# Microalgae production in wastewater treatment systems, anaerobic digestion and modelling using ADM1

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**Abstract**

An integrated microalgae-based system for urban wastewater treatment, microalgae production and bioenergy generation through anaerobic digestion was evaluated over a period of one year. The pilot HRAP was effective at removing COD (~ 80%) and ammonium (~ 95%) and robust, despite common variations in wastewater composition and weather conditions in the Mediterranean region. Biomass production showed a strong seasonality, reaching an annual average of 10 g TSS/$m^2$·d and the highest values in spring (23 g TSS/$m^2$·d). Conversely, the macromolecular composition was fairly constant (58% proteins, 22% carbohydrates and 20% lipids). Predominant microalgae species varied throughout the year, influencing biogas production. Indeed, the anaerobic biodegradability of harvested biomass was 20-25% in July-October 2012 and May-July 2013 and 25-38% in November 2012-April 2013. Adapting the content of particulate inert COD in Anaerobic Digestion Model No. 1 (ADM1) was crucial for model calibration. After adjustment, ADM1 was able to predict microalgae anaerobic digestion performance, which showed an average methane yield of 0.09 L $CH_4$/g COD at 15 days HRT and 0.16 L $CH_4$/g COD at 20 days HRT.

**Keywords:**

Algae; Biodegradability; Bioenergy; Biogas; High rate algal pond; Microalgal biomass

## 1. Introduction

Wastewater treatment plants (WWTP) based on microalgae raceway ponds (i.e. high rate algal ponds, HRAP) have been studied since the 1950's as a cost-effective alternative to conventional activated sludge systems, due to their low energy demand and simplicity of operation (Oswald and Gotaas, 1957). In microalgae-based systems two main mechanisms are involved in pollutants removal: i) direct or indirect transformation of pollutants by microalgae, e.g. nutrients assimilation and precipitation; and ii) enhancement of bacterial biodegradation by oxygen generated through microalgae photosynthesis (Rawat et al., 2011). Both mechanisms take place simultaneously through the so-called "algae-bacteria symbiosis" (Oswald and Gotaas, 1957). Since oxygen needed for organic matter removal is provided by microalgae photosynthesis, there is no need for mechanical aeration, as occurs in conventional activated sludge reactors. This is a major advantage, since aeration is the most energy consuming process in activated sludge systems, ranging from 60 to 80% of the total energy demand (Chachuat et al., 2005).

Another important benefit of microalgae-based systems is that produced biomass can be recovered and valorised for different purposes such as biofuels, bioplastics and non-food bioproducts production. Therefore, these systems may have a dual application: wastewater treatment along with microalgal biomass production (Olguín, 2012). In recent years, bioenergy generation through microalgae has been intensively studied and it is trending topic; however several processes for biomass production and harvesting must still be optimised for full-scale applications (Pittman et al., 2011).

Regarding downstream processing of microalgal biomass, anaerobic digestion for biogas production is a promising technology, already consolidated for sewage sludge treatment in conventional WWTP. However, the anaerobic digestion of microalgae is limited by its cell wall complexity, which hampers the hydrolysis step. Indeed, the methane yield of microalgae species (0.10-0.30 L $CH_4$/g VS) (González-Fernández et al., 2011) is relatively low if compared to other organic substrates, such as agricultural waste (up to 0.53 L $CH_4$/g VS) (Gunnaseelan, 1997).

Furthermore, experimental studies on the anaerobic digestion of microalgal biomass indicated that the methane yield was influenced by biomass characteristics (Passos et al., 2014; Passos and Ferrer, 2014). Biomass characteristics and dynamics (i.e. competition and dominance) in HRAP vary according to many factors, including environmental conditions (e.g. seasonality), operational properties (e.g. nutrient content and hydraulic retention time) and biological relationships (e.g. grazers and parasites) (Park et al., 2011). Therefore, it is composed by a mixed community, mostly formed by green microalgae species, where bacteria and other microorganisms coexist.

In order to understand the parameters limiting microalgae biodegradability mathematical modelling may be used, as a tool for increasing knowledge and predicting anaerobic digestion performance. The Anaerobic Digestion Model no.1 (ADM1) is a well-accepted biokinetic model used to describe the main processes taking place during anaerobic digestion (Batstone et al., 2002). Indeed, several studies have modelled the anaerobic digestion of different types of organic substrates using ADM1, such as sewage sludge (Astals et al., 2013) and agricultural waste (Zhou et al., 2011). To date, however, only one dealt with microalgal biomass anaerobic digestion (Mairet et al., 2011). In this work ADM1 showed good fitting with experimental data, however modelling hydrolysis with Contois kinetics was crucial (Mairet et al., 2011). In the original ADM1, hydrolysis rates are calculated using first order kinetics. Nonetheless, for complex substrates such as microalgae, hydrolysis may be better represented by the Contois model. In this manner, kinetics do not depend on the substrate concentration, but on the amount of substrate per biomass unit, which is associated to the growth of hydrolytic bacteria (Mairet et al., 2011; Vavilin et al., 2008).

This study was set out to investigate an integrated system for wastewater treatment, microalgae production and conversion to methane, and to identify the limitations of the process. Thus, the specific objectives were: 1) to analyse microalgal biomass production and composition treating urban wastewater in a pilot HRAP; 2) to quantify biogas production through anaerobic digestion of harvested biomass; and 3) to calibrate ADM1 for microalgae anaerobic digestion

modelling using experimental data from a continuous reactor. To this end, a pilot-scale HRAP and a continuous anaerobic digester were monitored during one year. The main novelty of this study is that it considers the whole system, from microalgae growth to biogas production, treating real urban wastewater.

## 2. Material and Methods

### *2.1 Microalgae-based wastewater treatment system*

The experimental set-up was located outdoors at the Department of Hydraulic, Maritime and Environmental Engineering of the Universitat Politècnica de Catalunya·BarcelonaTech (Barcelona, Spain) (Fig. 1). For the purposes of this study, the system was monitored over one year, from July 2012 to July 2013. Real wastewater from a nearby municipal sewer was continuously pumped and treated as follows. Firstly, wastewater was screened and stored in a homogenisation tank (1.2 $m^3$). From this tank a continuous wastewater flow of 180 L/d was conveyed to a primary settler with a surface area of 0.0255 $m^2$, a useful volume of 7 L, a hydraulic surface load influent rate of 7.05 m/d and a hydraulic retention time (HRT) of 0.9 h. The primary effluent was continuously discharged into the HRAP by means of a peristaltic pump with a flow rate of 60 L/d, while the excess effluent is discharged. The HRAP was built in PVC, it had a surface area of 1.54 $m^2$, a water height of 0.3 m, a useful volume of 0.47 $m^3$, and a HRT of 8 days. Microalgae contact with sunlight was enhanced through continuous stirring with a bladed paddle-wheel driven by an engine operated at 5 rpm, reaching an average flow velocity of 10 cm/s. Mixing also avoided biomass settling within the pond. Since the mixed liquor was under constant stirring and the HRT was 8 days, the system operated similarly to a completely mixed reactor.

In order to assess the HRAP wastewater treatment efficiency, chemical oxygen demand (COD) and ammonium nitrogen ($N\text{-}NH_4^+$) were analysed from HRAP influent and mixed liquor samples taken once a week. Microalgal biomass production was quantified from the concentration of total suspended solids (TSS) in the HRAP mixed liquor, on a weekly basis. Biomass production

was estimated as g TSS/$m^2$·d and calculated as an average per month. pH and temperature were monitored every weekday at 2 PM in the HRAP. Microscopic images of the mixed liquor in the HRAP were taken every 1-2 months over the year.

Microalgal biomass was harvested in a clarifier with a useful volume of 10 L, a surface area of 0.0255 $m^2$, a hydraulic surface load influent rate of 2.35 m/d and a HRT of 4 hours. 1 L of biomass was purged from the settler every weekday, which had a total solids (TS) concentration of 1.0-1.5% (w/w). Subsequently, purged biomass was thickened in gravity-settling cones for 24 hours to increase the TS concentration to 2.0-2.5% (w/w) before undergoing anaerobic digestion. Harvested biomass was characterised by the concentration of TS, volatile solids (VS), COD, Total Kjeldahl Nitrogen (TKN), N-$NH_4^+$ and pH, once a week. Its macromolecular composition was determined by the concentration of proteins, carbohydrates and lipids, once a week during a period of three months, since it appeared to be fairly constant.

### *2.2 Biochemical methane potential tests*

The anaerobic biodegradability of microalgal biomass was investigated in biochemical methane potential (BMP) tests carried out every 1-2 months (July, August, October and November 2012 and February, March, May and July 2013) in order to ease ADM1 model calibration.

Serum bottles had a total volume of 160 mL and a useful volume of 100 mL. Digestate from a full-scale anaerobic reactor treating sewage sludge in a WWTP near Barcelona (Spain) was used as inoculum. The substrate to inoculum ratio was 0.5 g $COD_s$/g $VS_i$ (Passos et al., 2013), and each bottle contained 5 g of COD. After adding the corresponding amount of microalgal biomass and digested sludge, bottles were filled with distilled water up to 100 mL, flushed with Helium gas, sealed with butyl rubber stoppers and incubated at 35 ºC until biogas production ceased. Biogas production was determined periodically by measuring the pressure increase with an electronic manometer (Greisinger GMH 3151). After each measurement gas was released until atmospheric pressure. Samples from the headspace volume were taken every 2-3 days to determine biogas

composition ($CH_4/CO_2$) by gas chromatography (GC). A blank treatment with only inoculum was used to quantify the amount of methane produced by endogenous respiration. The methane yield was calculated by subtracting the blank results to each trial, dividing by the amount of microalgal biomass (g VS) added to each bottle. The methane content in biogas was periodically analyzed by GC.

The anaerobic biodegradability of biomass was deduced from the net methane yield (mL $CH_4$/g COD) and the theoretical methane yield under standard conditions (350 mL $CH_4$/g $COD_{removed}$) (Eq. 1).

$$Anaerobic\ biodegradability\ (\%) = \frac{Methane\ yield\ (mL\ CH_4/gCOD)}{350\ mL\ CH_4/gCOD}\ 100 \qquad \text{(Eq. 1)}$$

***2.3 Anaerobic digester***

Biogas production from thickened microalgal biomass was studied in a continuous anaerobic digester from July 2012 to July 2013. The digester consisted of a continuous stirred tank reactor with a useful volume of 1.5 L and a total volume of 2 L. Mesophilic conditions (35 ± 2 ºC) were maintained by means of an electric heating cover (Selecta, Spain) and stirring was provided by a magnetic stirrer (Thermo Scientific). The reactor was sealed and supplied with an inlet, outlet, gas collector and temperature sensor. Biogas production was recorded daily by means of a water displacement system.

The anaerobic reactor was operated at 15 and 20 days hydraulic retention time (HRT) in order to optimise biogas production: 1) 15 days HRT (from July to October 2012) and 2) 20 days HRT (from December 2012 to June 2013). During the stabilisation period, when the reactor was switched from 15 to 20 days HRT, biogas production was not recorded (mid-October to November 2012). The reactor was operated on a continuous feeding basis: it was daily fed with 100 and 75 mL of biomass for the first and second periods, respectively. The same volume was daily purged from and added to the digesters.

165 The digester influent and effluent were characterised by the concentration of TS, VS, COD,
166 TKN, N-$NH_4$ and pH once a week. Volatile fatty acids (VFA) were analysed weekly by GC
167 (Agilent Technologies 7820A). The methane content in biogas was measured twice a week by GC.
168
169 ***2.4 Analytical methods***
170 Concerning the microalgae-based wastewater treatment system, solar radiation and ambient
171 temperature were obtained from a nearby meteorological station (Department of Astronomy and
172 Meteorology, University of Barcelona, http://www.infomet.am.ub.es). pH was analysed with a
173 Crison Portable 506 pH-meter. For evaluating wastewater treatment efficiency in terms of COD and
174 N-$NH_4^+$ removal, mixed liquor samples were filtrated (glass fiber filter 47 mm and average pore
175 size 1 μm) in order to exclude COD and $NH_4$-$N^+$ contents in biomass, which was subsequently
176 separated in the clarifier. COD was measured according to Standard Methods (APHA, AWWA;
177 WPCF, 1999), while $NH_4$-$N^+$ was measured according to the Solorzano method (Solorzano, 1969).
178 Regarding biomass production, TSS was determined from the mixed liquor following Standard
179 Methods (APHA, AWWA; WPCF, 1999).
180 With regards to harvested biomass, COD, TS, VS and TKN were measured according to
181 Standard Methods (APHA, AWWA; WPCF, 1999), $NH_4$-$N^+$ was measured according to the
182 Solorzano method (Solorzano, 1969) and pH was analysed with a Crison Portable 506 pH-meter.
183 Carbohydrate content was determined by phenol-sulphuric acid method after acid hydrolysis and
184 measured by spectrophotometry (Spectronic Genesys 8). Protein content was determined from the
185 TKN, using a TKN/protein conversion factor of 5.95 was used (López et al., 2010). Lipid content
186 was determined by the Soxhlet extraction method (APHA, AWWA; WPCF, 1999). Values were
187 expressed as percentage of lipids, carbohydrates and proteins over the VS content.
188 Microalgae identification was carried out by optic microscope examination (Axioskop 40
189 Zeiss, Germany), using a camera and Motic Image Plus 2.0 software. Microalgae were identified to
190 genus from classical specific literature (Bourrelly, 1966; Palmer, 1962).

For the anaerobic digestion, TS, VS, COD and TKN were analysed according to Standard Methods (APHA, AWWA; WPCF, 1999), while $N\text{-}NH_4$ was analysed according to the Solorzano method (Solorzano, 1969). pH was analysed with a Crison Portable 506 pH-meter. VFA were determined by GC (Agilent Technologies 7820A), according to the procedure described by Passos et al. (2013). Soluble samples for VFA and $N\text{-}NH_4$ analysis were obtained by centrifugation (UNICEN20, 4200 rpm, 8 min, 20 ºC) and filtration (glass fiber filter 47 mm and pore size 1 μm). The methane content in biogas was measured with a GC (Trace GC Thermo Finnigan) equipped with a Thermal Conductivity Detector, following the procedure described by Passos et al. (2013).

### *2.5 Modelling approach*

ADM1 was used to model the anaerobic digestion of microalgal biomass harvested from the HRAP. Simulations were carried out in MATLAB® using the ADM1 implementation of Rosen and Jeppsson (2006). As proposed by Mairet et al. (2011), the Contois model was used to describe microalgae hydrolysis. In order to describe the variability in microalgal biomass anaerobic biodegradability over the year evidenced by BMP tests, the fraction of particulate inert COD was not kept constant (Table 1). Adjusted values were defined during the model calibration. Conversely, the fraction of proteins (58%), carbohydrates (22%), and lipids (20%) was kept fairly constant during the whole period. Maximum specific hydrolysis rates for carbohydrates, proteins and lipids ($k_{hyd}$) were adjusted to 2.8, 1.3 and 2.7 $d^{-1}$, respectively, based on the values used by Mairet et al. (2011). Similarly, the Contois half saturation constants of hydrolysis for carbohydrates, proteins and lipids (0.50, 0.26 and 0.49 kg $COD/m^3$, respectively) were taken from Mairet et al. (2011). All other parameters were maintained as in the original ADM1.

## 3. Results and Discussion

### *3.1 Wastewater treatment*

The wastewater treatment efficiency of the HRAP was quite uniform throughout the year (Fig. 2).

COD and $NH_4$-N removal efficiencies were 60-92% and 94-99%, respectively, in accordance with previous studies in the pilot HRAP operated under the same conditions (Garcia et al., 2000; 2006). COD removal was the lowest (60-65%) during July of 2012 and 2013, since the influent concentration of COD was also the lowest (around 100 mg/L). In general, experimental results highlight the robustness of the technology, showing high removal efficiencies even in winter conditions and despite the variability in influent wastewater characteristics. For instance, influent COD ranged between 100 and 1020 mg/L (Fig. 2). Notwithstanding, effluent COD oscillated between 50 and 60 mg/L all over the year. Likewise, only slight variations in effluent $NH_4$-$N^+$ (0.3-4 mg/L) were registered, whereas influent $NH_4$-$N^+$ concentration (18-126 mg/L) varied considerably. In other studies, $NH_4$-$N^+$ removal in open ponds treating wastewater ranged from 60 and 99.5% (Batista et al., in press; Posadas et al., 2015; Sutherland et al., 2014). In HRAP, microalgae assimilation and ammonia stripping are the main nitrogen removal pathways (Arbib et al., 2013; García et al., 2006; Nurdogan and Oswald, 1995). In a previous study including an exhaustive nitrogen mass balance, it was demonstrated that stripping reached an overall removal ranging from 30 to 50%, while algal uptake removed approximately 25% of nitrogen. In that study nitrification was observed to be limited only to certain periods (especially winter) (García et al., 2000). Similarly, in our case, ammonia stripping may have played an important role because of the relatively high pH values (8-9).

### *3.2 Microalgal biomass production and characterisation*

Biomass production in the HRAP showed a seasonal pattern (Fig. 3). The highest microalgae production was observed in May (23 g TSS/$m^2$d), while the lowest was observed from October to December (around 3 g TSS/$m^2$d). Yearly average microalgae production was 10 g TSS/$m^2$d, somewhat lower than literature results ranging from 13 to 35 g TSS/$m^2$·d (Park et al., 2011). It is speculated that microalgae production was limited by inorganic carbon. In fact, urban wastewater has proportionally more available nutrients than carbon dioxide, which is a limiting factor for algal

243 growth (Craggs, 2005). This may be overcomed by $CO_2$ injection in HRAP (Park and Craggs,
244 2010).

245 The main characteristics of harvested microalgal biomass are summarised in Table 2.
246 Average macromolecular composition was 58% proteins, 22% carbohydrates and 20% lipids. These
247 results are similar to those found in pure cultures of green microalgae, such as *Scenedesmus*
248 *obliquus*: 50-56% of proteins, 12-14% of lipids and 10-17% of carbohydrates; and *Chlorella*
249 *vulgaris*: 51-58% of proteins, 14-22% of lipids and 12-17% of carbohydrates (Becker, 2004).

250 In general, taking into consideration the theoretical specific methane yield for each
251 macromolecular compound, namely 0.85 L $CH_4$/g VS for proteins, 0.42 L $CH_4$/g VS for
252 carbohydrates and 1.01 L $CH_4$/g VS for lipids (Sialve et al., 2009), microalgal biomass in the
253 present study had a theoretical specific methane yield of 0.40 L $CH_4$/g VS.

254 Microalgal biomass was periodically characterised by optical microscopy over the year.
255 Qualitative results showed that the main green microalgae species belonged to the genus
256 *Monoraphidium*, *Oocystis*, *Scenedesmus*, *Stigeoclonium* and diatoms of the genus *Nitzschia* sp. and
257 *Navicula* sp.; although dominant microalgae populations varied throughout the year (Fig. 4). This is
258 generally common in open ponds treating wastewater. According to previous literature,
259 predominant species in biomass grown in microalgae-based wastewater treatment systems have a
260 rigid cell wall, due to its adaptability to grow under variable ambient conditions, with grazers and
261 high organic content (Park et al., 2011). In our case, conspicuous ciliate protozoa and rotifer
262 populations grazing on microalgae were observed towards the last months (Fig. 5).

263 Since biogas production from microalgae has been proved to be species-specific, BMP tests
264 were carried out over the year in order to evaluate changes in biomass anaerobic biodegradability.
265 In fact, microalgal biomass methane yield varied between 72 and 128 mL $CH_4$/g COD and its
266 anaerobic biodegradability between 21 and 37% (Fig. 6). Towards the end of the experimental
267 period, when biomass was mainly composed by *Oocystis* sp. and diatoms (i.e. May and July 2013),
268 the anaerobic biodegradability was lower (around 20-25%) than in the period in which biomass was

composed by *Stigeoclonium* sp. and *Monoraphidium* sp. (around 30-40%) (i.e. November and February 2012) (Fig. 4). This confirms that methane yield depends on the species composing microalgal biomass in each moment, which may be highly variable in open systems. Variations in anaerobic biodegradability are mainly due to the characteristics of microalgae cell wall, which is the first membrane degraded by hydrolytic anaerobic bacteria. In fact, *Stigeoclonium* sp. and *Monoraphidium* sp. cell walls are composed by structural polysaccharide compounds, such as cellulose, hemicellulose and pectin (Dawes, 1966; Kim et al., 2014). These species are more biodegradable than *Oocystis* sp., which are composed by multiple external layers formed by structural polysaccharides and diatoms containing a resistant layer nanopatterned silica ($SiO_2$) (Passos and Ferrer, 2015). So even if the macromolecular composition of harvested biomass was fairly constant, individual compounds (i.e. types of carbohydrates) and the cell wall structure differ among species, which ultimately affect its anaerobic biodegradability. As a result, the methane yield changed over the year, concomitantly which variations in predominant microalgae species.

### *3.3 ADM1 calibration*

The high variability in biomass composition in these treatment systems makes modelling difficult. As already discussed, microalgal biomass anaerobic biodegradability was highly variable (Fig. 6), according to dominant microalgae species growing in the pilot HRAP. Therefore, in order to fit the model to experimental data, the fraction of particulate inert COD in microalgal biomass was modified over the studied period. In this case, inert COD consists in the fraction of organic matter which is not biodegraded due to the complexity of microalgae cell wall structure, which may be composed cellulose, hemicellulose or even silica as in the case of diatoms (Passos and Ferrer, 2015).

In accordance with this variation, the inert fraction of COD was set at 64% of the total COD from July to October 2012 and from May to July 2013; while it was set at 59% of the total COD from November 2012 to April 2013 (Table 1). These values are lower than those found in BMP

295 tests, which varied from 64 to 80% of non-biodegraded COD (Fig. 5). The reason for this is that
296 when BMP tests are carried out with non-acclimated inoculum, organic matter removal and
297 methane yield are lower than in continuous reactors with acclimated biomass (Batstone, et al.
298 2009).

299 The model calibration in terms of inert COD was in accordance with the characteristics of
300 harvested biomass. During the months where biomass was composed by microalgae species with
301 more complex cell structure, the model was calibrated with the highest fraction of particulate inert
302 COD to better fit experimental data (64%). As already mentioned, during this period microalgal
303 biomass was formed by *Oocystis* sp. and diatom species like *Nitzschia* sp, which are resistant to
304 hydrolytic degradation. This is in accordance with our previous studies, where low methane yield
305 was achieved through anaerobic digestion of microalgal biomass composed mainly by *Oocyistis* sp.
306 (Passos and Ferrer, 2015), as compared to periods with *Monoraphidium* sp. (Passos and Ferrer,
307 2014).

308

309 ***3.3 Anaerobic digestion performance and ADM1output***

310 After adjusting the fraction of inert COD, the model was applied to experimental data. As shown in
311 Figure 7, calibrated ADM1 was able to predict quite well microalgal biomass anaerobic digestion
312 performance (i.e. methane yield, COD and $N\text{-}NH_4^+$).

313 Microalgal biomass methane yield showed a high variability during the studied period
314 (0.06-0.23 L $CH_4$/g COD) (Fig. 7a). During the first months (July-October 2012), the anaerobic
315 reactor was operated at a HRT of 15 days and average microalgal biomass methane yield was 0.09 L
316 $CH_4$/g COD. When the reactor was operated at a HRT of 20 days, the methane yield increased to
317 around 0.16 L $CH_4$/g COD (78%). This indicates that a longer HRT was required to enhance the
318 anaerobic digestion performance, due to microalgae slow hydrolysis. In a previous study, *Chlorella*
319 *vulgaris* methane yield was 0.11 L $CH_4$/g COD when digested at 16 days HRT and 0.18 L $CH_4$/g
320 COD when digested at 28 days (60% increase) (Ras et al., 2011). On the other hand, microalgal

biomass grown in wastewater attained a methane yield of 0.25 L $CH_4$/g COD with a HRT of 30 days (Golueke et al., 1957). Apart from the HRT, microalgae anaerobic digestion is influenced by other factors, especially microalgal biomass characteristics, such as the cell wall structure. From December 2012 to July 2013, when the reactor was operated with the same HRT (20 days), the methane yield still showed high variability. In this period, average values fluctuated from 0.10 to 0.23 L $CH_4$/g COD. As discussed previously, low methane yield was associated to the presence of low biodegradable microalgae species. Microalgae anaerobic biodegradability is species-specific, i.e. it depends mainly on the characteristics and complexity of their cell wall structure (Passos et al., 2014). For this reason, adjusting the fraction of inert COD was crucial to model our experimental data with ADM1.

Overall, the average methane yield attained in the reactor was 0.20 L $CH_4$/g VS, lower than the theoretical specific methane yield (0.40 L $CH_4$/g VS) calculated from microalgal biomass macromolecular composition. This means that theoretically around 50% VS were digested. However, experimental results showed that VS removal was 25-40%, and COD removal was 17-40%. As can be seen in Fig. 6b, effluent COD was in average 15 g/L when the reactor was operated with a HRT of 15 days and varied from 11 to 15 g/L when the HRT was 20 days (Fig. 7b). Since the influent COD was approximate 18 g/L (Table 2), COD removal was 17% when the HRT was 15 days and 17-40% when the HRT was 20 days. Such variability highlights that the anaerobic biodegradability depended on operational conditions but also on the characteristics of harvested biomass.

Regarding ammonium nitrogen, an increase in effluent concentration (Fig. 7c) was observed during the period in which biomass had the highest anaerobic biodegradability (e.g. lowest content of inert COD (59%)). Since protein was the main macromolecule in biomass (58%) (Table 2), increased biomass anaerobic biodegradability incremented the concentration of inorganic nitrogen in the digestate (Fig. 6c). Nevertheless, ammonium nitrogen concentration reached maximums of 230 mg/L at 15 days HRT and 380 mg/L at 20 days HRT, which are far below toxic values of 1700

mg/L (Schwede et al., 2013). In terms of VFA, values ranged from 0 to 83 mg $COD_{eq}$/L at a HRT of 15 days and from 0 to 112 mg $COD_{eq}$/L at a HRT of 20 days.

To summarise, the main drawback of biogas production from biomass grown in HRAP was the variability and low biodegradability of microalgae-bacteria community. Since open ponds do not allow for species control, microalgae anaerobic digestion ought to be improved by operating the reactors at long HRT (i.e. 30 days) or by applying pretreatment techniques for enhancing particulate biomass hydrolysis. In this case, longer HRT may ease the hydrolysis of slowly biodegradable compounds, while pretreatment methods may solubilise particulate biomass previous to anaerobic digestion. This would be most important in periods were microalgal biomass was composed by species with a complex cell wall structure such as *Oocystis* sp. and diatoms.

## 4. Conclusions

This study analysed an integrated system for wastewater treatment, microalgae production and anaerobic digestion to produce biogas. The HRAP was robust in terms of wastewater treatment efficiency (~ 80% COD and 95% $N\text{-}NH_4^+$ removal), despite variations in wastewater composition and weather conditions. Biomass production showed a strong seasonality, reaching an annual average of 10 g TSS/$m^2$·d and the highest values in spring (23 g TSS/$m^2$·d). Conversely, the macromolecular composition was fairly constant (58% proteins, 22% carbohydrates and 20% lipids). The methane yield of harvested biomass was in average 0.09 and 0.16 L $CH_4$/g COD when the anaerobic reactor was operated at a HRT of 15 and 20 days, respectively. Variations in anaerobic digestion performance over the year were attributed to changes in dominant microalgae species, hence in their cell structure and anaerobic biodegradability. Therefore, adjustments on the fraction of particulate inert COD in ADM1 were needed for model fitting. By adjusting this parameter, the calibrated model satisfactory simulated the anaerobic digestion performance.

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**Table 1.** Values of modified parameters for ADM1 calibration.

| Parameter | Period | |
|---|---|---|
| | **Jul - Oct 2012 and May - Jul 2013** | **Nov 2012 - Apr 2013** |
| ***Stoichiometric parameters*** | | |
| COD fraction of particulate inert (%) | 64.0 | 59.0 |
| COD fraction of carbohydrates (%) | 7.8 | 8.9 |
| COD fraction of proteins (%) | 20.4 | 23.3 |
| COD fraction of lipids (%) | 6.8 | 7.8 |

**Table 2.** Average characteristics of harvested microalgal biomass. Mean values (Standard deviation).

| Parameter | Value |
|---|---|
| pH | 7.50 (0.40) |
| TS [% (w/w)] | 2.28 (0.36) |
| VS [% (w/w)] | 1.27 (0.21) |
| VS/TS (%) | 58.27 (2.74) |
| COD (g/L) | 17.82 (3.75) |
| TKN (g/L) | 1.00 (0.32) |
| $N\text{-}NH_4$ (mg/L) | 11.50 (2.54) |
| Proteins (% VS) | 58 (2.52) |
| Carbohydrates (% VS) | 22 (2.69) |
| Lipids (% VS) | 20 (1.33) |

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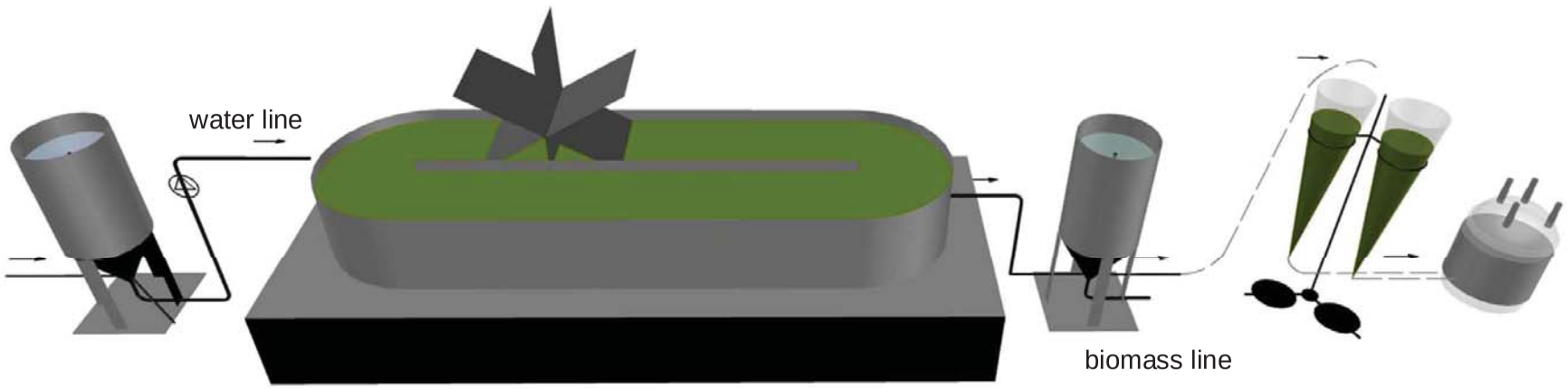


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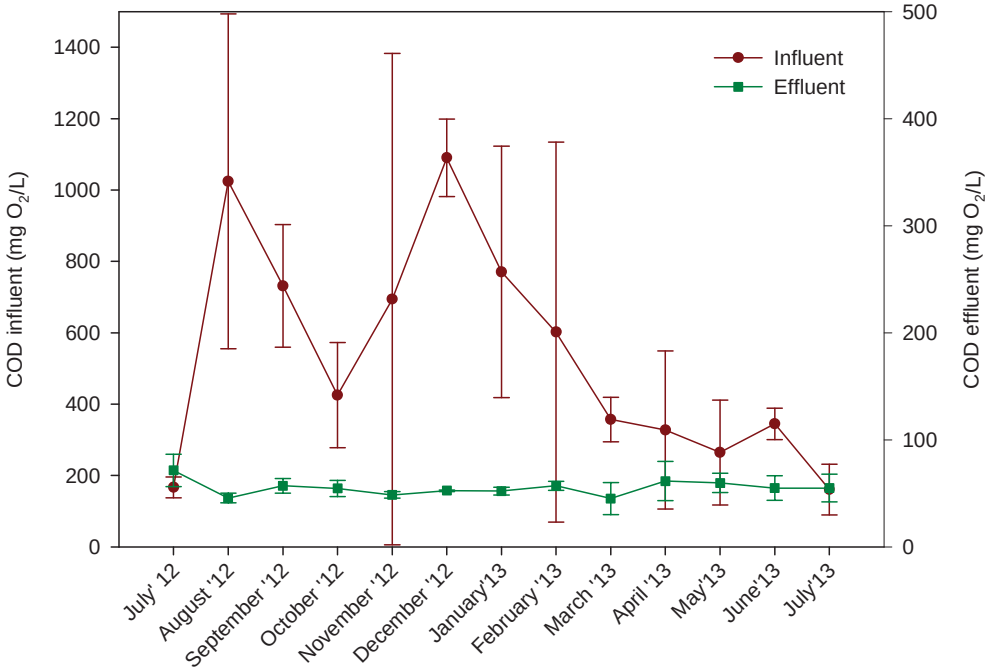
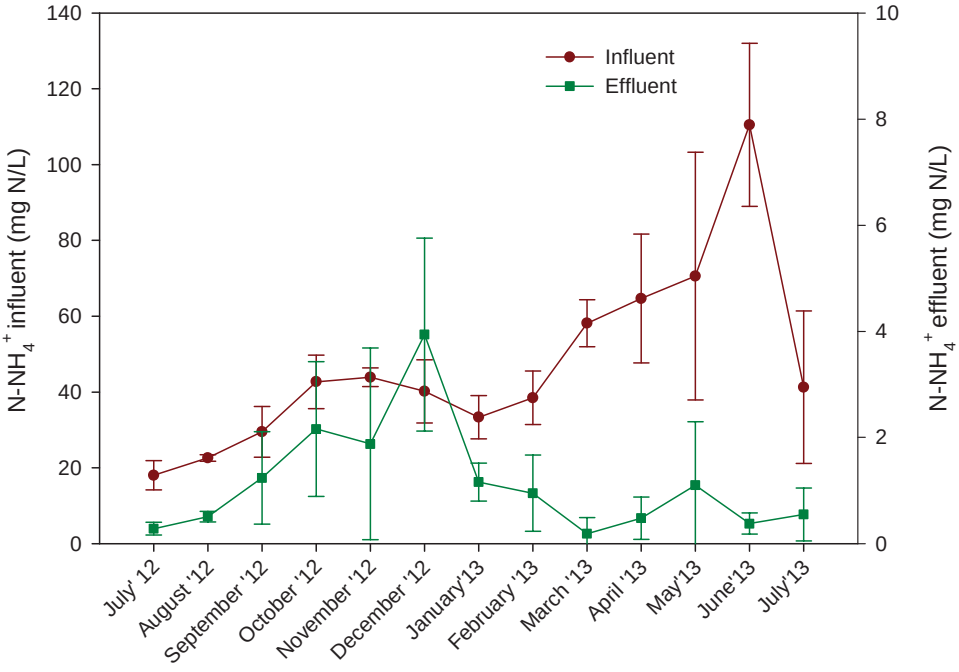
505 **Figure 1.** Schematic diagram of the process.




**(a)**




**(b)**

**Figure 2.** Chemical oxygen demand (COD) (a) and ammonium nitrogen ($N\text{-}NH_4^+$) (b) influent and effluent concentrations in the pilot HRAP over one year.

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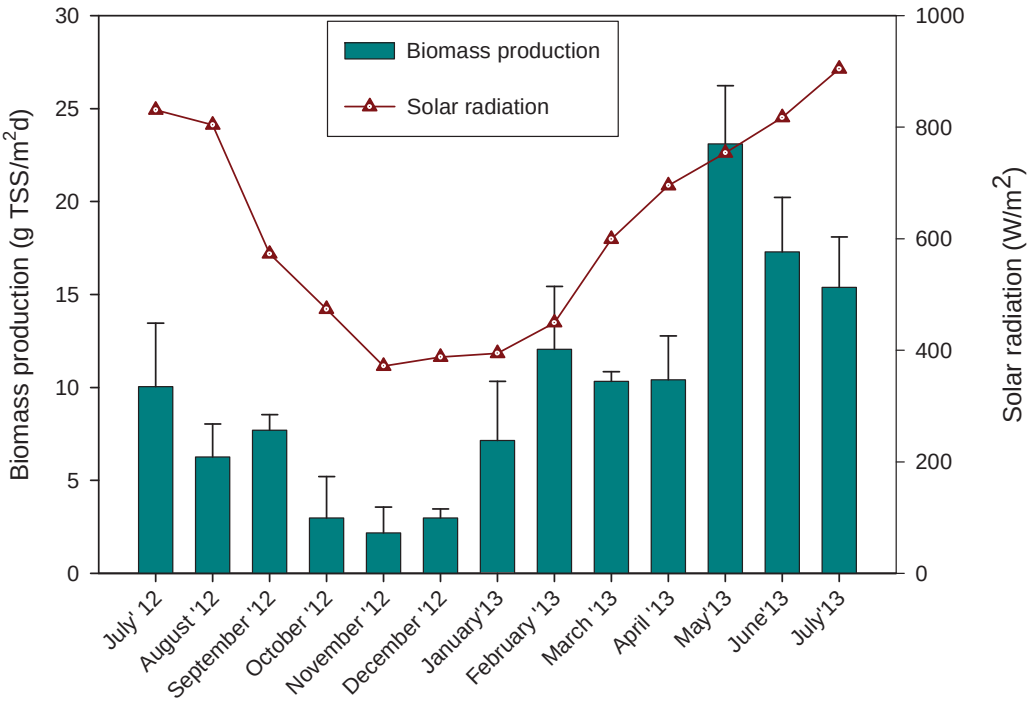


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514 **Figure 3.** Biomass production in the pilot HRAP and solar radiation over the year.

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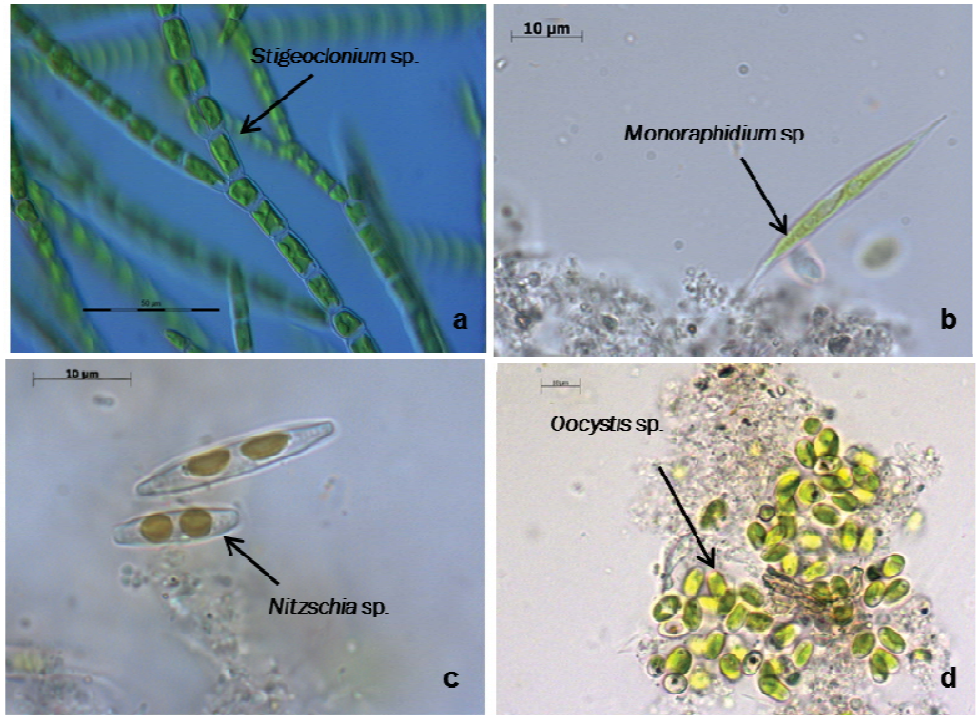


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517 **Figure 4.** Microalgae species grown in the pilot HRAP: a) green filamentous microalgae

518 *Stigeoclonium* sp. (November 2012); b) green microalgae *Monoraphidium* sp. (Febuary 2013); c)

519 diatom *Nitzschia* sp. (May and July 2013) and; d) green microalgae *Oocystis* sp. (July 2013).

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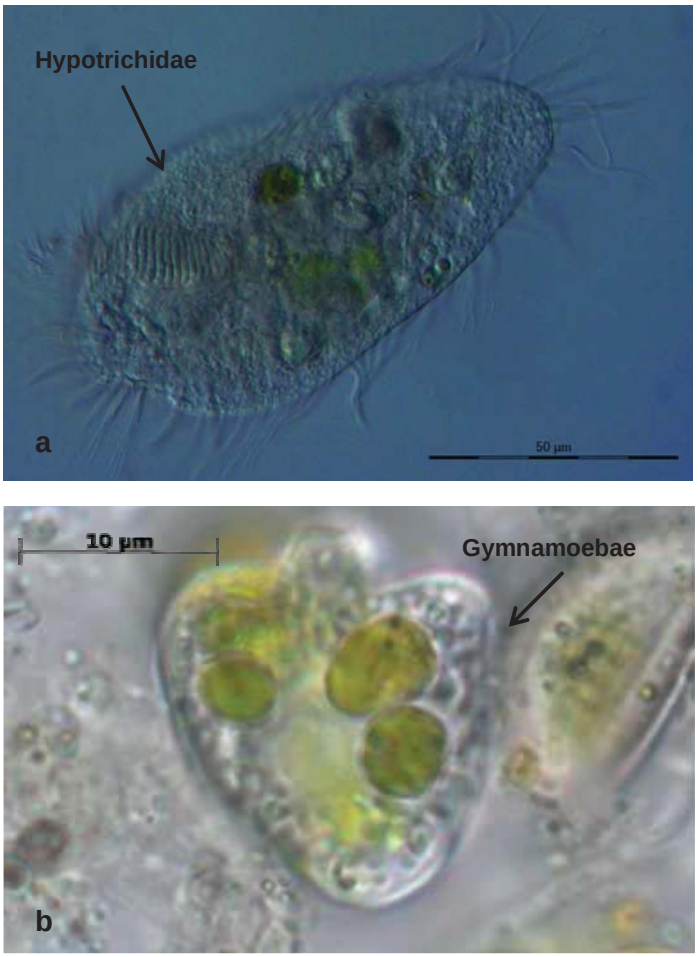
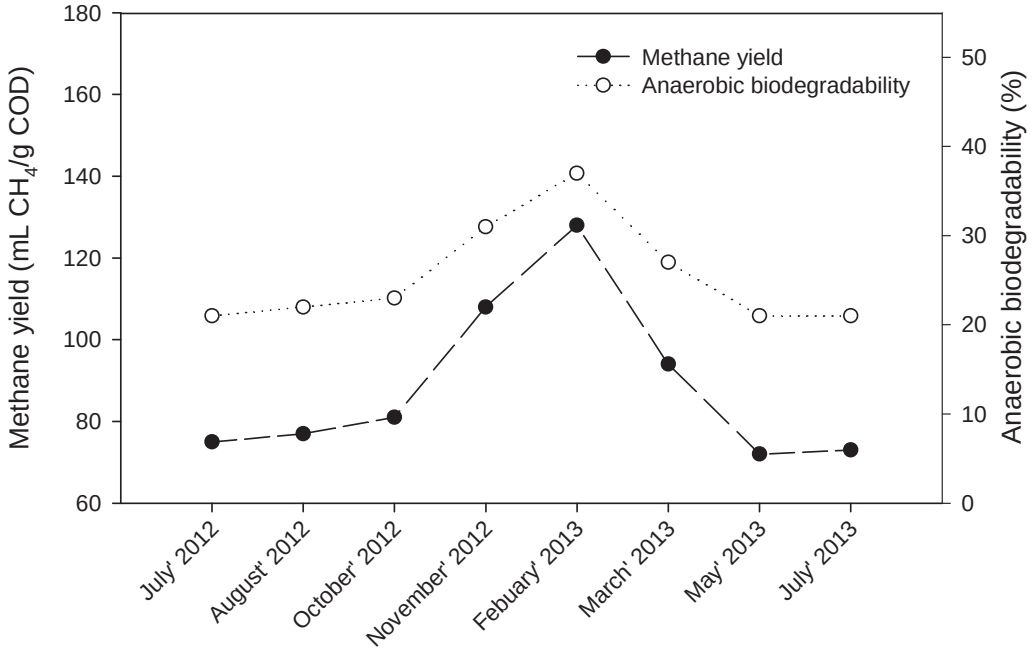



**Figure 5.** Protozoa observed in the pilot HRAP: a) ciliate protozoa belonging to Hypotrichidae; and b) Gymnamoebae. Both organisms predate microalgae species.

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525 **Figure 6.** Methane yield and anaerobic biodegradability of microalgal biomass over the year.

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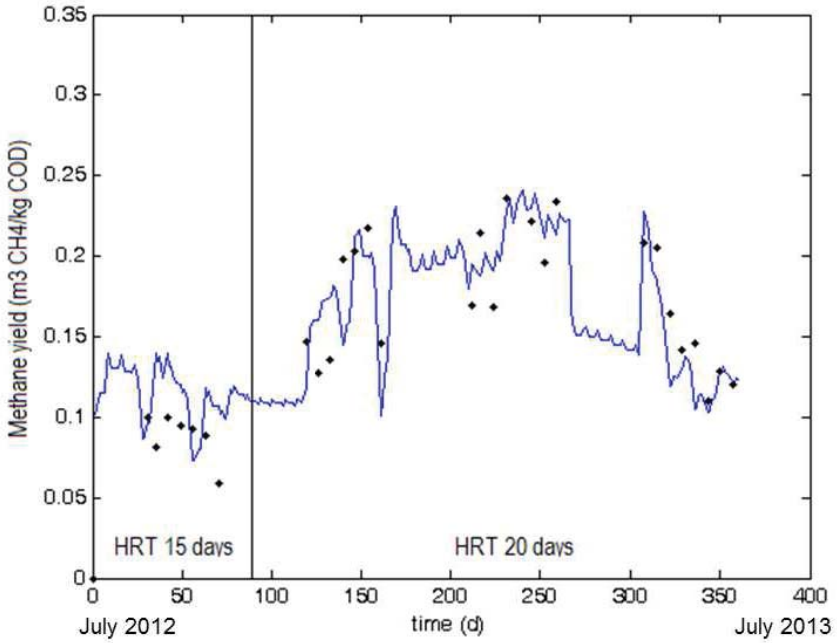


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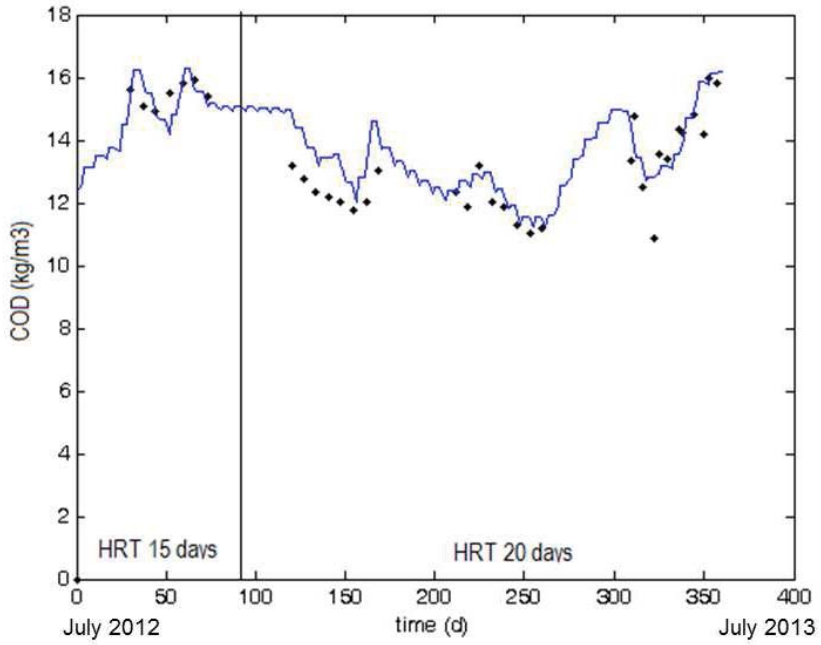
528 (a)

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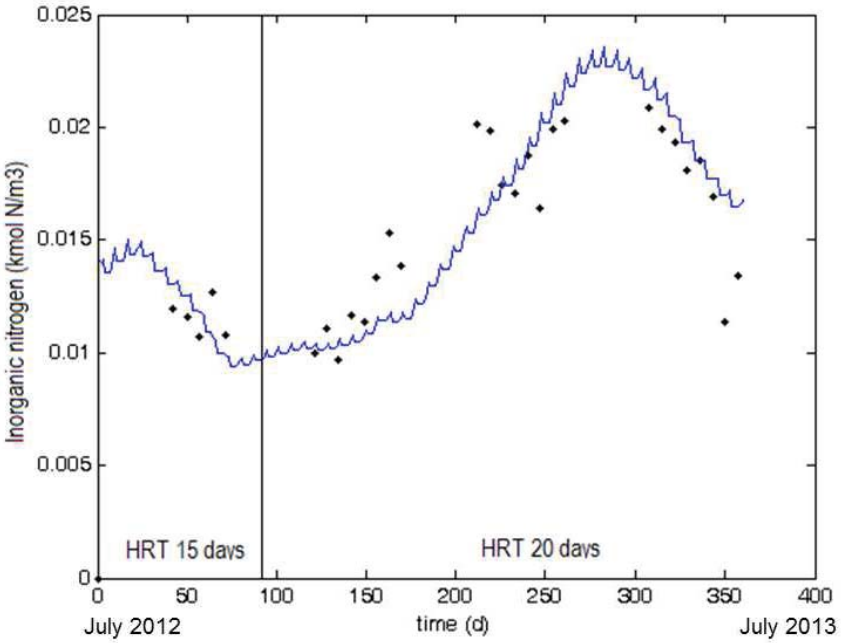
530 (b)




(c)

**Figure 7.** Weekly averages of microalgal biomass methane yield (a) and COD (b) and inorganic nitrogen (c) concentrations in the digestate. Experimental data (black spots) and model output (blue line).