale anaerobic reactor for WAS mono-digestion (1st and 2nd cycles), followed by WAS and algal co-digestion (3rd and 4th cycles), was tested. Table 2 presents the characterization of substrates and inoculum employed in cycles 1 to 4. Process stability was evaluated by monitoring the pH, alkalinity index, VFA concentration, and methane composition.

Figure 1

Table 2

3.2.1. Anaerobic mono-digestion of waste activated sludge

When finishing the 1st cycle, a volume of 31 L of the digestate was used as inoculum for the 2nd cycle. As shown in Table 3, methane yields of 155.4 and 132.9 NL kg VS^{-1} were generated in SBR cycles 1 and 2, respectively, consistent with values recorded by other authors (Arias et al., 2018). The biogas production profiles of cycles 1 and 2 (Fig. 2a) followed a similar trend (due to operation issues, biogas production in cycle 1 was quantified from the ninth day). However, biogas production in cycle 2 decreased from day 23 onwards, suggesting that a large part of the available biodegradable organic matter had been consumed. The VS elimination in cycle 1 was 58% higher than that in cycle 2 (Table 3), which may be associated with the different features of the WAS employed (WAS generated during the wine grape harvesting season possessed a higher organic matter content), as previously reported (Higgins et al., 2018). Regarding VFAs, the acetic acid concentration in cycle 1 increased from day 4 of digestion, maintaining a similar value up to day 19, and its concentration was reduced by the end of the cycle (Fig. 3a). The evolution of the acetic acid concentration in cycle 2 showed a similar pattern, and acetic acid was no longer detected after day 21, while the butyric acid concentration increased from day 18 to day 30, after which it tended to diminish (Fig. 3a and 3c), enhancing biogas production (Fig. 2a). Likewise, in both cycles, the alkalinity index (IA/PA ratio) generally remained below the threshold value of 0.4 (Astals et al., 2012; Martín-González et al., 2010), while the pH ranged between 7.7 and 8.3 (Fig. 4a). These values indicate stable performance of the reactor in both mono-digestions.

Figure 2

Figure 3

3.2.2. Anaerobic co-digestion of waste activated sludge and algal biomass

The third and fourth SBR cycles assessed the co-digestion of a mixture where microalgae were used as a co-substrate for WAS. In comparison to that in cycles 1 and 2, the biogas yield obtained in the 3rd cycle (137.2 NL kg VS^{-1}) was 27% and 16% lower, respectively (Fig. 2b). Furthermore, the slight elimination of VS (4%, Table 3) and major propionic acid accumulation (Fig. 3b) in cycle 3 suggest that the biomass was partially degraded and not converted into methane, showing limited process performance. However, despite these results, no inhibition was observed. Differences between cycles 3 and 4 could be explained by the use of a different inoculum, as in cycle 3, the inoculum might not have been adapted to the microalgal substrate. Additionally, the cycles had different proportions of microalgae, and a major microalgal content in cycle 4 led to a higher biogas yield. In addition, the biogas production rate was analysed by the kinetic constant with K_H = 0.0992 d^{-1}, indicating accelerated co-digestion in comparison to that in cycle 2 (Table 3). Nonetheless, despite the higher kinetics, the biogas yield was lower than that observed in mono-digestion cycles 1 and 2. This fact could be explained by improved hydrolysis of algal biomass by the hydrolytic bacteria in the WAS and the formation of non-biodegradable soluble materials, as also reported elsewhere (Scarcelli et al., 2020).

Significant improvements were attained in cycle 4, obtaining a 92% increase in biogas yield (264.1 NL biogas kg VS^{-1}) in comparison to that in cycle 3 (Fig. 2b) and consistent with results reported in previous WAS and microalgae co-digestion studies (Olsson et al., 2018; Wang and Park, 2015). VS was reduced by 35% (Table 3), attaining a better output than the other cycles. According to Wang et al. (2013), the VS reduction in WAS-microalgae co-digestion could be enhanced by algae addition. Thus, it is possible that co-digestion contributed to the degradation of some poorly degradable organic matter, as also reported by Olsson et al. (2018). Moreover, according to Fig. 3d and Fig. 4b, the system showed high stability in terms of pH (ranging from 7.5 - 8.9), VFAs (not detected), and the alkalinity index (remaining below the reference value). The co-substate addition in cycle 4 led to an increase in biogas yield by 41% and 61% in comparison to that in cycles 1 and 2, respectively. Moreover, the digestion rate was 1.8 - 2.9-fold higher in comparison with that in WAS mono-digestion. The lag phase (λ) estimated by the Gompertz model was longer during the co-digestion cycles (Table 3), which could be associated with the recalcitrance of the microalgal cell wall hampering hydrolysis (Klassen et al., 2016). Despite the lower hydrolysis rate estimated for cycle 4 (K_H = 0.061 d^{-1}), the maximum biogas production rate (R_{max}) was higher than that in the other cycles (Table 3). Co-digestion in the 4th cycle showed an improvement in biogas yield and anaerobic digestion performance (methane content and absence of VFAs). These outcomes demonstrate that the co-digestion strategy with microalgae prevents process instabilities, leads to a more robust process, and can markedly enhance WAS mono-digestion improving the biogas yield and VS reduction. Some authors have reported similar results when co-digesting sludge and algal biomass. A 10% increase in the biogas yield was reported when WAS and *Chlorella* sp. were co-digested in comparison with WAS alone (Wang and Park, 2015). Beltrán et al. (2016) obtained a 22% higher methane yield from WAS and *Chlorella sorokiniana* (ratio 75:25) co-digestion in comparison to WAS mono-digestion. Similarly, Mahdy et al. (2015) reported a higher methane yield when co-digesting three mixtures of *Chlorella vulgaris* and WAS. Nonetheless, other authors have previously reported co-digestion of these substrates, obtaining contradictory outcomes. Wang et al. (2013) reported that co-digestion of WAS and *Chlorella* sp. yielded similar biogas production to that with WAS. The co-digestion of a 25% *Scenedesmus dimorphus* and 75% sludge mixture reduced the methane yield by 3% in comparison to that of the sludge (Peng and Colosi, 2015). Likewise, Scarcelli et al. (2020) reported that despite the co-digestion of WAS and a microalgal consortium mainly composed of *Chlorella* sp. affecting the solubilisation of the substrate, it did not lead to a higher methane yield in comparison to that under WAS mono-digestion. Different methane yields obtained in activated sludge and microalgae co-digestion might have resulted from the diversity of algal species and their growth conditions as well as WAS features, which lead to differences in the digestibility of the substrates (Wang and Park, 2015). Overall, the operation of the anaerobic reactor in SBR mode showed adaptation to seasonal variations in WAS and microalgae production.

Table 3

Figure 4

Additionally, the generated biogas obtained from sludge and algae co-digestion could be upgraded to high biomethane to be used as biofuel in vehicles, providing an eco-friendly alternative for transportation inside agricultural lands (Fig. 1). Considering the fuel consumption of tractors 100% powered by methane (model New Holland T6.180 *Methane Power*) and data from the 4th SBR cycle, for an algal production of 0.63 g L^{-1} d^{-1}, a CH_4 yield of 7.7 L d^{-1} and a production of 2.8 m^3 CH_4 $year^{-1}$ (2 kg CH_4 y^{-1}) were estimated, which is enough to fuel the tractor to run 485 km per year. Other factors influencing tractor fuel consumption should be considered, such as the type of agricultural work performed, the features of the soils, weather conditions, and tractor mechanical maintenance. A full-scale, real case study (EU FP7 All-Gas project) performed in Chiclana (Spain) has efficiently validated the use of biomethane obtained from algal biomass cultivated in wastewater as car biofuel.

3.3. Plant growth bioassays

The effects of the four bio-wastes on plant biomass accumulation were assessed through bioassays. The GI was used to evaluate the phytotoxicity effect of the bio-wastes on the biomass production of lettuce. The studied bio-wastes for agricultural irrigation-fertilization included digestate from SBR cycle 1 (F1), digestate from SBR cycle 3 (F2), dry algal biomass (F3), and PBR effluent (F4). F1 and F2 can be considered biosolids since they were obtained after the anaerobic (co-)digestion of the sludge (Collivignarelli et al., 2019). When coupling microalgae tertiary wastewater treatment to the winery WWTP (Fig. 1), both F3 and F4 are sources of nutrients with the potential to be applied in the vineyards of the company. If anaerobic digestion is not implemented, these bio-wastes could be valorised. Besides, a commercial organic amendment was used as a commercial reference product (FC).

An application schedule was followed in the bioassays. A defined volume or amount of water and bio-wastes were applied to the pots considering the target dose of TN to be added and the TN content in the liquid and solid bio-wastes. In this sense, totals of 2.3 and 8.1 g of F3 and FC, respectively, were applied to the pots on day 1, and then water was added for irrigation according to the water demand of the plant. In the case of liquid bio-wastes (F1, F2, and F4), the same total volume of liquid bio-waste (350 mL) was periodically applied to the pots until day 13, and then, the pots were irrigated with water to restore solution losses by plant uptake and/or evaporation. Thus, F1 was applied at a dose 54% higher than that of F2 and F4.

3.3.1. Plant height and total fresh weight monitoring

The evolution of plant height and total plant (shoot and root) fresh weight on days 20, 27, and 40 is shown in Fig. 5a and Fig. 5b, respectively. Shoot plant weight and plant height were notably enhanced by both digestates (F1 and F2), achieving better outcomes than the other tested bio-wastes (Fig. 5b). Throughout the assay, F1 and F2 showed no significant differences ($p > 0.05$) in root and shoot weights, respectively. In contrast to the reference control, F1 significantly enhanced plant height by 66-78% ($p < 0.05$), while F2 improved it by 41-52% ($p < 0.05$) (Fig. 5a). Regarding root weight, both digestates had lower values throughout the assay in comparison to the reference control (reduced by 9-21% for F1 and by 6-17% for F2), showing no statistically significant differences ($p > 0.05$) (Fig. 5b).

When applying dry algal biomass (F3), the plant height was constant (16-17 cm) at the three sampling times (Fig. 5a). By day 40, the plant heights of pots treated with F3 were statistically similar to those of the reference control ($p > 0.05$) and F4 ($p > 0.05$) (Fig. 5a). Although the shoot fresh weight was 31% higher than that of the control ($p < 0.05$), the root weight of plants with F3 application was reduced by 1% ($p < 0.05$). The treated water obtained from the PBR effluent (F4) slightly improved plant height during the bioassay in comparison to that in the control, with no significant differences ($p > 0.05$). F4 strongly reduced lettuce total weight compared with that in the control (shoot weight was reduced by 25-48%, and root weight was reduced by 8-18%). By day 40, the root mass of the bio-wastes was reduced compared to that of the reference control ($p < 0.05$) (Fig. 5b), which could be explained by the lower nutrient content in reclaimed water. However, in recent years, the use of reclaimed water for agricultural irrigation has been encouraged, contributing to saving freshwater while applying the available nutrients to the soil (Delanka-Pedige et al., 2020).

Figure 5

3.3.2. Growth index

The influence of the four studied bio-wastes on the GI (expressed as a percentage of the total fresh plant mass with respect to the reference control) is shown in Fig. 6. After 40 days, the best performance in terms of the GI was obtained for digestates F1 and F2 (163% and 155% of the control, respectively). Meanwhile, the GI for the dry algal biomass (F3) and the PBR effluent (F4) were 121% and 81% with respect to the control, respectively. Hence, both digestates and the dry algal biomass attained a better outcome in terms of lettuce biomass accumulation in comparison to the reference material, suggesting a lack of phytotoxicity. The better outcome of F1 could be related to its application at a higher dose. The positive effects of F1 and F2 on plant biomass accumulation could be explained by the higher nitrogen available for plant assimilation in the liquid bio-wastes (574 mg TN L^{-1} in F1 and 374 mg TN L^{-1} in F2) in comparison to the other bio-wastes, with nitrogen being the main nutrient present in both digestates. Although the total phosphorus (TP) and total potassium (TK) contents in FC (2.9% of TP and 0.9% of TK) were higher than those in F1, F2 and F4 (0.02, 0.02, and 0.0004% of TP; and 0.13, 0.01, and 0.02% of TK in F1, F2, and F4, respectively), these nutrients require water to be soluble and bioavailable. As previously reported, the GI for F2 could be related to the reduced phytotoxicity of digestates obtained from co-digestion processes, having a dilution effect of inhibitory compounds (Solé-Bundó et al., 2017).

Figure 6

3.3.3. Other parameters monitoring

The heavy metal concentrations in the digestates were below the threshold values defined by the sludge European Directive (CEC, 2003, 1986). Concerning hygenisation, *E. coli* was found in both digestates at lower values than the proposed value ($5 \cdot 10^5$ colony forming units per gram) by the EU

Directive on spreading sludge on land (EC, 2009b). As reported by other authors, digestates could potentially be applied as biofertilizers and/or soil amendments (Guilayn et al., 2019; Wang and Lee, 2021).

Even when dry algal biomass had a similar TP and ammoniacal nitrogen content to those in the control, the nitrogen content in F3 was 3.5-fold higher (56 mg kg^{-1}), while the TK content was reduced by 33% (0.6%). Due to the ability of microalgae to fix nitrogen, they constitute an alternative and low-cost source of nitrogen (Dineshkumar et al., 2018). Algal biomass is considered a slow-release biofertilizer since N and P release from dry algal biomass changes over time (Mulbry et al., 2005). Thus, the application of algal biomass (F3) as a slowly released biofertilizer in agriculture could be a suitable option to offset mineral fertilizer application. Some authors have reported beneficial effects after the use of algal biomass as a biofertilizer. Coppens et al. (2016) reported the use of dry microalgal-bacterial flocs as biofertilizer for tomato cultivation, which showed an initial nitrogen availability of 7% and increased N mineralization by 11% and 25% after 21 and 95 days, respectively, ultimately yielding a plant growth rate equivalent to that when using organic fertilizers. Mulbry et al. (2005) applied dry algal biomass as a soil amendment, reporting an equivalent plant dry weight (corn and cucumber) and nutrient content to those under commercial fertilizer use. They reported that after 21 days, 30-33% of the TN and 39-75% of the TP in algal biomass was available for the plants. Diverse mineralization rates could be explained by different soil properties (type of soil, moisture content, pH, and microbial activity, among others). Biofertilizer from algal biomass is a higher-quality by-product than co-digestion digestates due to its high content of proteins and phytohormones (Arashiro et al., 2018). Another advantage of its application to soils is the absence of NH_3 volatilization (Mulbry et al., 2005), and it is assumed that algal biofertilizer is not lost through runoff or leaching (Fang et al., 2016).

Considering that the generation and application of these bio-wastes would be performed by the same company, facility, storage, transportation, and distribution costs would be negligible. In addition to the quality of the studied bio-wastes, the suitability of their application for fertilization and/or irrigation should meet the current regulations. Organic matter stability and heavy metal content in the soil should be assessed to determine the potential risks of metal accumulation and hygenisation in the soil-plant system (Alburquerque et al., 2012).

4. Conclusions

Microalgae efficiently removed nutrients from the secondary effluent. The co-digestion of WAS and harvested microalgae in SBR mode was 45-70% higher than that for WAS mono-digestion. Bioassays of the generated bio-wastes showed GIs of 163%, 155%, and 121% in comparison to the control for the mono-digestion digestate, co-digestion digestate, and dry algal biomass, respectively, highlighting their potential application as biofertilizers to the company´s arable lands.

This strategy contributes to moving towards the circular use of resources within the company, closing nutrient loops by anaerobic co-digestion of WAS and microalgae, generating biomass, and reusing recovered water in the vineyards.

E-supplementary data of this work can be found in online version of the paper.

Acknowledgments

This work has been funded by the Generalitat de Catalunya (ViTech Project TES/792/2017) and the Spanish State Research Agency (AEI) through the project BECAS (CTM2016-75587-C2-1-R). This work was proposed as a case study for the CYRUS project from the Programme entitled: Cooperation for innovation and the exchange of good practices. Strategic Partnerships for higher education (Project No: 2019-1-RO01-KA203-063773).

References

1. Alburquerque, J.A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M., Bernal, M.P., 2012. Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. Biomass and Bioenergy 40, 181–189. https://doi.org/10.1016/j.biombioe.2012.02.018
2. Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., Van Lier, J.B., 2009. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. Water Sci. Technol. 59, 927–934. https://doi.org/10.2166/wst.2009.040
3. APHA, 2008. Standard methods for the examination of water and wastewater, American Public Health Association. Washington.
4. Arashiro, L.T., Montero, N., Ferrer, I., Acién, F.G., Gómez, C., Garfí, M., 2018. Life cycle assessment of high rate algal ponds for wastewater treatment and resource recovery. Sci. Total Environ. 622–623, 1118–1130. https://doi.org/10.1016/j.scitotenv.2017.12.051
5. Arbib, Z., Ruiz, J., álvarez-Díaz, P., Garrido-Pérez, C., Barragan, J., Perales, J.A., 2013. Long term outdoor operation of a tubular airlift pilot photobioreactor and a high rate algal pond as tertiary treatment of urban wastewater. Ecol. Eng. 52, 143–153. https://doi.org/10.1016/j.ecoleng.2012.12.089
6. Arias, D.M., Solé-Bundó, M., Garfí, M., Ferrer, I., García, J., Uggetti, E., 2018. Integrating microalgae tertiary treatment into activated sludge systems for energy and nutrients recovery from wastewater. Bioresour. Technol. 247, 513–519. https://doi.org/10.1016/j.biortech.2017.09.123
7. Astals, S., Nolla-Ardèvol, V., Mata-Alvarez, J., 2012. Anaerobic co-digestion of pig manure and crude glycerol at mesophilic conditions: Biogas and digestate. Bioresour. Technol. 110, 63–70. https://doi.org/10.1016/j.biortech.2012.01.080
8. Avila, R., Carrero, E., Crivillés, E., Mercader, M., Vicent, T., Blánquez, P., 2020. Effects of low temperature thermal pretreatments in solubility and co-digestion of waste activated sludge and microalgae mixtures. Algal Res. 50, 101965. https://doi.org/10.1016/j.algal.2020.101965
9. Avila, R., Carrero, E., Vicent, T., Blánquez, P., 2021. Integration of enzymatic pretreatment and sludge co-digestion in biogas production from microalgae. Waste Manag. 124, 254–263. https://doi.org/10.1016/j.wasman.2021.01.042
10. Barroso Soares, R., Ferreira Martins, M., Franci Gonçalves, R., 2019. A conceptual scenario for the use of microalgae biomass for microgeneration in wastewater treatment plants. J. Environ. Manage. 252, 109639. https://doi.org/10.1016/j.jenvman.2019.109639
11. Beltrán, C., Jeison, D., Fermoso, F.G., Borja, R., 2016. Batch anaerobic co-digestion of

waste activated sludge and microalgae (*Chlorella sorokiniana*) at mesophilic temperature. J. Environ. Sci. Heal. Part A 51, 847–850. https://doi.org/10.1080/10934529.2016.1181456
12. CEC, 2003. Regulation (EC) No 2003/2003 Relating to Fertilisers. (Brussels, 13 October).
13. CEC, 1986. Council Directive 86/278/EEC on the Protection of the Environment, and in particular of the soil, when sewage sludge is used in agriculture. (Brussels, 12 June).
14. Collivignarelli, M.C., Abbà, A., Frattarola, A., Miino, M.C., Padovani, S., Katsoyiannis, I., Torretta, V., 2019. Legislation for the reuse of biosolids on agricultural land in Europe: Overview. Sustain. 11. https://doi.org/10.3390/su11216015
15. Coppens, J., Grunert, O., Van Den Hende, S., Vanhoutte, I., Boon, N., Haesaert, G., De Gelder, L., 2016. The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. J. Appl. Phycol. 28, 2367–2377. https://doi.org/10.1007/s10811-015-0775-2
16. Delanka-Pedige, H.M.K., Cheng, X., Munasinghe-Arachchige, S.P., Bandara, G.L.C.L., Zhang, Y., Xu, P., Schaub, T., Nirmalakhandan, N., 2020. Conventional vs. algal wastewater technologies: Reclamation of microbially safe water for agricultural reuse. Algal Res. 51. https://doi.org/10.1016/j.algal.2020.102022
17. Dineshkumar, R., Kumaravel, R., Gopalsamy, J., Sikder, M.N.A., Sampathkumar, P., 2018. Microalgae as Bio-fertilizers for Rice Growth and Seed Yield Productivity. Waste and Biomass Valorization 9, 793–800. https://doi.org/10.1007/s12649-017-9873-5
18. EC, 2016. Circular economy: New Regulation to boost the use of organic and waste-based fertilisers [WWW Document]. Eur. Commossion - Fact Sheet. URL https://ec.europa.eu/commission
19. EC, 2009a. Directive 2009/28/EC of 23 April 2009, Official Journal of the European Union.
20. EC, 2009b. Environmental, economic and social impacts of the use of sewage sludge on land. Final Report Part II: Report on Options and Impacts. Prepared by RPA, Milieu Ltd and WRc for the European Commission.
21. Fang, L.L., Valverde-Pérez, B., Damgaard, A., Plósz, B.G., Rygaard, M., 2016. Life cycle assessment as development and decision support tool for wastewater resource recovery technology. Water Res. 88, 538–549. https://doi.org/10.1016/j.watres.2015.10.016
22. Guilayn, F., Jimenez, J., Martel, J.-L., Rouez, M., Crest, M., Patureau, D., 2019. First fertilizing-value typology of digestates: A decision-making tool for regulation. Waste Manag. 86, 67–79. https://doi.org/10.1016/j.wasman.2019.01.032
23. Hagos, K., Zong, J., Li, D., Liu, C., Lu, X., 2017. Anaerobic co-digestion process for biogas production: Progress, challenges and perspectives. Renew. Sustain. Energy Rev. 76, 1485–1496. https://doi.org/10.1016/j.rser.2016.11.184
24. Higgins, B.T., Gennity, I., Fitzgerald, P.S., Ceballos, S.J., Fiehn, O., VanderGheynst, J.S., 2018. Algal–bacterial synergy in treatment of winery wastewater. npj Clean Water 1, 6. https://doi.org/10.1038/s41545-018-0005-y
25. Klassen, V., Blifernez-Klassen, O., Wobbe, L., Schlüter, A., Kruse, O., Mussgnug, J.H., 2016. Efficiency and biotechnological aspects of biogas production from microalgal substrates, Journal of Biotechnology. Elsevier. https://doi.org/10.1016/j.jbiotec.2016.07.015
26. Mahdy, A., Mendez, L., Ballesteros, M., González-Fernández, C., 2015. Algaculture integration in conventional wastewater treatment plants: Anaerobic digestion comparison of primary and secondary sludge with microalgae biomass. Bioresour. Technol. 184, 236–244. https://doi.org/10.1016/j.biortech.2014.09.145
27. Mann, J.E., Myers, J., 1968. On pigments, growth, and photosynthesis of *Phaeodactylum Tricornutm*. J. Phycol. 4, 349–355. https://doi.org/10.1111/j.1529-8817.1968.tb04707.x
28. Martín-González, L., Colturato, L.F.F., Font, X., Vicent, T., 2010. Anaerobic co-digestion of the organic fraction of municipal solid waste with FOG waste from a sewage treatment plant: Recovering a wasted methane potential and enhancing the biogas yield. Waste Manag. 30, 1854–1859. https://doi.org/10.1016/j.wasman.2010.03.029
29. Milledge, J.J., Nielsen, B. V., Maneein, S., Harvey, P.J., 2019. A brief review of anaerobic digestion of algae for BioEnergy. Energies. https://doi.org/10.3390/en12061166
30. Mulbry, W., Westhead, E.K., Pizarro, C., Sikora, L., 2005. Recycling of manure nutrients: Use of algal biomass from dairy manure treatment as a slow release fertilizer. Bioresour. Technol. 96, 451–458. https://doi.org/10.1016/j.biortech.2004.05.026
31. Nagarajan, D., Lee, D.J., Chen, C.Y., Chang, J.S., 2020. Resource recovery from wastewaters using microalgae-based approaches: A circular bioeconomy perspective.

Bioresour. Technol. https://doi.org/10.1016/j.biortech.2020.122817
32. Nielfa, A., Cano, R., Fdz-Polanco, M., 2015. Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. Biotechnol. Reports 5, 14–21. https://doi.org/10.1016/j.btre.2014.10.005
33. Olsson, J., Forkman, T., Gentili, F.G., Zambrano, J., Schwede, S., Thorin, E., Nehrenheim, E., 2018. Anaerobic co-digestion of sludge and microalgae grown in municipal wastewater - A feasibility study. Water Sci. Technol. 77, 682–694. https://doi.org/10.2166/wst.2017.583
34. Peng, S., Colosi, L.M., 2015. Anaerobic Digestion of Algae Biomass to Produce Energy during Wastewater Treatment. Water Environ. Res. 88, 29–39. https://doi.org/10.2175/106143014x14062131179195
35. Qu, W., Zhang, C., Chen, X., Ho, S.H., 2021. New concept in swine wastewater treatment: development of a self-sustaining synergetic microalgae-bacteria symbiosis (ABS) system to achieve environmental sustainability. J. Hazard. Mater. 418, 126264. https://doi.org/10.1016/j.jhazmat.2021.126264
36. Rajesh Banu, J., Preethi, Kavitha, S., Gunasekaran, M., Kumar, G., 2020. Microalgae based biorefinery promoting circular bioeconomy-techno economic and life-cycle analysis. Bioresour. Technol. 302, 122822. https://doi.org/10.1016/j.biortech.2020.122822
37. Sarpong, G., Gude, V.G., Magbanua, B.S., Truax, D.D., 2020. Evaluation of energy recovery potential in wastewater treatment based on codigestion and combined heat and power schemes. Energy Convers. Manag. 222, 113147. https://doi.org/10.1016/j.enconman.2020.113147
38. Scarcelli, P.G., Serejo, M.L., Paulo, P.L., Boncz, M.Á., 2020. Evaluation of biomethanization during co-digestion of thermally pretreated microalgae and waste activated sludge, and estimation of its kinetic parameters. Sci. Total Environ. 706, 135745. https://doi.org/10.1016/j.scitotenv.2019.135745
39. Sigurnjak, I., Vaneeckhaute, C., Michels, E., Ryckaert, B., Ghekiere, G., Tack, F.M.G., Meers, E., 2017. Fertilizer performance of liquid fraction of digestate as synthetic nitrogen substitute in silage maize cultivation for three consecutive years. Sci. Total Environ. 599–600, 1885–1894. https://doi.org/10.1016/j.scitotenv.2017.05.120
40. Solé-Bundó, M., Cucina, M., Folch, M., Tàpias, J., Gigliotti, G., Garfí, M., Ferrer, I., 2017. Assessing the agricultural reuse of the digestate from microalgae anaerobic digestion and co-digestion with sewage sludge. Sci. Total Environ. 586, 1–9. https://doi.org/10.1016/j.scitotenv.2017.02.006
41. Solé-Bundó, M., Passos, F., Romero-Güiza, M.S., Ferrer, I., Astals, S., 2019. Co-digestion strategies to enhance microalgae anaerobic digestion: A review. Renew. Sustain. Energy Rev. 112, 471–482. https://doi.org/10.1016/j.rser.2019.05.036
42. Wang, M., Park, C., 2015. Investigation of anaerobic digestion of *Chlorella* sp. and *Micractinium* sp. grown in high-nitrogen wastewater and their co-digestion with waste activated sludge. Biomass and Bioenergy 80, 30–37. https://doi.org/10.1016/j.biombioe.2015.04.028
43. Wang, M., Sahu, A.K., Rusten, B., Park, C., 2013. Anaerobic co-digestion of microalgae *Chlorella* sp. and waste activated sludge. Bioresour. Technol. 142, 585–590. https://doi.org/10.1016/j.biortech.2013.05.096
44. Wang, W., Lee, D.J., 2021. Valorization of anaerobic digestion digestate: A prospect review. Bioresour. Technol. 323, 124626. https://doi.org/10.1016/j.biortech.2020.124626
45. Wang, Y., Guo, W., Yen, H.W., Ho, S.H., Lo, Y.C., Cheng, C.L., Ren, N., Chang, J.S., 2015. Cultivation of *Chlorella vulgaris* JSC-6 with swine wastewater for simultaneous nutrient/COD removal and carbohydrate production. Bioresour. Technol. 198, 619–625. https://doi.org/10.1016/j.biortech.2015.09.067
46. Wollmann, F., Dietze, S., Ackermann, J., Bley, T., Walther, T., Steingroewer, J., Krujatz, F., 2019. Microalgae wastewater treatment: Biological and technological approaches. Eng. Life Sci. 19, 860–871. https://doi.org/10.1002/elsc.201900071

Figure 1. Schematic representation of the circular bioeconomy proposal for the winery company. References: WW = wastewater. WAS = waste activated sludge. WWTP = Wastewater Treatment Plant. WRRF = Water Resource Recovery Facility.

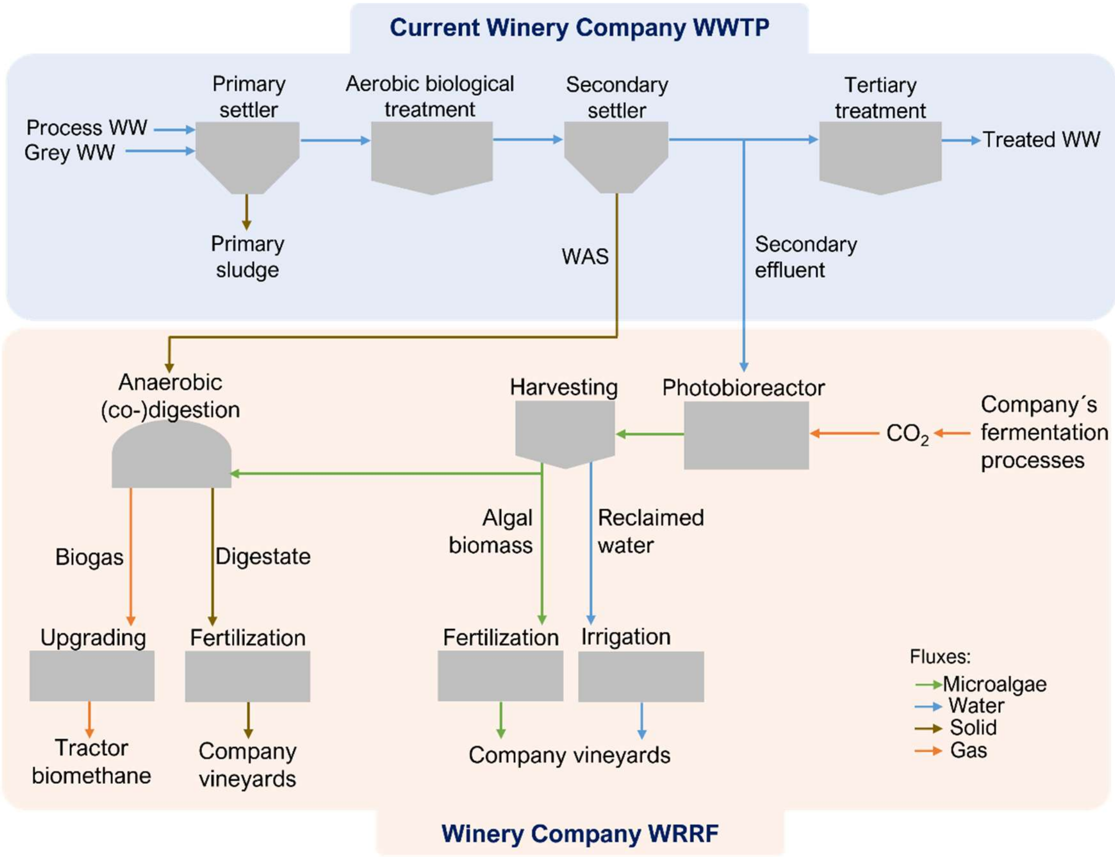
Figure 2. Biogas yield obtained in (a) SBR cycles 1 and 2 (WAS mono-digestion), and (b) cycles 3 and 4 (WAS and microalgae co-digestion). Dots represent experimental data and curves are data estimated by the Gompertz model.

Figure 3. (a) Acetic (HAc), (b) propionic (HPr), (c) butyric (HBu), and (d) total volatile fatty acids (TVFAs) concentration during the SBR cycles 1 (◆), 2 (■), and 3 (●). Note: no VFAs were detected in cycle 4.

Figure 4. pH and alkalinity index (intermediate alkalinity/partial alkalinity (IA/PA) ratio) evolution in (a) cycles 1 (pH-1, IA/PA-1) and 2 (pH-2, IA/PA-2), and (b) cycles 3 (pH-3, IA/PA-3) and 4 (pH-4, IA/PA-4).

Figure 5. Monitoring of (a) plant height, (b) shoot fresh and root fresh weight in bioassays after application of the target organic materials: digestate from WAS mono-digestion (F1), digestate from WAS and microalgae co-digestion (F2), dry algal biomass (F3), PBR effluent (F4), and an organic commercial amendment (FC), at days 20, 27, and 40 after sowing. Error bars represent the standard deviation of the mean (n = 7 at day 20, n = 8 at day 27, and n = 9 at day 40).

Figure 6. Effect of bio-wases on the growth index (expressed as a percentage of the total fresh plant mass with respect to the reference control -organic commercial amendment-) of lettuce (*Lactuca sativa*) at the end of the bioassay (day 40). References = F1, digestate from WAS mono-digestion; F2, digestate from WAS and microalgae co-digestion; F3, dry algal biomass; and F4, PBR effluent.



679

680 Figure 1

681

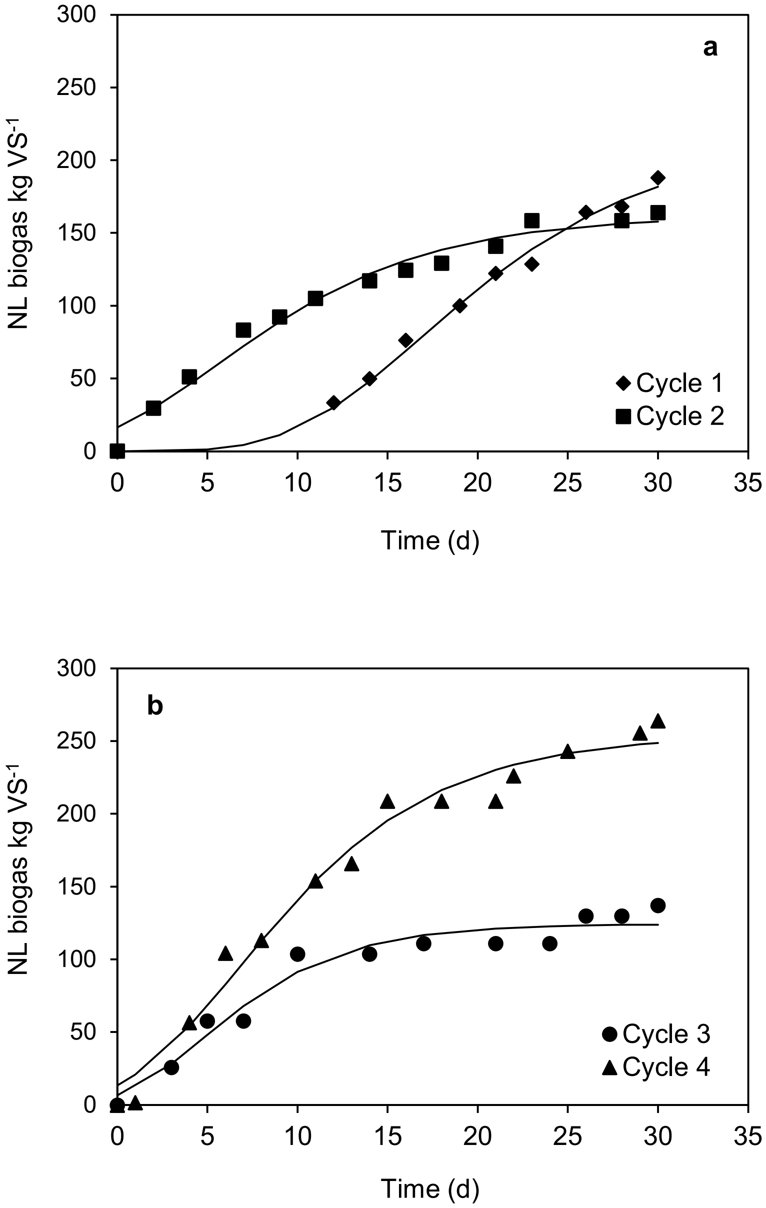
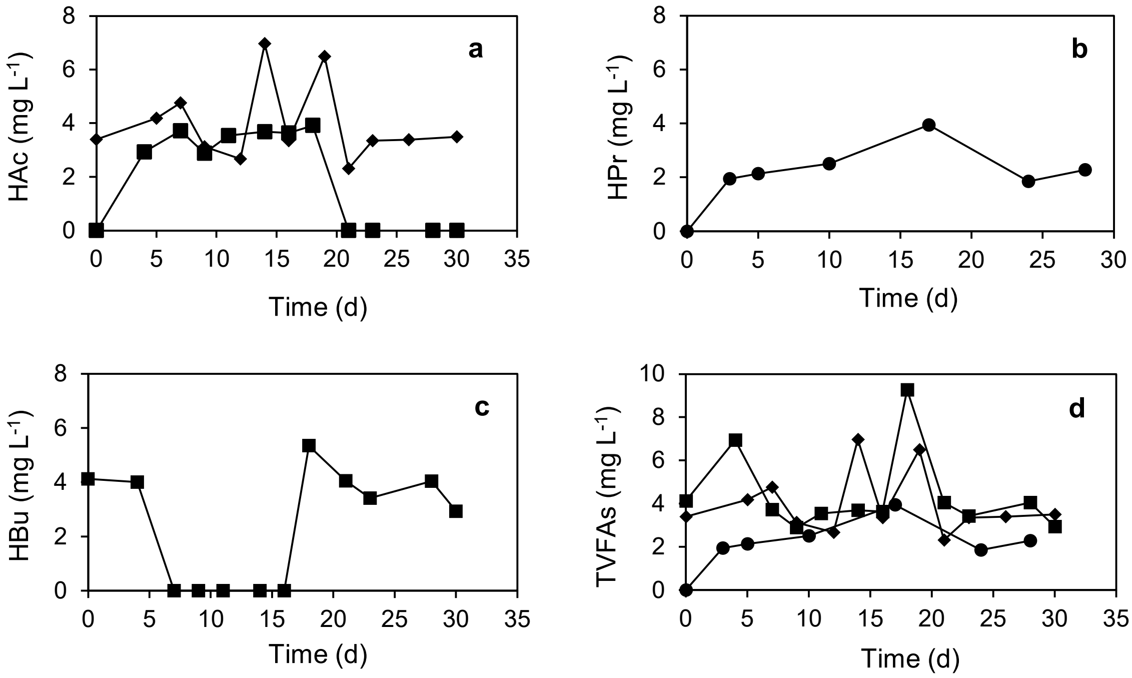

a
NL biogas kg VS-1
Time (d)
Cycle 1
Cycle 2
b
NL biogas kg VS-1
Time (d)
Cycle 3
Cycle 4

Figure 2



686

687 Figure 3

688

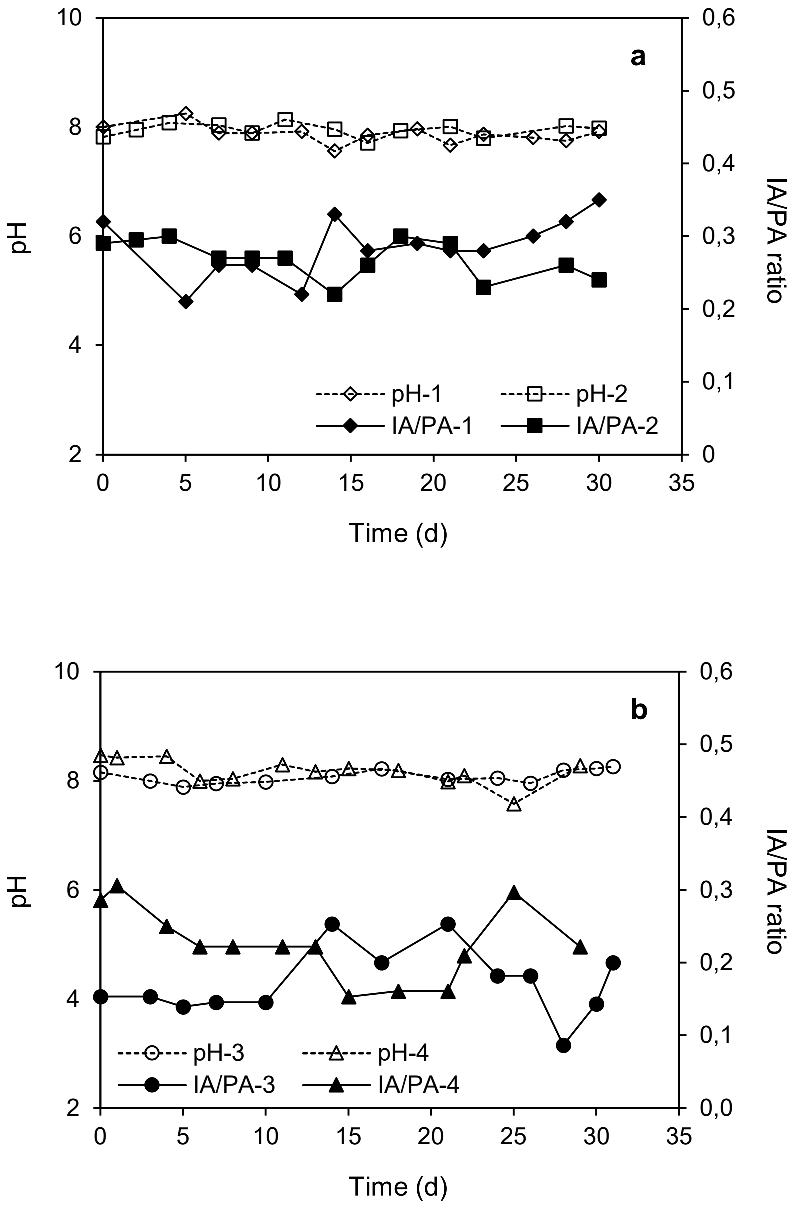

a
pH
IA/PA ratio
Time (d)
pH-1
pH-2
IA/PA-1
IA/PA-2
b
pH
IA/PA ratio
Time (d)
pH-3
pH-4
IA/PA-3
IA/PA-4

Figure 4

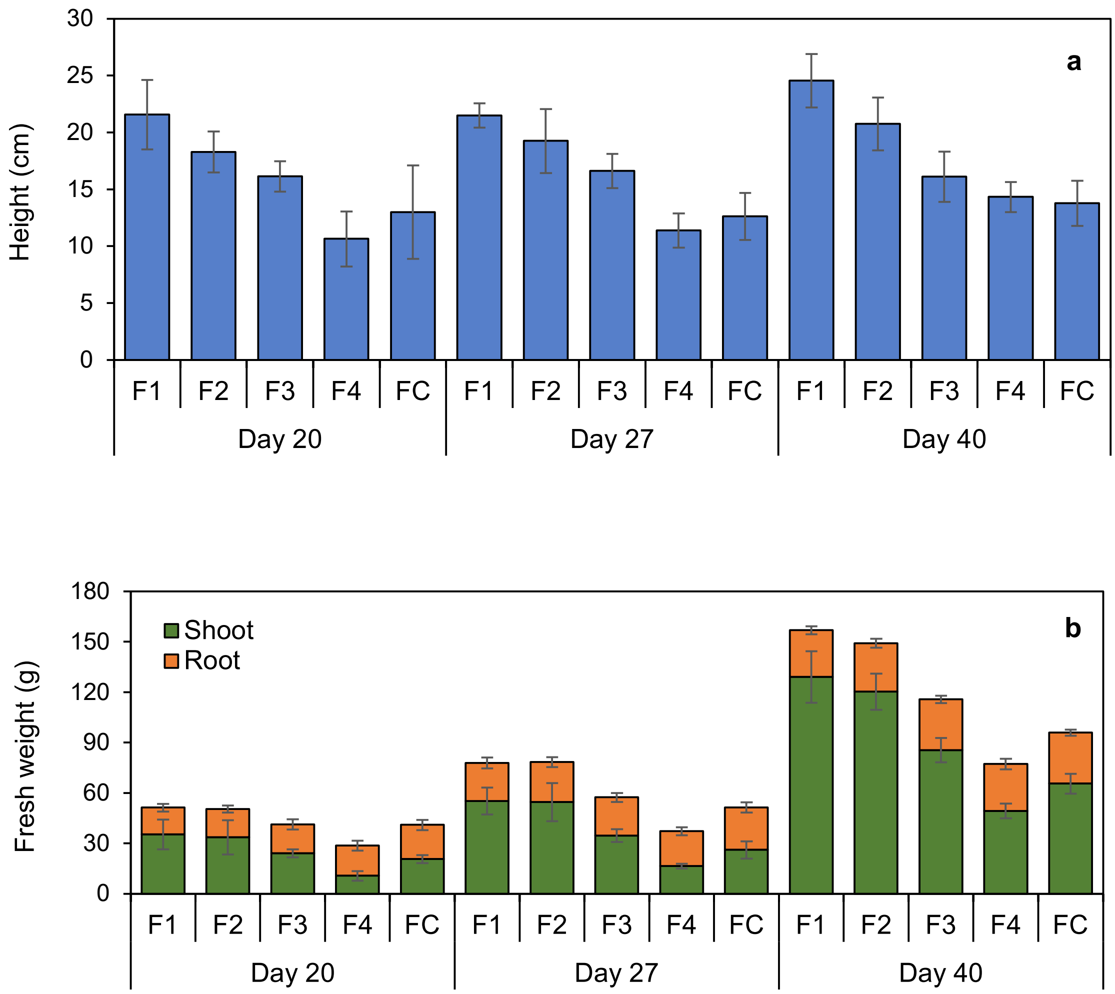

a
Height (cm)
30
25
20
15
10
5
0
F1 F2 F3 F4 FC F1 F2 F3 F4 FC F1 F2 F3 F4 FC
Day 20
Day 27
Day 40
b
Fresh weight (g)
180
150
120
90
60
30
0
Shoot
Root
F1 F2 F3 F4 FC F1 F2 F3 F4 FC F1 F2 F3 F4 FC
Day 20
Day 27
Day 40

Figure 5

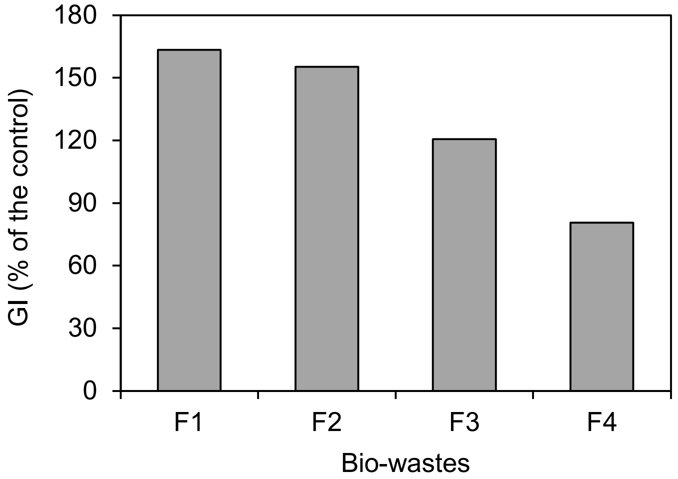


Figure 6

Table 1. Characterization of the PBR influent and the settled effluent.

Parameter	PBR influent	PBR effluent
COD (mg L^{-1})	81.4 ± 6.5	96.0 ± 0.0
TOC (mg L^{-1})	23.1 ± 5.5	19.9 ± 0.0
TS (mg L^{-1})	62.5 ± 27.6	59.0 ± 4.2
VS (mg L^{-1})	48.0 ± 21.2	49.0 ± 1.4
N-NH_4^+ (mg L^{-1})	4.8 ± 1.3	0.1 ± 0.0
TN (mg L^{-1})	7.9 ± 5.7	3.5 ± 1.4
P-PO_4^{-3} (mg L^{-1})	2.5 ± 0.0	0.2 ± 0.0
pH	8.1 ± 0.2	8.3 ± 0.0

Table 2. Initial characterisation of SBR cycles.

SBR cycle	1	2	3	4
Inoculum volume (L)	33.0	31.0*	33.0*	33.0*
Activated sludge volume (L)	15.0	15.0	14.4	16.2
Microalgae volume (L)	n.a.	n.a.	0.6	0.8
Activated sludge and microalgae mixture volume (L)	n.a.	n.a.	15	17
VS inoculum (g L^{-1})	3.6	7.3*	5.7*	4.5*
pH inoculum	7.9	7.8*	7.7*	7.6*
VS activated sludge (g L^{-1})	5.0	10.6	6.9	5.0
VS microalgae (g L^{-1})	n.a.	n.a.	0.4	1.9
VS activated sludge and microalgae mixture (g L^{-1})	n.a.	n.a.	6.6	4.8
Relation VS_i/VS_s	1.6	1.4	1.9	1.8
Relation activated sludge and microalgae mixture (v:v)	n.a.	n.a.	24.0	20.0

VS = volatile solids. VS_i = volatile solids in the inoculum. VS_s = volatile solids in the substrate(s).
* Coming from the previous cycle.

707 **Table 3.** Experimental results and estimated parameters from anaerobic SBR cycles.

Cycle	VS removal (%)	Net experimental CH_4 yield (NL CH_4 kg VS^{-1})	Methane content (%)	Gompertz model				Hydrolysis rate	
				P_{max} (NL biogas kg VS^{-1})	R_{max} (NL biogas kg VS^{-1} d^{-1})	λ (d)	r^2	K_H (d^{-1})	r^2
1	30	155.4	82.8 ± 3.6	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2	19	132.9	81.0 ± 0.0	162.1 ± 6.9	8.9 ± 0.9	-1.2 ± 0.9	0.997	0.0815	0.9928
3	4	108.7	79.2 ± 3.1	124.4 ± 5.1	10.3 ± 2.0	0.3 ± 1.2	0.955	0.0992	0.9600
4	35	225.8	85.5 ± 2.2	254.8 ± 10.3	15.0 ± 1.6	0.5 ± 0.9	0.979	0.0610	0.9874

708 n.a. = not applicable (Parameters from batch test 1 could not be estimated by the models since initial biogas production was not registered due to
709 operational issues).

710