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 Uggetti¹*, Joan García¹, Juan Antonio Álvarez², María Jesús García-Galán¹

¹GEMMA - 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

²AIMEN 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
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 m$^3$ 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$^3$ – 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.
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 lever 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.
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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tubes</td>
<td>16</td>
</tr>
<tr>
<td>Number of tanks</td>
<td>2</td>
</tr>
<tr>
<td>Tanks volume (m$^3$)</td>
<td>2.5</td>
</tr>
<tr>
<td>Tank surface (m)</td>
<td>5 x 1</td>
</tr>
<tr>
<td>Tubes volume (m$^3$)</td>
<td>9.2</td>
</tr>
<tr>
<td>Tube diameter (m)</td>
<td>0.125</td>
</tr>
<tr>
<td>Tube length (m)</td>
<td>47</td>
</tr>
<tr>
<td>Total PBR volume (m$^3$)</td>
<td>11.7</td>
</tr>
<tr>
<td>Design velocity inside tube (m/s)</td>
<td>0.25</td>
</tr>
<tr>
<td>Number of engines</td>
<td>2</td>
</tr>
<tr>
<td>Engine power (kW)</td>
<td>0.35</td>
</tr>
<tr>
<td>Retention time within the tubes at design velocity (min)</td>
<td>3.13</td>
</tr>
<tr>
<td>Retention time within the open tanks at design velocity (min)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

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 CO$_2$ 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 (Unrein et al., 2010; Yamamoto and Nakahara, 2005). A recent study by Ji et al. (Ji et al., 2017) demonstrated that green algae such as *Scenedesmus obliquus* or *Chlorella vulgaris* outcompeted cyanobacteria (*Mycrocystis aeruginosa*) under low CO$_2$ conditions and pH levels above 10. The injection is made through an air sparger placed in one of the open tanks after the paddlewheel. The pH of the mixed liquor is measured every five seconds with a pH probe, and when the measurement exceeds the pH 8.5 set point, a valve opens and CO$_2$ is bubbled into the PBR tubes. CO$_2$ 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 were 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 (see section 3.3.1).

Every day, from 2 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) are 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.

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 media. Thus, PBRs have the dual advantage of simultaneously producing microalgal biomass and treating wastewater without requiring external aeration.

### 2.3. Analytical methodology and PBRs follow up

Samples from the influent and from the 3 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). Analyses for orthophosphate (dissolved reactive phosphorus) ($\text{P-PO}_4^{3-}$), nitrite ($\text{N-NO}_2^-$) and nitrate ($\text{N-NO}_3^-$) were measured using an ion chromatograph DIONEX ICS1000 (Thermo-scientific, USA). Alkalinity was determined using the titration method 2320 B of Standards Methods (APHA/AWWA/WEF, 2012). Total suspended solids (TSS) and volatile suspended solids (VSS) were measured in triplicate in the influent and effluent of each PBRs following the gravimetric method 2540 C and 2540 D in Standard Methods (APHA/AWWA/WEF, 2012). Turbidity was measured in the mixed liquor of each PBR with a turbidity-meter (Hanna, USA). 2001). Temperature, pH and a dissolved oxygen (DO) 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/AWWA/WEF, 2012).

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 (Bourrelly, 1990; Palmer, 1962)

3. Preliminary results

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)
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.3±0.3</td>
</tr>
<tr>
<td>CE (mS/cm)</td>
<td>2.5±0.4</td>
</tr>
<tr>
<td>T (°C)</td>
<td>24.2±2.0</td>
</tr>
<tr>
<td>NH₄⁺-N (mg/L)</td>
<td>3.8±3.4</td>
</tr>
<tr>
<td>NO₂⁻-N (mg/L)</td>
<td>0.9±1.4</td>
</tr>
<tr>
<td>NO₃⁻-N (mg/L)</td>
<td>8.4±2.1</td>
</tr>
<tr>
<td>PO₄³⁻-N (mg/L)</td>
<td>0.8±1.1</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>78.5±48.9</td>
</tr>
<tr>
<td>VSS (mg/L)</td>
<td>27.3±12.0</td>
</tr>
</tbody>
</table>

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 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 3 PBRs, this would correspond to a biomass production of almost 2.2 kg VSS/d.

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)
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 nitrates and nitrates. Both N and P present in the influent were completely removed in the PBRs (Figure 5).
Figure 5. Average concentrations of a) ammonium, b) phosphorus and c) inorganic oxidized nitrogen (NOx) 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.

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; Garcia-Galán et al., 2018).

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)

<table>
<thead>
<tr>
<th>Time, month</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>Sept</th>
<th>Oct</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO4 concentration (mg/L)</td>
<td>0.0</td>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>NOx concentration (mg/L)</td>
<td>0</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

INFLUENT | EFFLUENT
<table>
<thead>
<tr>
<th>DATE</th>
<th>COD total [mg/L]</th>
<th>COD soluble (Average value) [mg/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>03/05/2017</td>
<td>62.1</td>
<td>33.37 ± 8.1</td>
</tr>
<tr>
<td>09/05/2017</td>
<td>154</td>
<td>181.10 ± 105.2</td>
</tr>
<tr>
<td>16/05/2017</td>
<td>-</td>
<td>85.13 ± 29.8</td>
</tr>
<tr>
<td>23/05/2017</td>
<td>110.5</td>
<td>137.60 ± 38.2</td>
</tr>
<tr>
<td>30/05/2017</td>
<td>157.7</td>
<td>157.37 ± 18.9</td>
</tr>
<tr>
<td>06/06/2017</td>
<td>98.4</td>
<td>162.43 ± 1.3</td>
</tr>
<tr>
<td>13/06/2017</td>
<td>139.3</td>
<td>260.67 ± 10.1</td>
</tr>
<tr>
<td>20/06/2017</td>
<td>58.5</td>
<td>142.47 ± 34.2</td>
</tr>
<tr>
<td>27/06/2017</td>
<td>159.3</td>
<td>107.40 ± 22.8</td>
</tr>
<tr>
<td>04/07/2017</td>
<td>144</td>
<td>122.93 ± 26.6</td>
</tr>
<tr>
<td>05/09/2017</td>
<td>64.6</td>
<td>148.93 ± 39.2</td>
</tr>
<tr>
<td>14/09/2017</td>
<td>50.3</td>
<td>59.47 ± 12.5</td>
</tr>
<tr>
<td>26/09/2017</td>
<td>25.6</td>
<td>40.77 ± 8.6</td>
</tr>
<tr>
<td>04/10/2017</td>
<td>-</td>
<td>44.50 ± 12.5</td>
</tr>
<tr>
<td>10/10/2017</td>
<td>69</td>
<td>75.90 ± 5.5</td>
</tr>
<tr>
<td>24/10/2017</td>
<td>68.5</td>
<td>54.00 ± 21.4</td>
</tr>
</tbody>
</table>

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 cf. *Cyclotella* sp., the green algae cf. *Oocystis* sp. and the cyanobacteria cf. *Synechocystis* sp.

In order to separate the biomass produced and to clarify the water treated, a lamella settler was installed after the 3 PBRs. The water stored in the 3 storage tanks is then pumped through the lamella settler, which is divided in 2 chambers: the first one is a mixing chamber, where the water gets in contact with a liquid coagulant (polyaluminum chloride liquid, with a 9% of aluminum (PAX-18) (provided by Kemira Water Solutions, Spain). The optimum dose is estimated weekly 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 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.
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., *Gloeothecce* 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 and 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. (Arias et al., 2018).

4.2 Biomethane

The biomass obtained in the PBRs is submitted to a thermal pretreatment and subsequently used as substrate in an anaerobic co-digestion (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 HRT of 20 days in the digester. Such proportion was selected during the start up in accordance with the results obtained by Arias et al. (2018), 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 a 90% in the biogas production during the AcoD process (Passos et al., 2013; Passos et al., 2015).

During the start-up stage, the organic loading rate introduced to the digester was low, (0.15-0.30 g SV/L·d). It is foreseen to increase this amount, firstly using settled secondary sludge as cosubstrate, 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.

<table>
<thead>
<tr>
<th></th>
<th>TS (g/L)</th>
<th>VS (g/L)</th>
<th>COD (g/L)</th>
<th>N-NH₄⁺ (mg/L)</th>
<th>Total P (mg/L)</th>
<th>TOC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Algae</td>
<td>2.85-11.6</td>
<td>1.23-40.8</td>
<td>0.38-7.01</td>
<td>0.5-84.1</td>
<td>1.5-80.2</td>
<td>93.7-170</td>
</tr>
<tr>
<td>Pretreated algae</td>
<td>2.75-14.7</td>
<td>1.14-5.74</td>
<td>1.29-6.31</td>
<td>4-29.1</td>
<td>41.5-110.1</td>
<td>67.8-553.4</td>
</tr>
<tr>
<td>Secondary sludge</td>
<td>3.06-7.44</td>
<td>1.29-6.14</td>
<td>0.25-24.9</td>
<td>3.3-135.9</td>
<td>11.8-307.9</td>
<td>311.4-476.4</td>
</tr>
<tr>
<td>Digestate</td>
<td>3.25-18.2</td>
<td>1.28-11.9</td>
<td>7.05-15.2</td>
<td>35.9-1115.32</td>
<td>77.8-408.7</td>
<td>343.2-827</td>
</tr>
</tbody>
</table>

It has recently been showed that microalgae biomass can be a potential co-substrate for biogas production together with municipal wastewater sludge significantly enhancing CH₄ yields (Mahdi et al., 2015, Arias et al., 2018). The biogas produced is stored in a gasometer (1,000 L), before the upgrading process, which aims 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 VOCs 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, 4m high, 20 cm of diameter) interconnected to one of the open tanks of one PBR that provides the mixed liquor. The biogas is injected on 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 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).

4.3 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 media (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 (around 35 kg TS/m² year) in order to ensure the proper plant acclimation, but
it will be gradually increased.

4.4 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 SolarSpring Gmbh (partners of the project) to provide drinking water. The process is based on low pressure ultrafiltration, 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 ultrafiltration, 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 on-line monitoring of pressure, flow and water level, turbidity and UV-radiation. The working flux is 500 L/h divided in 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.

### 4.5 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 and Sillanpää, 2010), and they have already been proved as a cost-effective solution for nutrients removal from wastewaters using industrial by-products (Garfí and 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. The two different coating compositions
were developed and tested, an inorganic and an organic-based one, both having the
property of stabilizing the adsorptive base materials, 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 minimizing the water use and
the energy requirements. The system allows to know real time water needs,
controlling cultivation key performance indicators such as pH, soil and air
temperature, soil characteristics, plant response etc by means of a Wireless Sensor
Network (WSNs, based on System-On-a-Chip devices) capable of reading and
transmitting the variables values. The crop selected is rapeseed (*Brassica napus*),
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/1000 m²
was planted at a depth of 1.5 cm, in sowing lines spaced 15 cm.

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

The operation and maintenance of the plant in Agròpolis 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 energetic costs of each system.
Finally, the data generated throughout the length of the project will be used to carry out life cycle assessments (LCA) 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 already showed 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 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 INCVER 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.

6. Conclusions

This paper presents the experimental microalgae-based treatment plant build 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 nitrates and nitrates). Further good results are expected in terms of bioplastics, biomethane and biofertilizers.
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.

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