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# Microalgal cultivation on digestate: Process efficiency and economics

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## ABSTRACT

This study aims at evaluating the process efficiency of an outdoor pilot-scale microalgae-based wastewater treatment system. Experimental results from two monitoring campaigns were analysed, showing that the system removed on average 85.1 % and 36.2 % of the influent ammoniacal nitrogen and orthophosphate, respectively, with an associated algal productivity of 9.5 g TSS·m<sup>-2</sup>·d<sup>-1</sup>. Based on pilot-scale results, a comprehensive techno-economic assessment was performed, allowing to calculate a biomass production cost of 4.3 €·kg TSS<sup>-1</sup> and a wastewater treatment cost of 2.7 €·m<sup>-3</sup> (corresponding to a nitrogen removal cost of 12.5 €·kg N<sup>-1</sup>). These costs turned out to be comparable with conventional wastewater treatment processes, thus recognizing the potential of microalgae cultivation on wastewaters as feasible alternative to conventional energy-demanding bioremediation systems and to expensive algal cultivation processes. A sensitivity and scenario analysis indicated that, under the most optimistic condition (20 % increase in the productivity, and 20 % OPEX and CAPEX reduction), biomass production and nitrogen removal costs could be further reduced of approximately 44 %.

## 1. Introduction

Wastewater treatment processes are of primary importance to allow for water reuse, combined with energy and resource recovery. These processes have been studied for more than a century, and the most widely used technology is nowadays the biological treatment based on the activated sludge process (ASP). This process achieves high removal efficiencies for wastewater macro pollutants (i.e., N, P and C compounds), but has strong environmental impacts, in terms of greenhouse gas emissions [1]. In addition, the ASP uses considerable amounts of energy since oxygen must be provided to aerobic bacteria for the oxidation of ammoniacal nitrogen and organic matter. Indeed, aeration in wastewater treatment plants is the major responsible for energy consumption [2]. Different solutions have been evaluated in the last decades, trying to find more suitable and environmentally-friendly processes [3]. Within this framework, microalgae-based wastewater treatment (MBWWT) processes can strongly reduce the CO<sub>2</sub> emissions and the energy requirement for water processing [4]. Indeed, microalgae and other phototrophic microorganisms can be exploited to remove nutrients using sunlight, directly producing O<sub>2</sub> for the biological treatment units and reducing the demand of external oxygenation [5,6].

Microalgae-bacteria consortia that develop in MBWWT are typically composed by different microalgae strains, and by nitrifying and heterotrophic bacteria. The algal-bacterial biomass that is generated in the process is suitable for several valorisation alternatives, including energy recovery as biogas or biodiesel, and nutrient recovery through the production of biofertilizers and biostimulants [4,7]. Microalgae can play a major role in a global bio-economy [8], but the production costs are generally high, dictated by the cost of synthetic nutrient media and of the installation and operation of closed photobioreactors (PBRs). Such high costs can practically make the process unfeasible, unless high-value compounds are produced [9]. On the other hand, using wastewater nutrients for growing microalgae in open ponds holds a remarkable potential to cut the production costs [10]. Among the wastewater streams that are available for microalgae cultivation, municipal and agro-industrial wastewaters can be effectively used, making both microalgae cultivation and wastewater treatment attractive [11,12]. The liquid fraction of the digestate that is generated by the anaerobic digestion of waste sludge in wastewater treatment plants or by animal manure in agricultural biogas plants is an interesting substrate for microalgae cultivation, as it can provide all the required nutrients for microalgae growth [13,14]. However, the technical and economic

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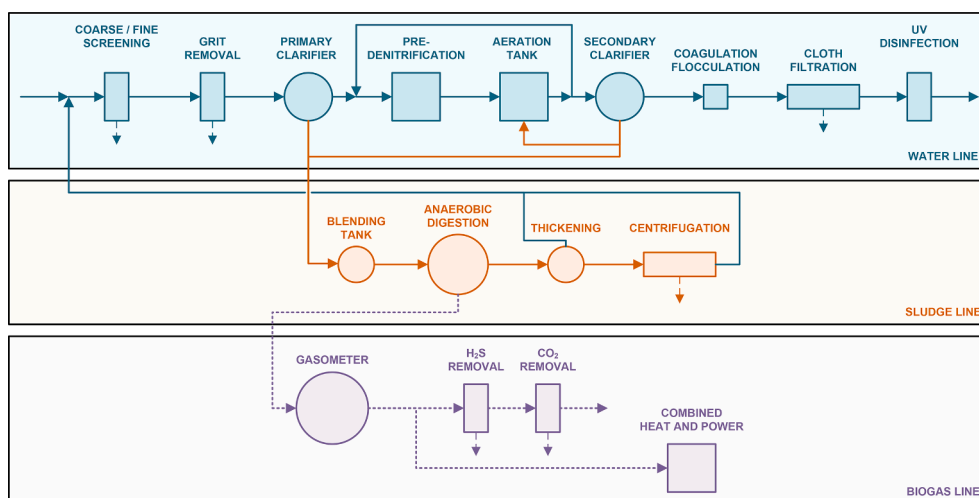


Fig. 1. Layout of the Bresso-Seveso WWTTP.

**Table 1**  
Characterisation of the digestate used to feed the microalgae cultivation unit.

PARAMETER	UNIT	YEAR 2020	YEAR 2021	AVERAGE
TAN	mg N·L <sup>-1</sup>	221 ± 29	183 ± 19	220 ± 45
NO <sub>2</sub>	mg N·L <sup>-1</sup>	0.2 ± 0.2	0.4 ± 0.3	0.3 ± 0.2
NO <sub>3</sub>	mg N·L <sup>-1</sup>	0.6 ± 0.2	0.1 ± 0.0	0.4 ± 0.6
TN	mg N·L <sup>-1</sup>	222 ± 3	184 ± 19	221 ± 45
PO <sub>4</sub> <sup>3-</sup>	mg P·L <sup>-1</sup>	8.1 ± 1.5	10.9 ± 1.4	8.9 ± 2.2
TP	mg P·L <sup>-1</sup>	10.1 ± 1.6	13.1 ± 1.1	11.5 ± 2.0
COD <sub>S</sub>	mg COD·L <sup>-1</sup>	122 ± 34	213 ± 55	151 ± 57
COD <sub>TOT</sub>	mg COD·L <sup>-1</sup>	290 ± 91	301 ± 40	296 ± 70
ALK/TAN	mol·mol <sup>-1</sup>	1.24 ± 0.25	1.57 ± 0.28	1.50 ± 0.30
OD <sub>680</sub>	–	0.15 ± 0.10	0.12 ± 0.06	0.12 ± 0.07
TSS	g TSS·L <sup>-1</sup>	0.23 ± 0.11	0.25 ± 0.09	0.22 ± 0.10
VSS	g VSS·L <sup>-1</sup>	0.15 ± 0.04	0.18 ± 0.05	0.16 ± 0.05
Turbidity	FAU	160 ± 92	90 ± 42	103 ± 76
pH	–	8.3 ± 0.3	8.6 ± 0.2	8.5 ± 0.3

feasibility of using digestate for producing microalgae at the industrial scale has not been demonstrated and quantified in available literature reports. This work aims at evaluating the process efficiency of a microalgae-based system for digestate treatment operating at middle-low latitudes, through the assessment of nutrient removal capabilities and biomass productivities at the pilot-scale. Based on the results obtained from two experimental campaigns, a comprehensive techno-economic assessment was performed, including all relevant cost items for the process. Thus, this study provides a realistic evaluation of the algal production cost at the full-scale by using recycled nutrients from the waste stream.

## 2. Materials and methods

### 2.1. Wastewater treatment plant and wastewater characteristics

#### 2.1.1. Wastewater treatment plant

The pilot-scale experimentation was conducted inside a wastewater treatment plant (WWTP) located in Milan, Italy (Latitude: 45°31'31.915"N, Longitude: 9°11'42.997"E), treating wastewater from 220,000 population equivalents (P.E.). The WWTP is composed by conventional water and sludge treatment lines. The water line includes: mechanical preliminary treatments (coarse and fine screening; sand, grit, and oil removal), primary treatment (settling), secondary treatments (denitrification/nitrification) and tertiary treatments (filtration and UV disinfection). The sludge line includes: anaerobic digestion (mesophilic digestion of mixed primary/secondary sludge), post-thickening, and sludge dewatering (polyelectrolyte-assisted

centrifugation). A dedicated biogas line is also present, where the biogas from anaerobic digestion is first stored in a gasometer and then either utilized in a combined heat and power (CHP) unit to cover part of the plant auto-consumption or upgraded to biomethane (desulphurization and hollow-fibre membrane filtration) for injection into the national gas grid; the CO<sub>2</sub> from biogas upgrading is available for microalgae cultivation on digestate. A schematic representation of the Bresso-Seveso WWTP is provided in Fig. 1.

#### 2.1.2. Digestate characteristics

The liquid fraction of the digestate (hereafter simply referred to as “digestate”) was used to feed the pilot-scale microalgae-bacteria cultivation unit. The digestate has suitable characteristics as microalgae growth medium (Table 1), due to the low content of suspended solids (resulting in a low turbidity and optical density), and the relatively low concentrations of Total Ammoniacal Nitrogen (TAN), as previously reported [13,15]. However, the concentration of total alkalinity (ALK) is low in the digestate, resulting in an ALK to TAN ratio that could make the inorganic carbon concentration limiting for the growth of microalgae and nitrifying bacteria [16]. Therefore, the digestate was supplemented with sodium bicarbonate.

### 2.2. Pilot-scale experimentation

#### 2.2.1. Microalgae cultivation unit

Microalgae cultivation was performed in a pilot-scale raceway pond (RWP) provided by SEAM Engineering S.r.l. and described in a previous work [15]. The RWP had a total surface of 5.8 m<sup>2</sup> with an adjustable gravity overflow drain set to 0.13 – 0.15 m, resulting in an overall volume of 0.75 – 0.87 m<sup>3</sup>. A paddlewheel with an adjustable revolution speed was used for mixing the algal suspension, being constantly operated at 4 RPM. The area covered by the paddlewheel was approximately 1.5 m<sup>2</sup>, so that the effective surface receiving the solar radiation is approximately 4.3 m<sup>2</sup> which was the surface used for calculating the areal biomass productivity. The RWP was covered by a greenhouse, protecting the culture from heavy rain events, and increasing the temperature during the wintertime. During spring, summer, and autumn, the lateral openings of the greenhouse were removed to prevent excessive temperatures. The influent digestate was stored in a container (1 m<sup>3</sup> volume) and fed to the RWP by a peristaltic pump. The RWP was sparged from the bottom with CO<sub>2</sub> (either a pure CO<sub>2</sub> cylinder, or to the reject CO<sub>2</sub> line from biogas upgrading) via a temporized electro-valve operating for 2 – 8 min·d<sup>-1</sup> with flowrates of 1.0 – 1.5 Nm<sup>3</sup>·h<sup>-1</sup>. The pond was equipped with a PLC, and with online probes for: temperature, Dissolved Oxygen (DO) concentration, pH, turbidity, TAN and NO<sub>x</sub>

**Table 2**

Main operational conditions of the raceway pond during the two monitoring campaigns.

PARAMETER	UNIT	MONITORING CAMPAIGN	
		YEAR 2020	YEAR 2021
Duration	d	103	210
Period	–	July – October	May – December
HRT	d	8.6 – 10	6
Liquid height	m	0.13 – 0.15	0.15
CO <sub>2</sub> source	–	Pure CO <sub>2</sub>	CO <sub>2</sub> from biogas upgrading
Corrected ALK/TAN ratio	–	1.20 – 1.73	1.95 – 2.60
pH of the suspension	–	5.8 – 8.2	7.0 – 8.2
Average daily pond temperature	°C	11.2 – 26.9	20.8 – 29.6
Pond thermal excursion	°C	1.0 – 13.3	0.6 – 9.3
Average daily irradiance	μE·m <sup>-2</sup> ·s <sup>-1</sup>	217 – 695	400 – 737

concentrations, and solar radiation.

### 2.2.2. Monitoring campaigns and operational conditions

The RWP was operated in two consecutive years (2020 and 2021), during periods typically characterized by favourable weather conditions for algal growth at these latitudes. The monitoring campaign of 2020 lasted for 103 days (from July to October), due to the limitations imposed by the COVID-19 pandemics, while in 2021 the pilot RWP was fully operative, and it was monitored for 210 days (from May to December). In both cases, the RWP was inoculated with a mixed microalgae-bacteria consortium, containing mainly *Chlorella*, *Scenedesmus*, and *Chlamydomonas* spp., and acclimated in batch conditions for two weeks with diluted digestate (1:2). Then the system was switched to the continuous feeding operation mode, with an HRT of 8.6 – 10 days in 2020 and of 6.0 days in 2021. The HRTs were calculated based on the influent digestate flowrate and the reactor volume ( $HRT = V \cdot Q_{IN}^{-1}$ ). Pure CO<sub>2</sub> was bubbled in the 2020 campaign, while the reject CO<sub>2</sub> stream

from biogas upgrading was used during 2021. The liquid discharge was conducted manually during 2020, resulting in a varying liquid height (from 0.13 to 0.15 m), while continuous outflow was allowed in 2021, when the RWP was operated at the liquid height of 0.15 m. The lateral coverage of the greenhouse was only mounted during December 2021. In both campaigns, the ALK to TAN ratio was modified by dosing NaHCO<sub>3</sub>, until reaching average values of  $1.3 \pm 0.2$  in the 2020 campaign, and  $2.1 \pm 0.5$  in the 2021 campaign. During 2021, the ALK to TAN ratio was increased compared to the previous year, to further increase inorganic carbon availability and sustain autotrophic (microalgae and nitrifying bacteria) growth, according to previous studies [16]. Table 2 summarizes the main operational conditions for the two monitoring campaigns.

Measured weather data were retrieved from the database of the regional agency for environmental protection, ARPA Lombardia (<https://www.arpalombardia.it>). The weather station is located 2 km away from the microalgae cultivation unit. The main environmental conditions (i.e., air temperature and irradiance) for the two monitoring campaigns are reported in Fig. 2. To convert the solar radiation data into the desired units, a conversion factor of  $2.105 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\cdot(\text{W}\cdot\text{m}^{-2})^{-1}$  was used, following Sulev & Ross [17] and coherently with previous works [18]. Weather data showed no statistical difference among the two monitoring campaigns (at the level of significance of 95 %), suggesting that the observed differences among the two campaigns are related to the process operation, rather than to environmental conditions.

### 2.2.3. Sampling and analytical methods

To properly characterize the dynamics of the algal suspension and the influent digestate, the following parameters were routinely analysed (every 5 – 9 days): pH, concentrations of nutrients and solids, optical properties, and physico-chemical characteristics. Nutrient analyses included: Total Ammoniacal Nitrogen ( $\text{TAN} = \text{N-NH}_4^+ + \text{N-NH}_3$ ), oxidized nitrogen forms ( $\text{N-NO}_2$  and  $\text{N-NO}_3$ ), orthophosphate ( $\text{P-PO}_4^{3-}$ ), total P ( $\text{P}_{\text{TOT}}$ ), soluble and total COD ( $\text{COD}_s$  and  $\text{COD}_{\text{TOT}}$ ). Optical

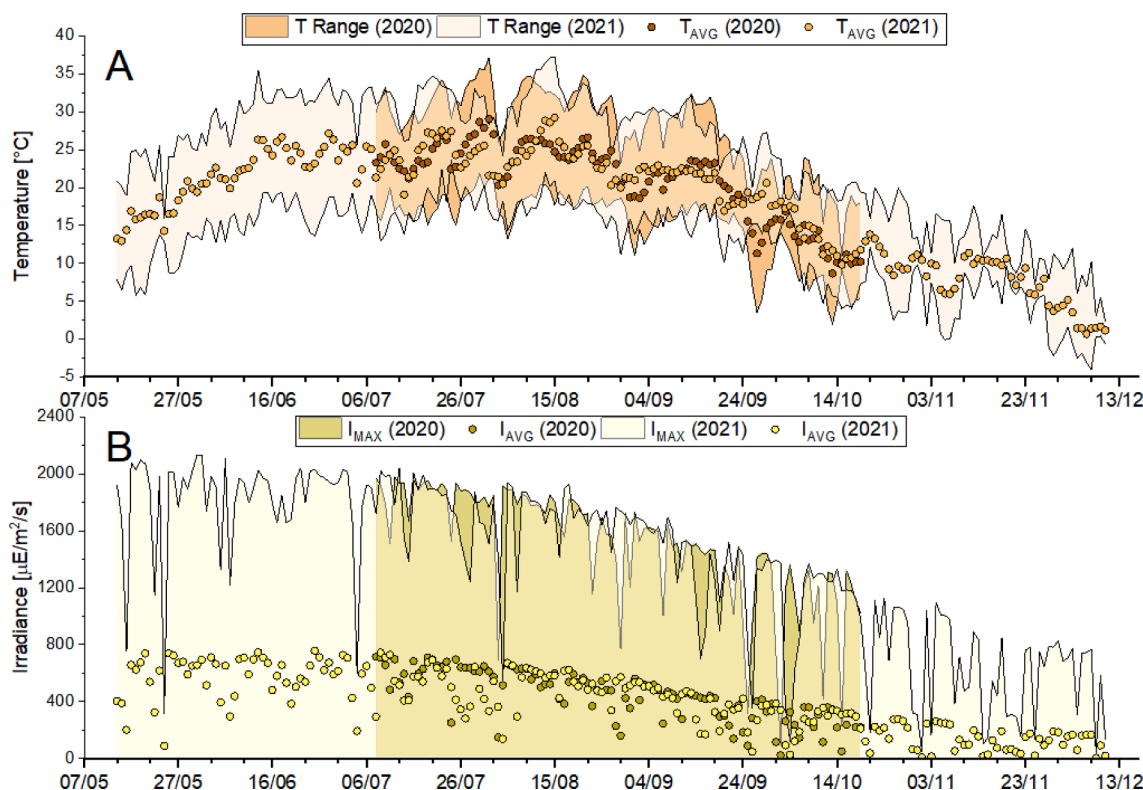


Fig. 2. Average daily air temperature (A) and irradiance (B) measured during the two monitoring campaigns.

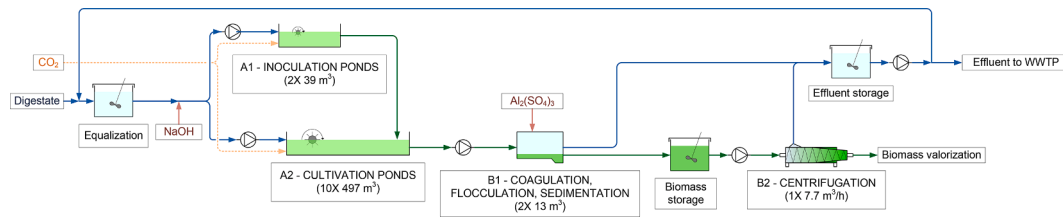


Fig. 3. Layout of the proposed microalgae cultivation plant.

characterization included turbidity and optical density at the wavelength of 680 nm ( $OD_{680}$ ). The physical–chemical properties analysed were pH, temperature, and electric conductivity. The growth of microalgae was monitored by algal counts and by the concentration of Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS).

As for the analytical methods, soluble nutrients, i.e., TAN,  $N-NO_2^-$ ,  $N-NO_3^-$ ,  $P-PO_4^{3-}$ , and  $COD_S$  were measured on membrane-filtered samples (cut-off: 0.45  $\mu m$ ), using commercial test kits (Hach Lange, test codes: LCK303, LCK342, LCK339, LCK348, and LCK314, respectively). Compounds to be measured in the particulate phase, including  $P_{TOT}$  and  $COD_{TOT}$  were measured on unfiltered samples after digestion (1 h at 100 °C and 2 h at 148 °C, respectively) in a thermostat, using the same test kits as for soluble compounds. Test kits were then read spectrophotometrically. The  $OD_{680}$  and turbidity were measured using the same instrument, in plastic cuvettes with light paths of 1 and 5 cm, respectively. Cell counts were determined using a Bürker chamber with 0.04 mm<sup>2</sup> squares, under a light microscope at 40X magnification. The microalgal species were contextually identified by visual examination. TSS and VSS were determined according to the Standard Methods for the Examination of Water and Wastewater [19]. Temperature, pH, and electric conductivity of the samples were measured immediately after sampling, using a portable multi-meter probe. The alkalinity was monitored on both the influent digestate and outlet samples of the RWP, by potentiometric titration with  $H_2SO_4$  (0.02 N), according to the Standard Methods [19].

#### 2.2.4. Calculations

Based on laboratory analyses, mass balances were calculated for each relevant parameter, by considering the RWP as a completely mixed reactor. This assumption was justified by the small scale of the pilot reactor [14,20]. The hydraulic balance of the RWP was considered to calculate the effluent flowrate, as follows (Equation (1)):

$$Q_{OUT}(t_i) = Q_{IN}(t_i) - Q_{EV}(t_i) \quad (1)$$

where:  $Q_{OUT}(t_i)$  is the effluent flowrate at the time step  $t_i$ ,  $Q_{IN}(t_i)$  is the influent flowrate, and  $Q_{EV}(t_i)$  is the evaporation rate calculated using the Buckingham model, as detailed in Pizzera et al. [14]. In the hydraulic balance, the contribution of the rainfall was neglected, since the RWP was always covered by the greenhouse roof. Once the influent and effluent flowrates were calculated, the rates of production or consumption for a generic soluble or particulate element (identified as X) could be evaluated (Equation (2)).

$$R_X(t_i) = \frac{\Delta X}{\Delta t} - \frac{Q_{AVG,IN}(t_i) \cdot X_{AVG,IN}(t_i)}{V_{AVG}(t_i)} + \frac{Q_{AVG,OUT}(t_i) \cdot X_{AVG,OUT}(t_i)}{V_{AVG}(t_i)} + \frac{X_{AVG,OUT}(t_i) \cdot \Delta V}{V_{AVG}(t_i) \cdot \Delta t} \quad (2)$$

where:  $R_X(t_i)$  [g X·m<sup>-3</sup>·d<sup>-1</sup>] is the transformation rate for the element X at the time  $t_i$ ,  $\Delta X = (X_i - X_{i-1})$  [g X·m<sup>-3</sup>] is the variation in the concentration of the element X in the time interval;  $\Delta t = (t_i - t_{i-1})$  [d],  $Q_{AVG,IN}(t_i)$  and  $Q_{AVG,OUT}(t_i)$  [m<sup>3</sup>·d<sup>-1</sup>] are the average values of the influent and effluent flowrates along the time interval, respectively,  $X_{AVG,IN}(t_i)$  and  $X_{AVG,OUT}(t_i)$  [g X·m<sup>-3</sup>] are the average values of the influent and effluent concentrations of X in the time interval, respectively, and  $V_{AVG}(t_i)$  [m<sup>3</sup>]

is the average value of the volume.

In case the RWP volume is constant, Equation (2), simplifies to Equation (3):

$$R_X(t_i) = \frac{\Delta X}{\Delta t} - \frac{Q_{AVG,IN}(t_i) \cdot X_{AVG,IN}(t_i)}{V} + \frac{Q_{AVG,OUT}(t_i) \cdot X_{AVG,OUT}(t_i)}{V} \quad (3)$$

In Equation (2) and Equation (3), production rates result as positive, while removal rates have negative sign. For simplicity, when referring to a nutrient removal rate (e.g., for  $R_{TAN}$  and  $R_{PO_4}$ ), the absolute value of such computation is reported. The volumetric biomass productivity ( $P_V$ ) was considered equal to the rate of TSS production ( $R_{TSS}$ ), in accordance with previous studies [14,15]. The areal biomass productivity ( $P_A$ , Equation (4)) was referred to the surface of the RWP, and since the paddlewheel had a large footprint, locally providing a significant shading of the culture,  $P_A$  was calculated by accounting for the effective area that received the solar radiation, as previously proposed [21]:

$$P_A(t_i) = P_V(t_i) \cdot \frac{V_{AVG}(t_i)}{A^*} = R_{TSS}(t_i) \cdot \frac{V_{AVG}(t_i)}{A^*} \quad (4)$$

where:  $P_A(t_i)$  [g TSS·m<sup>-2</sup>·d<sup>-1</sup>] is the areal biomass productivity at the time  $t_i$ ,  $P_V(t_i)$  [g TSS·m<sup>-3</sup>·d<sup>-1</sup>] is the volumetric biomass productivity,  $A^* = (A_{RWP} - A_{PW})$  [m<sup>2</sup>] is the illuminated area.

The photosynthetic efficiency was calculated according to previous studies [22], as shown in Equation (5).

$$\eta_{PHO} = \frac{E_{ALG}}{E_I} = \frac{P_A \cdot LHV_{ALG}}{E_I} \quad (5)$$

where:  $\eta_{PHO}$  [%] is the photosynthetic efficiency,  $E_{ALG}$  [MJ·m<sup>-2</sup>·d<sup>-1</sup>] is the amount of energy in the algal biomass,  $LHV_{ALG} = 21$  [MJ·kg DW<sup>-1</sup>] is the lower heating value of the algal biomass, and  $E_I$  [MJ·m<sup>-2</sup>·d<sup>-1</sup>] is the amount of energy received by the pond.

To quantify the amount of free ammonia nitrogen (FAN) produced in the system, the following relation was used [23,24], based on the actual TAN, pH and temperature measured in the RWP (Equation (6)).

$$FAN = \frac{TAN \cdot 10^{pH}}{10^{pH} + \exp\left(\frac{6.344}{T_p}\right)} \quad (6)$$

where:  $T_p$  [K] is the temperature of the raceway pond.

#### 2.2.5. Statistical methods

Correlations among the methods for evaluating the algal biomass and solids were assessed through Pearson's coefficients. To determine the statistical difference among weather data in the two monitoring campaigns, the paired  $t$ -test was run on comparable datasets (i.e., during the period in which data were available for both monitoring campaign, July – October). All analyses were performed at the significance level of 95 % (i.e., with  $\alpha = 0.05$ ), using the software OriginPro 2020b.

### 2.3. Techno-economic assessment

#### 2.3.1. Proposed layout for the integration of the algae-based process in the WWTP

For the integration of algal cultivation as a side-stream digestate

**Table 3**  
Main design hypothesis for the techno-economic assessment.

SECTION	PARAMETER	UNIT	VALUE	REFERENCE / NOTES
GENERAL DATA	Biomass composition	–	CH <sub>1.83</sub> O <sub>0.48</sub> N <sub>0.11</sub> P <sub>0.01</sub>	[20,35]
	N content in the algal biomass	% w/w	6.6	Based on biomass composition
	Digestate availability	m <sup>3</sup> .h <sup>-1</sup>	90	From WWTP data
	N concentration in the digestate	g.m <sup>-3</sup>	220	Based on pilot data
	Specific area requirement	m <sup>2</sup> .PE <sup>-1</sup>	0.5	[36,37]
	Total algae cultivation area required	ha	1.98	Based on the specific area requirement
	Net/gross area ratio for RWPs	–	0.8	[29]
	Operativity of the plant	d.y <sup>-1</sup>	274	Considering operativity from March to November
	Algal productivity	g TSS.m <sup>-2</sup> .d <sup>-1</sup>	9.9	Based on pilot data and photosynthetic efficiency
	Increase productivity coefficient	–	1.2	20 % gain due to higher irradiance without greenhouse
A1 – INOCULATION PONDS	Number of paddlewheels per RWPs	–	1	Assumed
	Area requirement	%	2	Of the total algae cultivation area
	Net area required	ha	0.04	Based on total area required
	Number of RWP modules	–	2	Based on module design
	Module length	m	49	Assumed
	Module width	m	4	Assumed
	Liquid height	m	0.2	Assumed
	Total volume	m <sup>3</sup>	78.4	Calculated
	Digestate treated	m <sup>3</sup> .d <sup>-1</sup>	7.5	Based on RWPs volume and HRT
	Effluent biomass concentration	g TSS.m <sup>-3</sup>	631	Based on treated digestate and productivity
A2 – CULTIVATION PONDS	Area requirement	%	98	On the total algae cultivation area
	Net area required	ha	1.94	Based on total area required
	Number of RWP modules	–	10	Based on module design
	Module length	m	153	Assumed
	Module width	m	13	Assumed
	Liquid height	m	0.25	Assumed
	Total volume	m <sup>3</sup>	4,972	Calculated
	Digestate treated	m <sup>3</sup> .d <sup>-1</sup>	365	Based on RWPs volume and HRT
	Effluent biomass concentration	g TSS.m <sup>-3</sup>	642	Based on treated digestate and productivity
	NaOH concentration required	mM NaOH	25	To correct influent alkalinity [16]
B1 – COAGULATION, FLOCCULATION, SEDIMENTATION	Flocculant added	–	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	Assumed
	Flocculant dosage	g Al.m <sup>-3</sup>	15	[31]
	Contact time	min	15	Assumed
	HRT in the coagulation zone	h	1.75	[30]
	Solids removal efficiency	%	95	[31]
	Effluent biomass concentration	kg TSS.m <sup>-3</sup>	5	[29]
	Flowrate treated	m <sup>3</sup> .d <sup>-1</sup>	46.1	Based on productivity and pre-concentration effluent
B2 – CENTRIFUGATION	Solids removal efficiency	%	95	[21]
	Effluent biomass concentration	kg TSS.m <sup>-3</sup>	200	[29]

treatment, the algae-bacteria unit is aimed at removing nutrients (N and P) from digestate, and at utilizing the CO<sub>2</sub> available from the full-scale biogas upgrading section converting it into potentially valuable biomass. As suggested by the data retrieved at the pilot scale, it was assumed that no specific pre-treatments were required for the liquid fraction of the digestate. Therefore, the plant only includes an algae/bacteria cultivation line and a subsequent biomass concentration, to produce an algal paste for further utilization/processing. As depicted in Fig. 3, the plant layout is composed by the following sections: (A) biomass production, including inoculation ponds (section A1) and cultivation ponds (section A2); (B) biomass harvesting, including biomass pre-concentration (section B1) and harvesting through centrifugation (section B2). The proposed process produces a highly concentrated algal biomass stream and a treated digestate stream. The treated digestate is then recirculated to the head of the water line. The produced algal-bacterial paste can be valorised in a biorefinery perspective, following different valorisation pathways [25–27]. The production of low-value products, such as bioplastics or biofuels (biogas or biodiesel) is typically adopted for the wastewater-grown biomass since it is not possible to valorise it as food or feed applications. The concurrent production of bio-stimulants for the agricultural market could be considered as an interesting alternative. However, only the biomass production is analysed in this section, aimed at evaluating the costs and feasibility of

scaling-up the production line, in view of a subsequent decision-making about the best valorisation alternative.

### 2.3.2. Techno-economic assessment

Relevant design hypotheses for each section composing the layout shown in Fig. 3 are presented below and further detailed in Table 3.

#### General design data

##### Section A – Biomass production

According to pilot-scale productivity and weather data for the considered location, the biomass production is calculated by considering an operativity of the plant of 9 months per year (from March to November, i.e., for 274 d.y<sup>-1</sup>). During this operational period, an average productivity of 9.9 g TSS.m<sup>-2</sup>.d<sup>-1</sup> was calculated, based on the average photosynthetic efficiency reached in the pilot-scale experiments ( $\eta_{\text{PHO}} = 1.83\%$ ) and local irradiance data. The obtained productivity was further incremented by 20 %, since in the scaled-up process no greenhouse is foreseen, and this will lead to a higher light availability [28]. The approach of considering a 20 % increase in the productivity is still conservative, since the irradiance reduction due to the greenhouse can reach up to 40 % (according to measured PAR data, data not shown). This would also compensate the eventual contribution of rainfall events, that are not explicitly considered in this analysis. As reported in previous works [21,29], low-cost systems are used for the inoculum and

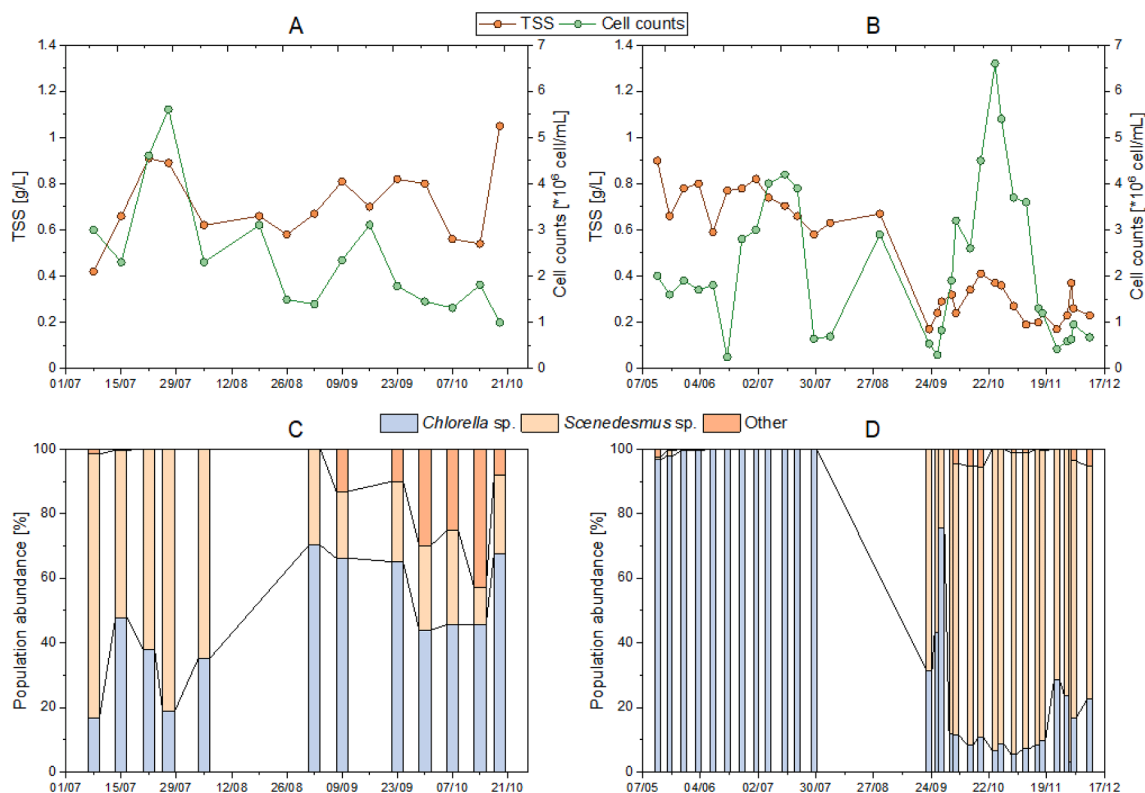


Fig. 4. Evolution of biomass concentration and microalgal communities: total suspended solids and microalgal cell counts for 2020 (A) and 2021 (B), microalgal population relative abundance in 2020 (C) and 2021 (D).

cultivation RWPs. The ponds are therefore constituted by a waterproof lining laid on the ground, and by concrete walls to hold the liner in place. RWPs are provided with a paddlewheel for mixing the algal suspension and with the instrumentation needed to monitor the state of the process, i.e., with DO, pH, and temperature probes connected to a PLC for remote monitoring. As for the sizing of inoculation and cultivation ponds, the required volume is calculated based on the maximum digestate availability and the imposed HRT (Table 3). The inoculum section (A1), which is aimed at guaranteeing a more rapid start-up of the plant in the event of occasional crashes, is constituted by 2 RWPs having a total length of 49 m, and a width of 4 m (total surface of 0.04 ha) and a liquid depth of 0.2 m (total volume of 78.4 m<sup>3</sup>). The biomass cultivation section (A2) is constituted by 10 RWPs with length of 153 m and width of 13 m (overall pond surface of 1.94 ha), and a liquid depth of 0.25 m (volume of 4972 m<sup>3</sup>). Regarding the arrangement of RWPs, a distance among ponds of 3 m was considered, to allow for maintenance operations and for placing piping and wiring. As suggested by pilot results, NaOH supplementation was considered to correct the ALK to TAN ratio of the influent digestate to 1.6 mol·mol<sup>-1</sup> [16]. Digestate feeding pumps (operated for 24 h·d<sup>-1</sup>) were designed according to conventional hydraulic criteria keeping into account friction head losses, and by considering that each feeding pump would serve two RWPs.

#### Section B – Biomass concentration and harvesting

The biomass pre-concentration section (B1) is assumed to include two coagulation, flocculation, and sedimentation (CFS) tanks, each serving five of the RWPs of section A2. Each CFS tanks is divided into a coagulation section with fast mixing, a flocculation zone with no mixing, and a calm zone realized by lamellar settling [21,30]. Based on previous lab-scale tests [31], aluminium sulphate (Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) is dosed in the coagulation zone to reach a concentration of 15 g Al·m<sup>-3</sup> and a contact time of 15 min is maintained, obtaining a theoretical S/L separation efficiency of 95 %. The section B1 is aimed at increasing the algae concentration up to 5 g TSS·L<sup>-1</sup>. Each CFS tank is fed by a feeding pump operated continuously. After each CFS tank, a harvesting pump is

employed to convey the pre-concentrated biomass to the subsequent harvesting unit (section B2), while a recirculation pump sends the liquid fraction back to section A or to the main line of the WWTP. In section B2, the pre-concentrated biomass stream is equalized in a storage tank, from which it is sent, by means of a pump, to a centrifuge operated for 6 h·d<sup>-1</sup>, to obtain an algal paste at a final concentration of 200 g TSS·L<sup>-1</sup>, coherently with previous findings [21,29]. After centrifugation, the liquid fraction is fed to a storage tank, from which the effluent is sent back to the main water line of the WWTP.

#### Calculation of CAPEX, OPEX and cost savings

To provide a realistic assessment of biomass production costs, capital expenditures (CAPEX) and operational expenditures (OPEX) were calculated for each section, as detailed below. When reliable estimates of the costs were not available, Lang multipliers were applied to provide a preliminary estimate of the overall plant cost, as proposed in several techno-economic assessments of chemical engineering plants and microalgal biorefineries [32,33]. Lang factors were applied to the following items of expenditure: installation costs, service facilities, construction expenses, engineering and supervision, contractor's fee, contingencies, and maintenance.

#### CAPEX calculation

The relevant CAPEX for this analysis are described in this section. The land costs include the acquisition of the land, and its preparation for installing the RWPs and the accessory elements. The land cost is assumed according to the average price of agricultural land in Lombardy for the year 2020 [34]. Equipment costs include the purchase of the following items: the paddlewheels, feeding and recirculation pumps, instrumentation/control units for the RWPs (for sections A1 and A2); the CFS tanks, mixers, and pumps (for section B1), and the centrifuge (for section B2). The depreciation of investments is evaluated considering a lifetime for the plant equal to 20 years and is based on Lang factors, according to Ruiz et al. [33].

Equipment costs for which direct quotations were not available, were scaled based on the cost of quoted reference items, according to the

Table 4

Comparison of biomass productivity and nutrient removal efficiencies obtained in this study with the literature.

REFERENCE	INFLUENT WASTEWATER	MAIN ALGAL GENERA	P <sub>A</sub> [g TSS·m <sup>-2</sup> ·d <sup>-1</sup> ]	η <sub>TAN</sub> [%]	η <sub>PO4</sub> [%]
[52]	Piggery WW	<i>Anabena, Dolichospermium</i>	11.3 – 28.8	93 – 96	52 – 58
[53]	Piggery WW	<i>Chlorella, Scenedesmus, Nitzschia</i>	–	96 – 99	–
[21]	Piggery WW	<i>Chlorella, Scenedesmus</i>	10.7 ± 6.5	90 ± 10	90 ± 27
[54]	Municipal WW	<i>Scenedesmus</i>	20 – 26	–	–
[48]	Municipal WW	<i>Pedastrium</i>	9–16.7	74 – 77	–
[55]	Digestate (food processing WW)	<i>Chlorella, Scenedesmus, Dictyosphaerium</i>	2 – 9	70–94	16 – 59
[49]	Digestate (food processing WW)	<i>Chlorella</i>	2.5 – 11.8	–	36 – 86
[46]	Digestate (solid waste)	<i>Chlorella, Scenedesmus, Synecococcus, Syneocystis</i>	1.2 – 6.9	15 – 92	78 – 97
[14]	Digestate (piggery WW)	<i>Chlorella, Scenedesmus</i>	3 – 16	80 ± 13	–
[56]	Digestate (piggery WW)	<i>Chlorella, Scenedesmus</i>	–	79 ± 15	–
[57]	Digestate (piggery WW)	<i>Scenedesmus, Chroococcus</i>	6.2 ± 1.0	99.7 ± 0.7	–
[16]	Digestate (piggery WW)	<i>Chlorella, Scenedesmus</i>	3.6 – 22	3 – 86	9 – 57
[58]	Digestate (piggery WW)	<i>Chlorella, Scenedesmus</i>	4.2	69	–
[59]	Digestate (municipal WW)	<i>Nannochloropsis</i>	5 – 14	20 – 60	20 – 85
[51]	Digestate (municipal WW)	<i>Chlorella</i>	15	99 – 100	85 – 92
[60]	Digestate (municipal WW)	<i>Ankistrodesmus, Micractinium</i>	3.8 – 8	–	32 – 50
[50]	Digestate (municipal WW)	<i>Scenedesmus</i>	24	55	–
[15]	Digestate (municipal WW)	<i>Chlorella, Scenedesmus, Chlamydomonas</i>	3.4 – 8.4	86 ± 7	71 ± 10
[61]	Digestate (municipal WW)	<i>Pseudoanabena, Nannochloropsis, Halamphora, Geitlerinema</i>	32.4	80 – 90	80 – 90
[62]	Digestate (municipal WW)	<i>Scenedesmus</i>	5 – 22.9	94	90.6
This study (year 2020)	Digestate (municipal WW)	<i>Chlorella, Scenedesmus</i>	11.8 ± 7.6	84.5 ± 7.8	36.9 ± 12.1
This study (year 2021)	Digestate (municipal WW)	<i>Chlorella, Scenedesmus</i>	8.4 ± 5.7	85.4 ± 8.1	35.8 ± 18.7
This study (overall)	Digestate (municipal WW)	<i>Chlorella, Scenedesmus</i>	9.5 ± 6.4	85.1 ± 7.9	36.2 ± 16.7

exponential rule that is typically adopted in chemical engineering projects (**Equation (7)**):

$$\frac{Cost_A}{Cost_B} = \left( \frac{Size_A}{Size_B} \right)^n \quad (7)$$

where: Cost<sub>A</sub> [€] and Size<sub>A</sub> are the cost and the size of a generic item to be quoted, Cost<sub>B</sub> [€] and Size<sub>B</sub> are the cost and size of a similar item for which a reliable quote is available, and n [-] is the cost exponent, typically varying in the range 0.45–0.85 [38,39].

#### OPEX calculation

Energy costs include the expenses for running all the electro-mechanic devices, i.e., the feeding pumps and the paddlewheels in sections A1 and A2, the mixers, harvesting and recirculation pumps, and the centrifuge in sections B1 and B2. The reagents costs include the NaOH required to correct the alkalinity in section B2 and the flocculant for section B1. Additional nutrients costs were not considered, since nitrogen and phosphorus are provided by the digestate, and CO<sub>2</sub> is freely available from the biogas upgrading section of the plant. Finally, labour costs are calculated accounting to similar case studies [21,40], and the microalgae production plant is operated by two field operators, a supervisor and a plant manager.

#### Cost savings and carbon credits

In the economic analysis, the saved cost due to the removal of TAN and PO<sub>4</sub><sup>3-</sup> was computed. To evaluate the cost saved due to ammoniacal nitrogen removal in the algae-bacteria unit, the TAN load that should not be oxidized in the main line of the WWTP was computed as the sum of the nitrogen in the algal biomass (assuming a nitrogen content in the algal biomass equal to 6.6 %, on a mass basis) and the nitrified TAN. Assuming an energy consumption for nitrogen removal in the WWTP of 6.5 kWh·kg N<sup>-1</sup> [41], the energy saving for nitrification could be calculated. The savings related to the removal of orthophosphate was calculated assuming a P removal cost of 2.4 €·kg P<sup>-1</sup> [42]. Finally, the credits obtained through the biofixation of CO<sub>2</sub> by the algae-bacteria consortium were quantified by assuming a stoichiometric content of 1.88 kg CO<sub>2</sub>·kg DW<sup>-1</sup> and a carbon credit of 83 €·t CO<sub>2</sub><sup>-1</sup>, i.e., equal to the 2022 average calculated from the EU Emissions Trading System data [43].

#### Cost actualization

To account for inflation, the actual value of a currency considering was calculated by using the European Union Consumer Price Index (EU-HICP) provided by Eurostat [44].

#### 2.3.3. Sensitivity and scenario analysis

A sensitivity analysis was conducted, by considering the variation of the most important parameters having a relevant impact on the final biomass production cost. These parameters are the biomass productivity (that can vary according to different plant operations and local climate conditions) as well as the overall OPEX and CAPEX. A variation of ± 20 % was considered for these parameters, to identify their effect on the final biomass production and wastewater treatment costs.

### 3. Results and discussion

#### 3.1. Pilot-scale microalgae cultivation and digestate treatment

##### 3.1.1. Microalgal productivity and communities

Fig. 4 shows the trends of the biomass parameters during the two monitoring campaigns. Results are expressed in terms of TSS and microscope cell counts. In 2020 campaign (Fig. 4A), after an initial growth period lasting approximately 20 days, biomass concentration was quite stable, reaching a TSS concentrations of approximately 0.55–0.80 g TSS·L<sup>-1</sup> (with microalgal cell counts of 4.6 – 5.6 · 10<sup>6</sup> cells·mL<sup>-1</sup>). During the last 20 days of operation, the biomass concentrations decreased, in response to the sub-optimal temperature and irradiance conditions. Indeed, during this period, the maximum daily irradiance reached in the outdoor system decreased well below 700 μmol·m<sup>-2</sup>·s<sup>-1</sup>, frequently not even reaching 200 μmol·m<sup>-2</sup>·s<sup>-1</sup>. Concurrently, the average daily temperature was always lower than 20 °C, reaching minimum values of 2 °C in October. During the 2021 monitoring campaign (Fig. 4B), from May to August, more stable conditions were achieved for biomass concentration, with average TSS concentrations of 0.59–0.90 g TSS·L<sup>-1</sup> (corresponding to optical densities of 0.5 – 0.8 and microalgae counts of 1.7 – 4.2 · 10<sup>6</sup> cells·mL<sup>-1</sup>). A sudden culture crash with algae flocculation was experienced at the end of September. This was probably due to the presence of residual flocculant in the influent digestate batch, though this hypothesis could not be experimentally verified. The rapid and unaccounted tendency of microalgae to flocculate was also combined to the appearance of microalgae predators (mainly, protozoa), that facilitated a further drop in the biomass concentration, up to exceptionally low values (below 0.1 g TSS·L<sup>-1</sup>, corresponding to a loss of approximately 83 % of the algal cells in the suspension). After this event, the system was inoculated again with an algal consortium that was previously grown in the laboratory (composed of *Chlorella* and *Scenedesmus* spp.) on a new batch of digestate. Following the re-inoculation, the



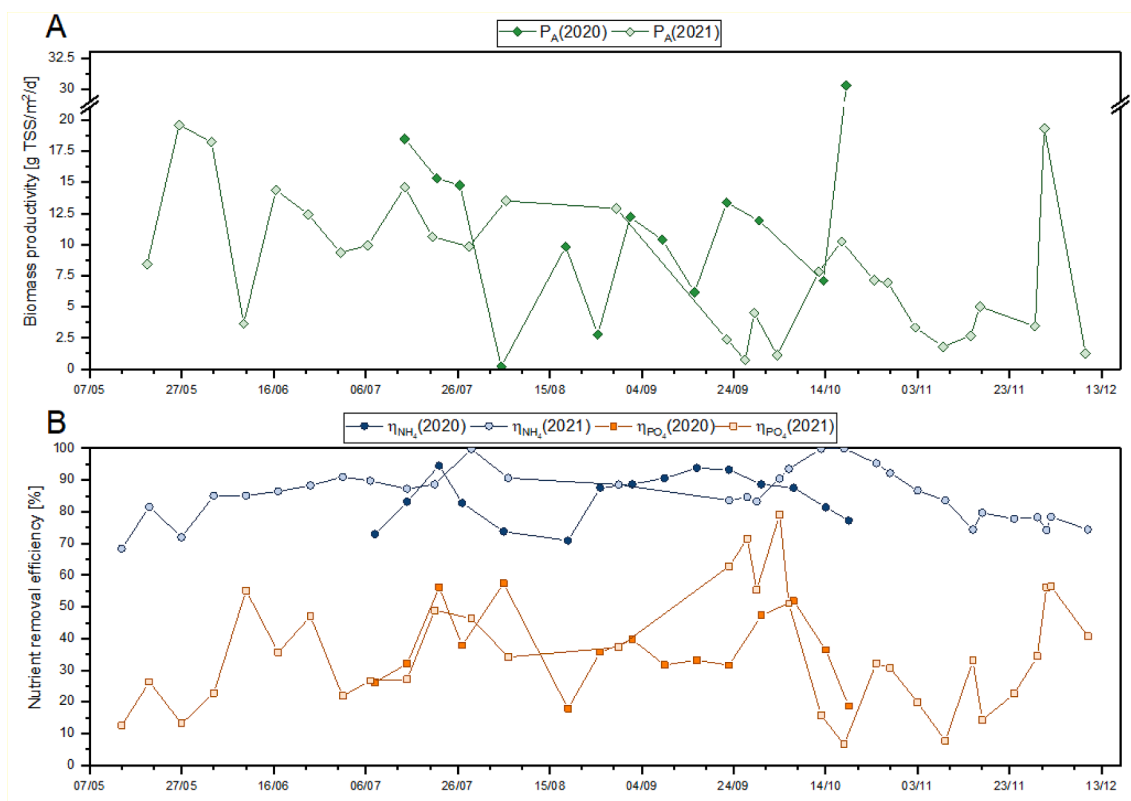


Fig. 5. Evolution of the algal biomass productivity (A) and nutrient removal efficiencies (B) during the two monitoring campaigns.

biomass concentration reached levels comparable, or even higher, than those obtained in during the same period of 2020. After reaching the highest number of cells in the suspension at the end of October ( $6.63 \cdot 10^6$  cells·mL<sup>-1</sup>), the biomass concentrations steadily decreased until December due to the sub-optimal weather conditions (average daily irradiance below  $100 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  and air temperature in the range  $0 - 6$  °C). The lateral panels of the greenhouse were then installed, and the biomass concentration experienced a slight increase until the end of the experimentation (up to  $0.23 - 0.37$  g TSS·L<sup>-1</sup>), due to the increased temperature in the pond.

Regarding the composition of the microalgae community, Fig. 4 shows that the dominant algae species were *Chlorella* and *Scenedesmus* spp., consistently with the results reported in the literature (Table 4). These two ubiquitous species are typically found in wastewater and digestate treatment systems [45,46]. During the first phase of 2020, *Scenedesmus* sp. was dominant while *Chlorella* sp. took over from September onward, together with other unidentified green algae species (up to 43 % of the total number of cells, Fig. 4B). In 2021, the occurrence of algae other than *Chlorella* and *Scenedesmus* spp. was negligible. A shift from the prevalence of *Scenedesmus* sp., to the complete dominance of *Chlorella* sp. was observed during the first two weeks; *Chlorella* prevailed until the end of September (Fig. 4D). After the system was restarted with the new inoculum (containing both species), *Scenedesmus* sp. became the dominant algal population, with peaks up to 90 % of the total number of cells. Concurrently, other species showed up, during the second part of the 2021 campaign, though remaining in very low concentrations (less than 5 % of the total algal biomass). Although the reasons for the prevalence of one genus over another remain to be defined, it can be assumed that environmental conditions (mostly, irradiance and temperature) are playing a key role.

Fig. 5 A-B reports the trend of the total biomass productivity ( $P_A$ ) during the two monitoring campaigns. As shown in Table 4, in both monitoring campaigns, the system achieved productivities that typically fall in the range of literature values. In 2020, an average productivity of

$11.8 \pm 7.6$  g TSS·m<sup>-2</sup>·d<sup>-1</sup> was reached, corresponding to approximately  $61.1 \pm 39.5$  g TSS·m<sup>-3</sup>·d<sup>-1</sup>. During this year, the photosynthetic efficiency was  $1.53 \pm 1.73$  %, that is comparable to the values reviewed by Norsker et al. [47]. In 2021, on average, a lower productivity was reached, since the campaign was extended until winter and because of the biomass concentration drop that occurred in September 2021. The average productivity considering the entire experimental period was  $8.4 \pm 5.7$  g TSS·m<sup>-2</sup>·d<sup>-1</sup> ( $41.6 \pm 28.1$  g TSS·m<sup>-3</sup>·d<sup>-1</sup>). However, a productivity higher than that obtained in 2020 was obtained when considering only the data before the culture crash ( $12.1 \pm 4.2$  g TSS·m<sup>-2</sup>·d<sup>-1</sup> until September). Considering the period following the culture crash (from September to December), the average productivity was  $5.2 \pm 4.8$  g TSS·m<sup>-2</sup>·d<sup>-1</sup>. On average, for the year 2021, the photosynthetic efficiency was  $1.97 \pm 2.85$  %. The values obtained in the present monitoring campaigns are comparable with the results obtained in the treatment of agro-zootechnical and urban wastewater [14,48,49], also being higher than the values previously obtained in the same treatment system [15]. In some cases, a higher productivity could be achieved using digestates derived from urban wastewater [50,51] or from piggyery wastewaters [21,52]. However, discrepancies with other studies in the literature are likely to be attributed to the different climate conditions as well as to the case specific wastewater characteristics.

### 3.1.2. Nutrient removal performance and efficiency

The concentrations of nitrogen compounds were monitored over time to calculate the removal rates and efficiencies of TAN and orthophosphates. During the two consecutive years, the nitrogen loading rate (NLR) was  $19.3 \pm 2.5$  and  $31.7 \pm 7.4$  g N·m<sup>-3</sup>·d<sup>-1</sup> in 2020 and 2021, respectively, resulting in a similar trend for nitrogen removal also due to the similar weather conditions recorded during the two years. A relatively low concentration of TAN (typically lower than  $50$  mg N·L<sup>-1</sup>) was always released with the effluent, while in a few cases, corresponding to the start-up phases or during sub-optimal weather conditions, the effluent TAN was slightly higher (up to a maximum of  $70$  mg N·L<sup>-1</sup>).

**Table 5**  
Main design hypothesis and results for CAPEX calculation.

SECTION	PARAMETER	UNIT	VALUE	REFERENCE / NOTES
GENERAL DATA	Specific cost of liner	€·m <sup>-2</sup>	4.7	[69]
	Specific cost of concrete	€·m <sup>-3</sup>	100	[21]
	Specific cost of pipes/wires	€·m <sup>-1</sup>	31	[70]
	Cost of civil works	€	42,089	Based on 70,000 € for 5.5 ha
	Piping length	m	594	Based on 3 m distance among RWPs
	Wiring length	m	297	Assumed as half of the piping length
	Cost of piping and wiring	€	27,244	Based on 3 m distance among RWPs
	Land cost	€·ha <sup>-1</sup>	47,996	Average agricultural land cost in Lombardy [34]
	Cost exponent for civil work, piping/wiring, pump, centrifuge, instrumentation	–	0.65	[39]
	Cost exponent for paddlewheels, mixers, tanks	–	0.45	[39]
	Number of pumps	.	13	Based on process layout
	Feeding, recirculation and harvesting pumps cost	€	23,572	Based on 1115 €·unit <sup>-1</sup> for 3.6 m <sup>3</sup> ·h <sup>-1</sup> pump
	Number of storage tanks	–	2	Based on process layout
	Cost for storage tanks	€	461	Based on 811 € for 754 m <sup>3</sup>
A1 – INOCULATION PONDS	Cost of paddlewheels	€	15,063	Based on 40,000 € for 2 acres installation
	Cost of liner	€	2,172	Calculated
	Height of concrete walls	m	0.3	Considering an allowance of 10 cm
	Width of concrete walls	m	0.03	Assumed
	Total concrete volume	m <sup>3</sup>	2.7	Calculated
A2 – CULTIVATION PONDS	Cost of paddlewheels	€	210,351	Based on 40,000 € for 2 acres installation
	Cost of liner	€	97,745	Calculated
	Height of concrete walls	m	0.3	Considering an allowance of 15 cm
	Width of concrete walls	m	0.05	Assumed
	Total concrete volume	m <sup>3</sup>	94.1	Calculated
B1 – COAGULATION, FLOCCULATION, SEDIMENTATION	Instrumentation cost	€	27,912	Based on 1000 €·unit <sup>-1</sup> for 100 m <sup>3</sup> RWP
	CFS tank cost	€	100,889	Based on 125,000 €·unit <sup>-1</sup> for 100 m <sup>3</sup> tank
B2 – CENTRIFUGATION	Mixer cost	€	8,071	Based on 10,000 €·unit <sup>-1</sup> for 100 m <sup>3</sup> tank
	Centrifuge cost	€	54,521	Based on 250,000 €·unit <sup>-1</sup> for 80.0 m <sup>3</sup> ·h <sup>-1</sup> centrifuge

These resulting performances suggest that the system could be efficiently adopted as a side-stream process for nitrogen removal from digestate, though effluent concentrations would not allow for the direct discharge of the treated digestate to the receiving water body. In both campaigns, a substantial proportion of the influent nitrogen was nitrified ( $80.6 \pm 37.0$  % in 2020 and  $60.0 \pm 31.8$  % in 2021), with high nitrification rates reaching an average value on the entire experimentation of  $29.9 \pm 10.2$  g N-NH<sub>4</sub><sup>+</sup>·m<sup>-3</sup>·d<sup>-1</sup>. Partial nitrification was observed, especially in 2020, as confirmed by the low concentrations of nitrate obtained (typically below 60 mg N·L<sup>-1</sup>). Only at the end of the 2021 trial, nitrification was almost complete, with an outlet concentration of nitrate over 200 mg N·L<sup>-1</sup> (i.e., up to 95% of the influent TAN), probably thanks to the combination of the higher ALK to TAN ratio in this year, and the milder competition between nitrifiers and microalgae, caused by the lower algal growth rate recorded in the same period. Nitrite accumulation in algae-bacteria systems treating anaerobic digestion effluents and other wastewaters has been previously observed in the literature [15,56], though a solid explanation of this phenomenon is still lacking. Partial nitrification is often attributed to the presence of FAN, that is formed in abundance at high pH, temperature, and TAN concentrations, which are common conditions in algae-bacteria systems. Free ammonia is indeed a strong inhibitor for the growth of autotrophic microorganisms, including microalgae and cyanobacteria [24,63], as well as AOB and NOB [64,65]. However, the maximum FAN concentration was always below 0.3 mg N·L<sup>-1</sup>, which is smaller than reported inhibitory levels for NOB [66]. Other factors, such as the competition for common nutrients may have played a role in shaping the nitrifying community. Inorganic carbon limitation is a possible explanation for the low NOB activity, as the digestate is characterized by an alkalinity shortage, making it difficult to store inorganic carbon in the bulk [16]. On the other hand, the tolerance to environmental conditions could have played a role in favoring AOB, as the temperature and DO concentration in the pond were closer to optimal values for AOB than for NOB [67].

On the overall, the system achieved high TAN removal efficiencies (Fig. 5C-D). During the experiments conducted in 2020, the TAN removal efficiency averaged  $84.5 \pm 7.8$  %, always being in the range 71–95 %, and a similar result was achieved in 2021, when the mean removal efficiency was  $85.4 \pm 8.1$  % (range: 68 – 100 %). The values obtained are consistent with previous work reported in the literature (Table 4). TAN removal was comparable, or even higher, than those reported in many previous studies describing the phyco-remediation of urban wastewater [48], digestates from municipal wastewaters [50,59], and digestates from agro-industrial wastewaters [14,56]. Only in a few cases, TAN removal reached higher values, mostly due to the combination of high HRTs and NLRs [52,53,57,62]. The calculation of oxygen production by microalgae and oxygen consumption by nitrifying bacteria also confirmed that the oxygen demand for TAN oxidation was mainly provided by photo-oxygenation and/or oxygen transfer, in complete absence of external aeration, allowing for a large energy saving and the reduction of operating costs for digestate nitrification (data not shown).

The trends in orthophosphate removal during the two monitoring campaigns are shown in Fig. 5C-D. The outlet PO<sub>4</sub><sup>3-</sup> concentrations were low (4–7 mg P·L<sup>-1</sup>), however it must be considered that the PLR was also quite low ( $0.72 \pm 0.13$  g P·m<sup>-3</sup>·d<sup>-1</sup> in 2020, and  $1.32 \pm 0.34$  g P·m<sup>-3</sup>·d<sup>-1</sup> in 2021), due to the low concentrations in the digestate. As shown in Fig. 5, the resulting removal efficiency was on average  $36.9 \pm 12.1$  % during 2020 (varying between 17.9 and 57.5 %), and  $35.8 \pm 18.7$  % during 2021 (with a wider range of variation of 6.5 – 79.1 %). In terms of PO<sub>4</sub><sup>3-</sup> removal rates, the system was able to remove an average of only  $0.31 \pm 0.29$  g P-PO<sub>4</sub><sup>3-</sup>·m<sup>-3</sup>·d<sup>-1</sup> in 2020, and  $0.52 \pm 0.44$  g P-PO<sub>4</sub><sup>3-</sup>·m<sup>-3</sup>·d<sup>-1</sup> in 2021. Therefore, it can be concluded that the algae/bacteria consortium was less efficient in P removal, as it is commonly reported in the literature (see Table 4), regardless of the reactor type, wastewater origin, and weather characteristics [55,59,60]. On the other hand, orthophosphate removal efficiencies obtained in some recent

**Table 6**  
Main design hypothesis and results for OPEX calculation.

SECTION	PARAMETER	UNIT	VALUE	REFERENCE / NOTES
GENERAL DATA	Cost of electricity	€·kWh <sup>-1</sup>	0.1171	Based on the average price for non-household consumers in Italy [34]
	Efficiency of paddlewheel motor	%	85	[29]
	Increased power consumption for CO <sub>2</sub> sparging	%	15	[29]
	Specific paddlewheel consumption	W·m <sup>-2</sup>	0.35	[29]
	Number of field operators	–	2	Assuming 5 RWP-operator <sup>-1</sup>
	Number of supervisors	–	1	Assumed
	Number of plant managers	–	1	Assumed
	Salary of field operators	€·h <sup>-1</sup>	13.1	Assuming a factor of 3 on a base salary of 4.4 €·h <sup>-1</sup>
	Salary of supervisors	€·h <sup>-1</sup>	18.8	Assuming a factor of 4.3 on a base salary of 4.4 €·h <sup>-1</sup>
	Salary of plant managers	€·h <sup>-1</sup>	29.3	Assuming a factor of 6.7 on a base salary of 4.4 €·h <sup>-1</sup>
	Work shift	h·d <sup>-1</sup>	6	Assumed
	Installed power for feeding, recirculation and harvesting pumps	kW	5.7	Calculated
	A1 – INOCULATION PONDS	Energy consumption for feeding, recirculation and harvesting pumps	kWh·y <sup>-1</sup>	37,615
Installed power for paddlewheels		kW	0.2	Calculated
A2 – CULTIVATION PONDS	Energy consumption for paddlewheels	kWh·y <sup>-1</sup>	1,233	Calculated
	Installed power for paddlewheels	kW	9.2	Calculated
B1 – COAGULATION, FLOCCULATION, SEDIMENTATION	Energy consumption for paddlewheels	kWh·y <sup>-1</sup>	60,423	Calculated
	NaOH required	kg·d <sup>-1</sup>	365	Calculated to reach 25 mM alkalinity in the digestate
	NaOH cost	€·kg <sup>-1</sup>	0.4	[16]
	Flocculant required	kg·d <sup>-1</sup>	5.5	Calculated
B2 – CENTRIFUGATION	Flocculant cost	€·kg <sup>-1</sup>	0.23	[71]
	Specific energy consumption for mixers	kWh·(m <sup>3</sup> ·d <sup>-1</sup> ) <sup>-1</sup>	0.0014	[29]
	Energy consumption for mixers	kWh·y <sup>-1</sup>	1,681	Calculated
B2 – CENTRIFUGATION	Specific energy consumption for centrifuge	kWh·m <sup>-3</sup>	1.3	[40]
	Energy consumption for centrifuge	kWh·y <sup>-1</sup>	65,689	Calculated

experiences were higher than those reported here [15,46,49], possibly due to the fact that, in these studies, PLRs were higher and the environmental conditions were more suitable for microalgae growth than in the current case study.

On the overall, it can be concluded that the results obtained in this study confirmed the suitability of the treatment system to efficiently remove ammoniacal nitrogen and valorise the chemical energy content of the digestate, while producing a biomass that is dominated by algal species typically rich in carbohydrates, lipids, and proteins [68], therefore being suitable for further valorisation. The variability of the effluent concentrations of nutrients, and especially the low removal efficiencies obtained for phosphates suggest that the system is mostly suitable for side-stream treatment at these latitudes, since low nutrient concentrations in the effluent cannot be guaranteed, and regulatory limits may be exceeded.

### 3.2. Techno-economic assessment and scenario analysis

Based on the pilot data provided in Section 2.3, a comprehensive techno-economic assessment was conducted, to evaluate the feasibility of scaling-up the process of microalgae cultivation on digestate by assessing costs of biomass production and of wastewater treatment. To calculate the savings of the WWTP, costs deriving from the oxidation of the TAN contained in the digestate and for phosphate removal were accounted on the baseline scenario. In the framework of the desirable implementation of a carbon-neutral economy, CO<sub>2</sub> compensation credits were also included. On the contrary, no revenues from biomass selling were considered, limiting the economic considerations to the biomass production only and allowing to identify the best option for biomass valorisation, according to the expected market price for different bio-products. The detailed CAPEX and OPEX costs resulting from the TEA of the baseline case are reported in Table 5 and Table 6, respectively.

#### 3.2.1. Baseline case study

By considering the expected average productivity based on the photosynthetic efficiency over the 274 d·y<sup>-1</sup> of plant operation (9.9 g TSS·m<sup>-2</sup>·d<sup>-1</sup>), a biomass production cost of 4.3 €·kg TSS<sup>-1</sup> is obtained (Table 7). This cost is comparable to the previous studies describing techno-economic assessments on microalgal cultivation in RWPs using wastewaters. For example, Al Ketife et al. [72] reported biomass production costs of 0.5 €·kg DW<sup>-1</sup>, when treating municipal wastewaters using *Nannochloropsis* sp., while a recent study comparing the economics of microalgal cultivation in different types of wastewaters in large-scale RWPs reported costs ranging from 0.4 to 0.8 €·kg DW<sup>-1</sup> [73]. Recently, a similar biomass production cost of 1.9 €·kg TSS<sup>-1</sup>, was reported for the treatment of piggery wastewaters in large scale farms, while at a lower scale, this cost increased up to 10.3 €·kg TSS<sup>-1</sup> [21]. It should be stressed that biomass production costs largely depend on the type of cultivation reactor considered, wastewater characteristics, climate, and available area, therefore a direct comparison is not straightforward [9,73]. For example, the production cost for open ponds are in the range 4.7 – 12.7 €·kg DW<sup>-1</sup> [74], while they raise to 98 – 610 €·kg DW<sup>-1</sup> for closed PBRs [75].

As shown in Fig. 6, the yearly cost is mostly due to OPEX (accounting for 285.2 k€·y<sup>-1</sup>, or 79.3 % of the yearly costs), with a minor contribution of CAPEX (74.4 k€·y<sup>-1</sup>, representing 20.7 % of yearly expenses). A more detailed analysis of the cost breakdown allows to highlight that the major contribution in terms of OPEX is given by labour, and overheads (42.8 % and 27.6 %, respectively), followed by materials, and maintenance (13.2 % and 7.3 %, respectively). In terms of CAPEX share, yearly costs mostly come from the investment depreciation (85.3 %), followed by the interests and land cost (6.8 % and 6.4 %, respectively). Regarding the overall benefits from nutrient and CO<sub>2</sub> removal (85.1 k€·y<sup>-1</sup>), nitrogen removal contributes to 81.2 % of the overall saving, followed by CO<sub>2</sub> fixation credits (11.6 %) and P removal (7.2 %).

In terms of digestate treatment, the cost for the proposed (Fig. 7)

Table 7

Summary of costs for the different scenarios identified from the sensitivity analysis (best-case: 20% increase in the productivity and 20% decrease in the OPEX and CAPEX; worst-case: 20% decrease in the productivity and 20% increase in the OPEX and CAPEX).

ITEM	PARAMETER	UNIT	BEST-CASE	BASELINE	WORST-CASE	REFERENCE / NOTES
DIRECT COSTS (DC)	Major equipment costs (MEC)	€	433,366	522,527	601,835	Costs for paddlewheels, concrete, liner, pumps, tanks, mixers, and centrifuge
	Installation costs	€	86,673	104,505	120,367	Calculated as 20 % of MEC
	Instrumentation and control	€	22,330	27,912	33,494	Equal to the costs for instrumentation and control
	Piping	€	18,163	18,163	18,163	Equal to the costs for piping
	Electrical	€	9,081	9,081	9,081	Equal to the costs for wiring
	Land improvement	€	33,671	42,089	50,507	Equal to the cost of civil works
	Service facilities	€	86,673	104,505	120,367	Calculated as 20 % of MEC
	Total DC	€	689,958	828,782	953,813	Calculated as the sum of DC
INDIRECT COSTS (IC)	Construction expenses	€	68,996	82,878	95,381	Calculated as 10 % of DC
	Engineering and supervision	€	130,010	156,758	180,550	Calculated as 30 % of MEC
OTHER COSTS (OC)	Total IC	€	199,006	239,636	275,932	Calculated as the sum of IC
	Contractor's fee	€	34,498	41,439	47,691	Calculated as 5 % of DC
CAPEX	MEC contingency	€	133,344	160,263	184,462	Calculated as 15 % of the sum of DC and IC
	Total OC	€	167,842	201,702	232,152	Calculated as the sum of OC
OPEX	Depreciation	€·y <sup>-1</sup>	52,840	63,506	73,095	Calculated as the sum of DC, IC and OC, divided by the lifetime of the plant
	Interest	€·y <sup>-1</sup>	4,227	5,080	5,848	Calculated as 8 % of the depreciation
	Property tax	€·y <sup>-1</sup>	571	686	789	Calculated as 1 % of the sum of depreciation and interest
	Insurance	€·y <sup>-1</sup>	342	412	474	Calculated as 0.6 % of the sum of depreciation and interest
	Land	€·y <sup>-1</sup>	3,801	4,752	5,702	Calculated as the cost of land divided by the lifetime of the plant
	Total CAPEX	€·y <sup>-1</sup>	61,782	74,435	85,907	Calculated as the sum of CAPEX
	Energy	€·y <sup>-1</sup>	16,083	20,104	24,125	Calculated from major equipment consumption
	Labour	€·y <sup>-1</sup>	97,706	122,133	146,559	Calculated as the sum of labour cost
	Raw materials	€·y <sup>-1</sup>	30,049	37,561	45,073	Calculated from mass balances
	Maintenance	€·y <sup>-1</sup>	13,868	20,901	28,888	Calculated as 4 % of MEC
SAVINGS	Operating supplies	€·y <sup>-1</sup>	148	231	332	Calculated as 0.4 % of the sum of electricity and raw materials
	Contingencies	€·y <sup>-1</sup>	3,606	5,634	8,113	Calculated as 15 % of the sum of raw materials
	Overheads	€·y <sup>-1</sup>	49,093	78,669	115,795	Calculated as 55 % of the sum of labour and maintenance
	Total OPEX	€·y <sup>-1</sup>	210,552	285,232	368,886	Calculated as the sum of OPEX
	Energy for nitrification	€·y <sup>-1</sup>	69,807	69,174	68,540	Calculated based on energy saved for nitrification
	P removal cost	€·y <sup>-1</sup>	6,096	6,096	6,096	Calculated based on phosphate removal efficiency
	CO <sub>2</sub> credits	€·y <sup>-1</sup>	11,849	9,875	7,900	Calculated based on the carbon credit
SPECIFIC COSTS	Total savings	€·y <sup>-1</sup>	87,753	85,144	82,535	Calculated as the sum of savings
	Biomass production costs	€·kg TSS <sub>1</sub> <sup>-1</sup>	2.4	4.3	7.4	Calculated based on the biomass productivity
	Digestate treatment cost	€·m <sup>-3</sup>	1.5	2.7	4.7	Calculated based on the treated digestate
	Nitrogen removal cost	€·kg N <sup>-1</sup>	7.0	12.5	21.1	Calculated based on the removed nitrogen

process is 2.7 €·m<sup>-3</sup>, that is well comparable to the results of Chaudry [73] who obtained treatment costs of 0.2–1.9 €·m<sup>-3</sup>, and of Ansari et al. [76], reporting a cost of 0.9 €·m<sup>-3</sup> for municipal wastewater treatment. Even if conventional wastewater treatment costs typically range from 0.1 to 0.5 €·m<sup>-3</sup> [77–79], these costs are strongly influenced by the specific influent composition and process configuration, so they are hardly comparable. When referring to a more robust indicator, such as the specific nitrogen removal cost, the proposed process seems to be a very promising technology with a cost of 12.5 €·kg N<sup>-1</sup>, which is similar to previously reported costs for piggery wastewater phyco-remediation, i.e., 4.3–23 €·kg N<sup>-1</sup> [21], and in the same range of conventional bioprocesses, typically ranging from 1.5 to 14.7 €·kg N<sup>-1</sup>, depending on the applied technology and influent characteristics [80,81].

### 3.2.2. Sensitivity and scenario analysis

The sensitivity analysis allowed to identify the parameters having the highest influence on the final biomass production cost. The variation of the productivity had a large impact on the final cost. Indeed, a 20 % increase in the productivity would result in a reduction of the production cost to 3.8 €·kg TSS<sup>-1</sup> (-13.3 % compared to the baseline scenario), while a 20 % decrease in the productivity would result in an increase of the cost up to 5.2 €·kg TSS<sup>-1</sup> (+19.7 %). On the other hand, increasing the total OPEX and CAPEX also had a strong effect of the biomass production cost, with the variation in total OPEX having more influence than the CAPEX. Indeed, a 20 % increase in the OPEX would result in a production cost of 5.6 €·kg TSS<sup>-1</sup> (corresponding to a 28.2 % increase),

while the same increment in the CAPEX would result in a cost of 4.7 €·kg TSS<sup>-1</sup> (i.e., a percentage increase of 7.6 %). When looking at the sensitivity results obtained decreasing the operational and capital expenditures of 20 %, the overall costs would reduce to 3.2 €·kg TSS<sup>-1</sup> and 4.0 €·kg TSS<sup>-1</sup>, respectively (representing a reduction of 25.7 % for the OPEX reduction, and 7.6 % when reducing the CAPEX).

Following the sensitivity analysis, two scenarios were considered in addition to the baseline case: i) a best-case scenario where a 20 % reduction in both OPEX and CAPEX are achieved, coupled to a 20 % increase in the productivity of the system, and ii) a worst-case scenario with a 20 % reduction in the productivity, coupled to the same increase for OPEX and CAPEX (Table 7). For the worst-case scenario, the biomass production cost can increase up to 7.4 €·kg TSS<sup>-1</sup>, while the digestate treatment cost and the specific nitrogen removal cost would reach 4.7 €·m<sup>-3</sup> and 21.1 €·kg N<sup>-1</sup>, respectively. On the contrary, in the best-case scenario, the biomass production cost decreases to 2.4 €·kg TSS<sup>-1</sup>, and the resulting digestate treatment and nitrogen removal cost drop to 1.5 €·kg m<sup>-3</sup> and 7.0 €·kg N<sup>-1</sup>, respectively. The results compare well to the most convenient biomass production costs reported to date in the literature [72,73,76,82,83]. This suggests that any effort in increasing the biomass productivity (e.g., by adopting mathematical growth models allowing to improve nutrient utilization or to better controlling the growth environment) would be compensated by a strong reduction in the production cost. In the same way, the related reduction of CAPEX and OPEX would have a very strong potential in spreading the promising phyco-remediation biotechnology, with the advantage of reducing

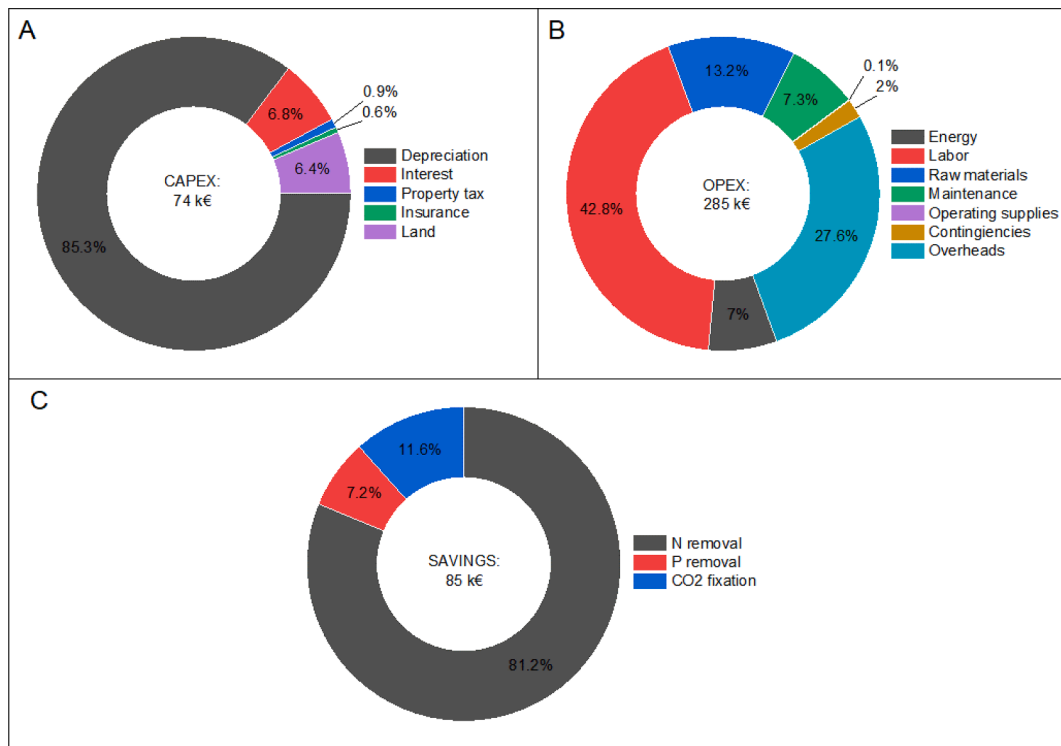


Fig. 6. Techno-economic assessment costs breakdown: A) CAPEX, B) OPEX, C) savings.

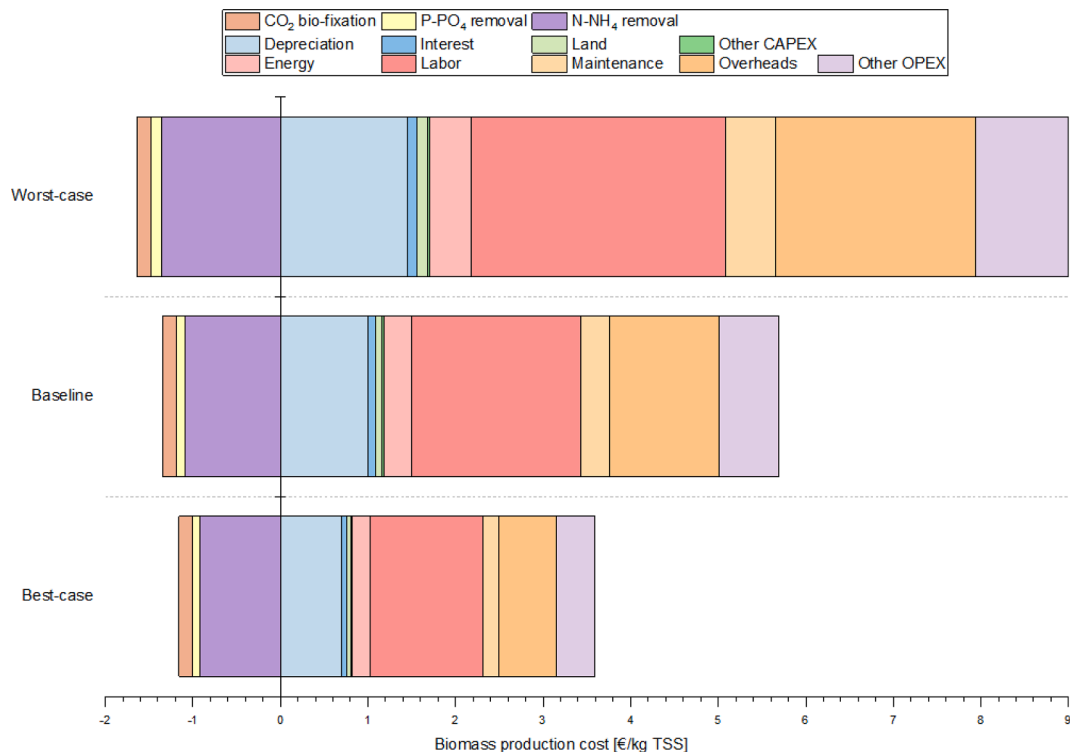


Fig. 7. Cost breakdown for the different scenarios identified from the sensitivity analysis (best-case: 20% increase in the productivity and 20% decrease in the OPEX and CAPEX; worst-case: 20% decrease in the productivity and 20% increase in the OPEX and CAPEX). "Other CAPEX" include: property tax and insurance; "Other OPEX" include: raw materials, operating supplies, and contingencies.

wastewater treatment costs and its environmental impacts, as well as promoting a circular and more sustainable utilization of the nutrients present in waste streams.

#### 4. Conclusions

In this work, a pilot-scale microalgae-bacteria system treating the liquid phase of digestate, located in Northern Italy, was operated side-stream within a municipal wastewater treatment plant, to collect performance data. The average biomass productivity was  $9.5 \pm 6.4 \text{ g TSS} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ , while TAN removal efficiency was  $85.1 \pm 7.9 \%$ . Referring to the overall wastewater treatment process, the microalgae-bacteria unit allowed to save a considerable amount of energy for nitrification, while producing valuable biomass to be valorised in a circular economy perspective. Based on the pilot-scale results, a comprehensive techno-economic assessment and sensitivity analysis was conducted to evaluate the costs and feasibility of scaling-up the process to 2 ha, which is the area required to treat the nutrients contained in the available digestate flowrate. The resulting biomass production cost is  $4.3 \text{ €} \cdot \text{kg TSS}^{-1}$ , thus allowing for its exploitation as biostimulants or other bioproducts generation. The related digestate treatment cost is  $2.7 \text{ €} \cdot \text{m}^{-3}$ , corresponding to a specific nitrogen removal cost of  $12.5 \text{ €} \cdot \text{kg N}^{-1}$ . These costs would eventually reduce of approximately 44 % under a best-case scenario, in which the productivity is increased, and both the OPEX and CAPEX are reduced by 20 %. The results suggest that scaling up the process makes its cost comparable to conventional bioprocesses. The results reported in this study provide an important contribution to evaluate the opportunity of integrating microalgae-bacteria cultivation in side-stream digestate treatment processes, in view of generating bioproducts at the industrial scale, while guaranteeing the valorisation of digestate nutrients.

#### Declaration of Competing Interest

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.

#### Data availability

Data will be made available on request.

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