

Feasibility analysis for reduction of carbon footprint in a wastewater treatment plant

*Original*

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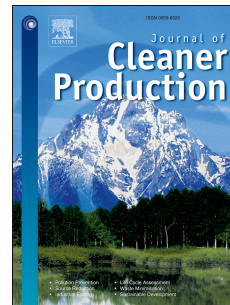
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## Feasibility analysis for reduction of carbon footprint in a wastewater treatment plant

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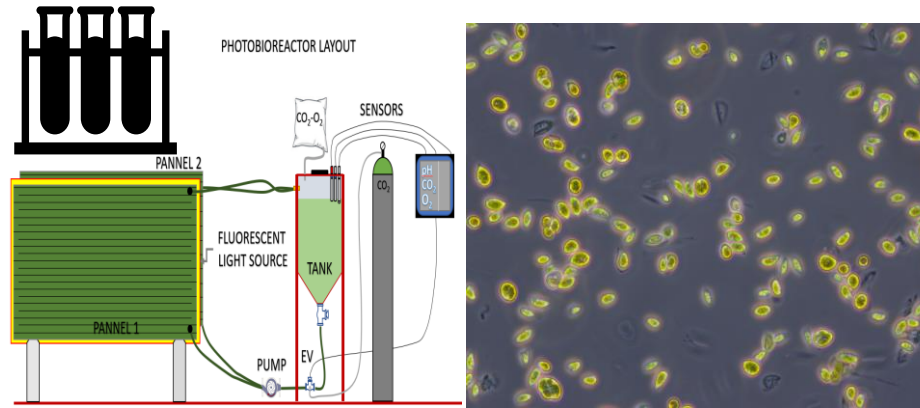
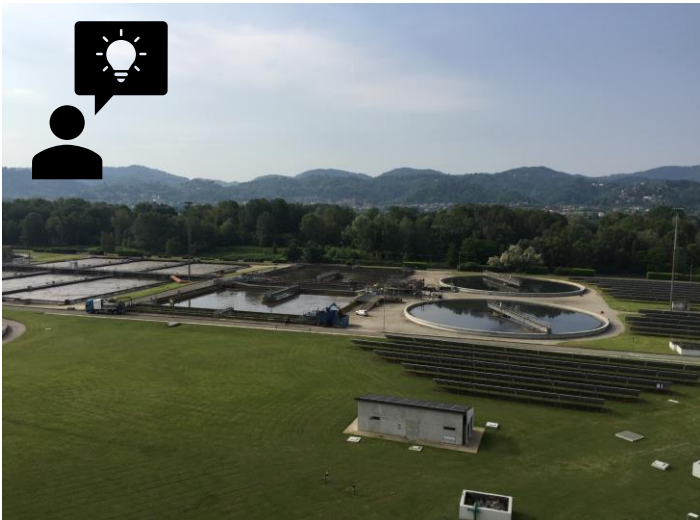
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# Microalgae CO<sub>2</sub> fixation

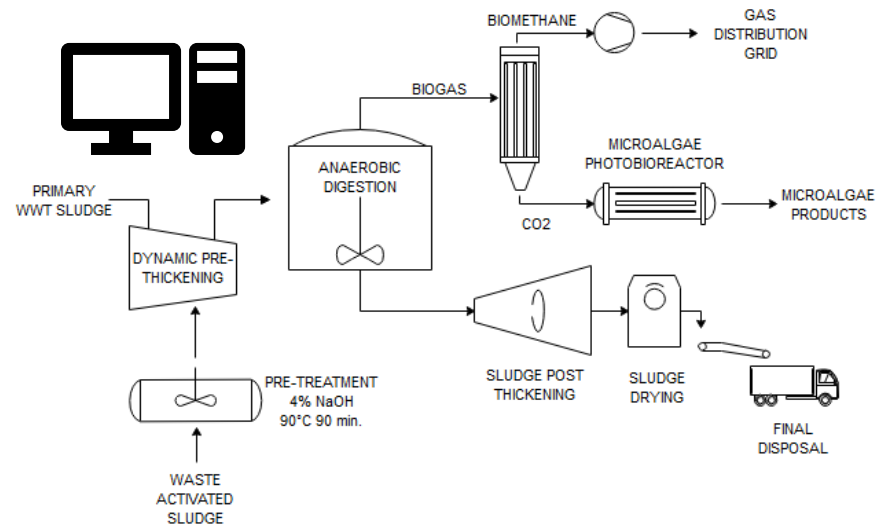
## Feasibility analysis



## Reduction



## MCBioCH4 model



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## Abstract

This study presents an integrated feasibility analysis approach to reduce the carbon footprint in the largest Italian wastewater treatment plant (WWTP). Firstly, a model-based feasibility analysis was carried out to assess the applicability of upgrading scenarios, for an ongoing anaerobic sludge digestion process. Application of dynamic sludge thickener, as well as hybrid thermo-alkali pre-treatment of waste activated sludge, were assessed to enhance the biogas production in the WWTP. Further, an implementation of the selective membranes was proposed and studies to upgrade the produced biogas in sludge treatment units to biomethane with an average efficiency of 98.6%. Model-based sludge pre-treatment and biogas upgrading strategies were developed and evaluated in terms of mass, energy, and greenhouse gas emission balance. The obtained results prove that practicing the proposed upgrading scenario can lead to an 18% improvement in biogas production and a significant reduction of thermal energy auto-consumption and total greenhouse gas emissions. In the second phase, the laboratory-based feasibility analysis was performed about the integration of microalgae technology into the current process of the WWTP. A planar photobioreactor was built to estimate the volumetric mass transfer coefficient ( $K_La$ ) and  $CO_2$  consumption of the reactor. By the use of 44 and 76  $\mu\text{mol}/\text{m}^2/\text{s}$  light intensities, the results show 80% and 70% reductions in total  $CO_2$ , respectively. The tested configuration guaranteed 11.763 and 27.943  $\text{mg}/\text{l}/\text{h}$   $CO_2$  consumptions, as well as 0.5775  $\text{h}^{-1}$  and 17.7  $\text{h}^{-1}$   $K_La$  values. Overall, the results prove that applications of the technologies proposed in this study can significantly reduce the carbon footprint of the WWTP.

**Keywords:** Anaerobic digestion; biomethane; carbon footprint; microalgae; sludge pre-treatment; sustainable wastewater treatment.

## 1. Introduction

During the past few years, wastewater treatment plants (WWTPs) have been adopting newly developed technologies for increasing reclamation efficiency, to comply with the discharge limits imposed by law, which become more restrictive year by year. The main concern of the WWT industry has always been to meet water quality standards to maintain public trust. Thus, WWTPs are typically designed to meet specific effluent requirements, with no significant energy efficiency considerations. As a result, few if any WWTPs were designed with energy-efficiency criteria in mind. This attitude has been changing in recent years, however, mainly because of the general

126 framework for the achievement of 2030-2050 goals defined for Climate and Energy by the  
127 European Union.

128 The most challenging aspect of WWTP energy optimization is finding a viable, economically  
129 feasible solution that can address several different objectives (e.g., effluent quality, energy  
130 consumption, and environmental aspects). In this regard, the whole treatment process must be  
131 considered and assessed under a multi-disciplinary perspective. The wastewater treatment process  
132 generates several energy and material flows that have a direct or indirect impact on the  
133 environment. The analysis of energy optimization scenarios must thus be supplemented with  
134 information on the emission balances associated with them (Magaril et al., 2017).

135 Presently, energy recovery through anaerobic digestion of sewage sludge represents a vital step  
136 toward the reduction of energy consumption in WWTPs. The biogas produced in the anaerobic  
137 digestion (AD) process can be used either for valorization in internal combustion engines, to  
138 provide electric and thermal energy, or for upgrading biogas to biomethane, for subsequent  
139 injection into the gas grid. Biomethane production is continuously increasing in the EU and  
140 worldwide, as it represents a more versatile energy vector than biogas. Biomethane can replace  
141 natural gas and be sent into the national gas transmission grid. Besides, recent regulations have  
142 introduced attractive economic subsidies for the production of biomethane (Paolini et al., 2018a).  
143 The most frequently used technologies for biogas upgrading are: pressurized water scrubbing –  
144 PWS, pressure swing absorption – PSA, chemical absorption with amine solutions – MEA,  
145 membrane permeation – MB and cryogenic separation – CRY (Ravina and Genon, 2015). The  
146 selection of the best technological solution in terms of energy consumption and environmental  
147 impacts requires a preliminary comparative analysis tailored to the case under study. The use of  
148 dedicated modeling tools may support such a selection.

149 The management of the off-gas produced by the biogas upgrading process also represents an open  
150 issue for plant operators. This off-gas mainly consists of the CO<sub>2</sub> initially contained in the biogas  
151 stream, with a minor amount of CH<sub>4</sub> that has not been recovered in the process. Some additional  
152 minor components, such as H<sub>2</sub>S and siloxanes, may also be present (Paolini et al., 2018b).  
153 Presently, operators of a biomethane plant are usually allowed to discharge off-gas into the  
154 atmosphere, up to the limits imposed by regulations. In this regard, an increasing interest is being  
155 shown in innovative technologies to recover the CO<sub>2</sub> contained in the biomethane off-gas. Among  
156 these, the use of microalgae as a biofilter for CO<sub>2</sub> is most promising. These microalgae organisms  
157 can be used to trap CO<sub>2</sub> coming from the exhaust gases, as they require carbon dioxide to perform  
158 the photosynthesis process. As a secondary benefit, microalgae can be used for the production of  
159 bioproducts. Although microalgae methods perform reasonably well, they are usually considered

160 expensive because they consume a relatively high quantity of energy if an artificial primary light  
161 source is used. Most of the other available techniques, however, need complex operating systems  
162 and produce unwanted end products that require additional treatment processes or create secondary  
163 pollution.

164 Furthermore, using these techniques, the CO<sub>2</sub> removed from the raw biogas is typically discharged  
165 into the atmosphere as a greenhouse gas (GHG), and most of these methods need preliminary H<sub>2</sub>S  
166 removal. To overcome all these limitations, recent studies (Nagarajan et al., 2019; Zabed et al.,  
167 2020) have considered the use of microalgae to upgrade biogas, thanks to their photosynthetic CO<sub>2</sub>  
168 reduction capacity. When microalgae are used for biogas upgrading, photosynthesis can convert  
169 CO<sub>2</sub> present in raw biogas into biomass and oxygen. Currently, microalgae culturing for CO<sub>2</sub> bio-  
170 fixation has gained considerable momentum due to its high photosynthetic rate that allows more  
171 efficient CO<sub>2</sub> bio-fixation than terrestrial plants. Although the potential of microalgae to contribute  
172 to services and commodities demand across the world is high, it is still necessary to eliminate a  
173 large number of bottlenecks related to its biological, engineering, and economic aspects (Richmond,  
174 2000).

175 In our previous study (Borzooei et al., 2019), a methodology was proposed to improve the energy  
176 balance of the largest WWTP in Italy, located at Castiglione Torinese. An integrated approach  
177 consisting of modeling and experimental works was applied to both water and sludge treatment  
178 lines, to minimize energy consumption and maximize renewable energy production. For the  
179 wastewater treatment line, a stepwise approach was reported that includes development, calibration,  
180 and implementation of the model to find the non-dominated and optimized performances of the  
181 WWTP. For the sludge line, a combination of thermal and chemical pre-treatments (hybrid pre-  
182 treatments) was reported to improve the capacity of waste-activated sludge (WAS) to produce  
183 methane and consequently enhance the energy recovery of the sludge line.

184 Optimization of the anaerobic digestion of sewage sludge is considered a worthwhile strategy  
185 because its advantage lies not only in cost savings but also in mitigating the environmental concerns  
186 posed by GHG emissions (Kim et al., 2015). The greatest challenge for the pre-treatment of biogas  
187 substrates is combining the right substrate composition with the right pre-treatment technology to  
188 increase the bioavailability of the substrate. Although this represents an open and extended research  
189 topic, few studies have focused on the comparative evaluation of the possible alternatives in terms  
190 of GHG emissions. Besides, considering the general GHG reduction policies and guidelines, the  
191 feasibility of optimization interventions must be evaluated together with CO<sub>2</sub> sequestration  
192 technologies.

193 In this study, mass, energy, and GHG balances of the sludge treatment section of the WWTP were  
194 analyzed, considering the energy optimization options elaborated in the study of Borzooei et al.  
195 (2019). The analysis started by focusing on the energy valorization of sewage sludge through  
196 anaerobic digestion. In this first stage, biomethane production as an alternative to on-site biogas  
197 combustion was evaluated, considering conventional upgrading technologies. In the second stage,  
198 the potential reduction of the CO<sub>2</sub> emitted via the off-gas was analyzed, considering microalgae bio-  
199 fixation technology. An experimental planar photobioreactor was used to evaluate the possibility of  
200 using microalgae to absorb the CO<sub>2</sub> in the off-gas coming from a WWTP. The final goal of the  
201 study was to provide relevant information toward the definition of the most environmentally  
202 friendly and energy-efficient integrated management scheme of WWTPs.

203 Greenhouse gas flow accounting of the entire sewage sludge treatment line was performed with the  
204 screening model MCBioCH<sub>4</sub> (acronym of the bio-methane computational model), developed by the  
205 authors (Ravina et al., 2019). In the framework of energy recovery optimization of sewage sludge  
206 management processes, the application of MCBioCH<sub>4</sub> aims at a triple target: i) estimating the  
207 productivity of biogas/biomethane in terms of achievable gas flow rates; ii) re-defining the  
208 anaerobic digestion section of the plant given the selected options; and iii) accounting for the whole  
209 environmental impact of the system on a cradle-to-grave basis, considering biogas/biomethane as an  
210 alternative energy source to fossil fuels. Also, using a planar photobioreactor custom-made by the  
211 research team specifically for this study allowed us to perform different experiments characterized  
212 by measuring the mass transfer coefficient and CO<sub>2</sub> consumption inside the reactor under two  
213 different artificial-light scenarios.

## 214 **2. Materials and Methods**

### 215 **2.1 Case study definition**

216 The case study involved a scenario of sludge digestion optimization at Castiglione Torinese  
217 WWTP. This scenario was compared with the actual operating configuration, here referred to as  
218 Scenario 0. Currently, the sludge pre-thickening process operating in the plant allows an increase of  
219 the TS content up to values in the order of 3%. Sludge is pumped and transferred to the digesters  
220 where anaerobic digestion takes place. Biogas is then injected into two combined heat and power  
221 (CHP) units having a nominal electric power of 1.44 MW each. The thermal energy produced by  
222 the CHP units is recovered through an internal closed-loop water circuit that receives heat from the  
223 CHP exhaust gases and transfers it to the digested sludge that is then re-circulated to the digesters  
224 inlet. The heat provided by the CHP units is not sufficient to increase the re-circulated sludge  
225 temperature to 38°C (designed temperature: the digesters work in mesophilic conditions). The  
226 sludge-drying line provides the required additional heat. The waste heat produced in this section is

227 transferred to the digestion process to fill the thermal energy gap. Thermal energy for the drying  
 228 line is provided by two boilers fueled by natural gas. It is estimated that 1 MW of heat can be  
 229 recovered from this section, with an exchange efficiency of around 85%. Electricity produced by  
 230 the CHP units is partly used to satisfy the consumption of the plant auxiliary systems, and the  
 231 remaining amount is sent into the national distribution grid. Internal electricity consumption of the  
 232 digestion and sludge treatment section was estimated to be around 8,000 MWh/y. Total biogas loss  
 233 from the process is estimated to be 2% (w/w) of the gross biogas production. At the exit of the  
 234 digestion process, the sludge undergoes a post-thickening and centrifugation process, with TS  
 235 content increased up to 5% and 25%, respectively. Part of the sludge (around 20,000 t/y) is  
 236 transferred to the drying line, while the remaining part is transferred outside the plant. For this  
 237 study, an average traveling distance outside the plant of 20 km was considered. This distance is  
 238 approximate, as the final destination of the digested sludge can vary depending on regulation and  
 239 market constraints (Kiselev et al., 2019).

240 In the alternative scenario (Scenario 1), a sludge pre-treatment with biomethane production was  
 241 considered. In Scenario 1, two main innovations are introduced in the sludge line of the WWTP.  
 242 The first is the installation of a dynamic sludge thickener, with the capacity of increasing the sludge  
 243 TS content to a value of 6.5%. Secondly, a pre-treatment of WAS entering the digestion process is  
 244 carried out. The process proceeds through a hybrid thermo-alkali treatment, where WAS are put in  
 245 contact with NaOH (4% of the TS content) at a temperature of 90°C for 90 minutes. Primary sludge  
 246 and WAS are mixed after the pre-treatment, and the mixture of the substrates is introduced into the  
 247 digesters. The biogas produced is upgraded, and biomethane is obtained. Scenario 1 simulates an  
 248 upgrading process with selective membranes that yields an average efficiency of 98.6%. The  
 249 specific electricity consumption of the upgrading process is estimated to be 0.3 kWh/m<sup>3</sup> of biogas  
 250 treated, according to Muñoz et al. (2015). It is assumed that the produced biomethane is injected  
 251 into the national gas distribution network, replacing an equivalent amount of natural gas. Under the  
 252 hypotheses of this scenario, a part of the thermal energy needed by the pre-treatment and digestion  
 253 stages is still provided by the sludge-drying line. The residual amount is provided by an external  
 254 energy source, a back-up boiler fueled by natural gas. The main input parameters and their  
 255 corresponding values considered in the simulations are reported in Table 1.

256 **Table 1.** Input values and parameters considered in the simulations

Input parameter/value	Scenario 0	Scenario 1
Primary sludge input flow (t/h)	66.1	30.5
Secondary sludge input flow (t/h)	35.6	16.4
TS input flow (t/h)	3.05	3.05

Primary sludge SMP (Nm <sup>3</sup> /kg VS)	0.280	0.280
Secondary sludge SMP (Nm <sup>3</sup> /kg VS)	0.090	0.245
Primary sludge TS content after pre-thickening (%)	3	6.5
Secondary sludge TS content after pre-thickening (%)	3	6.5
CH <sub>4</sub> content in biogas (%)	62	62
CH <sub>4</sub> loss from digestion and conversion processes (%)	2	1.33
Thermal energy auto-consumption (MWh/y)	35,650	20,610
Electricity auto-consumption (MWh/y)	8,000	11,770
CHP system efficiency (electric; thermal %)	42.0; 43.0	-
Upgrading system efficiency (%)	-	98.6
Emission factor for natural gas consumption/substitution (gCO <sub>2</sub> eq/kWh)	206	206
Emission factor for electricity substitution (Italian national grid) (gCO <sub>2</sub> eq/kWh)	337	337

257

## 258 **2.2 Computational model for evaluation of biogas and biomethane solutions**

259 MCBioCH<sub>4</sub> (acronym of the bio-methane computational model) is a standalone application  
 260 modeling mass, energy, and environmental balances of biogas/biomethane production plants on a  
 261 cradle-to-grave basis, i.e., from substrates production to biogas/biomethane end-use. The design of  
 262 MCBioCH<sub>4</sub> was explicitly addressed to support the preliminary evaluation of alternative plant  
 263 configurations and technological options. In this model, default datasets and assisted input  
 264 definitions were implemented in such a way as to help users in the interpretation of mass, energy,  
 265 and environmental balances.

266 The code was developed as a standalone application based on the MATLAB® software  
 267 (Mathworks, n.d.), and is provided with a user-friendly graphical users interface (GUI). Three  
 268 different modules were implemented in MCBioCH<sub>4</sub> for the calculation of mass, energy, and GHG  
 269 balance, respectively. Users can simulate four different options for biogas/biomethane energy  
 270 conversion:

- 271 • biogas combustion with cogeneration of electrical and thermal energy (option B-H);
- 272 • biogas combustion with the generation of electricity only (option B-NH);
- 273 • biomethane to be injected into the national grid (option M-G);
- 274 • biomethane to be used in transportation (option M-T).

275 If biogas combustion options are selected, the energy conversion by combustion in a commercial  
 276 cogeneration unit (endothermic engine) is simulated. The recovery of thermal energy can be  
 277 specified. Conversely, if biomethane scenarios are selected, the user is allowed to choose the  
 278 upgrading technology, as well as the main features of the upgrading system.

279 The following technologies are implemented: pressurized water scrubbing (PWS), pressure swing  
 280 absorption (PSA), chemical absorption with amine solutions (MEA) and membrane permeation

281 (MB). These are considered to be the most common and mature upgrading technologies currently  
 282 available (Ullah Khan et al., 2017). Other upgrading technologies, such as cryogenic separation  
 283 (CRY) or those based on carbon mineralization (alkaline with regeneration or bottom ash for biogas  
 284 upgrading), may be simulated by introducing customized values of electricity and thermal energy  
 285 specific consumption.

286 MCBioCH<sub>4</sub> is well structured with simple and clear dialog boxes to facilitate interaction with low-  
 287 expertise users. As crucial information for starting, the user is asked to input the daily mass flow of  
 288 substrates to be inserted into the digester. Other input parameters can either be provided as default  
 289 values or be specified by the user. The following sets of output can be obtained from the model:

- 290 • the detailed mass and energy balance of the system;
- 291 • the net mass flow and energy content of the biogas/biomethane stream;
- 292 • the GHG balance of the system, including a comparison with an equivalent system powered  
 293 by traditional (fossil) fuels. For further explanation about the developed model, Ravina et al. (2019)  
 294 should be consulted.

295

### 296 **2.3 Microalgae experimental setup**

297 Since there is no available commercial application or industrial standard for the technology for  
 298 upgrading biogas to biomethane production, this study investigated the application of an innovative  
 299 setup, in the following sections.

#### 300 **2.3.1 Microalgae preparation and culture medium**

301 The strain used for this work was *Scenedesmus obliquus* (SAG 276-3a), a green microalgae species  
 302 of the genus *Scenedesmus* that lives in freshwater, notable for the genetic coding of its  
 303 mitochondria. This strain has already been used in previous studies, with different aims (De Morais  
 304 et al., 2007; Tang et al., 2011; Ho et al., 2012a; Ho et al., 2012b; Franchino et al., 2013).  
 305 Microalgae were grown with BG-11 medium realized, using distilled water for small volumes and  
 306 tap water for larger ones.

307 **Table 2.** BG-11 medium composition

BG-11 medium		
COMPOUND	MOLECULAR FORMULA	CONCENTRATION [g/l]
Sodium Nitrate	NaNO <sub>3</sub>	1,5
Dipotassium Hydrogen Phosphate	K <sub>2</sub> HPO <sub>4</sub>	0,04
Magnesium Sulfate Heptahydrate	MgSO <sub>4</sub> · 7H <sub>2</sub> O	0,075
Calcium Chloride	CaCl <sub>2</sub>	0,036
Citric Acid	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub>	0,006
Ferric Ammonium Citrate	C <sub>6</sub> H <sub>11</sub> FeNO <sub>7</sub>	0,006

Na <sub>2</sub> EDTA	C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> Na <sub>2</sub> O <sub>8</sub> · 2H <sub>2</sub> O	0,001
Sodium carbonate	Na <sub>2</sub> CO <sub>3</sub>	0,02
Boric Acid	H <sub>3</sub> BO <sub>3</sub>	2,86 · 10 <sup>-3</sup>
Manganese Chloride Tetrahydrate	MnCl <sub>2</sub> · 4H <sub>2</sub> O	1,81 · 10 <sup>-3</sup>
Zinc Sulfate Heptahydrate	ZnSO <sub>4</sub> · 7H <sub>2</sub> O	0,222 · 10 <sup>-3</sup>
Molibdenum Sodium Oxide	MoNa <sub>2</sub> O <sub>4</sub> · 4H <sub>2</sub> O	0,39 · 10 <sup>-3</sup>
Copper Sulfate Pentahydrate	CuSO <sub>4</sub> · 5H <sub>2</sub> O	0,079 · 10 <sup>-3</sup>
Cobalt Nitrate Hexahydrate	Co(NO <sub>3</sub> ) <sub>2</sub> · 6H <sub>2</sub> O	0,049 · 10 <sup>-3</sup>

308

309 Strain banks usually send slant cultures. It is suggested to let the cultures grow in light conditions at  
310 20 - 25°C until micro-organisms cover the entire inclined surface of the agar. This process can take  
311 several weeks. Subsequently, microalgae are scraped from the surface of the agar and inoculated in  
312 a 400-ml glass bottle containing 100 ml of BG-11 solution. This bottle is placed on an orbital  
313 shaker to prevent sedimentation, and fluorescent lamps illuminate it. After two weeks, the strain  
314 volume is doubled, and an air sparging system is installed, modifying the bottle's plug. This system  
315 consists of a small air compressor connected through a plastic tube and a filter to an immersed  
316 micro-bubble diffuser that is placed inside the bottle. The plug has two holes: one for the inlet tube,  
317 one for the gas exit tube. A week later, algae have spent almost all nutrients present in the solution,  
318 so the culture volume is doubled again, reaching the maximum available capacity of the bottle.  
319 After this growth period, algae are centrifuged (4,000 rpm for 5 minutes) and re-suspended in 6  
320 bottles containing 400 ml of BG-11 solution each. The total volume of culture is now equal to 2.4 l,  
321 enough to proceed, after the required growth period, to the column inoculum. The column consists  
322 of a vertical polycarbonate tube measuring 20 cm in diameter, 120 cm in height, with a total  
323 capacity of 28 l. This reactor is illuminated by four vertical fluorescent lamps radially disposed of.  
324 CO<sub>2</sub> can be supplied in the form of air by a compressor or in pure form by a gas cylinder. Carbon  
325 dioxide flowrate is manually regulated according to optimal pH levels, with a maximum value of 2  
326 l/min. To enhance gas diffusion in the liquid phase, it is sparged through 4 micro-bubble diffusers  
327 fixed on the bottom of the column. Two plastic channels are disposed above the diffusers to  
328 enhance convective motions and thus mixing. This method forces gas bubbles to mix with liquid  
329 and go up inside the channels placed in the center of the column while the rest of the culture turns  
330 back down externally. Five of six bottles with a useful volume of 400 ml are used to inoculate the  
331 column, and the remaining one is centrifuged and re-suspended in 6 new bottles of the same  
332 capacity (400 ml). After a couple of weeks, the biomass concentration of the culture inside the  
333 column is sufficient to permit the inoculum inside the planar photobioreactor to be used for this  
334 study, in an initial configuration having a capacity of 100 l.

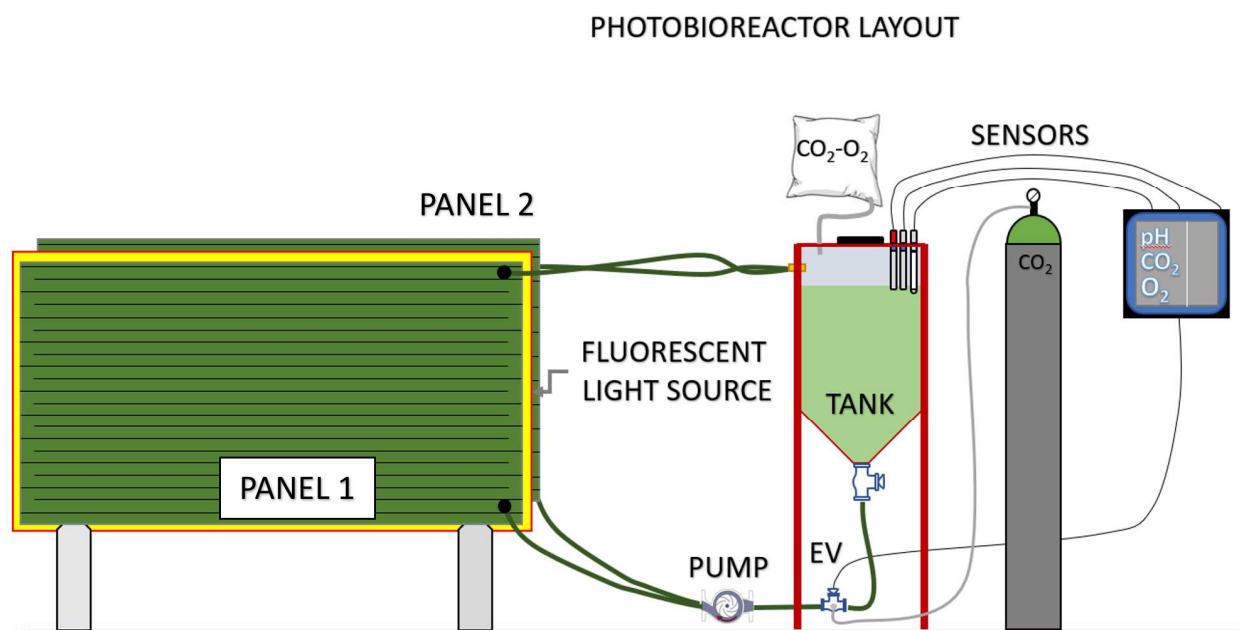
335

### 2.3.2. Experimental setup

336  
337 The presence of O<sub>2</sub> in the mixture can be hazardous due to flammability limits: in the case of CH<sub>4</sub>,  
338 DIPPR tables report concentration values between 5.0 to 15.0 vol % determined at 298 K and  
339 101,325 Pa. Higher temperatures and/or pressures will reduce the lower limit and raise the upper  
340 limit. However, the experiments (for safety reasons) are conducted using a pure source of CO<sub>2</sub>.  
341 Closed photobioreactors are designed to have larger optical cross-sectional areas to receive natural  
342 or artificial light (Lee et al., 1995; Morita et al., 2000). Microalgae strains can be cultivated year-  
343 round in continuous or semi-continuous culture mode and can obtain high cell density per unit area  
344 or volume as well as high CO<sub>2</sub> fixation rate by using PBRs (Giordano et al., 2005; Wang et al.,  
345 2012). Closed PBRs have many advantages over open ponds, including 1) easier control of  
346 parameters that affect algae growth; 2) relatively stable culture conditions; 3) aseptic operation; 4)  
347 capability of high-density cultivation; 5) high area/volume ratio to increase mass transfer efficiency  
348 with less space occupation, which significantly improves CO<sub>2</sub> fixation efficiency; 6) ability for the  
349 natural (or artificial) light source to be collected and distributed to the interior of the bioreactor  
350 using a collector and optical fiber, to obtain much higher light utilization; and 7) avoided or reduced  
351 water evaporation (Chisti, 2007; Wang et al., 2012; Cheng et al., 2013). To this end, a custom  
352 photobioreactor (PBR) was constructed and implemented in this study. This microalgae growing  
353 system is subdivided into two main parts: a photo stage loop and a mixing tank (Fig. 1). The first  
354 one exploits the photosynthetic efficiency of microalgae to maximize CO<sub>2</sub> absorption from the inlet  
355 gas; the second one ensures culture mixing and gas separation.

356 The photo stage loop is composed of up to 5 neon lamps of 58 W each, interposed between two 1.5-  
357 m<sup>2</sup> parallel alveolar flat panels. These panels are partitioned into a series of internal rectangular  
358 channels in which, thanks to a 45 W high-efficiency pump, culture flows from the bottom to the top.  
359 After that, the culture enters the mixing tank. The CO<sub>2</sub> enters the system just before the pump, using  
360 a solenoid valve managed by electronic control. The automatic control is linked with pH or  
361 dissolved CO<sub>2</sub> values. This CO<sub>2</sub> diffusion system should assure a high gas-liquid mass transfer  
362 coefficient, and thus a better absorption of CO<sub>2</sub> from microalgae. The compact design of the pilot  
363 PBR guarantees optimal light utilization permitting high K values while taking up little volume,  
364 also allowing the scaling-up of the plant merely by increasing the number of these modules in  
365 parallel. Oxygen, dissolved CO<sub>2</sub>, and pH probes are fixed on the plug of the first tank and connected  
366 to a Mettler-Toledo® multi-parameter transmitter. This device controls the solenoid valve for CO<sub>2</sub>  
367 injection, maintaining a pH level between 6.7 and 6.9. The upper part of the tank is sealed, and the  
368 gas released over time from the liquid surface is stored inside a 5L Tedlar bag. This bag is changed  
369 every day, and the stored gas analyzed with a GA-5000 gas analyzer to determine CO<sub>2</sub> presence.

370 Biomass can be extracted from the bottom of the tank while nutrients are inserted from the top. The  
 371 fed-batch regime is manually achieved by substituting 16.6 l of algal medium with the same  
 372 quantity of fresh nutrients three times a week. In this way, the culture medium is replaced after six  
 373 interventions (i.e., two weeks). This substitution volume is calculated considering a growth rate of  
 374 0.06 1/day obtained during a batch-growing curve and evaluated according to Shuler & Kargi  
 375 (2002), to maintain biomass concentration stability.



376  
 377 **Fig. 1.** Photobioreactor layout with the indication of main components

### 378 2.3.3. Data processing

380 Measurements of biomass growth are taken both before and after the medium substitution through  
 381 two procedures: absorbance and dry weight. The first one is obtained using a UNICAM® Helios- $\alpha$   
 382 spectrometer on three samples: pure, 50%, and 25% (dilution with distilled water). Dry weight  
 383 concentrations are the result of a 378 K evaporation process in a fan-assisted oven for 48 h. Three  
 384 crucibles containing microalgal broth are utilized for this process, then samples are weighed using  
 385 an analytical balance; mean value and standard deviation are obtained.

386 The global gas-liquid mass transfer coefficient for carbon dioxide  $K_{La}(\text{CO}_2)$  is measured by  
 387 adjusting the unsteady-state method for aerobic cultures of microorganisms proposed by Genon  
 388 (1993). This modified method can be applied to reactors containing living cultures of  
 389 photosynthetic organisms and permit the measuring of the  $K_{La}$  value as well as culture  $\text{CO}_2$   
 390 consumption. The last value is significant: it reveals the real performances and efficiencies of the  
 391 system. It depends on irradiance (and consequently on emission spectrum) and biomass  
 392 concentration inside the culture g/l. Volumetric  $\text{CO}_2$  consumption can be defined as:

$$r = \frac{G_{gas,in} x_{CO_2,in} - G_{gas,out} x_{CO_2,out}}{V} \quad [1]$$

393  
394 where  $r$  is the volumetric CO<sub>2</sub> consumption [mg/l/s],  $G_{gas,in}$  and  $G_{gas,out}$  are the gas flowrates at the  
395 inlet and the outlet [mg/s], respectively,  $x_{CO_2,in}$  and  $x_{CO_2,out}$  are the mass fractions of inlet and outlet  
396 gas flows [-], respectively, and  $V$  is the illuminated volume of culture [l].

397 Starting from the regime conditions of CO<sub>2</sub> concentration in the liquid phase, the carbon source  
398 obtained by CO<sub>2</sub> injection is interrupted. In this way, the culture is constrained to consume the  
399 carbon dioxide dissolved in liquid. The following equation can describe this process

$$r + \frac{dc_L(t)}{dt} = 0 \quad [2]$$

400 where  $r$  is the volumetric CO<sub>2</sub> consumption [mg/l/s],  $c_L(t)$  is the CO<sub>2</sub> concentration in the liquid  
401 phase [mg/l], and  $t$  is time [s].

402 This shows a linear decrease of dissolved CO<sub>2</sub> concentration in the culture medium. After this first  
403 step, when the linear trend stabilizes, CO<sub>2</sub> injection starts again until regime conditions are reached.

404 The equation below can describe this situation:

$$k_{La}(c_{\infty}^* - c_L(t)) = r + \frac{dc_L(t)}{dt} \quad [3]$$

405 where  $k_{La}$  is the global gas-liquid mass transfer coefficient [h<sup>-1</sup>],  $c_{\infty}^*$  is the CO<sub>2</sub> concentration in the  
406 liquid phase at  $t=\infty$  [mg/l],  $c_L$  is the CO<sub>2</sub> concentration in the liquid phase at time  $t$  [mg/l],  $r$  is the  
407 volumetric CO<sub>2</sub> consumption [mg/l/s], and  $t$  is time [s].

408 Concentration values are calculated by an InPro® 5000i CO<sub>2</sub> probe connected to a Mettler Toledo®  
409 M-800 multi-parameter transmitter and recorded by a Kobold® electronic multi-channel data  
410 logger. The probe is placed both in the collection container of the tank's plug (only one tank will be  
411 used for these first experiments) and in the lower part of the tank, near the pump's inlet tube. In this  
412 way, different values of CO<sub>2</sub> concentrations in the liquid between these two setups permit us to  
413 evaluate run-off system efficiency.  
414  
415

416

### 417 **3. Results and discussion**

#### 418 **3.1 Application of the MCBioCH4 model**

419 The results obtained by simulating the two scenarios with the MCBioCH4 model are reported in  
420 Tables 3-4 and Figures 2-3. These results take into account the outcomes of the pre-treatment tests  
421 reported in Borzooei et al. (2019). The innovations introduced by Scenario 1 trigger two critical  
422 positive impacts on the overall energy and mass balance of the sludge line of the WWTP. First, the  
423 installation of an effective thickener allows a reduction of the sludge volume entering the digestion  
424 process. The simulation shows that, in Scenario 1, the number of digesters can be reduced from 6 to  
425 4. This reduction in volume brings three main positive consequences to the system (Table 3):

- 426           ● The thermal energy spent for pre-heating of substrates is 41% lower than in the present  
427           system;
- 428           ● Heat dispersion from the digesters is 21% lower than in the present system;
- 429           ● A lower amount of energy (-20%) is needed to handle and transfer the digested sludge to  
430           final disposal and use.

431 The other positive impact brought by Scenario 1 is the increased specific methane production  
432 (SMP) provided by the application of the pre-treatment. Table 3 shows that net biogas production in  
433 Scenario 1 is around 18% higher than in the present system. An amount of 5,000 t/y of biomethane  
434 is produced and injected into the natural gas distribution grid. Assuming a conversion efficiency of  
435 90%, this corresponds to replacing 63,740 MWh/y of natural gas with biomethane (Table 3). In  
436 Scenario 1, the methane released in the upgrading process causes an increase in total methane losses  
437 from the overall process (+59%). Electricity consumption is also higher in Scenario 1, because of  
438 the energy needed to upgrade biogas to biomethane (+47%). The upgrading process consumes  
439 3,604 MWh/y of electricity. Electricity consumption of other types of equipment of the digestion  
440 process amounts to an additional 8,162 MWh/y. Electricity consumption of the advanced post-  
441 thickener is not significant, though, being around 162 MWh/y. The results confirm that the heat  
442 recovered from the sludge drying process is not sufficient to cover the internal demand for thermal  
443 energy. For this reason, an external source of heat is needed. This external source is represented by  
444 a boiler fueled by natural gas, which is expected to cover the remaining 28% of the demand.

445 **Table 3.** Mass and energy balance of sludge digestion scenarios simulated with the MCBioCH4 model

Input parameter/value	Scenario 0	Scenario 1	Difference
Biogas production (t/y)	11,456	13,539	+18%
Gross biogas energy content (MWh/y)	60,773	71,828	+18%
Thermal energy internal demand for pre-heating of substrates	33,728	20,236	-41%
Thermal energy internal demand for compensation of digesters dispersion	1,928	1,542	-21%
Internal electricity demand, total	8,000	11,768	+47%
Net thermal energy production (MWh/y)	26,514	63,740 <sup>1</sup>	+140%
Net electricity production (MWh/y)	25,454	-	-100%
Thermal energy auto-consumption covered by biogas/biomethane (%)	59	-	-59%
Thermal energy auto-consumption covered by drying line (%)	41	72	+31%
Electricity auto-consumption covered by biogas/biomethane (%)	100	0	-100%
Thermal energy auto-consumption covered by external source (%)	0	28	+28%
Electricity auto-consumption covered by external source (%)	0	100	+100%
Energy consumption for digestate handling/transfer (MWh/y)	371.7	296.6	-20%

Total CH <sub>4</sub> loss from the process (t/y)	87.0	138.7	+59%
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446 <sup>†</sup> Considering a grid-to-final use efficiency of 0.9

447

448 The total greenhouse gas balance provided by the environmental module of the MCBioCH<sub>4</sub> model  
 449 is reported in Table 4. This table compares the simulated scenarios in terms of GHG emissions. The  
 450 results show that both the present and the alternative configurations have favorable balances,  
 451 meaning that avoided emissions for the substitution of natural gas and electricity are higher than the  
 452 emissions produced for process maintenance. The introduction of sludge pre-treatment and the  
 453 advanced thickening stage (Scenario 1) are expected to improve the general environmental balance  
 454 of the plant. Specific Equivalent CO<sub>2</sub> emission is expected to decrease from -0.278 t CO<sub>2eq</sub>/t biogas  
 455 to -0.394 t CO<sub>2eq</sub>/t biogas (from -3,182 t CO<sub>2eq</sub>/y to -5,333 t CO<sub>2eq</sub>/y, -41%). Scenario 1 thus results  
 456 in a lower GHG impact. Among previous studies, Remy et al., (2013) calculated the GHG balance  
 457 of different options of a sludge treatment process in a large WWTP in Berlin (1.5 million of  
 458 population equivalents, PE, assuming a mean COD load of 120 g · PE<sup>-1</sup> · d<sup>-1</sup>). Overall, the existing  
 459 sludge treatment line has a carbon footprint of --11.6 kg CO<sub>2eq</sub> · PECOD-1 · y-1), corresponding to  
 460 -17,400 tCO<sub>2eq</sub>/y. However, unlike in the present study, the final sludge disposal options were  
 461 considered. Without considering sludge disposal ways, the GHG balance yields a value of -6,900  
 462 tCO<sub>2eq</sub>/y. Another study by Houillon and Jolliet (2005) considered six wastewater sludge treatment  
 463 scenarios applied to a 300,000 PE WWTP. The results showed that, depending on the process and  
 464 sludge management, the GHG balance could shift from -100 kgCO<sub>2eq</sub>/t of dry matter (DM) to 500  
 465 kgCO<sub>2eq</sub>/t DM. If represented in the same unit, this study shows a range of -84 – -140 kgCO<sub>2eq</sub>/t  
 466 DM.

467 **Table 4.** Environmental balance of sludge digestion scenarios simulated with the MCBioCH<sub>4</sub> model

Input parameter/value	Scenario 0		Scenario 1		Difference
	t CO <sub>2eq</sub> /y	t CO <sub>2eq</sub> /m <sup>3</sup> biogas y	t CO <sub>2eq</sub> /y	t CO <sub>2eq</sub> /m <sup>3</sup> biogas y	
Total CH <sub>4</sub> loss from the process	2,437	0.213	3,883	0.287	+34%
Total CO <sub>2</sub> loss from the process	147	0.013	115	0.008	-39%
Net electricity production	883	-0.514	-	-	-
Biomethane replacing natural gas	-	-	-14,594	-1.078	-
Thermal energy auto-consumption covered by external source	-	-	1,203	0.089	+100%
Electricity auto-consumption covered by external source	-	-	3,967	0.293	+100%
Energy consumption for digestate handling/transfer	117	0.010	93	0.007	-30%
Produced GHG emissions	2,701	0.236	9,261	0.684	+180%
Avoided GHG emissions	-5,883	-0.514	-14,594	-1.078	-109%

GHG emission balance	-3,182	-0.278	-5,333	-0.394	-41%
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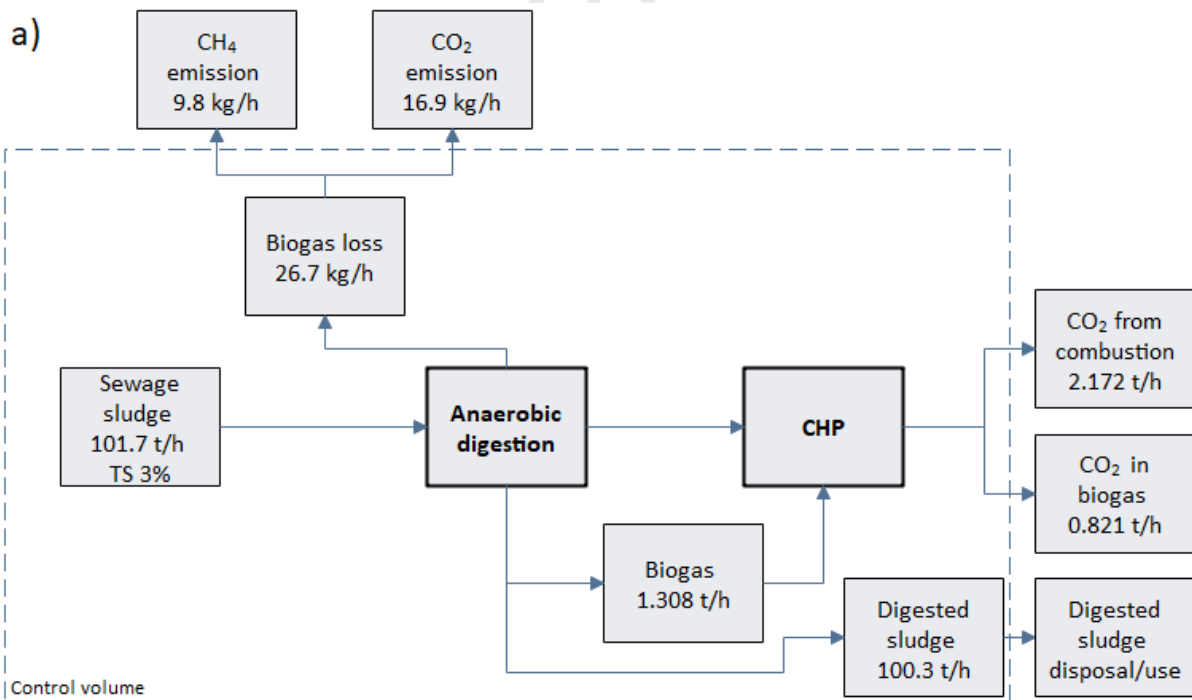
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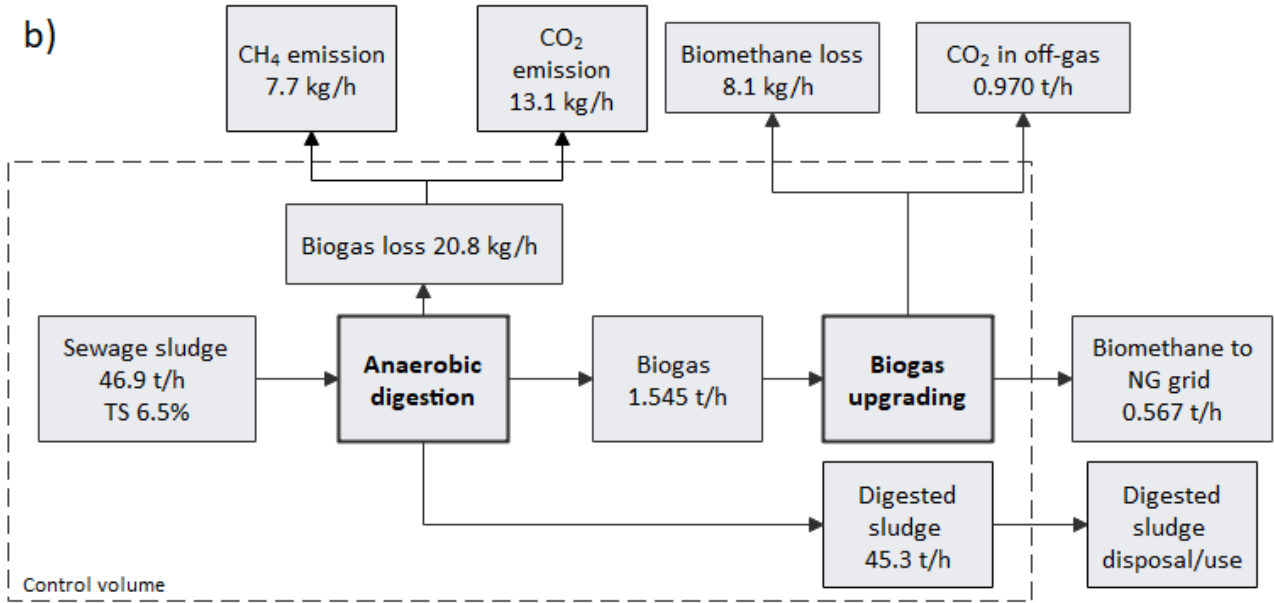
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The results reported herein also show that the production of biomethane would allow optimum exploitation of the energy contained in the sludge, as it would be directly introduced into the natural gas distribution grid. However, energy would not be produced onsite; thus, external sources of electricity and heat would be needed to satisfy the process of auto-consumption. On the one hand, this represents a limitation of the biomethane option. On the other hand, it is expected that indirect emissions due to electricity consumption will constantly be decreasing shortly, due to the higher share of renewable sources (Italian Ministry of Economic Development, 2017). Considering the subsidies recently introduced by Italian regulations, this configuration is also the most economically feasible solution. Nevertheless, the economic balance of the proposed solutions should be evaluated in future studies. To achieve the common general GHG reduction objectives, a higher level of process integration must be met. Sludge optimization and digestion scenarios must thus be evaluated together with the feasibility of microalgae carbon sequestration interventions proposed in the following.



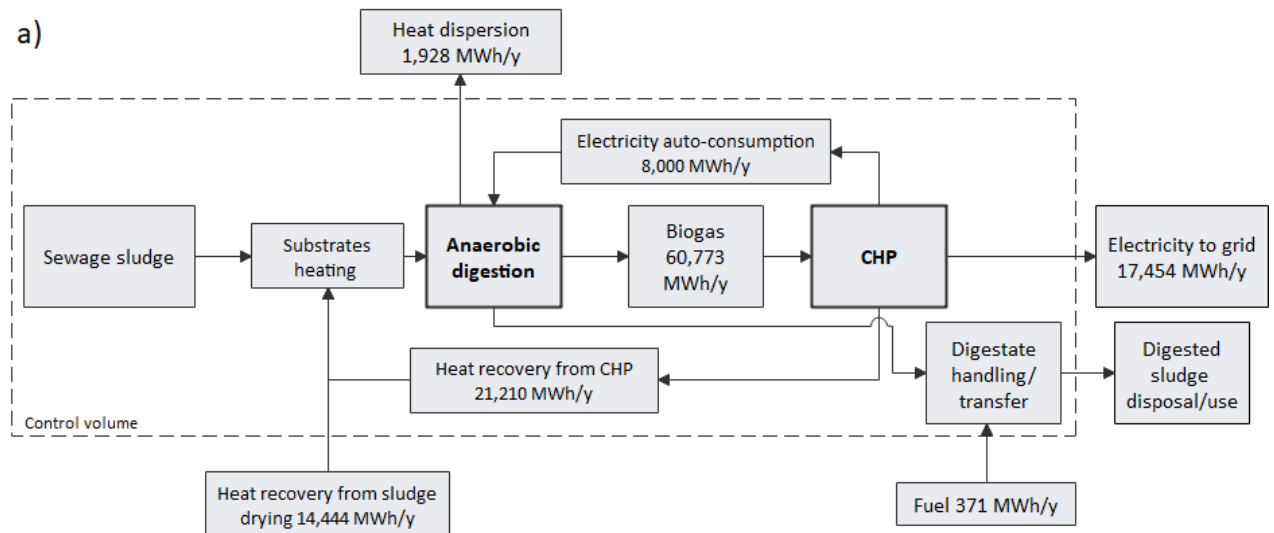
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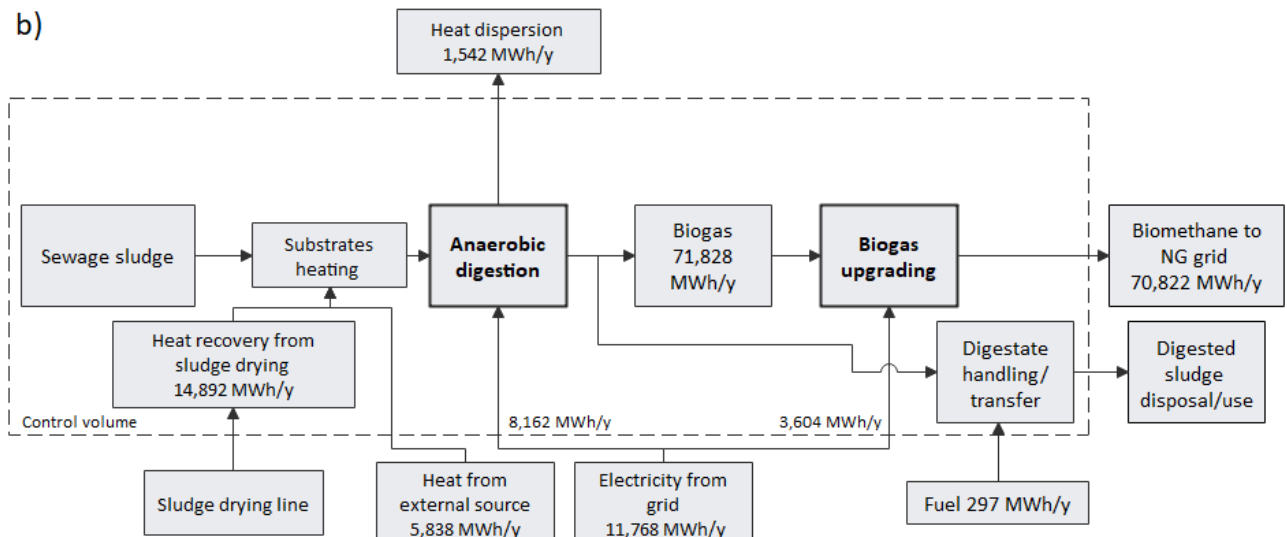


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Fig. 2. Mass balances of Scenario 0 (a) and Scenario 1 (b)



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493 **Fig. 3.** Energy balances of Scenario 0 (a) and Scenario 1 (b)

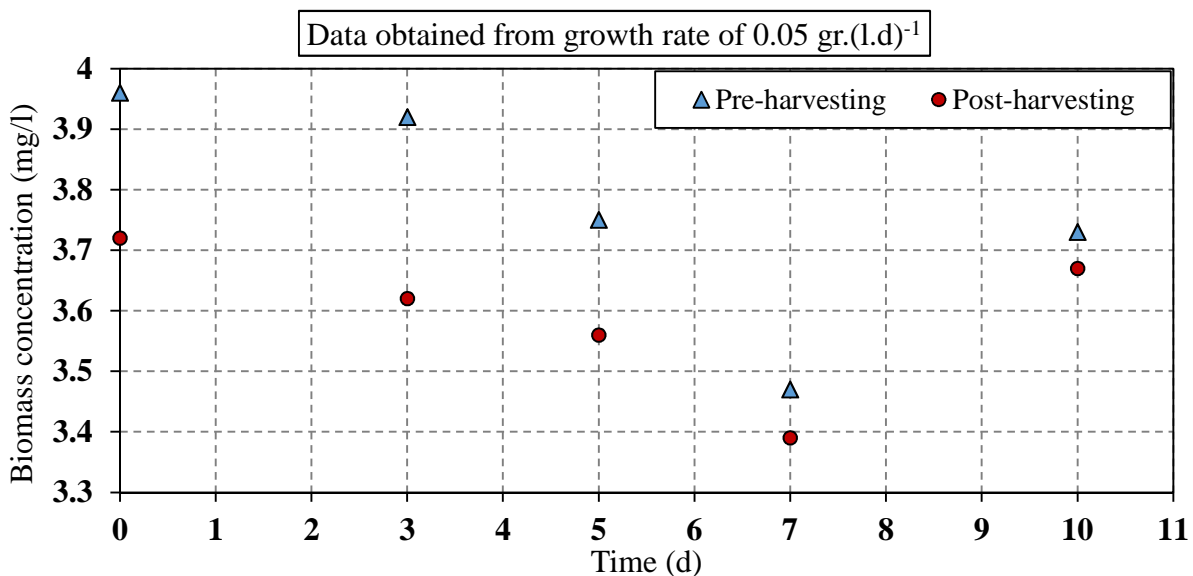
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### 495 **3.2 Application of microalgae CO<sub>2</sub> fixation**

496 Microalgae growth was tested using the already described PBR system with a total volume  
 497 maintained at 100 L. During the growth stage; biomass concentration is measured. The illumination  
 498 system is composed of 5 equally spaced fluorescent lamps placed between the two panels. This light  
 499 source can supply around  $76 \mu\text{mol}/\text{m}^2\text{s}$ . Growth curves of this first phase show increasing values for  
 500 a period lasting about 30 days, after which, without the addition of nutrients, the strain reaches its  
 501 concentration asymptote. This value can vary depending on growing conditions like illuminance,  
 502 pH, temperature, CO<sub>2</sub>, and nutrient concentrations. If a shortage of nutrients persists, biomass  
 503 concentration starts to decrease rapidly, as the last part of the curve shows. As previously noted, the  
 504 mean biomass productivity calculated is equal to 0.06 g/l day.

505 Continuous operation is achieved, as described in the materials and methods section (Figure 4).

506 During this phase, illumination is provided by only three of five fluorescent lamps providing around  
 507  $44 \mu\text{mol}/\text{m}^2\text{s}$ . Growth curves of continuous operation look stair-stepped due to medium substitution  
 508 in the fed-batch method that occurs every Monday, Wednesday, and Friday; in this way, the time  
 509 distance between two replacements may be either 2 or 3 days. This interval difference can be noted  
 510 in the graph below: over the weekend, the culture grows more consistently. Biomass concentration  
 511 remains quite constant during continuous operation; it is possible to detect 4 g/l concentration  
 512 asymptote in these conditions of illumination (3 fluorescent lamps).

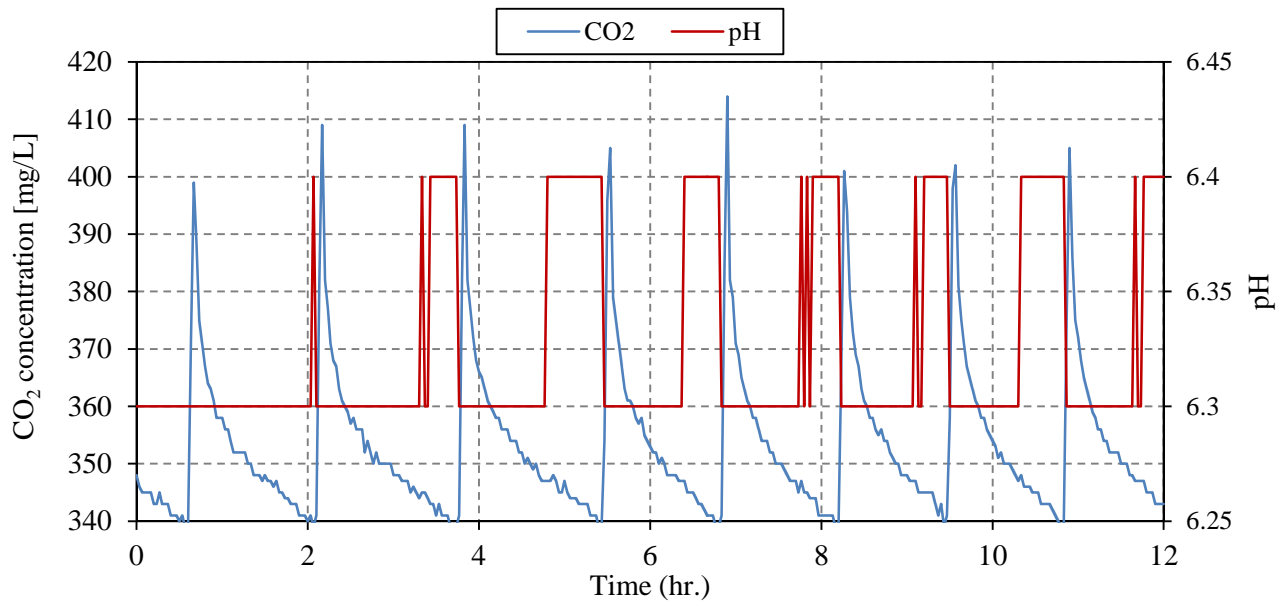


513

514 **Fig. 4.** Growth trends during continuous operation in fed-batch feeding mode

515

516 As for CO<sub>2</sub> regulation, two approaches have been used: indirect regulation of the pH level and  
 517 direct control of the CO<sub>2</sub> concentration. Both showed high stability, but the direct method permits  
 518 the maintenance of desired concentration values more accurately.



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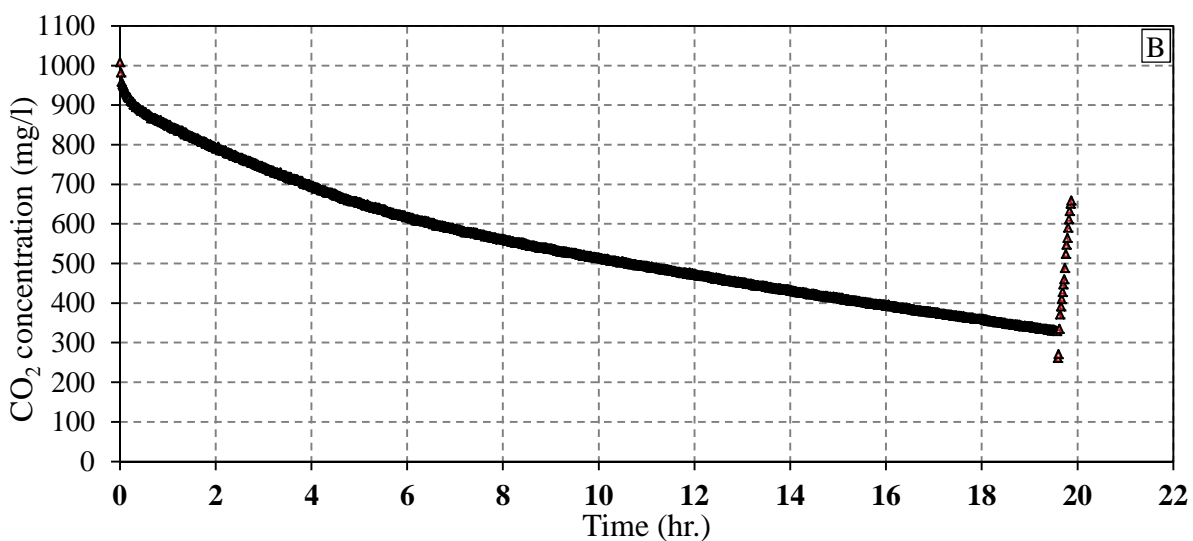
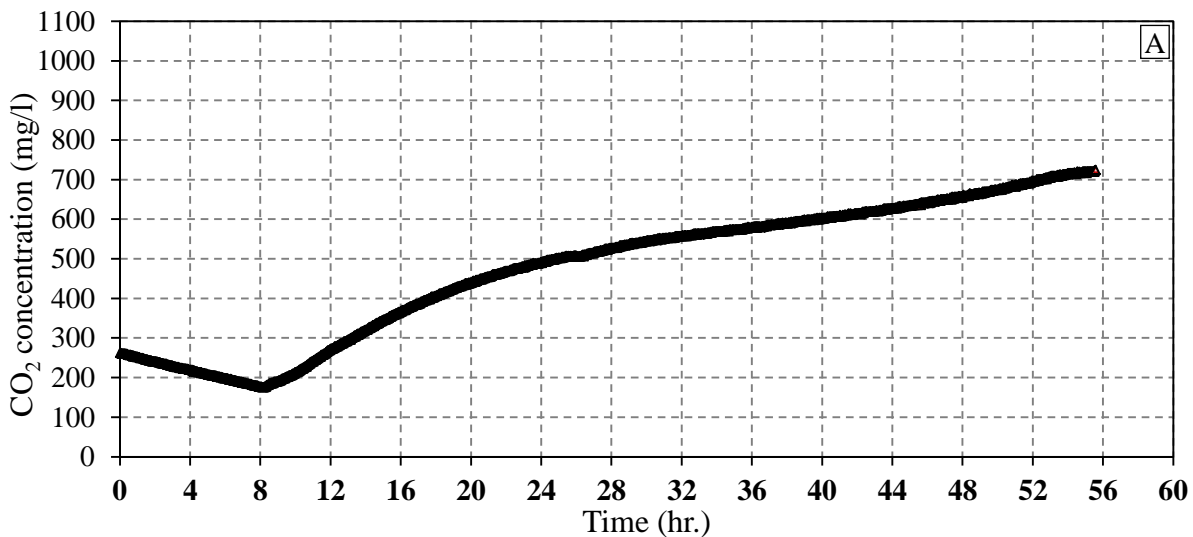
520 **Fig. 5.** CO<sub>2</sub> and pH trends with regulation based on the dissolved CO<sub>2</sub> values coming from the CO<sub>2</sub> sensors placed  
 521 inside the microalgae culture

522

523 Fig. 5 shows CO<sub>2</sub> and pH trends with this type of regulation. The data collected from the  
 524 respirometry tests are visible in Fig. 6, and they present two trends: the first one, a descending  
 525 phase, indicates the respirometry of the system; the second one, showing an ascending pattern, is  
 526 strictly related to the evaluation of  $K_{La}$  as described in the materials and methods paragraph. The  
 527 angular coefficient obtained from the descending phase of the graph is the value of  $r$ , which is equal  
 528 to the volumetric consumption of CO<sub>2</sub> caused by the microalgae. The data collected during the  
 529 ascending phase were used to evaluate the  $K_{La}$  using Equation 3. The trend of this curve is directly  
 530 related to the CO<sub>2</sub> input flow rate and the ability of the system to transfer the gas phase into a liquid  
 531 one. Trials conducted for  $K_{La}$  and CO<sub>2</sub> consumption were performed as previously explained using  
 532 two illumination configurations: 3 and 5 fluorescent lamps. The first test (3 fluorescent lamps and  
 533 biomass concentration around 3.93 g/l) reported the following values: a CO<sub>2</sub> consumption of 11.763  
 534 mg/l/h and a  $K_{La}$  value of 0.5775 h<sup>-1</sup>. The first, considering a light-exposed volume of 50 l,  
 535 corresponds to 7.72 l<sub>CO2</sub>/day under normal conditions. The fixation rate may also give information  
 536 about the microalgae's growth, knowing their approximate molecular formula.  $K_{La}$ , tested by  
 537 injecting 0.5 l<sub>CO2</sub>/m, returned lower values than expected: this result can be interpreted as a prompt  
 538 response of the system to variations in the liquid's CO<sub>2</sub> concentration due to an essential presence of  
 539 microorganisms. This means high carbon dioxide utilization and hence low dispersion in the

540 environment.  $K_{La}$  depends on the quantity of gas injected into the system per time unit; for this  
 541 reason, the second experiment is conducted with a higher  $CO_2$  flowrate since higher illumination is  
 542 planned, and therefore higher biomass concentration is expected.

543 The second test (5 fluorescent lamps and biomass concentration of around 4.5 g/l) reported a  $CO_2$   
 544 consumption of 27.943 mg/l/h and a  $K_{La}$  value of  $17.7 h^{-1}$ . The first one corresponds, considering a  
 545 light-exposed volume of 50 l, to 18.33  $l_{CO_2}/day$  under normal conditions. The second one, tested by  
 546 injecting 2  $l_{CO_2}/min$ , shows the strong dependence of this coefficient on the inlet gas flowrate.  
 547 These data demonstrate the ability to perform fast regulation in the system's  $CO_2$  concentration and  
 548 guarantee optimal carbon-feed to the culture. An appropriate  $K_{La}$  value can be decisive in the  
 549 optimization of gas and liquid flow rates, and therefore of energy consumption.



551

552

553 **Fig. 6.** Dissolved  $CO_2$  trends inside the PBR during the 3 (A) and 5 (B) fluorescent lamps tests. The inlet  $CO_2$   
 554 flows were set equal to 0.5  $L_{CO_2}/min$  in case A and 2  $L_{CO_2}/min$  in case B.

555 Furthermore, to determine the CO<sub>2</sub> reduction efficiency of the PBR system, the gas stored in the  
556 Tedlar bags was analyzed with a gas analyzer. The total decrease of CO<sub>2</sub> exiting the system was  
557 recorded as around 80% in the first case and about 70% in the second one, starting from a source of  
558 CO<sub>2</sub>. The only comparison that can be made between the obtained data and other studies is with the  
559 work of Meier et al. (2017), as very few experimental works have been performed using an  
560 experimental setup similar to the one proposed in this article. In that work the authors obtained  
561 identical outcomes, although with significant differences, like the layout of the system was not quite  
562 the same: light saturation was achieved with a photon flux equal to 500  $\mu\text{mol}/\text{m}^2\text{s}$ , and the CO<sub>2</sub>  
563 mass coefficient was not directly measured but was obtained through an analytical relationship with  
564 an oxygen coefficient. One of the most significant differences of the proposed experimental setup  
565 compared to reported methodologies is the way the CO<sub>2</sub> is fed and controlled in the system, which  
566 allows an exact gas dosage. Impressive results were also obtained by using a single-stage closed  
567 PBR with a biomass concentration around two different photoperiods: one equal to 24 h of light and  
568 the other with alternating light/dark periods of 12:12, using an autotrophic *Scenedesmus* culture.  
569 With these experimental setups, Prandini et al. (2016) obtained a reduction of CO<sub>2</sub> equal to 99% ca.  
570 and 70% ca. respectively, but the concentration of oxygen inside the microalgae substrate was so  
571 high as to be considered a limiting growth factor. The other two experimental studies are presented  
572 in the literature by Basu et al. (2015) and Thiansathit et al. (2015), using small-scale PBRs. Both  
573 studies were performed using *Scenedesmus obliquus* under autotrophic conditions; in the first case,  
574 the strain was grown inside an open cylindrical glass tube PBR with alternating light/dark periods  
575 of 14:10 and the second one used a 5.3L translucent cylindrical plastic tank and alternating  
576 light/dark periods of 16:8. The carbon uptake by microalgae was reported, based on the hours of  
577 continuous CO<sub>2</sub> supply, in a range from 10.23% (12 hr) to 2.54% (24 hr) in the first experiment. In  
578 contrast, in the work of Thiansathit et al. (2015), the carbon uptake was recorded at a value of  
579 around 7%. Several un-controlled growing factors negatively influenced the experiments. In a  
580 recent study (Rodero et al., 2019) with consideration for industrial upscaling. In their work, the  
581 authors elaborated and tested a hybrid system composed of an open pond growing stage and a  
582 washing column dedicated to biogas upgrading with microalgae. The system used a mixed culture  
583 of microalgae and bacteria, allowing a CO<sub>2</sub> reduction in the inlet biogas ranging from 60 to almost  
584 100%. This result, on the one hand, allows the industrial implementation of this technology, and on  
585 the other hand, sacrifices the biomass quality that must be considered a by-product in the best case  
586 or waste in the worst one. With the reported data, it is becoming evident that the results of biogas  
587 purification via microalgae are close to those of chemical absorption processes, although biogas  
588 purification yield does need to be enhanced through optimization strategy. Some balances can be

589 evaluated by considering that microalgae biomass is made up of about 55% carbon, that the  
590 estimated growth rate is 0.06 g/l, and the carbon absorption rate of 0.037 g/l d. Consequently, the  
591 CO<sub>2</sub> removal rate can be evaluated as equal to 0.135 g/l d. Based on the obtained results, it is  
592 expected that the integration of microalgae technologies would bring additional advantages to  
593 WWTP energy optimization and reduction of GHG emissions, as for 1 ton of biomass produced,  
594 about 2 tons of CO<sub>2</sub> will get fixed.

595

## 596 **Conclusion**

597 This study offers an integrated experimental and modeling feasibility analysis assessing possible  
598 opportunities to minimize the carbon footprint of the largest Italian WWTP. The proposed  
599 methodology includes a scenario analysis for improving the biogas production in sludge treatment  
600 units by the use of special pre-treatment techniques as well as upgrading biogas to biomethane. The  
601 implementation of a sludge thickener to increase the total solids (TS) content of the sludge was  
602 considered. The production of biomethane would allow optimum exploitation of the energy  
603 contained in the sludge, as it would be directly introduced into the natural gas distribution grid. The  
604 calculation of the environmental balance showed that the innovations presented in this study would  
605 reduce the GHG emissions of the sludge treatment line of the plant by around 40%. In the second  
606 part of the study, the investigation of using a custom-made planar photobioreactor, measuring the  
607 mass transfer coefficient and CO<sub>2</sub> consumption under two different artificial light scenarios, was  
608 reported. Regarding the test conducted with microalgae, the system achieved optimal conditions for  
609 microalgae growth and reached high values of biomass concentration in the culture, competing with  
610 the best technologies in this industrial sector. These tests demonstrated the possibility of rapid  
611 intervention in carbon dioxide regulation and the capability to maintain optimal carbon-feed to the  
612 culture. A further study about the energy cost, various illumination sources, and compatibility in  
613 terms of mass balance with sludge treatment units is suggested for scaling up the proposed setup  
614 into industrial application. This study demonstrates how increasing the level of integration among  
615 processes is one key factor toward energy savings and lower environmental impacts in WWTPs.

616

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- Model-based sludge pre-treatment and biogas upgrading scenarios are evaluated in a WWTP
- Various upgrading scenarios are studied and compared in terms of mass, energy, and GHG balance
- Application of dynamic sludge thickener, hybrid thermo-alkali sludge pre-treatment and biomethane production are proposed
- Use of an experimental microalgae technology is considered for CO<sub>2</sub> fixation
- Experimental setup is proposed to evaluate the  $K_{La}$  of CO<sub>2</sub> in the microalgae system

Journal Pre-proof

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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