# Growth of *Tetraselmis suecica* and *Dunaliella tertiolecta* in aquaculture wastewater: numerical simulation with the BIO_ALGAE model

**Authors:** Valeria Andreotti[1,2], Alessandro Solimeno[1], Anuta Chindris[2], Francesca Marazzi[3] and Joan García[1]

[1]GEMMA-Group of Environmental Engineering and Microbiology, Department of Civil and Environmental Engineering, Universitat Politècnica de Catalunya BarcelonaTech, c/Jordi Girona, 1-3, Building D1, E-08034, Barcelona, Spain.

[2]International Marine Centre IMC, Loc. Sa Mardini 09170, Torregrande (OR) Italy.

[3]DISAT, University of Milano-Bicocca, Piazza della Scienza 1, 20126, Milano, Italy,

**Correspondence**: V. Andreotti, GEMMA-Group of Environmental Engineering and Microbiology, Department of Civil and Environmental Engineering, Universitat Politècnica de Catalunya BarcelonaTech, c/Jordi Girona, 1-3, Building D1, E-08034, Barcelona, Spain.

E-mail: valeria.andreotti@upc.edu

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**Abstract**

In the field of aquaculture, the main microalgae application is animal nutrition, in which they can be used as an unprocessed component, or as dried material for feed preparations. Also, microalgae can have the potential to assimilate the main nutrients dissolved in aquaculture wastewater and therefore can help in the treatment and at the same time producing valuable biomass. The aim of this study was to calibrate the microalgae-bacteria model BIO_ALGAE to simulate the uptake of nutrients (nitrogen, phosphorus) and the biomass production of *Tetraselmis suecica* and *Dunaliella tertiolecta* grown in aquaculture wastewater. The microalgae were cultivated in batch conditions for 7 days using 120 L vertical column photobioreactors. In the first 4 days, after which the algal density reached a steady state the average biomass production was 83.7 ± 4.4 mg/L/d for *T. suecica* and 56.4 ± 5.1 mg/L/d for *D. tertiolecta*. The two species were able to remove more than 96% of Dissolved Inorganic Nitrogen (DIN) and Dissolved Inorganic Phosphorus (DIP). The total lipid content was analyzed at the end of the 7 days, *T. suecica* and *D. tertiolecta* had different lipid content: 75.8 ± 1.6% and 23.2 ± 2.0%, respectively.

The BIO_ALGAE model fits very well the experimental data of both species in terms of biomass and nutrient uptake and could be an effective tool to predict the production of microalgae using aquaculture wastewater as growth media, obtaining at the same time the removal of nutrients from wastewater and the production of biomass to be used as feed.

## 1. Introduction

In the last four decades microalgae based biotechnology has developed constantly [1]. Microalgae have the capacity to remove the macronutrients dissolved in wastewater, in particular, nitrogen and phosphorus, and produce at the same time biomass that can be used as such or as source of valuable compounds [2, 3].

Recently, some studies have been carried out to explore the use of microalgae for the treatment of aquaculture wastewater and the production of biomass [4-10]. Aquaculture wastewater is composed in particular of nitrogenous components (ammonia, nitrite, nitrate), phosphorus and organic carbon [11,12,7,13]. Its composition is related to the nature and quantity of feed, from the species being reared, and from the type of system in operation. In aquaculture, microalgae are used also as feed additive in the commercial rearing or as live food for many aquatic animals in freshwater and in marine systems [14,15]. Microalgae are therefore the source of fatty acids, proteins, essential amino acids and pigments and for this reason, they have an important nutritional role for marine animals [16]. The composition of microalgal cells depends on the conditions of the culture [17-22], in particular on the culture age, on the light characteristics and intensity, on nutrient source and availability, and on the cell density [23].

The yield of commercially valuable products from microalgae could be improved by inducing environmental stress conditions [9]. It was demonstrated that lipid accumulation in microalgae cells increases under nutrient-deficient conditions [24] and can reach 85% of the dry weight [25-27]. Mata et al. [14] reported that for marine microalgae the total lipid content per dry mass values is species-specific and can vary from 22.7 to 29.7% in *Nannochloropsis oculata*, from 7% to 40% in *Isochrysis galbana* and from 8.5 to 23% in *Tetraselmis suecica*. Other studies showed that the macromolecular content is related to the growth phase of the culture [28-30].

Due to their nutritional value, two unicellular green marine microalgae *Dunaliella* and *Tetraselmis* have been used in aquaculture as feed for live preys of fish larvae, for Peneid

shrimp larvae, and *Tetraselmis* also for bivalve mollusk larvae [1]. *Dunaliella tertiolecta* is simple to cultivate, is highly salt tolerant, [31] and has been reported to have a lipid concentration of 36–42% [32]. In addition, it was demonstrated that *Dunaliella spp.* are able to increase their lipid accumulation when nitrogen starvation occurs [33,34,31]. Chen et al. [31] identified the nutritional requirements for *D. tertiolecta* growth and neutral lipid production in a synthetic medium and showed that this organism was able to use either ammonium or nitrate as a nitrogen source. As to phosphorus, starvation seems to have little effect on growth and lipid accumulation, apparently due to intracellular phosphate storage [31].

*Tetraselmis spp*. are able to accumulate lipids (approximately 20-30% on dry weight basis) and tolerate a wide range of environmental conditions [35,27].

In aquaculture wastewater *T. suecica* and *D. tertiolecta* showed a similar pattern of nutrient assimilation, being able to remove more than 90% inorganic nitrogen and inorganic phosphorus after 2 and 1 days, respectively [10].

Due to the influence of many parameters, such as nutrient availability, light, oxygen, and temperature, it is not easy to predict the growth of microalgae, but mathematical models offer the possibility to study microalgae growth in different bioreactors [38]. Several models have been developed to predict algal productivity and nutrient removal efficiency in synthetic media and in urban wastewater [39-44]. For aquaculture wastewater, fewer experiences are reported [45,46], and a mathematical model has not yet been implemented and calibrated.

The aim of this study was, therefore, to implement and calibrate the microalgae-bacteria mechanistic model BIO_ALGAE [44] for aquaculture wastewater in order to simulate the uptake of nutrients (N, P) and the biomass production of *T. suecica* and *D. tertiolecta*. The total lipid content was also investigated at the end of the experiment.

## 2. Materials and methods

*2.1. Microalgae and wastewater*

Microalgae were obtained from the Agency for Agricultural Research in Sardinia (AGRIS, Italy) and sourced from the Culture Collection for Algae and Protozoa (CCAP: Oban, Scotland, UK).

Inocula were grown in fully controlled photobioreactors (6 L volume), with natural seawater (NSW) enriched with Guillard F/2 medium [47,48]. The culture procedures and the photobioreactors operation were carried out according to Saiu et al. [49].

The aquaculture wastewater (AW) was obtained from a grey mullet fish hatchery located in the International Marine Centre - IMC Foundation (Oristano, Sardinia, Italy), where fish were reared in a recirculating aquaculture system (RAS) consisting of 4 tanks of 2000 L volume each [10]. The tanks were monitored daily, the seawater temperature was maintained at 20.3 ± 1.9 °C, salinity was 36.6 ± 1.0 g/L, DO (dissolved oxygen) 8.1 ± 1.2 mg/L and pH 7.5 ± 0.1. Weekly, 30% of the water in the tanks was discharged and replaced by clean seawater, and a part of the outflowing 30% (AW) was used as culture medium for microalgae experiments. Average concentrations of nitrate nitrogen (mg/L), nitrite nitrogen (mg/L), ammonium nitrogen (mg/L) and orthophosphate (mg/L) were 3.32 ± 0.17, 0.11 ± 0.02, 0.28 ± 0.05 and 0.63 ± 0.01, respectively.

*2.2. Analyses*

$NO_3^-$-N, $NO_2^-$-N, $NH_4^+$-N and $PO_4^{3-}$P concentrations were measured by an automatic chemical analyzer μCHEM based on Loop Flow Analysis (Systea, Italy). Microalgal concentration was measured as mg TSS/L, according to the method used by Saiu et al. [49] for seawater culture samples. Algal growth was assessed by following the TSS data collected during the exponential growth phase. The specific microalgal growth rate (μmax in $d^{-1}$) was calculated as the slope of the line fitting the TSS mg/L data plotted in a log [TSS(t)/TSS(0)] versus time graph.

Lipids were extracted from the biomass collected at the end of each experiment. 100 mg of microalgae, previously lyophilized at -80 °C, were suspended in 10 mL of chloroform-methanol 2:1 according to Folch et al. [50]. The solution was vortex mixed for 30 s, sonicated for other 30 s and then centrifuged at 3.000 rpm for 5 min. The liquid fraction was filtered using GF/C filter paper in a funnel and the remaining solids were re-extracted with 5 mL of chloroform–methanol 2:1 [51]. The solvent was removed by evaporation and after that the lipid content was determined gravimetrically. The percent lipid content was calculated with reference to the weight of dry biomass [51].

The lipid productivity in mg/L/d was calculated according to Singh et al. [52]:

Lipid productivity (mg/L/d) = Biomass productivity (mg/L/d) * (Lipid content % /100).

### *2.3. Culture systems and photobioreactors*

To start the experiments, aliquots of microalgae suspensions were collected from the 6 L photobioreactors in the exponential growth phase when the microalgal concentration was approximately 0.13 g TSS/L.

Two completely mixed bubble column photobioreactors of 120 L were used in batch condition for 7 days. Four consecutive replicates for each species were done.

As the experiments were carried out in different periods, the nutrient concentrations of AW used for the two species were not exactly the same, as shown in Table 1.

Table 1. Nutrient concentrations in the AW used for the two microalgal species (mg/L). Values are expressed as mean ± SE (n=4).

| | *T. suecica* | *D. Tertiolecta* |
|---|---|---|
| $NO_2^-$-N | 0.073±0.001 | 0.156±0.009 |
| $NO_3^-$-N | 3.755±0.016 | 2.878±0.038 |
| $NH_4^+$-N | 0.144±0.001 | 0.408±0.031 |
| $PO_4^{3}$-P | 0.657±0.002 | 0.613±0.018 |

Light was provided by fluorescent lamps (Cool Daylight - 58W/865 Lumilux) for 24/24. Photosynthetically active radiation (PAR) was 150 (μmol/s/$m^2$) in the external part of photobioreactor. The cultures were maintained at constant temperature (23°C). Dissolved oxygen (DO) concentration was 8.0 ± 2 mg/L and pH was 8.0 ± 2. PH and were measured every 10 minutes. The airflow was constant at 2 $m^3$/h.

A sample of each culture was collected daily to analyze the microalgae growth and the nutrient concentrations in the culture medium.

*2.4. Statistical analysis*

Data analysis was performed using R Studio (Version 1.0.153 – © 2009-2017 R Studio, Inc.). Differences in the removal efficiencies and biomass as mg TSS/L among microalgae species were analyzed using all 4 replicates (R1 to R4). Normality and homogeneity of data were examined using Shapiro Wilk's W test. The statistical significance of differences between the two algal species was determined for all the measured parameters by the Kruskal-Wallis test (p-values <0.05).

All data are expressed as mean±SE.

*2.5. BIO_ALGAE model*

BIO_ALGAE model has been described in Solimeno et al. [44] and was used to simulate mixed cultures of microalgae and bacteria. This model was implemented in COMSOL Multiphysics™ v5.3 software and was basically constructed through the RWQM1 [53], with modifications of ASM3 [54]. The kinetic expressions of BIO_ALGAE are based on Monod type functions for carbon, nitrogen and phosphorus limitation. C was included as limiting factor because in some cases, namely when intense photosynthesis raises pH to very high values, $CO_2$ can be no more available as it turns to carbonate. This model is applicable for waste stabilization ponds, high

rate algal ponds, and photobioreactors.

The model considers the 19 components (6 particulate and 13 dissolved) included in the common nomenclature of the International Water Association (IWA) model [44]. Particulate and dissolved components implicated as variables in the physical, chemical and biokinetic processes are described in our previous works Solimeno et al. [43, 44, 55].

To simplify presentation of the simulation results, Tables S1 and S2 in Supplementary Material (SM) present the biokinetic processes and the matrix of stoichiometric parameters. Values of biokinetic, physical and chemical parameters are shown in SM, Tables S3-S4. Mathematical expressions of the stoichiometric coefficients of each process are also shown in SM, Table S5.

## 3. Results

### *3.1. Nutrient removal and biomass production*

At the beginning of experiments, the concentrations of *T. suecica* and *D. tertiolecta* were 96.9 ± 4.7 mg TSS/L and 88.1 ± 6.7 mg TSS/L, respectively. As shown in Figure 1, the growth of the two microalgae had similar trends, but the statistical analysis demonstrated a significant difference between them for biomass production ($p < 0.05$).

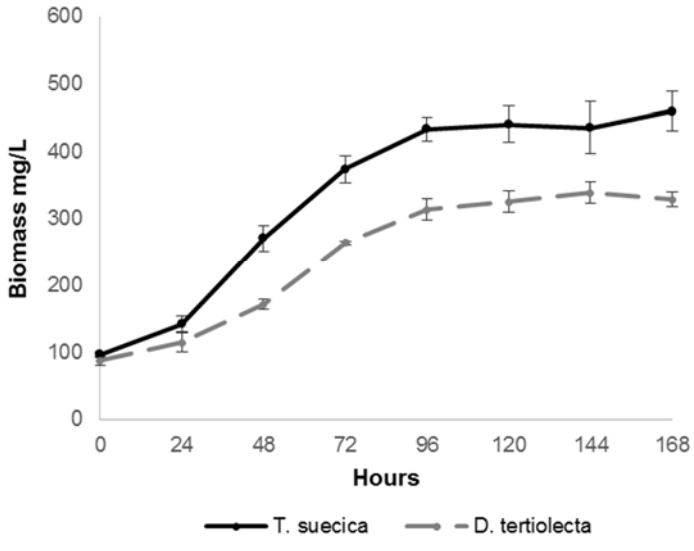

Fig. 1. Biomass algal concentration measured as mg TSS/L (mean (n=4) ± standard error) for *T. suecica* and *D. tertiolecta* during the experiments.

*T. suecica* showed a better performance in terms of biomass productivity in batch culture (reaching a maximum of 460.0 ± 29.8 mg TSS/L at the end of the experiment) than *D. tertiolecta* (329.4 ± 11.0 mg TSS/L). This is also confirmed by the daily biomass production during the 7 days, that was 65.7 ± 4.3 mg/L/d for *T. suecica* and 47.1 ± 1.6 mg/L/d for *D. tertiolecta*. In both cases, the exponential phase lasted 96 hours. In that time range, the density reached 433.8 ± 17.4 and 313.8 ± 15.8 mg TSS/L for *T. suecica* and for *D. tertiolecta,* respectively (Figure 1). The biomass production per day in this phase was 83.8 ± 4.4 mg/L/d for *T. suecica* and 56.4 ± 5.1 mg/L/d for *D. tertiolecta*.

The specific microalgal growth rate (μ in $d^{-1}$) was 0.16 $d^{-1}$ for *T. suecica* and 0.15 $d^{-1}$ for *D. tertiolecta.*

Figures 2 and 3 show the decrease of dissolved inorganic nitrogen (DIN) and phosphorus (DIP) concentrations, during the 7 days of treatment, for the two cultures, respectively. DIN was the sum of $NO_2^-$ -N, $NO_3^-$ -N and $NH_4^+$-N in mg/L, while DIP was the total dissolved orthophosphate ($PO_4^{3-}$-P mg/L).

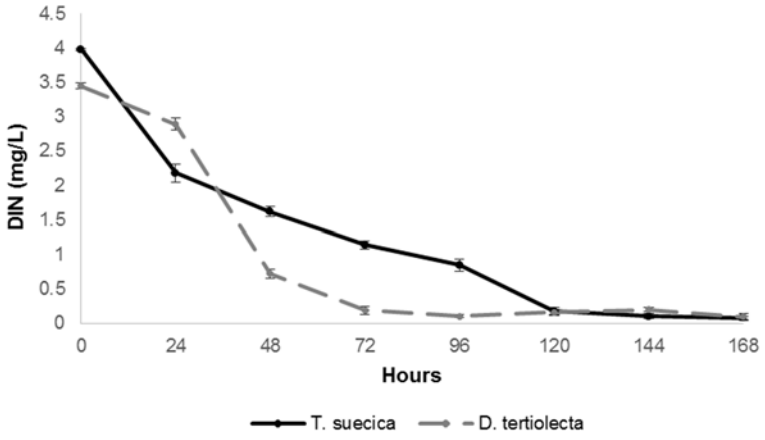


Fig. 2. Decrease in concentration (mg/L) of Dissolved Inorganic Nitrogen (DIN) for *Tetraselmis suecica* and *Dunaliella tertiolecta,* (n=4).

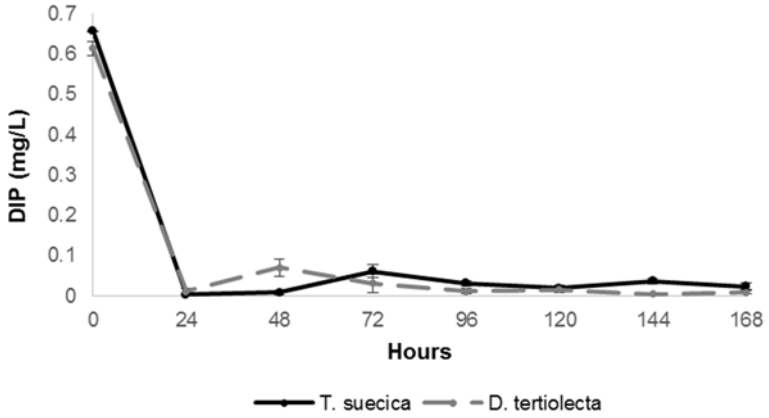


Fig. 3. Decrease in concentration (mg/L) of Dissolved Inorganic Phosphorous (DIP) for *Tetraselmis suecica* and *Dunaliella tertiolecta*

In 7 days, the total DIN removal efficiency % was 98 ± 0.6% for *T. suecica*, and 97 ± 1.5% for *D. tertiolecta*. During the exponential growth phase, the daily removal rate was 0.88 ± 0.05 mg N /L/d *T. suecica*, and 0.96 ± 0.01 mg N /L/d for *D. tertiolecta* ($p > 0.05$). The complete removal occurred after 72 hours in the case of *D. tertiolecta* and after 120 hours in the case of *T. suecica*. The total DIP removal efficiency was similar for the two species: 97 ± 1.2% for *T. suecica* and 99 ± 0.7 % and *D. tertiolecta* respectively ($p > 0.05$). As also shown in Figure 3, the DIP was completely removed after 24 hours in both cases, with a removal rate in the exponential phase of 0.81 ± 0.05 and 0.93 ± 0.02 mg/L/d for *T. suecica* and *D. tertiolecta*, respectively.

The total lipid content after 7 days was very different in the two species, being 75.8 ± 1.6% in the biomass of *T. suecica*, while only 23.2 ± 2.0% in the biomass of *D. tertiolecta*. The lipid accumulation rate was also lower for *D. tertiolecta* (11.1 mg/L/d) than for *T. suecica* (49.8 mg/L/d).

*3.2. Implementation of BIO_ALGAE model*

The model was calibrated using the data for the 7 days of batch experimentation and it was conducted comparing simulated and experimental data curves. For the calibration, only two replicates of experimental data (R1-R2) were used. Unlike the original model [44] that considers relevant features such as light attenuation, photorespiration and temperature dependency, for this experiment, light and temperature were constant and, thus, were not considered as growth limiting factors. The initial values of the parameters of concern are shown in Table 2.

Table 2. Values of the parameters of concern at the beginning of the experiment. All components are described in Solimeno et al. [44].

| Component | Concentration | Units |
|---|---|---|
| $S_{NO3}$ | 2.98 | gN-$NO_3$ $m^{-3}$ |
| $S_{NO2}$ | 0.14 | gN-$NO_2$ $m^{-3}$ |
| $S_{NH3}$ | 0.41 | gN-$NH_3$ $m^{-3}$ |
| $S_{NH4}$ | 1.6 | gN-$NH_4$ $m^{-3}$ |
| $S_{PO4}$ | 0.65 | gP-$PO_4$ $m^{-3}$ |
| $S_{CO2}$ | 0.145 | gC-$CO_2$ $m^{-3}$ |
| $S_{CO3}$ | 0.866 | gC-$CO_3$ $m^{-3}$ |
| $S_{HCO3}$ | 35.00 | gC-$HCO_3$ $m^{-3}$ |
| $S_H$ | $1.78\ 10^{-9}$ | gH $m^{-3}$ |
| $S_{OH}$ | $4.69\ 10^{-6}$ | gH-OH $m^{-3}$ |
| $S_S$ | 2 | gCOD $m^{-3}$ |
| $S_{O2}$ | 8.74 | g$O_2$ $m^{-3}$ |
| $S_I$ | 8 | gCOD $m^{-3}$ |
| $X_H$ | 1 | gCOD $m^{-3}$ |
| $X_I$ | 10 | gCOD $m^{-3}$ |
| $X_S$ | 1 | gCOD $m^{-3}$ |
| $X_{AOB}$ | 0.05 | gCOD $m^{-3}$ |
| $X_{NOB}$ | 0.05 | gCOD $m^{-3}$ |
| $X_{ALG}$ | 80 | gTSS $m^{-3}$ |

The kinetic expressions of BIO_ALGAE are based on Monod type functions. The Monod equations do not consider the variable "cell quota" (intracellular nutrient concentration), as the Droop model does [56]. This variable is important if the growth depends also (or chiefly) on a stored intra-cellular pool of nutrient, and not only on the nutrients available in the growth media, as in the Monod equations. In fact, BIO_ALGAE has been developed for microalgae growing in urban wastewaters, where normally the availability of nutrients is high. Nutrients in AW have much lower concentrations than in urban wastewater, so they can have a completely different influence on growth than in urban wastewaters. In fact, in most experimental works microalgae cultivation in AW included nutrient addition to increase production [5,57]. On the contrary, in our work N and P in AW were depleted in few days, but no nutrient addition was provided and algal growth did not stop. This suggested that growth was more closely related to the intra-cellular nutrient concentration than to the external one [58] and this, in turn, could depend on the fact that the algal biomass used for the experiment had been previously grown in a nutrient-rich medium. The use of nutrient rich inoculum for batch experiments could preclude to find the correct relationships between external nutrient concentrations and algal growth. As one of the aims of the work was to calibrate BIO_ALGAE model in order to use it to predict algal growth in batch experiments as a function of nutrient availability, the theoretical initial concentrations able to sustain the observed growth were calculated according to external data [58]. The model has been programmed to have an input of N and P in the system. Various concentrations were tested to obtain the amount of biomass indicated in the experimental data. The obtained data (19 mg $NO_3^-$-N /L and 8 mg $PO_4^{3-}$-P /L) were then used as input for the calibration of the model.

For the calibration, the sum of $NO_2^-$ -N and $NO_3^-$ -N was used. The experimental data on biomass were expressed as Total Suspended Solids (TSS), while the simulation provided both TSS and by $X_{ALG}$ (mg TSS algal biomass/L). TSS is the sum of all particulate components including microalgae and bacteria biomass, and $X_{ALG}$ is the concentration of microalgae (mg

TSS algal biomass/L) [43,44].

The comparison between experimental and simulated data show how for *T. suecica* the two curves $X_{ALG}$ and TSS follow quite well the same pattern of the experimental data (R1-R2) (Figure 4A). After 50 hours some differences between the two curves can be observed, but these differences are not statistical significant ($p > 0.05$). After 72 hours the maximum values (nearly 400 mg/L) were reached and after that a slow decrease occurred, so that a true steady state did not take place. As previously told, at the end of the experiment the simulated and experimental data did not agree anymore. For *D. tertiolecta* (Figure 4B) the predicted curves were very similar to the experimental ones ($p > 0.05$), but their shape was different from those derived from *T. suecica* experiments. In the first 24 hours, no lag-phase was observed and the biomass density increased, even if slowly. Between 24 and 48 hours, the data show a sort of steady state while the exponential growth occurred between 48 and 96 hours, when TSS and $X_{ALG}$ reached their maxima (just a little lower than for *T. suecica*), to keep nearly constant afterwards (Figure 4B).

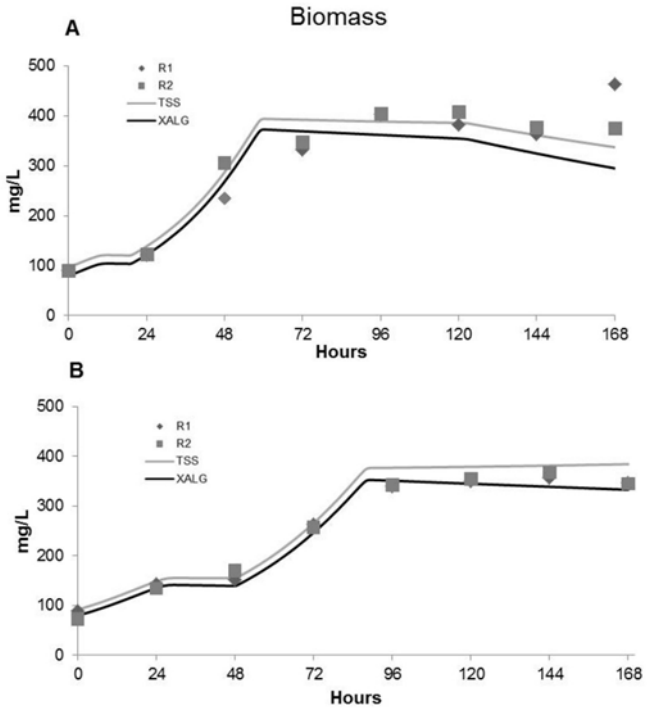


Figure 4. Trend of biomass concentration with time in the experimental trial (mg TSS/L, average of the two

replicates) and according to BIO_ALGAE simulation (TSS and $X_{ALG}$) for *T. suecica* (A) and for *D. tertiolecta* (B).

As to nutrient removal, the simulations of the sum of $NO_3^-$ -N + $NO_2^-$ -N and the $PO_4^{3-}$-P, represent quite well the experimental data in *T. suecica* (Figure 5A). Instead, in *D. tertiolecta* the simulation curve of $NO_3^-$ -N + $NO_2^-$ -N has a rapid decrease at 24 hours, while in the real data the concentrations of these nutrients begins to drop after 48 hours (Figure 5B). Simulated phosphorus concentrations fitted well the experimental data for the two microalgae, although these data showed a non-constant distribution after 24 hours.

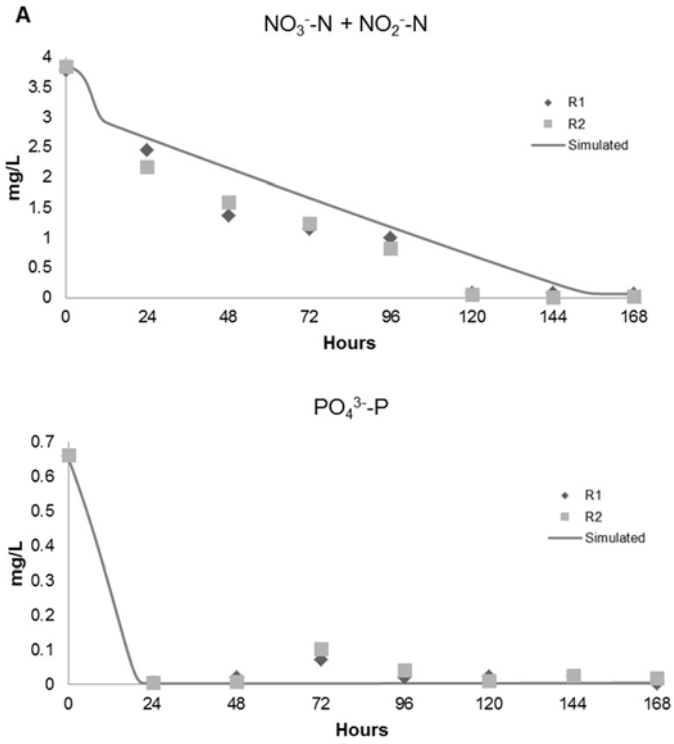


Figure 5A: Nutrient removal for *T. suecica,* experimental data (R1, R2) and BIO_ALGAE simulation curves in mg/L.

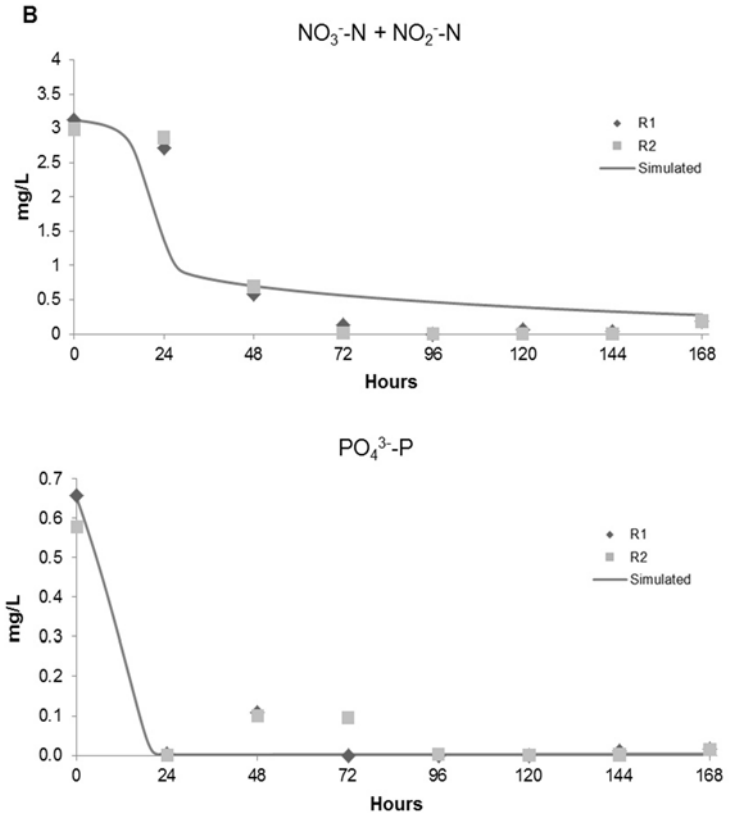


Figure 5B. Nutrient removal for *D. tertiolecta,* experimental data (R1, R2) and BIO_ALGAE simulation curves in mg/L.

## 4. Discussion

This work has confirmed that aquaculture wastewater is suitable for the cultivation of *T. suecica* and *D. tertiolecta*. In a previous work with reactors of 6 L and the same AW, biomass production was of 86.14 ± 5 mg/L/d for *T. suecica*, and 54.26 ± 5 mg/L/d for *D. tertiolecta* [10], while in the present work, with 120 L reactors, biomass production was lower for *T. suecica* (65.71 ± 4.25 mg/L/d) and similar for *D. tertiolecta* (47.05 ±1.57 mg/L/d). This small variation could depend on the different nutrients concentrations of the wastewater in two experiments. However, the biomass production was not affected by the low nitrogen values in aquaculture. This is also confirmed in a recent study, in which a *D. tertiolecta* cell size increase was observed under nitrogen starvation conditions [31]. The results obtained by Michels et al.

[5], instead, range between 0.46 and 0.52 g/L/d of biomass production with extra addition of phosphorus in the aquaculture wastewater. Gao et al. [8], cultivated *Chlorella vulgaris* and *Scenedesmus obliquus* in aquaculture wastewater and obtained a quite low biomass production: 7.3 and 6.2 mg/L/d respectively. Khatoon et al. [59] made a comparison between *Tetraselmis chuii* growth in aquaculture wastewater and in a synthetic medium and observed no significant differences ($p > 0.05$) in term of biomass production in two different medium.

In this study, AW was analyzed for presence of nitrates, nitrites, ammonia and phosphates that are essential for microalgae cultivation. For both species the removal efficiency exceeded 95% for DIN and DIP, reaching higher levels than in the previous study [10] and in various literature data. Michels et al. [5] showed that *T. suecica* has a removal efficiency of 49.4% for N and 99.0% for P in AW, while Lowrey et al. [60] used *Tetraselmis sp*. in a dairy wastewater obtaining a reduction of 51% of total nitrogen, and of 40% of total phosphorus. Wu et al. [61] cultivated *D. tertiolecta* in a saline sewage (13±0.2 mg/L of Nitrate as nitrogen mg/L and 14.7±0.1 mg/L of Orthophosphate) and the removal percentage was 60 ± 5.4% for nitrate and 70 ± 13.5% for orthophosphate and after 6 days. The better results obtained in the present study could depend on the initial concentration of nutrients in the wastewater and microalgae strains used [62]. However, further studies on the microalgae growth in AW must be carried out, because of its suitability in effluents, that can be species-specific and no microalgae should be neglected [63]. For example, recent studies [64,10] demonstrated that *Isochrysis galbana* have a lower productivity than *T. suecica* when cultivated in the same aquaculture wastewater. On the contrary, instead, Freire et al. [65] and Zheng et al. [66] have successfully cultivated the *Isochrysis* genus in fish farm effluents.

Nowadays, very few studies analyzed the microalgal biomass composition produced in aquaculture wastewater. Ansari et al. [9] have obtained a total lipid percentage of 30.85% for *Scenedesmus obliquus*, 31.85% for *Chlorella sorokiniana*, and 35.90% for *Ankistrodesmus falcatus* grown in aquaculture wastewater. Another recent study [57] extracted from *C.*

*sorokiniana* cultivated in aquaculture wastewater the 39.1% of lipids and calculated a daily production of 138.17 mg/L/d. *T. suecica* cultivated in artificial seawater showed a different response to nutrient deprivation, with a lipid content of 22% in the nitrogen-starved culture, 27% under nitrogen and phosphorus starvation and 29% in a culture with a sufficient content of nutrients [67]. Furthermore, this species cultivated in f/2 culture medium has a lipid content of 4.85% [68]. The lipid productivity for *T. suecica* observed in this study was higher compared to the previously reported studies, and this result allows us to confirm that these wastewaters are suitable for the production of lipids in *T. suecica*. *Dunaliella sp* is also known to respond to nitrogen starvation by increasing lipid production [33,34]. The nitrogen and phosphorous content were lower in our wastewaters than in synthetic media and this is likely to have caused a nutrient stress and the consequent reduction of microalgal growth and increase of lipid concentration [57].

The BIO_ALGAE model was able to fit very well both species in terms of biomass and nutrients uptake and this meant a good agreement between our real data and simulations.

As represented in a classical growth curve, even in this study, the biomass continues to grow during a few days after nutrient exhaustion. Despite this, we were able to adapt the BIO_ALGAE model that has been appropriate to represent also the effect of macronutrients, such as nitrogen or phosphorus, on the growth rate.

After 80 hours, that corresponds approximately of the begin of the stationary phase, it was necessary to calculate a new input of nutrients in the culture to simulate the real data. The amount of internal nutrients was calculated on the basis of experimental data, according to confirm by Lemesle et al. [58]. Indeed, experimental data show that biomass continues to increase even if the nutrients are completely removed, as well as the simulation curve that has an exponential phase in about 50 hours.

In starvation conditions, as in our experimentation, the growth rate of the biomass can, therefore, be related to the internal concentration of the limiting element [69]. As an example, the correlation between maximum uptake velocities and cell quota for limiting nutrient may need to be modified if phosphate or iron are limiting factors because of the greater potential for luxury uptake of phosphorus and iron relative to nitrogen [70,71]. Chen et al. [31] have showed that *D. tertiolecta* had internal phosphate stores sufficient for the synthesis of lipids in phosphate-deficient cultures. This model was developed for municipal wastewater with a high concentration of nitrogen and phosphorous, while in AW, N and P content is limited and dependent on a number of factors including the area used for culture, the bred species, production level and the profile of the waterbody [72]. In particular, the content of these nutrients in the feed have decreased, especially for N [72]. Despite this, the simulation curves of the nutrient removal ($NO_3^-$ -N + $NO_2^-$ -N and $PO_4^{3-}$P) represent accurately the experimental data for two microalgae.

It is already demonstrated that these microalgae species are able to compete with other microorganism, in particular, ciliates [73-75, 10] and for this reason, any sterilization process were done. In this way, avoiding pretreatment and sterilization of wastewater, the management costs would be reduced, as well as energy and manual labor.

**5. Conclusion**

In this paper, it was demonstrated that *T. suecica* e *D. tertiolecta* are suitable for upscale in vertical column photobioreactors with a volume of 120 liters. Using aquaculture wastewater as culture medium it was obtained a removal of nutrient (N and P) greater than 95%. Moreover, *T. suecica* has been able of produce more than 75% of total lipid content, while *D. tertiolecta* only 23% and it is possible to confirm that nitrogen stress has disproportionate effects on growth and lipid content, with a difference between species. These microalgae are valid candidates for a second use in aquaculture systems as live-feed for hatchery-grown of herbivorous and filter feeders [76]. Despite this, further studies are necessary to analyze the protein and lipid

composition of these species.

In this study, it was also proved for the first time the applicability of the BIO_ALGAE model to simulate the growth of these microalgae and the assimilation of nutrients in aquaculture wastewater. The model was calibrated by comparing simulated results to experimental data during 7 days of batch experiment. The results of the calibration indicate that the model was able to reproduce quite well the assimilation of nutrient, but further modifications are necessary as concern the biomass production.

The possibility of applying BIO_ALGAE model to predict use of microalgae for wastewater treatment and the biomass production using for feed in aquaculture is a new aspect that should be developed with further studies. The next approach in order to better understand the wastewater aquaculture treatment with microalgae will be to predict the growth and nutrient uptake using the model in a continuous system.

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## Author contributions

V. Andreotti collected the data, interpreted the results and drafted the article, A. Chindris contributed in the experimental setup, microalgae production plant and laboratory analysis. F. Marazzi contributed to data collection and laboratory analysis. A. Solimeno applied the experimental data to the mathematical model. Manuscript revision and approval: F. Marazzi, A. Solimeno, A. Chindris, J. Garcia.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

## Statement of informed consent

Authors declare that there is “No conflicts, informed consent, human or animal rights applicable” in this study.

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