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# The potential of different marine microalgae species to recycle nutrients from recirculating aquaculture systems (RAS) fish farms and produce feed additives

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ABSTRACT

This study researched the use of six microalgae species (*N. gaditana*, *P. lutheri*, *I. galbana*, *T. chuii*, *P. tricornutum* and *C. gracilis*) and a bloom to treat effluent from a marine fish farm and produce quality biomass. More specifically, simulated water from a recirculating aquaculture system (RAS) was used. Microalgae culture was carried out under controlled conditions using 18 L bubble column photoreactors under batch and semi-continuous operation. The main parameters analysed were micronutritional requirements, biomass productivity, nutrient removal rate (nitrogen and phosphorus), biomass composition, and quality. Also, based on the results obtained, a quantitative classification of the microalgae was carried out. The results showed that all microalgae required at least trace metals. In certain species, the addition of vitamins was also required for viable cultivation. In the case of biomass productivity under batch operation, values were between 67 mg $L^{-1}$ $d^{-1}$ and 7 mg $L^{-1}$ $d^{-1}$ using *T. chuii* and *C. gracilis*, respectively, and between 71 mg $L^{-1}$ $d^{-1}$ and 9 mg $L^{-1}$ $d^{-1}$ using *T. chuii* and *N. gaditana* under semi-continuous operation. In the case of total dissolved phosphorus removal, no differences were found between species, reaching in all cases final concentrations <0.01 mg $L^{-1}$. Total dissolved nitrogen removal rate varied between species and operating conditions, being the highest obtained using *T. chuii* under semi-continuous operation (12.6 mg $L^{-1}$ $d^{-1}$) and the lowest with *C. gracilis* batch operation (0.15 mg $L^{-1}$ $d^{-1}$). Biomass composition in terms of protein and lipids varied between species and operating conditions, but quality in terms of amino acids and fatty acids profile remained homogeneous in all cases. Finally, according to the developed score methodology, *I. galbana* was the microalgae with the highest biomass production score, while *T. chuii* was for wastewater treatment.

## 1. Introduction

Aquaculture is an essential food industry, providing a source of animal protein for more frequent consumption and lower production costs [1]. The annual consumption of fish as an animal source of protein per capita has increased by 1.5%, reaching 20.5 kg in 2018 [2]. According to the Food and Agriculture Organization of the United Nations (FAO) [2], world fish production reached 179 million tons in 2018, of which 46% came from aquaculture, this is equivalent to a value of USD 250 billion.

The aquaculture industry is moving forward, but still faces challenges, one of the most critical, sustainability. The main environmental issues facing aquaculture are the dependence on the wild fish meal (FM) and fish oil (FO) for feed production and the environmental degradation resulting from aquaculture activity [2].

In 2018, 18.1% of the global fish catch was destined to produce fish meal and fish oil; that is over 18 million tons [2]. The ratio between wild fish caught for FM and FO production and aquaculture fish production is called the fish In-fish Out (FIFO) ratio [3]. During the last few years, much effort has been made to reduce further the rate [4]. The Marine Ingredients Association (IFFO) published a list of the evolution of the FIFO rate [5], in the case of marine fish, it decreased from 1.48 in 2000 to 0.53 in 2015. The latest data for overall aquaculture was 0.22 in 2015.

Fish meal and oil are a protein, essential amino acids, essential lipids (PUFAS) and energy source [6]. Therefore, the foods that replace them should meet nutritional requirements and others related to digestibility, palatability, or skin colour of the fish. The most commonly used

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ingredients to replace FM and FO are terrestrial vegetables such as soybean, wheat, corn or rapeseed [7]. However, adverse effects such as reduced palatability, reduced intestinal health or enzymatic activity of fish have been reported [8]. Besides, the composition of essential amino acids (AA) and the profile of fatty acids (FA) must also be considered. Plant-based fish meal and fish oil substitutes can be limiting in sulfur-containing amino acids [9] and deficient in certain PUFAs [10].

For this reason, unconventional foods such as insects [11] or microalgae [12] are under research to reduce the use of fish meal and fish oil. Microalgae are widely used in aquaculture as inducers of biological activities, as dyes and as live feed for zooplankton, bivalves, crustaceans and larvae. Their interest as a fish feed additive and fish meal substitute has increased because they are a sustainable, stable and profitable source of protein and lipids and are natural food for marine and freshwater organisms [13]. The nutritional characteristics of microalgae depend on the species and the culturing conditions. The quality of proteins in all microalgae is high; however, not all microalgae contain EPA and DHA, key PUFAs in feed diets [13]. Therefore, some authors have considered that blended microalgae diets can achieve an optimal balance in which all nutritional requirements are met [14,15].

The interest in the use of recirculating aquaculture systems (RAS) is increasing because it decreases water use, carbon footprint and reduces nitrogen in the form of ammonium and nitrite [16,17], generating less environmental impact. Traditional RAS consists of at least one nitrification unit, a solids removal system and a disinfection unit. The latest studies were focused on increasing nutrient (nitrogen and phosphorus) and solids removal efficiency. More specifically, there is a keen interest in recovering phosphorus and ensuring denitrification in order that nitrogen does not accumulate in the system [18,19]. Microalgae biotechnology is a superior interest technology since it removes nitrogen and phosphorus from water while generating valuable biomass. In wastewater treatment, it is a widely researched technology [20] with experiences at high levels of technology readiness (technology readiness levels, TRL). An example is the FP7 European All-Gas Project (http://www.all-gas.eu; TRL = 6), in which one of the largest microalgae wastewater treatment facility has been developed.

The interest in the treatment of aquaculture streams using microalgae biotechnology is increasing [21]. However, the potential of different species is not systematically explored in a single study under identical conditions. In the previous studies, the treatment of aquaculture streams is evaluated based on growth kinetics and proximal composition [22]. However, other parameters are essential in the environmental and techno-economic viability of the process such as the harvestability, the kinetics of consumption of N and P or the micro nutritional requirements.

This study aims to select the optimal species for cultivating microalgae using marine RAS stream through a multi-criteria decision system including the following parameters: biomass productivity, nutrient removal kinetic, nutritional requirements, harvestability and biomass composition (proximal analysis, fatty acids, amino acids). For this, growth and nutrient removal kinetic under batch and semi-continuous operation of the following microalgae species were studied: *Nannochloropsis gaditana*, *Pavlova lutheri*, *Tetraselmis chuii*, *Isochrysis galbana*, *Phaeodactylum tricornutum* and *Chaetoceros gracilis*. A bloom of microalgae, obtained from a marine aquaculture stream, was also studied. Micronutritional demand of these species was studied as well.

## 2. Material and methods

### 2.1. Culture media

A RAS stream was simulated with the same characteristics obtained in the fish farm located in the Andalusian Aquaculture Technology Center (CTAQUA) in El Puerto de Santa Maria (Cadiz, Spain) in which juvenile sea bass were grown. The facility sampled included 18 tanks of 300 L with a fish density of 8.4 kg $m^{-3}$. The RAS consisted of an aerobic nitrification biofilter, a drum filter, a skimmer and a UV disinfection unit (Fig. S1). During the experiments, micronutrients (vitamins, trace metals or silicates) were added to the simulated culture medium at a concentration according to a previous test (Section 2.2.2). The characteristics of the simulated water matrix were, therefore, as described in Table 1. The average represents the measurement of seven samples from each experiment.

### 2.2. Experimental setup

#### 2.2.1. Microalgae inoculum and bloom isolation

The species used were *Nannochloropsis gaditana*, *Pavlova lutheri*, *Isochrysis galbana*, *Tetraselmis chuii*, *Phaeodactylum tricornutum* and *Chaetoceros gracilis* from the culture collection of the Laboratory of Marine Culture (University of Cadiz). The bloom was produced by adding f/2 culture medium [23] to water from the stream of the aquaculture facility. Bloom characterisation was carried out by using Image Stream X Mark II (Amnis Corporation, Seattle, USA) imaging flow cytometer (INMAR, Universidad de Cádiz). The results showed uniformity in terms of microalgae population (>99%) (Fig. S2, A). The average cell size: diameter (3.4 ± 0.9 μm), length (3.8 ± 1.6 μm), thickness (2.7 ± 0.9–3.4 ± 0.9 μm) and width (3.2 ± 0.9 μm) is in accordance with the round morphology observed (Fig. S2, B). Due to the size, shape, green pigmentation, chloroplast distribution and the presence of pyrenoid, it was determined that the bloom had a high coincidence with the genus *Parachlorella* (Chlorophyta, Trebouxiophyceae). All the inocula were conserved using one-litre reactors (Fig. S3) in a room at constant temperature (22 ± 1 °C) and 24 h photosynthetic photon flux density (PPFD) (100 μmol $m^{-2}$ $s^{-1}$).

#### 2.2.2. Micronutrient tests

Micronutrient requirements were determined using 1 L (borosilicate-Pyrex) bottles. The experiments were performed at constant temperature (22 ± 1 °C), controlled lighting by two fluorescent lamps (36 W, 6500 K, PPFD = 100 μmol $m^{-2}$ $s^{-1}$, T8) without photoperiod (24 h light) and fixed aeration (1 air volume per liquid volume per minute, 0.45 μm filtered ambient air). The inoculum lacking metals, silicates and vitamins was mixed with the synthetic aquaculture stream. The initial biomass concentration of the mixture, measured as total suspended solids (TSS), in all cases was 30 ± 10 mg TSS $L^{-1}$. The mixture was divided into four bottles named +M+V (with vitamins and trace metals at a concentration of the culture medium f/2), -M+V (vitamins only), +M-V (trace metals only) and a control, -M-V (no trace metals or vitamins). In the case of the diatoms, *P. tricornutum* and *C. gracilis*, the effect of silicates (+S) was also included.

**Table 1**
Characteristics of the simulated recirculating aquaculture stream. Average and standard deviation ($n$ = 7).

| Parameter | |
|---|---|
| TDN (mg $L^{-1}$) | 11.5 ± 1.2 |
| N-$NO_3^-$ (mg $L^{-1}$) | 4.5 ± 0.7 |
| N-$NO_2^-$ (mg $L^{-1}$) | 1.6 ± 0.2 |
| N-$NH_4^+$ (mg $L^{-1}$) | 6.1 ± 0.4 |
| TDP (mg $L^{-1}$) | 0.28 ± 0.10 |
| DOC (mg $L^{-1}$) | 4.7 ± 2.2 |
| pH | 7.9 ± 0.3 |
| Conductivity (mS $cm^{-1}$) | 50.0 ± 0.2 |
| Salinity (‰) | 38 |
| Micronutrients | [a] |

TDN, total dissolved nitrogen.
TDP, total dissolved phosphorus.
DOC, dissolved organic carbon.
[a] In the case that micronutrients were added, the concentration was that of f/2 medium.

#### 2.2.3. Growth and nutrient removal test

The tests were performed on three 18 L (20 cm diameter) bubble columns (Fig. S4) placed in a culture chamber at constant temperature (22 ± 1 °C). Prior to the experiments, there was a pre-adaptation of the cultures in 8 L reactors in which simulated RAS stream was used. The ambient air, previously filtered (0.45 μm) was bubbled from the bottom at a flow rate of 2 L $min^{-1}$. No photoperiod was set (24-h illumination) and warm light (2700–3200 K) LED panels (40 W) was used to generate a PPFD of 131 μmol $m^{-2}$ $s^{-1}$ along 95% of the length of the reactor. Initial biomass concentration of the mixture was 29 ± 11 mg TSS $L^{-1}$. Samples were taken using a tap located at the base. Evaporation was compensated daily by distilled water. A gauze plug was placed at the top to facilitate gas exchange and prevent external contamination. A triplicate was carried out during the batch experiment while only one reactor was used during the semi-continuous experiment. The experiments were interspersed between the different microalgae, as shown in Fig. S4.

### 2.3. Chemical analysis and methods

The biomass concentration was measured indirectly through a calibration curve of total suspended solids vs optical density at 680 nm ($OD_{680nm}$). The calibration curves were positively correlated ($R^2 > 0.99$) and were performed on each microalga in exponentially growing phase. Daily, pH, $OD_{680nm}$, photosynthetic activity (quantum yield, QY) were analysed, and samples were taken for nutrient and DOC analysis in triplicate. The pH was measured with a GLP 21 CRISON sensor. Quantum yield (QY), also called light-processing efficiency ($F_v/F_m$) [24], was analysed using a FluorPen FP100 (Photon Systems Instruments). The GLP 32 CRISON electrochemical analyser was used to determine conductivity, and an ATAGO (S/Mill-E) handheld refractometer was used for salinity. PAR irradiation was measured using an Apogee MQ100 Quantum Meter (Apogee Instruments, INC). TSS was measured gravimetrically in triplicate according to the standard method 2540 [25] and washing the filter with distilled water after filtering the sample to remove salts.

For water quality analysis, during batch, samples were taken from each of the reactors (triplicate, $n = 3$) while in semi-continuous, one sample from the last three days of operation was analysed ($n = 3$). Samples for analysis of dissolved organic carbon (DOC), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were filtered (0.45 μm) and stored in plastic bottles at −20 °C. DOC was measured by Shimadzu TOC-L analyser in the non-purgeable organic carbon mode. TDN and TDP were determined by the method proposed by Köthe and Bitsch [26]. The method is based on the oxidation of all nitrogen and phosphorus compounds (organic and inorganics) to nitrates ($NO_3^-$) and phosphates ($PO_4^{3-}$) and the subsequent determination of these anions. Dissolved inorganic nitrogen (DIN) corresponds to the sum of the N-$NO_3^-$, N-$NO_2^-$ and N-$NH_4^+$. These species were analysed in 24 h using the standard methods 4500- $NO_2^-$ and 4500-$NH_3$ [25]. The quantification of N-$NO_3^-$ in seawater was performed using the Spectroquant® colourimetric kit test (Code 1.14942.0001, Merck). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and DIN. P-$PO_4^{3-}$ was analysed using the standard method 4500-P [25].

After harvesting the biomass, the samples were rinsed with distilled water until the conductivity of the supernatant was <400 μS $cm^{-1}$. Biomass was characterised after been freeze-dried using a Freeze Dryer LyoAlfa15 (Telstar). The characterisation consisted of a triple analysis of each triplicate for the samples obtained at the end of the batch and semi-continuous operation. Total soluble proteins determination was carried out using the BioRad DC Lowry reagent kit, based on the traditional Lowry procedure [27]. Carbohydrates and lipids were determined following the protocols of Dubois et al. [28] and the phospho-vanillin spectrophotometric method [29], respectively. Elemental analysis and phosphorus were measured once per triplicate. Elemental analysis (C, H, S, and total particulate nitrogen TPN) was performed using Thermo Scientific FLASH 2000 Elemental Analyser. Total particulate phosphorus (TPP) was determined, previous acid digestion using a heating block (DigiPREP JR, SCP-Science), by a plasma-atomic emission spectrometry (ICP-AS, Iris Intrepid model, Thermo Elemental). For the fatty acid (FA) and amino acid (AA) profile, a composite sample from the three batch reactors and a composite sample from the last three days of semi-continuous operation were analysed. FA profile was carried out by transesterification using Lepage and Roy method [30] and mass spectrometry coupled to gas chromatography. AA profile was determined using fluorescence detection and an AccQ·Tag Ultra Derivation Kit [31].

### 2.4. Data analysis

#### 2.4.1. Biomass production

Verhulst's [32] kinetic logistic equation was used to model biomass growth in batch experiments.

$$\frac{X = X_0 \bullet X_{max} \bullet e^{\mu_{max} \cdot t}}{X_{max} - X_0 + X_0 e^{\mu_{max} \cdot t}} \quad (1)$$

where $X_0$ and X are biomass concentration (mg TSS $L^{-1}$) at instant $t = 0$ (d) and t, respectively, $X_{max}$ is the highest biomass concentration in the reactor (mg SS $L^{-1}$), and $\mu_{max}$ is the specific growth rate ($d^{-1}$).

Productivity under batch operation ($P_b$, mg $L^{-1}$ $d^{-1}$) was calculated according to Ruiz et al. [33] (Eq. (2)).

$$\frac{P_b = \mu_{max} \bullet (0.9 \bullet X_{max} - 1.1 \bullet X_0}{\frac{ln\left(\frac{9 \bullet (X_{max} - 1.1 \bullet X_0)}{1.1 \bullet X_0}\right)}{}} \quad (2)$$

For semi-continuous operation, the initial biomass concentration ($X_i$) after feeding the reactor was stated to determine the volume of the reactor to be harvested. From a mass balance in the reactor operated in a semi-continuous at steady-state combined with the Verhulst growth equation, $X_i$ (mg $L^{-1}$), $X_e$ (mg $L^{-1}$), hydraulic retention time (HRT, d) and productivity ($P_{sc}$, mg $L^{-1}$ $d^{-1}$) were determined using Eqs. (3), (4), (5) and (6) from Villar-Navarro et al. [34] for maximum productivity.

$$X_i = \frac{X_{max}}{1 + e^{\mu_{max} \cdot 0.5 \cdot t_F}} \quad (3)$$

$$\frac{X_e = X_{max} \bullet e^{\mu_{max} \cdot 0.5 \cdot t_F}}{1 + e^{\mu_{max} \cdot 0.5 \cdot t_F}} \quad (4)$$

$$\frac{HRT = t_F \bullet e^{\mu_{max} \cdot 0.5 \cdot t_F}}{e^{\mu_{max} \cdot 0.5 \cdot t_F} - 1} \quad (5)$$

$$P_{SC} = \frac{X_e - X_i}{t_F} \quad (6)$$

$X_e$ and $X_i$ are the biomass concentration in the reactor before and after feeding the photobioreactor (mg $L^{-1}$), and $t_F$ is the time elapsed between feedings (d).

#### 2.4.2. Nutrients removal

Batch nutrient removal kinetics ($k_s$, $d^{-1}$) was calculated using three different models, Log-linear (Eq. (7)), Log-linear + shoulder (Eq. (8)) and Log-linear + tail (Eq. (9)). The tool GinaFit [35] was used to model the results.

$$S = S_0 \bullet e^{-k_S \cdot t} \quad (7)$$

$$S = S_0 \bullet e^{(-k_S \cdot t)} \bullet \frac{e^{(k_S \cdot SL)}}{1 + (e^{(k_S \cdot SL)} - 1) \bullet} e^{(k_S \cdot t)} \quad (8)$$

$$S = (S_0 - S_{res}) \bullet e^{(-k_S \cdot t)} + S_{res} \quad (9)$$

Where S is the substrate, nitrogen or phosphorus, concentration (mg $L^{-1}$) at an instant t (d), $S_0$ is the initial nutrient concentration (mg $L^{-1}$),

and $k_S$ (being S = N or P) represents the first-order kinetic constant of substrate removal ($d^{-1}$).

Eq. (10) was used to determine, according to Villar-Navarro et al. [34], from a mass balance in the reactor and considering a first-order substrate removal kinetic, the predicted nutrient concentration in the effluent ($S_{e\text{-}c}$) during semi-continuous operation.

$$S_{e-c} = S_0 \bullet \left[ \frac{\frac{t_F}{HRT}}{e^{-k_s \bullet t_F} - \left(1 - \frac{t_F}{HRT}\right)} \right] \quad (10)$$

Eq. (11) describes the substrate consumption rate when the substrate availability is half ($CR_{1/2\text{-}S}$, mg $L^{-1}$ $d^{-1}$) under batch operation.

$$CR_{1/2-S} = \frac{dS}{dt} = k_S \bullet S = k_S \bullet \frac{S_0 - S_{na}}{2} \quad (11)$$

Being $S_{na}$, the unassimilable dissolved substrate concentration.

In the semi-continuous operation, and according to a previous study [34], the substrate removal rate ($R_c$, mg $L^{-1}$ $d^{-1}$) and nitrogen (total particulate nitrogen, TPN) and phosphorus (total particulate phosphorus, TPP) concentration in biomass of dry weight (%) were calculated using Eqs. (12) and (13), respectively.

$$R_C = \frac{S_0 - S_e}{t_F} \quad (12)$$

$$TPN_c \text{ or } TPP_c = \frac{S_i - S_e}{X_e - X_i} \bullet 100 \quad (13)$$

$S_e$ is the nutrient concentration in the effluent (mg $L^{-1}$).

The percentage of nitrogen input to the reactor that is removed by stripping ($\%_{NS}$) has been calculated with the ratio mass flow rate of nitrogen by stripping ($\dot{m}_{NS}$, mg $d^{-1}$) and the mass flow rate of dissolved nitrogen removed by microalgae ($\dot{m}_0 - \dot{m}_e$, mg $d^{-1}$) (Eq. (14)).

$$\%_{Ns} = \frac{\dot{m}_{NS}}{\dot{m}_0 - \dot{m}_e} \bullet 100 \quad (14)$$

Eq. (15) was used to calculate the mass flow rate of nitrogen removed by stripping ($\dot{m}_{NS}$, mg $d^{-1}$).

$$\dot{m}_{NS} = Q_0 \cdot (N_0 - N_e - N_x \cdot X_e) \quad (15)$$

where $Q_0$ is the feed flow rate (L $d^{-1}$), $N_0$ is the dissolved nitrogen concentration in the feed (mg $L^{-1}$), $N_e$ is the dissolved nitrogen concentration in the effluent (mg $L^{-1}$), $N_x$ is the nitrogen concentration in the biomass (% TPN), and $X_e$ is the biomass concentration in the effluent (mg $L^{-1}$).

### 2.5. Statistical analysis

Nutrient and biomass concentrations data were adjusted to each kinetic model using the "solver" tool of Microsoft Excel to minimise residues squared. It uses a generalised reduced gradient algorithm implemented and the GRG2, a non-linear programming algorithm [36].

Confidence interval (CI) was calculated at a significance level $\alpha = 0.05$ for mean samples with student's *t*-test distribution. Normality of the data was determined by means of Shapiro-Wilk test ($p > 0.05$) using R software.

## 3. Results and discussion

### 3.1. Micronutritional requirements experiments

A preliminary test was carried out to know if micronutrients could limit microalgae growth using fish farm effluent as culturing media. In the case of *N. gaditana*, *I. galbana*, *T. chuii* and the bloom, only the effect of vitamins and metals was studied while diatoms (*P. tricornutum* and *C. gracilis*) include silicates.

As an example, Fig. 1 represents the evolution of *I. galbana* concentration under the four different conditions tested. Microalgae growth kinetics is higher and similar in those experiments in which metals were added regardless of whether the medium contained vitamins or not. For brevity, the figures of the rest microalgae are shown in supplementary data (Fig. S5).

In quantitative terms, the maximum productivity reached in all the batch experiments was calculated (Table 2) by modelling the data using the Verhulst growth model Eq. (1). When the productivity with the effluent with no micronutrients addition (-M-V-S) is compared with the rest of the experiments, it can be observed that the addition of trace metals almost double biomass productivity. On the other hand, the addition of vitamins does not generate a substantial increase in the microalgae growth kinetics. Concerning the silicates, like the vitamins, the results indicate that they do not appear to be a limiting growth factor of the diatoms.

These results are in accordance to previous studies that indicated that the addition of iron, one of the trace metals, increases the microalgae biomass since it is one of the trace metals involved in enzymatic reactions in photosystem I and II [37]. Previous studies also indicate that microalgae do not need the addition of vitamins or silicates for cultivation [38] and that the vitamin requirement is different depending on the species [39].

### 3.2. Biomass evolution

Fig. 2 shows the evolution of *I. galbana* biomass concentration under batch and semi-continuous operation. The evolution of the rest of the microalgae species is shown in Fig. S6. Maximum biomass concentration ($X_{max}$) and specific growth rate ($\mu_{max}$) (Table 3), in batch, were determined by adjusting the experimental data to the Verhulst growth equation (Eq. (1)) while maximum batch biomass productivity was calculated using Eq. (2). For *I. galbana*, $X_{max}$ and $\mu_{max}$ reached 220 ± 8 mg $L^{-1}$ and 0.015 ± 0.001 $d^{-1}$, respectively. In general, *T. chuii* was the microalgae with the highest $\mu_{max}$ (0.049 $d^{-1}$) and productivity (67 mg $L^{-1}$ $d^{-1}$). On the contrary, *N. gaditana* and *C. gracilis* were the microalgae with the lowest $\mu_{max}$ (0.015 and 0.016 $d^{-1}$). Moreover, *C. gracilis* was also the one with the lowest $X_{max}$ and productivity (52 mg $L^{-1}$ and 7 mg $L^{-1}$ $d^{-1}$) in batch. These results are in line with those obtained by other authors, with average $\mu_{max}$ values around 0.038 $d^{-1}$ for *I. galbana* [40], 0.030 $d^{-1}$ for *T. chuii* [22] and 0.040 $h^{-1}$ for *P. tricornutum* [41]. In the case of productivity, the bibliographical values, between 52 mg $L^{-1}$ $d^{-1}$ and 133 mg $L^{-1}$ $d^{-1}$ [42] for marine microalgae species, are slightly higher than those obtained in this study. Probably because the concentration of nutrients (11.5 ± 1.2 mg TDN $L^{-1}$; 0.28 ± 0.10 mg TDP $L^{-1}$) is lower than those found in the culture media (35 mg TDN $L^{-1}$; 2.3 mg TDP $L^{-1}$) used in that study, suggesting that it could be one of the growth-limiting factors of the culture.

The batch lasted between 10 and 15 days for all the microalgae. Once the batch was completed, semi-continuous production began. The operating parameters of the semi-continuous operation, $X_i$ (Eq. (3)), $X_e$ (Eq. (4)) and HRT (Eq. (5)) to obtain the maximum productivity, were calculated from the kinetic parameters obtained during the batch. In the case of *I. galbana*, the semi-continuous operation lasted 24 days. During the first ten days, the feeding was of simulated aquaculture effluent plus trace metals (according to the results of Section 3.1). However, growth decreased dramatically on days 23, 24 and 25 (Fig. 2). Therefore, it was decided to add vitamins to find out if they were indispensable for *I. galbana*. In fact, adding trace metals plus vitamins, $X_i$ (61 ± 2, $\alpha = 0.05$) and $X_e$ (96 ± 2, $\alpha = 0.05$) kept stable values until the end of the experiment. Both, *N. gaditana*, *P. lutheri* and *C. gracilis* also needed to add vitamins and trace metals in the long term. In the case of the bloom, vitamins were added on time. *T. chuii* and *P. tricornutum* were the only two species with constant growth without vitamins. Therefore, batch trials provide valuable information for microalgae cultivation. However, to confirm the micronutritional requirements of different species in the

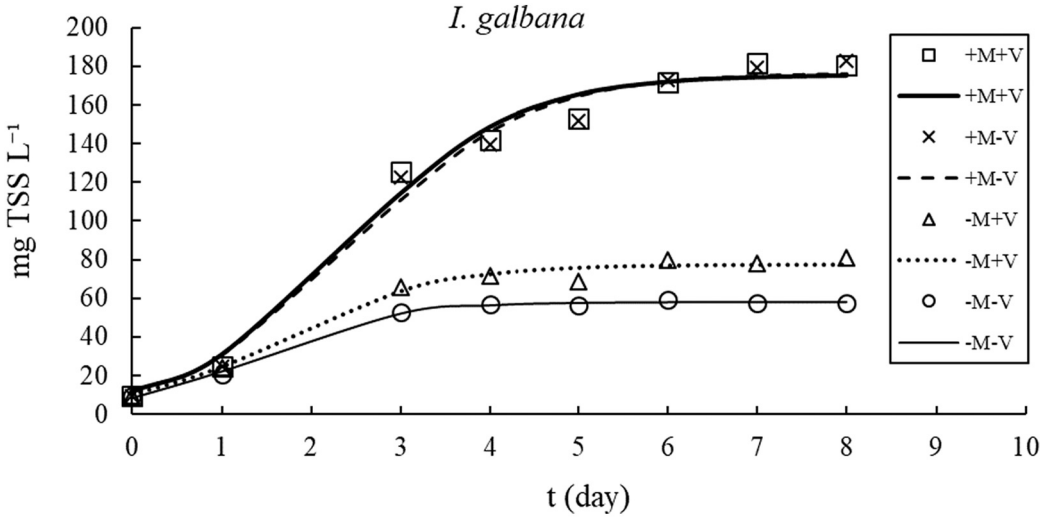


**Fig. 1.** Micronutritional requirements experiment for *Isochrysis galbana* (IG): no vitamins or metals (-M-V), only vitamins (-M+V), only metals (+M-V) and vitamins and metals (+M+V). Dots represent experimental data and lines the predicted values.

**Table 2**
Maximum batch productivity ($P_b$, mg $L^{-1}$ $d^{-1}$) of all microalgae using vitamins (V), metals (M) and silicates (S).

| | *N. gaditana* | *I. galbana* | *T. chuii* | *P. tricornutum* | *C. gracilis* | Bloom |
|---|---|---|---|---|---|---|
| -M-V-S | 19.86 | 19.57 | 60.38 | 16.50 | 12.94 | 19.56 |
| +M-V-S | 51.32 | 39.24 | 106.04 | 25.12 | 25.90 | 39.85 |
| +M+V-S | 50.08 | 40.31 | 106.04 | 26.29 | 25.90 | 43.07 |
| -M+V-S | 18.91 | 22.18 | 60.38 | 20.04 | 16.39 | 28.01 |
| +M+V+S | NE | NE | NE | 26.72 | 22.72 | NE |
| +M-V+S | NE | NE | NE | 24.67 | 26.40 | NE |
| -M+V+S | NE | NE | NE | 14.67 | 22.68 | NE |
| -M-V+S | NE | NE | NE | 15.78 | 14.30 | NE |

NE, not evaluated.

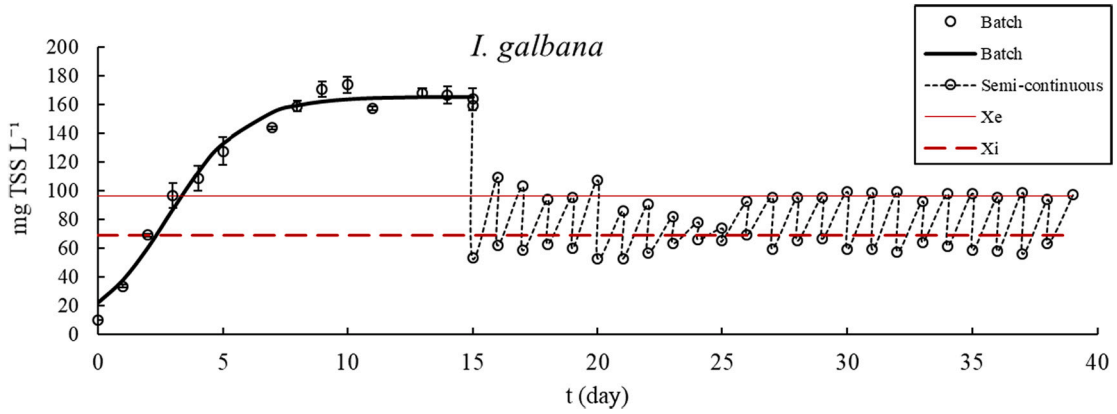


**Fig. 2.** Biomass concentration evolution of *Isochrysis galbana* during batch (average and standard deviation, $n = 3$) and semi-continuous operation. In batch, dots represent experimental data and lines predicted values. $X_i$ and $X_e$ are the values calculated for the semi-continuous stage using the batch kinetic parameters (Eqs. (2) and (3)).

long term, it is essential to carry out experiments under continuous or semi-continuous operation.

Under semi-continuous, the reactors were fed daily with an adequate volume to reach the value $X_i$ calculated with the model (Eq. (3)). The average values of $X_e$, $X_i$ and HRT of all semi-continuous experiments (24 days) can be found in Table 4. Comparing the predicted values of $X_e$ with the experimental ones, it was adjusted for all microalgae except *N. gaditana*, *P. lutheri*, *T. chuii* and the bloom with a difference between 8 and 39 mg $L^{-1}$. The experimental HRT ranged from 2.3 d (*P. tricornutum*) to 9.0 d (*N. gaditana*), being typical for microalgae from 3 to 10 d [43].

Fig. 3 shows the predicted (Eq. (6)) and experimentally observed productivity for all microalgae. They can be divided into three groups, those with low observed productivities (*N. gaditana*, *P. lutheri* and *C. gracilis*; 9.25–10.2 mg $L^{-1}$ $d^{-1}$), medium (*I. galbana*, *P. tricornutum* and the bloom; 28.9–36.6 mg $L^{-1}$ $d^{-1}$) and high (*T. chuii*; 70.6 mg $L^{-1}$ $d^{-1}$). Other authors [44,45] also observed productivities in semi-continuous operation close to those obtained in this study for *Pavlova lutheri* (12–15 mg $L^{-1}$ $d^{-1}$), *Nannochloropsis sp.* (12–20 mg $L^{-1}$ $d^{-1}$), and *T. chuii* (62–72 mg $L^{-1}$ $d^{-1}$). *I. galbana*, productivity was 59% higher than that described by other authors [44].

**Table 3**
Batch kinetics model parameters. Average and standard deviation ($n = 3$).

| | $\mu_{max}$ ($h^{-1}$) | $X_{max}$ (mg $L^{-1}$) | Productivity (mg $L^{-1}$ $d^{-1}$) | $R^2$ |
|---|---|---|---|---|
| *N. gaditana* | 0.015 ± 0.001 | 220 ± 8 | 23 ± 1 | 0.995 |
| *P. lutheri* | 0.022 ± 0.005 | 240 ± 58 | 33 ± 1 | 0.994 |
| *I. galbana* | 0.028 ± 0.004 | 166 ± 4 | 28 ± 3 | 0.983 |
| *T. chuii* | 0.049 ± 0.001 | 247 ± 3 | 67 ± 1 | 0.994 |
| *P. tricornutum* | 0.046 ± 0.001 | 94 ± 7 | 32 ± 1 | 0.966 |
| *C. gracilis* | 0.016 ± 0.003 | 52 ± 6 | 7 ± 1 | 0.950 |
| Bloom | 0.038 ± 0.002 | 178 ± 3 | 42 ± 2 | 0.990 |

$\mu_{max}$, specific growth rate.
$X_{max}$, highest biomass concentration in the reactor.

**Table 4**
Semi-continuous operation parameters. Average and confidence interval ($\alpha = 0.05$, $n = 16$). In brackets, the predicted values calculated with Eqs. (3), (4) and (5).

| | $X_i$ (mg $L^{-1}$) | $X_e$ (mg $L^{-1}$) | HRT (d) |
|---|---|---|---|
| *N. gaditana* | 100 ± 1 [100] | 109 ± 1 [120] | 9.0 ± 0.1 [6.2] |
| *P. lutheri* | 84 ± 8 [104] | 91 ± 5 [135] | 6.1 ± 0.2 [4.4] |
| *I. galbana* | 61 ± 2 [69] | 96 ± 2 [97] | 3.4 ± 0.2 [3.5] |
| *T. chuii* | 74 ± 2 [86] | 143 ± 7 [159] | 2.7 ± 0.1 [2.3] |
| *P. tricornutum* | 36 ± 1 [34] | 64 ± 3 [60] | 2.3 ± 0.1 [2.3] |
| *C. gracilis* | 22 ± 1 [24] | 29 ± 1 [29] | 6.1 ± 0.1 [5.7] |
| Bloom | 63 ± 1 [69] | 96 ± 5 [109] | 3.4 ± 0.4 [2.7] |

$X_i$, biomass concentration after feeding the reactor.
$X_e$, biomass concentration before feeding the reactor.
HRT, hydraulic retention time.

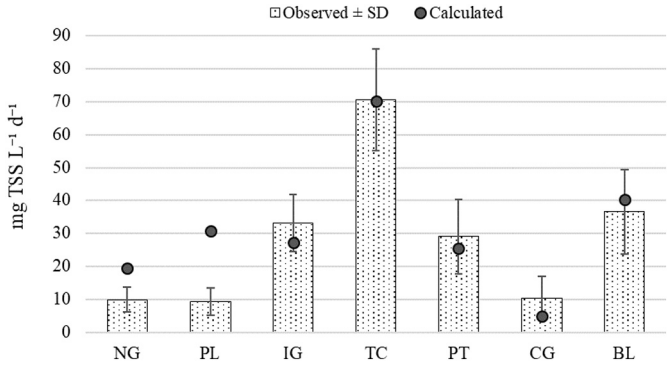


**Fig. 3.** Productivity (mg $L^{-1}$ $d^{-1}$) under semi-continuous operation. Average standard deviation (SD, $n = 25$). Dots represent the predicted productivity, calculated by Eq. (6). NG, *Nannochloropsis gaditana*; PL, *Pavlova lutheri*; IG, *Isochrysis galbana*; TC, *Tetraselmis chuii*, PT, *Phaeodactylum tricornutum*; CG, *Chaetoceros gracilis*; BL, bloom.

### 3.3. Nutrient removal

During the first hour, in the batch experiment, 80 to 97% TDP removal occurs for all species except *N. gaditana*, which decreased by 19%. In that case, 94% removal (0.02 mg TDP $L^{-1}$) was reached at 21 h. As Table 5 shows, phosphorus removal first-order kinetic constant ($k_P$) ranged from 35.4 to 42.6 $d^{-1}$ (Table 5), being the lowest 0.131 $d^{-1}$ (*N. gaditana*). In this case, 94% removal was achieved in 21 h (0.02 mg $L^{-1}$). The consumption rate when half of the substrate is available ($CR_{1/2\text{-}TDP}$) range between 4.68 and 7.67 mg $L^{-1}$ $d^{-1}$ for all microalgae except for *N. gaditana*, 0.02 mg $L^{-1}$ $d^{-1}$ (Table S1). In all cases, there was no residual TDP in the effluent at the end of the batch. These removal values were similar to those found in other studies with similar marine species (*Dunaliella* sp., *Tetraselmis* sp. and *Nannochloropsis* sp.), between 0.78 and 6.66 mg $P\text{-}PO_4^{3-}$ $L^{-1}$ $d^{-1}$ [42,46]. Furthermore, one of the studies [46] indicates that the high removal rates can be explained due to phosphorus precipitation which occurs at pH > 8, as is the case in all the experiments (Fig. S7).

Fig. 4 shows the evolution of the nitrogen species during the batch operation for *I. galbana*. It is observed that ammonium was the first nitrogen species to be consumed. Once the $N\text{-}NH_4^+$ intake was total, nitrate and nitrite started to be removed (day four). On day seven, dissolved inorganic nitrogen (DIN) decreased to 0.5 mg $L^{-1}$ and dissolved organic nitrogen (DON) began to increase. From day nine all TDN was DON.

"Log-linear", "Log-linear + shoulder" and, in the case of TDN/DIN removal, the "Log-linear + tail" models were used (Table 5). It was noted that $N\text{-}NH_4^+$ was the preferred nitrogen species for all microalgae as it was the first substrate to be consumed, with no shoulder in six of the seven species tested. The first-order kinetic removal constant ($k_N$) varied from 0.531 (*C. gracilis*) to 2.352 (*T. chuii*) $d^{-1}$, being the average 1.483 $d^{-1}$. In the case of nitrate, four out of seven microalgae presented a shoulder between 3.3 and 5.5 days and the $k_N$ range from 0.016 (*C. gracilis*) to 2.261 $d^{-1}$ (*T. chuii*), being the average 0.898 $d^{-1}$. Five out of seven microalgae presented shoulder for nitrite consumption, between 6.2 and 11.1 days. Thus, a sequential consumption of nitrogen species was observed in which nitrite was the third species to decrease in concentration and did so at a kinetic removal constant comparable to that of nitrate or ammonium, between 0.011 (*C. gracilis*) and 2.250 $d^{-1}$ (*I. galbana*), being the average 1.074 $d^{-1}$.

In the case of the TDN, $k_N$ varied from 0.045 $d^{-1}$ (*C. gracilis*) to 1.607 $d^{-1}$ (*T. chuii*), being the average 0.556 $d^{-1}$. These values are within the values (1.13–1.33 $d^{-1}$) founded by other authors [47]. Six out of seven microalgae showed a residual value ($N_{res}$), which indicated that there was non-assimilable nitrogen. This non-assimilable nitrogen corresponds to the DON generated by the microalgae, except for *N. gaditana* and *P. tricornutum*, where residual DIN concentrations were observed at the end of the batch experiment (Table 5). In the case of *C. gracilis*, only 54% of TDN was consumed. Table S1 shows the substrate consumption rate when the substrate is halfway through ($CR_{1/2\text{-}TDN}$). The highest consumption was achieved with *T. chuii* (8.70 mg $L^{-1}$ $d^{-1}$). *I. galbana*, the bloom and *N. gaditana* obtained similar values, 2.71, 2.62 and 2.12 mg $L^{-1}$ $d^{-1}$, respectively. The lowest values were found for *P. lutheri* (1.49 mg $L^{-1}$ $d^{-1}$), *P. tricornutum* (1.40 mg $L^{-1}$ $d^{-1}$) and, finally, *C. gracilis* (0.15 mg $L^{-1}$ $d^{-1}$). Sacristan del Alva et al. [42] and Schulze et al. [46] found similar values for TDN removal rates using marine microalgae under batch operation, from 2.79–7.34 mg N $L^{-1}$ $d^{-1}$.

Finally, Figs. S5, S6 and S7 show the evolution of pH, QY and DOC during the batch. The average initial pH was 7.9 ± 0.3. In all cases, the pH > 8 during the first seven days and, in some cases, it reached 9.82 (*I. galbana*). In most cases, a pattern of rising pH was observed during the first four days of the experiment, which correspond to the highest photosynthetic activity days. This pattern can also be observed in Fig. S8, where the QY increased during these days. In the case of dissolved organic carbon (DOC) (Fig. S9), all microalgae tended to increase concentration over time. In some microalgae, DOC concentration remained around 10 mg $L^{-1}$ (*N. gaditana*, *P. tricornutum*, the bloom), *T. chuii* and *C. gracilis* reached 15 mg $L^{-1}$, and *P. lutheri* and *I. galbana* exceeded 30 mg $L^{-1}$. Although many authors described DOC removal in microalgae reactors [42,47], in this study, an increase was observed in all experiments. This phenomenon was explained by Kim et al. [48]. They linked an increase in DOC using *Tetraselmis* sp. to the secretion of polysaccharides. Furthermore, these authors also found that the microalgae can reuse the release of DOC at some point of the growth, as occurred with the bloom (Fig. S9).

The quality of the effluent in terms of TDN, TDP, DOC and pH during semi-continuous operation is presented in Table S2. The effluent with

**Table 5**
Nutrient removal kinetics of nitrogen ($k_N$) and phosphorus ($k_P$). Average and standard error ($n = 3$).

| | | *N. gaditana* | *P. lutheri* | *I. galbana* | *T. chuii* | *P. tricornutum* | *C. gracilis* | Bloom |
|---|---|---|---|---|---|---|---|---|
| $N\text{-}NO_3^-$ | $k_N$ ($d^{-1}$) | 0.622 ± 0.242 | 0.607 ± 0.137 | 1.838 ± 0.178 | 2.261 ± 0.524 | 0.066 ± 0.003 | 0.016 ± 0.003 | 0.876 ± 0.215 |
| | SL (d) | 3.5 ± 2.4 | 5.5 ± 1.0 | 5.1 ± 0.3 | n.d. | n.d. | n.d. | 3.3 ± 0.7 |
| | $N_{res}$ (mg $L^{-1}$) | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| | $R^2$ | 0.920 | 0.945 | 0.996 | 0.903 | 0.982 | 0.909 | 0.977 |
| $N\text{-}NO_2^-$ | $k_N$ ($d^{-1}$) | 1.716 ± 0.278 | 1.488 ± 0.042 | 2.250 ± 0.053 | 1.546 ± 0.504 | 0.163 ± 0.027 | 0.011 ± 0.002 | 0.344 ± 0.148 |
| | SL (d) | 6.4 ± 0.4 | 7.8 ± 0.1 | 6.5 ± 0.1 | n.d. | 11.1 ± 0.9 | n.d. | 6.2 ± 1.1 |
| | $N_{res}$ (mg $L^{-1}$) | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| | $R^2$ | 0.986 | 0.999 | 0.999 | 0.825 | 0.985 | 0.911 | 0.947 |
| $N\text{-}NH_4^+$ | $k_N$ ($d^{-1}$) | 2.228 ± 0.152 | 1.035 ± 0.120 | 1.404 ± 0.106 | 2.352 ± 0.091 | 1.366 ± 0.233 | 0.531 ± 0.053 | 1.644 ± 0.312 |
| | SL (d) | n.d. | n.d. | n.d. | n.d. | n.d. | 5.7 ± 1.1 | n.d. |
| | $N_{res}$ (mg $L^{-1}$) | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| | $R^2$ | 0.982 | 0.961 | 0.978 | 0.994 | 0.920 | 0.983 | 0.933 |
| DIN | $k_N$ ($d^{-1}$) | 0.504 ± 0.103 | 0.493 ± 0.053 | 2.076 ± 0.337 | 2.177 ± 0.253 | 0.238 ± 0.032 | 0.051 ± 0.003 | 0.285 ± 0.030 |
| | SL (d) | n.d. | 4.1 ± 0.6 | 5.8 ± 0.4 | n.d. | n.d. | n.d. | n.d. |
| | $N_{res}$ (mg $L^{-1}$) | 0.18 | n.d. | n.d. | n.d. | 2.32 | n.d. | n.d. |
| | $R^2$ | 0.956 | 0.989 | 0.984 | 0.987 | 0.985 | 0.979 | 0.947 |
| TDN | $k_N$ ($d^{-1}$) | 0.431 ± 0.049 | 0.276 ± 0.020 | 0.563 ± 0.084 | 1.607 ± 0.360 | 0.382 ± 0.072 | 0.045 ± 0.005 | 0.590 ± 0.055 |
| | SL (d) | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| | $N_{res}$ (mg $L^{-1}$) | 0.53 | 1.96 | 1.75 | 1.10 | 2.88 | n.d. | 1.50 |
| | $R^2$ | 0.978 | 0.993 | 0.965 | 0.933 | 0.954 | 0.911 | 0.986 |
| TDP | $k_P$ ($d^{-1}$) | 0.131 ± 0.022 | 41.806 ± 15.522 | 42.65 ± 18.83 | 35.368 ± 0.223 | 38.683 ± 21.696 | 42.281 ± 24.411 | 36.031 ± 18.027 |
| | SL (d) | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| | $N_{res}$ (mg $L^{-1}$) | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| | $R^2$ | 0.980 | 0.879 | 0.837 | 0.999 | 0.761 | 0.750 | 0.800 |

TDN, total dissolved nitrogen; DIN, dissolved inorganic nitrogen; TDP, total dissolved phosphorus; SL, shoulder length; $N_{res}$, residual value.
n.d., not detected.

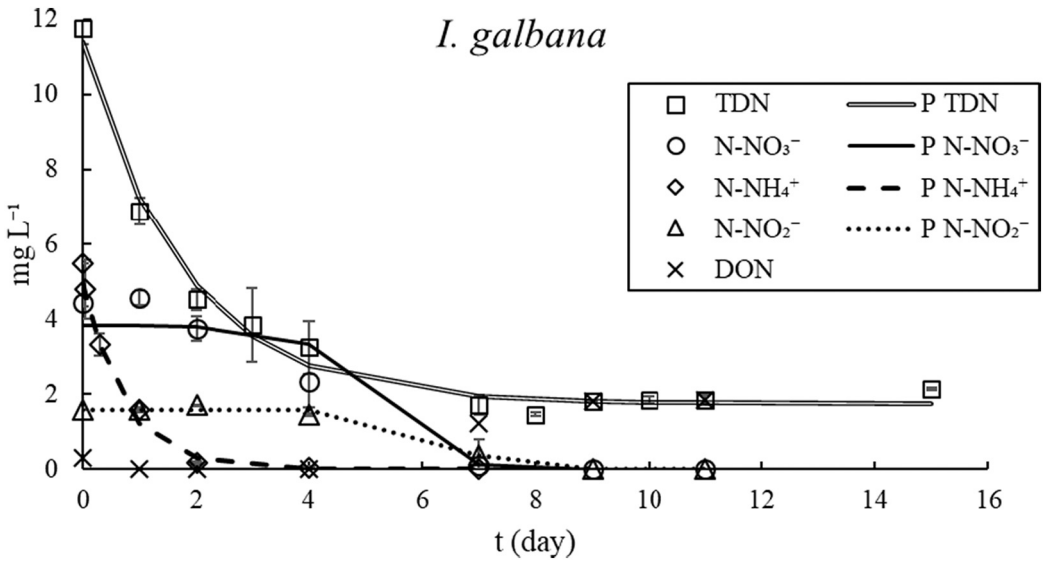


**Fig. 4.** Evolution of nitrogen concentration during batch experiment testing *I. galbana*. Dots are experimental, and lines are predicted (P) values. Average and standard deviation ($n = 3$). TDN, total dissolved nitrogen; DON, dissolved organic nitrogen.

the lowest concentration of TDN and TDP was that obtained with *T. chuii* (TDN = 0.76 ± 0.04 mg $L^{-1}$; TDP = 0.01 ± 0.00 mg $L^{-1}$), followed by *I. galbana* (TDN = 2.39 ± 0.12 mg $L^{-1}$; TDP = 0.00 ± 0.01 mg $L^{-1}$). DOC concentration varied between 4.72 (*P. tricornutum*) and 10.81 mg $L^{-1}$ (*N. gaditana*) and the pH between 8.35 (*C. gracilis*) and 9.14 (*I. galbana*).

Biomass concentration of nitrogen and phosphorus was calculated (Eq. (13)) and compared with the analysed values. In the case of the TPP, differences between observed and calculated values were below 0.06%. The analysed phosphorus biomass content varied between 0.14 (*T. chuii*) and 0.32% TPP (the bloom), within the typical values (0.1–1.2% TPP) [49]. The experimental nitrogen content ranged from 3.79 (*T. chuii*) to 5.45% TPN (*I. galbana*), while usually varying between 2.73 and 8.60% TPN for marine species [50]. Differences between observed and calculated values of 1.8–6.8% TPN were found. This mismatch means that a part of the nitrogen was removed in an abiotic way. Stripping is proposed as a secondary removal route since the possibility of denitrification [43] is low due to the high pH (>8.35) and the low DOC (<10.8 mg $L^{-1}$) in all cases. Besides, denitrification processes were discarded, as there was no photoperiod, and the reactors were aerated, so the presence of oxygen was continuous. The percentage of stripping of total removed nitrogen was calculated according to the Eq. (14). Average nitrogen removal by stripping of 51 ± 4% for all microalgae was observed (Table S2), which is in line with the results obtained by other authors (40%) [51].

Finally, observed ($R_o$) and calculated (Eq. (12)) ($R_c$) removal of TDN and TDP was compared during the semi-continuous operation. In the case of the TDP, no significant differences (CI; α = 0.05) were found between calculated and experimental values except in *N. gaditana* (50%)

(Table S2). Fig. 5 shows the results of the observed and calculated removal rates for TDN under semi-continuous. In this case, the observed and calculated values also matched, so it can be considered that the prediction is reasonably suitable. In terms of observed values, the highest removal rate (mg TDN $L^{-1}$ $d^{-1}$) was obtained with *T. chuii* (12.59 ± 0.59), followed in equal parts by *N. gaditana* (11.16 ± 0.41) and *I. galbana* (10.18 ± 1.25), then the bloom (8.10 ± 0.28), *P. tricornutum* (6.94 ± 0.87) and finally *C. gracilis* (1.52 ± 0.13). These values were close to the range described by other authors (3.85–7.07 mg N $L^{-1}$ $d^{-1}$) using similar marine microalgae [52,53].

### 3.4. Biomass composition

Table 6 shows biomass composition of dry weight (%) of all microalgae under batch (B) and semi-continuous (SC) operation. Table S3 shows elemental analysis (C, H, N, S) and phosphorus content. The species with the highest total soluble protein (TSP) content was *I. galbana* (SC), followed by *P. tricornutum* (SC). The lowest TSP content was found in *P. tricornutum* (B), *T. chuii* (B) and *N. gaditana* (B). The species with the highest content of total lipids were *N. gaditana* (B) and the bloom (B). Those with the lowest content were *T. chuii* (B and SC) and *C. gracilis* (SC). *P. lutheri* (B) and *I. galbana* (B) were the species with the highest content of soluble carbohydrates and *I. galbana* (SC) and *P. tricornutum* (B) the ones with the lowest percentages. Considering that the results for proteins and carbohydrates are soluble and the total content would be higher, the results are within the range obtained by other authors for all microalgae. It should be noted that it is only indicated in the studies for *P. lutheri* [54] and *C. gracilis* [55] that biomass was obtained at the end of the exponential stage or under batch production. The other consulted references for the rest species [13,54,56], were not indicated under which type of operation the biomass was obtained.

Regarding TSP, there was an increase from 17 (*C. gracilis*) to 107% (*P. tricornutum*) under semi-continuous operation. However, the percentage of lipids was higher under batch than under semi-continuous operation, between 24 (*C. gracilis*) and 45% (*T. chuii*). These results were previously founded by other authors [57,58], who suggested that during nitrogen and phosphorus starvation (at stationary phase) microalgae tend to accumulate lipids, while under non-limiting nitrogen conditions (under semi-continuous operation) proteins synthesis is promoted.

Table 7 shows the fatty acid (FA) profile of microalgae cultured in simulated fish farming effluent under batch and semi-continuous operation. Since the fatty acids of the microalgae would replace fish oil (FO), the fatty acid profile of FO was indicated as well [59]. The values of FA

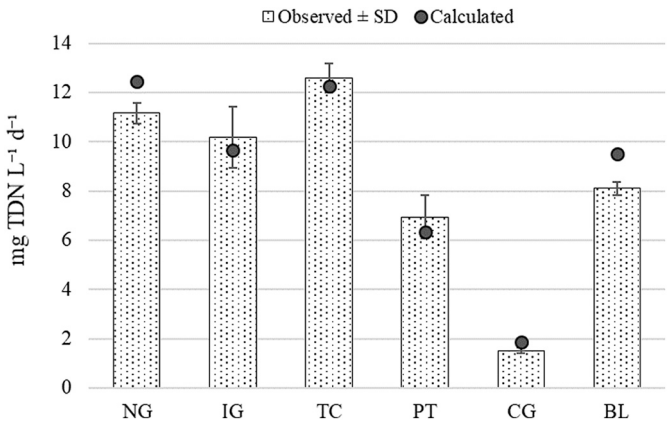


**Fig. 5.** Observed and calculated total dissolved nitrogen (TDN) removal (mg TDN $L^{-1}$ $d^{-1}$). Average and standard deviation (SD), $n = 3$ for observed values. NG, *N. gaditana*; IG, *I. galbana*; TC, *T. chuii*; PT, *P. tricornutum*; CG, *C. gracilis*; BL, bloom.

**Table 6**
Gross composition of microalgae. Average and confidence interval ($\alpha = 0.05$, $n = 9$) of total soluble proteins (TSP), total lipids (TL) and total soluble carbohydrates (TSC) of the six microalgae species and the bloom cultured under batch (B) and semi-continuous (SC) operation.

| | | TSP (%) | TL (%) | TSC (%) |
|---|---|---|---|---|
| *N. gaditana* | B | 14.3 ± 2.3 | 29.7 ± 1.0 | 16.3 ± 1.7 |
| | SC | 20.3 ± 0.3 | 17.5 ± 0.5 | 20.7 ± 3.6 |
| *P. lutheri* | B | 27.7 ± 3.0 | 18.6 ± 0.9 | 32.9 ± 2.8 |
| | SC | NE | NE | NE |
| *I. galbana* | B | 19.2 ± 1.2 | 20.0 ± 1.2 | 30.6 ± 2.9 |
| | SC | 36.4 ± 0.8 | 14.6 ± 0.5 | 6.9 ± 1.0 |
| *T. chuii* | B | 14.2 ± 0.6 | 10.7 ± 0.3 | 25.4 ± 1.1 |
| | SC | 23.0 ± 2.8 | 5.7 ± 0.7 | 24.4 ± 7.1 |
| *P. tricornutum* | B | 13.8 ± 0.4 | 18.3 ± 0.7 | 8.5 ± 0.8 |
| | SC | 28.6 ± 0.6 | 11.6 ± 0.6 | 12.3 ± 1.1 |
| *C. gracilis* | B | 18.4 ± 0.3 | 14.0 ± 1.5 | 16.8 ± 6.1 |
| | SC | 21.1 ± 1.5 | 11.3 ± 0.3 | 13.0 ± 3.6 |
| Bloom | B | 16.6 ± 0.5 | 23.2 ± 0.9 | 23.7 ± 1.0 |
| | SC | 20.1 ± 1.5 | 15.1 ± 1.3 | 17.5 ± 0.8 |

NE, not evaluated.

that reach the minimum value referring to FO were highlighted. The last two rows included the sum of the values highlighted from the 16 FA (n/1, FA) and the three groups of FA (n/3, of ΣFA): saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The sample with the highest number of coincidences was *C. gracilis* (SC) with seven of the 16 FA of FO, including arachidonic acid (ARA). Another five samples with six of the 16 FA followed it. The *N. gaditana* biomass obtained in batch was the one with the weakest fatty acid profile with respect to FO.

Regarding the three large groups of FA, almost all the samples had an adequate SFA and MUFA profile. Only *N. gaditana* (SC) and the bloom (SC) also met optimal PUFA values. About the essential PUFAs, ARA, EPA and DHA, these results differed from what other authors [56,60,61] have described in terms of content (ARA: 0.0–3.3%; EPA: 1.2–28.4%; DHA: 0.0–12.7%), but not in the proportion between them. Although the percentage was equal or lower, in all species, the EPA predominated over ARA and DHA, not in *I. galbana* (B and SC) where DHA was predominant, which coincides with that observed by other authors for the FA profile of this specie [61]. No clear pattern related with the feeding regime was observed in the distribution of these PUFA: in four of the six microalgae, a higher concentration of ARA, EPA or DHA was observed under batch operation. For *T. chuii* and *C. gracilis*, their proportion increased slightly during semi-continuous operation.

Finally, Table 8 shows the amino acid (AA) profile of different microalgae together with a fish meal (FM) AA profile [62]. Values above the values of the FM profile were highlighted. Also, two rows were added in which the number of matches (n/9 of Essential-AA and n/17 of Total-AA) compared to the FM reference values. In this case, the profiles were very similar, between five and seven matches of the nine essential AA and between 10 and 13 matches of the total AA were founded. If each amino acid is taken into account, all microalgae reached optimum levels of leucine (Leu), phenylalanine (Phe), threonine (Thr), valine (Val), and serine (Ser). On the contrary, only two samples reached glycine (Gly) and histidine (His) optimum values, and all samples were below optimum values of methionine (Met) and lysine (Lys). Furthermore, no differences were observed between the number of matches under batch and semi-continuous operation. In general terms, Leu and arginine (Arg) were the predominant essential AA, and His and Met were the least abundant. The most frequent non-essential AA were glutamic acid (Glu) and aspartic acid (Asp), and the least was cysteine (Cys).

A recent review by Kolmakova and Kolmakov [63] determined the predominant amino acids in microalgae and discussed the differences between species. As observed in this study, an essential amino acid profile of microalgae contains a high percentage of Leu and Arg and low Met and His. The most remarkable differences were found with

**Table 7**
Fatty acid (FA) profile (% of total) of six microalgae and a bloom under batch (B) and semi-continuous operation and fish oil (FO) profile [61]. The highlighted values indicate that they are above the reference value (FO). The sum of highlighted values is indicated in the n-matches rows: "n/16 of FA" and "n/3, of FA".

| | | *N. gaditana* | | *P. lutheri* | *I. galbana* | | *T. chuii* | | *P. tricornutum* | | *C. gracilis* | | Bloom | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FA (% of total) | Fish oil | B | SC | B | B | SC | B | SC | B | SC | B | SC | B | SC |
| 14:0 | 7.2 | 2.6 | 2.3 | **8.1** | **17.9** | **23.7** | n.d. | n.d. | 2.5 | **7.9** | **11.0** | **16.9** | 1.0 | 0.4 |
| 15:0 | – | 0.4 | 0.2 | 0.5 | 0.4 | 0.4 | n.d. | n.d. | 0.3 | 0.5 | 0.8 | 0.8 | 0.1 | 0.1 |
| 16:0 | 17.8 | **50.3** | **38.6** | **39.3** | **19.1** | **24.1** | **31.1** | **36.4** | **31.0** | **45.9** | **37.1** | **36.7** | **36.8** | **29.8** |
| 16:1 n-9 | 9.8 | **36.6** | 2.7 | **32.9** | 3.1 | 5.7 | n.d. | n.d. | **53.6** | **36.5** | **36.0** | **32.8** | 8.2 | 2.7 |
| 16:2 | – | 0.3 | 0.2 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 0.6 | 0.6 | 0.2 | n.d. |
| 17:0 | – | 0.3 | 0.2 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 0.3 | 0.3 |
| 18:0 | 3.9 | 2.0 | **4.6** | 0.9 | n.d. | n.d. | n.d. | n.d. | 0.8 | 1.5 | 1.1 | 1.5 | **5.2** | **4.1** |
| 18:1 n-7 | – | 0.4 | 2.0 | 1.8 | 1.4 | 2.2 | 7.5 | 7.0 | 2.3 | 0.5 | 1.1 | 0.5 | 2.1 | 2.3 |
| 18:1 n-9 | 12.0 | 5.7 | **15.7** | 3.1 | **36.5** | **25.6** | **44.3** | **28.3** | 5.3 | 2.5 | 9.3 | 4.7 | **25.2** | **22.2** |
| 18:2 n-6 | 1.1 | 0.7 | **16.0** | **1.8** | **2.7** | **3.6** | **3.3** | **5.7** | 0.3 | 0.4 | 0.9 | **1.4** | **14.3** | **20.3** |
| 18:3 n-3 | – | n.d. | 16.4 | 0.6 | 2.7 | 2.3 | 7.9 | 14.9 | n.d. | n.d. | n.d. | n.d. | 6.4 | 17.7 |
| 18:3 n-6 | 0.8 | n.d. | n.d. | 0.3 | n.d. | n.d. | 0.6 | n.d. | n.d. | n.d. | n.d. | 0.4 | n.d. | n.d. |
| 20:0 | 0.3 | n.d. | **0.6** | **0.8** | **9.1** | **7.4** | **1.7** | **2.5** | **0.3** | **0.4** | **0.5** | **0.7** | n.d. | n.d. |
| 20:1 | 1.9 | n.d. | n.d. | n.d. | n.d. | n.d. | **2.1** | **3.3** | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 20:4 n-6 ARA | 0.3 | **0.3** | n.d. | **0.6** | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | **0.3** | 0.1 | n.d. |
| 20:5 n-3 EPA | 18.3 | 0.4 | n.d. | 6.6 | n.d. | n.d. | 1.5 | 2.0 | 3.0 | 1.5 | 1.0 | 2.2 | 0.2 | n.d. |
| 22:0 | 0.1 | n.d. | **0.1** | n.d. | **0.7** | **1.0** | n.d. | n.d. | n.d. | **0.6** | n.d. | n.d. | n.d. | n.d. |
| 22:1 n-9 | 1.4 | n.d. | n.d. | n.d. | 0.2 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| 22:6 n-3 DHA | 8.5 | n.d. | 0.4 | 2.7 | 6.0 | 4.1 | n.d. | n.d. | n.d. | n.d. | 0.5 | n.d. | n.d. | n.d. |
| 24:0 | 0.1 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | **0.4** | **1.5** | **0.3** | **0.4** | n.d. | n.d. |
| 24:1 | – | n.d. | 0.1 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | 0.3 | n.d. | n.d. | n.d. | n.d. |
| *Other* | 16.5 | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Σ SFA | 29.4 | **55.6** | **46.4** | **49.6** | **47.2** | **56.6** | **32.9** | **38.9** | **35.4** | **58.2** | **50.7** | **57.1** | **43.3** | **34.8** |
| Σ MUFA | 25.1 | **42.6** | 20.5 | **37.8** | **41.3** | **33.4** | **53.9** | **38.6** | **61.3** | **39.9** | **46.4** | **38.1** | **35.5** | **27.1** |
| Σ PUFA | 32.9 | 1.8 | **33.1** | 12.6 | 11.5 | 10.0 | 13.3 | 22.5 | 3.3 | 1.9 | 2.9 | 4.9 | 21.2 | **38.0** |
| n-Matches (n/16, of FA) | | 3 | 6 | 6 | 6 | 6 | 5 | 5 | 4 | 6 | 5 | 7 | 4 | 4 |
| n-Matches (n/3, of ΣFA) | | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 |

ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, poly-unsaturated fatty acids.
n.d., not detected.

**Table 8**
Amino acid (AA) profile (g 100 $g^{-1}$ protein) of six microalgae and a bloom under batch (B) and semi-continuous operation and a fish meal profile [64]. The highlighted values indicate that they are above the reference value (FM). The sum of highlighted values is indicated in the n-matches rows: "n/9 of Essential-AA" and "n/17 of Total-AA".

| | | *N. gaditana* | | *P. lutheri* | *I. galbana* | | *T. chuii* | | *P. tricornutum* | | *C. gracilis* | | Bloom | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| g 100 $g^{-1}$ protein | Fish meal | B | SC | B | B | SC | B | SC | B | SC | B | SC | B | SC |
| Essential amino acids | | | | | | | | | | | | | | |
| Arg | 6.5 | **7.8** | **9.6** | **6.5** | 6.2 | **6.7** | **12.3** | **17.1** | **6.7** | **8.5** | **7.9** | 5.6 | **9.9** | **7.2** |
| His | 2.4 | 1.7 | 1.7 | 1.6 | 1.4 | 1.8 | 1.6 | 1.1 | 1.1 | **2.5** | 1.2 | 1.5 | 1.5 | 2.0 |
| Ile | 4.2 | **4.5** | 4.0 | 4.1 | **4.8** | **4.8** | 4.0 | 3.7 | **4.7** | **5.2** | **4.2** | **5.1** | 4.0 | **4.1** |
| Leu | 7.2 | **9.4** | **8.8** | **9.7** | **9.8** | **9.6** | **8.6** | **8.0** | **8.3** | **10.7** | **7.3** | **9.0** | **8.9** | **8.8** |
| Lys | 7.5 | 5.4 | 6.2 | 6.3 | 6.7 | 5.4 | 4.6 | 6.4 | 5.5 | 2.6 | 4.4 | 6.2 | 6.1 | 6.8 |
| Met | 2.8 | 1.4 | 1.2 | 1.4 | 2.2 | 2.4 | 2.4 | 1.9 | 1.0 | 1.0 | 1.3 | 1.3 | 1.9 | 2.0 |
| Phe | 3.9 | **5.1** | **4.7** | **4.5** | **5.2** | **6.3** | **5.8** | **4.4** | **4.9** | **14.1** | **4.9** | **5.0** | **4.5** | **4.5** |
| Thr | 4.1 | **5.7** | **5.0** | **5.0** | **5.6** | **5.5** | **5.6** | **4.9** | **6.0** | **5.7** | **5.1** | **5.1** | **5.3** | **5.0** |
| Val | 5.0 | **6.7** | **6.4** | **6.5** | **6.5** | **6.2** | **6.0** | **5.5** | **6.8** | **7.4** | **5.7** | **6.2** | **6.7** | **6.5** |
| Non-essential amino acids | | | | | | | | | | | | | | |
| Ser | 3.9 | **5.7** | **5.1** | **4.9** | **5.6** | **5.4** | **6.6** | **4.6** | **5.6** | **6.0** | **5.4** | **6.4** | **5.0** | **4.8** |
| Gly | 6.2 | 5.8 | 4.9 | 5.1 | 5.0 | 5.3 | **6.5** | 4.3 | 4.6 | **9.2** | 4.8 | 5.0 | 4.8 | 4.8 |
| Asp | 9.1 | 8.9 | **9.9** | **11.6** | **11.2** | **11.0** | **8.1** | **9.3** | **14.2** | 2.2 | **14.7** | **13.4** | **10.7** | **10.0** |
| Glu | 12.7 | 10.5 | **14.0** | **14.1** | **13.1** | 12.4 | 11.6 | **16.0** | **15.2** | 2.7 | **19.2** | **13.8** | **13.4** | **12.7** |
| Ala | 6.2 | **7.9** | **7.3** | **10.0** | **8.4** | **7.7** | **6.5** | **6.4** | **7.6** | 5.9 | **6.3** | **7.2** | **7.6** | **7.6** |
| Pro | 4.0 | **7.8** | **6.0** | **4.6** | **4.7** | **4.7** | **4.6** | 3.5 | **4.0** | **4.5** | 3.6 | **5.7** | **5.9** | **5.8** |
| Cys | 0.8 | 0.7 | **0.9** | 0.7 | **0.8** | **0.9** | **0.9** | **0.8** | 0.6 | **3.2** | 0.7 | 0.5 | **1.1** | **0.8** |
| Tyr | 3.1 | **5.1** | **4.3** | **3.5** | 2.6 | **4.0** | **4.2** | 2.2 | 2.9 | **8.6** | **3.2** | 2.9 | 2.6 | **6.5** |
| n-Matches (n/9 of Essential-AA) | | 6 | 5 | 5 | 5 | 6 | 5 | 5 | 6 | 7 | 6 | 5 | 5 | 6 |
| n-Matches (n/17 of Total-AA) | | 10 | 12 | 11 | 11 | 12 | 12 | 10 | 11 | 12 | 11 | 10 | 11 | 13 |

Arg, arginine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val, valine; Ser, serine; Gly, glycine; Asp, aspartate (aspartic acid); Glu, glutamate (glutamic acid); Ala, alanine; Pro, proline; Cys, cysteine; Tyr, tyrosine.

cyanobacteria, whose Val and Leu content was higher, the latter being related to toxin synthesis. A predominance of Glu and Asp and a lower tyrosine (Tyr) presence (Cys not analysed) were observed. However, differences in AA composition were also found within taxonomic groups. These differences were generally attributed to the characteristics of the culture: light and dark cycles, turbulence, salinity, pH or temperature. A previous study [44] found no significant difference between batch and semi-continuous operation using marine microalgae.

### 3.5. Microalgae ratings

In Table 9 a scoring matrix has been compiled taking into account the parameters previously studied under semi-continuous operation: micronutrients requirements, biomass productivity, nitrogen and phosphorus removal kinetics, protein and lipid content, protein and lipid quality. Also, two columns were added with the average value and the coefficient of variation (CV) for each of the parameters studied. In the case of micronutritional requirements, no numerical value was indicated, just if during the semi-continuous operation, the addition of vitamins (+V) or trace metals (+M) was necessary. Each indicator was standardized dividing the results obtained by the highest, since in all cases the higher, the better result.

Firstly, it can be distinguished that the parameter with the highest variability in the scores was biomass productivity (CV = 77.5%), followed by nitrogen removal (CV = 46.9%). Next, it was observed a lower variability among the microalgae in phosphorus removal, protein and lipid content (CV = 25.8–32.5%). Finally, the most stable parameters were those concerning lipid and protein quality (CV = 14.4–18.3%). Regarding the average score per parameter, biomass production was not optimal, as the average was the lowest of all and stood at 0.40, with values ranging between 0.13 and 1.00. Nitrogen removal and protein and lipid content were at an intermediate-high average score (0.67–0.71) although, in the case of nitrogen removal, the variability was high. Finally, microalgae perform better in phosphorus removal and protein and lipid quality (0.79–0.81) with more homogeneous scores.

Considering biomass productivity, the microalgae with the highest score was *T. chuii*. The bloom, *I. galbana* and *P. tricornutum* were the next most valuable species, being 41–52% less productive. Finally, *N. gaditana*, *P. lutheri* and *C. gracilis* obtained values below 0.14. In the case of nitrogen removal, four patterns were observed. *T. chuii* had the highest removal rate followed by *N. gaditana* and *I. galbana*. The third group was formed by the bloom and *P. tricornutum*, with values between 0.64 and 0.55. Finally, the least efficient microalgae were *C. gracilis*, with a removal rate of 12% compared to that obtained with *T. chuii*. The bloom and *T. chuii* had the highest scores for phosphorus removal. *I. galbana*, *P. tricornutum* and *C. gracilis* followed these. Finally, *N. gaditana* obtained the lowest value. Regarding biomass composition, the microalgae with the highest score for protein content was *I. galbana*, while *P. tricornutum* the second-highest scoring species. Finally, *T. chuii*, *C. gracilis*, *N. gaditana* and the bloom were the furthest from the highest score. For lipid content, four groups were observed: *N. gaditana* was the optimal microalgae. In second place came *I. galbana* and the bloom. Below, *P. tricornutum* and *C. gracilis*. The microalgae with the lowest lipid content compared to the highest was *T. chuii*. For protein quality, the highest scores were obtained with *P. tricornutum*, followed by *I. galbana* and the bloom. Finally, *N. gaditana*, *T. chuii* and *C. gracilis* scored the lowest, 0.76. *C. gracilis* scored highest for lipid quality. In second place were *N. gaditana*, *I. galbana* and *P. tricornutum*, with a score of 0.86. Finally, *T. chuii* and the bloom came last. Even so, high-quality values for both proteins and lipids (scores above 0.5) were achieved in all cases.

The microalgae with the highest global score was *I. galbana* (5.68), followed by *T. chuii* (5.31). *P. tricornutum*, the bloom and *N. gaditana* were in the middle of the table with scores in the range of 5.08 and 4.54. In the last place, with the lowest score was *C. gracilis* (3.97). Taking into account the number of times they reached the maximum score (1.00), it should be noted that *T. chuii* obtained the maximum score twice and that all species (except *P. lutheri*) were the best in some parameter.

Finally, another classification was established depending on the uses of microalgae. In the case of water treatment, the values obtained for nitrogen and phosphorus removal were added together. In this case, the microalgae with the highest score were *T. chuii* (1.91). In second place was *I. galbana* (1.66) and the bloom (1.64). The worst was *C. gracilis* (0.91). Furthermore, for water treatment, low biomass productivity is an advantage to produce less sludge as possible. Therefore, *I. galbana* and the bloom would be more suitable than *T. chuii*. Also, the bloom would be a good option as, on a larger scale production system with open photobioreactors as High Rate Algae Ponds (HRAP), it is less affected by environmental changes (seasonal and diurnal variation) or biological contamination (zooplankton, fungi or other native algae) [64]. For protein production, the productivity score and the scores for protein content and quality were added together. The same was calculated in the case the target was lipid production. For protein production, *T. chuii* (2.35) and *I. galbana* (2.32) were the best, closely followed by *P. tricornutum* (2.22) and the bloom (1.93). The worst-ranked were *C. gracilis* (1.44) and *N. gaditana* (1.42). For lipids, no significant differences were observed: *I. galbana* scored highest (2.16) followed by *T. chuii* (2.05), *N. gaditana* (2.00), *P. tricornutum* (1.93) and the bloom (1.92). The last was *C. gracilis* (1.73). Overall, for biomass *I. galbana* was the most balanced, followed by *T. chuii*. Although the bloom is not the best, it shows itself as an attractive intermediate option for all parameters.

Although other studies did not mention a similar classification, it is observed that commonly *T. chuii* is widely used in research about fish farm effluents treatment [22,65,66] while *I. galbana* or *P. tricornutum* are highly valued for their biomass quality [44,54,67,68].

## 4. Conclusions

When aquaculture effluents were combined with microalgae biotechnology micronutrient requirements depends not only on the species but also on the reactor operation (batch or semi-continuous). The addition of trace metals is indispensable in all cases and can double biomass productivity. The proposed methodology using Verhulst batch growth kinetic parameters is an accurate and straightforward procedure to predict microalgae productivity in photo-bioreactors operating under semi-continuous. Phosphorus removal was the same for all microalgae under different operating conditions, reaching almost 100% efficiency. Nitrogen removal varied depending on the species and

**Table 9**
Score matrix of the six microalgae species and the Protein quality refers to the number of essential amino acids (n/9) that match the essential amino acids in fish meal [64]. Lipid quality refers to the number of fatty acids (n/16) matches the fish oil profile [61].

| | *N. gaditana* | *P. lutheri* | *I. galbana* | *T. chuii* | *P. tricornutum* | *C. gracilis* | Bloom | Average | CV (%) |
|---|---|---|---|---|---|---|---|---|---|
| Micronutritional requirements | +M+V | +M+V | +M+V | +M | +M | +M+V | +M+V | | |
| Biomass productivity | 0.14 | 0.13 | 0.47 | 1.00 | 0.41 | 0.14 | 0.52 | 0.40 | 77.5 |
| Nitrogen removal | 0.89 | NE | 0.81 | 1.00 | 0.55 | 0.12 | 0.64 | 0.67 | 46.9 |
| Phosphorus removal | 0.38 | NE | 0.85 | 0.91 | 0.79 | 0.79 | 1.00 | 0.79 | 27.1 |
| Proteins content | 0.56 | NE | 1.00 | 0.64 | 0.81 | 0.58 | 0.56 | 0.69 | 25.8 |
| Lipids content | 1.00 | NE | 0.83 | 0.33 | 0.67 | 0.61 | 0.83 | 0.71 | 32.5 |
| Proteins quality | 0.71 | NE | 0.86 | 0.71 | 1.00 | 0.71 | 0.86 | 0.81 | 14.4 |
| Lipids quality | 0.86 | NE | 0.86 | 0.71 | 0.86 | 1.00 | 0.57 | 0.81 | 18.3 |
| **Sum** | **4.54** | **NE** | **5.68** | **5.31** | **5.08** | **3.97** | **4.98** | | |

+M, trace metals added; +V, vitamins added; CV, coefficient of variation.
NE, not evaluated.

operating condition, between 54 and 94% in batch and 36 and 94% in semi-continuous. Predictions for nitrogen and phosphorus removal were fulfilled, and the importance of abiotic nitrogen removal by stripping was analysed, more specifically, an average of 72% of the $N\text{-}NH_4^+$ entering the system. While significant differences in protein and lipid composition were observed between species and operating conditions, protein (amino acid profile) and lipid (fatty acid profile) quality were homogeneous in all cases. Finally, a scoring matrix was developed that summed up all the results and ranked the microalgae depending on the objective, with *I. galbana* and *T. chuii* being the microalgae with the highest scores for biomass production with wastewater and wastewater treatment respectively.

## CRediT authorship contribution statement

**Elena Villar-Navarro**: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft; **Carmen Garrido-Pérez**: Conceptualization, Methodology, Formal analysis, Writing – review & editing; **José A. Perales**: Conceptualization, Data curation, Writing – original draft, Funding acquisition.

## Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

## 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2021.102389.

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