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Towards energy neutral microalgae-based wastewater treatment plants

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ABSTRACT: The aim of this study was to assess the energy balance of a hypothetical microalgae-based wastewater treatment plant (10,000 PE) located in the Mediterranean Region, where harvested microalgal biomass and primary sludge would be co-digested to produce biogas and bioenergy. The assessment was based on experimental results obtained over one year in pilot high rate algal ponds followed by anaerobic digesters for biogas production from harvested microalgal biomass and primary sludge. The energy balance compared four scenarios: 1) anaerobic co-digestion of microalgal biomass and primary sludge, and cogeneration from biogas in a combined with heat and power (CHP) unit; 2) co-digestion with thermal pretreatment of microalgal biomass, and cogeneration from biogas in a CHP unit; 3) co-digestion and heat generation from biogas in a boiler; and 4) co-digestion with thermal pretreatment of microalgal biomass, and heat generation from biogas in a boiler. According to the results, when biogas was used to cogenerate electricity and heat (scenarios 1 and 2), the electricity balance was always positive, and the best results were obtained with pretreated microalgal biomass (scenario 2). Similarly, the heat balance was always positive when biomass was thermally pretreated (scenario 2). On the other hand, when biogas was only used to produce heat (scenarios 3 and 4), heat requirements were covered during the whole year. The sensibility analysis of the scenarios with pretreatment (2 and 4) confirmed that the microalgae-based WWTP would be energy neutral or even net energy producer.

Keywords:

Anaerobic co-digestion; Energy balance; High rate algal pond; Microalgal biomass; Wastewater

1. Introduction

The wastewater treatment sector has considerably evolved over the past decades showing a huge increase in treatment facilities based on conventional wastewater treatment systems [1]. However, energy requirements for these conventional technologies (such as activated sludge) are about 1 kWh/m^3 [2], which represents a high energy consumption. Furthermore, it has been estimated that aeration is responsible for more than 60% of the total energy consumption of activated sludge processes [3]. Thus, energy devoted to wastewater treatment must be significantly reduced to cut down both environmental impacts and costs. Besides, the final effluent and by-products from wastewater treatment facilities are currently regarded as wastes with no value. To make wastewater treatment self-sufficient, it is necessary to shift from the current model of sanitation towards a new one in which wastewater treatment systems will become a low energy demanding industry, able to generate marketable products rather than wastes.

In this new scenario, microalgae-based wastewater treatment systems (such as high rate algal ponds (HRAPs)) are an alternative in suitable cases (e.g. enough surface area available and high solar radiation) with low-energy demand, which produces microalgal biomass that could be used as bioenergy feedstock [4]. HRAPs were developed in the late 1950s in California [5] and used since then to treat a wide variety of municipal, industrial and agricultural wastewaters [6]. In such systems, microalgae photosynthesis provides the oxygen required by heterotrophic bacteria to oxidise organic matter without external aeration [7]. Since these systems do not require mechanical aeration, they only consume around 0.02 kWh/m^3 [8]. This corresponds to a saving of more than 50% of the energy applied to the mechanical aeration of an activated sludge reactor. Furthermore, microalgal biomass produced in HRAPs could be digested to produce biogas and cover the energy requirements for wastewater treatment [9]. It was estimated that between 800-1400 GJ/ha year could be produced from microalgae-based wastewater treatment plants (WWTPs), which could be used to provide sufficient energy for medium (10,000 PE) and small-scale systems (2,000 PE) [10]. Furthermore, the sludge from the primary treatment could be co-digested to increase the biogas and bioenergy production. In spite of the increasing interest in HRAPs and anaerobic digestion of microalgal biomass, their full-scale implementation for bioenergy generation in WWTPs has yet to be exploited. Since the wastewater treatment capacity has been widely proved, the following step towards the dissemination of these systems is the evaluation of energy aspects in an integrated system, including biogas production from by-products (microalgae and sludge).

The aim of this study was to assess the energy balance of a hypothetical microalgae-based WWTP (10,000 PE) with anaerobic co-digestion of harvested microalgal biomass and primary sludge. For the first time, a year-round energy assessment of a microalgae-based WWTP was undertaken based on experimental data on biomass and biogas production. These data were gathered over one year in pilot HRAPs followed by anaerobic digesters, and were used to evaluate the energy balance of four different scenarios (with or without microalgae biomass thermal pretreatment, and a cogeneration unit or a boiler for biogas conversion). This scenario analysis allows establishing the conditions for the WWTP to be energy self-sufficient. To the best of the authors' knowledge, this is the first study evaluating the energy balance of a microalgae-based wastewater treatment system, including the co-digestion of microalgae and primary sludge with or without microalgae thermal pretreatment.

2. Experimental section

2.1. Pilot plant

Two pilot HRAPs located outdoors on the roof of the building of the Group of Environmental Engineering and Microbiology-GEMMA (Department of Civil and Environmental Engineering of the Universitat Politècnica de Catalunya·BarcelonaTech (Barcelona, Spain)) were monitored over one and a half years (from July 2012 to December 2013). In this pilot plant, wastewater from a municipal sewer was daily pumped to a homogenisation tank (1.2 m^3), where it was screened and stored for a few hours (not relevant for wastewater quality). From this tank, wastewater flowed continuously (180 L/d) to a primary settler (7 L, 0.0255 m^2), with a critical settling velocity of 7 m/d and a hydraulic retention time (HRT) of 1 h. Following, the primary effluent was pumped to the two parallel HRAPs working each at different HRT (4 and 8 days), corresponding to flow rates of 120 and 60 L/d. Both HRAPs (from now on referred to as 4 days-HRAP and 8 days-HRAP) were built in PVC and had a surface area of 1.54 m^2, a water depth of 0.3 m and a useful volume of 0.47 m^3. A paddle-wheel driven by an engine operated at 5 rpm ensured a flow velocity of 10 cm/s. Microalgal biomass grown in the HRAPs was harvested in two secondary settlers with a useful volume of 10 L, a surface area of 0.0255 m^2, a critical settling velocity of 4.7 and 2.4 m/d, and a HRT of 2 and 4 hours for the 4 days-HRAP and 8 days-HRAP, respectively. Around 1-1.5 L of biomass with a total solids concentration of 0.7-1.5% (w/w) (depending on the period of the year) was harvested from each settler every weekday. More details on the microalgae composition can be found in Gutiérrez et al. [11]. Subsequently, harvested microalgal biomass was thickened in gravity settling cones for 24 h to increase the solids concentration to 2.5% (w/w), before undergoing anaerobic co-digestion. A fraction of this thickened microalgae biomass was thermally pretreated. To this end, a 250 mL-glass bottle was filled with 150 mL of thickened biomass and placed in an incubator at 75 °C under continuous stirring for 10h [12]. Afterwards, pretreated and non-pretreated thickened biomass was co-digested with primary sludge in two identical lab-scale anaerobic digesters (1.5 L). Due to the low flow rate of primary sludge of the pilot-scale primary settlers, primary sludge was collected from a municipal WWTP near Barcelona and had an average volatile solids (VS) concentration of 28.5 g/L. The reactors were fed with a mixture of 75% primary sludge and 25% microalgal biomass (pretreated and non-pretreated) on a VS basis. This proportion was selected based on the optimal one among several conditions of co-digestion in biochemical methane potential (BMP) tests [13]. Continuous lab-scale reactors were operated under mesophilic conditions (37 ± 1 °C) by an electric heating cover (Selecta, Spain) at a HRT of 20 days. The biomass flow rate varied from 14.6 (December) to 110 m^3/d (April). Constant mixing was provided by a magnetic stirrer (Thermo Scientific).

2.2. Experimental procedures

Microalgal biomass production was quantified once a week by determining the concentration of total suspended solids (TSS) from a grab sample of the HRAPs mixed liquor collected at 10 am. Monthly average biomass production was calculated in terms of g TSS/m^2·d, from daily production estimated for each week (Eq. (1)).

$$Microalgal\ biomass\ production = \frac{TSS(Q - Q_E + Q_P)}{A} \tag{1}$$

where TSS is the total suspended solids concentration of the HRAPs mixed liquor (mg

TSS/L), Q is the wastewater flow rate (L/d), Q_E is the evaporation rate (L/d), Q_P is the precipitation rate (L/d) and A is the surface area of the pilot HRAPs (m^2). The evaporation rate was calculated following Eq. (2).

$$Q_E = \frac{E_p A}{7} \tag{2}$$

where E_p is the potential evaporation between weekly samples (mm), calculated from Turc's formula (Eq. (3)). Note that the 7 in Eq. (2) is necessary to change from weekly to daily evaporation rate.

$$E_p = a(R + 50)\frac{Ta}{Ta+15} \tag{3}$$

where R is the average solar radiation in a week (cal/cm^2d), T_a is the average air temperature in a week (°C), and a is the dimensionless coefficient verying depending on the numbers of days elapsed between sampling (in this case 0.091, which is the value corresponding to 7 days between sapling). In general the precipitation rate was negligible in comparison to the other flows.

Filtered HRAPs mixed liquor, which has the same nutrients and dissolved organic matter concentrations as the secondary settler effluent, was used to analyse the soluble chemical oxygen demand (sCOD) and ammonium nitrogen (NH_4^+-N) concentrations, as indicators of wastewater treatment efficiency. Thus, COD removal was calculated from the difference between the concentrations in unfiltered samples of the primary effluent and filtered samples of the HRAPs mixed liquor (glass fiber filters of 47 mm and average pore size 1 μm). The wastewater treatment efficiency was weekly monitored during the whole experimental period. COD was analysed according to Standard Methods [14] and NH_4^+-N was measured according to the Solorzano method [15]. All analyses were performed in triplicate and averages were used to give data shown in this paper.

Solar radiation, air temperature and precipitation data were obtained from a nearby meteorological station (Department of Astronomy and Meteorology, University of Barcelona, http://infomet.am.ub.es).

Experimental results were used to determine the best HRT for wastewater treatment (which is the primary goal of the HRAPs) and the linked microalgal biomass production over the year. In general, as lower the HRT the higher the biomass production, but effluent water quality has to be maintained.

2.3. Energy assessment

The best HRAPs operation conditions (4 days of HRT from March to October and 8 days of HRT from November to February) were then used to perform the year-round energy assessment of a hypothetical full-scale WWTP located in the Mediterranean region.

To this aim, four scenarios were considered:

(1) HRAPs followed by anaerobic co-digestion of harvested microalgal biomass and primary sludge, and a combined heat and power (CHP) unit for biogas conversion;

(2) HRAPs followed by thermal pretreatment of microalgal biomass, anaerobic co-digestion with primary sludge, and a CHP unit for biogas conversion;

(3) HRAPs followed by anaerobic co-digestion of harvested microalgal biomass and primary sludge, and a boiler for biogas conversion;

(4) HRAPs followed by thermal pretreatment of microalgal biomass, anaerobic co-digestion with primary sludge, and a boiler for biogas conversion.

In scenarios 1 and 2, both electricity and heat would be generated from biogas, while in scenarios 3 and 4 all the biogas would be used to generate heat, and the electricity requirements of the WWTP would have to be supplied by another source (ideally from renewable energy). Ideally scenarios 1 and 2 are preferred, but in case they are not possible, scenarios 3 and 4 represent a suitable alternative (at least to recover energy as heat).

Monthly average microalgal biomass production, environmental parameters and wastewater treatment performance obtained in experimental HRAPs over one year (from January to December 2013) were used for the energy assessment (Appendix, Table A1). In addition, other experimental data required for the energy assessment were taken from our previous studies: (i) harvesting efficiency and harvested biomass concentration from Gutiérrez et al. [11] and; (ii) methane yield with and without thermal pretreatment from Solé et al. [13]. All the values used for the energy assessment are summarised in Table 1.

Table 1

Parameters used for the energy assessment of a microalgae-based WWTP.

Parameter	**Unit**	**4 days- HRAP**	**8 days-HRAP**	**Reference**
WWTP capacity	PE	10,000	10,000	This study
Wastewater generation	L/PE·d	150	150	This study
Wastewater flow rate (Q)	m^3/d	1,500	1,500	This study
$\boldsymbol{E_{input,HRAP}}$				
Number of HRAP	-	2	4	Calculated
Channel width (W)	m	12	12	Calculated
Channel length (L)	m	625	625	Calculated
HRAP surface area (A)	m^2	7,500	7,500	Calculated
Water depth (d)	m	0.4	0.4	Sutherland *et al* [16]
Water velocity (υ)	m/s	0.15	0.15	Lundquist *et al* [17]
Water flow rate in motion (Q_w)	m^3/s	0.48	0.48	Calculated
Manning friction factor (n)	-	0.025	0.025	Lundquist *et al* [17]
Specific weight of water at 20 ºC (γ)	kN/m^3	9.78	9.78	Metcalf and Eddy, [2]
Paddle-wheel efficiency (ε)	%	50	50	Lundquist *et al* [17]
$\boldsymbol{E_{input,ADelectricity}}$				
Digester influent flow rate (Q_b)	m^3/d	19 - 55	15 - 31	Calculated
Digester hydraulic retention time (HRT_d)	day	20 – 60	34 – 57	Calculated

Digester nominal volume (V_d)	m^3	1473	1473	Calculated
Energy consumption for pumping (θ)	kJ/m^3	1,800	1,800	Lu *et al.* [18]
Energy consumption rate for mixing (ω)	$kJ/m^3 \cdot d$	300	300	Lu *et al.* [18]
$E_{input,ADheat}$				
Density of digester influent (ρ)	kg/m^3	1,000	1,000	Metcalf and Eddy, [2]
Specific heat of digester influent (γ)	kJ/kg ºC	4.18	4.18	Metcalf and Eddy, [2]
Ambient temperature (T_a)	ºC	10 – 26	10 – 26	This study
Anaerobic digestion temperature (T_d)	ºC	35	35	Assumed
Pretreatment temperature (T_d)	ºC	75	75	Passos and Ferrer, [12]
Heat transfer coefficient (k)	$W/m^2 \cdot {}^oC$	1	1	Metcalf and Eddy, [2]
Heat recovery efficiency (ϕ)	-	0.85	0.85	Lu *et al.* [18]
Surface area of the digester wall (A_d)	m^2	569	569	Calculated
E_{output}				
Microalgal biomass production (P_m)	$g\ TSS/m^2 \cdot d$	5.4 – 41.2	5.6 – 23.1	This study
Microalgal biomass harvesting efficiency (φ)	-	0.89	0.76	Gutiérrez *et al.* [11]
VS daily production of microalgal biomass	kg VS/day	117-340	90-192	This study
VS daily production of primary sludge	kg VS/day	351-1021	270-576	Solé *et al.* [13]
Lower heating value of methane (ξ)	$kWh/m^3\ CH_4$	10	10	Metcalf and Eddy, [2]
Methane yield (Y)	$m^3 CH_4/kg\ VS$	0.32	0.32	Solé *et al.* [13]
Methane yield with microalgal biomass pretreatment (Y)	$m^3 CH_4/kg\ VS$	0.46	0.46	Solé *et al.* [13]
Electricity generation efficiency of the CHP unit (η_1)	-	0.35	0.35	Assumed
Heat generation efficiency of the CHP unit (η_2)	-	0.55	0.55	Assumed
Heat generation efficiency of the boiler (η_2)	-	0.90	0.90	Assumed

The considered hypothetical microalgae-based WWTP would treat a wastewater flow rate of 1,500 m^3/d, corresponding to approximately 10,000 PE. Both the HRAPs system and digester designs were based on our experimental results. Concerning the HRAPs sizing, the total volume was determined by multiplying the flow rate (1,500 m^3/d) by the highest HRT (8 days). The total volume of water (12,000 m^3, when the system operated at 8 days of HRT) was divided by a fixed water depth (0.4 m, in accordance with Sutherland et al. [16]), obtaining a total surface area of 3 ha. The system would be composed of four parallel HRAPs (7,500 m^2 each) in the typical form of raceways with two channels and two reversals (937 m long and 8 m wide). Only two of these HRAPs would operate in warm periods with a HRT of 4 days (from March to October); while the four HRAPs would operate during cold periods with a HRT of 8 days (from November to February).

2.3.1. *Energy input*

The energy consumption included: (1) electricity for the HRAPs paddle-wheel and (2) electricity and heat for the anaerobic digester. The energy input for wastewater pretreatment, primary and secondary settlers was assumed to be negligible in the context of the present study, in comparison to other necessary inputs [2]. Note that these inputs usually represent less of the 10% of the total energy of WWTPs [19]. The electricity input for the paddle-wheel was calculated from Eq. (4) [17].

$$E_{\text{input,HRAP electricity}} = \frac{Q_w\ \gamma\ (\Delta d_{\text{channels}} + \Delta d_{\text{reversals}}) 24}{A\ \varepsilon} \tag{4}$$

where $E_{\text{input,HRAP electricity}}$ is the input electricity for the HRAPs (kWh/d), Q_w is the mixed liquor flow rate in motion (m^3/s), γ is the specific weight of water at 20 °C (kN/m^3), $\Delta d_{\text{reversals}}$ is the head loss in reversals (m), $\Delta d_{\text{channels}}$ is the head loss in channels (m), A is HRAPs surface area (m^2) and ε is the paddle-wheel efficiency.

The flow of mixed liquor in motion (Q_w) corresponded to the flow rate through the transversal area of the HRAPs (Eq. (5)).

$$Q_w = \upsilon \cdot d \cdot W \tag{5}$$

where υ is the water velocity (m/s), d is the water depth (m) and W is the channel width (m).

The head loss in channels and reversals was calculated according to Eq. (6) and (7), respectively [17].

$$\Delta d_{\mathrm{channels}} = \frac{\upsilon^2 L}{\left(\frac{1.428}{n}\right)^2 \left(\frac{d\,W}{W+2d}\right)^{1.26}} \tag{6}$$

where $\Delta d_{\mathrm{channels}}$ is head loss in channels (m), L is the channel length (m) and n is the Manning friction factor.

$$\Delta d_{\mathrm{reversals}} = 2\,\frac{\upsilon^2}{2g} \tag{7}$$

where $\Delta d_{\mathrm{reversals}}$ is the head loss in reversals (m) and g is the gravitational force (m/s^2).

The electricity input was multiplied by the number of HRAPs operating in each period (two from March to October and four from November to February).

The energy required for anaerobic digestion was calculated as the electricity and heat input for the system. The nominal volume of the anaerobic digester was determined considering the maximum microalgal biomass flow rate observed over the year (i.e. the average from the month of April), and adding the primary sludge flow rate. This represents a flow rate of 55 m^3/d.

The microalgal biomass flow rate was determined from the weekly biomass production (Eq. 1, which ranged from 14.1 to 27.2 g TSS/m^2d in the 4 days-HRAP, and from 5.6 to 12.1 g TSS/m^2d in the 8 days-HRAP). Note that these big ranges of production were mostly related to changes in solar radiation, and to less extend to temperature. Biomass production was expressed as volatile solids after harvesting and concentration in the settlers and gravity cones (g VS/m^2d). For this, microalgal biomass harvesting efficiency values used in this study ranged between 76 and 89%, while monthly average efficiency values were taken from Gutiérrez et al. [11]. Moreover, an average ratio of 70% VS/TS was considered. Thickened microalgal biomass had an average volatile solids concentration of 17.5 g VS/L.

According to this, the flow rate was calculated following Eq. (8).

$$\mathrm{Q_{mb}} = \frac{\mathrm{B_p\ A\ \varphi}}{\mathrm{VS_{mb}}} \tag{8}$$

where Q_{mb} is the estimated thickened microalgal biomass flow rate (m^3/d), B_p is the microalgal biomass production (g VS/m^2d), A is the HRAPs surface area (m^2), φ is the biomass harvesting efficiency and VS_{mb} is the thickened microalgal biomass volatile solids concentration (g VS/m^3).

The thickened primary sludge flow rate was calculated by adding 75% of primary sludge on a mass VS basis (Eq. (9)). Thickened primary sludge had an average concentration of 28.5 g VS/L.

$$\mathrm{Q_{ps}} = 3 \times \frac{\mathrm{VS_{mb}}}{\mathrm{VS_{ps}}} \times \mathrm{Q_{mb}} \tag{9}$$

where Q_{ps} is the thickened primary sludge flow rate (m^3/d), and VS_{ps} is the thickened primary sludge VS concentration (g VS/L). Finally, the total flow rate was calculated as the sum of the thickened microalgal biomass and the thickened primary sludge flow rates (Eq. (10)).

$$\mathrm{Q_b} = \mathrm{Q_{mb}} + \mathrm{Q_{ps}} \tag{10}$$

where Q_b is the total flow rate to the digester (m^3/d).

The highest flow rate to the digester (55 m^3/d) was then considered for sizing the digester, which attained a useful volume of 1105.5 m^3 and a total volume of 1474.0 m^3 by setting a HRT of 20 days (Eq. (11)).

$$V_d = \mathrm{Q_b}\ \mathrm{HRT}_d \tag{11}$$

where V_d is the digester nominal volume (m^3), and HRT_d is the digester hydraulic retention time (day). Consequently, the HRT of the anaerobic digester varied over the year depending on the total flow rate (Q_b), being 20 and 75.8 days for the maximum and minimum influent flow rate, respectively.

The electricity input for the anaerobic digester included mixing and pumping (Eq. (12)).

$$\mathrm{E}_{\mathrm{input,AD\ electricity}} = \mathrm{Q_b}\ \theta + V_d\ \omega\ 0.000278 \qquad (12)$$

where $E_{input,ADelectricity}$ is the input electricity for anaerobic digestion (kWh/d); ϴ is the electricity consumption for pumping (kJ/m^3) [18]; ω is the electricity consumption for mixing (kJ/m^3d) [18]; and 0.000278 is the conversion factor from kJ to kWh.

The heat input for the anaerobic digestion was calculated as the energy required for heating the digester influent from ambient temperature (T_a) to digestion temperature (T_d) (Eq. (13)). The monthly average air temperature of Barcelona (Spain) was considered. The density (ρ) and specific heat (γ) of digester influent were assumed to be the same as those of water, 1,000 kg/m^3 and 4.18 kJ/kg°C, respectively. Heat losses through the digester wall were calculated considering a heat transfer coefficient (k) of 1 W/m^2d [2] corresponding to an insulated digester [2].

$$\mathrm{E}_{\mathrm{input,AD\ heat}} = [\rho\ \mathrm{Q_b}\ \gamma\ (\mathrm{T_d} - \mathrm{T_a}) + \mathrm{k}\ \mathrm{A_d}\ (\mathrm{T_d} - \mathrm{T_a})\ 86.4]\ 0.000278 \qquad (13)$$

where $E_{input,\ AD\ heat}$ is the input heat for the anaerobic digestion (kWh/d); ρ is the digester influent density (kg/m^3); γ is the digester influent specific heat (kJ/kg°C); T_d is the anaerobic digestion temperature (°C); T_a is the air temperature (°C); k is the heat transfer coefficient

(W/m^2°C); A_d is the surface area of the digester wall (m^2); and 0.000278 is the conversion factor from kJ to kWh.

Concerning the pretreatment scenarios (2 and 4), a low temperature pretreatment (75 °C) was considered, as proposed by Passos and Ferrer [12]. In such scenarios, input heat was recalculated as the energy required for heating the influent microalgal biomass from ambient temperature (T_a) to the pretreatment temperature (T_p), and subtracting the energy recovered by cooling down the biomass from the pretreatment temperature (T_p) to the digestion temperature (T_d). Besides, the heat requirement for rising up primary sludge temperature from T_a to T_d was also accounted for Eq. (14).

$$E'_{\mathrm{input,AD\ heat}} = \left[\rho\, Q_{mb}\, \gamma\, (T_d - T_a) - \rho\, Q_{mb}\, \gamma\, \left(T_p - T_d\right)\phi + \rho\, Q_{ps}\, \gamma\, (T_d - T_a) + k\, A_d\, (T_d - T_a)\, 86.4\right] 0.000278 \tag{14}$$

where $E'_{\mathrm{input,\ AD\ heat}}$ is the input heat for the anaerobic digestion with microalgal biomass pretreatment (kWh/d); T_p is the pretreatment temperature (°C); ϕ is the heat recovery efficiency; and 0.000278 is the conversion factor from kJ to kWh.

2.3.2. *Energy output*

The energy output was calculated from experimental results on methane production from microalgal biomass and primary sludge co-digestion [13]. According to this, the average methane yield of mesophilic lab-scale digesters operated at 20 days of HRT was 0.32 m^3 CH_4/kg VS without pretreatment (scenarios 1 and 3), and 0.46 m^3 CH_4/Kg VS with microalgal biomass thermal pretreatment [13]. From the biogas produced, electricity would only be cogenerated in scenarios 1 and 2, while in scenarios 3 and 4 electricity would have to be supplied by another renewable energy source (e.g. solar panels).

The electricity output was calculated from the average methane yield (Eq. (15)). A lower calorific value of methane (ξ) of 10 kWh/m^3 CH_4 [2] and an electricity conversion efficiency of the CHP unit of 35% were considered (η_1).

$$E_{output,electricity} = (P_b Y \xi \eta_1) \tag{15}$$

where $E_{output,\ AD\ electricity}$ is the output electricity from biogas (kWh/d); P_b is the VS production with which the digester is fed (kg VS/d) (microalgae biomass production plus primary sludge production); Y is the average methane yield (m^3 CH_4/kg VS); ξ is the lower calorific value of methane (kWh/m^3 CH_4); and η_1 is the efficiency for electricity generation.

Similarly, heat production was calculated according to Eq. (16). The heat conversion efficiency (η_2) was assumed to be 55% in the CHP unit (scenarios 1 and 2) and 90% in the boiler (scenarios 3 and 4).

$$E_{output,heat} = (P_b Y \xi \eta_2) \tag{16}$$

where $E_{output,\ AD\ heat}$ is the output heat from biogas (kWh/d); and η_2 is the efficiency for heat generation.

2.3.3. Net energy ratio

Finally, the net energy ratio (NER) of electricity ($NER_{electricity}$) and heat (NER_{heat}) were calculated as the energy output (energy produced by the system) over the energy input (energy consumed by the system) (Eq. (17) and (18)). Values higher than 1 indicate net energy production.

$$NER_{electricity} = \frac{\mathrm{E_{output,electricity}}}{\mathrm{E_{input,AD\ electricity}} + \mathrm{E_{input,HRAP\ electricity}}} \tag{17}$$

$$NER_{heat} = \frac{\mathrm{E_{output,heat}}}{\mathrm{E_{input,AD\ heat}}} \tag{18}$$

The NER of the four scenarios was evaluated on both seasonal and monthly basis.

2.4. Sensitivity analysis

A sensitivity analysis was performed to evaluate how the uncertainty on input parameters may influence the results. Hence, the following crucial parameters were taken into account: methane yield (Y); electricity generation efficiency (η_1); heat generation efficiency (η_2); energy consumption for pumping (θ); energy consumption rate for mixing (ω); heat transfer coefficient (k) and heat recovery efficiency (ϕ). A variation of ± 10% was considered for all parameters, with the exception of the heat transfer coefficient (k) and the heat recovery efficiency (ϕ). For these parameters the following values were considered: 3-5 $W/m^2 \cdot {}^oC$ and 0.50-0.65, respectively [2]. The annual average NERs of electricity and heat were calculated for the scenarios 2 and 4 (HRAPs followed by thermal pretreatment of microalgal biomass, anaerobic co-digestion with primary sludge and a CHP unit or a boiler for biogas conversion, respectively).

2.5. Statistical analysis

COD and NH_4^+-N removals, along with microalgal biomass production from the 4 days-HRAP and 8 days-HRAP, were compared by means of the Student's paired t test using Minitab 17.0 software. $p=0.05$ was set as the level of statistical significance.

3. Results and discussion

3.1. Experimental results

3.1.1. Wastewater treatment

Data gathered over one and a half years of experiments were divided into four periods corresponding to the seasons in the Mediterranean Region. The wastewater treatment efficiency of both HRAPs varied seasonally, according to variations on the primary effluent composition and weather conditions (Appendix, Table A1).

COD removal efficiencies showed no significant differences ($p>0.05$) between HRAPs operating at different HRT, reaching average values of 36-96 % in the 4 days-HRAP (Appendix, Fig. A1a) and 47-96 % in the 8 days-HRAP (Appendix, Fig. A1b). Note that changes in COD removal efficiency were mostly due to variations in influent COD, while effluent COD maintained quite constant (Appendix, Fig. A1a). Average effluent COD concentrations were 60 mg O_2/L in the 4 days-HRAP and 55 mg O_2/L in the 8 days-HRAP (Appendix, Table A1b).

Concerning NH_4^+-N removal efficiencies, significant differences were observed between the HRAPs ($p<0.05$). In the 8 days-HRAP, only slight variations on the NH_4^+-N concentration (between 0.2-4 mg/L) were registered over the year (Supporting information, Fig. S2b), leading to consistent NH_4^+-N removal efficiencies over 95%. In the 4 days-HRAP, high fluctuations on NH_4^+-N concentration led to lower removal efficiencies (83% in average), mainly in periods with low temperatures (e.g. winter and autumn) (Supporting information, Fig. S2a). Indeed, the higher NH_4^+-N load of the 4 days-HRAP, which received twice the flow rate of the 8 days-HRAP, was not completely removed in autumn and winter when temperatures were low [20].

3.1.2. Biomass production

Average microalgal biomass production for the 4 days-HRAP and the 8 days-HRAP is plotted in Fig. 1. The profile of biomass production followed the same trend in both HRAPs. As it can be observed, microalgal biomass production showed seasonal variations, following the same trend as the solar radiation. Differences between biomass productions in both HRAPs were statistically significant ($p<0.05$) and varied from 14.1 to 27.2 g TSS/m^2d in the 4 days-HRAP, and from 5.6 to 12.1 g TSS/m^2d in the 8 days-HRAP.

Biomass productions obtained during the last year of experimentation (from January to December 2013) were used for the energy assessment. In this period, average concentrations of 230 and 332 mg TSS/L were obtained in the 4 days- HRAP and the 8 days-HRAP, respectively. Even though the biomass concentration remained higher in the HRAP with longer HRT during the whole year, the average microalgal biomass production (Eq. (1)) was lower (in average 17.5 g TSS/m^2 d in the 4 days-HRAP vs. 13 g TSS/m^2d in the 8 days-HRAP), although differences were not statistically significant ($p>0.05$). These results are in accordance with those reported by Park and Craggs [21] on a 5 months-experiment in two full-scale HRAPs operated at 4 and 8 days-HRT with CO_2 injection. The authors observed higher biomass concentrations along with lower microalgal biomass productions for the 8 days-HRAP (549 mg VSS/L and 16 g VSS/ m^2d) as compared to the 4 days-HRAP (341 mg

VSS/L and 21 g VSS/m^{2}d). Values higher than those observed in the present study may be attributed to the summer conditions of the experiment and CO_2 injection, preventing carbon limitation and pH control. In the present study, the peak of production in both HRAPs was measured in spring (28 and 17 g TSS/m^{2}d for the 4 days- and 8 days-HRAP, respectively), along with a high average solar radiation (474 W/m^2 in June). In this period, the 4 days-HRAP biomass production was similar to the annual maximum literature values (25-30 g TSS/m^{2}d) [21,22]. Comparing seasonal variations, the productions obtained in the 4 days-HRAP and 8 days-HRAP were similar during cold periods, while higher differences were observed in spring and summer. Note that the very high production observed in April 2013 in the 4 days-HRAP was due mostly to biomass detachment from the walls and the bottom of the pond. This should be considered as an exceptional case.

To sum up, the results obtained indicate that short HRT (4 days) in warm periods with high solar radiation may ensure both wastewater treatment and higher microalgal biomass production (average value of 20 g TSS/m^2), while longer HRT (8 days) would be necessary during cold periods with low solar radiation to guarantee the wastewater treatment performance.

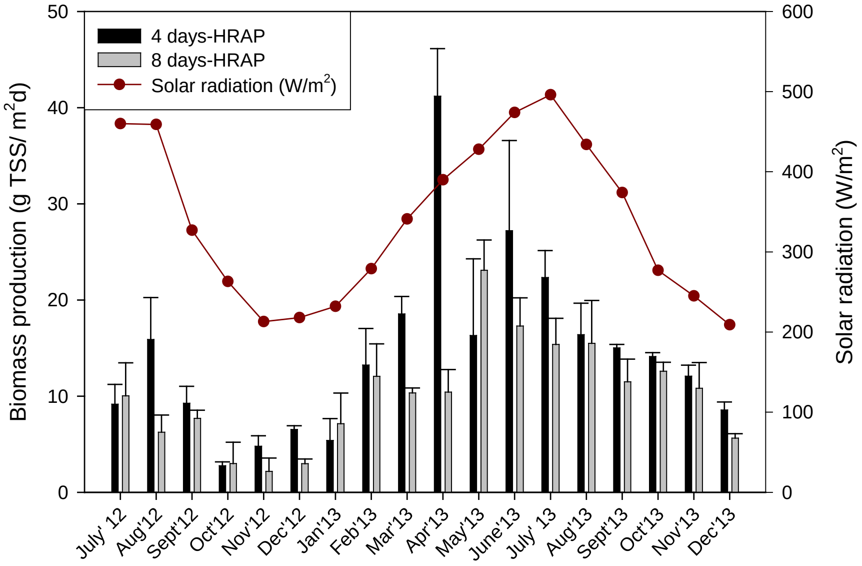


Fig. 1. Average monthly microalgal biomass production in the 4 days-HRAP (black bars) and the 8 days-HRAP (gray bars). The line represents the average monthly solar radiation.

3.2. Energy assessment

The objective of the energy assessment was to determine under which conditions the system would be energy neutral or even net energy producer (NER>1). To this aim, values of microalgal biomass production were taken from the experimental-plant HRAPs operating at 4 days HRT in the cold season (from November to February) and at 10 days HRT in the warm season (from March to October) (Appendix). The seasonal energy balance of the four scenarios, with and without cogeneration and microalgal biomass pretreatment is summarised in Table 2. As can be seen all average NER values are positive and therefore indicative of net energy production.

Table 2
Results of the average seasonal energy assessment of a microalgae-based WWTP in the following scenarios: (1) HRAPs followed by anaerobic co-digestion of microalgal biomass and primary sludge, and a CHP unit for biogas conversion; (2) HRAPs followed by thermal pretreatment of microalgal biomass, anaerobic co-digestion with primary sludge, and a CHP unit for biogas conversion; (3) HRAPs followed by anaerobic co-digestion of microalgal biomass and primary sludge, and a boiler for biogas conversion; (4) HRAPs followed by thermal pretreatment of microalgal biomass, anaerobic co-digestion with primary sludge, and a boiler for biogas

conversion. Standard deviations in brackets. Note that $E_{\text{input, HRAP electricity}}$ does not have standard deviation since values were the same for different months is each season.

	Parameter	Winter	Spring	Summer	Autumn
Scenario 1	$E_{\text{input, HRAP electricity}}$ (kWh/d)	21	10	10	21
	$E_{\text{input, AD electricity}}$ (kWh/d)	104.6 (2.1)	112.4 (5.2)	104.9 (2.8)	102.5 (2.5)
	$E_{\text{output, electricity}}$ (kWh/d)	685.1 (117.5)	1114.9 (288.4)	700.1 (156.0)	566.1 (137.1)
	$NER_{\text{electricity}}$	5.59 (0.96)	8.94 (2.01)	6.03 (1.18)	4.68 (0.91)
	$E_{\text{input, AD heat}}$ (kWh/d)	1058.0 (149.9)	1023.0 (340.0)	434.4 (37.2)	718.1 (173.5)
	$E_{\text{output, heat}}$ (kWh/d)	1076.7 (184.7)	1752.0 (453.2)	1100.2 (245.1)	889.5 (215.4)
	NER_{heat}	1.01 (0.10)	1.75 (0.33)	2.50 (0.33)	1.29 (0.29)
Scenario 2	$E_{\text{input, HRAP electricity}}$ (kWh/d)	21	10	10	21
	$E_{\text{input, AD electricity}}$ (kWh/d)	104.6 (2.1)	112.4 (5.2)	104.9 (2.8)	102.5 (2.5)
	$E_{\text{output, electricity}}$ (kWh/d)	984.9 (168.9)	1602.6 (414.6)	1006.4 (224.2)	813.7 (197.1)
	$NER_{\text{electricity}}$	8.03 (1.38)	12.85 (2.89)	8.66 (1.70)	6.73 (1.31)
	$E_{\text{input, AD heat}}$ (kWh/d)	1055.6 (174.2)	1136.7 (349.4)	484.7 (43.2)	776.5 (169.2)
	$E_{\text{output, heat}}$ (kWh/d)	1547.7 (265.5)	2518.4 (651.5)	1581.6 (352.3)	1278.7 (309.7)
	NER_{heat}	1.46 (0.09)	2.24 (0.37)	3.24 (0.46)	1.68 (0.31)
Scenario 3	$E_{\text{input, AD heat}}$ (kWh/d)	1058.0 (149.9)	1023.0 (340.0)	434.4 (37.2)	718.1 (173.5)
	$E_{\text{output, heat}}$ (kWh/d)	1761.8 (302.2)	2866.8 (741.7)	1800.4 (401.0)	1455.6 (352.5)
	NER_{heat}	1.66 (0.16)	2.87 (0.54)	4.10 (0.54)	2.11 (0.47)
Scenario 4	$E_{\text{input, AD heat}}$ (kWh/d)	1055.6 (174.2)	1136.7 (349.4)	484.7 (43.2)	776.5 (169.2)
	$E_{\text{output, heat}}$ (kWh/d)	2532.6 (434.4)	4121.1 (1066.2)	2588.0 (576.5)	2092.4 (506.7)
	NER_{heat}	2.39 (0.15)	3.67 (0.61)	5.31 (0.75)	2.75 (0.50)

NER results are different when evaluated in a monthly basis (Fig. 2). In the cogeneration scenarios (1 and 2), electricity and heat balances were evaluated separately (Fig. 2a and 2b). The $NER_{\text{electricity}}$ was higher than 1 during the whole year for both scenarios, with and without pretreatment (Fig. 2a), meaning that the electricity generation exceeded the electricity requirements of the system. While the electricity input for the HRAP (i.e. mixing) and anaerobic digester (i.e. pumping and stirring) was always lower than 140 kWh/d, the electricity output ranged between 570 and 1120 kWh/d for Scenario 1 and between 820 and 1600 kWh/d for Scenario 2, depending on the biomass production in the HRAPs (Table 2a). This means that even when microalgal biomass had the lowest production (5.6 g TSS/m^{2}d), the electricity balance was positive (i.e. 282 and 458 kWh/d for scenarios 1 and 2, respectively).

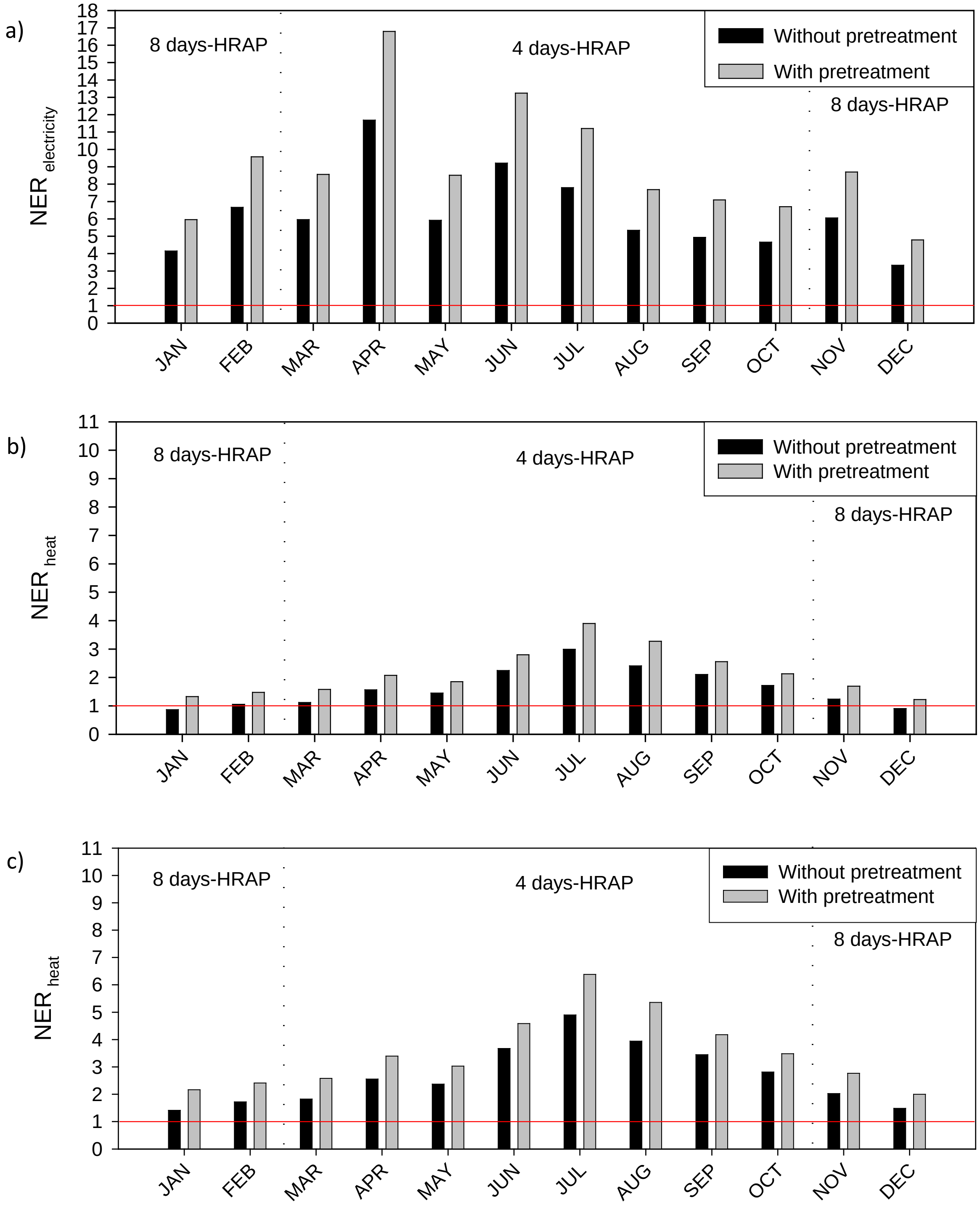


Fig. 2. Results of the average monthly net energy ratio (NER) assessment of a microalgae-based WWTP: $NER_{electricity}$ (a), $NER_{,heat}$ with a CHP unit (b) and with a boiler (c), without microalgal biomass pretreatment (black bars) and with microalgal biomass thermal pretreatment (gray bars).

Despite the positive results for electricity balance estimation, anaerobic digestion also requires heat (Fig. 2b). Results from Table 2a show that the heat input was from 3 to 8-times higher (485-1140 kWh/d) than the electricity input (around 140 kWh/d). The heat output depends on the VS production and methane yield and, therefore, it was lowest during the winter season. Even so, the NER_{heat} was lower than 1 in winter and higher than 1 during the rest of the year (scenario 1). This winter limitation was overcome by implementing a pretreatment step to enhance the microalgal biomass methane yield (scenario 2). According to Solé et al. [13] a low temperature pretreatment (75°C) increased the methane yield by 44% (to 0.32-0.46 m^3 CH_4/kg VS). By incorporating this pretreatment in the energy assessment (scenario 2), the system became net heat producer during the whole year (Fig. 2b). Indeed, the heat output (1280-2520 kWh/d) (Table 2a) increased the NER_{heat} from nearly 1.0-2.5 (scenario 1) to 1.4-3.2 (scenario 2).

The other scenarios (3 and 4) considered that all the biogas produced via anaerobic digestion would be converted into heat (Fig. 2c). In this case, the electricity needed to run the system (~ 100 kWh/d) would have to be supplied by other renewable energy technologies (such as solar panels). In scenario 3, heat production increased by around 60% (e.g. from 1750 to 2870 kWh/d during spring) (Table 2b). This contribution made the system net heat producer during the whole year, since the NER_{heat} ranged from 1.7 to 4.1 (Fig. 2c). Furthermore, when pretreatment was considered (scenario 4), the NER_{heat} increased further, reaching values from 2.4 to 5.3 (Table 2b).

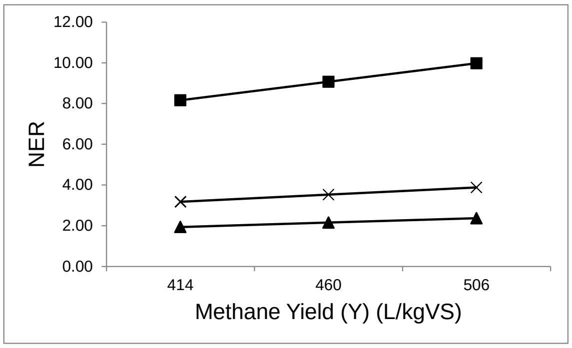
It is worth mentioning that the proportion of primary sludge and microalgal biomass used (i.e. 75/25% in a mass VS basis) was selected according to a previous study evaluating several co-digestion conditions [13]. The mentioned study also showed that the higher the amount of primary sludge in the mixture, the higher the methane yield obtained in the co-digestion process, since it is a more biodegradable substrate compared to microalgae. Therefore, in a full realistic scenario, in which all primary sludge and microalgal biomass would be co-digested in the reactor, the proportion of both substrates would vary throughout the year, and will be different than the 75/25% proportion. A calculation using values of primary sludge production collected from a real WWTP and microalgal biomass from our pilot-scale HRAPs, shows that during winter an average proportion of 30/70% of microalgal biomass/primary sludge would be harvested, while during summer this proportion would be 60/40%. Therefore the proportion achieved in winter is very similar to that considered in the present paper. On the contrary, in summer the higher proportion of microalgae suggests that the methane yield in the co-digestion process would be probably lower than that used in this study (and therefore methane production would be also lower). Nonetheless, according to the results of our study this does not represent a great limitation, because during the summer months the WWTP would also have a higher biomass flow rate (biomass production), and therefore this would balance the decrease in methane yield.

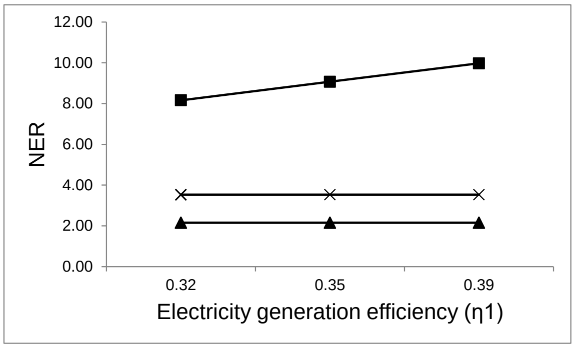
Moreover, to further advance in closing the loop in the WWTP, residual biomass after anaerobic digestion may be reused for agricultural purposes. In fact, co-digestate from pretreated microalgae and primary sludge showed suitable content in terms of organic matter and micronutrients (especially organic and ammonium nitrogen) for soil amendment [22]. In this work, no phytotoxicity was observed when digestate was diluted (10% v/v) and heavy metals were below threshold established by the European legislation. Moreover, thermal pretreatment applied to microalgal biomass improved hygienisation, obtaining absence of *E. coli* [22].

On the whole, it can be concluded that a microalgae-based WWTP in which all the biogas produced via anaerobic co-digestion of microalgal biomass with primary sludge is converted to heat and electricity by cogeneration would be energy self-sufficient if a pretreatment step is implemented (scenario 2). If biogas is only converted to heat, the system would be heat self-sufficient with and without pretreatment (scenarios 3 and 4). The best alternative would then depend on the cost of each process and governmental incentives for cogeneration and electricity injection to the grid.

3.3. Sensitivity analysis

The results of the sensitivity analysis are shown in Fig. 3. It shows limited and almost linear variations of the results for all the parameters analysed. It should be noticed that the most sensitive parameters were the heat transfer coefficient (k) and the heat recovery efficiency (ϕ). Nevertheless, even if the most pessimistic scenarios were considered for these parameters (i.e. heat transfer coefficient (k) equal to 5 W/m^2 ºC, and heat recovery efficiency (ϕ) equal to 0.5), the microalgae-based WWTP would remain net energy producer (NER>1) for scenarios 2 and 4 (HRAPs followed by thermal pretreatment of microalgal biomass, anaerobic co-digestion together with primary sludge and a CHP unit or a boiler for biogas conversion).





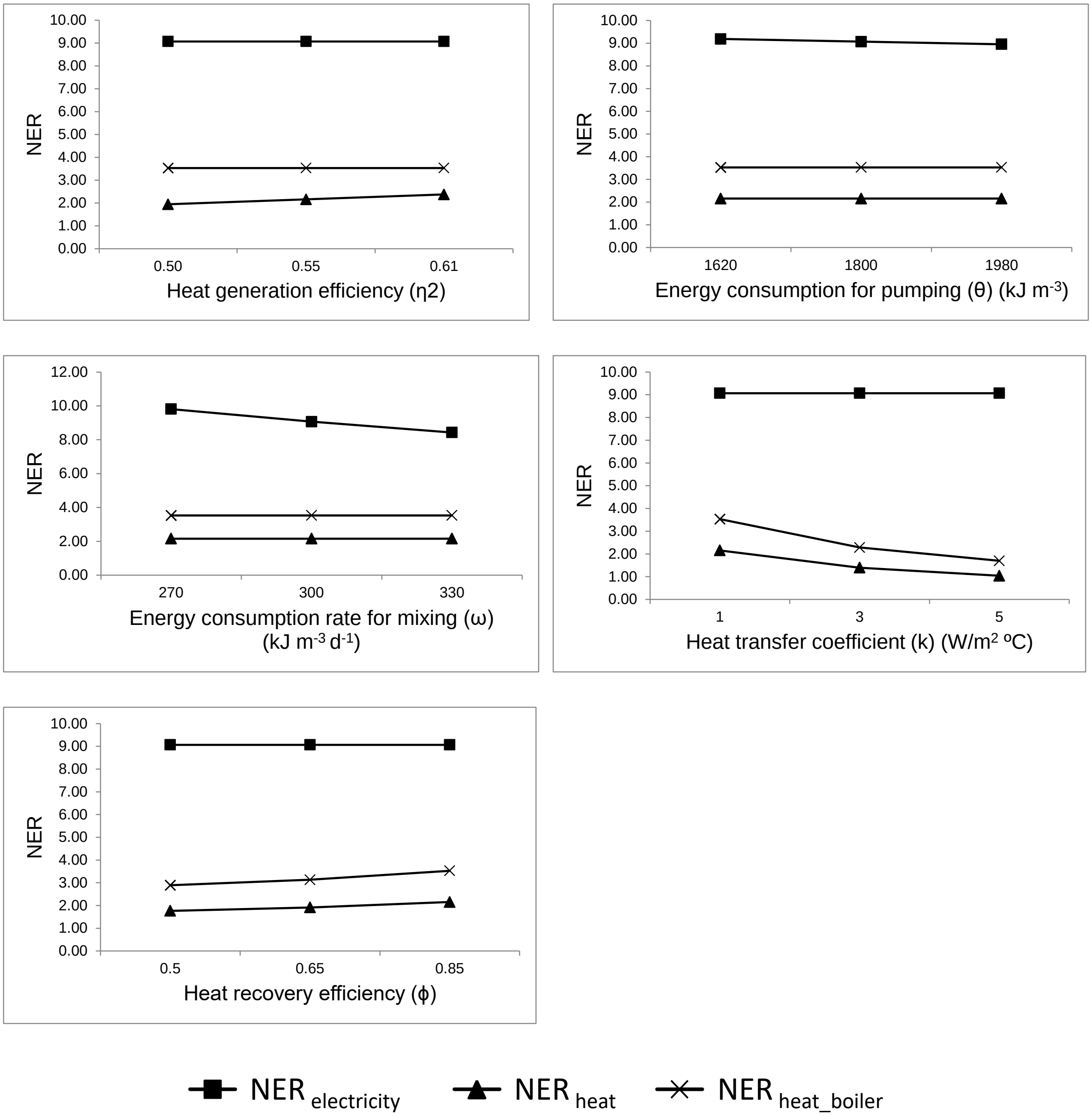


Fig. 3. Results of the sensitivity analysis on the annual average net electricity ratio (NER $_{electricity}$), net heat ratio with a CHP unit (NER $_{heat}$) and with a boiler (NER $_{heat\_boiler}$), with microalgal biomass thermal pretreatment (scenarios 2 and 4).

4. Conclusions

From the results obtained in experimental HRAPs and anaerobic digesters, an energy assessment was undertaken to evaluate the suitability of microalgae-based systems by applying anaerobic co-digestion of microalgal biomass (with and without thermal pretreatment) and primary sludge. The energy assessment of a hypothetical 10,000 PE microalgae-based WWTP with anaerobic co-digestion located in a Mediterranean Region showed a positive energy balance for electricity, which increased further if biomass pretreatment was applied before anaerobic co-digestion. On the other hand, the energy assessment of the system became net heat producer during the whole year only if pretreatment was applied. If all the energy produced was used for heating providing electricity from other renewable sources, heat requirements were covered during the whole year increasing the heat production by some 60%. Although transfer coefficient and the heat recovery efficiency were considered the most sensitive factors for achieving a positive energy balance, the microalgae-based WWTP would remain net energy producer in systems applying thermal pretreatment of microalgal biomass, anaerobic co-digestion together with primary sludge and a CHP unit or a boiler for biogas conversion.

Author contributions

F.P. and R.G. collected data from the experimental plant and performed the energy assessment in collaboration with E.U. and I.F. M.G. analyzed data and performed the sensitivity analysis. J.G. supervised the scientific work. All authors edited and approved the final manuscript.

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Conflict of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication. No conflicts, informed consent, human or animal rights applicable.

References

[1] W.W. Li, H.Q. Yu, Z. He, Towards sustainable wastewater treatment by using microbial fuel cells-centered technologies, Energy Environ. Sci. 7 (2013) 911–924. doi:10.1039/C3EE43106A.

[2] Eddy Metcalf, Wastewater Engineering. Treatment and Reuse, 4 edn, McGraw-Hill, New York, 2004.

[3] B. Chachuat, N. Roche, M.A. Latifi, Long-term optimal aeration strategies for small-size alternating activated sludge treatment plants, Chem. Eng. Process. Process Intensif. 44 (2005) 591–604.

[4] R.J. Craggs, S. Heubeck, T.J. Lundquist, J.R. Benemann, Algae biofuel fromwastewater treatment high rate algal ponds, Water Sci. Technol. 63 (2011) 660–665.

[5] W.J. Oswald, C.G. Golueke., Biological transformation of solar energy., Adv. Appl. Microbiol. 2 (1960) 223–262.

[6] L. Christenson, R. Sims, Production and harvesting of microalgae for wastewater

treatment, biofuels and bioproducts, Biotechnol. Adv. 29 (2011) 686–702. doi:10.1016/j.biotechadv.2011.05.015.

[7] E. Sforza, E.A. Ramos-Tercero, B. Gris, F. Bettin, A. Milani, A. Bertucco, Integration of Chlorella protothecoides production in wastewater treatment plant: From lab measurements to process design, Algal Res. 6 (2014) 223–233. doi:10.1016/j.algal.2014.06.002.

[8] J. García, Eliminació de matèria orgánica i nutrients en llacunas d'alt rendiment, Universitat de Barcelona, 1996.

[9] Y. Shen, J.L. Linville, M. Urgun-Demirtas, M.M. Mintz, S.W. Snyder, An overview of biogas production and utilization at full-scale wastewater treatment plants (WWTPs) in the United States: Challenges and opportunities towards energy-neutral WWTPs, Renew. Sustain. Energy Rev. 50 (2015) 346–362. doi:10.1016/j.rser.2015.04.129.

[10] A. Mehrabadi, R. Craggs, M. Farid, Wastewater treatment high rate algal ponds (WWT HRAP) for low-cost biofuel production, Bioresour. Technol. 184 (2014) 202–214. doi:10.1016/j.biortech.2014.11.004.

[11] R. Gutiérrez, I. Ferrer, A. Gonzalo, H. Salvadó, J. García, E. Uggetti, Microalgae recycling improves biomass recovery from wastewater treatment high rate algal ponds, Water Res. (2016) 539–549.

[12] F. Passos, I. Ferrer, Microalgae conversion to biogas: Thermal pretreatment contribution on net energy production, Environ. Sci. Technol. 48 (2014) 7171–7178. doi:10.1021/es500982v.

[13] M. Solé, F. Passos, M. Garfí, I. Ferrer, Microalgae and primary sludge co-digestion: effect of thermal pretreatment, energy balance and digestate characteristics., in: 14th

World Congr. Anaerob. Dig., Viña del Mar, Chile, 2015.

[14] APHA-AWWA-WPCF, Standard Methods for the Examination of Water and Wastewater, 20th ed., Washington, 1999.

[15] L. Solorzano, Determination of ammonia in natural waters by the phenolhypochlorite method, Limnol. Ocean. 14 (1969) 799–801.

[16] D.L. Sutherland, M.H. Turnbull, R.J. Craggs, Increased pond depth improves algal productivity and nutrient removal in wastewater treatment high rate algal ponds, Water Res. 53 (2014) 271–281. doi:10.1016/j.watres.2014.01.025.

[17] T.J. Lundquist, I.C. Woertz, N.W.T. Quinn, J.R. Beneman, A Realistic Technology and Engineering Assessment of Algae Biofuel Production. Algae Biofuels Assessment Workshops., Institute Berkeley, California, January 15-16., 2010.

[18] J. Lu, H.N. Gavala, I.V. Skiadas, Z. Mladenovska, B.K. Ahring, Improving anaerobic sewage sludge digestion by implementation of a hyperthermophilic prehydrolisis step, J. Environ. Manage. 88 (2008) 881–889.

[19] G. Tchobanoglous, Wastewater treatment trends in the 21st century. The 3rd International Sede Boqer Conference on Water Technologies 2012 Advanced Technologies in Water Management, in: Ben-Gurion University of the Negev, Israel, 2012.

[20] J. García, R. Mujeriego, M. Hernández-Mariné, High rate algal pond operating strategies for urban wastewater nitrogen removal., J. Appl. Phycol. 12 (2000) 331–339.

[21] J.B.K. Park, R.J. Craggs, Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition, Water Sci. Technol. 61 (2010) 633–639. doi:10.2166/wst.2010.951.

[22] M. Solé-Bundó, M. Cucina, M. Folch, J. Tàpias, G. Gigliotti, M. Garfí, I. Ferrer, Assessing the Agricultural Reuse of the Digestate From Microalgae Anaerobic Digestion and Co-Digestion With Sewage Sludge, Sci. Total Environ. 586 (2017) 1–9

Appendix

The following information is presented in the Appendix: the characterization of the primary effluent and mixed liquor from both HRAPs (Table A1); and the COD and NH_4^+-N concentrations and removals from both HRAPs (Fig. A1 and A2).

Table A1. Characterisation of the primary effluent (a) and the mixed liquor from the 4 days-HRAP and the 8 days-HRAP (b) over the experiment. Average values (± s.d.) from samples taken at 10 PM.

(a)

Parameter	**Summer'12 (Jul-Sept) n (daily) = 31 n (weekly) = 10**	**Autumn'12 (Oct-Dec) n (daily) = 39 n (weekly) = 15**	**Winter'13 (Jan –Mar) n (daily) = 28 n (weekly) = 9**	**Spring'13 (Apr - Jun) n (daily) = 35 n (weekly) = 13**	**Summer'13 (Jul-Sep) n (daily) = 31 n (weekly) = 10**	**Autumn'13 (Oct-Dec) n (daily) = 39 n (weekly) = 15**
Temperature (°C)	28 (2)	18 (4)	16 (4)	25 (3)	28 (2)	20 (3)
pH	7.6 (0.2)	7.7(0.3)	8.3 (0.2)	7.8 (0.3)	7.9 (0.1)	7.7 (0.2)
DO (mg/L)	0.8 (1.2)	3.2 (1.7)	2.6 (2)	2.7 (1.4)	1.2 (0.9)	6.4 (2.4)
COD (mg/L)	641 (223)	736 (315)	576 (315)	312 (138)	254 (53)	295 (106)
NH_4^+-N (mg/L)	23 (4)	42(6)	43 (6)	82 (24)	36 (25)	23 (10)

(b)

Parameter	**Summer'12 (Jul-Sep) n (daily) = 31 n (weekly) = 10**		**Autumn'12 (Oct-Dec) n (daily) = 39 n (weekly) = 15**		**Winter'13 (Jan –Mar) n (daily) = 28 n (weekly) = 9**		**Spring'13 (Apr - Jun) n (daily) = 35 n (weekly) = 13**		**Summer'13 (Jul-Sep) n (daily) = 31 n (weekly) = 10**		**Autumn'13 (Oct-Dec) n (daily) = 39 n (weekly) = 15**	
	4 days-HRAP	8 days-HRAP	4 days-HRAP	8 days-HRAP	4 days-HRAP	8 days-HRAP	4 days-HRAP	8 days-HRAP	4 days-HRAP	8 days-HRAP	4 days-HRAP	8 days-HRAP
Temperature (°C)	24.9 (2.3)	24.5 (2.1)	12.0 (2.9)	11.7 (2.9)	9.2 (1.8)	9.2 (1.8)	20.0 (1.7)	20.0 (1.7)	24.8 (2.0)	24.6 (1.9)	16.1 (3.0)	16.1 (2.9)
pH	8.4 (0.3)	9.2 (0.5)	7.9 (0.2)	8.2 (0.4)	8.4 (0.3)	8.5 (0.2)	8.3 (0.3)	8.2 (0.3)	8.7 (0.4)	9.0 (0.3)	8.2 (0.3)	8.8 (0.3)
DO (mg/L)	10.7 (3.2)	13.4 (3.9)	8.7 (0.9)	10.0 (1.4)	8.3 (1.5)	10.5 (1.1)	8.2 (2.2)	8.0 (1.4)	8.9 (1.2)	10.4 (1.7)	9.6 (1.1)	11.5 (1.7)

SCOD (mg/L)	53 (8)	58 (9)	57 (7)	52 (4)	61 (12)	51 (8)	66 (14)	59 (13)	54 (8)	59 (9)	69 (10)	54 (7)
NH_4^+-N (mg/L)	2.6 (2.0)	0.7 (0.4)	11.3 (3.3)	2.7 (0.4)	17.6 (3.5)	0.8 (0.5)	0.8 (0.5)	0.7 (0.6)	0.8 (0.3)	0.4 (0.3)	2.9 (2.6)	0.7 (0.9)
Microalgae production (g TSS/m^2d)	11.4 (2.7)	8.0 (2.0)	4.7 (0.6)	2.7 (1.4)	12.4 (2.6)	9.4 (2.4)	28.2 (7.4)	16.9 (2.8)	17.9 (2.1)	14.1 (3.2)	11.6 (0.8)	9.7 (1.4)

Note: DO: dissolved oxygen, COD: chemical oxygen demand, SCOD: soluble chemical oxygen demand and NH_4^+-N: ammonium nitrogen

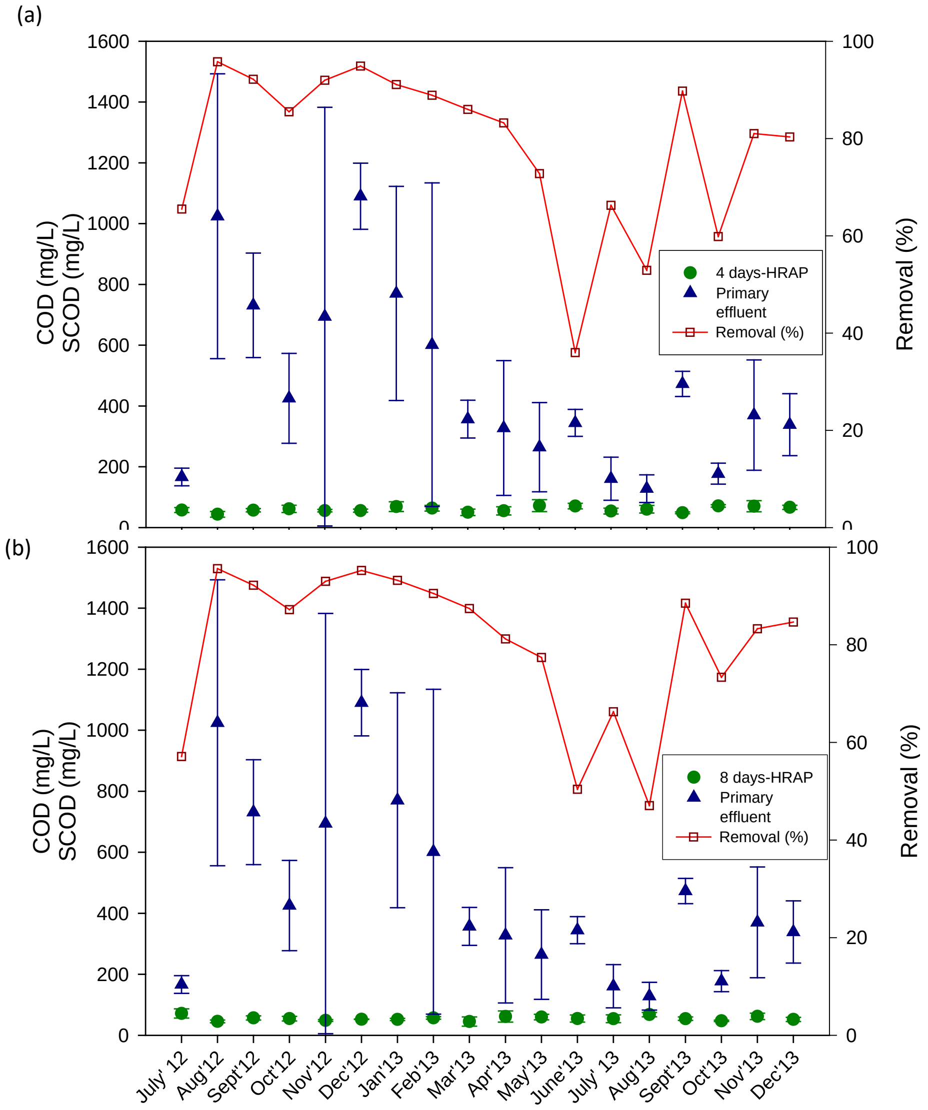

Figure A1. Chemical oxygen demand (COD) from the primary effluent (blue triangles) and soluble chemical oxygen demand (SCOD) from mixed liquor (green dots) of 4 days-HRAP (a) and 8 days-HRAP (b).The red line represents the COD removal efficiency.

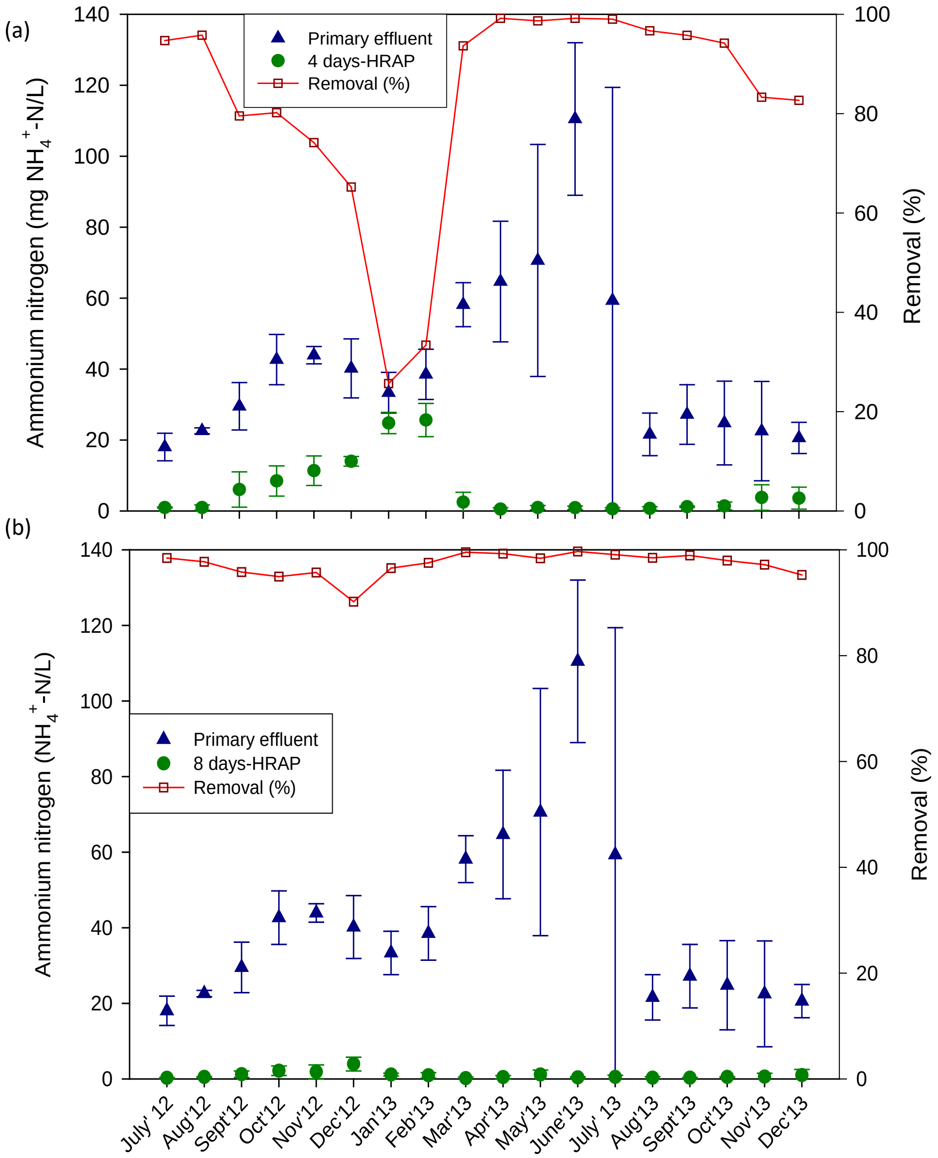

Figure A2. Ammonium nitrogen (NH_4^+-N) from the primary effluent (blue triangles) and mixed liquor (green dots) of the 4 days-HRAP (a) and 8 days-HRAP (b).The red line represents the NH_4^+-N removal efficiency.