

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

**Short title: Microalgae culture converting wastewater to bioproducts**

Enrica Uggetti<sup>1,\*</sup>, Joan García<sup>1</sup>, Juan Antonio Álvarez<sup>2</sup>, María Jesús García-Galán<sup>1</sup>

<sup>1</sup>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, E-08034, Barcelona, Spain

<sup>2</sup>AIMEN Technology Centre, c/ Relva, 27 A – Torneiros, Porriño, 36410, –  
Pontevedra, Spain

\* Corresponding author:

Tel.: +34 934016465

Fax: +34 934017357

E-mail address: [enrica.uggetti@upc.edu](mailto:enrica.uggetti@upc.edu)

## **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 m<sup>3</sup> 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 m<sup>3</sup> 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 m<sup>3</sup> – 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.

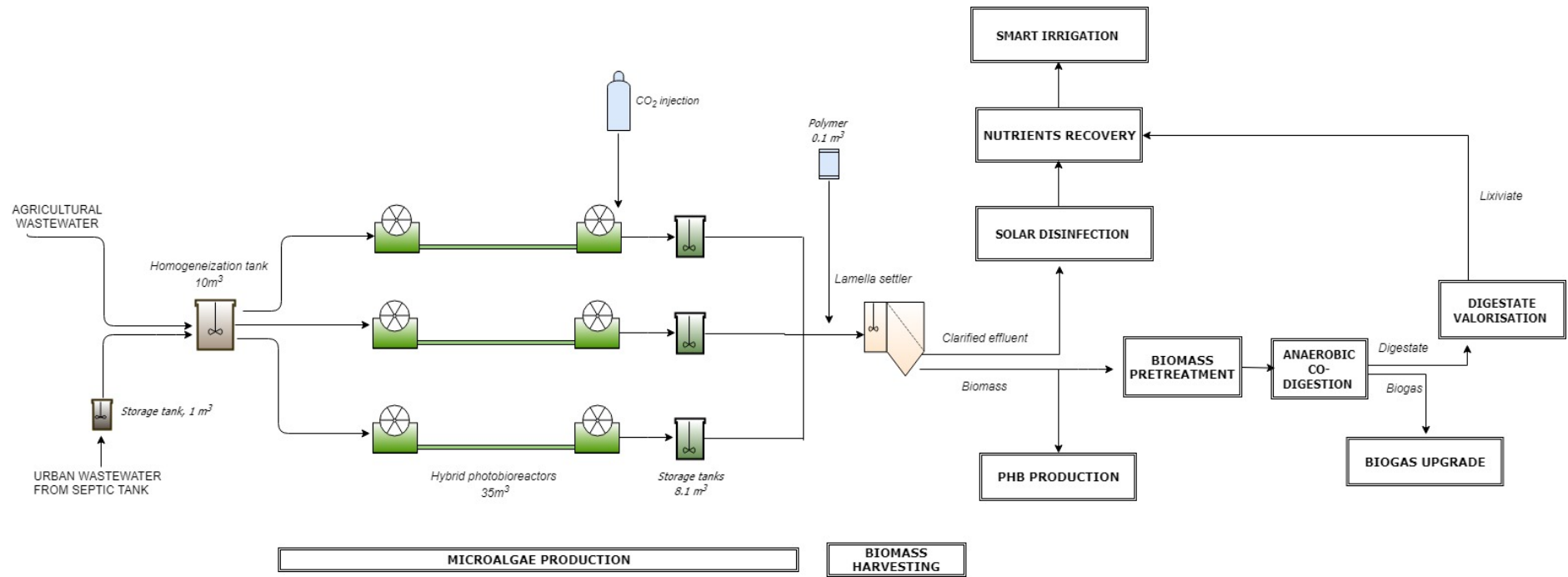
The novelty of this plant resides in changing the paradigm of considering wastewater as a disposable waste, to start regarding it as a valuable resource, from which new added-value products can be obtained and proficiently used. To our knowledge this is the first plant built at this scale coupling microalgae-based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



1

2

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

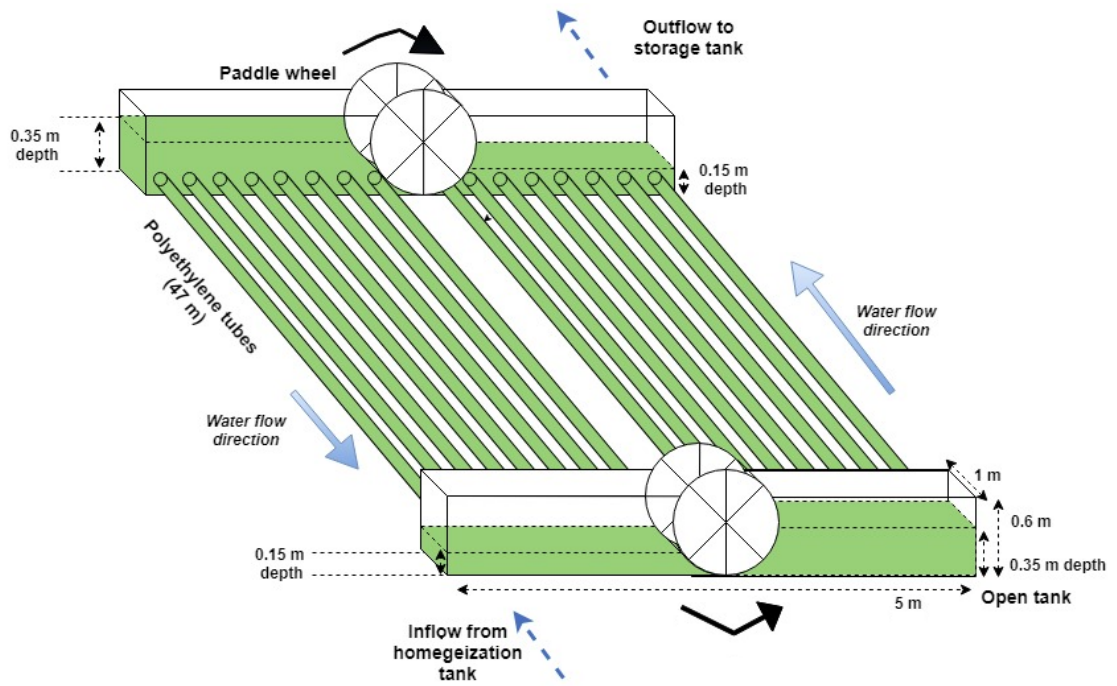
## 2.2 Photobioreactor design and operation

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 m<sup>3</sup>. 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 m<sup>3</sup> (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.



28

29



30



31

32

33

34

35

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 <sup>3</sup> )	2.5
Tank surface (m)	5 x 1
Tubes volume (m <sup>3</sup> )	9.2
Tube diameter (m)	0.125
Tube length (m)	47
Total PBR volume (m <sup>3</sup> )	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

37

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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> is bubbled into the PBR tubes. CO<sub>2</sub>  
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 m<sup>3</sup>/d each (6.9 m<sup>3</sup>/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 m<sup>3</sup> of agricultural wastewater and 0.5 m<sup>3</sup> 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 m<sup>3</sup>/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) ( $\text{P-PO}_4^{3-}$ ), nitrite ( $\text{N-NO}_2^-$ ) and  
82 nitrate ( $\text{N-NO}_3^-$ ) 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 )

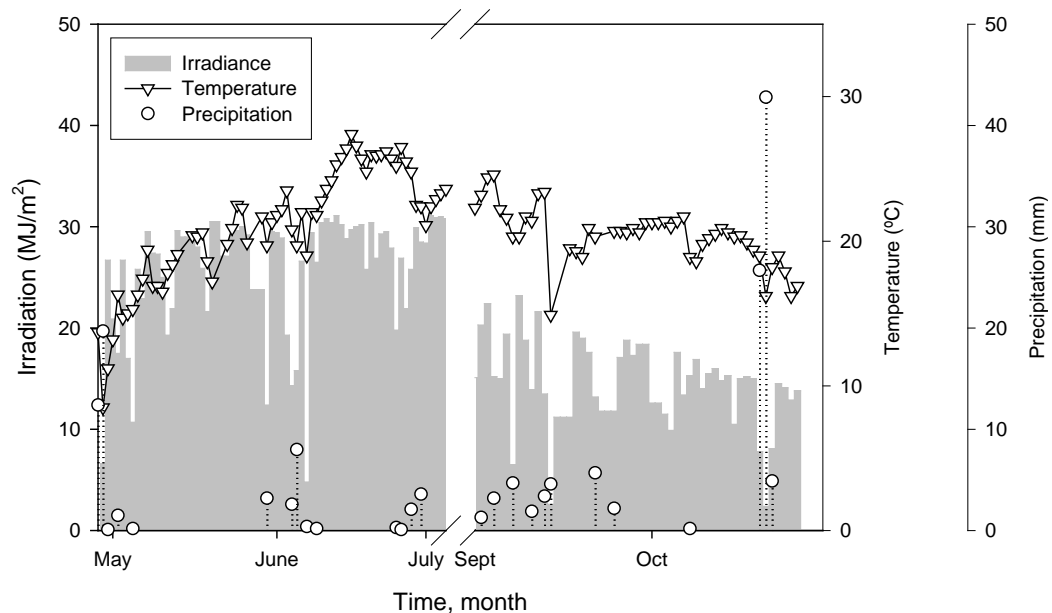
---

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

101

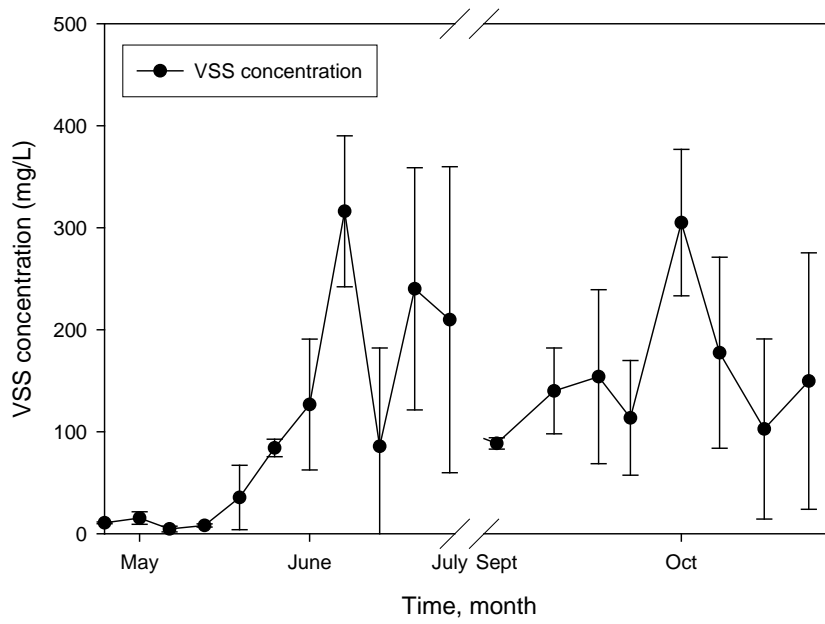
102

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.



110  
 111  
 112

Figure 3. Daily irradiance (MJ/m<sup>2</sup>), 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](http://www.meteo.cat/observacions/xema/dades))



113

114

115

116

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

117

Concerning wastewater treatment, the concentration of nutrients in the influent

118

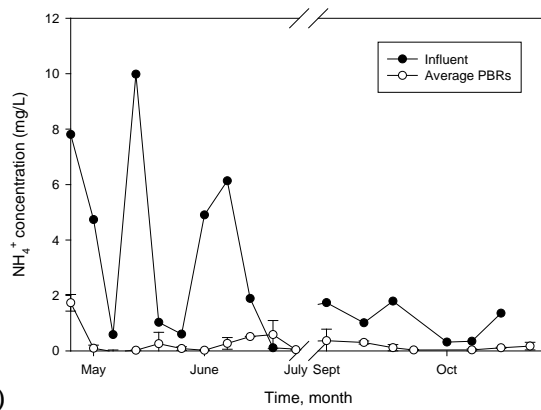
was always < 2 mg/L for phosphates, < 10 mg/L for ammonia and <15 mg/L for

119

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

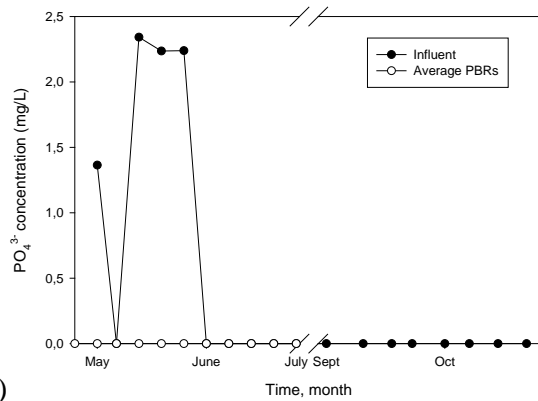
120

in the PBRs (Figure 5).



121

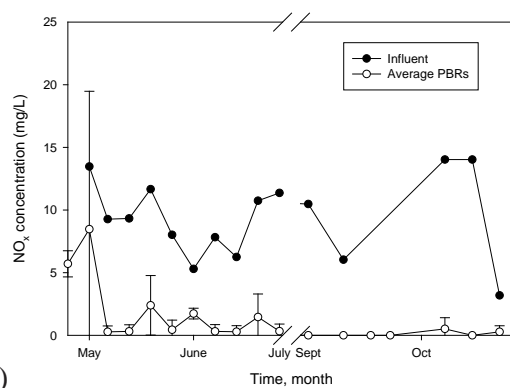
a)



122

b)

123



124

c)

125

126

127

128

129

Figure 5. Average concentrations of a) ammonium, b) phosphorus and c) inorganic oxidized nitrogen (NO<sub>x</sub>) 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.

130

As observed in Table 3, the CODs concentrations measured in the mixed liquor

131

were usually higher than the total COD concentrations at the influent feedstock, and

132

so elimination rates were negative. It has been demonstrated that a fraction of

133

photosynthetically fixed carbon is released during microalgae growth as dissolved or

134

carbon, and it usually corresponds to a 5-30% of the carbon fixed by photosynthesis.

135

The higher COD values in the mixed liquor of the PBR could be attributed to this

136

DOM exudation, but also to the low organic matter biodegradability of the

137

wastewater influent. Similar results were observed in recent studies (Arbib et al.,

138

2013; García-Galán et al., 2018).

139

**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)

140

141

INFLUENT

EFFLUENT

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

142

143

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.

158



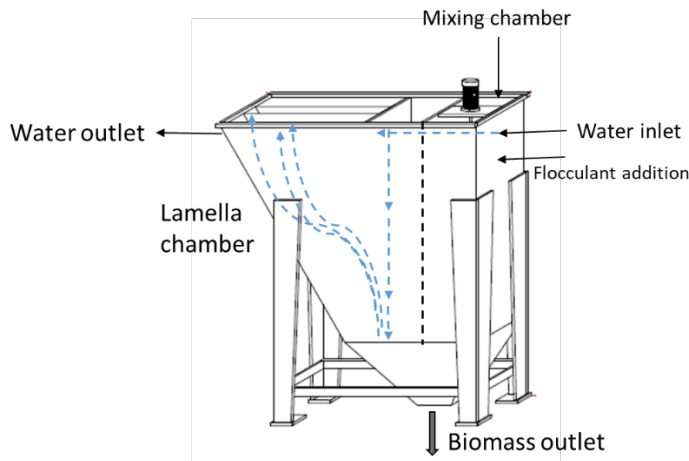


Figure 6. Scheme of the lamella settler.

## 4. Bioproducts generation

### 4.1 Bioplastic accumulation in wastewater-born cyanobacteria

As mentioned in Section 3.1, the three PBRs in the Agròpolis site are fed with a mixture of urban and agricultural wastewater in order to obtain a concentration of nutrients appropriate to select cyanobacteria. The interest of 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 prosthesis is now being investigated. The most commonly occurring polymer within the PHAs family is the 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

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.

212  
213

**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 <sub>4</sub> <sup>+</sup> (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

214  
215

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 CH<sub>4</sub> 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 CH<sub>4</sub> 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 CO<sub>2</sub>, H<sub>2</sub>S, NH<sub>3</sub> 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 CO<sub>2</sub> 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 m<sup>2</sup>), 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/m<sup>2</sup>). 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/m<sup>2</sup> 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  $\mu\text{m}$  (total  
256 membrane surface up to 6  $\text{m}^2$ ). For both membranes, a pretreatment is set up based  
257 on a pre-filter (100  $\mu\text{m}$ ) and a media filter (5  $\mu\text{m}$ ). 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 m<sup>2</sup>). 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/m<sup>2</sup>. A recommended amount of 300-400 g/1000 m<sup>2</sup>  
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).

### 339 **References**

340 Arias, D.M., Uggetti, E., García-Galán, M.J., García, J. 2017 Cultivation and  
341 selection of cyanobacteria in a closed photobioreactor used for secondary effluent  
342 and digestate treatment. *Science of the Total Environment*, **587-588**, 157–167.

343 Arias, D., Solé-Bundó, M., Garfí, M., Ferrer, I., García, J., Uggetti, E. 2018  
344 Integrating microalgae tertiary treatment into activated sludge systems for energy and  
345 nutrients recovery from wastewater. *Bioresource Technology*, **247**, 513-519.

346 Arias, D.M., Uggetti, E., García-Galán, M.J., García, J. 2017 Production of  
347 polyhydroxybutyrates and carbohydrates in wastewater-borne cyanobacteria: effect  
348 of nutrients limitation and photoperiods. *New Biotechnonology*, (under revision).

349 Bhatnagar, A., Sillanpää, M. 2010 Utilization of agro-industrial and municipal  
350 waste materials as potential adsorbents for water treatment-a review. *Chemical*  
351 *Engineering*, **157**, 277–296.



352 Balaji, S., Gopi, K., Muthuvelan, B. 2013 A review on production of poly  $\beta$   
353 hydroxybutyrates from cyanobacteria for the production of bio plastics. *Algal*  
354 *Research*, **2**, 278-285.

355 Drog, B. 2015 Photo-autotrophic Production of Poly(hydroxyalkanoates) in  
356 Cyanobacteria. *Chemical Biochemistry Engineering Quarterly*, **29**, 145–156.

357 EC 2000 Water Framework Directive 2000/60/EC of the European Parliament  
358 and of the Council establishing a framework for the Community action in the field of  
359 water policy. [http://ec.europa.eu/environment/water/water-](http://ec.europa.eu/environment/water/water-framework/index_en.html)  
360 [framework/index\\_en.html](http://ec.europa.eu/environment/water/water-framework/index_en.html).

361 EC 2007 Communication from the Commission to the European Parliament and  
362 the Council - Addressing the challenge of water scarcity and droughts in the  
363 European Union (COM/2007/0414 final).

364 [http://ec.europa.eu/environment/water/quantity/eu\\_action.htm#2007\\_com](http://ec.europa.eu/environment/water/quantity/eu_action.htm#2007_com)

365 EC 2017 EC Water Reuse- Background and policy context, 2017.  
366 <http://ec.europa.eu/environment/water/reuse.htm>

367 Garfí, M., Puigagut, J. 2016 Reusing industrial by-products to enhance  
368 phosphorus removal in waste stabilization ponds: laboratory approach. *Desalination*  
369 *and Water Treatment*, **57**, 1857-1864.

370 Garfí, M., Flores L., Ferrer, I. 2017 Life Cycle Assessment of wastewater  
371 treatment systems for small communities: Activated sludge, constructed wetlands and  
372 high rate algal ponds. *Journal of Cleaner Production*, **161**, 211-219.

373 Koller, M., Marsalek, L. 2015 Cyanobacterial Polyhydroxyalkanoate Production:  
374 Status Quo and Quo Vadis? *Current Biotechnology*, **04**, 1–1.

375 Mahdy, A., Mendez, L., Ballesterosa, M., González-Fernández, C. 2015  
376 Algalculture integration in conventional wastewater treatment plants: Anaerobic

377 digestion comparison of primary and secondary sludge with microalgae biomass.  
378 *Bioresource Technology*, **184**, 236-244.

379 Passos, F., García, J., Ferrer, I. 2013 Impact of low temperature pretreatment on  
380 the anaerobic digestion of microalgal biomass. *Bioresource Technology*, **138**, 79-86.

381 Passos, F., Carretero, J., Ferrer, I. 2015 Comparing pretreatment methods for  
382 improving microalgae anaerobic digestion: Thermal, hydrothermal, microwave and  
383 ultrasound. *Chemical Engineering Journal*, **279**, 667-672.

384 Solé-Bundó, M., Cucina, M., Folch, M., Tàpias, J., Gigliotti, G., Garfí, M.,  
385 Ferrer, I. 2017 Assessing the agricultural reuse of the digestate from microalgae  
386 anaerobic digestion and co-digestion with sewage sludge. *Science Total*  
387 *Environment*, **586**, 1-9.

388 Stal, L. 1992 Poly(hydroxyalkanoate) in cyanobacteria: an overview. *FEMS*  
389 *Microbiology Letter*, **103**, 169-18.

390 Terumi Arashiro L., Montero, N., Ferrer, I., Ación, F.G., Gómez, C., Garfí, M.  
391 2018 Life cycle assessment of high rate algal ponds for wastewater treatment and  
392 resource recovery. *Science Total Environment*, **622-623**, 1118-1130.

393 Toledo-Cerventes, A., Lebrero, R., Cavinato, C., Muñoz, R. 2017 Biogas  
394 upgrading using algal-bacterial processes. In: *Microalgae-Based Biofuels and*  
395 *Bioproducts: From Feedstock Cultivation to End Products*, Muñoz and Gonzalez-  
396 Fernandez (eds.), 1<sup>st</sup> ed., Woodhead Publishing, 283-304.

397 Uggetti, E., Ferrer, I., Llorens, E., García, J. 2010 Sludge treatment wetlands: A  
398 review on the state of the art. *Bioresource Technology*, **101** (9), 2905-2912.