

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

Short title: Microalgae culture converting wastewater to bioproducts

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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 photobioreactor (PBR) 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 (PHBs), 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 resource recovery process more than a waste treatment.

Keywords: wastewater, circular economy, bioplastics, microalgal biomass, closed systems.

1. Introduction

Basically, all kind of human activities are putting water resources under a constant pressure: global warming, overexploitation 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 thirty 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 an 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 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 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 attention of 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, research and technological centers from 7 European countries, with a proved expertise in wastewater and organic waste treatment and bio-products generation. The project started in June 2016 and research and demonstrative activities will be developed during the following 3 years in 3 different experimental sites located in Spain (Barcelona, Almería-Cádiz) and Germany (Leipzig). The project Technology Readiness Level (TRL) 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 Agrópolis (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.

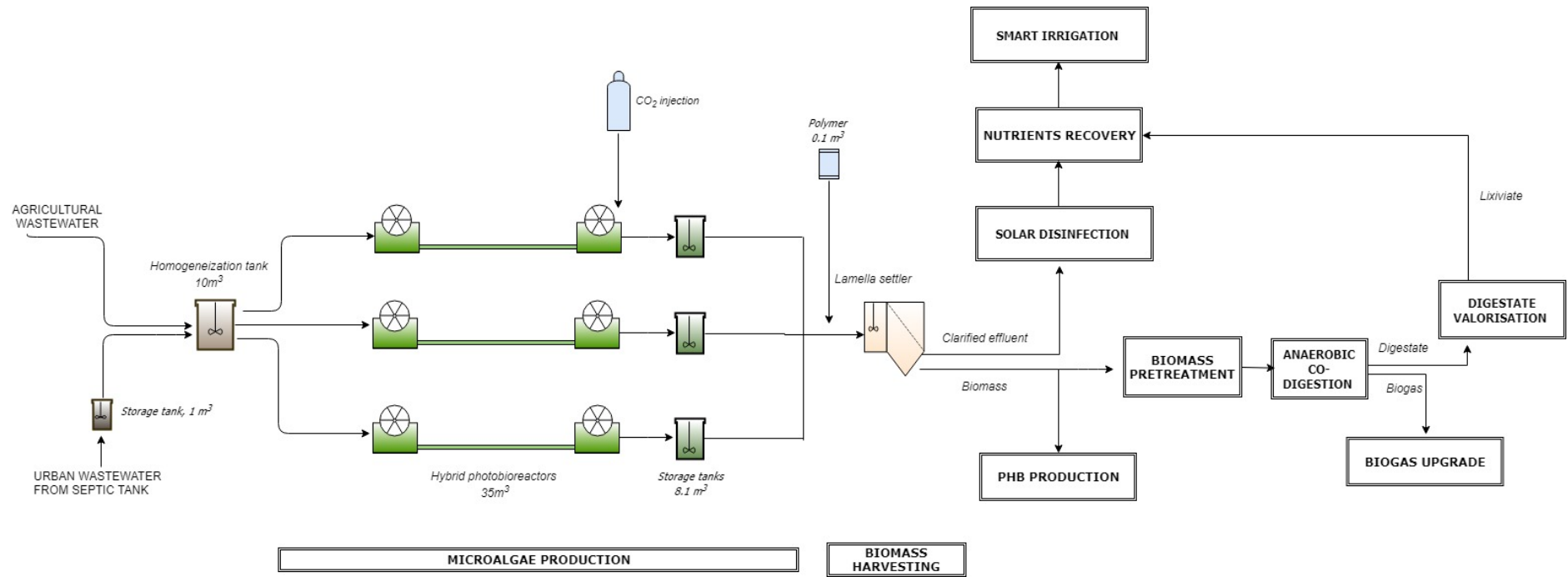
The novelty of this plant resides in changing the paradigm of considering wastewater as a disposable waste, to start regarding it as a valuable resource, from which new added-value products can be obtained and proficiently used. To our knowledge this is the first plant built at this scale coupling microalgae-based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

2. Materials and methods

2.1 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 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 form of 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 obtained.



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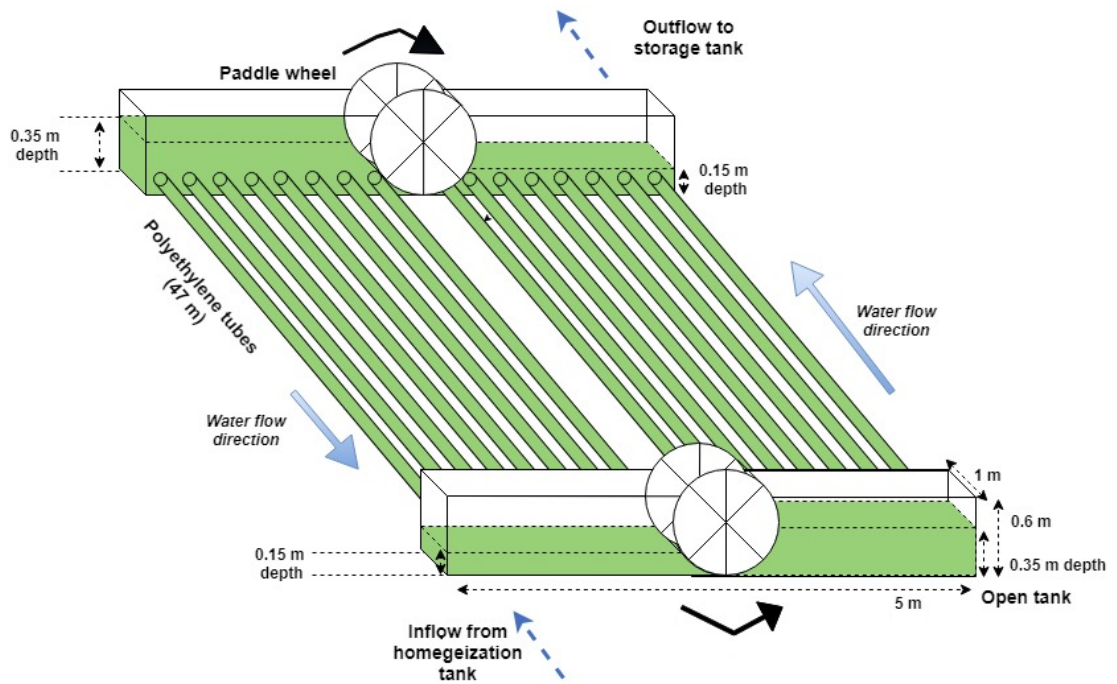
Figure 1. Scheme of the INCOVER plant located at the Agròpolis campus, Viladecans, Barcelona.

2.2 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 2 open tanks made from polypropylene (5 m long x 1 m width x 0.6 m height) connected between them through 16 low density polyethylene tubes (125 mm diameter and 47 m length). The useful volume is approximately 11.7 m³. The tubes lie down on a waterproof covering sheet in order to ensure separation from the ground. Both open tanks ensure and favour the homogenous distribution and mixing of the liquor and also the release of the exceeding dissolved oxygen accumulated along the closed tubes. In each open tank, a paddle-wheel with six blades (1 m width x 0.35 m long) 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 paddlewheels 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 8 tubes from the deep side of one tank to the shallow side of the opposite one. Here the flow is moved by the paddlewheels to the deeper part of the tank and then it returns to the shallow side of the first tank through the other 8 tubes, and so on. Technical characteristics of PBRs are summarized in Table 1.

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

Table 1. Technical characteristics of each of the photobioreactors.

Parameter	Value
Number of tubes	16
Number of tanks	2
Tanks volume (m ³)	2.5
Tank surface (m)	5 x 1
Tubes volume (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

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38 In order to control PBR operation, online sensors of pH, dissolved oxygen and
39 temperature (Hach Lange Spain S.L.) were installed in one of the two open tanks of
40 each PBR. In the mixed liquor, the pH value is approximately maintained below 8.5
41 through controlled CO₂ addition. This pH boundary was selected based on the results
42 of previous works that reported a pH preference of cyanobacteria ranging from 8 to
43 9 (Unrein et al., 2010; Yamamoto and Nakahara, 2005). A recent study by Ji et al. (Ji
44 et al., 2017) demonstrated that green algae such as *Scenedesmus obliquus* or
45 *Chlorella vulgaris* outcompeted cyanobacteria (*Mycrocystis aeruginosa*) under low
46 CO₂ conditions and pH levels above 10. The injection is made through an air sparger
47 placed in one of the open tanks after the paddlewheel. The pH of the mixed liquor is
48 measured every five seconds with a pH probe, and when the measurement exceeds
49 the pH 8.5 set point, a valve opens and CO₂ is bubbled into the PBR tubes. CO₂
50 injection stops as soon as pH 8.5 is reached again.

51 The three PBRs were installed in winter 2017. They were inoculated at the end
52 of April with a mixed culture of microalgae and bacteria grown in urban wastewater.

53 Approximately, 10 L were added to each PBR, with a volatile suspended solids (VSS)
54 concentration of 223 mg/L. From that moment on, PBRs have been operating in
55 parallel, fed with 2.3 m³/d each (6.9 m³/d in total) of a mixture of agricultural and
56 urban wastewater at a ratio of approximately 5:1 (see section 3.3.1).

57 Every day, from 2 to 5 a.m., 6.4 m³ of agricultural wastewater and 0.5 m³ of urban
58 wastewater (the latter has been previously treated in an aerated septic tank) are
59 pumped into the homogenization tank, where they are mixed in order to reach an
60 influent with the suitable nutrients concentration for cyanobacteria growth. Then, at
61 5 a.m. the feeding operation starts: firstly, 2.3 m³/d of mixed liquor are pumped out
62 from each PBR to one storage tank. After that (from 7 a.m. on), the same volume of
63 influent from the homogenization tank is pumped into each PBR. This operation takes
64 place in the early morning in order to have nutrients available for biomass growth
65 during the day.

66 During the microalgae-based wastewater treatment, microalgae biomass grows
67 thanks to the solar radiation and the nutrients (mostly nitrogen (N) and phosphorus
68 (P)) present in the influent wastewater. At the same time and through photosynthesis,
69 microalgae generate the oxygen needed by the bacteria to aerobically degrade the
70 organic contaminants present in that media. Thus, PBRs have the dual advantage of
71 simultaneously producing microalgae biomass and treating wastewater without
72 requiring external aeration.

73 **2.3. Analytical methodology and PBRs follow up**

74 Samples from the influent and from the 3 PBRs are collected weekly and
75 immediately taken to the laboratory, where they are analyzed to determine: 1)
76 biomass concentration and production as VSS and 2) water quality in terms of

77 ammonium and phosphorus concentrations. Sampling was paused during the month
78 of August.

79 All parameters, with the exception of Chlorophyll *a*, were analyzed in both
80 influent wastewater and effluent (mixed liquor of each PBR). Analyses for
81 orthophosphate (dissolved reactive phosphorus) (P-PO_4^{3-}), nitrite (N-NO_2^-) and
82 nitrate (N-NO_3^-) were measured using an ion chromatograph DIONEX ICS1000
83 (Thermo-scientific, USA). Alkalinity was determined using the titration method 2320
84 B of Standards Methods (APHA/AWWA/WEF, 2012). Total suspended solids (TSS)
85 and volatile suspended solids (VSS) were measured in triplicate in the influent and
86 effluent of each PBRs following the gravimetric method 2540 C and 2540 D in
87 Standard Methods (APHA/AWWA/WEF, 2012). Turbidity was measured in the
88 mixed liquor of each PBR with a turbidity-meter (Hanna, USA). 2001). Temperature,
89 pH and a dissolved oxygen (DO) were measured directly in the open tanks of each
90 PBR, where the corresponding sensors are introduced in the mixed liquor.
91 Chlorophyll *a* (procedure 10200 H) and total and soluble chemical oxygen demand
92 (CODs) were determined also following Standard Methods (APHA/AWWA/WEF,
93 2012).

94 Mixed liquor samples were regularly examined under an optic microscope
95 (Motic, China) for qualitative evaluation of microalgae populations and to determine
96 the cyanobacteria and microalgae abundance. Taxonomic books were employed for
97 identification of both microalgae and cyanobacteria species (Bourelly, 1990; Palmer,
98 1962)

99 **3. Preliminary results**

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

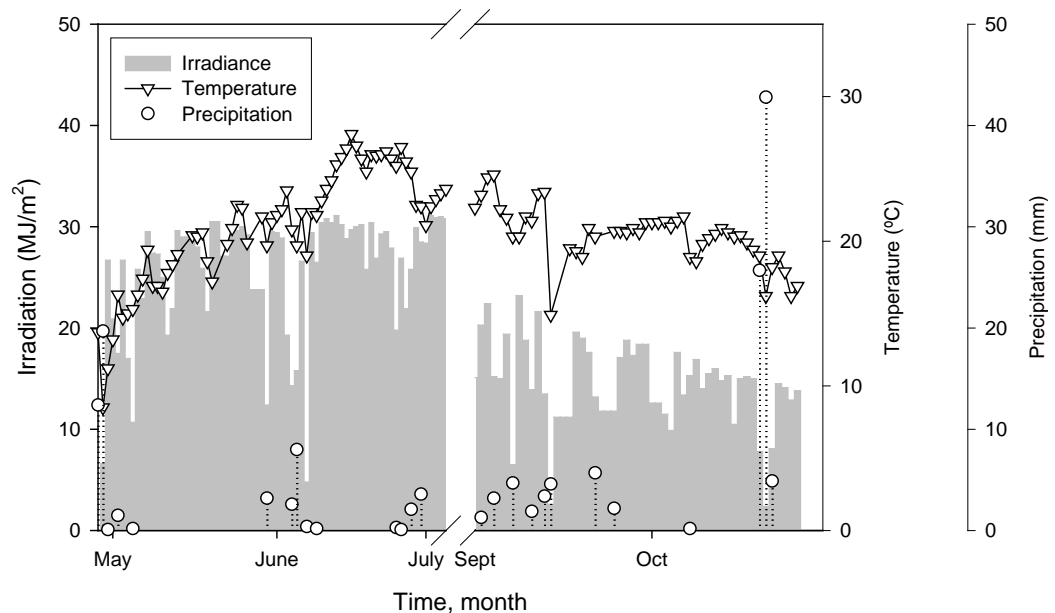
Table 2. Physico-chemical characterization of the influent wastewater (from April till
October 2017, n=19)

Parameter	Value
pH	8.3±0.3
CE (mS/cm)	2.5±0.4
T (°C)	24.2±2.0
NH ₄ ⁺ -N (mg/L)	3.8±3.4
NO ₂ ⁻ -N (mg/L)	0.9±1.4
NO ₃ ⁻ -N (mg/L)	8.4±2.1
PO ₄ ³⁻ -N (mg/L)	0.8±1.1
TSS (mg/L)	78.5±48.9
VSS (mg/L)	27.3±12.0

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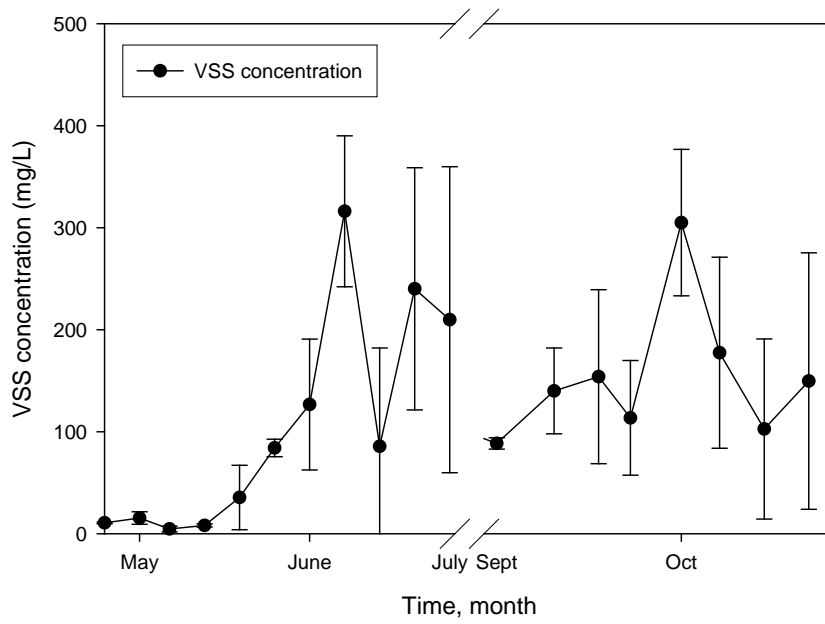
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103 Figure 3 shows the irradiance, temperature and precipitation registered on site
 104 during this first period. The biomass concentration during the first months of
 105 operation (April to October) is shown in Figure 4 and expressed as VSS (mg/L). After
 106 inoculation, around 20 days were needed to detect an increase in the volatile solids
 107 concentration; from that day onwards, the concentration increased constantly until
 108 reaching concentrations up to 320 mg VSS/L (Figure 4). Considering the operation
 109 of the 3 PBRs, this would correspond to a biomass production of almost 2.2 kg VSS/d.



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Figure 3. Daily irradiance (MJ/m²), average air temperature (°C) and precipitation (mm) registered during the first 6 months of operation of the plant. (Source: www.meteo.cat/observacions/xema/dades)



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Figure 4. Biomass concentration measured as VSS (mean and st.dev.) achieved in the 3 photobioreactors during the first 6 months of operation.

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Concerning wastewater treatment, the concentration of nutrients in the influent

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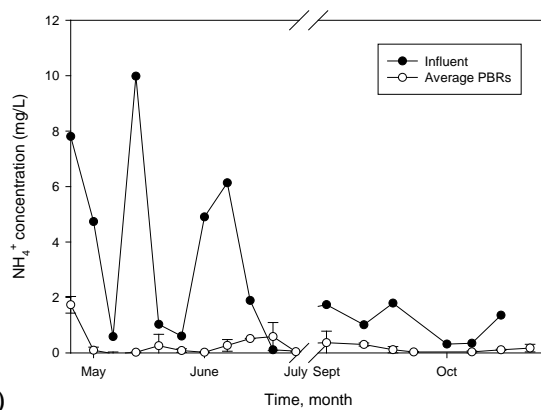
was always < 2 mg/L for phosphates, < 10 mg/L for ammonia and <15 mg/L for

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nitrites and nitrates. Both N and P present in the influent were completely removed

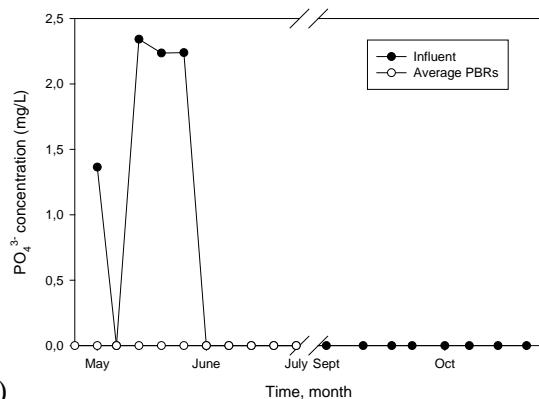
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in the PBRs (Figure 5).



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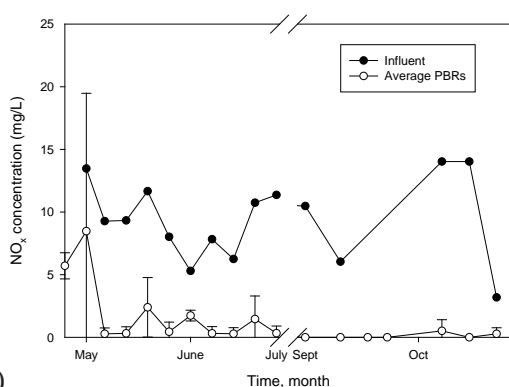
a)



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b)

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c)

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Figure 5. Average concentrations of a) ammonium, b) phosphorus and c) inorganic oxidized nitrogen (NO_x) recorded in the influent and in the 3 photobioreactors during the first months of operation. Note: the standard deviation is not showed for the influent because only one sample was analyzed.

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As observed in Table 3, the CODs concentrations measured in the mixed liquor were usually higher than 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 or carbon, and it usually corresponds to a 5-30% of the carbon fixed by photosynthesis. The higher COD values in the mixed liquor of the PBR could be attributed to this DOM exudation, but also to the low organic matter biodegradability of the wastewater influent. Similar results were observed in recent studies (Arbib et al., 2013; García-Galán et al., 2018).

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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 till October 2017, n=16)

INFLUENT

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DATE	COD total [mg/L]	COD soluble (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

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

148 In order to separate the biomass produced and to clarify the water treated, a
149 lamella settler was installed after the 3 PBRS. The water stored in the 3 storage tanks
150 is then pumped through the lamella settler, which is divided in 2 chambers: the first
151 one is a mixing chamber, where the water gets in contact with a liquid coagulant
152 (polyaluminum chloride liquid, with a 9% of aluminum (PAX-18) (provided by
153 Kemira Water Solutions, Spain). The optimum dose is estimated weekly performing
154 jar tests with the corresponding mixed liquor, and it is usually between 2 and 5 mg/L.
155 After the mixing chamber, the water passes to a second chamber where lamella favors
156 the solids settling at the bottom whereas water is discharged in the upper part of the
157 settler (Figure 6). The settler currently works at a rate of 400 L per hour.

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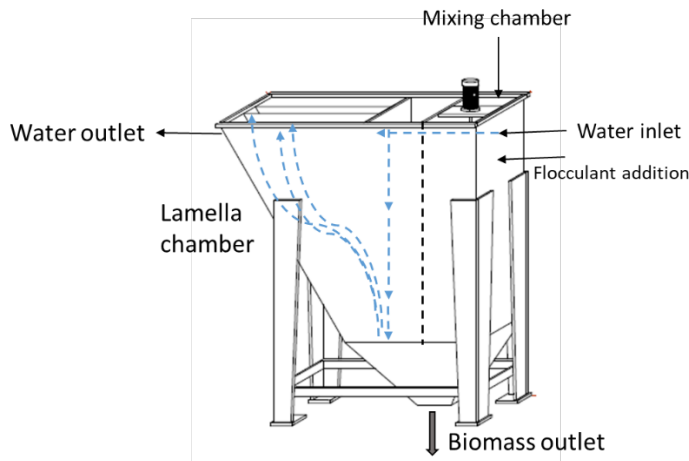


Figure 6. Scheme of the lamella settler.

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162 4. Bioproducts generation

163 4.1 Bioplastic accumulation in wastewater-born cyanobacteria

164 As mentioned in Section 3.1, the three PBRs in the Agròpolis site are fed with a
 165 mixture of urban and agricultural wastewater in order to obtain a concentration of
 166 nutrients appropriate to select cyanobacteria. The interest of growing cyanobacteria
 167 is because these prokaryotic photosynthetic microorganisms have the potential to
 168 assimilate and store glycogen, cyanophycin (amino acid polymer), polyphosphates
 169 and polyhydroxyalkanoates (PHAs) (Stal, 1992). PHAs are an interesting alternative
 170 to ordinary plastics from the petrochemical industry, and are nowadays used for
 171 packaging, and their use in biomedicine for prosthesis is now being investigated. The
 172 most commonly occurring polymer within the PHAs family is the
 173 polyhydroxybutyrate (PHB) and its accumulation has been demonstrated for several
 174 cyanobacteria such as *Spirulina* sp., *Aphanothece* sp., *Gloeothece* sp., and
 175 *Synechococcus* spp. (Balaji et al., 2013). The novelty in the INCOVER plant resides
 176 in the use of wastewater to select cyanobacteria from a mixed microalgae culture,
 177 instead of using pure or genetically modified cultures in usually expensive processes.
 178 Indeed, most of the studies dealing with the production of PHBs from cyanobacteria

179 are based on pure or genetically modified cultures (Drosg, 2015; Koller and
180 Marsalek, 2015), using sterile medium substrates in expensive and highly controlled
181 processes. Once the cyanobacteria are selected in the PBRs, they will be submitted to
182 nutrients stress in lab experiments in order to discern the best conditions to promote
183 PHB accumulation, following the recent study by Arias et al. (Arias et al., 2018).

184 **4.2 Biomethane**

185 The biomass obtained in the PBRs is submitted to a thermal pretreatment and
186 subsequently used as substrate in an anaerobic co-digestion (AcoD) process. A
187 thickener is used after the lamella settler to increase solids concentration of the
188 separated biomass; this harvesting unit (lamella settler and thickener) has been
189 designed in order to increase TSS concentration from 3-8 to 30 g/L. After this step,
190 the biomass is directed to the AcoD unit, which consists of a thermal pretreatment
191 tank (19 L) and an anaerobic digester (1,000 L), together with the corresponding
192 storage tanks for the biomass and co-substrate. Due to the proximity of the
193 wastewater treatment plant of Gavá, secondary sludge was selected as co-substrate.
194 The inoculum was sewage activated sludge from the same plant (100 L). Considering
195 the biomass volume obtained from the PBR, the digester is now operated at a working
196 volume of 400 L. The microalgae biomass undergoes thermal pretreatment at 75 °C
197 during 20 hours before being introduced in the digester, currently at a loading rate of
198 7.5 L/d together with 7.5 L/d of co-substrate (1:1, v/v), resulting in a HRT of 20 days
199 in the digester. Such proportion was selected during the start up in accordance with
200 the results obtained by Arias et al. (2018), who demonstrated good results in term of
201 methane content and biogas production rates working at 50% of microalgae and 50%
202 of co-substrate (activated sludge). It has also been demonstrated in previous studies
203 that thermal pretreatment improves the solubility of the biomass, leading to an

204 increase of a 90% in the biogas production during the AcoD process (Passos et al.,
205 2013; Passos et al., 2015).

206 During the start-up stage, the organic loading rate introduced to the digester was
207 low, (0.15-0.30 g SV/L·d). It is foreseen to increase this amount, firstly using settled
208 secondary sludge as cosubstrate, with a higher concentration of organic matter and
209 also improving the operation of the PBRs and the harvesting of the biomass achieved
210 in the settler. Characterization of the feedstock and products of the AcoD unit is given
211 in Table 4.

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Table 4. Characterization of the different feedstocks and products used in the AcoD unit. Analysis were carried out from the 20th of June till the 7th of November.

	TS (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

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216 It has recently been showed that microalgae biomass can be a potential co-
217 substrate for biogas production together with municipal wastewater sludge
218 significantly enhancing CH₄ yields (Mahdi et al., 2015, Arias et al., 2018). The
219 biogas produced is stored in a gasometer (1,000 L), before the upgrading process,
220 which aims at increasing the CH₄ content of the biogas from 60-70% up to 99%, so
221 that it can be used directly as biofuel. In the upgrading process, mixed liquor of the
222 PBR is used as absorption solution to simultaneously remove CO₂, H₂S, NH₃ and
223 VOCs from biogas at a low energy cost. The biogas produced at the INCOVER plant
224 is therefore sparged through a fine bubble plate diffuser in an absorption bubble
225 column (PVC, 4m high, 20 cm of diameter) interconnected to one of the open tanks
226 of one PBR that provides the mixed liquor. The biogas is injected on the bottom of

227 the column, while mixed liquor from the PBR is circulating countercurrent from the
228 top to the bottom. Within the column, photosynthetic CO₂ assimilation concomitant
229 with the aerobic oxidation of the other trace biogas pollutants aforementioned will
230 take place (Toledo-Cervantes et al., 2017). The absorption column has been designed
231 by a research group of the Universidad de Valladolid (partners of the project).

232 **4.3 Biofertilizers**

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

249 **4.4 Reclaimed water**

250 Getting back to the water treatment line, the clarified water from the lamella
251 settler and thickener is pumped to a solar driven disinfection system designed and

252 developed by SolarSpring Gmbh (partners of the project) to provide drinking water.
253 The process is based on low pressure ultrafiltration, which requires low energy. The
254 system consists of different capillary membranes made of polyethersulfone with a
255 capillar diameter of 0,9 and 1.5 mm and a pore diameter around 0.02 μm (total
256 membrane surface up to 6 m^2). For both membranes, a pretreatment is set up based
257 on a pre-filter (100 μm) and a media filter (5 μm). After the ultrafiltration, water
258 passes through a post-treatment consisting of activated carbon filters and an
259 integrated UV-C reactor for disinfection, finally reaching a storage tank. There is also
260 a solar generator for independent power supply and various sensors for on-line
261 monitoring of pressure, flow and water level, turbidity and UV-radiation. The
262 working flux is 500 L/h divided in two parallel lines (dual system), one used as a
263 reference system and monitored, and the other used for potential implementations
264 depending on the results. This way, efficiencies under different settings can be
265 compared.

266 **4.5 Nutrients recovery**

267 After the solar-driven UF and disinfection, the water treated in the PBRs is fed
268 to three adsorption columns filled with sol-gel coating developed by researchers from
269 Aarhus University and the Danish Technological Institute (partners of the project) in
270 order to recover N and P. Adsorption processes are attractive for nutrient removal
271 due to the simplicity of operation, their low cost and the possibility of resource
272 recovery from wastewaters (Bhatnagar and Sillanpää, 2010), and they have already
273 been proved as a cost-effective solution for nutrients removal from wastewaters using
274 industrial by-products (Garfí and Puigagut, 2016). Calcite and crushed autoclaved
275 aerated concrete were tested as base materials in the INCOVER plant, due mainly to
276 their high affinity for P and their low cost. The two different coating compositions

277 were developed and tested, an inorganic and an organic-based one, both having the
278 property of stabilizing the adsorptive base materials, avoiding its disintegration in
279 contact with water. They also allow the penetration and absorption of P, and even
280 enhance the capacity of the base material, and prevent the biofouling of the adsorptive
281 material.

282 Finally, the water produced is used to irrigate a small area of agricultural land
283 within the Agròpolis facilities (around 250 m²). A smart irrigation system has been
284 deployed by FINT (partners of the project) that allows minimizing the water use and
285 the energy requirements. The system allows to know real time water needs,
286 controlling cultivation key performance indicators such as pH, soil and air
287 temperature, soil characteristics, plant response etc by means of a Wireless Sensor
288 Network (WSNs, based on System-On-a-Chip devices) capable of reading and
289 transmitting the variables values. The crop selected is rapeseed (*Brassica napus*),
290 autumn-winter cycle crop already known for producing food (mustard) and feed oil
291 from its oilseeds, and currently considered a new crop for bioenergy production.
292 Sowing took place at the end of September 2017 and the rapeseed population is
293 initially aimed to be 40-45 plants/m². A recommended amount of 300-400 g/1000 m²
294 was planted at a depth of 1.5 cm, in sowing lines spaced 15 cm.

295 **5. Monitoring and decision support system decisions**

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

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

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

320

321 **6. Conclusions**

322 This paper presents the experimental microalgae-based treatment plant build under the
323 European project INCOVER. The preliminary results are encouraging, showing biomass
324 production of almost 2.2 kg VSS/d and satisfactory wastewater treatment performances
325 (< 2 mg/L for phosphates, < 10 mg/L for ammonia and <15 mg/L for nitrates and nitrates).
326 Further good results are expected in terms of bioplastics, biomethane and biofertilizers

327 production. This plant presents a sustainable approach for wastewater management, by
328 changing the paradigm of considering wastewater as a disposable waste, to start regarding
329 it as a valuable resource, from which new added-value products can be obtained and
330 proficiently used.

331 **Acknowledgments**

332 The INCOVER project has received funding from the European Union's Horizon
333 2020 research and innovation programme under grant agreement No. 689242. The
334 dissemination of results herein reflects only the author's view and the Commission is
335 not responsible for any use that may be made of the information it contains. M.J.
336 García and E. Uggetti would like to thank the Spanish Ministry of Industry and
337 Economy for their research grants (IJCF-2014-22767 and IJCI-2014-21594,
338 respectively).

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