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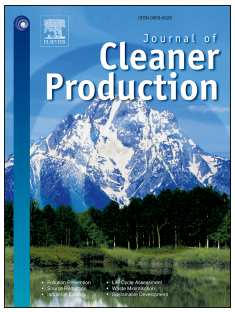
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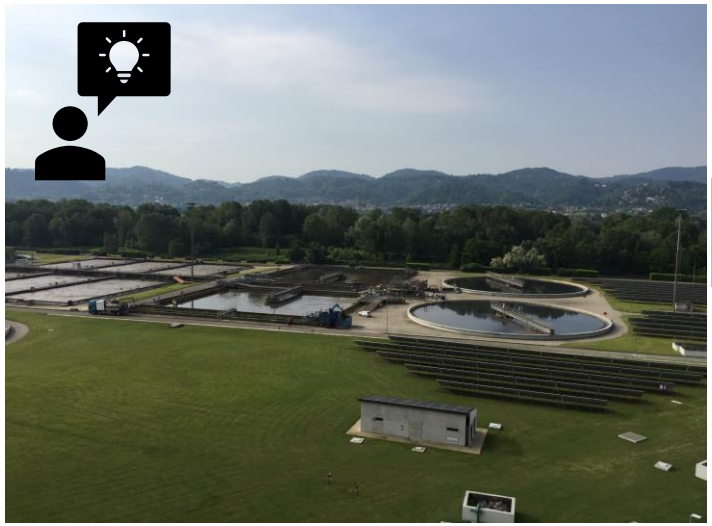
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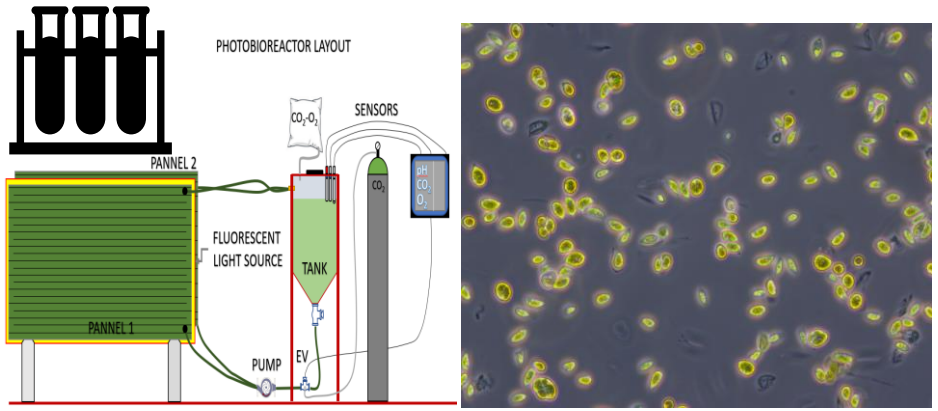
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# Microalgae $CO_2$ fixation

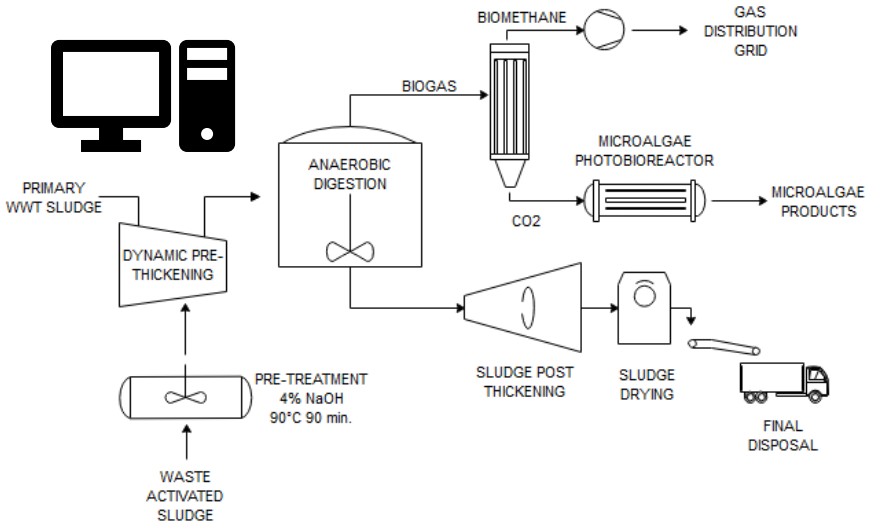
## Feasibility analysis








## MCBioCH4 model




## Reduction



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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 µmol/$m^2$/s light intensities, the results show 80% and 70% reductions in total $CO_2$, respectively. The tested configuration guaranteed 11.763 and 27.943 mg/l/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

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

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

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

The management of the off-gas produced by the biogas upgrading process also represents an open issue for plant operators. This off-gas mainly consists of the $CO_2$ initially contained in the biogas stream, with a minor amount of $CH_4$ that has not been recovered in the process. Some additional minor components, such as $H_2S$ and siloxanes, may also be present (Paolini et al., 2018b). Presently, operators of a biomethane plant are usually allowed to discharge off-gas into the atmosphere, up to the limits imposed by regulations. In this regard, an increasing interest is being shown in innovative technologies to recover the $CO_2$ contained in the biomethane off-gas. Among these, the use of microalgae as a biofilter for $CO_2$ is most promising. These microalgae organisms can be used to trap $CO_2$ coming from the exhaust gases, as they require carbon dioxide to perform the photosynthesis process. As a secondary benefit, microalgae can be used for the production of bioproducts. Although microalgae methods perform reasonably well, they are usually considered

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

Furthermore, using these techniques, the $CO_2$ removed from the raw biogas is typically discharged into the atmosphere as a greenhouse gas (GHG), and most of these methods need preliminary $H_2S$ removal. To overcome all these limitations, recent studies (Nagarajan et al., 2019; Zabed et al., 2020) have considered the use of microalgae to upgrade biogas, thanks to their photosynthetic $CO_2$ reduction capacity. When microalgae are used for biogas upgrading, photosynthesis can convert $CO_2$ present in raw biogas into biomass and oxygen. Currently, microalgae culturing for $CO_2$ bio-fixation has gained considerable momentum due to its high photosynthetic rate that allows more efficient $CO_2$ bio-fixation than terrestrial plants. Although the potential of microalgae to contribute to services and commodities demand across the world is high, it is still necessary to eliminate a large number of bottlenecks related to its biological, engineering, and economic aspects (Richmond, 2000).

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

Optimization of the anaerobic digestion of sewage sludge is considered a worthwhile strategy because its advantage lies not only in cost savings but also in mitigating the environmental concerns posed by GHG emissions (Kim et al., 2015). The greatest challenge for the pre-treatment of biogas substrates is combining the right substrate composition with the right pre-treatment technology to increase the bioavailability of the substrate. Although this represents an open and extended research topic, few studies have focused on the comparative evaluation of the possible alternatives in terms of GHG emissions. Besides, considering the general GHG reduction policies and guidelines, the feasibility of optimization interventions must be evaluated together with $CO_2$ sequestration technologies.

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In this study, mass, energy, and GHG balances of the sludge treatment section of the WWTP were analyzed, considering the energy optimization options elaborated in the study of Borzooei et al. (2019). The analysis started by focusing on the energy valorization of sewage sludge through anaerobic digestion. In this first stage, biomethane production as an alternative to on-site biogas combustion was evaluated, considering conventional upgrading technologies. In the second stage, the potential reduction of the $CO_2$ emitted via the off-gas was analyzed, considering microalgae bio-fixation technology. An experimental planar photobioreactor was used to evaluate the possibility of using microalgae to absorb the $CO_2$ in the off-gas coming from a WWTP. The final goal of the study was to provide relevant information toward the definition of the most environmentally friendly and energy-efficient integrated management scheme of WWTPs.

Greenhouse gas flow accounting of the entire sewage sludge treatment line was performed with the screening model MCBioCH4 (acronym of the bio-methane computational model), developed by the authors (Ravina et al., 2019). In the framework of energy recovery optimization of sewage sludge management processes, the application of MCBioCH4 aims at a triple target: i) estimating the productivity of biogas/biomethane in terms of achievable gas flow rates; ii) re-defining the anaerobic digestion section of the plant given the selected options; and iii) accounting for the whole environmental impact of the system on a cradle-to-grave basis, considering biogas/biomethane as an alternative energy source to fossil fuels. Also, using a planar photobioreactor custom-made by the research team specifically for this study allowed us to perform different experiments characterized by measuring the mass transfer coefficient and $CO_2$ consumption inside the reactor under two different artificial-light scenarios.

## 2. Materials and Methods

### 2.1 Case study definition

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

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

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

**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 |

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| | | |
|---|---|---|
| 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 ($gCO_2eq/kWh$) | 206 | 206 |
| Emission factor for electricity substitution (Italian national grid) ($gCO_2eq/kWh$) | 337 | 337 |

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

MCBioCH4 (acronym of the bio-methane computational model) is a standalone application modeling mass, energy, and environmental balances of biogas/biomethane production plants on a cradle-to-grave basis, i.e., from substrates production to biogas/biomethane end-use. The design of MCBioCH4 was explicitly addressed to support the preliminary evaluation of alternative plant configurations and technological options. In this model, default datasets and assisted input definitions were implemented in such a way as to help users in the interpretation of mass, energy, and environmental balances.

The code was developed as a standalone application based on the MATLAB® software (Mathworks, n.d.), and is provided with a user-friendly graphical users interface (GUI). Three different modules were implemented in MCBioCH4 for the calculation of mass, energy, and GHG balance, respectively. Users can simulate four different options for biogas/biomethane energy conversion:

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

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

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

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

MCBioCH4 is well structured with simple and clear dialog boxes to facilitate interaction with low-expertise users. As crucial information for starting, the user is asked to input the daily mass flow of substrates to be inserted into the digester. Other input parameters can either be provided as default values or be specified by the user. The following sets of output can be obtained from the model:

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

## 2.3 Microalgae experimental setup

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

### 2.3.1 Microalgae preparation and culture medium

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

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

| **BG-11 medium** | | |
|---|---|---|
| COMPOUND | MOLECULAR FORMULA | CONCENTRATION [g/l] |
| Sodium Nitrate | $NaNO_3$ | 1,5 |
| Dipotassium Hydrogen Phosphate | $K_2HPO_4$ | 0,04 |
| Magnesium Sulfate Heptahydrate | $MgSO_4 \cdot 7H_2O$ | 0,075 |
| Calcium Chloride | $CaCl_2$ | 0,036 |
| Citric Acid | $C_6H_8O_7$ | 0,006 |
| Ferric Ammonium Citrate | $C_6H_{11}FeNO_7$ | 0,006 |

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| | | |
|---|---|---|
| $Na_2EDTA$ | $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ | 0,001 |
| Sodium carbonate | $Na_2CO_3$ | 0,02 |
| Boric Acid | $H_3BO_3$ | $2,86 \cdot 10^{-3}$ |
| Manganese Chloride Tetrahydrate | $MnCl_2 \cdot 4H_2O$ | $1,81 \cdot 10^{-3}$ |
| Zinc Sulfate Heptahydrate | $ZnSO_4 \cdot 7H_2O$ | $0,222 \cdot 10^{-3}$ |
| Molibdenum Sodium Oxide | $MoNa_2O_4 \cdot 4H_2O$ | $0,39 \cdot 10^{-3}$ |
| Copper Sulfate Pentahydrate | $CuSO4 \cdot 5H_2O$ | $0,079 \cdot 10^{-3}$ |
| Cobalt Nitrate Hexahydrate | $Co(NO3)2 \cdot 6H_2O$ | $0,049 \cdot 10^{-3}$ |

Strain banks usually send slant cultures. It is suggested to let the cultures grow in light conditions at 20 - 25°C until micro-organisms cover the entire inclined surface of the agar. This process can take several weeks. Subsequently, microalgae are scraped from the surface of the agar and inoculated in a 400-ml glass bottle containing 100 ml of BG-11 solution. This bottle is placed on an orbital shaker to prevent sedimentation, and fluorescent lamps illuminate it. After two weeks, the strain volume is doubled, and an air sparging system is installed, modifying the bottle's plug. This system consists of a small air compressor connected through a plastic tube and a filter to an immersed micro-bubble diffuser that is placed inside the bottle.The plug has two holes: one for the inlet tube, one for the gas exit tube. A week later, algae have spent almost all nutrients present in the solution, so the culture volume is doubled again, reaching the maximum available capacity of the bottle. After this growth period, algae are centrifuged (4,000 rpm for 5 minutes) and re-suspended in 6 bottles containing 400 ml of BG-11 solution each. The total volume of culture is now equal to 2.4 l, enough to proceed, after the required growth period, to the column inoculum. The column consists of a vertical polycarbonate tube measuring 20 cm in diameter, 120 cm in height, with a total capacity of 28 l. This reactor is illuminated by four vertical fluorescent lamps radially disposed of. $CO_2$ can be supplied in the form of air by a compressor or in pure form by a gas cylinder. Carbon dioxide flowrate is manually regulated according to optimal pH levels, with a maximum value of 2 l/min. To enhance gas diffusion in the liquid phase, it is sparged through 4 micro-bubble diffusers fixed on the bottom of the column. Two plastic channels are disposed above the diffusers to enhance convective motions and thus mixing. This method forces gas bubbles to mix with liquid and go up inside the channels placed in the center of the column while the rest of the culture turns back down externally. Five of six bottles with a useful volume of 400 ml are used to inoculate the column, and the remaining one is centrifuged and re-suspended in 6 new bottles of the same capacity (400 ml). After a couple of weeks, the biomass concentration of the culture inside the column is sufficient to permit the inoculum inside the planar photobioreactor to be used for this study, in an initial configuration having a capacity of 100 l.

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### 2.3.2. Experimental setup

The presence of $O_2$ in the mixture can be hazardous due to flammability limits: in the case of $CH_4$, 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 $CO_2$. 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 $CO_2$ 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 $CO_2$ 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 $CO_2$ 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-$m^2$ 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 $CO_2$ enters the system just before the pump, using a solenoid valve managed by electronic control. The automatic control is linked with pH or dissolved $CO_2$ values. This $CO_2$ diffusion system should assure a high gas-liquid mass transfer coefficient, and thus a better absorption of $CO_2$ 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 $CO_2$, 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 $CO_2$ 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 $CO_2$ presence.

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

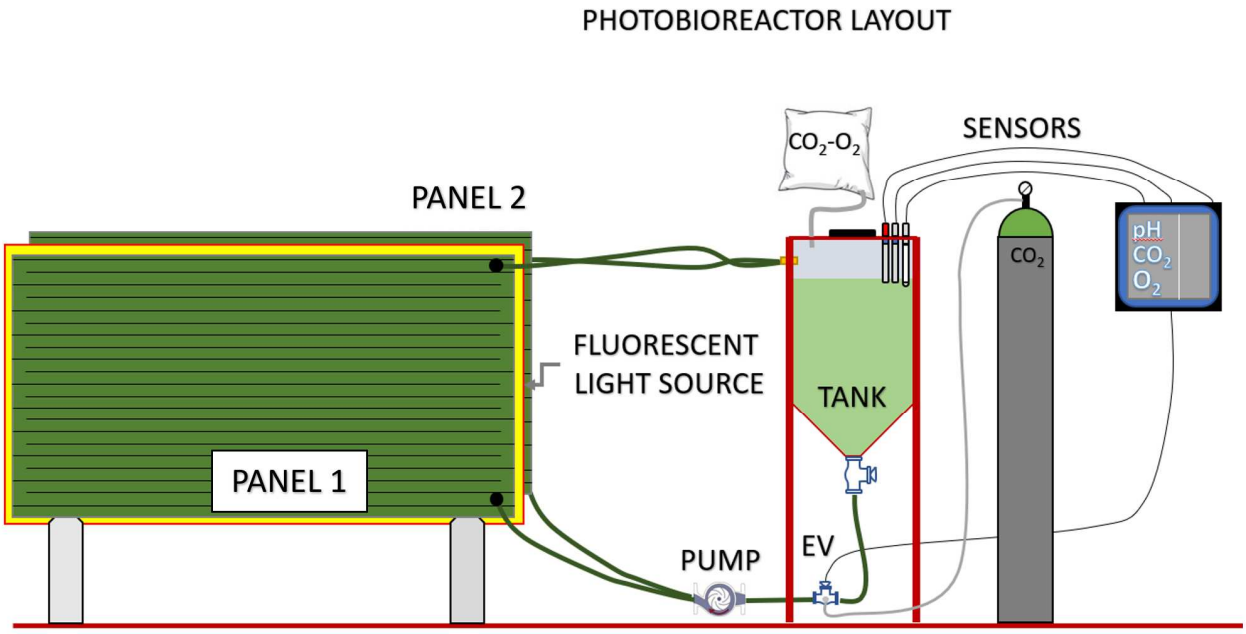



**Fig. 1.** Photobioreactor layout with the indication of main components

### 2.3.3. Data processing

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

The global gas-liquid mass transfer coefficient for carbon dioxide $K_{La}(CO_2)$ is measured by adjusting the unsteady-state method for aerobic cultures of microorganisms proposed by Genon (1993). This modified method can be applied to reactors containing living cultures of photosynthetic organisms and permit the measuring of the $K_{La}$ value as well as culture $CO_2$ consumption. The last value is significant: it reveals the real performances and efficiencies of the system. It depends on irradiance (and consequently on emission spectrum) and biomass concentration inside the culture g/l. Volumetric $CO_2$ consumption can be defined as:

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$$r = \frac{G_{gas,in}\, x_{CO2,in} - G_{gas,out}\, x_{CO2,out}}{V} \qquad [1]$$

where $r$ is the volumetric $CO_2$ consumption [mg/l/s], $G_{gas,in}$ and $G_{gas,out}$ are the gas flowrates at the inlet and the outlet [mg/s], respectively, $x_{CO2,in}$ and $x_{CO2,out}$ are the mass fractions of inlet and outlet gas flows [-], respectively, and V is the illuminated volume of culture [l].

Starting from the regime conditions of $CO_2$ concentration in the liquid phase, the carbon source obtained by $CO_2$ injection is interrupted. In this way, the culture is constrained to consume the carbon dioxide dissolved in liquid. The following equation can describe this process

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

where $r$ is the volumetric $CO_2$ consumption [mg/l/s], $c_L(t)$ is the $CO_2$ concentration in the liquid phase [mg/l], and $t$ is time [s].

This shows a linear decrease of dissolved $CO_2$ concentration in the culture medium. After this first step, when the linear trend stabilizes, $CO_2$ injection starts again until regime conditions are reached. The equation below can describe this situation:

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

where $k_{La}$ is the global gas-liquid mass transfer coefficient [$h^{-1}$], $c_\infty^*$ is the $CO_2$ concentration in the liquid phase at t=∞ [mg/l], $c_L$ is the $CO_2$ concentration in the liquid phase at time t [mg/l], $r$ is the volumetric $CO_2$ consumption [mg/l/s], and $t$ is time [s].

Concentration values are calculated by an InPro® 5000i $CO_2$ probe connected to a Mettler Toledo® M-800 multi-parameter transmitter and recorded by a Kobold® electronic multi-channel data logger. The probe is placed both in the collection container of the tank's plug (only one tank will be used for these first experiments) and in the lower part of the tank, near the pump's inlet tube. In this way, different values of $CO_2$ concentrations in the liquid between these two setups permit us to evaluate run-off system efficiency.

## 3. Results and discussion

### 3.1 Application of the MCBioCH4 model

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

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

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

**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% |

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| | | | |
|---|---|---|---|
| Total $CH_4$ loss from the process (t/y) | 87.0 | 138.7 | +59% |

[1] *Considering a grid-to-final use efficiency of 0.9*

The total greenhouse gas balance provided by the environmental module of the MCBioCH4 model is reported in Table 4. This table compares the simulated scenarios in terms of GHG emissions. The results show that both the present and the alternative configurations have favorable balances, meaning that avoided emissions for the substitution of natural gas and electricity are higher than the emissions produced for process maintenance. The introduction of sludge pre-treatment and the advanced thickening stage (Scenario 1) are expected to improve the general environmental balance of the plant. Specific Equivalent $CO_2$ emission is expected to decrease from -0.278 t $CO_{2eq}$/t biogas 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 in a lower GHG impact. Among previous studies, Remy et al., (2013) calculated the GHG balance of different options of a sludge treatment process in a large WWTP in Berlin (1.5 million of population equivalents, PE, assuming a mean COD load of 120 g · $PE^{-1}$ · $d^{-1}$). Overall, the existing sludge treatment line has a carbon footprint of --11.6 kg $CO_2$eq · PECOD-1 · y-1), corresponding to -17,400 t$CO_2$eq/y. However, unlike in the present study, the final sludge disposal options were considered. Without considering sludge disposal ways, the GHG balance yields a value of -6,900 t$CO_{2eq}$/y. Another study by Houillon and Jolliet (2005) considered six wastewater sludge treatment scenarios applied to a 300,000 PE WWTP. The results showed that, depending on the process and sludge management, the GHG balance could shift from -100 kg$CO_{2eq}$/t of dry matter (DM) to 500 kg$CO_{2eq}$/t DM. If represented in the same unit, this study shows a range of -84 – -140 kg$CO_{2eq}$/t DM.

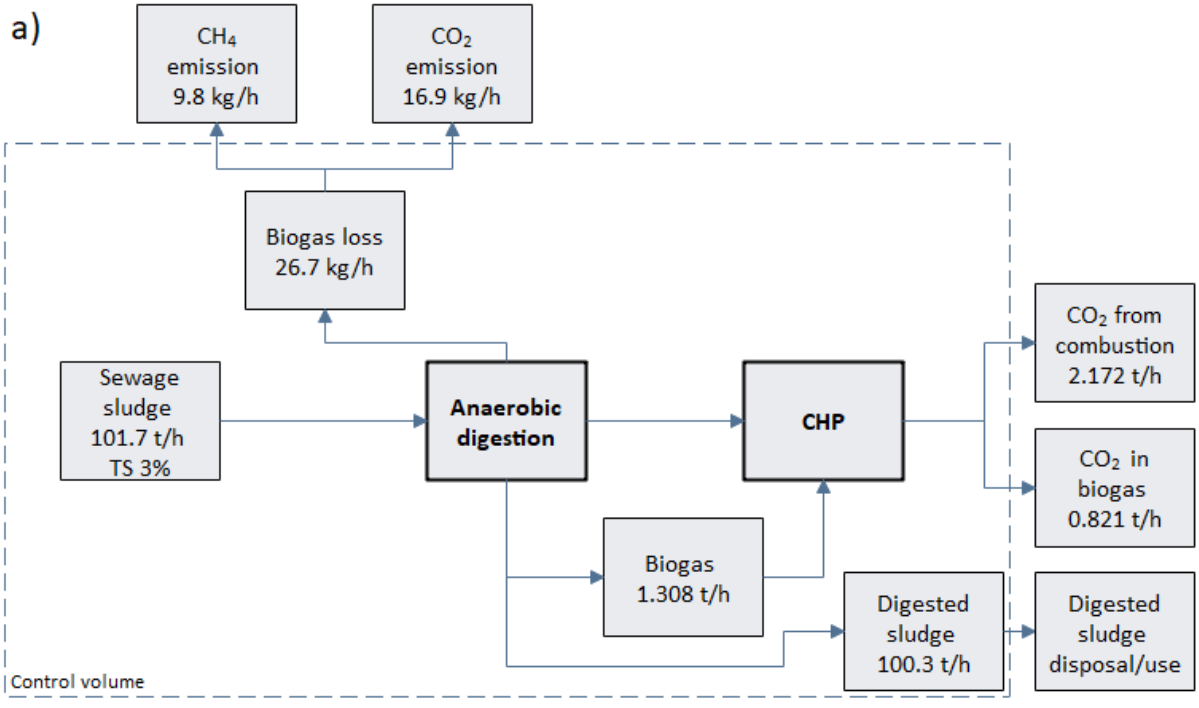
**Table 4.** Environmental balance of sludge digestion scenarios simulated with the MCBioCH4 model

| **Input parameter/value** | **Scenario 0** | | **Scenario 1** | | **Difference** |
|---|---|---|---|---|---|
| | *t $CO2_{eq}$/y* | *t $CO2_{eq}$/$m^3$ biogas y* | *t $CO2_{eq}$/y* | *t $CO2_{eq}$/$m^3$ 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% |

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| | | | | | |
|---|---|---|---|---|---|
| GHG emission balance | -3,182 | -0.278 | -5,333 | -0.394 | -41% |

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.



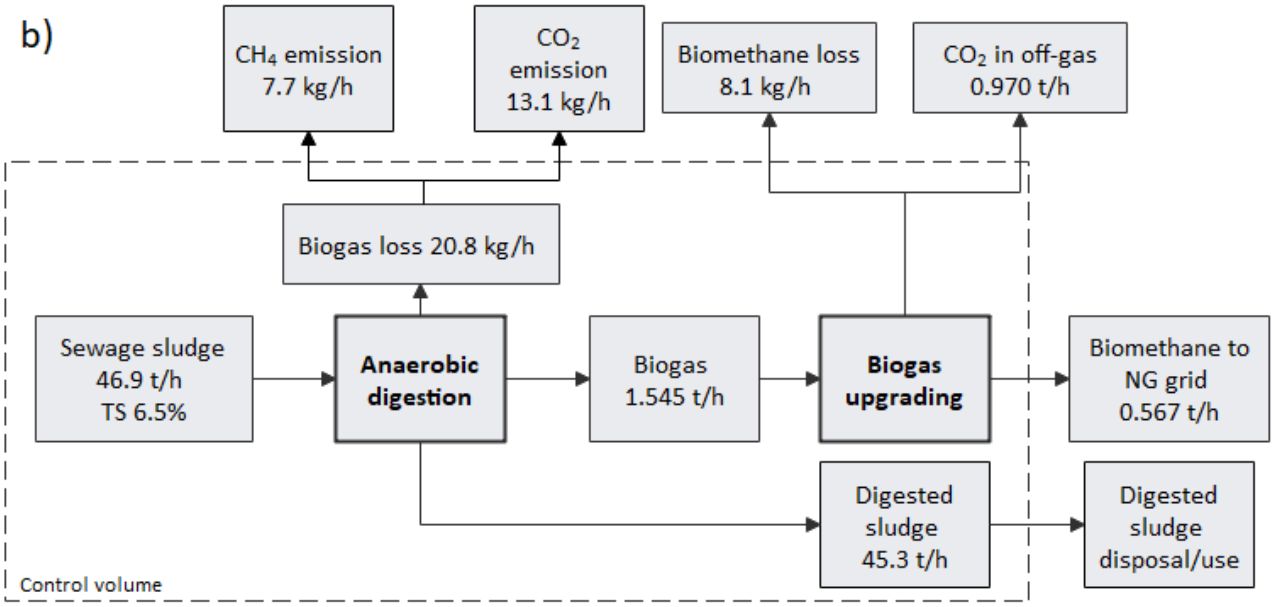
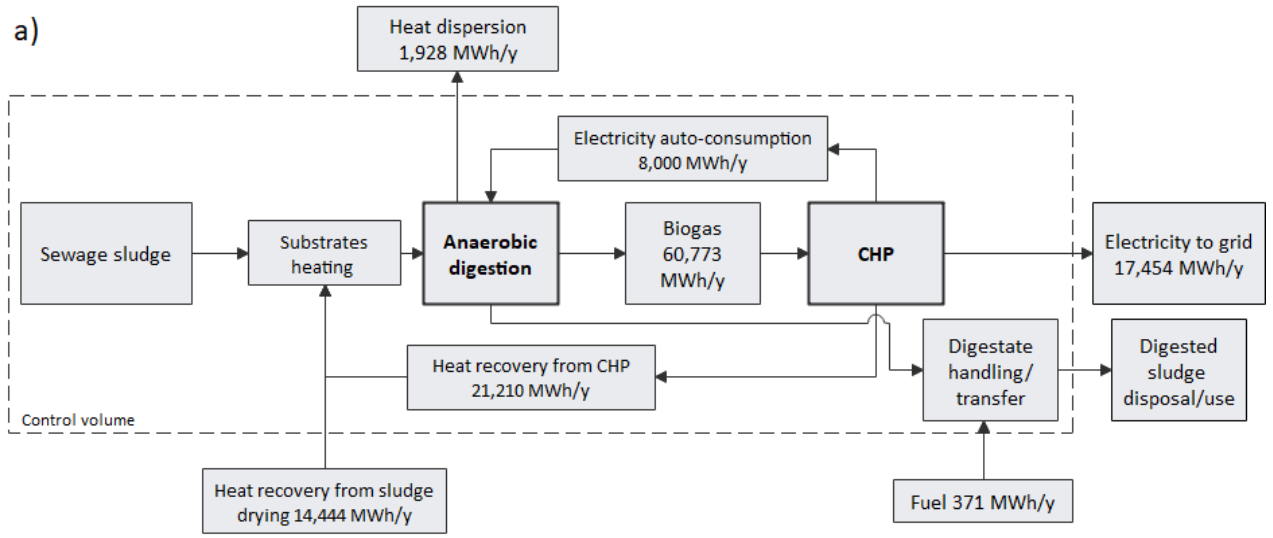
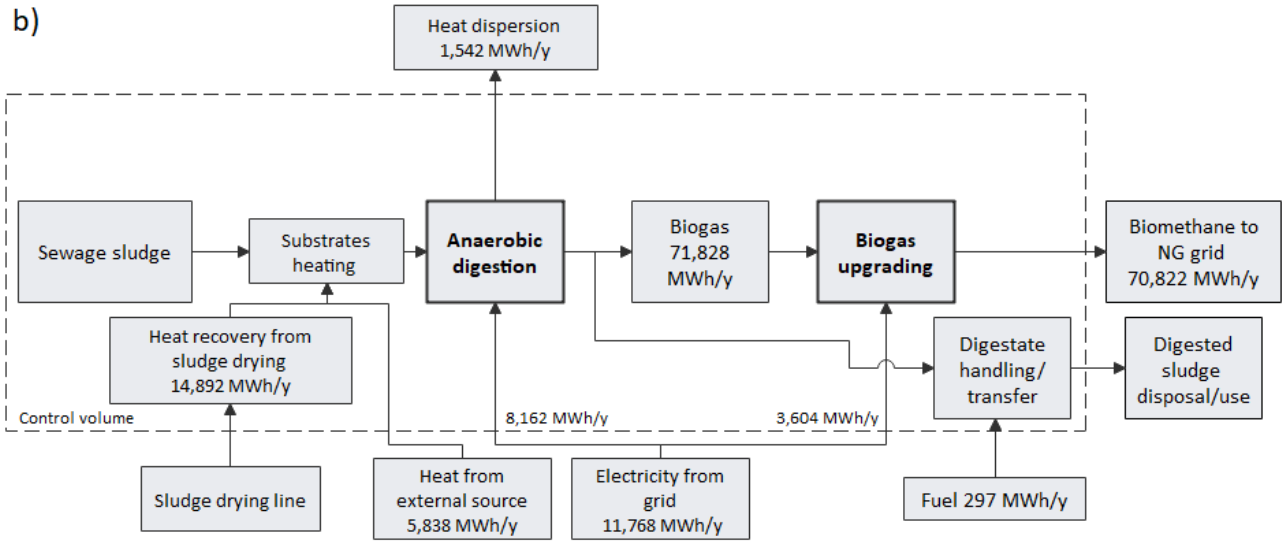



**Fig. 2.** Mass balances of Scenario 0 (a) and Scenario 1 (b)



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

### 3.2 Application of microalgae $CO_2$ fixation

Microalgae growth was tested using the already described PBR system with a total volume maintained at 100 L. During the growth stage; biomass concentration is measured. The illumination system is composed of 5 equally spaced fluorescent lamps placed between the two panels. This light source can supply around 76 μmol/$m^2$s. Growth curves of this first phase show increasing values for a period lasting about 30 days, after which, without the addition of nutrients, the strain reaches its concentration asymptote. This value can vary depending on growing conditions like illuminance, pH, temperature, $CO_2$, and nutrient concentrations. If a shortage of nutrients persists, biomass concentration starts to decrease rapidly, as the last part of the curve shows. As previously noted, the mean biomass productivity calculated is equal to 0.06 g/l day.

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

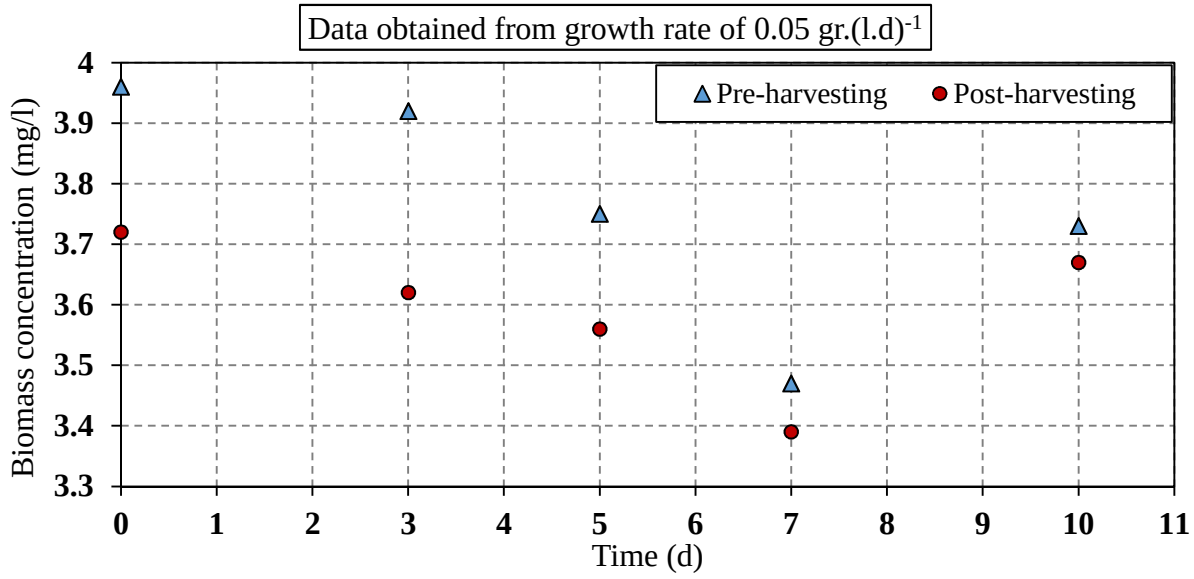



**Fig. 4.** Growth trends during continuous operation in fed-batch feeding mode

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As for $CO_2$ regulation, two approaches have been used: indirect regulation of the pH level and direct control of the $CO_2$ concentration. Both showed high stability, but the direct method permits the maintenance of desired concentration values more accurately.

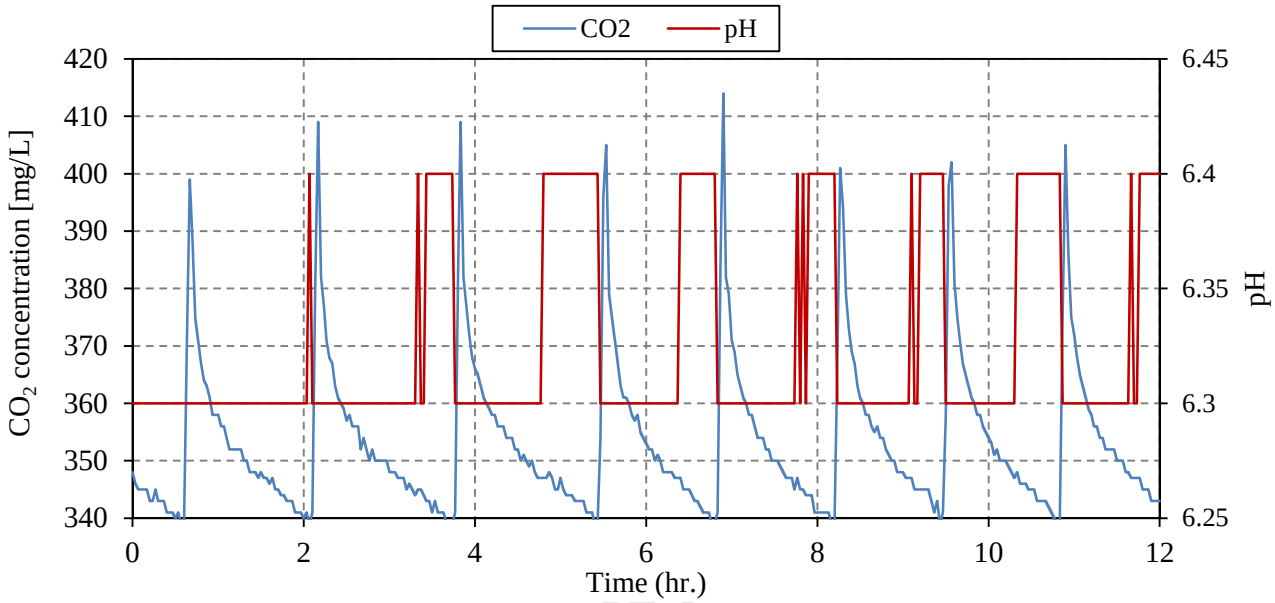



**Fig. 5.** $CO_2$ and pH trends with regulation based on the dissolved $CO_2$ values coming from the $CO_2$ sensors placed inside the microalgae culture

Fig. 5 shows $CO_2$ and pH trends with this type of regulation. The data collected from the respirometry tests are visible in Fig. 6, and they present two trends: the first one, a descending phase, indicates the respirometry of the system; the second one, showing an ascending pattern, is strictly related to the evaluation of $K_{La}$ as described in the materials and methods paragraph. The angular coefficient obtained from the descending phase of the graph is the value of r, which is equal to the volumetric consumption of $CO_2$ caused by the microalgae. The data collected during the ascending phase were used to evaluate the $K_{La}$ using Equation 3. The trend of this curve is directly related to the $CO_2$ input flow rate and the ability of the system to transfer the gas phase into a liquid one. Trials conducted for $K_La$ and $CO_2$ consumption were performed as previously explained using two illumination configurations: 3 and 5 fluorescent lamps. The first test (3 fluorescent lamps and biomass concentration around 3.93 g/l) reported the following values: a $CO_2$ consumption of 11.763 mg/l/h and a $K_La$ value of 0.5775 $h^{-1}$. The first, considering a light-exposed volume of 50 l, corresponds to 7.72 $l_{CO2}$/day under normal conditions. The fixation rate may also give information about the microalgae's growth, knowing their approximate molecular formula. $K_La$, tested by injecting 0.5 $L_{CO2}$/m, returned lower values than expected: this result can be interpreted as a prompt response of the system to variations in the liquid's $CO_2$ concentration due to an essential presence of microorganisms. This means high carbon dioxide utilization and hence low dispersion in the

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

The second test (5 fluorescent lamps and biomass concentration of around 4.5 g/l) reported a $CO_2$ consumption of 27.943 mg/l/h and a $K_La$ value of 17.7 $h^{-1}$. The first one corresponds, considering a light-exposed volume of 50 l, to 18.33 $l_{CO2}$/day under normal conditions. The second one, tested by injecting 2 $l_{CO2}$/min, shows the strong dependence of this coefficient on the inlet gas flowrate. These data demonstrate the ability to perform fast regulation in the system's $CO_2$ concentration and guarantee optimal carbon-feed to the culture. An appropriate $K_La$ value can be decisive in the optimization of gas and liquid flow rates, and therefore of energy consumption.

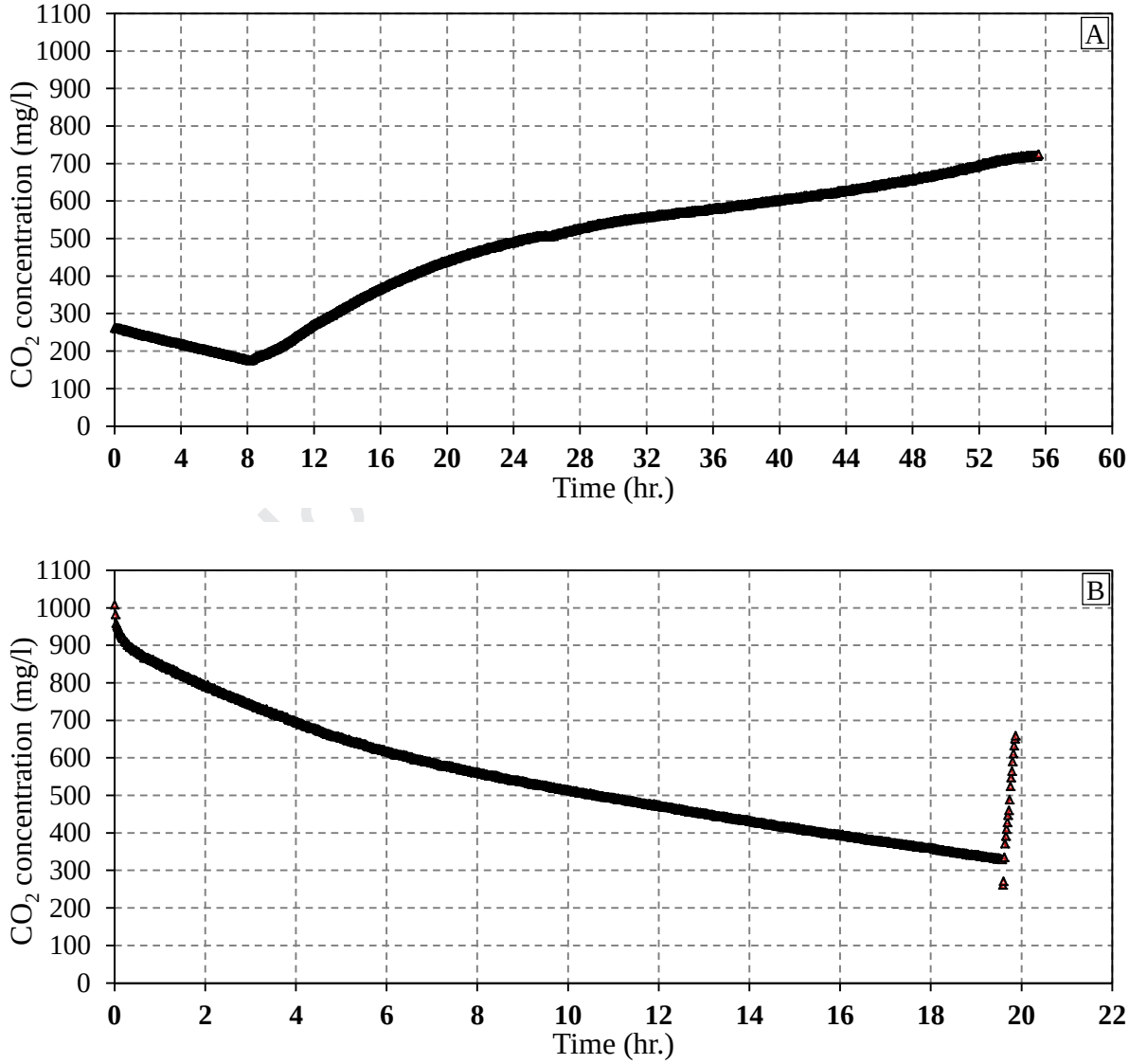



**Fig. 6.** Dissolved $CO_2$ trends inside the PBR during the 3 (A) and 5 (B) fluorescent lamps tests. The inlet $CO_2$ flows were set equal to 0.5 $L_{CO2}$/min in case A and 2 $L_{CO2}$/min in case B.

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Furthermore, to determine the $CO_2$ reduction efficiency of the PBR system, the gas stored in the Tedlar bags was analyzed with a gas analyzer. The total decrease of $CO_2$ exiting the system was recorded as around 80% in the first case and about 70% in the second one, starting from a source of $CO_2$. The only comparison that can be made between the obtained data and other studies is with the work of Meier et al. (2017), as very few experimental works have been performed using an experimental setup similar to the one proposed in this article. In that work the authors obtained identical outcomes, although with significant differences, like the layout of the system was not quite the same: light saturation was achieved with a photon flux equal to 500 μmol/$m^2$s, and the $CO_2$ mass coefficient was not directly measured but was obtained through an analytical relationship with an oxygen coefficient. One of the most significant differences of the proposed experimental setup compared to reported methodologies is the way the $CO_2$ is fed and controlled in the system, which allows an exact gas dosage. Impressive results were also obtained by using a single-stage closed PBR with a biomass concentration around two different photoperiods: one equal to 24 h of light and the other with alternating light/dark periods of 12:12, using an autotrophic *Scenedesmus* culture. With these experimental setups, Prandini et al. (2016) obtained a reduction of $CO_2$ equal to 99% ca. and 70% ca. respectively, but the concentration of oxygen inside the microalgae substrate was so high as to be considered a limiting growth factor. The other two experimental studies are presented in the literature by Basu et al. (2015) and Thiansathit et al. (2015), using small-scale PBRs. Both studies were performed using *Scenedesmus obliquus* under autotrophic conditions; in the first case, the strain was grown inside an open cylindrical glass tube PBR with alternating light/dark periods of 14:10 and the second one used a 5.3L translucent cylindrical plastic tank and alternating light/dark periods of 16:8. The carbon uptake by microalgae was reported, based on the hours of continuous $CO_2$ supply, in a range from 10.23% (12 hr) to 2.54% (24 hr) in the first experiment. In contrast, in the work of Thiansathit et al. (2015), the carbon uptake was recorded at a value of around 7%. Several un-controlled growing factors negatively influenced the experiments. In a recent study (Rodero et al., 2019) with consideration for industrial upscaling. In their work, the authors elaborated and tested a hybrid system composed of an open pond growing stage and a washing column dedicated to biogas upgrading with microalgae. The system used a mixed culture of microalgae and bacteria, allowing a $CO_2$ reduction in the inlet biogas ranging from 60 to almost 100%. This result, on the one hand, allows the industrial implementation of this technology, and on the other hand, sacrifices the biomass quality that must be considered a by-product in the best case or waste in the worst one. With the reported data, it is becoming evident that the results of biogas purification via microalgae are close to those of chemical absorption processes, although biogas purification yield does need to be enhanced through optimization strategy. Some balances can be

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evaluated by considering that microalgae biomass is made up of about 55% carbon, that the estimated growth rate is 0.06 g/l, and the carbon absorption rate of 0.037 g/l d. Consequently, the $CO_2$ removal rate can be evaluated as equal to 0.135 g/l d. Based on the obtained results, it is expected that the integration of microalgae technologies would bring additional advantages to WWTP energy optimization and reduction of GHG emissions, as for 1 ton of biomass produced, about 2 tons of $CO_2$ will get fixed.

## Conclusion

This study offers an integrated experimental and modeling feasibility analysis assessing possible opportunities to minimize the carbon footprint of the largest Italian WWTP. The proposed methodology includes a scenario analysis for improving the biogas production in sludge treatment units by the use of special pre-treatment techniques as well as upgrading biogas to biomethane. The implementation of a sludge thickener to increase the total solids (TS) content of the sludge was considered. 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. The calculation of the environmental balance showed that the innovations presented in this study would reduce the GHG emissions of the sludge treatment line of the plant by around 40%. In the second part of the study, the investigation of using a custom-made planar photobioreactor, measuring the mass transfer coefficient and $CO_2$ consumption under two different artificial light scenarios, was reported. Regarding the test conducted with microalgae, the system achieved optimal conditions for microalgae growth and reached high values of biomass concentration in the culture, competing with the best technologies in this industrial sector. These tests demonstrated the possibility of rapid intervention in carbon dioxide regulation and the capability to maintain optimal carbon-feed to the culture. A further study about the energy cost, various illumination sources, and compatibility in terms of mass balance with sludge treatment units is suggested for scaling up the proposed setup into industrial application. This study demonstrates how increasing the level of integration among processes is one key factor toward energy savings and lower environmental impacts in WWTPs.

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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 $CO_2$ fixation
- Experimental setup is proposed to evaluate the $K_{La}$ of $CO_2$ in the microalgae system

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**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.

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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.

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

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