

Start-up of a microalgae-based treatment system within the biorefinery concept: from wastewater to bioproducts

Enrica Uggetti, Joan García, Juan Antonio Álvarez and
María Jesús García-Galán

ABSTRACT

Within the European project INCOVER, an experimental microalgae-based treatment system has been built for wastewater reuse and added-value products generation. This article describes this new experimental plant and the start-up stage, starting from the new design of three semi-closed horizontal photobioreactors with low energy requirements for microalgae cultivation (30 m³ total), using agricultural runoff and urban wastewater as feedstock. The inflow nutrients concentration is adjusted to select cyanobacteria, microalgae able to accumulate polyhydroxybutyrates, which can be used for bioplastics production. Part of the harvested biomass is used as substrate for anaerobic co-digestion (AcoD) with secondary sludge to obtain biogas. This biogas is then cleaned in an absorption column to reach methane concentration up to 99%. The digestate from the AcoD is further processed in sludge wetlands for stabilization and biofertilizer production. On the other hand, treated water undergoes ultrafiltration and disinfection through a solar-driven process, then it is pumped through absorption materials to recover nutrients, and eventually applied in an agricultural field to grow energy crops by means of a smart irrigation system. This plant presents a sustainable approach for wastewater management, which can be seen as a resource recovery process more than a waste treatment.

Key words | bioplastics, circular economy, closed systems, microalgal biomass, wastewater

Enrica Uggetti (corresponding author)

Joan García

María Jesús García-Galán

GEMMA – Environmental Engineering and Microbiology Research Group, Department of Civil and Environmental Engineering, Universitat Politècnica de Catalunya-BarcelonaTech, c/ Jordi Girona 1-3, Building D1, Barcelona E-08034, Spain
E-mail: enrica.uggetti@upc.edu

Juan Antonio Álvarez

AIMEN Technology Centre, c/ Relva, 27 A – Torneiros, Porriño, Pontevedra 36410, Spain

INTRODUCTION

Basically, all kind of human activities are putting water resources under a constant pressure: global warming, over-exploitation and pollution of freshwater resources, urbanization and also an increasing competition between various user groups. The 2007 European Communication on Water Scarcity and Droughts (EC 2007) highlighted that climate change and increasing population would raise frequency and severity of water scarcity and drought events. Over the past 30 years, droughts have dramatically increased in number and intensity in the EU and at least 11% of the European population and 17% of its territory have been affected by water scarcity to date (EC 2007). Moreover, only half of the European countries meet the Water Framework Directive (EC 2000), that foresaw a good ecological status of water bodies by 2015 through an adequate wastewater treatment, making major additional wastewater treatment solutions necessary. It is therefore

mandatory to put effort in the wastewater treatment sector to look for new technological solutions environmentally and economically feasible in order to overcome this gap.

Nowadays, treated water is mostly discharged into surface waters or into the ground instead of being reused. Despite that during the last years reclaimed water reuse has been encouraged for agricultural irrigation as well as other uses, only a small amount of treated wastewater is currently recycled. At present, about 1 billion m³ of treated urban wastewater is reused annually, which accounts for approximately 2.4% of the treated urban wastewater effluents and less than 0.5% of annual EU freshwater withdrawals. However, the EU potential is estimated in the order of 6 billion m³ – six times the current volume. Cyprus and Malta already reuse more than 90% and 60% of their wastewater respectively, while Greece, Italy

doi: 10.2166/wst.2018.195

and Spain reuse between 5 and 12% of their effluents, clearly indicating a huge potential for further uptake (EC 2017).

In this context, the solution to the increasing water scarcity lies partially in the search for and the implementation of new alternative wastewater technologies with low cost and energy consumption, able to generate reusable water and new products and resources instead of residues. The need for a radical change in the wastewater sector has driven the European Commission to promote innovative ideas in this field. A recent example is the project INCOVER: 'Innovative Eco-Technologies for Resource Recovery from Wastewater' (<http://incover-project.eu/> GA 689242), that aims to resolve the need for new wastewater technologies by promoting water reuse, changing the paradigm of considering wastewater as only a waste and regarding it as a valuable resource, from which new added-value products can be obtained. The project is coordinated by the AIMEN Technology Center (Spain) and is being carried out through the collaboration of 18 partners, including companies, universities, and research and technological centers from seven European countries, with a proven expertise in wastewater and organic waste treatment and bioproducts generation. The project started in June 2016 and research and demonstration activities will be developed during the following 3 years in three different experimental sites located in Spain (Barcelona, Almería-Cádiz) and Germany (Leipzig). The project 'technology readiness level' to be achieved is 7, implying a complete demonstration in a real environment.

The aim of this article is to describe the implementation and start-up stage of the experimental plant designed and built by

the Group of Environmental Engineering and Microbiology of the Universitat Politècnica de Catalunya-BarcelonaTech (GEMMA-UPC), and located at the UPC site Agròpolis (Viladecans, Barcelona). Special focus will be given to the design and operation of the microalgae production system. The results obtained during the first months of operation of the different technologies installed are shown and discussed.

The novelty of this plant resides in changing the paradigm of considering wastewater as a disposable waste, to start regarding it as a valuable resource, from which new added-value products can be obtained and proficiently used. To our knowledge this is the first plant built at this scale coupling microalgae-based wastewater treatment with generation of bioplastics, biomethane, biofertilizers and clean water coupled with smart irrigation.

MATERIALS AND METHODS

Agròpolis experimental plant

In the Agròpolis plant (Figure 1), a combination of agricultural runoff and urban wastewater is treated by means of a mixed culture containing mostly microalgae and cyanobacteria growing in three horizontal hybrid (semi-closed) tubular photobioreactors (PBRs). In the culture, heterotrophic bacteria are also growing but at lower rates than the other microbial groups due to the low organic matter concentration of the influent. After biomass separation, treated water is submitted to solar energy-powered ultrafiltration (UF) and disinfection, then to nutrient recovery by means of

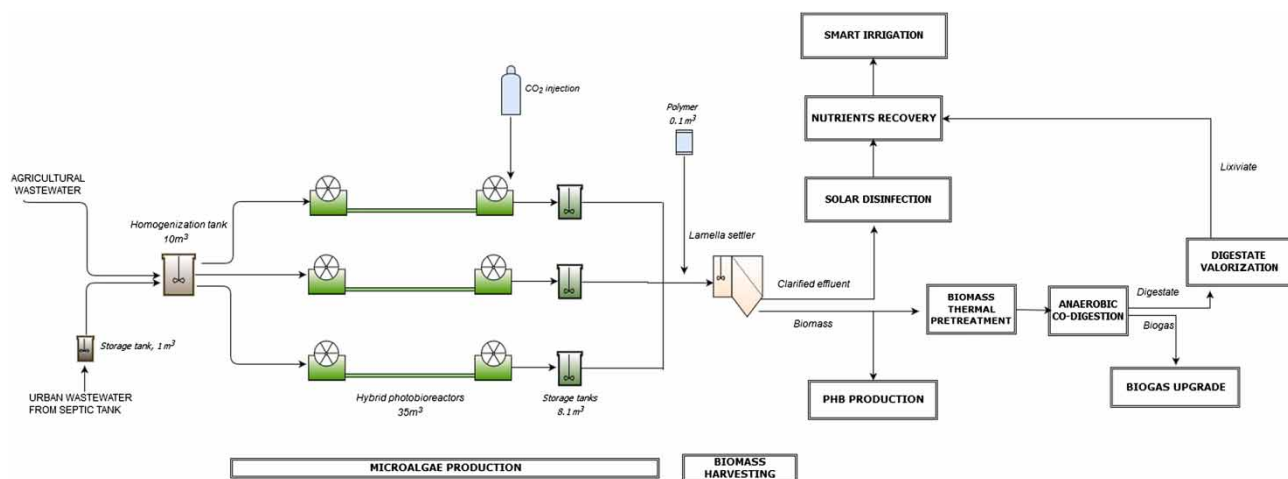


Figure 1 | Scheme of the INCOVER plant located at the Agròpolis campus, Viladecans, Barcelona.

three adsorption columns and finally reused in a smart irrigation system to grow energy crops (i.e. rapeseed). Concomitantly, the biomass obtained is submitted to anaerobic co-digestion (AcoD) with secondary sludge to produce biogas, which will be enriched in an absorption column filled with the mixed liquor from one of the PBRs. The digestate produced in the anaerobic digester is also treated in sludge treatment wetlands in order to produce biofertilizers.

Part of the biomass obtained from the PBRs is analyzed in the laboratory to determine the abundance of cyanobacteria and the content of bioplastics (in the form of polyhydroxybutyrate (PHB)) accumulated. Experiments regarding the best conditions to obtain cyanobacteria-dominated cultures and to achieve the highest accumulation of PHBs are currently being carried out. The operation of the PBRs will be adjusted after the results are obtained.

Photobioreactor design and operation

The PBRs were conceived, designed and constructed by the GEMMA Research Group (Universitat Politècnica de Catalunya-BarcelonaTech) in collaboration with the company Disoltech S.L. Each reactor (Figure 2) consists of two open tanks made from polypropylene (5 m length \times 1 m width \times 0.6 m height) connected by 16 low density polyethylene tubes (125 mm diameter and 47 m length). The useful volume is approximately 11.7 m³. The tubes lie on a waterproof covering sheet in order to ensure separation from the ground. Both open tanks ensure and favor the homogeneous distribution and mixing of the liquor and also the release of the excess dissolved oxygen accumulated along the closed tubes. In each open tank, a paddle wheel with six blades (1 m width \times 0.35 m length) is installed 1.8 m away from the external edge and at 3 cm height from the bottom. An engine (0.35 kW) connected to each paddle wheel provides a turning speed which can be changed from 0 to 12 rpm. Usually PBRs are operated with turning speeds ranging from 9 to 12 rpm to ensure turbulent flow inside the tubes. The water level is fixed at approximately 0.15 m before the paddle wheel and 0.35 m after it. The total working volume in each open tank is 1.25 m³ (approximately 20% of the volume of the PBR is due to the tanks). Each tank has a dam, which assists in maintaining two different surface water levels within the tank. In each tank, the mixed liquor is moved by the paddle wheels from the shallow water level sector to the deep one. Afterwards, thanks to the different water levels

(approximately 0.20 m of variation), the mixed liquor flows by gravity through eight tubes from the deep side of one tank to the shallow side of the opposite one. Here the flow is moved by the paddle wheels to the deeper part of the tank and then it returns to the shallow side of the first tank through the other eight tubes, and so on. Technical characteristics of PBRs are summarized in Table 1.

In order to control PBR operation, online sensors of pH, dissolved oxygen and temperature (Hach Lange Spain S.L.) were installed in one of the two open tanks of each PBR. In the mixed liquor, the pH value is approximately maintained below 8.5 through controlled CO₂ addition. This pH boundary was selected based on the results of previous works that reported a pH preference of cyanobacteria ranging from 8 to 9 (Yamamoto & Nakahara 2005; Unrein *et al.* 2010). A recent study by Ji *et al.* (2017) demonstrated that green algae such as *Scenedesmus obliquus* or *Chlorella vulgaris* outcompeted cyanobacteria (*Microcystis aeruginosa*) under low CO₂ conditions and pH levels above 10. The injection is made through an air sparger placed in one of the open tanks after the paddle wheel. The pH of the mixed liquor is measured every 5 seconds with a pH probe, and when the measurement exceeds the pH 8.5 set point, a valve opens and CO₂ is bubbled into the PBR tubes. CO₂ injection stops as soon as pH 8.5 is reached again.

The three PBRs were installed in winter 2017. They were inoculated at the end of April with a mixed culture of microalgae and bacteria grown in urban wastewater. Approximately, 10 L was added to each PBR, with a volatile suspended solids (VSS) concentration of 223 mg/L. From that moment on, PBRs have been operating in parallel, fed with 2.3 m³/d each (6.9 m³/d in total) of a mixture of agricultural and urban wastewater at a ratio of approximately 5:1.

Every day, from 2 a.m. to 5 a.m., 6.4 m³ of agricultural wastewater and 0.5 m³ of urban wastewater (the latter has been previously treated in an aerated septic tank) is pumped into the homogenization tank, where they are mixed in order to reach an influent with the suitable nutrients concentration for cyanobacteria growth. Then, at 5 a.m. the feeding operation starts: firstly, 2.3 m³/d of mixed liquor are pumped out from each PBR to one storage tank. After that (from 7 a.m. on), the same volume of influent from the homogenization tank is pumped into each PBR. This operation takes place in the early morning in order to have nutrients available for biomass growth during the day.

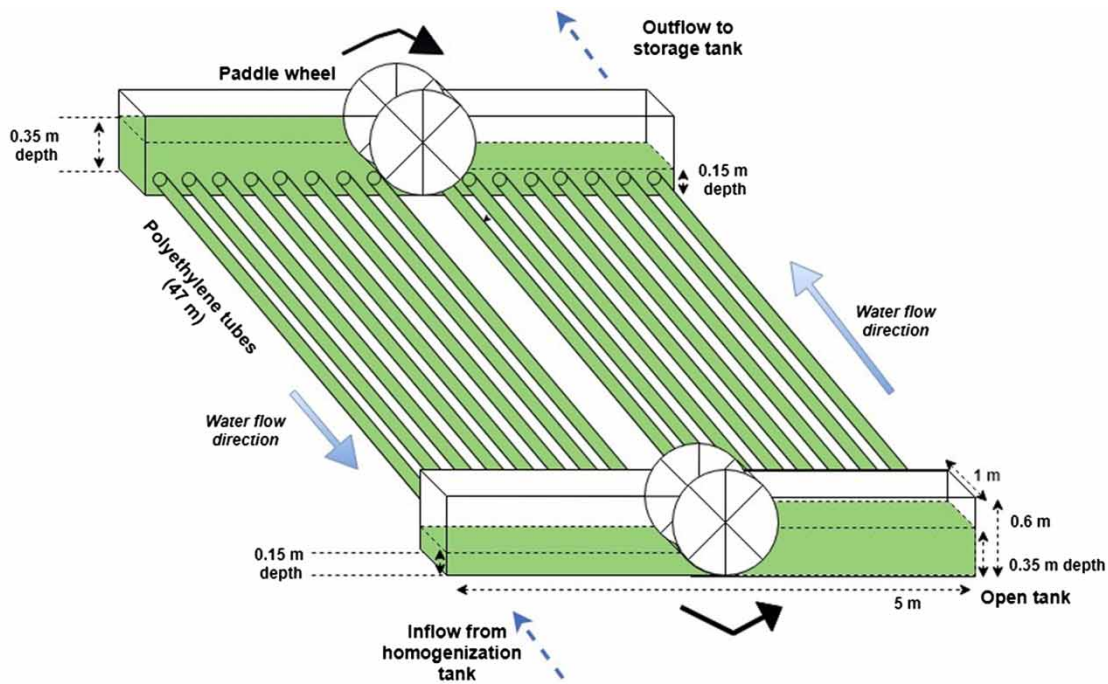


Figure 2 | Scheme of one photobioreactor and picture of the experimental plant with the three photobioreactors installed and operating.

During the microalgae-based wastewater treatment, microalgae biomass grows thanks to the solar radiation and the nutrients (mostly nitrogen (N) and phosphorus

(P)) present in the influent wastewater. At the same time and through photosynthesis, microalgae generate the oxygen needed by the bacteria to aerobically degrade the

organic contaminants present in that medium. Thus, PBRs have the dual advantage of simultaneously producing microalgae biomass and treating wastewater without requiring external aeration.

Analytical methodology and PBRs follow-up

Samples from the influent and from the three PBRs are collected weekly and immediately taken to the laboratory, where they are analyzed to determine: (1) biomass concentration and production as VSS and (2) water quality in terms of ammonium and phosphorus concentrations. Sampling was paused during the month of August.

All parameters, with the exception of chlorophyll *a*, were analyzed in both influent wastewater and effluent (mixed liquor of each PBR). Orthophosphate (dissolved reactive phosphorus) (P-PO_4^{3-}), nitrite (N-NO_2^-) and nitrate (N-NO_3^-) were measured using an ion chromatograph, Dionex ICS1000 (Thermo Scientific, USA). Alkalinity was determined using the titration method 2320 B of *Standard Methods* (APHA et al. 2001). Total suspended solids (TSS) and VSS were measured in triplicate in the influent and effluent of each PBR following the gravimetric method 2540 C and 2540 D in *Standard Methods* (APHA et al. 2001). Turbidity was measured in the mixed liquor of each PBR with a turbidity-meter (Hanna, USA). Temperature, pH and dissolved oxygen were measured directly in the open tanks of each PBR, where the corresponding sensors are introduced in the mixed liquor. Chlorophyll *a* (procedure 10200 H) and total and soluble chemical oxygen demand (CODs) were determined also following *Standard Methods* (APHA et al. 2001).

Mixed liquor samples were regularly examined under an optic microscope (Motic, China) for qualitative evaluation of microalgae populations and to determine the cyanobacteria and microalgae abundance. Taxonomic books were employed for identification of both microalgae and cyanobacteria species (Palmer 1962; Bourrelly 1985).

PRELIMINARY RESULTS

The physico-chemical characterization of the influent is shown in Table 2.

Figure 3 shows the irradiance, temperature and precipitation registered on site during this first period. The biomass concentration during the first months of operation (April to October) is shown in Figure 4 and expressed as VSS (mg/L). After inoculation, around 20 days were

Table 1 | Technical characteristics of each of the photobioreactors

Parameter	Value
Number of tubes	16
Number of tanks	2
Total volume of tanks (m^3)	2.5
Tank surface (m)	5×1
Total volume of tubes (m^3)	9.2
Tube diameter (m)	0.125
Tube length (m)	47
Total PBR volume (m^3)	11.7
Design velocity inside tube (m/s)	0.25
Number of engines	2
Engine power (kW)	0.35
Retention time within the tubes at design velocity (min)	3.13
Retention time within the open tanks at design velocity (min)	0.87

Table 2 | Physico-chemical characterization of the influent wastewater (from April until October 2017, $n = 19$)

Parameter	Value
pH	8.3 ± 0.3
Electrical conductivity (mS/cm)	2.5 ± 0.4
T ($^{\circ}\text{C}$)	24.2 ± 2.0
$\text{NH}_4^+\text{-N}$ (mg/L)	3.8 ± 3.4
$\text{NO}_2^-\text{-N}$ (mg/L)	0.9 ± 1.4
$\text{NO}_3^-\text{-N}$ (mg/L)	8.4 ± 2.1
$\text{PO}_4^{3-}\text{-N}$ (mg/L)	0.8 ± 1.1
TSS (mg/L)	78.5 ± 48.9
VSS (mg/L)	27.3 ± 12.0

needed to detect an increase in the volatile solids concentration; from that day onwards, the concentration increased constantly until reaching concentrations up to 320 mg VSS/L (Figure 4). Considering the operation of the three PBRs, this would correspond to a biomass production of almost 2.2 kg VSS/d.

Concerning wastewater treatment, the concentration of nutrients in the influent was always <2 mg/L for phosphates, <10 mg/L for ammonia and <15 mg/L for nitrites and nitrates. Both N and P present in the influent were completely removed in the PBRs (Figure 5).

As observed in Table 3, the CODs concentrations measured in the mixed liquor were usually higher than

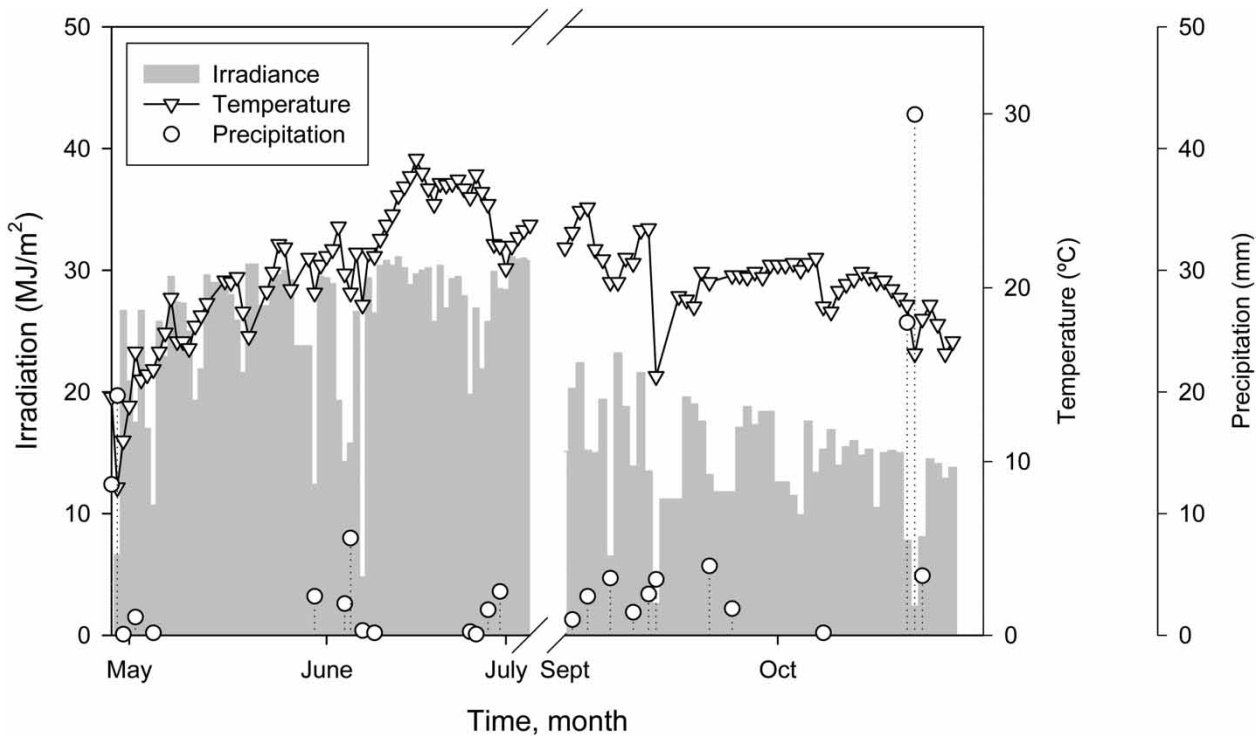


Figure 3 | Daily irradiance, average air temperature and precipitation registered during the first 6 months of operation of the plant (Source: www.meteo.cat/observacions/xema/dades).

the total COD concentrations at the influent feedstock, and so elimination rates were negative. It has been demonstrated that a fraction of photosynthetically fixed carbon is released during microalgae growth as dissolved carbon, and it usually corresponds to 5–30% of the carbon fixed by photosynthesis. The higher COD values in the mixed liquor of the PBR could be attributed to this dissolved

organic matter exudation, but also to the low organic matter biodegradability of the wastewater influent. Similar results were observed in recent studies (Zouhayr *et al.* 2013; García-Galán *et al.* 2018).

Regarding the species observed in the mixed liquor of the PBRs by optical microscopy, qualitative identification showed that the most frequent species were the diatom *Cyclotella* sp., the green algae *Oocystis* sp. and the cyanobacteria *Synechocystis* sp.

In order to separate the biomass produced and to clarify the water treated, a lamella settler was installed after the three PBRs. The water stored in the three storage tanks is then pumped through the lamella settler, which is divided into two chambers: the first one is a mixing chamber, where the water gets in contact with a liquid coagulant (polyaluminum chloride liquid, with 9% of aluminum (PAX-18), provided by Kemira Water Solutions, Spain). The optimum dose is estimated weekly by performing jar tests with the corresponding mixed liquor, and it is usually between 2 and 5 mg/L. After the mixing chamber, the water passes to a second chamber where the lamella favors the solids settling at the bottom whereas water is discharged in the upper part of the settler (Figure 6). The settler currently works at a rate of 400 L per hour.

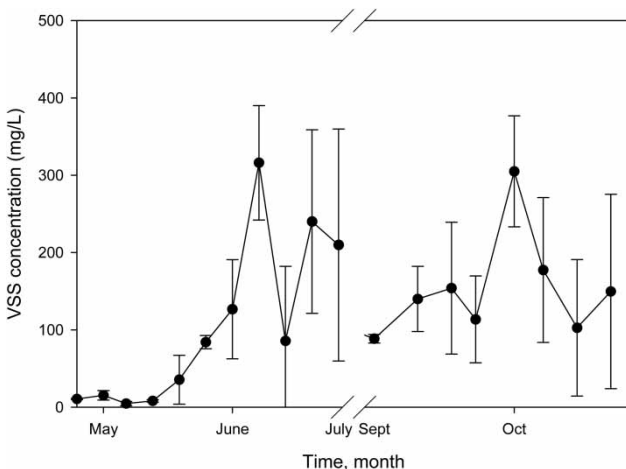


Figure 4 | Biomass concentration measured as VSS (mean and standard deviation) achieved in the three photobioreactors during the first 6 months of operation.

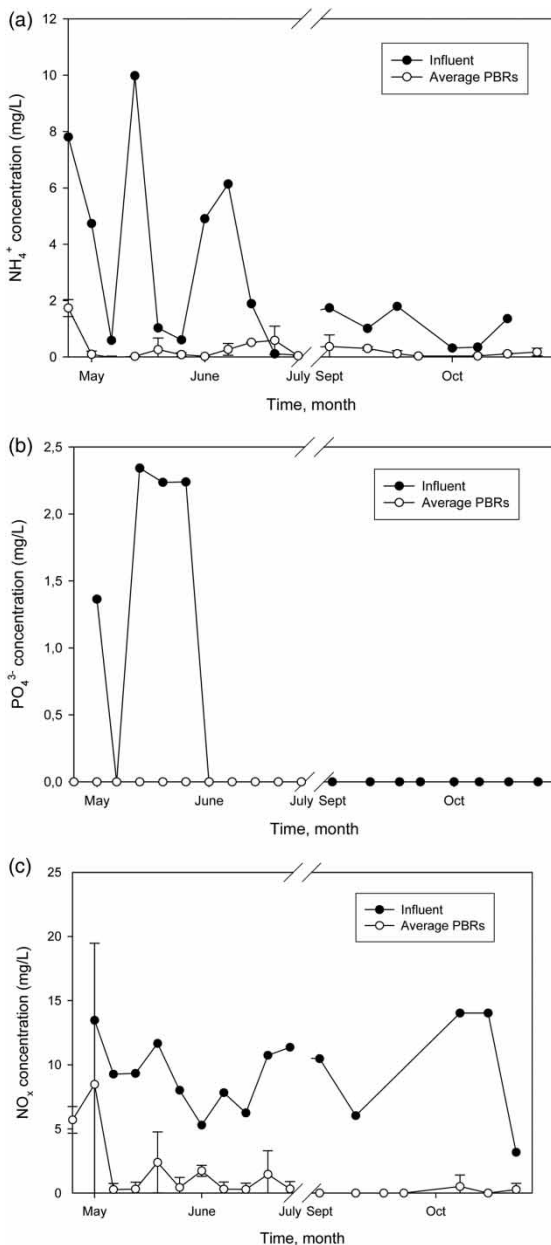


Figure 5 | Average concentrations of (a) ammonium, (b) phosphorus and (c) inorganic oxidized nitrogen (NO_x) recorded in the influent and in the three photobioreactors during the first months of operation. Note: the standard deviation is not shown for the influent because only one sample was analyzed.

BIOPRODUCTS GENERATION

Bioplastic accumulation in wastewater-borne cyanobacteria

As mentioned previously, the three PBRs in the Agròpolis site are fed with a mixture of urban and agricultural

Table 3 | Total and soluble COD values observed in the mixed influent wastewater and the mixed liquor of the PBRs (given as average value) (from April until October 2017, $n = 16$)

Date	Influent total COD (mg/L)	Effluent soluble COD (average value) (mg/L)
03/05/2017	62.1	33.37 ± 8.1
09/05/2017	154	181.10 ± 105.2
16/05/2017	–	85.13 ± 29.8
23/05/2017	110.5	137.60 ± 38.2
30/05/2017	157.7	157.37 ± 18.9
06/06/2017	98.4	162.43 ± 1.3
13/06/2017	139.3	260.67 ± 10.1
20/06/2017	58.5	142.47 ± 34.2
27/06/2017	159.3	107.40 ± 22.8
04/07/2017	144	122.93 ± 26.6
05/09/2017	64.6	148.93 ± 39.2
14/09/2017	50.3	59.47 ± 12.5
26/09/2017	25.6	40.77 ± 8.6
04/10/2017	–	44.50 ± 12.5
10/10/2017	69	75.90 ± 5.5
24/10/2017	68.5	54.00 ± 21.4

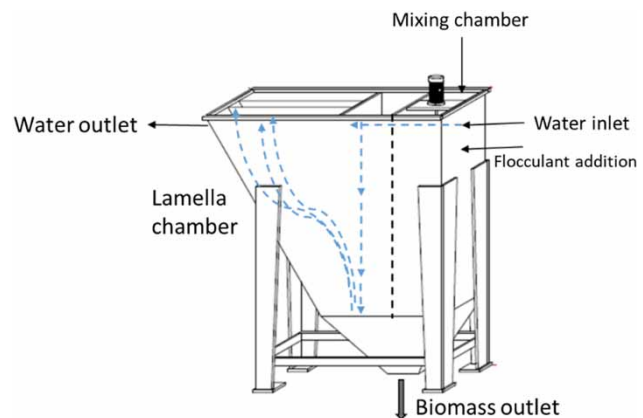


Figure 6 | Scheme of the lamella settler.

wastewater in order to obtain a concentration of nutrients appropriate to select cyanobacteria. The interest in growing cyanobacteria is because these prokaryotic photosynthetic microorganisms have the potential to assimilate and store glycogen, cyanophycin (amino acid polymer), polyphosphates and polyhydroxyalkanoates (PHAs) (Stal 1992). PHAs are an interesting alternative to ordinary plastics from the petrochemical industry, and are nowadays used for packaging, and their use in biomedicine for prostheses

is now being investigated. The most commonly occurring polymer within the PHAs family is polyhydroxybutyrate (PHB) and its accumulation has been demonstrated for several cyanobacteria such as *Spirulina* sp., *Aphanothece* sp., *Gloeothece* sp., and *Synechococcus* spp. (Balaji et al. 2013). The novelty in the INCOVER plant resides in the use of wastewater to select cyanobacteria from a mixed microalgae culture, instead of using pure or genetically modified cultures in usually expensive processes. Indeed, most of the studies dealing with the production of PHBs from cyanobacteria are based on pure or genetically modified cultures (Drosg 2015; Koller & Marsalek 2015), using sterile medium substrates in expensive and highly controlled processes. Once the cyanobacteria are selected in the PBRs, they will be submitted to nutrients stress in lab experiments in order to discern the best conditions to promote PHB accumulation, following the recent study by (Arias et al. 2017a).

Biomethane

The biomass obtained in the PBRs is submitted to a thermal pretreatment and subsequently used as substrate in an AcoD process. A thickener is used after the lamella settler to increase solids concentration of the separated biomass; this harvesting unit (lamella settler and thickener) has been designed in order to increase TSS concentration from 3–8 to 30 g/L. After this step, the biomass is directed to the AcoD unit, which consists of a thermal pretreatment tank (19 L) and an anaerobic digester (1,000 L), together with the corresponding storage tanks for the biomass and co-substrate. Due to the proximity of the wastewater treatment plant of Gavá, secondary sludge was selected as co-substrate. The inoculum was sewage activated sludge from the same plant (100 L). Considering the biomass volume obtained from the PBR, the digester is now operated at a working volume of 400 L. The microalgae biomass undergoes thermal pretreatment at 75 °C during 20 hours before being introduced in the digester, currently at a loading rate

of 7.5 L/d together with 7.5 L/d of co-substrate (1:1, v/v), resulting in a hydraulic retention time of 20 days in the digester. Such a proportion was selected during the start-up in accordance with the results obtained by Arias et al. (2017b), who demonstrated good results in term of methane content and biogas production rates working at 50% of microalgae and 50% of co-substrate (activated sludge). It has also been demonstrated in previous studies that thermal pretreatment improves the solubility of the biomass, leading to an increase of 90% in the biogas production during the AcoD process (Passos et al. 2013, 2015).

During the start-up stage, the organic loading rate introduced to the digester was low (0.15–0.30 g VS/L.d). This amount could be increased using settled secondary sludge as co-substrate, with a higher concentration of organic matter and also improving the operation of the PBRs and the harvesting of the biomass achieved in the settler. Characterization of the feedstock and products of the AcoD unit is given in Table 4.

It has recently been shown that microalgae biomass can be a potential co-substrate for biogas production together with municipal wastewater sludge, significantly enhancing CH₄ yields (Mahdy et al. 2015; Arias et al. 2018). The biogas produced is stored in a gasometer (1,000 L), before the upgrading process, aimed at increasing the CH₄ content of the biogas from 60–70% up to 99%, so that it can be used directly as biofuel. In the upgrading process, mixed liquor of the PBR is used as absorption solution to simultaneously remove CO₂, H₂S, NH₃ and volatile organic chemicals from biogas at a low energy cost. The biogas produced at the INCOVER plant is therefore sparged through a fine bubble plate diffuser in an absorption bubble column (PVC, 4 m high, 20 cm diameter) interconnected to one of the open tanks of one PBR that provides the mixed liquor. The biogas is injected at the bottom of the column, while mixed liquor from the PBR is circulating countercurrent from the top to the bottom. Within the column, photosynthetic CO₂ assimilation concomitant with the aerobic

Table 4 | Characterization of the different feedstocks and products used in the AcoD unit

	Total solids (g/L)	VS (g/L)	COD (g/L)	N-NH ₄ (mg/L)	Total P (mg/L)	TOC (mg/L)
Algae	2.85–11.6	1.23–40.8	0.38–7.01	0.5–84.1	1.5–80.2	93.7–170
Pretreated algae	2.75–14.7	1.14–5.74	1.29–6.31	4–29.1	41.5–110.1	67.8–553.4
Secondary sludge	3.06–7.44	1.29–6.14	0.25–24.9	3.3–135.9	11.8–307.9	311.4–476.4
Digestate	3.25–18.2	1.28–11.9	7.05–15.2	35.9–1115.32	77.8–408.7	343.2–827

Analyses were carried out from 20 June to 7 November.

oxidation of the other trace biogas pollutants aforementioned will take place (Toledo-Cervantes *et al.* 2017). The absorption column has been designed by a research group of the Universidad de Valladolid (partners of the project).

Biofertilizers

The sludge produced from the anaerobic digester (digestate) is a product rich in nutrients. The recent study from Solé-Bundó *et al.* (2017) evaluated the quality of microalgae digestate for agricultural reuse highlighting that, even if the digestate seems to be apt for agricultural reuse, a higher stabilization degree would be suitable before its application. For this reason, the digestate produced in the INCOVER plant is treated in a sludge treatment wetland (6 m²), which has proved to be highly efficient for sludge dewatering and stabilization in previous studies (Uggetti *et al.* 2010). The system, designed by Centre for Recirkulering (partner of the project), consists of a granular medium (0.45 m depth) planted with *Phragmites australis* (5 plants/m²). The drainage system for the discharge effluent is installed embedded in the coarse gravel layer at the bottom of the bed. In addition, vertical pipes for the aeration of the gravel bed are connected to the drainage system to allow the flow of air to improve aeration in the bed. Currently, the system has been loaded with water to ensure the establishment of the plants; once this stage is over, the planned solids load rate will be low (total solids of around 35 kg/m² year) in order to ensure the proper plant acclimation, but it will be gradually increased.

Reclaimed water

Getting back to the water treatment line, the clarified water from the lamella settler and thickener is pumped to a solar-driven disinfection system designed and developed by Solar-Spring GmbH (partners of the project) to provide drinking water. The process is based on low pressure UF, which requires low energy. The system consists of different capillary membranes made of polyethersulfone with a capillary diameter of 0, 9 and 1.5 mm and a pore diameter around 0.02 µm (total membrane surface up to 6 m²). For both membranes, a pretreatment is set up based on a pre-filter (100 µm) and a media filter (5 µm). After the UF, water passes through a post-treatment consisting of activated carbon filters and an integrated UV-C reactor for disinfection, finally reaching a storage tank. There is also a solar generator for independent power supply and various sensors

for online monitoring of pressure, flow, water level, turbidity and UV-radiation. The working flux is 500 L/h divided into two parallel lines (dual system), one used as a reference system and monitored, and the other used for potential implementations depending on the results. This way, efficiencies under different settings can be compared.

Nutrients recovery

After the solar-driven UF and disinfection, the water treated in the PBRs is fed to three adsorption columns filled with sol-gel coating developed by researchers from Aarhus University and the Danish Technological Institute (partners of the project) in order to recover N and P. Adsorption processes are attractive for nutrient removal due to the simplicity of operation, their low cost and the possibility of resource recovery from wastewaters (Bhatnagar & Sillanpää 2010), and they have already been proved as a cost-effective solution for nutrients removal from wastewaters using industrial by-products (Garfí & Puigagut 2016). Calcite and crushed autoclaved aerated concrete were tested as base materials in the INCOVER plant, due mainly to their high affinity for P and their low cost. Two different coating compositions were developed and tested, an inorganic- and an organic-based one, both having the property of stabilizing the adsorptive base material, avoiding its disintegration in contact with water. They also allow the penetration and absorption of P, and even enhance the capacity of the base material, and prevent the biofouling of the adsorptive material.

Finally, the water produced is used to irrigate a small area of agricultural land within the Agròpolis facilities (around 250 m²). A smart irrigation system has been deployed by FINT (partners of the project) that allows minimization of the water use and the energy requirements. The system allows real time water needs to be known, and cultivation key performance indicators such as pH, soil and air temperature, soil characteristics and plant response to be controlled by means of a wireless sensor network (based on system-on-a-chip devices) capable of reading and transmitting the variables' values. The crop selected is rapeseed (*Brassica napus*), an autumn-winter cycle crop already known for producing food (mustard) and feed oil from its oilseeds, and currently considered a new crop for bioenergy production. Sowing took place at the end of September 2017 and the rapeseed population is initially aimed to be 40–45 plants/m². A recommended amount of 300–400 g/1,000 m² was planted at a depth of 1.5 cm, in sowing lines spaced 15 cm apart.

MONITORING AND DECISION SUPPORT SYSTEM DECISIONS

The operation and maintenance of the plant in Agrópolis is under constant optimization for biomass, bioplastics and biomethane production. Novel sampling methodologies, based on optical sensing, are currently being implemented as *in-situ* sensors which will allow for a constant monitoring of physico-chemical parameters, aiming for a better system control and data management. This also implies a significant reduction of the energy costs of each system.

Finally, the data generated throughout the length of the project will be used to carry out life cycle assessments of the technological solutions proposed, in order to assess the impacts associated with the functioning, economical feasibility and productivity of each study (bioplastics production, treated wastewater, etc.). Previous studies focusing on the life cycle assessment of microalgal-based wastewater systems have already shown encouraging results (Garfí *et al.* 2017; Terumi Arashiro *et al.* 2018). For instance, Terumi Arashiro *et al.* (2018) highlighted that microalgae systems were more environmentally friendly when coupled with biogas production than when coupled with biofertilizer production. From a wastewater point of view, when comparing microalgae systems with activated sludge and constructed wetlands, microalgae high rate algal ponds appeared as the less expensive alternative, being the most suitable solution from an economic point of view (Garfí *et al.* 2017). The data gathered from the INCOVER plant will be useful to further improve information about the impact of the whole technology.

Eventually, and following the life cycle approaches, a decision support system will be developed within the framework of the INCOVER project, to favor the selection of a low cost, feasible wastewater treatment, always considering a holistic perspective for water management.

CONCLUSIONS

This paper presents the experimental microalgae-based treatment plant built under the European project INCOVER. The preliminary results are encouraging, showing biomass production of almost 2.2 kg VSS/d and satisfactory wastewater treatment performances (<2 mg/L for phosphates, <10 mg/L for ammonia and <15 mg/L for nitrites and nitrates). Further good results are expected in terms of bioplastics, biomethane and biofertilizer production. This plant presents a sustainable approach for

wastewater management, by changing the paradigm of considering wastewater as a disposable waste, to start regarding it as a valuable resource, from which new added-value products can be obtained and proficiently used.

ACKNOWLEDGEMENTS

The INCOVER project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 689242. The dissemination of results herein reflects only the authors' view and the Commission is not responsible for any use that may be made of the information it contains. M. J. García and E. Uggetti would like to thank the Spanish Ministry of Industry and Economy for their research grants (IJCF-2014-22767 and IJCI-2014-21594, respectively).

REFERENCES

- APHA, AWWA & WPCF 2001 *Standard Methods for the Examination of Water and Wastewater*, 20th edn. Washington.
- Arias, D. M., Uggetti, E., García-Galán, M. J. & García, J. 2017a Production of polyhydroxybutyrates and carbohydrates in wastewater-borne cyanobacteria: effect of nutrients limitation and photoperiods. *New Biotechnology* **42**, 1–11.
- Arias, D. M., Uggetti, E., García-Galán, M. J. & García, J. 2017b Cultivation and selection of cyanobacteria in a closed photobioreactor used for secondary effluent and digestate treatment. *Science of the Total Environment* **587–588**, 157–167.
- Arias, D., 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. *Bioresource Technology* **247**, 513–519.
- Balaji, S., Gopi, K. & Muthuvelan, B. 2013 A review on production of poly β hydroxybutyrates from cyanobacteria for the production of bio plastics. *Algal Research* **2**, 278–285.
- Bhatnagar, A. & Sillanpää, M. 2010 Utilization of agro-industrial and municipal waste materials as potential adsorbents for water treatment – a review. *Chemical Engineering* **157**, 277–296.
- Bourrelly, P. 1985 Les algues d'eau douce. Les algues vertes. *Reviue Hydrobiologie Tropicale* **17** (2), 101–115.
- Drosg, B. 2015 Photo-autotrophic production of poly (hydroxyalkanoates) in cyanobacteria. *Chemical Biochemistry Engineering Quarterly* **29**, 145–156.
- EC 2000 *Water Framework Directive 2000/60/EC of the European Parliament and of the Council Establishing a Framework for the Community Action in the Field of Water Policy*. http://ec.europa.eu/environment/water/water-framework/index_en.html.
- EC 2007 *Communication from the Commission to the European Parliament and the Council – Addressing the Challenge of*

- Water Scarcity and Droughts in the European Union (COM/2007/0414 Final)*. http://ec.europa.eu/environment/water/quantity/eu_action.htm#2007_com.
- EC 2017 *EC Water Reuse: Background and Policy Context, 2017*. <http://ec.europa.eu/environment/water/reuse.htm>.
- García-Galán, M. J., Gutiérrez, R., Uggetti, E., Maramoros, V., García, J. & Ferrer, I. 2018 Use of full-scale hybrid horizontal tubular photobioreactors to process agricultural runoff. *Biosystem Engineering* **116**, 138–149.
- Garfí, M. & Puigagut, J. 2016 Reusing industrial by-products to enhance phosphorus removal in waste stabilization ponds: laboratory approach. *Desalination and Water Treatment* **57**, 1857–1864.
- Garfí, M., Flores, L. & Ferrer, I. 2017 Life cycle assessment of wastewater treatment systems for small communities: activated sludge, constructed wetlands and high rate algal ponds. *Journal of Cleaner Production* **161**, 211–219.
- Ji, X., Verspagen, J., Stomp, M. & Huisman, J. 2017 Competition between cyanobacteria and green algae at low versus elevated CO₂: who will win, and why? *Journal of Experimental Botany* **68** (14), 3815–3828.
- Koller, M. & Marsalek, L. 2015 Cyanobacterial polyhydroxyalkanoate production: status quo and quo vadis? *Current Biotechnology* **4**, 1–1.
- Mahdy, A., Mendez, L., Ballesterosa, M. & González-Fernández, C. 2015 Algal culture integration in conventional wastewater treatment plants: anaerobic digestion comparison of primary and secondary sludge with microalgae biomass. *Bioresource Technology* **184**, 236–244.
- Palmer, C. M. 1962 Algas en los abastecimientos de agua. Manual ilustrado acerca de la identificación, importancia y control de las algas en los abastecimientos de agua. Editorial Interamericana, Mexico.
- Passos, F., García, J. & Ferrer, I. 2013 Impact of low temperature pretreatment on the anaerobic digestion of microalgal biomass. *Bioresource Technology* **138**, 79–86.
- Passos, F., Carretero, J. & Ferrer, I. 2015 Comparing pretreatment methods for improving microalgae anaerobic digestion: thermal, hydrothermal, microwave and ultrasound. *Chemical Engineering Journal* **279**, 667–672.
- 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. *Science of the Total Environment* **586**, 1–9.
- Stal, L. 1992 Poly(hydroxyalkanoate) in cyanobacteria: an overview. *FEMS Microbiology Letters* **103**, 169–118.
- Terumi Arashiro, L., Montero, N., Ferrer, I., Acien, F. G., Gómez, C. & Garfí, M. 2018 Life cycle assessment of high rate algal ponds for wastewater treatment and resource recovery. *Science of the Total Environment* **622–623**, 1118–1130.
- Toledo-Cervantes, A., Lebrero, R., Cavinato, C. & Muñoz, R. 2017 Biogas upgrading using algal-bacterial processes. In: *Microalgae-Based Biofuels and Bioproducts: From Feedstock Cultivation to End Products*, 1st edn (R. Muñoz & C. Gonzalez-Fernandez, eds). Woodhead Publishing, Cambridge, UK, pp. 283–304.
- Uggetti, E., Ferrer, I., Llorens, E. & García, J. 2010 Sludge treatment wetlands: a review on the state of the art. *Bioresource Technology* **101** (9), 2905–2912.
- Unrein, F., O'Farrell, I., Izaguirre, I., Sinistro, R., dos Santos Afonso, M. & Tell, G. 2010 Phytoplankton response to pH rise in a N-limited floodplain lake: relevance of N₂-fixing heterocystous cyanobacteria. *Aquatic Sciences* **72** (2), 179–190.
- Yamamoto, Y. & Nakahara, H. 2005 The formation and degradation of cyanobacterium *Aphanizomenon flos-aquae* blooms: the importance of pH, water temperature, and day length. *Limnology* **6** (1), 1–6.
- Zouhayr, A., Ruiz, J., Álvarez-Díaz, P., Garrido-Pérez, C., Barragan, J. & Perales, J. A. 2013 Effect of pH control by means of flue gas addition on three different photobioreactors treating urban wastewater in long-term operation. *Ecological Engineering* **57**, 226–235.

First received 31 October 2017; accepted in revised form 16 April 2018. Available online 27 April 2018