

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

Original

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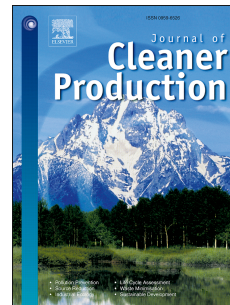
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Sina Borzooei, Giuseppe Campo, Alberto Cerutti, Lorenza Meucci, Deborah Panepinto, Marco Ravina, Vincenzo Riggio, Barbara Ruffino, Gerardo Scibilia, Mariachiara Zanetti



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Sina Borzooei

Politecnico di Torino,
DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
Abruzzi 24, 10129 Torino (Italy)
sina.borzooei@polito.it

Giuseppe Campo

Politecnico di Torino,
DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
Abruzzi 24, 10129 Torino (Italy)
giuseppe.campo@polito.it

Alberto Cerutti

Politecnico di Torino,
DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
Abruzzi 24, 10129 Torino (Italy)
alberto.cerutti@polito.it

Lorenza Meucci

SMAT S.p.A. (Società Metropolitana Acque Torino), Corso XI Febbraio 14, 10152 Torino (Italy)
lorenza.meucci@smatorino.it

Deborah Panepinto

Politecnico di Torino,
DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
Abruzzi 24, 10129 Torino (Italy)
deborah.panepinto@polito.it

Marco Ravina*

Politecnico di Torino,
DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
Abruzzi 24, 10129 Torino (Italy)
marco.ravina@polito.it

Vincenzo Riggio

Politecnico di Torino,
DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
Abruzzi 24, 10129 Torino (Italy)
vincenzo.riggio@polito.it

Barbara Ruffino

Politecnico di Torino,
DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
Abruzzi 24, 10129 Torino (Italy)
barbara.ruffino@polito.it

Gerardo Scibilia

Research Center, Società Metropolitana Acque Torino S.p.A., Viale Maestri del Lavoro, 4 – 10127
Torino (Italy)
gerardo.scibilia@smatorino.it

Mariachiara Zanetti

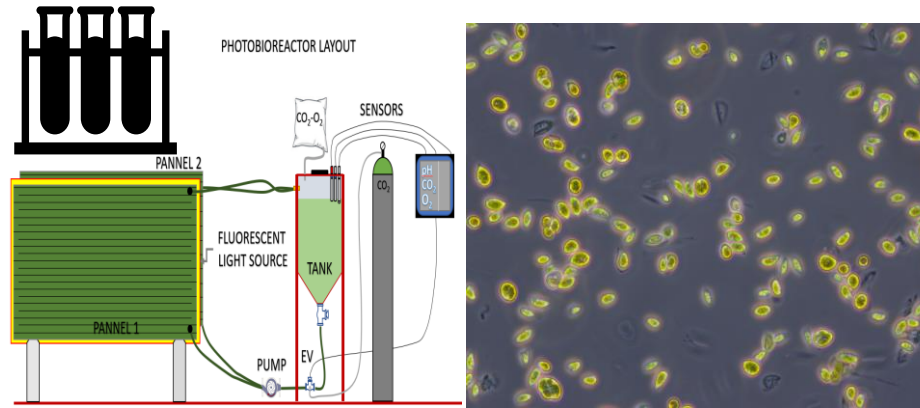
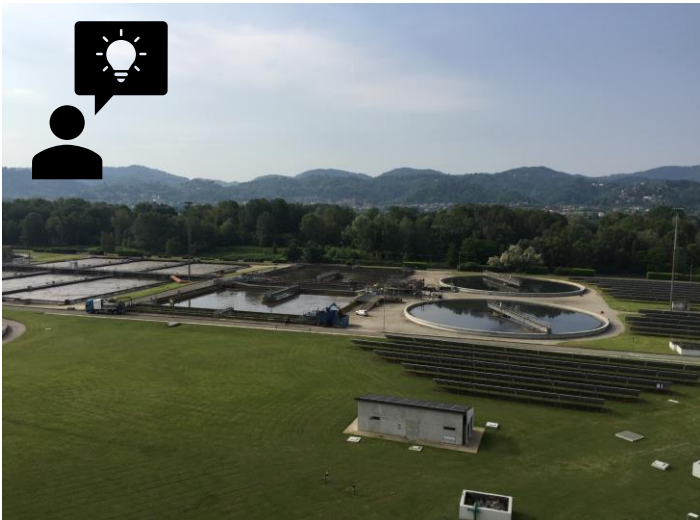
Politecnico di Torino,
DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
Abruzzi 24, 10129 Torino (Italy)
mariachiara.zanetti@polito.it

*Corresponding author

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Microalgae CO₂ fixation

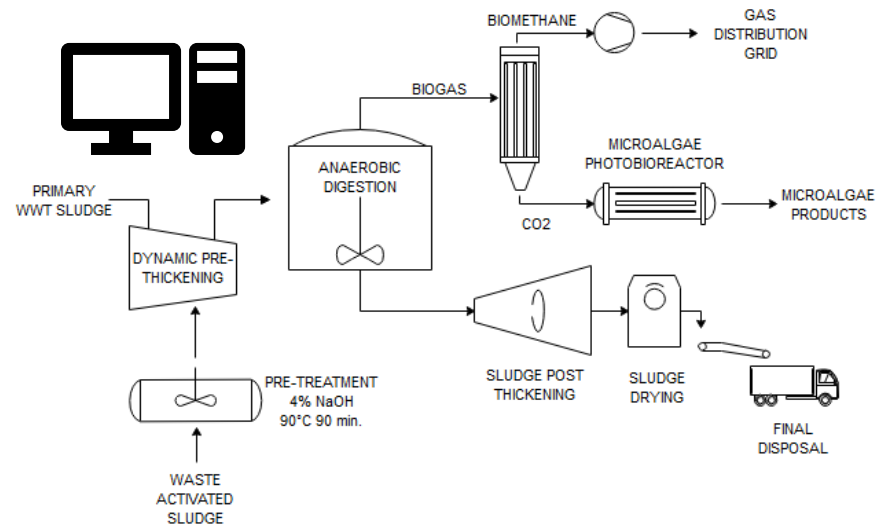
Feasibility analysis



Reduction



MCBioCH₄ model



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5 **Sina Borzooei**

6 Politecnico di Torino,
7 DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
8 Abruzzi 24, 10129 Torino (Italy)
9 sina.borzooei@polito.it

10
11 **Giuseppe Campo**

12 Politecnico di Torino,
13 DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
14 Abruzzi 24, 10129 Torino (Italy)
15 giuseppe.campo@polito.it

16
17 **Alberto Cerutti**

18 Politecnico di Torino,
19 DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
20 Abruzzi 24, 10129 Torino (Italy)
21 alberto.cerutti@polito.it

22
23 **Lorenza Meucci**

24 SMAT S.p.A. (Società Metropolitana Acque Torino), Corso XI Febbraio 14, 10152 Torino (Italy)
25 lorenza.meucci@smatorino.it

26
27 **Deborah Panepinto**

28 Politecnico di Torino,
29 DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
30 Abruzzi 24, 10129 Torino (Italy)
31 deborah.panepinto@polito.it

32
33 **Marco Ravina***

34 Politecnico di Torino,
35 DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
36 Abruzzi 24, 10129 Torino (Italy)
37 marco.ravina@polito.it

38
39 **Vincenzo Riggio**

40 Politecnico di Torino,
41 DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
42 Abruzzi 24, 10129 Torino (Italy)
43 vincenzo.riggio@polito.it

44
45 **Barbara Ruffino**

46 Politecnico di Torino,
47 DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
48 Abruzzi 24, 10129 Torino (Italy)

49 barbara.ruffino@polito.it

50
51 **Gerardo Scibilia**

52 Research Center, Società Metropolitana Acque Torino S.p.A., Viale Maestri del Lavoro, 4 – 10127
53 Torino (Italy)
54 gerardo.scibilia@smatorino.it

55
56 **Mariachiara Zanetti**

57 Politecnico di Torino,
58 DIATI (Department of Environment, Land and Infrastructure Engineering) Corso Duca degli
59 Abruzzi 24, 10129 Torino (Italy)
60 mariachiara.zanetti@polito.it

61
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63 *Corresponding author

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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 h^{-1} and 17.7 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 CO₂ initially contained in the biogas
151 stream, with a minor amount of CH₄ that has not been recovered in the process. Some additional
152 minor components, such as H₂S 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 CO₂ contained in the biomethane off-gas. Among
156 these, the use of microalgae as a biofilter for CO₂ is most promising. These microalgae organisms
157 can be used to trap CO₂ 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 CO₂ removed from the raw biogas is typically discharged
165 into the atmosphere as a greenhouse gas (GHG), and most of these methods need preliminary H₂S
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 CO₂
168 reduction capacity. When microalgae are used for biogas upgrading, photosynthesis can convert
169 CO₂ present in raw biogas into biomass and oxygen. Currently, microalgae culturing for CO₂ bio-
170 fixation has gained considerable momentum due to its high photosynthetic rate that allows more
171 efficient CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 MCBioCH₄ (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 MCBioCH₄ 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 CO₂ 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/m³ 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 ³ /kg VS)	0.280	0.280
Secondary sludge SMP (Nm ³ /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 ₄ content in biogas (%)	62	62
CH ₄ 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 ₂ eq/kWh)	206	206
Emission factor for electricity substitution (Italian national grid) (gCO ₂ eq/kWh)	337	337

257

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

259 MCBioCH₄ (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 MCBioCH₄ 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 MCBioCH₄ 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 MCBioCH₄ 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 ₃	1,5
Dipotassium Hydrogen Phosphate	K ₂ HPO ₄	0,04
Magnesium Sulfate Heptahydrate	MgSO ₄ · 7H ₂ O	0,075
Calcium Chloride	CaCl ₂	0,036
Citric Acid	C ₆ H ₈ O ₇	0,006
Ferric Ammonium Citrate	C ₆ H ₁₁ FeNO ₇	0,006

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

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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 CO₂ 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.

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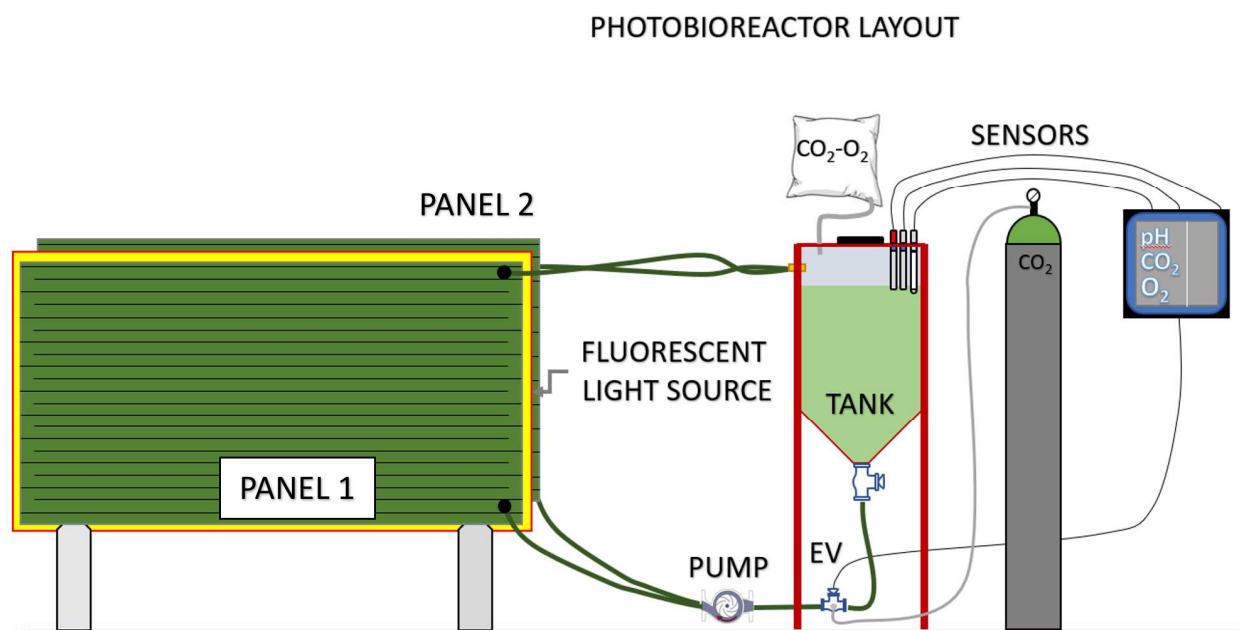
2.3.2. Experimental setup

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The presence of O₂ in the mixture can be hazardous due to flammability limits: in the case of CH₄, DIPPR tables report concentration values between 5.0 to 15.0 vol % determined at 298 K and 101,325 Pa. Higher temperatures and/or pressures will reduce the lower limit and raise the upper limit. However, the experiments (for safety reasons) are conducted using a pure source of CO₂. Closed photobioreactors are designed to have larger optical cross-sectional areas to receive natural or artificial light (Lee et al., 1995; Morita et al., 2000). Microalgae strains can be cultivated year-round in continuous or semi-continuous culture mode and can obtain high cell density per unit area or volume as well as high CO₂ fixation rate by using PBRs (Giordano et al., 2005; Wang et al., 2012). Closed PBRs have many advantages over open ponds, including 1) easier control of parameters that affect algae growth; 2) relatively stable culture conditions; 3) aseptic operation; 4) capability of high-density cultivation; 5) high area/volume ratio to increase mass transfer efficiency with less space occupation, which significantly improves CO₂ fixation efficiency; 6) ability for the natural (or artificial) light source to be collected and distributed to the interior of the bioreactor using a collector and optical fiber, to obtain much higher light utilization; and 7) avoided or reduced water evaporation (Chisti, 2007; Wang et al., 2012; Cheng et al., 2013). To this end, a custom photobioreactor (PBR) was constructed and implemented in this study. This microalgae growing system is subdivided into two main parts: a photo stage loop and a mixing tank (Fig. 1). The first one exploits the photosynthetic efficiency of microalgae to maximize CO₂ absorption from the inlet gas; the second one ensures culture mixing and gas separation.

The photo stage loop is composed of up to 5 neon lamps of 58 W each, interposed between two 1.5-m² parallel alveolar flat panels. These panels are partitioned into a series of internal rectangular channels in which, thanks to a 45 W high-efficiency pump, culture flows from the bottom to the top. After that, the culture enters the mixing tank. The CO₂ enters the system just before the pump, using a solenoid valve managed by electronic control. The automatic control is linked with pH or dissolved CO₂ values. This CO₂ diffusion system should assure a high gas-liquid mass transfer coefficient, and thus a better absorption of CO₂ from microalgae. The compact design of the pilot PBR guarantees optimal light utilization permitting high K values while taking up little volume, also allowing the scaling-up of the plant merely by increasing the number of these modules in parallel. Oxygen, dissolved CO₂, and pH probes are fixed on the plug of the first tank and connected to a Mettler-Toledo® multi-parameter transmitter. This device controls the solenoid valve for CO₂ injection, maintaining a pH level between 6.7 and 6.9. The upper part of the tank is sealed, and the gas released over time from the liquid surface is stored inside a 5L Tedlar bag. This bag is changed every day, and the stored gas analyzed with a GA-5000 gas analyzer to determine CO₂ 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- α
 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 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 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 CO₂ 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 CO₂ concentration in the liquid phase, the carbon source
398 obtained by CO₂ 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 CO₂ consumption [mg/l/s], $c_L(t)$ is the CO₂ concentration in the liquid
401 phase [mg/l], and t is time [s].

402 This shows a linear decrease of dissolved CO₂ concentration in the culture medium. After this first
403 step, when the linear trend stabilizes, CO₂ 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⁻¹], c_{∞}^* is the CO₂ concentration in the
406 liquid phase at $t=\infty$ [mg/l], c_L is the CO₂ concentration in the liquid phase at time t [mg/l], r is the
407 volumetric CO₂ consumption [mg/l/s], and t is time [s].

408 Concentration values are calculated by an InPro® 5000i CO₂ 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 CO₂ concentrations in the liquid between these two setups permit us to
413 evaluate run-off system efficiency.
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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 ¹	+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 ₄ loss from the process (t/y)	87.0	138.7	+59%
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446 [†] Considering a grid-to-final use efficiency of 0.9
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448 The total greenhouse gas balance provided by the environmental module of the MCBioCH₄ 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 CO₂ emission is expected to decrease from -0.278 t CO_{2eq}/t biogas
455 to -0.394 t CO_{2eq}/t biogas (from -3,182 t CO_{2eq}/y to -5,333 t CO_{2eq}/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⁻¹ · d⁻¹). Overall, the existing
459 sludge treatment line has a carbon footprint of --11.6 kg CO_{2eq} · PECOD-1 · y-1), corresponding to
460 -17,400 tCO_{2eq}/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_{2eq}/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_{2eq}/t of dry matter (DM) to 500
465 kgCO_{2eq}/t DM. If represented in the same unit, this study shows a range of -84 – -140 kgCO_{2eq}/t
466 DM.

467 **Table 4.** Environmental balance of sludge digestion scenarios simulated with the MCBioCH₄ model

Input parameter/value	Scenario 0		Scenario 1		Difference
	t CO _{2eq} /y	t CO _{2eq} /m ³ biogas y	t CO _{2eq} /y	t CO _{2eq} /m ³ biogas y	
Total CH ₄ loss from the process	2,437	0.213	3,883	0.287	+34%
Total CO ₂ 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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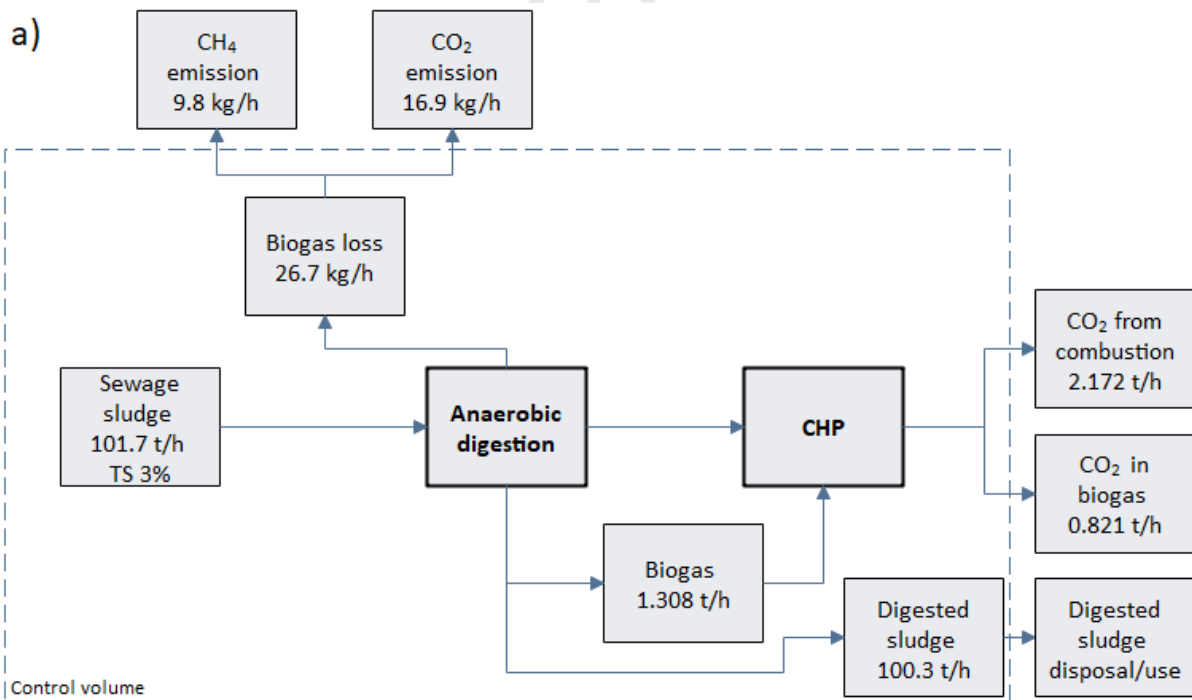
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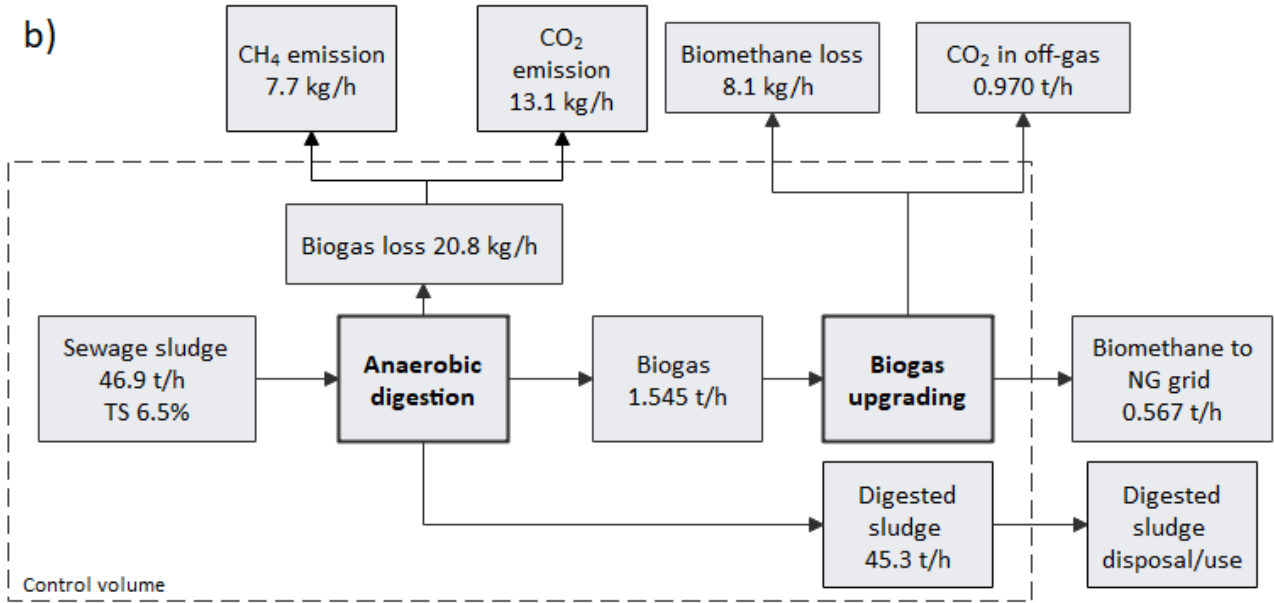
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The results reported herein also show that the production of biomethane would allow optimum exploitation of the energy contained in the sludge, as it would be directly introduced into the natural gas distribution grid. However, energy would not be 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.



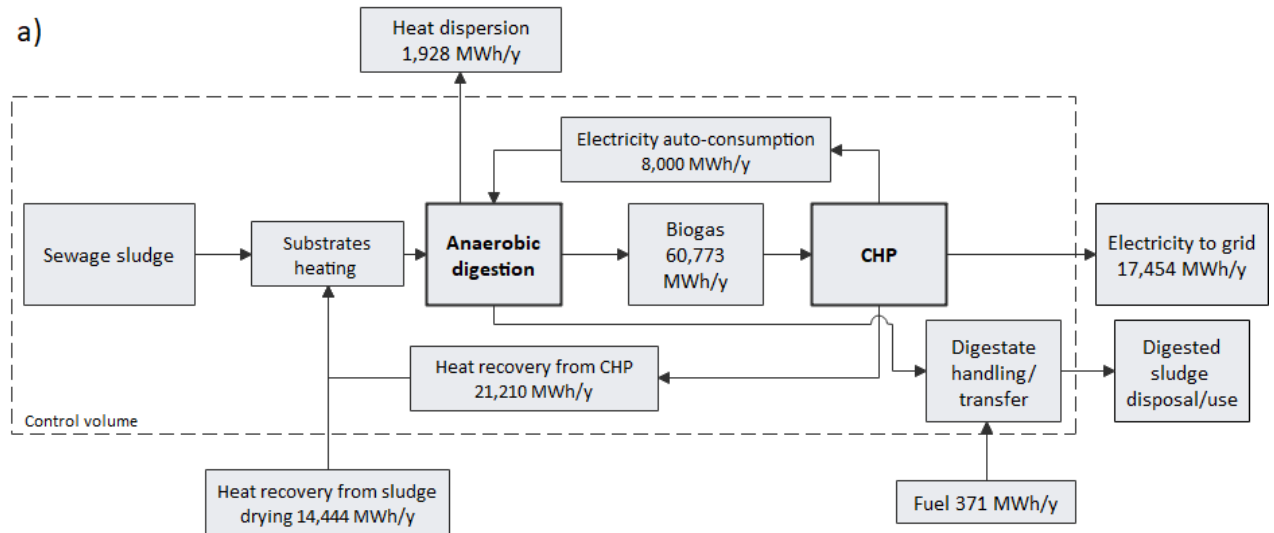
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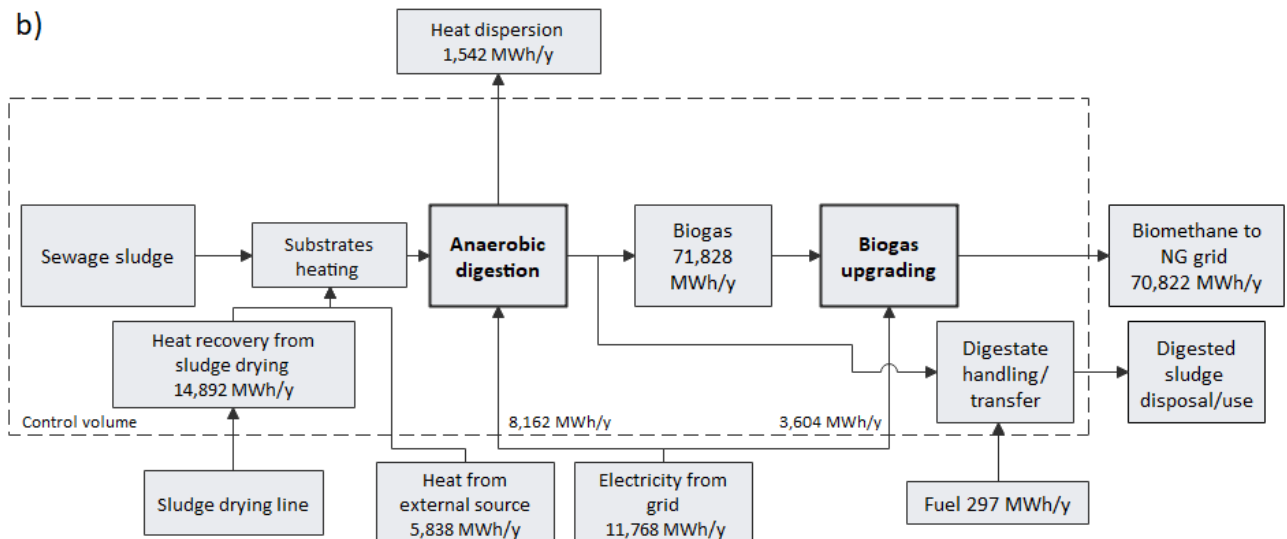


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Fig. 2. Mass balances of Scenario 0 (a) and Scenario 1 (b)



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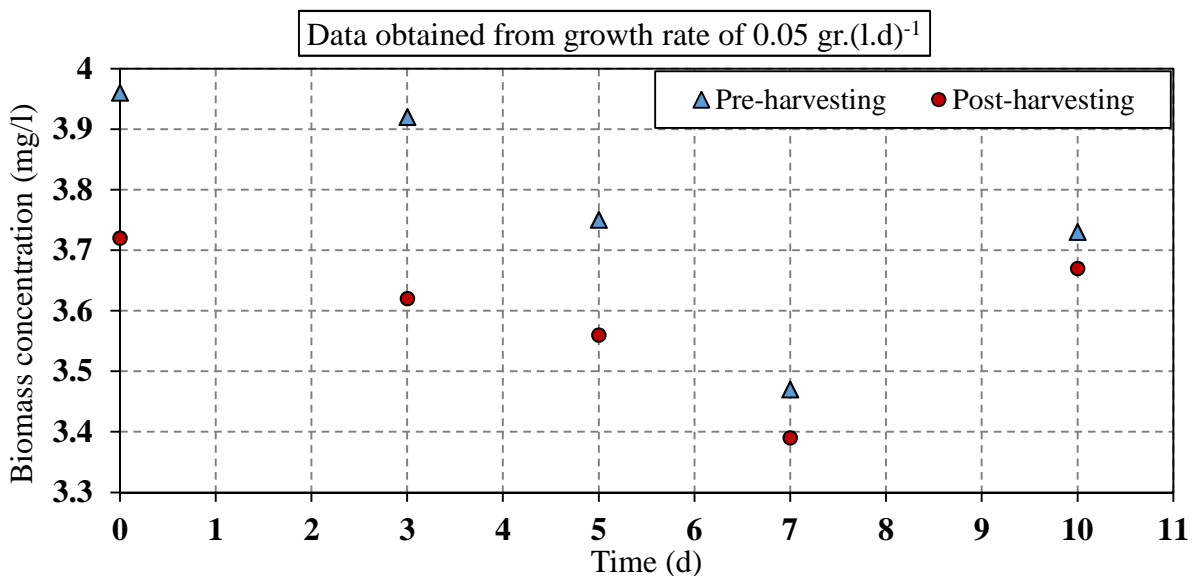
493 **Fig. 3.** Energy balances of Scenario 0 (a) and Scenario 1 (b)

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495 3.2 Application of microalgae CO₂ 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, CO₂, 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).

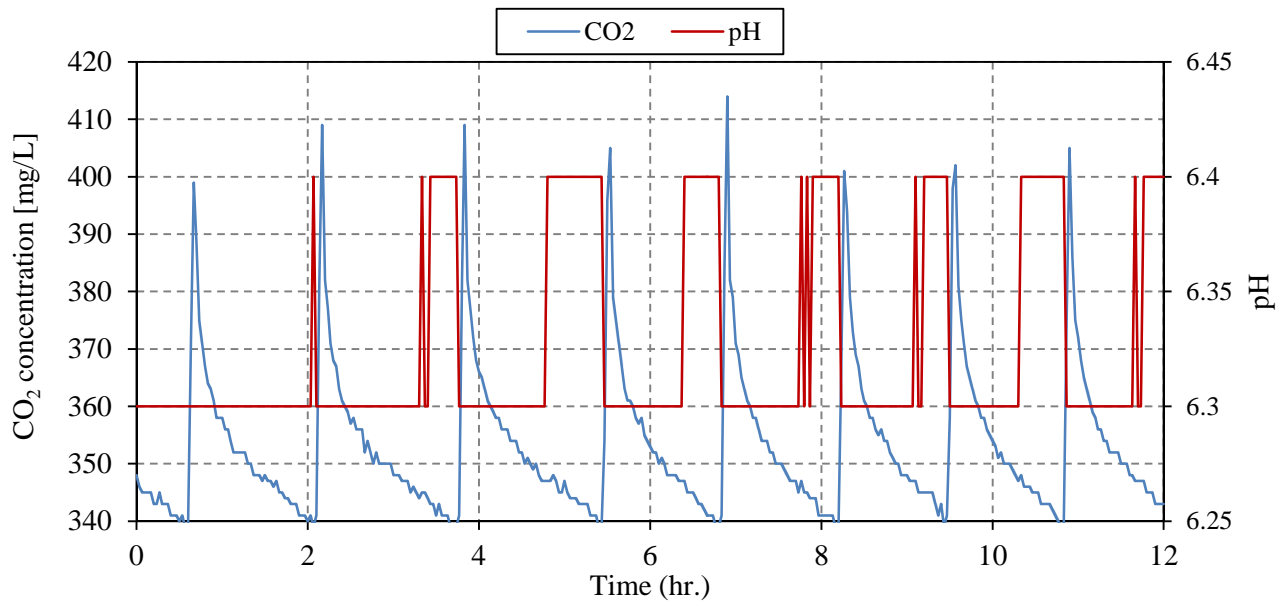


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514 **Fig. 4.** Growth trends during continuous operation in fed-batch feeding mode

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516 As for CO₂ regulation, two approaches have been used: indirect regulation of the pH level and
 517 direct control of the CO₂ concentration. Both showed high stability, but the direct method permits
 518 the maintenance of desired concentration values more accurately.



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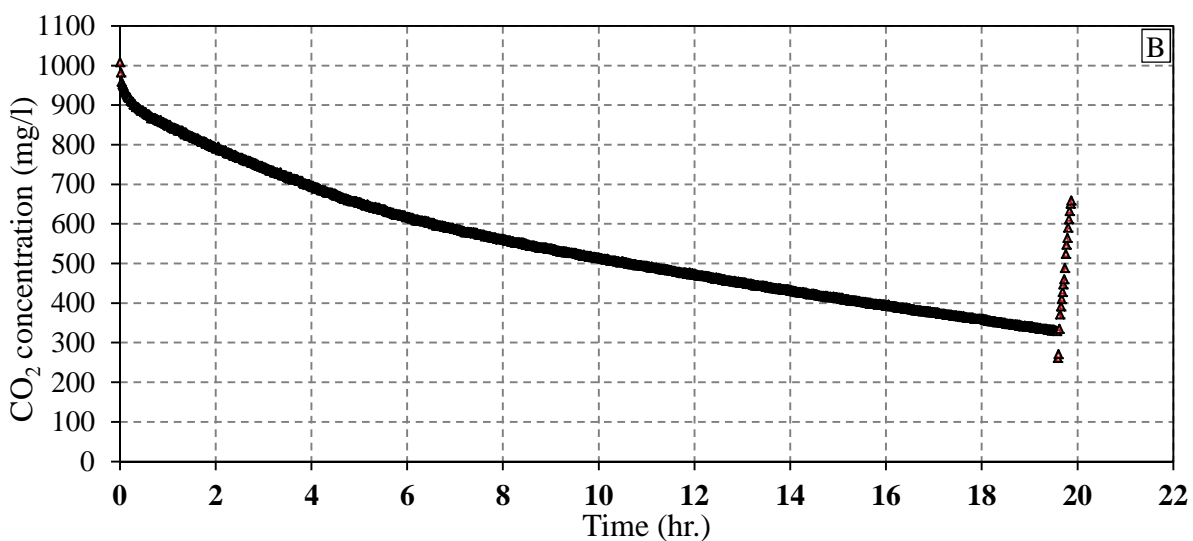
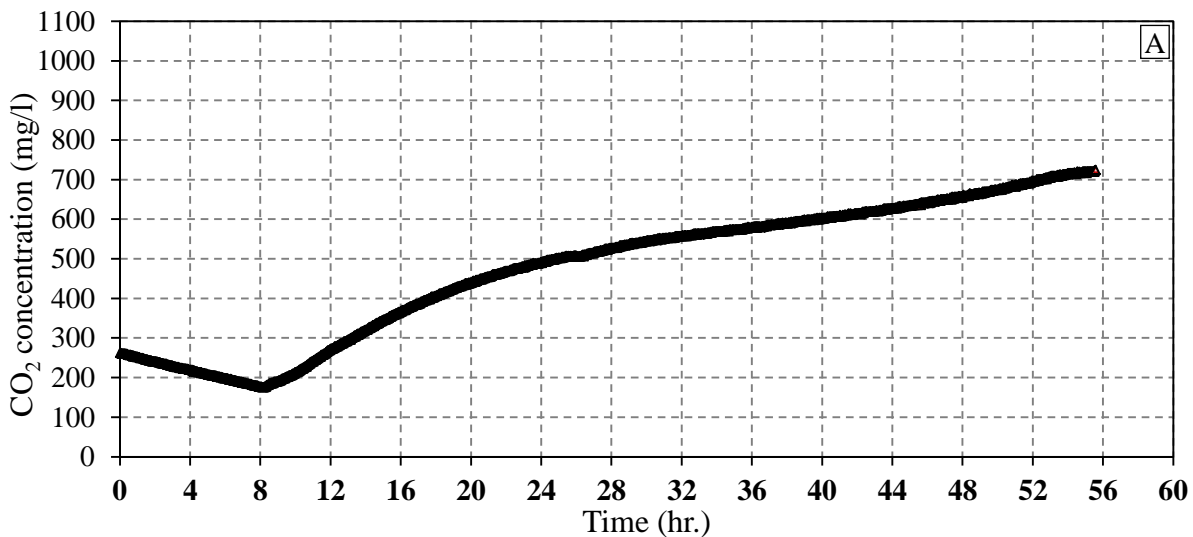
520 **Fig. 5.** CO₂ and pH trends with regulation based on the dissolved CO₂ values coming from the CO₂ sensors placed
 521 inside the microalgae culture

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523 Fig. 5 shows CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ consumption of 11.763
 534 mg/l/h and a K_{La} value of 0.5775 h⁻¹. The first, considering a light-exposed volume of 50 l,
 535 corresponds to 7.72 l_{CO2}/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_{CO2}/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 CO₂ 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 CO₂ reduction efficiency of the PBR system, the gas stored in the
556 Tedlar bags was analyzed with a gas analyzer. The total decrease of CO₂ 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 CO₂. 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 CO₂
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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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

617 **References**

- 618 Basu, S., Sarma Roy, A., Ghoshal, A.K., Mohanty, K., 2015. Operational strategies for maximizing
619 CO₂ utilization efficiency by the novel microalga *Scenedesmus obliquus* SA1 cultivated in
620 lab scale photobioreactor. *Algal Res.* 12, 249–257.
- 621 Borzooei, S., Campo, G., Cerutti, A., Meucci, L., Panepinto, D., Ravina, M., Riggio, V., Ruffino,
622 B., Scibilia, G., Zanetti, M., 2019. Optimization of the wastewater treatment plant: From
623 energy saving to environmental impact mitigation. *Science of The Total Environment* 691,
624 1182–1189. <https://doi.org/10.1016/j.scitotenv.2019.07.241>

- 625 Cheng, J., Huang, Y., Feng, J., Sun, J., Zhou, J., Cen, K., 2013. Improving CO₂ fixation efficiency
626 by optimizing *Chlorella* PY-ZU1 culture conditions in sequential bioreactors. *Bioresour*
627 *Technol* 144, 321–7.
- 628 Chisti, Y., 2007. Biodiesel from microalgae. *Biotechnol Adv* 25, 294–306.
- 629 De Morais, M.G., Vieira Costa J.A., 2007. Biofixation of carbon dioxide by *Spirulina* sp. and
630 *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *Journal of*
631 *Biotechnology* 129, 439-445
- 632 Franchino M., Comino E., Bona F., Riggio V.A., 2013. Growth of three microalgae strains and
633 nutrient removal from agro-zootechnical digestate. *Chemosphere* 92, 738-744
- 634 Genon, G., 1993. *Processi Biologici Industriali*. Torino: C.L.U.T
- 635 Giordano, M., Beardall, J., Raven, J.A., 2005. CO₂ concentrating mechanisms in algae:
636 mechanisms, environmental modulation, and evolution. *Annu Rev Plant Biol* 56, 99–131.
- 637 Ho S., Lu W., Chang J., 2012. Photobioreactor strategies for improving the CO₂ fixation efficiency
638 of indigenous *Scenedesmus obliquus* CNW-N: Statistical optimization of CO₂ feeding,
639 illumination, and operation mode. *Bioresource Technology* 105, 106-113
- 640 Ho S., Chen C., Chang J., 2012. Effect of light intensity and nitrogen starvation on CO₂ fixation
641 and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-
642 N. *Bioresource Technology* 113, 244-252
- 643 Houillon, G., Jolliet, O., 2005. Life cycle assessment of processes for the treatment of wastewater
644 urban sludge: energy and global warming analysis. *Journal of Cleaner Production* 13, 287–
645 299. <https://doi.org/10.1016/j.jclepro.2004.02.022>
- 646 Italian Ministry of Economic Development, Ministry of Environment, 2017. National Energy
647 Strategy. URL [https://www.mise.gov.it/images/stories/documenti/Testo-integrale-SEN-](https://www.mise.gov.it/images/stories/documenti/Testo-integrale-SEN-2017.pdf)
648 [2017.pdf](https://www.mise.gov.it/images/stories/documenti/Testo-integrale-SEN-2017.pdf) (accessed 3.24.20).
- 649 Kim, D., Bowen, J.D., Ozelkan, E.C., 2015. Optimization of wastewater treatment plant operation
650 for greenhouse gas mitigation. *Journal of Environmental Management* 163, 39–48.
651 <https://doi.org/10.1016/j.jenvman.2015.07.005>
- 652 Kiselev, A., Magaril, E., Magaril, R., Panepinto, D., Ravina, M., Zanetti, M.C., 2019. Towards
653 Circular Economy: Evaluation of Sewage Sludge Biogas Solutions. *Resources* 8, 91.
654 <https://doi.org/10.3390/resources8020091>
- 655 Lee, Y-K., Ding, S-Y, Low, C-S, Chang, Y-C, Forday, W.L., Chew, P-C, 1995. Design and
656 performance of an α -type tubular photobioreactor for mass cultivation of microalgae. *J Appl*
657 *Phycol* 7, 47–51.
- 658 Magaril, E., Magaril, R., Panepinto, D., Genon, G., Ravina, M., Trushkova, L., Chiara Zanetti, M.,
659 2017. Production and utilization of energy and climate adaptation: Global tasks and local
660 routes. *Int. J. SDP* 12, 1326–1337. <https://doi.org/10.2495/SDP-V12-N8-1326-1337>
- 661 Mathworks, n.d. Mathwors Inc. [WWW Document]. URL <https://mathworks.com/> (accessed
662 1.17.20).
- 663 Meier L., Barros P., Torres A., Vilchez C., Jeison D., 2017. Photosynthetic biogas upgrading using
664 microalgae: Effect of light/dark photoperiod. *Renewable Energy* 106, 17-23
- 665 Morita, M., Watanabe, Y., Saiki, H., 2000. Investigation of photobioreactor design for enhancing
666 the photosynthetic productivity of microalgae. *Biotechnol Bioeng* 69, 693–8.
- 667 Muñoz, R., Meier, L., Diaz, I., Jeison, D., 2015. A review on the state-of-the-art of
668 physical/chemical and biological technologies for biogas upgrading. *Rev Environ Sci*
669 *Biotechnol* 14, 727–759. <https://doi.org/10.1007/s11157-015-9379-1>
- 670 Nagarajan, D., Lee, D.-J., Chang, J.-S., 2019. Integration of anaerobic digestion and microalgal
671 cultivation for digestate bioremediation and biogas upgrading. *Bioresource Technology* 290,
672 121804. <https://doi.org/10.1016/j.biortech.2019.121804>
- 673 Paolini, V., Petracchini, F., Carnevale, M., Gallucci, F., Perilli, M., Esposito, G., Segreto, M.,
674 Occulti, L.G., Scaglione, D., Ianniello, A., Frattoni, M., 2018a. Characterisation and

- 675 cleaning of biogas from sewage sludge for biomethane production. *Journal of*
676 *Environmental Management* 217, 288–296. <https://doi.org/10.1016/j.jenvman.2018.03.113>
- 677 Paolini, V., Petracchini, F., Segreto, M., Tomassetti, L., Naja, N., Cecinato, A., 2018b.
678 Environmental impact of biogas: A short review of current knowledge. *Journal of*
679 *Environmental Science and Health, Part A* 53, 899–906.
680 <https://doi.org/10.1080/10934529.2018.1459076>
- 681 Prandini, J.M., Busi, S.M.L., Mezzari, M.P., Pirolli, M., Michelon, W., Moreira Soares, H., 2016.
682 Enhancement of nutrient removal from swine wastewater digestate coupled to biogas
683 purification by microalgae *Scenedesmus* spp. *Bioresour Technol.* 202, 67–75.
- 684 Ravina, M., Genon, G., 2015. Global and local emissions of a biogas plant considering the
685 production of biomethane as an alternative end-use solution. *Journal of Cleaner Production*
686 102, 115–126. <https://doi.org/10.1016/j.jclepro.2015.04.056>
- 687 Shuler, M., Kargi, F., 2002. *Bioprocess Engineering: Basic Concepts*. Prentice Hall.
- 688 Ravina, M., Castellana, C., Panepinto, D., Zanetti, M.C., 2019. MCBioCH4: A computational
689 model for biogas and biomethane evaluation. *Journal of Cleaner Production* 227, 739–747.
690 <https://doi.org/10.1016/j.jclepro.2019.04.224>
- 691 Remy, C., Lesjean, B., Waschnewski, J., 2013. Identifying energy and carbon footprint optimization
692 potentials of a sludge treatment line with Life Cycle Assessment. *Water Science and*
693 *Technology* 67, 63–73. <https://doi.org/10.2166/wst.2012.529>
- 694 Richmond, A., 2000. Microalgal biotechnology at the turn of the millennium: a personal view. *J*
695 *Appl Phycol* 12:441–451
- 696 Tang D., Han W., Li P., Miao X., Zhong J., 2011. CO₂ biofixation and fatty acid composition of
697 *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO₂ levels.
698 *Bioresource Technology* 102, 3071–3076
- 699 Thiansathit, W., Keener, T.C., Khang, S.J., Ratpukdi, T., Hovichitr, P., 2015. The kinetics of
700 *Scenedesmus obliquus* microalgae growth utilizing carbon dioxide gas from biogas.
701 *Biomass Bioenergy* 76, 79–85.
- 702 Ullah Khan, I., Hafiz Dzarfan Othman, M., Hashim, H., Matsuura, T., Ismail, A.F., Rezaei-
703 DashtArzhandi, M., Wan Azelee, I., 2017. Biogas as a renewable energy fuel – A review of
704 biogas upgrading, utilisation and storage. *Energy Conversion and Management* 150, 277–
705 294. <https://doi.org/10.1016/j.enconman.2017.08.035>
- 706 Wang, B., Lan, C.Q., Horsman, M., 2012. Closed photobioreactors for production of microalgal
707 biomasses. *Biotechnol Adv* 30, 904–12.
- 708 Zabed, H.M., Akter, S., Yun, J., Zhang, G., Zhang, Y., Qi, X., 2020. Biogas from microalgae:
709 Technologies, challenges and opportunities. *Renewable and Sustainable Energy Reviews*
710 117, 109503. <https://doi.org/10.1016/j.rser.2019.109503>
- 711

- 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 CO₂ fixation
- Experimental setup is proposed to evaluate the K_{La} of CO₂ in the microalgae system

Journal Pre-proof

Sina Borzooei Methodology, Software, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, **Giuseppe Campo** Methodology, Software, Formal analysis, Investigation, Resources, Data Curation, **Alberto Cerutti** Methodology, Formal analysis, Investigation, Resources, Data Curation, **Lorenza Meucci** Conceptualization, Methodology, Validation, Data Curation, Supervision, Project administration, Funding acquisition, **Deborah Panepinto** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, **Marco Ravina** Methodology, Software, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, **Vincenzo Riggio** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, **Barbara Ruffino** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data Curation, Supervision, **Gerardo Scibilia** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, **Mariachiara Zanetti** Conceptualization, Methodology, Validation, Resources, Supervision, Project administration, Funding acquisition.

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: