Bioremediation of aquaculture wastewater from *Mugil cephalus* (Linnaeus, 1758) with different microalgae species

[1]Valeria Andreotti[a,b*], Anuta Chindris[b], Gianni Brundu[b,c], Dario Vallainc[b,c], Matteo Francavilla[d], Joan García[a]

[a] 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.

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

[c] Department of Ecological and Biological Sciences (DEB) "Tuscia University", Viale dell' Università, snc 01100, Viterbo, Italy.

[d] Star*AgroEnergy Research Unit, Department of Agriculture, Food and Environment, University of Foggia, Via Napoli, 25, 71122 Foggia, Italy.

[1] **Correspondence to**: Valeria Andreotti, International Marine Centre IMC, Loc. Sa Mardini 09170, Torregrande (OR) Italy.

E-mail: v.andreotti@fondazioneimc.it

Abstract

Current aquaculture practices have a detrimental impact on the environment, in particular due to the release of high concentration of nitrogen and phosphorus that can induce eutrophication. This study investigates and compares the capacity of three microalgae species *Tetraselmis suecica*, *Isochrysis galbana* and *Dunaliella tertiolecta*, in the bioremediation of grey mullet *Mugil cephalus* wastewater.

The experiment was conducted in batch conditions for 7 days using completely mixed bubble column photobioreactors. After two days, *T. suecica* and *D. tertiolecta* were able to remove more than 90% of Dissolved Inorganic Nitrogen (DIN) and Dissolved Inorganic Phosphorous (DIP), whereas *I. galbana* removed only 32% and 79% of DIN and DIP, respectively. A higher biomass yield resulted for *T. suecica* (0.60 ± 0.03 g/L, mean ± SE).

This study confirms the potential to employ *T. suecica* in an Integrated Multi Trophic Aquaculture system for bioremediation of wastewater and identifies *D. tertiolecta* as another valid candidate species. Moreover, these species can growth in unsterilized culture media, and this reduces energy consumption, costs and efforts.

Keywords: phytoremediation, biotreatment, bioreactors, wastewater, algae.

1.1 Introduction

Aquaculture is one of the fastest-growing food producing sectors in the world, providing almost about 50% of all fish for human consumption; within 2030, this share is projected to rise to 62% (FAO, 2014). On the other hand, aquaculture represents one of the major contributors to the increasing levels of dissolved and particulate nutrients in the aquatic ecosystems (Lamprianidou et al., 2015). A high nutrient loading into the aquatic environment, in particular nitrogen and phosphorus may cause eutrophication, oxygen depletion and siltation (Burford et al., 2003).

With the aim to reduce the impacts of traditional aquaculture, several Countries around the world are developing Integrated Multi-Trophic Aquaculture (IMTA) systems, which re-uses the wastewaters for the growth of micro and macroalgae. Indeed, aquaculture wastewater provides nutrients (ammonia, nitrite, nitrate, dissolved organic nitrogen and phosphate) (Converti et al., 2006; Soletto et al., 2005; Abe et al., 2002) which can be used for the production of microalgae. The uptake of dissolved nutrients by microalgae is considered as the main way to remove nitrogen in aquaculture wastewaters (Attasat et al., 2013; Sirakov et al., 2013).

Previous studies showed that it is possible to remove nutrients from wastewater (fishes and shrimp production plants) employing microalgae and macroalgae as key elements in biological treatments (Gao et al., 2016; Michels et al., 2014; Sirakov and Velichkova, 2014; Bartoli et al., 2005; Borges et al., 2005; Lefebvre et al., 2004; Hussenot et al., 1998; Lefebvre et al., 1996; Hammouda et al., 1995; Shpigel et al., 1993).

This phycoremediation is an eco-friendly method that offers the advantage to be a low-cost way to nutrient removal (Mulbry et al., 2008). In addition, the biomass produced through bioremediation could have multi-purpose uses including fuels, fertilizers, fine chemicals production and feed in aquaculture (Mulbry et al., 2006; Vilchez et al., 1997).

One of the most common microalgae species employed in aquaculture bioremediation wastewater is *Tetraselmis* spp. (Michels et al., 2014; Sirakov and Velichkova, 2014; Borges et al., 2005). A recent study Michels et al., (2014) showed for the first time that it is possible to use *Tetraselmis suecica* for the nutrient assimilation of fishfarm wastewater throughout its cultivation in controlled photobioreactors.

The aim of this study is to evaluate and compare the capability of *T. suecica*, *Isochrysis galbana* and *Dunaliella tertiolecta*, widely used in aquaculture as feed for rotifers (Mason 1963), echinoderms (Brundu et al., 2016a, 2016b; Paredes et al., 2015; De La Uz et al., 2013; Azad et al., 2011; Miller and Emlet 1999; Zamora and Stotz 1994;), filter feeders (Nevejan et al., 2003; Carboni et al., 2016) and fin fishes (Fabregas et al., 1986), for the removal of dissolved inorganic nutrients (nitrogen and phosphorous) of wastewater aquaculture. We evaluate the biomass yield of these species in controlled bubble column annular photobioreactors, by using untreated mullet wastewater as culture medium. Contrarily to previous studies that sterilized the wastewater before its use for bioremediation to eliminate zooplankton, bacteria and suspended solids (Michels et al., 2014), we avoided the use of expensive pre-treatment procedures as filtration and

sterilization, aiming to reduce the costs of seawater treatment and simulate more real operation conditions of a wastewater treatment system.

2.1 Materials and methods

2.1.1 Aquaculture wastewater

Aquaculture wastewater was provided by an experimental fish hatchery located in the International Marine Centre - IMC Foundation (Oristano, Sardinia, Italy). Juveniles of grey mullet *Mugil cephalus* (Linnaeus, 1758) were obtained in laboratory and reared in a recirculating aquaculture system (RAS) consisting of 4 tanks of 2000 L volume. In this system, the tanks were linked in a single biological (trickling filter) and cartridge mechanical filter (10 µm) and supplied with UV lamp (UVPE5, 80 W) and protein skimmer (Panaque). Temperature was maintained at 23 ± 2 °C (mean ± SE) with a chiller (TECO TR60, 0.91 Kw) and natural photoperiod (14/10 L/D) was adopted (Figure 1).

Natural seawater (NSW) at 37.0 ± 1.0 ppt salinity was previously micro-filtered (0.5 µm) and UV lamp sterilized. Juveniles of 0.35 ± 0.43 g body weight (BW) were fed at 3% BW per day with the commercial formulated feed for sea fish supplied by Skretting SpA (PERLA LARVA) composed of 62% crude protein, 11% crude oils and fats, 9% crude ash, 0.8% crude fiber and 1.2% crude phosphorus. Fishes were stocked at an average density of 0.5 g body weight/L.

Tanks were monitored daily for checking mortality; the uneaten food and faeces were siphoned out twice a week for maintaining good water quality. A 30% water exchange was weekly performed, and a part of this 30% was

employed as wastewater in our experiment. Wastewater was taken at the inlet of the tank, after UV lamp.

2.1.2 Microalgae culture

The microalgae species were provided by the Agency for Agricultural Research in Sardinia (AGRIS) and sourced from the Culture Collection for Algae and Protozoa (CCAP: Oban, Scotland). Pre-culture inocula were permanently kept in Erlenmeyer flasks in Pyrex glass with total capacity of 2 L, closed with cotton and covered with gauze and aluminum foil. NSW was autoclaved at 121 °C for 30 min and enriched with Guillard F/2 medium (Guillard 1975; Guillard and Ryther 1962). Cultures were exposed to a constant illumination (155 μmol/s/m^2) provided by 4 fluorescent lamps (OSRAM type Natura). Continuous aeration 3 L/min was supplied by peristaltic pump (ECOH Air Pump) and temperature was maintained at 23 °C by air conditioning.

2.1.3 Experimental design

Nutrient uptake and biomass production of *T. suecica*, *I. galbana* and *D. tertiolecta* were evaluated during seven days in batch conditions using two completely mixed bubble column photobioreactors of 6 L; five runs were done for a total of three replicates per treatment.

Lighting system was composed by four neon daylight lamp (four fluorescent lamps type cool daylight, OSRAM Lumilux FQ 24W/865), with light

intensity of 100 μmol/s/m^2. This system was monitored with a Programmable Logic Controller (PLC) that it is a device that performs discrete or continuous control logic in process plant or factory environments (Figure 2). These controllers are hardware and software engineered microcomputers, used to provide industrial control operations (Netto et al., 2013). Reactors were equipped with temperature and aeration regulation control system; temperature was maintained at 23 °C, aeration was ensured by a blower at flow rate of 3 L/min. On the contrary, pH was not controlled and resulted at 7.7 ± 0.2. Phytoplankton laboratory-culture methods and photobiorectors operation were adopted according to Saiu et al., (2016).

Microalgae growth was measured as dry weight biomass (DW) (Clasceri *et al.* 1999). DW was measured once a day in 40 mL of water sample previously filtered through 0.45 μm Whatman fiber-glass. After filtration, filters were washed with 20 mL of deionized water to remove salts and dried in an oven at 105 °C until constant weight, following Saiu et al., (2016). The supernatant liquid fraction obtained after filtration was used for nitrate, nitrite, ammonia and phosphorous analysis. In order to monitor the microalgae nutrient uptake, nutrients were daily analysed by an automatic chemical analyzer μCHEM based on Loop Flow Analysis (Systea, Italy). Microalgae removal efficiencies of Dissolved Inorganic Nitrogen (DIN) and Dissolved Inorganic Phosphorous (DIP) were calculated according to the method used by Michels et al., (2014), as follow:

N removal efficiency (%) = ((DIN influent - DIN effluent) / DIN influent) x 100

P removal efficiency (%) = ((DIP influent - DIP effluent / DIP influent) x 100

DIN values were calculated as the sum of nitrite (NO_2^-), nitrate (NO_3^-) and ammonia (NH_4^+), while DIP corresponded to the total dissolved phosphate (PO_4^{3-}).

2.1.4 Statistical analysis

Data were analyzed by Statistica 6.1 StatSoft, Inc. (2004). Differences in the removal efficiences among phytoplankton species were analysed using analysis of variance (ANOVA). Shapiro Wilk's W test was used to verify the normality of the data distribution and Levene's test was used to verify the homogeneity of variances. Biomass was analyzed using repeated-measures ANOVA, with species as independent factor and days as repeated factor. Tukey's honestly-significant difference (HSD) test was used to evaluate all pair-wise treatment comparisons ($p < 0.05$).

3.1 Results

The nutrient concentration of the wastewater was regularly measured before each experiments (Table 1). It was possible to observe that the composition of wastewater was very similar in each experiment, being nitrate the N species with the higher concentration.

3.1.1 Nutrients removal efficiency

At the end of the experiment a clearly higher DIN removal efficiency ($p < 0.001$, two-way ANOVA) resulted for *T. suecica* (94.4 ± 1.0%, mean ± SE) and *D. tertiolecta* (95.4 ± 0.3%) in comparison with *I. galbana* (66.0 ± 1.5%). There were not statistical differences between the three species in the removal of DIP at the end of the experiments (Table 2).

T. suecica and *D. tertiolecta* showed a similar pattern of nutrient uptake (Figure 3 A, 3 C). Both species removed more than 90% of DIN and DIP after 2 and 1 day, respectively. On the contrary, *I. galbana* showed a slower nutrient uptake, lower than 35% and 80% removal for DIN and DIP, respectively, after 2 days (Figure 3 B). The nutrient uptake of DIN showed significant differences ($p < 0.001$) between *I. galbana* and the other two phytoplankton species (Repeated-measures ANOVA).

3.1.2 Biomass yield

Ciliate protozoan *Paramecium* spp. was observed in all cultures through the duration of the experiement, but we did not evaluate the abundance of this species. This was mainly due to lack of the wastewater pre-treatment procedures (i.e. filtration and sterilization). We found a significant difference in biomass yield among the three species (Repeated measures ANOVA, $p < 0.001$). *T. suecica* resulted in a higher DW (0.57 ± 0.02 g/L, mean ± SE) than *I. galbana* (0.12 ± 0.01 g/L) from 3 days up to the end of the experiment, 0.60 ± 0.03 g/L for *T. suecica* and 0.16 ± 0.02 g/L for *I.*

206 *galbana*. We found no difference between *D. tertiolecta* and the other two
207 species (Figure 4).
208
209 ## 4.1 Discussion
210 In this study, we tested the capability of three microalgae species to remove
211 nutrients dissolved in the wastewater of a hatchery pilot rearing system of
212 *M. cephalus*. We found two out of three species, *T. suecica* and *D.*
213 *tertiolecta*, able to remove more than 90% of the DIN and DIP after two
214 days of treatment. Differently, the phytoplankton species *I. galbana*
215 employed 7 days to remove 92% of DIN, while DIP were not completely
216 removed at the end of the experiment (66%).
217 This is the first time that the *D. tertiolecta* was used as aquaculture
218 wastewater species, while previous studies obtained efficient results by
219 using *T. suecica*. Michels et al., (2014) showed that with a biomass
220 concentration of 0.5 g/L, *T. suecica* resulted in a removal efficiency of
221 49.4% for N and 99.0% for P, after 15 days and using continuously operated
222 tubular photobioreactor. Michels et al., (2014) obtained an higher N removal
223 efficiency (95.7 ± 1.0%) after addition of extra orthophosphate to
224 compensate the insufficient amount of DIP in the wastewater. Culturing *T.*
225 *suecica* under batch condition, on the contrary, Borges et al., (2005)
226 obtained a maximum P removal of only 52-63% at 8 days, even after
227 nutrient (+N) ratio correction.
228 The growth of microalgae is influenced by the culture medium composition
229 and variables such as temperature, light intensity and pH (Molina *et al.*

1991). Moreover, it was previously observed that other factors are determinant for the growth of phytoplankton, as the N:P ratio. Once microalgae reaches the stationary phase, indeed, Molina *et al.* (1991) observed that the biomass concentration increases with the N:P ratio up to different levelling-off values, which depends upon temperature, with concentration remaining nearly constant for values beyond this point. At 25 °C, the N:P levelling-off value registered by Molina *et al.* (1991) for *Tetraselmis* spp. (10) is lower than values registered in the wastewater used for this study, 18 for *D. tertiolecta*, 16.3 for *I. galbana* and 32 for *T. suecica*.

In this study, the highest biomass yield (DW) was obtained with *T. suecica*, 0.6 ± 0.06 g/L, while 0.38 ± 0.06 and 0.16 ± 0.04 g/L was recorded for *D. tertiolecta* and *I. galbana*, respectively, at the end of the experiment. We hypothesize that these differences were due to a diverse species-specific cell size; according to FAO (2004), indeed, *T. suecica* has the largest median cell volume (300 μm^3), followed by *D. tertiolecta* (170 μm^3) and *I. galbana* (40-50 μm^3).

I. galbana is not suitable for the nutrient removal of *M. cephalus* aquaculture wastewater. According with Borges et al., (2005) *I. galbana* resulted in a low biomass yield and removal efficiency of DIN and DIP. We hypothesize that the ciliate *Paramecium* spp. influenced negatively the growth of *I. galbana*, because this organism effectively feeds on other live microorganisms (Wichterman 1986). *Paramecium* spp. was observed also in the cultures of *T. suecica* and *D. tertiolecta*, but the presence of this protozoan did not seem to affect the growth of these phytoplankton species.

I. galbana is smaller than the other two species, therefore it could be a more easy prey for the zooplankton. Moreover, it has been previously reported a large spectrum of antimicrobial activity and antibiotic substances of the genus *Tetraselmis* spp. (Austin et al., 1992; Austin and Day 1990) and *Dunaliella* spp. (Chang et al., 1993), which could limit the negative effects of *Paramecium* spp. on the growth of cultures. When aquaculture wastewater is used as a nutrient source for algae, sterilization may be necessary to minimize the negative effects of bacteria and other organisms on the algae growth (Cai et al., 2013; Stein 1979). However, sterilization process increases the capital cost of the algae cultivation system, representing a negative point for an efficient phytoplankton bioremediation system at large scale. Microalgae production, indeed, must be a low cost system, easily installable and maintainable (Cai et al., 2013). Avoiding to pre-treat and sterilize the wastewater, as in our experiment, reflects in a reduction of management costs, as manual labour and energy. Moreover, it was demonstrated that microalgae cultures with protozoans such as *Paramecium* spp. represent suitable diets for fish fries (FAO 1980).

During last decade, research efforts have been focused towards the development of more efficient, higher surface-to-volume ratio photobioreactors for microalgae cultivation (Tredici 2004; Rodolfi et al., 2008). This is the first study that compared the ability of these three microalgae species in nutrient removal of aquaculture wastewater by using controlled bubble column annular photobioreactors. Gao et al., (2016) recently tested *Chlorella vulgaris* and *Scenedesmus obliquus* cultivated in shrimp *Penaeus vannamei Boone* wastewater, in batch conditions and by using photobioreactors. A better performance in the biomass production was

recorded for *C. vulgaris* (7.3 mg/L/day) in comparison with *S. obliquus* (6.2 mg/L/day).

5.1 Conclusion

This study confirmes the potential of *T. suecica* in the assimilation of nutrients dissolved in aquaculture wastewater and in the production of biomass. *D. tertiolecta* also resulted suitable for bioremediation, removing more than 90% of dissolved inorganic nitrogen and phosphorous. Differently from *I. galbana*, *T. suecica* and *D. tertiolecta* are able to grow well in no sterilized culture media contaminated with bacteria and zooplankton (*Paramecium* spp.), reflecting in the potential to reduce manual labour and energy costs for pre-treatment of culture medium in a phytoplankton bioremediation system.

T. suecica and *D. tertiolecta* are valid candidate for the employement in IMTA systems. They can be cultivated for bioremediation of finfish or shrimp wastewater and biomass produced can be re-used as live-feed for hatchery-grown of herbivorous and filter feeders (Alsull and Omar 2012; Michels et al., 2014). Nevertheless, further studies will be needed to assess the biochemical composition of these phytoplankton species cultivated in aquaculture wastewater and to evaluate their effects as live-feed.

6.1 Acknowledgments

This research was supported by the Sardinia Research plan activity, Art. 26 of LR 37/98, “Experimental systems for microalgae biomass production, applications for aquaculture”.

We thank Paolo Mossone, Stefano Guerzoni, Maura Baroli and Stefano Marras for their technical support and the IMC staff for the help in the laboratory experiments.

7.1 References

Abe, K., Imamaki, A. and Hirano, M. (2002). Removal of nitrate, nitrite, ammonium and phosphate ions from water by the aerial microalga *Trentepholia aurea*. *Journal of Applied Phycology,* **14**, 129-134.

Alsull, M. and Omar, W. (2012). Responses of *Tetraselmis sp*. and *Nannochloropsis sp*. isolated from Penang National Park Coastal Waters, Malaysia, to the combined influences of salinity, light and nitrogen limitation. *International Conference on Chemical, Ecology and Environmental Sciences,* (ICEES'2012) March 17-18, Bangkok, pp. 142-145.

Attasat, S., Wanichpongpan, P. and Ruenglertpanyakul, W. (2013). Cultivation of microalgae (*Oscillatoria okeni* and *Chlorella vulgaris*) using tilapia-pond effluent and a comparison of their biomass removal efficiency. *Water Science & Technology*, **67**, 271-277.

Austin, B. and Day, J. G. (1990). Inhibition of prawn pathogenic Vibrio spp. by a commercial spray dried preparation of *Tetraselmis suecica*. *Aquaculture*, **90**, 389-392.

Austin, B., Bauder, E. and Stobie, M. B. C. (1992). Inhibition of bacterial fish pathogens by *Tetraselmsis suecica*. *Journal of Fish Diseases,* **15**, 55–61.

Azad, A. K., Pearce, C. M. and McKinley R.S. (2011). Influence of microalgal species and dietary rations on larval development and survival of the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson, 1857). *Aquaculture,* **322–323**, 210–217.

Bartoli, M., Nizzoli, D., Naldi, M., Vezzulli, L., Porrello, S., Lenzi, M. and Viaroli, P., (2005). Inorganic nitrogen control in wastewater treatment ponds from a fish farm (Orbetello, Italy): Denitrification versus Ulva uptake. *Marine Pollution*

337 *Bulletin,* **50**, 1386-1397.
338 Borges, M.P., Silva, P., Moreira, L. and Soares, R. (2005). Integration of consumer-
339 targeted microalgal production with marine fish effluent biofiltration – a strategy
340 for mariculture sustainability. *Journal of Applied Phycology,* **17,** 187-197.
341 Brundu, G., Vallainc, D., Baroli, M., Figus, A.M., Pinna, A., Carboni, S. (2016a).
342 Effects of on-demand feeding on sea urchin larvae (*Paracentrotus lividus*;
343 Lamarck, 1816), development, survival and microalgae utilization. *Aquaculture*
344 *Research,* doi:10.1111/are.12990.
345 Brundu, G., Vian Monleón, L., Vallainc, D. and Carboni, S. (2016b). Effects of larval
346 diet and metamorphosis cue on survival and growth of sea urchin post-larvae
347 (*Paracentrotus lividus*; Lamarck, 1816). *Aquaculture,* **465,** 265-270.
348 Burford, M. A., Costanzo, S. D., Dennison, W. C., Jackson, C. J., Jones, A. B.,
349 McKinnon, A. D., Preston, N. P., Trott, L. A. (2003). Asynthesis of dominant
350 ecological processes in intensive shrimp pond sand adjacent coastal
351 environments in NE Australia. *Marine Pollution Bulletin,* **46,** 1456-1469.
352 Cai, T., Park, S. Y. and Li, Y. (2013). Nutrient recovery from wastewater streams by
353 microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews,*
354 **19,** 360-369.
355 Carboni, S., Clegg, S. H. and Hughes, A. D. (2016). The use of biorefinery by-products
356 and natural detritus as feed sources for oysters (*Crassostrea gigas*) juveniles.
357 *Aquaculture,* **464,** 392–398.
358 Chang, T., Ohta, S., Ikegami, N., Miyata, H., Kashimoto, T. and Kondo, M. (1993).
359 Antibiotic substances produced by a marine green alga, D*unaliella primolecta*.
360 *Bioresource Technology,* **44**, 149-153.
361 Clasceri, L., Greenberg, A. and Eaton, A. (1999). Standard Methods for the Examination
362 of Water and Wastewater, 20th ed by Amer Public Health Assn, pp. 1931.

Converti, A., Scapazzoni, S., Lodi, A. and Carvalho, J. C. M. (2006). Ammonium and urea removal by *Spirulina platensis*. *Journal of Industrial Microbiology and Biotechnology*, **33**, 8-16.

De La Uz, S., Carrasco, J. F., Rodríguez, C. and Anadón, N. (2013). Metamorphosis, growth and survival of early juveniles of *Paracentrotus lividus* (Echinodermata: Echinoidea): Effects of larval diet and settlement inducers. *Cahiers de Biologie Marine*, **54**, 691-695.

Fabregas, J., Herrero, C., Abalde, J., Liaño, R. and Cabezas, B. (1986). Biomasa production and biochemical variability of the marine microalga *Dunaliella tertiolecta* (Butchner) with high nutrient concentrations. *Aquaculture*, **53**, 187-199.

FAO Global Aquaculture Production Volume and Value Statistics Database Updated to 2012. *Fisheries and Aquaculture Department*, March 2014, pp. 4 (2014a).

FAO Hatchery culture of bivalves. A practical manual, 2004. *FAO Fisheries Technical Paper*, ed by Alessandro Lovatelli Inland Water Resources and Aquaculture Department, Rome, pp. 177.

Gao, F., Li, C., Yanga, Z. H., Zenga, G. M., Feng, L. J., Liu, J., Liu, M. and Cai, H. W. (2016). Continuous microalgae cultivation in aquaculture wastewater by a membrane photobioreactor for biomass production and nutrients removal. *Ecological Engineering*, **92**, 55–61.

Guillard, R. R. L. and Ryther, J. H. (1962). Studies of marine planktonic diatoms *I. Cyclotella nana* (Hustedt) and *Detonula confervacea* (cleve). *Canadian Journal of Microbiology*. **8**, 229-239.

Guillard, R. R. L. (1975). Culture of phytoplankton for feeding marine invertebrates, in

388 *Culture of Marine Invertebrate Animals*, ed by Smith WL and Chanley MH,
389 Plenum Press, New York, pp. 26-60.
390 Hammouda, O., Gaber, A. and Abdel-Raouf, N., (1995). Microalgae and wastewater
391 treatment. *Ecotoxicology and Environmental Safety*, **31**, 205-210.
392 Hussenot, J., Lefebvre, S. and Brossard, N. (1998). Open-air treatment of wastewater
393 from land-based marine fish farms in extensive and intensive systems: current
394 technology and future perspectives. *Aquatic Living Resources*, **11**, 297-304.
395 Lamprianidou, F., Telfer, T. and Ross, L.G. (2015). A model for optimization of the
396 productivity and bioremediation efficiency of marine integrated multitrophic
397 aquaculture. *Estuarine, Coastal and Shelf Science*, **164**, 253-264.
398 Lefebvre, S., Hussenot, J. and Brossard, N. (1996). Water treatment of land-based fish
399 farm effluents by outdoor culture of marine diatoms. *Journal of applied*
400 *Phycology*, **8**, 193-200.
401 Lefebvre, S., Probert, I., Lefrançois, C. and Hussenot, J. (2004). Outdoor phytoplankton
402 continuous culture in a marine fish phytoplankton- bivalve integrated system:
403 combined effects of dilution rate and ambient conditions on growth rate, biomass
404 and nutrient cycling. *Aquaculture*, **240**, 211-231.
405 Mason, D. T., 1963. The growth response of *Artemia salina* to various feeding regimes.
406 *Crustaceana* 5: 138-150.
407 Michels, M. H. A., Vaskoska, M., Vermuë, M.H. and Wijffels, R.H. (2014). Growth of
408 *Tetraselmis suecica* in a tubular photobioreactor on wastewater from a fish farm.
409 *Water research*, **65**, 290-296.
410 Miller, B. A., and Emlet, R. B. (1999). Development of newly metamorphosed juvenile
411 sea urchins (*Strongylocentrotus franciscanus* and *S. purpuratus*): morphology,
412 the effects of temperature and larval food ration, and a method for determining
413 age. *Journal of Experimental Marine Biology and Ecology*, **235**, 67–90.

Molina, E., Martínez, E., Sánchez, S., García, F. and Contreras, A. (1991). The influence of temperature and the initial N: P ratio on the growth of microalgae *Tetraselmis sp. Process Biochemistry,* **26**, 183-187.

Mulbry, W., Kondrad, S. and Pizarro, P. (2006). Biofertilizers from algal treatment of dairy and swine manure effluents: characterization of algal biomass as slow release fertilizer. *Journal of Vegetable Science,* **12**, 107-125.

Mulbry, W., Kondrad, S., Pizarro, C. and Kebede-Westhead, E. (2008). Treatment of dairy manure effluent using freshwater algae: algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. *Bioresource Technology*, **99**, 8137-8142.

Netto, R., Bagri, A. (2013). Programmable Logic Controllers. *International Journal of Computer Applications,* **77**, 27-31.

Nevejana, N., Saeza, I., Gajardoa, G. and Sorgeloos, P. (2003). Supplementation of EPA and DHA emulsions to a *Dunaliella tertiolecta* diet: effect on growth and lipid composition of scallop larvae, *Argopecten purpuratus* (Lamarck, 1819). *Aquaculture,* **217**, 613 – 632.

Paredes, E., Bellas, J. and Costas, D., (2015). Sea urchin (*Paracentrotus lividus*) larval rearing - Culture from cryopreserved embryos. *Aquaculture,* **437**, 366-369.

Rodolfi, L., Zittelli, G. C., Bassi, N., Padovani, G., Biondi, N., Bonini, G. and Tredici, M. R. (2008). Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology Bioengineering,* **102(1)**, 100–112.

Saiu, G., Pistis, A., Chindris, A., Grosso, M., Baroli, M. and Scano, E. A. (2016). Study of the growth parameters of the *Nannochloropsis oculata* for the nitrogen and phosphorus removal from wastewater through design of experiment approach. *Chemical engineering transactions*, Vol. 49.

Shpigel, M., Neori, A., Popper, D. M. And Gordin, H. (1993). A proposed model for "environmentally clean" land-based culture of fish, bivalves and seaweeds. *Aquaculture,* **117**, 115-128.

Sirakov, I., Velichkova, K., Beev, G., and Staykov, Y. (2013). The influence of organic carbon on bioremediation process of wastewater originate from aquaculture with use of microalgae from genera *Botryococcus* and *Scenedesmus*. *Agricultural science and technology* **5**, 443-447.

Sirakov, I.N., and Velichkova, K. N. (2014). Bioremediation of wastewater originate from aquaculture and biomass production from microalgae species *Nannochloropsis oculata* and *Tetraselmis chuii*. *Bulgarian Journal of Agricultural Science*, **20**, 66-72.

Soletto, D., Binaghi, L., Lodi, A., Carvalho, J.C.M. and Converti, A. (2005). Batch and fed-batch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. *Aquaculture*, **243**, 217-224.

Stein, J.R. (1979). Handbook of Phycological Methods. *Culture Methods and Growth Measurements* ed by Stein J. R. Cambridge University Press, Cambridge, pp. 448.

Tredici, M. R. (2004). Mass production of microalgae: photobioreactors. In *Handbook of Microalgal Cultures, Biotechnology and Applied Phycology,* ed by Richmond A,. Blackwell, Oxford, pp. 178–214.

Vilchez, C., Garbayo, I., Lobato, M.V., Vega, J. M. (1997). Microalgae-mediated chemicals production and wastes removal (Review). *Enzyme and Microbial Technology* **20**, 562-572.

Wichterman, R. (1986). The Biology of Paramecium, in *Springer US*. Plenum Press, New York, pp. 599.

Woynarovich, E. and Horváth, L. (1980). The artificial propagation of warm-water

finfishes - a manual for extension. FAO *Fish.Tech.Pap*., 201:183 p.

Zamora, S. and Stotz, W. (1994). Cultivo masivo en laboratorio de juveniles de erizo *Loxechinus albus* (Molina, 1782), (Echinodermata: Echinoidea). Investigación Pesquera **38**, 37–54.

Table 1. Nutrients dissolved in the *Mugil cephalus* wastewater. Values are expressed as mean ± SE (n= 3).

	Tetraselmis suecica	***Dunaliella tertiolecta***	***Isochrysis galbana***
NO_3^- -N (mg/L)	4.1 ± 0.4	4.2 ± 0.1	4.2 ± 0.4
NO_2^- -N (mg/L)	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
NH_4^+ -N (mg/L)	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
PO_4^{3-} -P (mg/L)	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.1

Table 2. Influent and effluent DIN and DIP values (mg/L) and removal efficiency (%) of *Tetraselmis suecica*, *Dunaliella tertiolecta* and *Isochrysis galbana*. Values are expressed as mean ± SE (n= 3). Superscripts indicate significant differences among species.

	Tetraselmis suecica	***Dunaliella tertiolecta***	***Isochrysis galbana***
DIN Influent (mg/L)	4.5 ± 0.5	4.6 ± 0.1	4.6 ± 0.5
DIN Effluent (mg/L)	0.3 ± 0.1	0.2 ± 0.1	1.6 ± 0.1
DIN %	94.4 ± 1.0 [a]	95.4 ± 0.3 [a]	66.0 ± 1.5 [b]
DIP Influent (mg/L)	0.3 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
DIP Effluent (mg/L)	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
DIP %	96.0 ± 2.5	91.2 ± 2.3	91.9 ± 4.0

Figure 1: Recirculating aquaculture system (RAS) for rearing of juvenile grey mullets *Mugil cephalus*, consisting of four circular fiberglass tanks with 2000 L volume (V1, V2, V3 and V4). The system was equipped with biological (BF) and mechanical filter (MF), protein skimmer (PS), chiller (C) and UV lamp (UV). Dotted arrow = seawater outlet; continuous arrow = seawater intake.

Figure 2: Bubble column annular photobioreactors of 6 L volume (R1 and R2) used for the growth of phytoplankton, supplied with LIGHT, Programmable Logic Controller (PLC), gentle aeration (AIR), probes for temperature (T) and pH (pH).

Figure 3: Nutrient uptake (%) of Dissolved Inorganic Nitrogen (DIN) and Dissolved Inorganic Phosphorous (DIP) for *Tetraselmis suecica* (A), *Isochrysis galbana* (B) and *Dunaliella tertiolecta* (C), during 7 days. Values are expressed as mean ± SE (n= 3).

Figure 4: Microalgal growth curves as DW (g/L) of *Tetraselmis suecica*, *Isochrysis galbana* and *Dunaliella tertiolecta*, during 7 days. Values are expressed as mean ± SE (n= 3). Superscripts indicate significant differences among species.

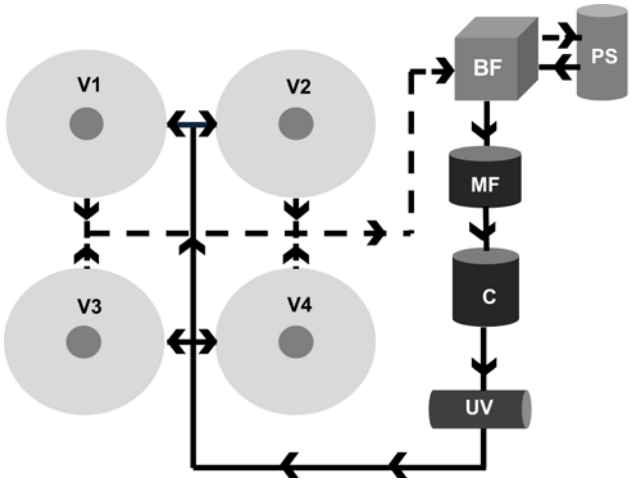

Figure 2

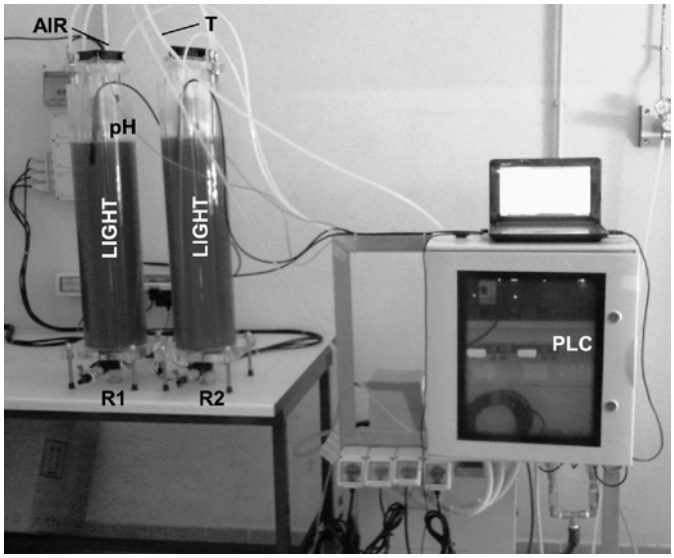

Figure 2

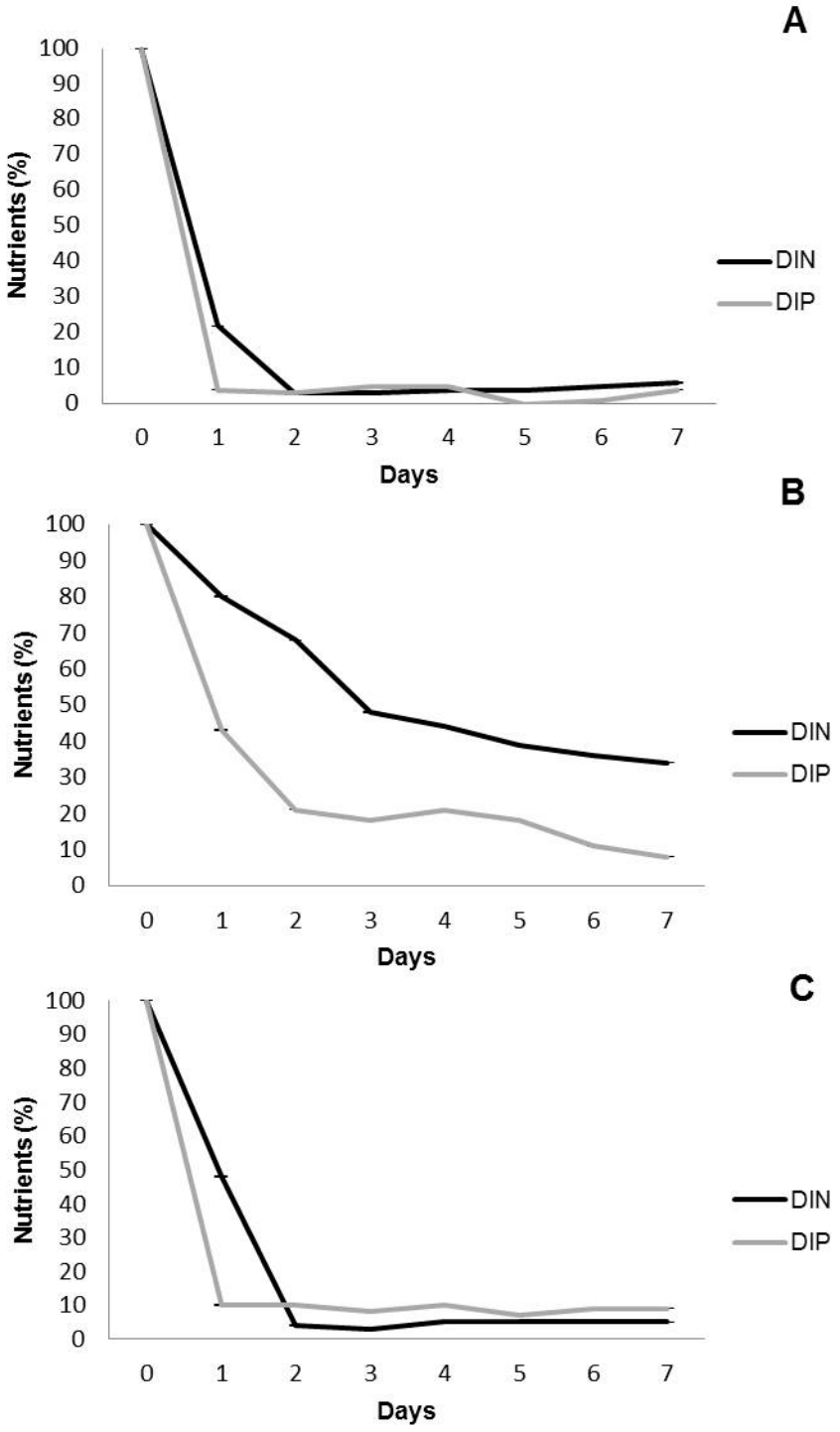
A
100
90
80
70
60
50
40
30
20
10
0
Nutrients (%)
0 1 2 3 4 5 6 7
Days
DIN
DIP
B
100
90
80
70
60
50
40
30
20
10
0
Nutrients (%)
0 1 2 3 4 5 6 7
Days
DIN
DIP
C
100
90
80
70
60
50
40
30
20
10
0
Nutrients (%)
0 1 2 3 4 5 6 7
Days
DIN
DIP

Figure 3

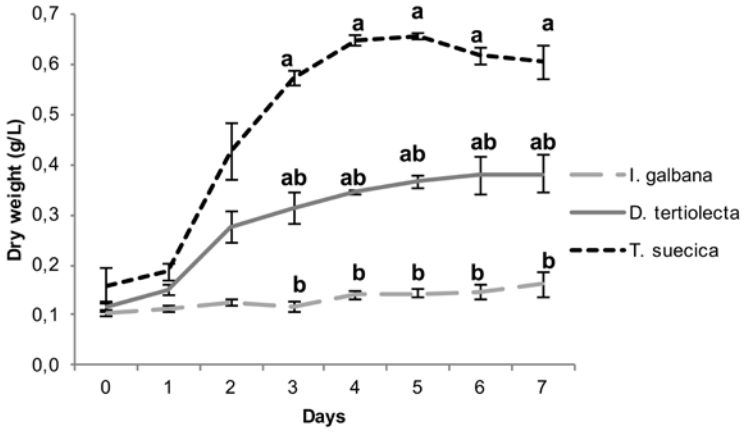
Dry weight (g/L)
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
0
1
2
3
4
5
6
7
Days
a
a
a
a
a
ab
ab
ab
ab
ab
b
b
b
b
b
I. galbana
D. tertiolecta
T. suecica

Figure 4