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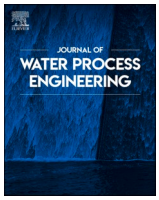

Utilization of native Chlorella strain in laboratory-scale raceway reactor for synthetic wastewater treatment: A study in batch and continuous modes with multi-substrate modeling

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ABSTRACT

Despite of the possibility to include microalgae in civil wastewater treatment process, the practice is still not common due to the lack of available instruments to implement it. In this study, a straightforward comprehensive approach for dealing with microalgal wastewater treatment involving an original kinetic model is proposed. A first set of batch cultures of a native strain of *Chlorella* was firstly carried out to obtain the kinetic parameters: maximum growth factor (μ_{max}) and half-saturation constant (K_s), for each limiting nutrient. Maximum growth factor values of 0.0279 for PO_4^{3-}, 0.0319 h^{-1} for NH_4^+, 0.0352 h^{-1} for glucose, and 0.0263 h^{-1} for the overall medium were found. Regarding the K_s, values of 1.08 mg L^{-1}, 27.70 mg L^{-1}, 1.34 mg L^{-1}, were found for PO_4^{3-}, NH_4^+ and glucose respectively, and a value of 2.8 % of the total nutrients for the overall medium. These parameters were used to set a multi-substrate kinetic model able to predict the growth in batch and in continuous operation within a laboratory scale raceway reactor. The removal capabilities of the microalgae for each addressed pollutant were evaluated in a batch system and in a continuous system at dilution rates of 0.0025, 0.005, 0.01 and 0.015 h^{-1}. This comprehensive approach represents a significant step towards addressing the continuous treatment of wastewater utilizing microalgae.

1. Introduction

During recent decades the human population has experienced remarkable growth, undergoing unprecedented demographic changes in a relatively short span of time. The intricate interplay between the increase of human population and the expansion of industrial activities has intensified the adverse consequences for the environment, leading to contamination of freshwater bodies, soil degradation, and the release of pollutants. In response to these pressing global challenges, the remediation and responsible management of wastewaters have assumed an extremely important significance in the areas of environmental science, policy-making, and public consciousness. Several methods for eliminating specific compounds were proposed before [1].

Microalgae may play an important role in the remediation of wastewaters [2–5], both civil and industrial originating from different processes [6–8]. They represent a diverse group of photosynthetic microorganisms which includes a vast range of species across different phyla. Microalgae also exhibit the remarkable ability to grow in diverse types of wastewater and efficiently remove nitrogen and phosphorous from these environments [9]. This feature makes them a viable option for integration into traditional wastewater treatment plants. The conventional wastewater treatment process typically comprises three stages: a primary treatment in which sand and debris are removed from the wastewaters, a secondary treatment in which an aerobic biological depuration is obtained through oxidation ponds and a tertiary treatment which usually decreases the concentration of pollutants such as nitrogen and phosphorous. The core of the process lies in the secondary treatment phase, where heterotrophic bacteria utilize oxygen to ferment organic carbon. The employment of microalgae during secondary or tertiary treatment of different kinds of wastewater has been proposed, mainly because of their ability to contribute to the degradation of polluting compounds [10,11]. In oxidation ponds, microalgae may operate in synergy with the activated sludge [12]. Microalgae and bacteria may have diverse relationship (mutualism, commensalism, parasitism and antagonism) and they may interchange substances and genes [13]. The presence of bacteria in the culture could inhibit the microalgal growth,

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in a competitive mechanism, but often they constitute consortia in which each specie has a specific role [14]. Precisely, in addition to nitrogen and phosphorous removal, the use of microalgae in wastewater treatment offers several advantages, including oxygen production for bacterial degradation of matrix compounds [15] and CO_2 removal from the atmosphere through photosynthesis. Importantly, the utilization of microalgae for oxygenation in oxidation ponds has been shown to be more cost-effective than traditional methods [16]. Microalgae may perform bioremediation of several kind of molecules by using several degradation mechanisms as recently summarized by Abdelfattah et al. [17]; in biosorption, a passive process, negatively charged molecules in microalgal biomass interact with various kind of positively charged pollutants, including heavy metals. In bioaccumulation, an active process, the pollutants from biosorption process enter the cells with an associated energy consumption and may be metabolized by the microorganism. In biodegradation, complex compounds are degraded in simpler building blocks; this mechanism involves a cascade of enzymes and is a multi-step process.

Another advantage of employing microalgae for wastewater treatment is that the biomass obtained after the treatment may accumulate high quantities of high-value compounds [10]. Additionally the debris of the process may be employed in alternative valorisation routes [18], thus integrating in a bio-refinery system.

Although the literature has provided examples of microalgae-based wastewater treatment plants at both lab-scale and pilot-scale [19–21], their industrial-scale adoption has been limited due to the lack of reliable and reproducible parameters that significantly influence growth, nutrient removal, and system scalability. Consequently, there is a pressing need for reliable tools which may help in the design and optimization of reactors for industrial operations. In the case of treating municipal sewage, outdoor reactors known as raceway ponds are typically employed [22].

In order to understand and predict wastewater treatment process and operate it within the desired conditions, kinetic models are a valuable instrument. Various attempts to model microalgal wastewater treatment have been done, but often they result in complex models with several inputs, which may be complex to apply. Recent examples are reported for wastewater remediation by *Desmodesmus* sp. and *Scenedesmus obliquus* and by *Chlorella vulgaris* [23,24]. Essential kinetic parameters are needed also for designing and appropriately sizing the raceway reactors, such as the maximum specific growth rate (μ_{max}) and the substrate constant (K_s). Typically, these parameters are derived from batch experiments in which microalgae are inoculated in a photobioreactor, with an initial known quantity of nutrients. In these experiments, the cellular concentration increases over time while the nutrients concentration decreases. The cellular growth rate for each batch culture in combination to the initial substrate concentration, when appropriately treated, gives information about kinetic parameters. The kinetic parameters may be used to appropriately size continuous systems, i.e. where the wastewater stream is continuously fed to the culture. It is worth noting that continuous systems are the ones predominantly utilized in industrial settings, with consequential practical applications. Cellular growth may be described by recurring to growth models. While the Monod equation is a widely used kinetic model for single-limiting-substrate situations [25], few examples exist of multi-substrate models applied to microalgal wastewater treatment [23,26]. Recent research by Ribeiro Lopes et al. proposes a procedure to develop a kinetic model to assess wastewater treatment by microalgae by a multi-substrate model considering organic carbon, nitrogen and phosphorous. Differently from the present research, the procedure comprehends more complex modeling (n-th order model for contaminant removal, Monod model for cellular growth), in addition to the application of a specific algorithm, making its application complex [27].

In this work, we propose an approach to study and predict the wastewater treatment by microalgae, using an autochthonous microalgal strain, present in the Mediterranean sea, that was previously isolated and characterized [28]. This approach is simple and involves obtaining of kinetic parameters from batch cultivation within Erlenmeyer flasks and applying them in an original approach for a multi-substrate model with the aim of addressing the main pollutants contained in the wastewater considered. The practical steps of the process and the original model are presented. The proposed strategy appeared very promising, and future research could explore its combination with tools tailored for co-cultivation of heterotrophic bacteria for even more effective wastewater treatment.

2. Material and methods

2.1. Microalgal growth

Microalgae *Chlorella* sp. *CW2*, previously isolated and characterized [28], was grown in a synthetic wastewater with the composition indicated in Table 1:

The synthetic growth medium was mixed by means of magnetic stirring before sterilizing. A pre-culture was prepared for each sample by inoculating 10 mL of sample from a stock culture in 100 mL of synthetic sewage. When cells were in late exponential phase (typically after about 8 cultivation days), 10 mL of the cell suspension were taken to inoculate the main culture in the sewage medium. 150 mL of culture were grown in 250 mL Erlenmeyer flasks placed in an oscillating incubator (Corning Lse) under a 200 μmol m^{-2} s^{-1} photon flux at 27 °C. Light intensity was measured with a Delta Ohm-HD 9021 quantometer equipped with a Photosynthetic Active Radiation (PAR) probe (Delta Ohm LP 9021 PAR). During the cultivation period, which lasted for 3 days, the concentration of the microalgal suspension was regularly monitored at least 2 times a day. This was achieved by measuring the absorbance of the culture at 750 nm using a spectrophotometer (Cary 60 UV–Vis, Agilent Technologies). An experimental correlation between the dry weight (DW) and the optical absorbance at 750 nm was obtained as follows: $DW=0.2315*Abs_{750nm}+0.0264$, where DW is the concentration of microalgae in g L^{-1} and Abs_{750nm} is the absorbance at 750 nm. The R^2 was 0.9897.

To obtain the kinetic parameters, microalgae were cultivated in biological triplicate using 250 mL Erlenmeyer flasks. The employed growth medium was obtained from the original synthetic wastewater recipe with varying concentrations of glucose from 2.2 mg L^{-1} to 300.0 mg L^{-1} (series GLUC), phosphate from 0.6 mg L^{-1} to 56.4 mg L^{-1} (series PO_4^-), ammonium from 9.6 mg L^{-1} to 153 mg L^{-1} (series NH_4^+). Additionally, another series of cultivations was carried out by growing the microalgae in diluted media. This dilution process was conducted gradually, starting from the original 100 % limiting nutrients concentration and decreasing down to 0.5 % (series STD). Each different dilution level was set-up in triplicate. By conducting these cultivations, we aimed to examine the effect of nutrient limitation on microalgae

Table 1
Composition of the synthetic wastewater used for the cultivation of Chlorella sp. CW2.

Component	Concentration [mg L^{-1}]
Glucose	221.7
NH_4Cl	153.0
$NaNO_3$	160.0
$CaCl_2{\cdot}(2H_2O)$	165.0
Na_2SO_4	63.37
K_2HPO_4	56.37
$NaHCO_3$	200.0
EDTA (Na_2 salt)	6.37
$FeCl_3{\cdot}(6\ H_2O)$	4.85
$CuSO_4{\cdot}(5\ H_2O)$	0.02
$ZnSO_4{\cdot}(7\ H_2O)$	0.03
$CoCl_2{\cdot}(6\ H_2O)$	0.02
$MnCl_2$ (4 H_2O)	0.28
Na_2MoO_4 (2 H_2O)	0.01

growth and to explore how their growth behaviour responds to different levels of nutrient availability. A summary scheme of the employed growth conditions is provided in Table 2.

The original growth medium was instead applied for the cultivations in the raceway reactor and for a single cultivation performed in triplicate within shaking flasks realised to validate the growth model.

2.2. Technique of analysis on the synthetic sewage

For the measurement of pH and conductivity in the original medium, a pHmeter equipped with a conductivity probe was employed (CRISON MM 41).

The content of carbon and in particular Inorganic Carbon (IC) and Non-Purgeable Organic Carbon (NPOC), was assessed by a TOCL CSH/CSN analyzer Shimadzu.

The content of glucose was analysed through High-performance liquid chromatography (HPLC). The suspension was filtered through 0.45 μm membranes (CA, Millipore) and sugars were analysed by means of a HPLC Dionex UltiMate 3000 equipped with a column Rezex ROA – Organic acid H^+ operating at 60 °C and using 0.6 mL min^{-1} of a 5 mM H_2SO_4 aqueous solution as eluent. The ion (phosphate and ammonium) content was analysed through ionic chromatography (IC, Metrohm 882 Compact IC plus). For the anionic chromatography, a Metrosep A Supp 5 was employed using a 0.7 mL min^{-1} of a Na_2CO_3 3.2 mM and $NaHCO_3$ 1 mM aqueous solution. A Metrosep C4–250/4.0 was employed for the cationic chromatography using a 1 mL min^{-1} of a H_3PO_4 5.5 mM aqueous solution. For each analysis, the sample was adequately diluted and filtered through a CHROMAFIX cartridge packed with C18 silica in order to remove all the organic content. Lastly, for cultivations in the raceway reactor, online sensors were used with the controller WTW MIQ/TC 2020 3G. For the analysis of turbidity, pH, oxygen concentration and temperature the sensors FDO 700 IQ, SensoLyt 700 IQ, ViSo-Turb 700 IQ were respectively employed. The turbidity was converted in cellular concentration X (mg L^{-1}) through a calibration curve for the sake of clarity.

2.3. Set-up of the raceway reactor

A raceway laboratory reactor was constructed using 5 mm thick-polymethyl methacrylate (PMMA) plates. As shown in Fig. 1, the reactor had a length of 780 mm and a width of 200 mm. To facilitate mixing and circulation, a paddle wheel, motor-driven with adjustable velocity, was installed on one side of the reactor. The operational volume of the reactor ranged from 1L to 13 L; during long-time operation the volume was daily adjusted by adding water. The system was enlightened under a 200 μmol m^{-2} s^{-1} photon flux and the temperature of the room was kept constant at 27 °C.

According to Sompech et al. and to Belay [29,30], maintaining the flow velocity in a range of 0.2 m s^{-1} to 0.6 m s^{-1} is sufficient to ensure turbulence and prevent the sedimentation of algal cells. For this reason, the impeller velocity was consistently set at more than 45–50 g min^{-1}, corresponding to a flow velocity of 0.3–0.4 m s^{-1}. The Reynolds number within this velocity range was determined based on the calculations outlined in [29] with some modifications, as:

Table 2
Schematic representation of growth conditions utilized in batch experiments to determine kinetic parameters under varying nutrient limitations.

Series	Limiting nutrient	Concentration tested	Unit
GLUC	Glucose	6.7, 13.3, 22.2, 222.0, 300.0	ppm
PO_4^-	Phosphate	0.6, 1.7, 3.4, 5.6, 25.0, 56.4	ppm
NH_4^+	Ammonium	9.6, 15.0, 20.0, 65.0, 153.0	ppm
STD	Glucose+phosphate+ammonium	0.5, 1.0, 10.0, 35.0, 70.0, 100.0	%

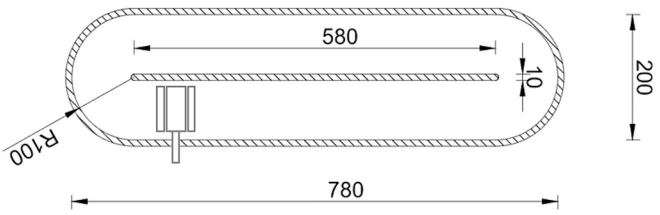

Fig. 1. Raceway pond top view.

$$Re = \frac{\rho u R_h}{\mu} \quad (1)$$

where 'ρ' (kg m^{-3}) is the fluid density, 'u' (m s^{-1}) is the superficial velocity in the channel, 'R_h' (in m) is the hydraulic radius of the channel and 'μ' (Pa s) is the fluid viscosity.

This resulted in $Re = 10{,}300$, meeting the objective of achieving full turbulence with a Reynolds number greater than 8000 [31].

Furthermore, the mixing time inside the raceway reactor was measured by introducing a 0.1 M NaCl solution in a specific spot of the raceway reactor and measuring the stabilization of the signal with a conductometer (WTW, Profiline Cond 3310). As shown in Fig. S1 of supplementary material, it resulted in about 12 s.

Given that, during continuous operation, the applied dilution rate (D), i.e. the ratio between the inlet flow rate and the reactor volume, ranged from 0.0025 to 0.015 h^{-1}, leading to an average residence time (τ) ranging from 400 to 66.7 h, the mixing time was significantly lower than τ. This suggests that the reactor can be characterized as truly operating under perfectly mixed conditions.

In order to vary the dilution rate, a different inlet flow rate was applied to the reactor, that was respectively 0.5, 1, 2 and 3 mL min^{-1}, leading to a dilution rate (D) of 0.0025, 0.005, 0.01 and 0.015 h^{-1}. The inlet flow rate was regulated by a peristaltic dosing pump (Seko Kronos 50, Rieti, Italy).

2.4. Determination of the kinetic parameters

To determine the kinetic parameters, μ_{max} and K_s, for the species *Chlorella* sp. grown into shaking flasks under different limiting nutrients, an experimental approach was employed to obtain kinetic parameters from batch data. The specific growth rate μ may be expressed by the Monod equation as follows:

$$\mu = \frac{\mu_{max} S}{K_s + S} \quad (2)$$

where S is the concentration of nutrient, and μ_{max} and K_s are the kinetic parameters. This equation describes the relationship between the concentration of limiting substrate and the specific growth rate μ.

The specific growth rate μ was calculated while the cells were in exponential phase, by using the following expression:

$$\mu = \frac{lnX - lnX_0}{t - t_0} \quad (3)$$

where X_0 is the biomass concentration at time t_0 and X is the biomass concentration at time t.

The kinetic parameters were estimated through a Langmuir Plot [32]. To obtain the kinetic parameters, μ_{max} and K_s, Eq. (2) can be rewritten according to Langmuir [25] as:

$$\frac{S}{\mu} = \frac{1}{\mu_{max}} S + \frac{K_s}{\mu_{max}} \quad (4)$$

A Langmuir plot of S/μ versus S should result in a straight line, and the kinetic parameters can be obtained from its slope ($1/\mu_{max}$) and intercept (K_s/μ_{max}).

To obtain the kinetic parameters of the Monod equation, batch cultivations were conducted in synthetic sewage with varying nutrient concentrations, as detailed in paragraph 2.3.

2.5. Modeling

An approach considering the nutrient concentration over discrete time intervals was used to model cell concentration within batches of cultures cultivated both in Erlenmeyer flasks and raceway reactors. Within each interval, the formula

$$X = X_i e^{(\mu - kd)\Delta T} \tag{5}$$

was applied, where X is the cells concentration (g L^{-1}), X_i represents the initial cell concentration for the specific time interval, μ is the growth factor at the prevailing nutrient concentration of that time interval (h^{-1}), kd is the death factor and ΔT is the duration of the time interval (h). For each time interval, the specific growth factor μ was calculated using a modified version of a multi-substrate Monod model previously proposed by Durruty and co-workers [33] as follows:

$$\mu = \frac{1}{3}\left(\frac{\mu_{maxNH_4^+}}{K_{maxNH_4^+} S_{NH_4^+}} + \frac{\mu_{maxPO_4^{3-}}}{K_{PO_4^{3-}} S_{PO_4^{3-}}} + \frac{\mu_{maxgluc}}{K_{gluc} S_{gluc}}\right) \tag{6}$$

where $\mu_{max[i]}$ and $K_{max[i]}$ are the relevant parameters of each nutrient calculated as outlined in Section 2.4 and S_{NH4+}, S_{PO43-} and S_{gluc} are the concentrations of each nutrient during the considered time interval.

To model the cell concentration (X) and the cell productivity (Qx) in continuous cell culture, the following equations, according to Doran [25], were applied:

$$X = Y_{xs}\left(S_i - \frac{DK_s}{\mu_{max} - D}\right) \tag{7}$$

$$Q_x = X\,D \tag{8}$$

where Y_{xs} is the biomass yield from substrate and S_i is the initial substrate concentration. These last equations used the kinetic parameters derived from the STD series (Table 1).

3. Results and discussion

3.1. Kinetic parameter determination through batch cultivations in shaking flasks

To determine the kinetic parameters, four series of batch cultivations were conducted within Erlenmeyer flasks for 3 days at a fixed temperature and light intensity (27 °C and 200 μmol m^{-2} s^{-1}, respectively). These cultures were set up in biological triplicate to ensure accuracy and reliability. The growth trend of the microalgae *Chlorella* sp. *CW2* is depicted in Fig. 2. Temporal evolution shows typical growth curves, exhibiting in all the experiments an exponential growth phase followed in some cases by a lag phase. The curves highlight the influence of varying nutrient concentrations on the growth dynamics. In general, the algae exhibited more robust growth when the limiting nutrient concentration was elevated, while it progressively diminished as the nutrient concentration decreased. It's worth noting that the STD series (Fig. 2 d) was designed by cultivating the microalgae in progressively diluted nutrient media. This series started with the original 100 % nutrient concentration and gradually decreased to a mere 0.5 %. Notably, in the series of cultures focused on glucose (referred to as the GLUC series) (Fig. 2 a), where glucose acted as the limiting nutrient, the growth patterns among varying concentrations were relatively more comparable than those of other cultivation series, in which a marked and progressive divergence in growth patterns was observed as nutrient concentrations ranged from higher to lower levels. This is reflected also in the difference in growth rate μ for each of the cultivations.

As described in paragraph 2.4, by using the calculated growth rate in each of the cultivations, a Langmuir plot was obtained for each cultivation series. In this way, the kinetic parameters, maximum growth factor μ_{max} and half-saturation constant K_s, were calculated for each limiting nutrient. They are plotted, together with the measured growth rates μ, for each cultivation series in Fig. 3.

As depicted in the Fig. 3, the maximum specific growth rate (μ_{max}) values resulted in 0.0279, 0.0319, 0.0352, and 0.0263 h^{-1} for the

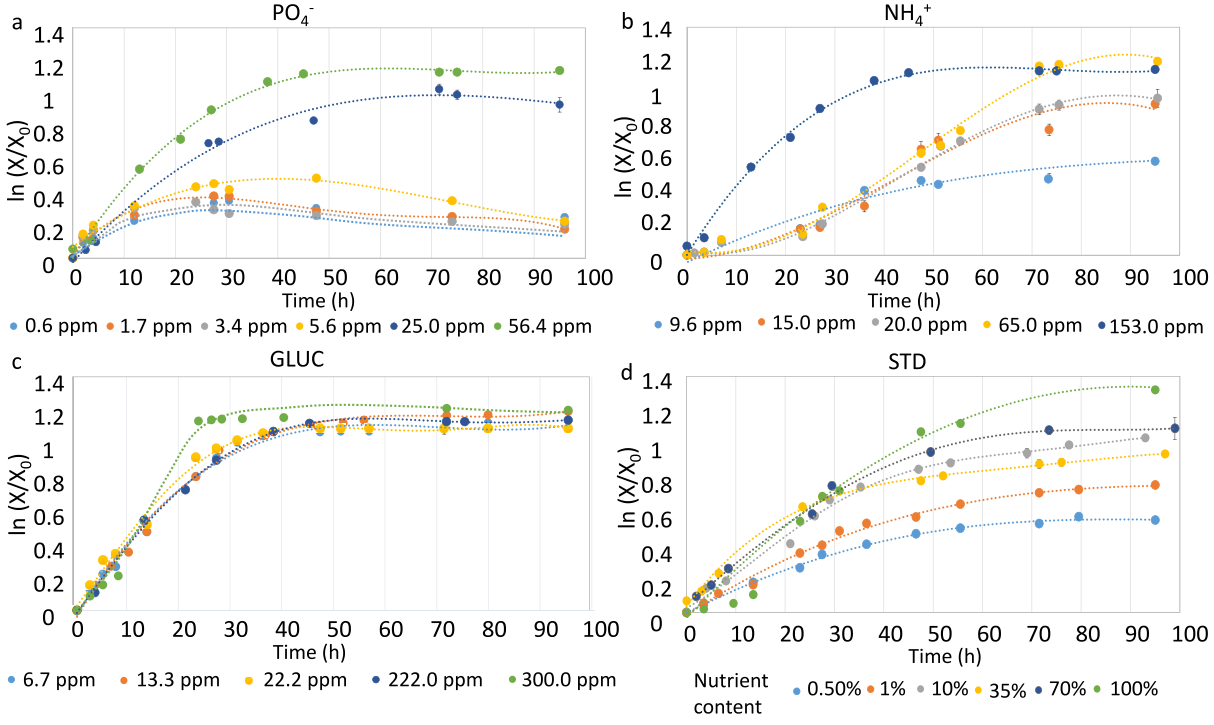

Fig. 2. Growth of microalga Chlorella sp. CW2 at 27 °C in different limiting nutrients concentrations.

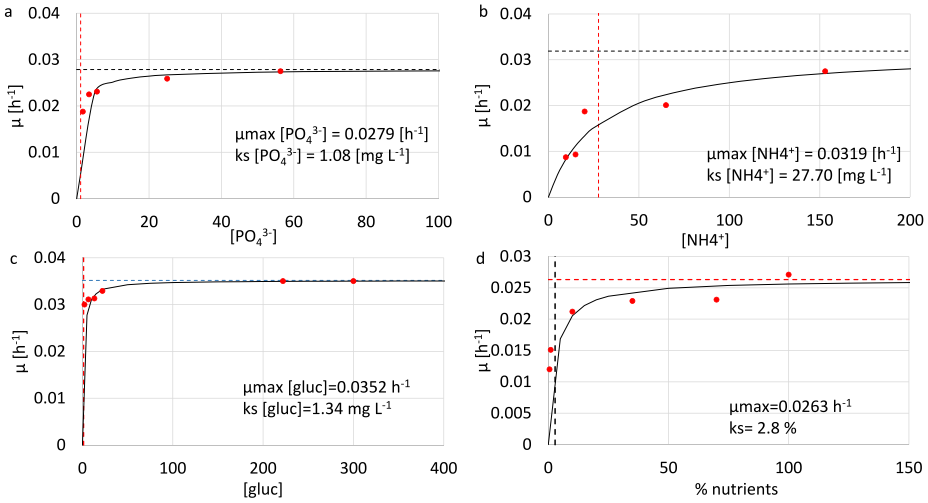

Fig. 3. Specific growth rate versus different limiting nutrient concentrations of the microalga Chlorella sp. CW2. red circles, experimental data; line, Monod equation.

respective series of PO_4^{3-}, NH_4^+, Gluc, and STD. These results perfectly fall within the range observed by other researchers [34–36], indicating consistency with existing studies. It is worth noting that the μ_{max} calculated for the STD series matches with the one calculated for the PO_4^{3-} series, which is also the lowest among the others. In fact, for the STD series (Fig. 3 d), the calculated μ_{max} cannot surpass any of the other values.

It is interesting to note the similarities between the approach used in this study and that of De Bhowmick et al. [34]. Both studies used similar methods, and De Bhowmick et al. also noticed changes in the growth parameters of *Chlorella minutissima* depending on the different combinations of elements present in synthetic wastewater [34]. Sforza et al. estimated similar values of μ_{max} by using a respirometric approach on *Chlorella protothecoides* in a synthetic medium [35]. For *Chlorella vulgaris* cultivated in sago wastewater, Vishnu Priya et al. estimated growth rates using various models, and the range we found in our study matches their results [36].

Additionally, in this study we calculated the half-saturation constants for the various limiting nutrients from the Langmuir plot as described by Doran [25]. The calculated values (see Fig. 3) were 1.08 mg L^{-1} for PO_4^{3-}, 27.70 mg L^{-1} for NH_4^+, 1.34 mg L^{-1} for Gluc, and 2.8 % of the total nutrients in the STD series. Interestingly, half-saturation constant for phosphate matches that found previously by Sforza et al. for phosphorous. Similarly, the value for ammonium falls within the same order of magnitude as their result for nitrogen. It's worth noting that the observed discrepancy in the half-saturation constant values could be attributed to the distinction between ammonium specifically and nitrogen as a whole in the cited study [35]. By observing the data, it appears evident that the microalgae used in this study, exhibits lower half-saturation constants in relation to glucose and phosphate when compared to ammonium. This suggests that a higher quantity of ammonium is needed to support the cellular process. In fact, nitrogen, which generally ranges from 1 to 10 % of cell dry weight, is an essential constituent of all proteins in algal cells. Consequently, the demand for nitrogen is greater than that for phosphorous, which, under optimal conditions, can accumulate up to 1 % of the dry weight [37]. Furthermore, a lower half saturation constant indicates that the microorganism can achieve a high growth rate even when nutrient concentrations are low; this trait, particularly evident for glucose and phosphate in this algae, provides a competitive advantage for this particular microorganism, which was isolated from active sludges of a municipal sewage treatment plant [28]. This adaptation likely helped it compete effectively with other organisms in its environment.

In conclusion, the data derived from the conducted experiments may be employed as kinetic parameters to enhance models which describe the microalgae growth, in particular of the *Chlorella* genus, and the removal capacity of the main nutrients, which are ammonium, glucose and phosphorous. This predictive capability holds significant potential for estimating the outcomes of microalgae cultivation in wastewater environments. This includes assessing both the production of biomass and the efficiency of nutrient removal.

3.2. Laboratory-scale raceway reactor batch cultivation and modeling

The data obtained from the cultivation within the laboratory-scale raceway reactor in batch conditions are shown in Fig. 4. The figure presents information about cellular concentration, oxygen concentration and pH evolution. The cellular concentration (X) showed an initial lag phase followed by a subsequent increase connected to the exponential phase. The cultivation was extended enough to observe a stationary phase subsequent to the exponential phase. The amount of algae produced during the batch cultivation falls in the range founded by other researchers when cultivating *Chlorella* in similar conditions [38,39]. Simultaneously with the rise of cellular concentration, an increase of the oxygen concentration is observed, caused by the photosynthetic activity of the cells. At the same time, a slow decline of the pH is observed starting from day 7 of cultivation from pH = 7 to pH = 6. This decrease is typical when ammonia is used as source of nitrogen: the pH could drop significantly during active growth due to the release of H^+ ions [37]. A similar occurrence was observed in the treatment of real swine wastewaters with *Chlorella,* where ammonia was used as nitrogen source [38].

Data about the concentration evolution of pollutants during batch cultivation, depicted in Fig. 5, provide important insights about the efficiency of the wastewater treatment. They reveal that the overall efficiency of abatement is satisfactory, as indicated by the reduction of phosphate, inorganic carbon (IC), ammonium and glucose by −73 %, −91 %, −77 % and −100 % respectively. The abatement of nutrients is comparable to what found by other researchers before [39,40].

However, the reduction of organic carbon (referred to as NPOC) is not as effective. This can be due to the production of certain organic compounds by the algae themselves which are released into the growth medium. This phenomenon was previously observed in another study where the chemical oxygen demand (COD) of an actual civil sewage, in which microalgae were cultivated, increased [21]. In the current research we extended this observation by analysing the IC and the NPOC. This allowed to distinguish between the contributions of inorganic and organic carbon. In fact, inorganic carbon (due to the presence

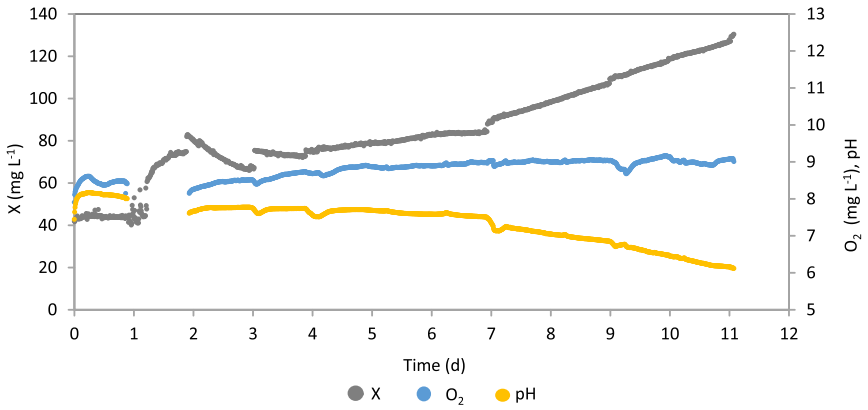

Fig. 4. Variations in cellular concentration (X), oxygen concentration (O_2) and pH trends during batch cultivation within the laboratory-scale raceway reactor.

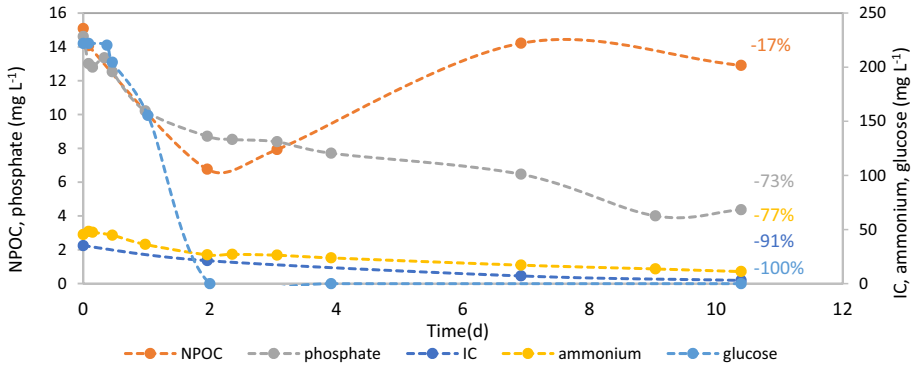

Fig. 5. Nutrients removal during batch cultivation in the raceway laboratory reactor. NPOC stands for Non-Purgeable Organic Carbon; IC stands for Inorganic Carbon.

of bicarbonate in the growth medium) is efficiently reduced, while organic carbon is not. Given that glucose, which contributes to the organic carbon, is completely depleted after just 2 days of cultivation, and considering that no other forms of organic carbon were introduced into the medium, we may conclude that organic compounds were produced by the microorganisms themselves. This highlights the effectiveness of combining sewage treatment via algae with heterotrophic bacteria. These bacteria, in fact, can utilize the organic compounds released by the microalgae into the medium and are supported by the oxygen produced by the algae. This approach was already employed with success before [28,40].

The cellular concentration data from the batch cultivation were modelled following the approach outlined in paragraph 2.5. Briefly, the concentration of each nutrient was considered over discrete time intervals to model cell concentration. This was achieved by employing a specific growth factor μ calculated for each time interval by using Eq. 6, which employed the kinetic parameters derived for each nutrient as described in paragraph 3.1. It is important to highlight that, unlike the Monod equation, the suggested model is a multi-substrate model. While numerous multi-substrate models exist [41], they tend to be complex. In contrast, the proposed model is straightforward and user-friendly for application in all scales. The modeling approach was applied to the batch growth within the raceway reactor and to the batch growth that occurred in Erlenmeyer flasks, both employing the standard version of the synthetic sewage. The results of Eq. 5 and the fitting of the experimental data are shown in Fig. 6.

The fitting of the experimental data derived from the shaking flask cultivation displays a high degree of accuracy. In contrast, the fitting of the other batch cultivation conducted within the raceway reactor exhibits a slightly diminished performance. This variance could derive from the kinetic parameters being determined within the shaking flasks, consequently leading to more accurate predictive outcomes in that

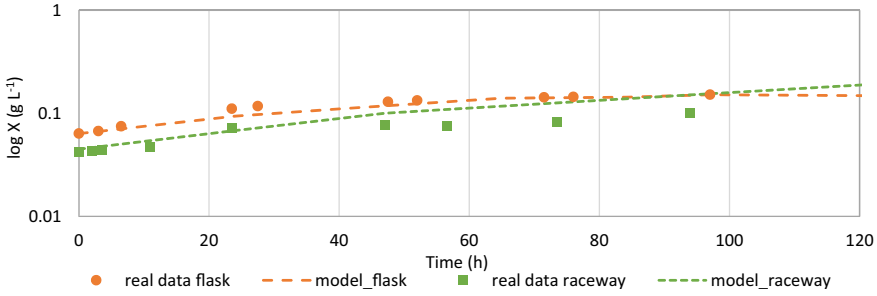

Fig. 6. Comparison between model prediction (Eq. 5 with kd = 0.3 μmax) and experimental data of Chlorella sp. CW2 growth curves in batch raceway reactor and shaking flask: "orange" shaking flask, "green" raceway photobioreactor. Dotted lines, model predictions; symbols, experimental data.

context. It's plausible that the algae's kinetics are slightly reduced in the raceway reactor, possibly due to inefficiencies in mass transfer or in light distribution, or to the not-axenic conditions. Another aspect to consider is that, while the model excels in predicting the exponential phase growth, it becomes less reliable as it approaches the stationary phase. This phenomenon has been previously observed by other researchers [42]. Another possible explanation for this behaviour could be attributed to a decrease in measured biomass due to precipitation. Specifically, when the algae concentration inside the reactor increased, it can lead to flocculation events. It seems that the reactor's circulation velocity may not be adequate considering the algae agglomerates, resulting in precipitation occurrences.

3.3. Continuous cultivation in laboratory raceway system and model

The data obtained from the cultivation within the laboratory-scale raceway reactor during continuous cultivation are shown in Fig. 4. The figure presents information about cellular concentration, oxygen concentration and pH evolution in different dilution rate conditions. It's important to highlight that the selection of the specific dilution rates applied to the reactor was not arbitrary, but it was based on the maximum global specific growth rate (μ_{max}) derived from Eq. 6 as described in paragraph 3.1. Notably, the cellular concentration decreased as the dilution rate increased, in line with the expectations. This phenomenon is due to the fact that, with a constant reactor volume, an increase in the dilution rate results in higher inlet and outlet flow rates, leading to the convective removal of cells from the reactor. As a matter of fact, when the convective kinetics surpass the growth kinetics ($D > D_{crit}$), a washout occurs, causing all the cells to be carried out from the reactor. However, in our specific case, this did not occur, as the applied dilution rate (D) remained significantly below the critical D value. Similar findings were obtained by other researchers [43]. Recently, Gonzalo Ibrahim et al. observed that under a hydraulic retention time of 3 days, equivalent to a dilution rate of 0.0138 h^{-1}, an indoor microalgae-based wastewater treatment system did not experience cells washing out, with a value similar to what found in the present work [44]. In continuous cultivation at a steady state, the critical dilution rate (D) value is very close to μ_{max} [25]. In this particular case, the calculated μ_{max} was 0.0263 h^{-1} (Fig. 3 d). Consequently, the chosen operating conditions were set below this value to ensure stable continuous cultivation. Lastly, as it can be seen in Fig. 7, no significant alterations were observed in the evolution of oxygen concentration and pH levels, which ranged between 7 and 8 for all the duration of the experiment.

In Fig. 8, data about the concentration evolution of pollutants are presented for continuous cultivation under varying dilution rates (D). As it can be seen, the evolution of inorganic carbon, ammonium, and phosphate exhibits a consistent pattern, with concentrations increasing as the dilution rate (D) increases. This trend can be readily explained by the lower concentration of cells within the reactor at higher D values. Consequently, for these pollutants, the most effective abatement of −63 % for inorganic carbon, −54 % for phosphate, and − 75 % for ammonium are achieved at the lowest D value of 0.0025 h^{-1}. In contrast, the organic carbon (NPOC) shows an inverse relationship, with its concentration decreasing as D increases, in concordance with the quantity of cells present in the reactor. This phenomenon further supports the notion that the cells produce certain organic compounds that are released into the growth medium. As concerns the glucose, it demonstrates effective reduction under all the tested dilution rates. Similar observations are reported by other researchers with a similar approach to the treatment of an anaerobic effluent by an indigenous strain of *Chlorella* [45].

Fig. 9 displays the average cellular concentration (X) and average cellular productivity (Qx) under the four different dilution rates tested and the proposed model predictions. As previously observed in Fig. 7, it is evident that the cellular concentration X decreased when dilution rate D increased. At the same time, cellular productivity, given by Eq. (8), increased with an increasing D. These trends are in line with the expectations set by the model represented in Fig. 9 by the dotted lines. This model is described by Eq. (7) and employs the kinetic parameters derived from batch cultivation of the series STD, which are shown in Fig. 3d. While the model may not precisely predict values for the higher tested dilution rates, it effectively captures the overall trends. Any deviations in the predictions could be attributed to variations in the cultivation conditions between the raceway laboratory reactor and the Erlenmeyer flasks, as discussed earlier. Notably, the kinetic parameters were calculated based on data obtained from Erlenmeyer flask cultivations, and differences between the two cultivation methods may arise from variations in factors such as light irradiance distribution and mass transfer kinetics.

Considering the relatively slow growth kinetics involved in the process, it is clear that lower dilution rates are most suitable for operating raceway reactors with the specific microalgae used in this study, particularly when the objective is to treat wastewater and reduce nutrient concentrations. This necessitates the use of treatment plants with large volume in the real scale, especially when dealing with substantial inflow rates. Employing lower dilution rates ensures longer residence times and is advantageous for the treatment process. Conversely, when operations prioritize high biomass production, as demonstrated in Fig. 9b, higher dilution rates are recommended.

4. Conclusion

In this study, we propose a simple and novel approach involving an

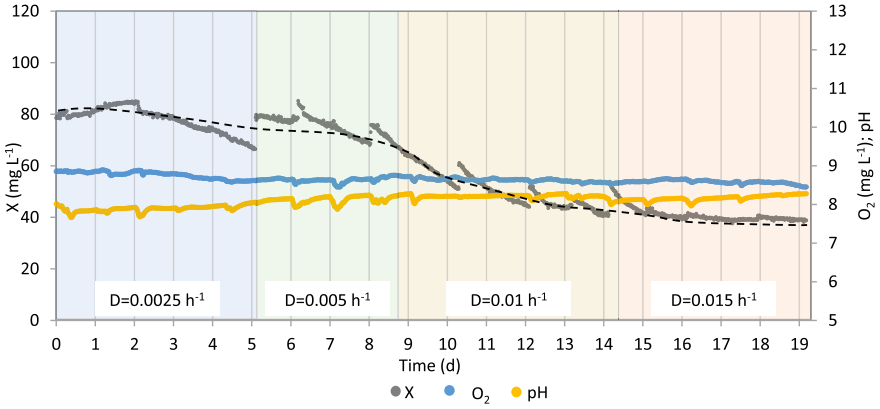

Fig. 7. Variations in cellular concentration (X), oxygen concentration (O_2) and pH trends during continuous cultivation within the laboratory-scale raceway reactor under different dilution rates (D). Dotted line represents the average cellular concentration.

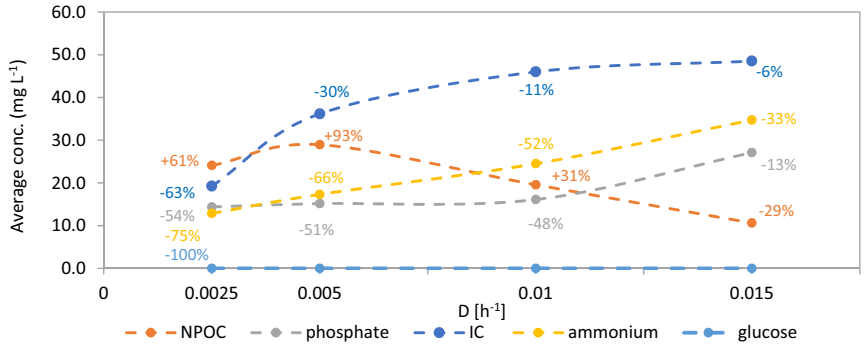

Fig. 8. Nutrient concentration during continuous cultivation in the raceway laboratory reactor under different dilution rates (D). NPOC stands for Non-Purgeable Organic Carbon; IC stands for Inorganic Carbon.

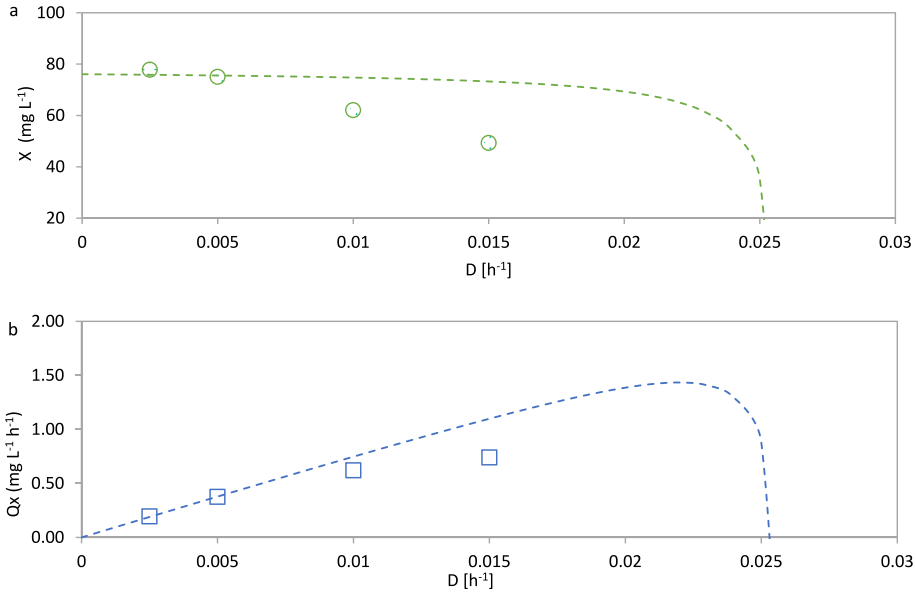

Fig. 9. Cellular concentration X (a) and cellular productivity (Qx) (b) versus dilution rate (D). Dotted lines, model predictions; symbols, experimental data.

original kinetic model for addressing the wastewater treatment using microalgae. The model was employed to predict batch mode operations in different reactor geometries. The obtained kinetic parameters were also employed to determine the proper dilution rates (D) to be applied in continuous operation and to predict the biomass concentration (X) and biomass productivity (Q_x). Dilution rates from 0.0025 to 0.015 h^{-1} were tested and results revealed that while the highest adopted dilution rate is optimal for biomass production, the most efficient removal of nutrients is achieved at lower dilution rates. Specifically, at D = 0.0025 h^{-1}, we achieved removal efficiencies of −63 % for inorganic carbon, −54 % for phosphate, and 75 % for ammonium.

In conclusion, this work demonstrates the feasibility of using microalgae, especially of native strains, for civil wastewater treatment, with continuous operational mode being the preferred approach. The used approach shows promise, and future research could explore its combination with co-cultivation of heterotrophic bacteria for even more effective wastewater treatment.

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CRediT authorship contribution statement

Serena Lima: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Alessandro Cosenza:** Software, Supervision, Writing – review & editing. **Giuseppe Caputo:** Supervision, Writing – review & editing. **Franco Grisafi:** Supervision, Writing – review & editing. **Francesca Scargiali:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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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