PHOSPHORUS AND METAL REMOVAL COMBINED WITH LIPID PRODUCTION BY THE GREEN MICROALGA *Desmodesmus* sp.: AN INTEGRATED APPROACH

[1]Rugnini L., [2]Costa G., [1]Congestri R., [3]Antonaroli S., [4]Sanità di Toppi, L., [1]Bruno L.

[1]LBA-Laboratory of Biology of Algae (Lorenza.Rugnini@uniroma2.it; roberta.congestri@uniroma2.it; laura.bruno@uniroma2.it), Dept. of Biology, +390672595989,University of Rome "Tor Vergata", via Cracovia 1, 00133 Rome, Italy [2]Laboratory of Environmental Engineering (costa@ing.uniroma2.it), Dept. Civil Engineering and Computer Science Engineering, University of Rome "Tor Vergata", via del Politecnico, 00133 Rome, Italy [3] Department of Chemical Sciences and Technologies (antonaro@uniroma2.it), University of Rome "Tor Vergata", via della Ricerca Scientifica snc, 00133 Rome, Italy [4] Department of Biology (luigi.sanita@unipi.it), University of Pisa, Via L. Ghini 13, 56126 Pisa, Italy

Corresponding author: Lorenza.Rugnini@uniroma2.it

ABSTRACT

This work focused on the potential of *Desmodesmus* sp. to be employed for wastewater bioremediation and biodiesel production. The green microalga was grown in a culture medium with a phosphorus (P) content of 4.55 mg L^{-1} simulating an industrial effluent; it was also exposed to a bimetal solution of copper (Cu) and nickel (Ni) for 2 days. P removal was between 94 and 100%. After 2 days of exposure to metals, 94% of Cu and 85% of Ni were removed by *Desmodesmus* sp. Adsorption tests showed that the green microalga was able to remove up to 90% of Cu and 43% of Ni in less than 30 minutes. The presence of metals decreased the lipid yield, but biodiesel quality from the biomass obtained from metal exposed samples was higher than that grown without metals. This result revealed that this technology could offer a new alternative solution to environmental pollution and carbon-neutral fuel generation.

Key words: green microalgae; bioremediation; photobioreactor; P removal; metal biosorption; biodiesel

1. Introduction

Even in regions where specific integrated policies for the protection of water environments have been established, such as the EU's Water Framework Directive (WFD) (2000/60/EC), the quality of surface water bodies is still hampered by anthropogenic activities causing diffuse and/or point-source emissions of both organic and inorganic pollutants. Indeed, almost twenty years after the adoption of the WFD, 47% of EU surface waters did not reach a "good ecological status" (Voulvoulis et al., 2017). The presence in the water of various organic, as well as inorganic nutrients such as nitrogen (N) and phosphorus (P), can lead to eutrophication, while the presence of metals and metalloids, due for example to mining operations, both for energy production and consumer goods (Torres et al., 2017), may lead to potential risks to human and ecosystem health. Some metals are micronutrients necessary for living organisms (e.g. Zn, Cu, Mn, Ni, and Co), while others have unknown biological functions (e.g. Cd, Pb, and Hg). Conventional physico-chemical methods for the treatment of wastewater containing high concentrations of nutrients and metals prove often ineffective or require high energy input, capital investment and operational costs (Chan et al., 2013). Phycoremediation is a process defined as the utilization of microalgae in the treatment of polluted wastewater (Jais et al., 2017). This process contributes significantly to the removal of nutrients and metals from wastewater, especially for pollutant concentrations between 1 and 100 mg L^{-1}, where chemical and physical methods such as chemical precipitation, electrolytic recovery, adsorption/ion exchange, solvent extraction and membranes are not fully satisfactory (Jais et al., 2017). Green microalgae remove N and P from wastewater through assimilation, while metal ions are removed through '*biosorption*' (involving both adsorption and absorption) as defined by Gadd (2008). Phycoremediation technologies present an additional asset besides wastewater treatment, i.e. they lead to the production of microalgal biomass (Gupta et al., 2016, 2017; Yang et al., 2015). Such biomass has gained an increasing interest due to its great potential for different biotechnological applications in the fields of energy, nanotechnology and environment (Bruno et al., 2012; De Angelis et al., 2016; Di Pippo et al., 2013; Gismondi et al., 2016). In particular, microalgal biomass produced during wastewater treatment can be used as feedstock for the production of a variety of biofuels such as biodiesel, bio-methane, ethanol, hydrogen, etc. (Chisti 2007; Gupta et al., 2016). During the past few decades, biofuels have attracted tremendous attention due to limited stock of fossil fuels, and the necessity to reduce the continuously increasing greenhouse gas emissions contributing to climate change (Gupta et al., 2017). The development of carbon-neutral biofuel is generally based on two primary concerns: environmental sustainability and economic viability. Only algal biodiesel has been estimated to present the potential to fulfil the global requirement of biofuels for transport (Chisti 2007, 2008) with little impact on the carbon

footprint. Wastewaters provide a sustainable means for microalgal biofuel production; however, not all algae can survive in these harsh and extreme environments. Even if suitable, different environmental stresses related to polluted wastewater, especially the toxicity caused by metals, would significantly affect the growth of the algae (Torricelli et al., 2004; Yang et al., 2015; Kumar et al., 2015) biomass production and lipid yield, as well as high production costs. Only a few works tried to combine lipid production with both P and metal removal, but these studies were limited to the construction of system dynamics models and the prediction of lipid production (Richards and Mullins, 2013). Based on a literature survey there is a lack of studies that address and quantify the impact of individual contaminants on microalgae grown in wastewater with the aim of identifying promising feedstock candidates for biofuel production (Yang et al., 2015; Jais et al., 2017). Thus, this work focused on the potential of the green microalga *Desmodesmus* sp. to be used for bioremediation of wastewater laden with P, copper (Cu) and nickel (Ni). Moreover, the effects of Cu and Ni on lipid accumulation for biodiesel production were evaluated. This approach could reduce the cost of algal biofuel by increasing the intrinsic algal biomass value with the ultimate purpose to find a cost-effective and eco-friendly method for biofuel production and wastewater bioremediation.

2. Materials and Methods

2.1 Microalgal strain

The strain of *Desmodesmus* sp. was isolated by serial dilutions and plate streaking from the outflow of a secondary sedimentation tank of a municipal wastewater treatment plant (WWTP) located south of Rome (Italy), where P concentration of the treated water was between 1 and 6 mg L^{-1}. The sludge of the secondary sedimentation tank where *Desmodesmus* sp. was isolated from contained the following concentrations of copper (509 mg kg^{-1}), nickel (21 mg kg^{-1}), chromium (29 mg kg^{-1}), cadmium (3 mg kg^{-1}) and lead (7 mg kg^{-1}), indicating the presence of these metals in the wastewater treated in the plant. The isolated strain was maintained at lab-scale in BG11 medium at 18 ± 2 °C and 55 ± 1.6 µmol photon m^{-2} s^{-1}. The green microalga was assigned to the 'VRUC-University of Rome Tor Vergata Culture Collection' (Castenholz 2001) with the code VRUC281.

2.2 Experimental set-up

The experiments (*Run1* and *Run2*) were carried out by using a 10 L photobioreactor (PBR; polyethylene bag). In both *Runs*, *Desmodesmus* sp. was grown in BG11 medium with a modified P concentration in the form of K_2HPO_4 (4.55 ± 0.01 mg L^{-1}) adjusted to simulate municipal or industrial wastewater effluents (Water Environment Federation, 2010).

In *Run1*, 500 mL of a culture of *Desmodesmus* sp. were inoculated in 9.5 L of liquid medium (initial density 0.180 ± 0.002 gDW L^{-1}). The PBR was operated at 18 ± 2 °C and was constantly illuminated with a light intensity of 26 ± 2.0 µmol photon m^{-2} s^{-1}; atmospheric air mixing was provided by a peristaltic pump without extra CO_2 addition. Growth was monitored for 21 days, until the stationary phase was reached. During growth, the biomass concentration, chlorophyll *a* (Chl *a*) and P content of the liquid medium were recorded every 2 or 4 days. Then, 9 L of biomass were harvested by sedimentation, reducing the culture pH below 3.0 using 1.0 M HCl. The remaining algal biomass of *Desmodesmus* sp. (about 500 mL; initial density 0.400 ± 0.002 gDW L^{-1}) was used as an inoculum for a new-growth experiment (*Run2*) in the same culture conditions, but with an acute exposure of cells to Cu and Ni (9.8 and 7.4 mg L^{-1}, respectively). Cu and Ni solutions (from 1000 mg L^{-1} stock solutions of $CuSO_4*5H_2O$ and $NiSO_4$, respectively) were added to the culture after 12 days and the biomass was collected after 2 days. The growth of the microalgae, the metal removal efficiency and the effect of the metal presence on cell lipid accumulation were evaluated.

2.3 Analytical procedures

Microalgal growth was calculated regularly by measuring the optical density (OD) of the cultures at a wavelength of 560 nm (OD_{560}) using a spectrophotometer (Beckman DU-65 Spectrophotometer). The OD_{560} values were converted into biomass dry weight (DW) concentration (g L^{-1}), based on a linear relationship between OD_{560} and DW ($p < 0.001$), which was obtained after extensive data analysis (Rugnini et al., 2017), according to the following equation:

Dry weight (g L^{-1}) = 0.622 *x* OD_{560} + 0.082 ($R^2 = 0.973$).

To determine the Chl *a* concentration, 2 ml of the algal suspension were centrifuged at 5000 rpm for 10 min and the supernatant was discarded. The pellet was re-suspended in 2 mL of methanol (95%) and incubated at 4 °C for 2 hours. Then, samples were centrifuged again and the OD (at 665 and 650 nm) of the supernatant measured; the Chl *a* concentration in the extract was calculated according to MacKinney (1941):

Chl *a* (mg L^{-1}) = (16.5×OD_{665}) − (8.3×OD_{650}).

Total phosphorus (TP, mg L^{-1}) analysis was conducted on 50 mL of filtered culture media using the blue molybdate method (Murphy et al., 1962; Valderrama 1981). The absorbance of the complex with blue coloration was spectrophotometrically measured at 882 nm. Standards were diluted from 100 mg L^{-1} KH_2PO_4 stock solutions to produce a standard curve. P removal efficiency was calculated using Eq. (1):

$$P_{\%} = \frac{P_0 - P_t}{P_0} \quad (1)$$

where P_0 is the initial P concentration and P_t is the corresponding P concentration after *t* days of cultivation (Aslan and Kapdan, 2006; Ji et al., 2013). Dry weight, Chl *a* and P removal efficiency are means of triplicates.

2.4 Metal removal investigations

The evaluation of the metal removal ability of the strain was carried out in duplicates using both living and non-living biomass. The living biomass tests were carried out exposing *Desmodesmus* sp. grown in *Run2* to the bimetal solution of Cu (9.8 mg L^{-1}) and Ni (7.4 mg L^{-1}) for 2 days. The tests on non-living microalgae were carried out exposing freeze-dried biomass collected from *Run1* or *Run2* (0.5 gDW L^{-1}) for 30 minutes to the same metal solution added in the liquid medium of *Run2*. At the end of both types of tests the microalgal suspensions were centrifuged and filtered (0.45 µm) and, after acidification with nitric acid, metal concentrations in the liquid media were analysed by ICP-OES employing an Agilent 710-ES spectrometer. The metal removal efficiency (E, %) was calculated using Eq. 2 (Chen et al., 2012):

$$E = \frac{(C_0 - C_f) \times 100}{C_0} \quad (2)$$

where C_0 and C_f are the initial and the final concentrations of metals (mg L^{-1}) in the liquid solution, respectively. Metal uptake (*q*, mg gDW^{-1}) was calculated on the basis of the liquid solution concentration values at the beginning and at the end of the metal exposure, following Eq. 3 (Yang et al., 2015):

$$q = \frac{V \times (C_0 - C_f)}{M} \quad (3)$$

where V is the volume of the solution (L) and M the mass of dry biomass (gDW) at the end of the experiments. Cu concentration in BG11 medium was taken into account for the calculation of all removal efficiencies and metal uptakes.

2.5 Lipid extraction

Biomass harvested in *Run1* and *Run2* was freeze-dried and used for lipid extraction. Fatty acids methyl esters (FAMEs) suitable for biodiesel production were obtained by *in situ* trans-esterification as described in Gismondi et al. (2016). The FAME content was estimated as the percentage of esterified lipids per grams of dry biomass. The FAME profile was determined using a Gas Chromatographer (Shimadzu, GC-2010 Plus) with flame ionization detector (CG/FID). 1 µl of each sample was injected into the column (30 m x 0.25 mm x 0.25 m film thickness) with a temperature program starting from 170 °C for 3 min, increasing of 3 °C/min to 240 °C final, held for 20 min. The split ratio was 80:20 and injection temperature 280 °C, helium was used as carrier

gas. The run time for every single sample was 60 min. FAMEs were identified by comparing the retention time with that of the standard Supelco 37 Component FAME mix (Sigma-Aldrich).

2.6 Biodiesel properties from FAME profiles

The molecular characteristics of FAMEs influence the parameters defining biodiesel qualities and properties such as CN (cetane number), IV (iodine value), CFPP (cold filter plugging point) and oxidation stability. These parameters were calculated using the empirical equations previously reported by Gismondi et al. (2016). The CN, saponification value (SV) and IV can be calculated based on the FAME profile using Eqs. (4) – (6) shown below:

$$CN = 46.3 + \left(\frac{5458}{SV}\right) - (0.225 * IV) \quad (4)$$

The SV (KOH g^{-1}) and IV (g $I_2 100 g^{-1}$) of fat are predicted following Eqs. (5) and (6), respectively:

$$SV = \Sigma \left(\frac{560 * N}{M}\right) \quad (5)$$

$$IV = \Sigma \left(\frac{254 * ND}{M}\right) \quad (6)$$

where N is the percentage of each FA component, M is the FA molecular mass and D is the number of double bonds. In addition, the degree of unsaturation (DU) was calculated based on Eq. (7) reported by Ramos et al. (2009), as the amount of monounsaturated (MUFA) and polyunsaturated (PUFA) FAs present in the microalgae oil.

$$DU = MUFA + 2\ (PUFA) \quad (7)$$

The long-chain saturated factor (LCSF) was also estimated through Eq. (8). This factor was directly used to calculate the Cold Filter Plugging Point (CFPP) through Eq. (9). These two factors are both related to chain saturation and length of FAMEs.

$$LCSF = (0.1 * C_{16}) + (0.5 * C_{18}) + (1 * C_{20}) + (2 * C_{24}) \quad (8)$$

$$CFPP = (3.1417 * LCSF) - 16.477 \quad (9)$$

2.7. Statistical analysis

The experimental data were statistically analysed using the GraphPad Prism software, version 7.03 (USA), via the Student's *t*-test. Differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1 Growth curves and P removal

Several studies demonstrated the potential of using microalgae isolates from WWTPs and other hypereutrophic systems to remove contaminants (Rugnini et al., 2017; Samorì et al., 2013). This

may be related to the fact that microalgal isolates obtained from polluted environments (such as the strain of *Desmodesmus* sp. employed in this study) might be more suitable for nutrients and metal removal than those grown in otherwise clean environments, exhibiting a great potential to tolerate and remove contaminants (Abou-Shanab et al., 2013; Rugnini et al., 2017). The growth of *Desmodesmus* sp. in BG11 with modified P-content was investigated, as illustrated in Fig. 1. The data obtained showed a conventional growth trend (expressed as Chl *a* concentration, red curve), with an exponential phase in *Run1* (Fig. 1a) lasting from day 6 to 21. The maximum biomass concentration and Chl *a* concentration were 0.61 ± 0.001 g L^{-1} and 22.3 ± 0.05 mg L^{-1}, respectively, with a biomass productivity of 0.02 ± 0.001 g L^{-1} day^{-1}. In *Run2* (Fig. 1b), *Desmodesmus* sp. showed a more rapid growth in comparison to *Run1* ($p < 0.05$), reaching a maximum biomass concentration of 0.73 ± 0.001 g L^{-1} at day 10 and a biomass productivity of 0.05 ± 0.002 g L^{-1} day^{-1}. Initial biomass concentration employed in *Run2* (0.40 ± 0.002 g L^{-1}) was twice that of *Run1* (0.18 ± 0.002 g L^{-1}); it is well known that cultures started with exponentially-growing microalgal inocula (as the one obtained from *Run1*) have shorter lag phases (as shown in *Run2*), reducing the time required for cultivation up-scaling (D'Este et al., 2017). In a previous study using *Desmodesmus* sp. (Rugnini et al., 2017), we found that metal concentrations higher than 7.0 mg L^{-1} represented a threshold level and the growth rate of *Desmodesmus* sp. was reduced by 55% when the cultures were chronically exposed to metals (12 days). In this study, the green microalga showed only a slight decrease (between 4 and 7%) in biomass production and Chl *a* concentration during the exposure to metal concentrations of 9.8 mgCu L^{-1} and 7.4 mgNi L^{-1} for 48 hours. So, even if toxic effects of the metals occurred, that can be responsible of a block in cell division and inactivation of PSII reaction centres (Torres et al., 2017; Yang et al., 2015), the reduction of the time of exposure allowed to maintain overall biomass production necessary for lipid extraction.

Fig.1 (1.5 column fitting image)

Both in *Run1* and *Run2*, *Desmodesmus* sp. was grown in modified BG11 with P concentration simulating the content of a WWTP effluent. The total P-content (TP) was measured every 2 days in both *Runs*. In *Run1* (Fig. 2a), the P content decreased rapidly from 4.55 ± 0.01 mg L^{-1} to 1.79 ± 0.001 mg L^{-1} within 8 days, which means that the P removal efficiency reached 61% with a P uptake of 0.12 mgP gDW^{-1}, and 100% removal after 21 days. In *Run2* (Fig. 2b), a 64% P removal efficiency was reached in 2 days, probably due to the initial higher biomass concentration

222 employed, while the remaining P concentration after 14 days was 0.17 ± 0.002 mg L^{-1} (96%
223 removal efficiency, 0.24 mgP gDW^{-1}). Moreover, the addition of Cu and Ni in *Run2* did not affect
224 the P removal efficiency of *Desmodesmus* sp. ($p < 0.05$), that increased from 91 (0.55 mgP gDW^{-1})
225 to 96% (0.24 mgP gDW^{-1}) during the exposure to the metals for 2 days.
226
227 **Fig. 2 (1.5 column fitting image)**
228
229 The European Water Framework Directive (2000/60 EC) states that P concentrations must be
230 reduced to the regulatory level of 2 mg L^{-1} for effluent discharge in water bodies, and below 1 mg L^{-1}
231 in sensitive aquatic ecosystems. Evaluating the potential of *Desmodesmus* sp. for wastewater
232 treatment, the value of 1 mg L^{-1} was achieved in 10 days in *Run1* and in less than 7 days in *Run2*,
233 with a final removal efficiency of 100% and 96%, respectively. The removal efficiencies achieved
234 by *Desmodesmus* sp. in this study were comparable to those of other species under the same
235 nutrient concentrations. Ji et al. (2013) reported a 51% P removal efficiency in 4 days by
236 *Desmodesmus* sp. EJ9-6 grown in diluted anaerobic digestion water (10%, TP_{in}= 4.5 mg L^{-1}) and P
237 was completely removed in 14 days, similar to our results for *Run2*. Song et al. (2014) showed that
238 *Scenedesmus* sp. SDEC-8 was able to remove 99% of P in 10 days, when the initial TP varied
239 between 4 and 8 mg L^{-1}. *Chlorella vulgaris* resulted to be able to remove up to 78% of P in 10 days,
240 when the initial TP was less than 7.7 mg L^{-1} and illumination was provided continuously (Aslan and
241 Kapdan, 2006). Under high irradiance (440 μmol photon m^{-2} s^{-1}), *Desmodesmus communis*
242 completely removed P in 24 hours when the P concentration was 1.7 mg L^{-1} and in 3 days when an
243 initial concentration of 4.5 mg L^{-1} was adopted (Samorì et al., 2013). Light is one of the most
244 important factors influencing the uptake of P by algae (Laliberte et al., 1997). According to
245 Sukačovà et al. (2015), the efficiency of P removal by microalgal biofilm (TP_{in}= 4 mg L^{-1}) was
246 lower in experiments with a day-night light regime than in ones with a continuous light regime
247 (74% and 97%, respectively). Boelee et al. (2014) reported that P removal from wastewater by a
248 phototrophic biofilm increased during the day and decreased with decreasing light intensity, until no
249 removal occurred during the night. These results suggest that the P removal rate could be increased
250 by imposing a sunlight regime during the day and artificial light during the night in microalgal
251 based wastewater treatments.

252 *3.2 Metal removal*

253 The biosorption ability of *Desmodesmus* sp. exposed concurrently to Cu (9.8 mg L^{-1}) and Ni (7.4
254 mg L^{-1}) is shown in Tab.1. As can been inferred, living cells of *Desmodesmus* sp. in *Run2* were able
255 to remove 94.3% of Cu and 85.2% within the 2 days of exposure to the metals. In this condition,

256 living biomass exhibited a higher biosorption capacity for Cu and Ni compared to the dry biomass
257 obtained from both *Run1* and *Run2* ($p < 0.05$), as bioaccumulation also involves active intracellular
258 uptake of the metal ions (Kumar et al., 2015; Markou et al., 2015). Dry biomass was incubated for
259 30 minutes with the same metal solution used for the living biomass, and results showed that
260 biomass from *Run1* (not exposed to metals during growth) adsorbed 89.8% and 42.9% of Cu and
261 Ni, respectively. For dry biomass obtained from *Run2* (exposed to metals for 2 days during its
262 growth), the removal efficiency decreased of about 30-33% in comparison to that obtained
263 employing *Run1* grown biomass. A possible explanation for this is that saturation of free binding
264 sites occurred in the biomass during growth in *Run2* and thus the freeze-dried biomass used in the
265 second experiment resulted less efficient in metal removal than that of *Run1* not exposed to the
266 metals previously. Metal ions are taken up by microalgae in a two-stage process: (I) a rapid passive
267 adsorption occurring at the cell surface, and (II) a much slower absorption in which metal ions are
268 transported across living cells into the cytoplasm, with posterior binding to intracellular compounds
269 (Kumar et al., 2015; Anastopoulous et al., 2015). Metal adsorption by functional groups on the cell
270 surface anyhow occurs both in living and non-living cells; this hence may explain the decrease in
271 the number of free binding sites of biomass from *Run2* compared to that of *Run1*. Furthermore,
272 biosorption of metals onto microalgal biomass is significantly affected by the presence of other
273 metals/co-ions in solution, owing to competitive interactions between them and the adsorption
274 binding sites on the cell surface (Monteiro et al., 2009). Several studies (Micheletti et al., 2008;
275 Kumar et al., 2015; Rugnini et al., 2017) also reported that in multi-metal solutions containing Cu
276 and Ni, metal affinity is Cu > Ni. In all the tested conditions, the data showed that the removal of
277 Cu was higher than that of Ni, with maximum uptake capacities of 13.18 mgCu gDW^{-1} and 9.04
278 mgNi gDW^{-1} in living biomass and 0.88 mgCu gDW^{-1} and 0.32 mgNi gDW^{-1} in dry biomass from
279 *Run1* ($p < 0.05$). Lau et al. (1999) reported that Cu was generally preferred to Ni by *Chlorella*
280 *miniata* and *C. vulgaris* due to its stronger binding strength and larger ionic radius compared to Ni,
281 which favoured a more covalent interaction between the metal ion and the ligands, particularly the
282 carboxylate groups of algal cell walls. Despite the fact that microalgae represent a feasible option
283 for metal removal in WWTP, studies involving multi-metal solutions are scarce, even if more
284 representative of real environmental conditions than single metal studies (Anastopoulos and Kyzas,
285 2015; Monteiro et al., 2009). Ajayan et al. (2015) evaluated the ability of *Scenedesmus* sp. to grow
286 and remove metals such as Cu, Cr, Pb and Zn in a tannery wastewater. Their results revealed that
287 living algal biomass during the growth period (12 days) not only reduced the pollution load of
288 metals (Cr-81.2–96%, Cu-73.2–98%, Pb-75–98% and Zn-65–98%) but also that of nutrients
289 (N>44.3% and P >95%), in agreement with the results we obtained employing *Desmodesmus* sp. in

290 *Run2* (Fig. 2b; Tab. 1). Biosorption of Cu and Ni (100 mg L^{-1}) by the cyanobacterium *Arthrospira*
291 *platensis* (living and dry biomass) was studied by Markou et al., (2015) who found a lower uptake
292 capacity in the living form of the cyanobacterium for both Cu and Ni, than that reported here. On
293 the other hand, Markou et al. (2015) reported a four and nine times higher adsorption capacity of *A.*
294 *platensis* dry biomass than that obtained in *Run1* and *Run2*, respectively.

295 **Tab. 1**

296 *3.3 Lipid extraction from the microalgae biomass*
297 The lipid content of algae is dependent on several factors, including nutrient availability in the
298 growth media, salinity, light intensity, metals and a variety of other contaminants that have the
299 potential to induce stress (Adams et al., 2013; Torres et al., 2017). Moreover, inorganic
300 contaminants such as metals induce oxidative stress that leads to lipid accumulation as a defence
301 mechanism (Fan et al., 2014). The lipid percentage (% dry weight of the biomass) of the freeze-
302 dried biomass of *Desmodesmus* sp. grown in absence (*Run1*) or in presence of metals (*Run2*) is
303 shown in Table 2. The highest lipid percentage of 17.6% was found for the culture grown without
304 Cu and Ni, whereas the cultures with Cu (9.8 mg L^{-1}) and Ni (7.4 mg L^{-1}) supply showed a lower
305 lipid percentage (12.9%). Different authors reported that metal stress increased lipid yields (Yang et
306 al., 2015; Napan et al., 2015), i.e. Torres et al. (2017) demonstrated that Ni consistently yielded
307 high lipid content in *Nannochloropsis salina*, while high concentrations of Cu had damaging effects
308 on lipid content. Cu may enhance the activities of antioxidant enzymes and compete with Ni to bind
309 to membrane proteins in order to protect the cells, similarly to what is reported for higher plants
310 (Hamed et al., 2017). Thus, we suppose that the lower lipid yield obtained from *Run2* biomass in
311 comparison to the one of *Run1*, can be due to the relatively high concentration of Cu used in this
312 study ($p < 0.05$). Even if lipid yields for *Desmodesmus* sp. in these culture conditions were not so
313 high for industrial biodiesel production (oleaginous species can store more than 50% of their
314 biomass as lipids), they proved similar to those reported by Gressler et al. (2013) for *Desmodesmus*
315 sp. biomass grown with the addition of CO_2 (18.73 ± 0.25%) or without additional CO_2 (12.00 ±
316 0.28%). Even Komolafe et al. (2014) reported a lipid yield of about 13% by *Desmodesmus* sp.,
317 lower compared to 21.2%, 19.7% obtained from *Desmosdesmus* cultivated in photobioreactors (6 L
318 volumes) by Jaimes-Duarte et al. (2012) and Wu et al. (2012), respectively.
319 Biodiesel quality is related to the fatty acid (FA) composition and is determined by the fatty acids'
320 degree of saturation. FAME profiles of *Desmodesmus* sp. grown in absence (*Run1*) or in presence
321 of Cu and Ni for 2 days (*Run2*), reported in Tab.2, showed that carbon chains ranged from C14 to
322 C22. The quantification of FAMEs revealed the abundance of unsaturated palmitic acid (C16:0;

28.58% and 35.61% in *Run1* and *Run2*, respectively), monounsaturated oleic acid (C18:1; up to 34.03% in *Run2*) and monounsaturated linoleic acid (C18:2; 27.08% and 10.23% in *Run1* and *Run2*, respectively). Long chain saturated and monounsaturated FA (MUFA) are suitable for biodiesel, as they improve oxidative stability without greatly affected its cold flow properties (Mandotra et al., 2014). Biodiesel obtained from *Desmodesmus* sp. grown in *Run2* contained large amounts of saturated and monounsaturated FAMEs (80.60%), twice the amount obtained for *Run1* (42.54%), due to the effect of Cu that increases overall neutral lipid accumulation because of the down-regulation of fatty acid desaturase enzymes (Ghafari et al., 2016). According to the European standard EN 14214 (Tab. 3), for an ideal biodiesel the percentage of linolenic acid (C18:3) and polyunsaturated FA (PUFA) (≥4 double bond) should not exceed 12% and 1% respectively (Gouveia et al., 2017). In this study, the C18:3 contributed to 2.76% and 0.69% of the total FAMEs in *Run1* and *Run2*, respectively, whereas PUFA with ≥4 double bonds were completely absent in *Run1* and were less than 1% in *Run2*. Lipid quality was also evaluated according to different parameters such as CN, IV, SV and CFPP. The CN is one of the important fuel properties of biodiesel which is highly influenced by the FA profile (see Tab. 2 and 3). A high CN value is an indicator of better combustion, low nitrous oxide emissions and easier start-up of the engine (Knothe, 2012). The CN value of *Desmodesmus* sp. grown in presence of metals was better than that obtained in *Run1*, 55.07 compared to 37.91, and in accordance with the requirements of EU and US standards. Higher CN values were reported for palm biodiesel (61), rich in esters of palmitic and stearic acids (Ramos et al., 2009), the green alga *Scenedesmus* sp. (59.57) (Talebi et al., 2013) and some cyanobacteria such as *Anabaena augstumalis*, *Nostoc* sp. and *Calothrix* sp. (71.68, 71.56 and 71.87, respectively) (Gismondi et al., 2016). The IV is used to determine the degree of unsaturation of biodiesel oil. The more the double bonds in the fatty acid chain, the higher is the IV of the oil (Knothe, 2012). The SV is the measure of the milligrams of potassium hydroxide (KOH) required to completely saponify one gram of oil. In the present study, the IV was found to be 83.32 g I_2 100 g^{-1} in *Run2*, slightly less than the value obtained with *Scenedesmus abundans* grown in a large scale (20 L) custom made photobioreactor (94.06 g I_2 100 g^{-1}) (Mandotra et al., 2014). Data showed that according to IV, biodiesel obtained from *Desmodesmus* sp. in *Run2* is better than soybean (128 g I_2 100 g^{-1}), sunflower seed (132 g I_2 100 g^{-1}) and peanut (97 g I_2 100 g^{-1}) as reported by Predojević et al. (2012). When grown in *Run1*, without exposure to metal stress as in *Run2,* the CN was 37.91 and IV increased to 156 g I_2 100 g^{-1}, not satisfying neither European nor American biodiesel standards (Giakoumis 2013; Ramos et al., 2009). The SV was found to be 202.82 mg KOH g^{-1} and 198.34 mg KOH g^{-1} in *Run1* and *Run2*, respectively, which is equal or slightly less than the SV of *S. abundans* (202.02 mg KOH g^{-1}) and *Chlorella luteoviridis* (207.91 mg KOH g^{-1}) found by

Mandotra et al., (2014) and Osundeko et al. (2013). The CFPP indicates the cold flow property of biodiesel and presents a lower value in relation to the presence of unsaturated fatty acids. The EN 14214 standard does not mention a low-temperature limit in its list of specifications, indicating that it is a Country specific parameter ranging from -45 °C to 4.5 °C in Artic and temperate climates. The CFFP values reported here both satisfied the parameter requirements of the EN 14214, with a minimum value of -7.50 °C in *Run1* and -5.29 °C in *Run2*. These data were in accordance with those reported by Alvarez-Diaz et al. (2015) where the CFFP of *Scenedesmus obliquus* grown in a two-stage cultivation system was always below 0°C. The results of these analyses show that even if the presence of Cu and Ni reduced the lipid yield, the quality of the biodiesel obtained from *Desmodesmus* sp. in *Run2* was higher than in *Run1* because of the high amounts of C16 and C18 and of all the parameters reported above.

Tab. 2

Tab. 3

4. Conclusions

The results reported in this study suggest that *Desmodesmus* sp. could be successfully used for wastewater bioremediation of phosphorus and metal removal. Biomass obtained as by-product could be further employed as metal adsorbent and as feedstock for biofuel production. In these growth systems, *Desmodesmus* sp. was able to remove more than 90% of total phosphorus and demonstrated a good biosorption ability for both Cu and Ni by living biomass, with a removal efficiency around 90% in less than 2 days of exposure. Data revealed that dry biomass of *Desmodesmus* sp. could be used as adsorbent material, with a biosorption ability of 90% and 43% for Cu and Ni, respectively, in just 30 minutes. Even biomass previously exposed to the metals during growth showed the ability to adsorb metals, suggesting the potential to employ the same biomass for more than one cycle of sorption. Good quality biodiesel obtained from biomass grown in presence of Cu and Ni supports the possibility to use metal-polluted wastewater for massive cultivation of microalgae, offering a new alternative solution to environmental pollution problems and carbon- neutral fuel availability.

Acknowledgement

This research was partially supported by Italian Ministry of University and Scientific Research (Miur, PRIN 2015, Prot. 20158HTL58).

389

390

391

5.6 References

Abou-Shanab, R.A.I, Ji, M.K., Kim, H.C., Paeng, K.J., Jeon, B.H., 2013. Microalgal species growing on piggery wastewater as a valuable candidate for nutrient removal and biodiesel production. J Environ Manage *115*: 257-264.

Adams, C., Godfrey, V., Wahlen, B., Seefeldt, L., Bugbee, B., 2013. Understanding precision nitrogen stress to optimize the growth and lipid content trade off in oleaginous green microalgae. Bioresource Technol *131*: 188–194.

Ajayan, K.M., Selvaraju, M., Unnikannan, P., Sruthi, P., 2015. Phycoremediation of Tannery Wastewater Using Microalgae *Scenedesmus* Species. Int J Phycoremediat *17:* 907-916.

Álvarez-Díaz, P.D., Ruiz, J., Arbib, Z., Barragán, J., Garrido-Pérez. M.C., Perales, J.A., 2015. Wastewater treatment and biodiesel production by *Scenedesmus obliquus* in a two-stage cultivation process. Bioresource Technol *181*: 90–96.

Anastopoulos, I., Kyzas, G.Z., 2015. Progress in batch biosorption of heavy metals onto algae. Journal of Molecular Liquids *209*: 77–86.

Aslan, S., Kapdan, I.K., 2006. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecol Eng *28*: 64-70.

Boelee, N.C., Janssen, M., Temmink. H., Shrestha, R., Buisman, C.J., Wijffels, R.H., 2014. Nutrient removal and biomass production in an outdoor pilot-scale phototrophic biofilm reactor for effluent polishing. Appl Biochem Biotech *172*: 405-412.

Bruno, L., Di Pippo, F, Antonaroli, S, Gismondi, A, Valentini, C, Albertano, P., 2012. Characterization for biofilm-forming cyanobacteria for biomass and lipid production. J Appl Microbiol *113*: 1052–1064, DOI: 10.1111/j.1365-2672.2012.05416.

Castenholz, R.W., 2001. Phylum BX. Cyanobacteria. Oxygenic Photosynthetic Bacteria. In: Bergey's Manual of Systematic Bacteriology, 2nd edn. Springer, New York, pp 473-487.

Chan, A., Salsali, H., McBean, E., 2013. Heavy Metal Removal (Copper and Zinc) in Secondary Effluent from Wastewater Treatment Plants by Microalgae. ACS Sustainable Chem Eng *2*: 130−137.

Chen, C.Y., Chang, H.W., Kao, P.C., Pan, J.L., Chang, J.S., 2012. Biosorption of cadmium by CO_2 fixing microalga *Scenedesmus obliquus* CNW-N. Bioresource Technol *105,* 74-80.

Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol Adv *25*:294–306.

Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol. Trends Biotechnol *26*(3):126–131.

D'este, M., Alvarado-Morales, M., Angelidaki, I., 2017. *Laminaria digitata* as potential carbon source in heterotrophic microalgae cultivation for the production of fish feed supplement. Algal Res *26*: 1-7.

De Angelis, R., Melino, S., Prosposito, P., Casalboni, M., Lamastra, F.R., Nanni, F., Bruno, L., Congestri, R., 2016. The diatom *Staurosirella pinnata* for photoactive material production. PLOS ONE DOI:10.1371/journal.pone.0165571.

Di Pippo, F., Ellwood, N.T.W., Gismondi, A., Bruno, L., Rossi, F., Magni, P., De Philippis, R., 2013. Characterization of exopolysaccharides produced by seven biofilm-forming cyanobacterial

strains for biotechnological applications. J Appl Phycol *25*: 1697–1708, DOI 10.1007/s10811-013-0028-1.

Fan, J., Cui, Y., Wan, M., Wang, W., Li, Y., 2014. Lipid accumulation and biosynthesis genes response of the oleaginous *Chlorella pyrenoidosa* under three nutrition stressors. Biotechnol. Biofuels *7*(1): 1.

Gadd, G.M., 2008. Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biol *84:* 13-28.

Ghafari, M., Rashidi, B., Haznedaroglu, B.Z., 2016. Effects of macro and micronutrients on neutral lipid accumulation in oleaginous microalgae. Biofuels, DOI: 10.1080/17597269.2016.1221644

Giakoumis, E.G., 2013. A statical investigation of biodiesel physical and chemical properties, and their correlation with the degree of unsaturation. Renew Energy *50*: 858-878.

Gismondi, A., Di Pippo, F., Bruno, L., Antonaroli, S., Congestri R., 2016. Phosphorus removal coupled to bioenergy production by three cyanobacterial isolates in a biofilm dynamic growth system. I J Phytorem *18*(9), 869–876.

Gouveia, L., Oliveira, A.C., Congestri, R., Bruno, L., Soare, A.T., Menezes, R.S., Filho, N.R.A., Tzoveni, I., 2017. Biodiesel from microalgae. In: In: Gonzalez-Fernadez, C., Munoz, R. (Eds), Microalgae-Based Biofuels and Bioproducts, From Feedstock Cultivation to End-Products, pp 235-258.

Gressler, P., Bjerka, T., Schneider, R., Souza, M., Lobo, E., Zappe, A., Corbellini, V., Moraes, M., 2013. Cultivation of *Desmodesmus subspicatus* in a tubular photobioreactor for bioremediation and microalgae oil production. Environ Technol *35*(2): 209-219.

Gupta, S.K., Ansari, F.A., Shriwastav, A., Sahoo, N.K., Rawat, I., Bux, F., 2016. Dual role of *Chlorella sorokiniana* and *Scenedesmus obliquus* for comprehensive wastewater treatment and biomass production for bio-fuels. J Clean Prod *115*: 255-264.

Gupta, S.K., Sriwastav, A., Ansari, F.A., Nasr, M., Nema, A.K., 2017. Phycoremediation: An Eco-friendly Algal Technology for Bioremediation and Bioenergy Production. © Springer Nature Singapore Pte Ltd. 2017 431 K. Bauddh et al. (eds.), Phytoremediation Potential of Bioenergy Plants, DOI 10.1007/978-981-10-3084-0_18.

Hamed, S.M., Selim, S., Klock, G., AbdElgawad, M., 2017. Sensitivity of two green microalgae to copper stress: Growth, oxidative and antioxidants analyses. Ecotox Environ Safe *144*: 19–25.

Jaimes-Duarte, D., Soler-Mendoza, W., Valasco-Mendoza, J., Munoz-penaloza, Y., Urbina-Suarez, N., 2012. Characterization chlorophytas microalgae with potential in the production of lipids for biofuels. CT&F–Cienc. Technol. Futuro. *5*:93–102.

Jais, N.M., Mohamed, R.M.S.R., Al-Gheethi, A.A., Amir Hashim, M.K., 2017. The dual roles of phycoremediation of wet market wastewater for nutrients and heavy metals removal and microalgae biomass production. Clean Techn Environ Policy *19*: 37–52.

Ji, M.K., Reda, A.I., Shanaba, A., Kima, S.K., Salama, E.S., Lee, S.H., Kabrad, A.N., Lee, Y.S., Hong, S., Jeon, B.H., 2013. Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO_2 for nutrient removal and biomass production. Ecol Eng *58*: 142– 148.

Knothe, G., 2012. Fuel properties of highly polyunsaturated fatty acid methyl esters. Prediction of fuel properties of algal biodiesel. Energy fuels *26*, 5265-5273.

Komolafe, O., Orta, S.B.V., Monje-Ramirez, I., Noguez, I.Y., Harvey, A.P., Ledesma, M.T.O., 2014. Biodiesel production from indigenous microalgae grown in wastewater. Bioresource Technol *154*: 297–304.

Kumar, K.S., Dahms, H.U., Won, E.J., Lee, J.S., Shin, K.H., 2015. Microalgae- A promising tool for heavy metal remediation. Ecotox Environ Safe *113*, 329-352.

Laliberte, G., Lessard, P., de la Noie, J., 1997. Effect of phosphorus addition on nutrient removal from wastewater with the cyanobacterium *Phormidium bohneri*. Bioresource Technol *59*: 227-233.

Lau, P.S., Lee, H.Y., Tsang, C.C.K., Tam, N.F.Y., Wong, Y.S., 1999. Effect of metal interference, pH and temperature on Cu and Ni biosorption by *Chlorella vulgaris* and *Chlorella miniata*. Environ Technol *20*: 953–961.

MacKinney, G., 1941. Absorption of light by chlorophyll solutions. The Journal of Biological Chemistry *140,* 315-322.

Mandotra, S.K., Kumar, P., Suseela, M.R., Ramteke, P.W., 2014. Fresh water green microalga *Scenedesmus abundans*: A potential feedstock for high quality biodiesel production. Bioresource Technol 156: 42-47.

Markou, G., Mitrogiannis, D., Celekli, A., Bozkurt, H., Georgakakis, D., Chrysikopoulos, C.V., 2015. Biosorption of Cu^{2+} and Ni^{2+} by *Arthrospira platensis* with different biochemical compositions. Chem Eng J *259*: 806–813.

Micheletti, E., Colica, G., Viti, C., Tamagini, P., De Philippis, R., 2008. Selectivity in the heavy metal removal by exopolysaccharide-producing cyanobacteria. J Appl Microbiol *105*: 88–94.

Monteiro, C.M., Castro, P.M.L., Malcata, F.X., 2009. Use of the microalga *Scenedesmus obliquus* to remove cadmium cations from aqueous solutions. World J Microbiol Biotechnol *25*: 1573–1578.

Murphy J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta *27*: 31-36.

Napan, K., Teng, L., Quin, J.C., Wood, B.D., 2015. Impact of heavy metals from flue gas integration with microalgae production. Algal Res *8*: 83–88.

Osundeko, O., Davis, H., Pittman, J.K., 2013. Oxidative stress-tolerant microalgae strains are highly efficient for biofuel feedstock production on wastewater. Biomass Bioenergy *56*, 284–294.

Predojević, Z., Škrbić, B., Durišić-Mladenović, N., 2012. Transesterification of linoleic and oleic sunflower oils to biodiesel using CaO as a solid base catalyst. J Serb Chem Soc *77*, 815–832.

Ramos, M.J., Fernàndez, C.M., Casa, A., Rodrìguez, L.,Pérez, A., 2009. Influence of fatty acid composition of raw materials on biodiesel properties. Bioresource Technol 100, 261-268.

Richards, R.G., Mullins, B.J., 2013. Using microalgae for combined lipid production and heavy metal removal from leachate. Ecol Model *249*: 59–67.

Rugnini, L., Costa, G., Congestri, R., Bruno, L., 2017. Testing of two different strains of green microalgae for Cu and Ni removal from aqueous media. Sci Tot Environ *601–602*: 959–967.

Samorì, G., Samorì, C., Guerrini, F., Pistocchi, R., 2013. Growth and nitrogen removal capacity of Desmodesmus communis and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: Part I. Water Res *79*: 791-801.

Song, M., Pei, H., Hua, W., Zhang, S., Maa, G., Han, L., Ji, Y., 2014. Identification and characterization of a freshwater microalga *Scenedesmus* SDEC-8 for nutrient removal and biodiesel production. Bioresource Technol *162*: 129–135.

Sukacova, K., Trtilek, M., Rataj, T., 2015. Phosphorus removal using a microalgal biofilm in a new biofilm photobioreactor for tertiary wastewater treatment. Water Res *71*: 55-63.

Talebi, A.F., Mohtashami, S.K., Tabatabaei, M., Tohidfar, M., Bagheri, A., Zeinalabedini, M., Bakhtiari, S., 2013. Fatty acids profiling: a selective criterion for screening microalgae strains for biodiesel production. Algal Res *2*:258–267.

Torres, E.M., Hess, D., McNeil, B.T., Guy, T., Quinn, J.C., 2017. Impact of inorganic contaminants on microalgae productivity and bioremediation potential. Ecotox Environ Safe *139*: 367–376.

Torricelli, E., Gorbi, G., Pawlik-Skowrońska, B., Sanità di Toppi, L., Corradi, M.G., 2004. Cadmium tolerance, cysteine and thiol peptide levels in wild type and chromium-tolerant strains of *Scenedesmus acutus* (Chlorophyceae). Aquat Toxicol *68*: 315-323.

Valderrama, J.C., 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar Chem *10*:109–122.

Voulvoulis, N., Arpon, K.D., Giakoumis, T., 2017. The EU Water Framework Directive: From great expectations to problems with implementation. Sci Tot Environ *575*: 358-366.

Wu, L.F., Chen, P.C., Huang, A.P., Lee, C.M., 2012. The feasibility of biodiesel production by microalgae using industrial wastewater. Bioresource Technol *113*: 14–18.

Yang, J.S., Cao, J., Xing, G.L., Yuan, H.L., 2015. Lipid production combined with biosorption and bioaccumulation of cadmium, copper, manganese and zinc by oleaginous microalgae *Chlorella minutissima* UTEX2341. Bioresource Technol *175:* 537-544.

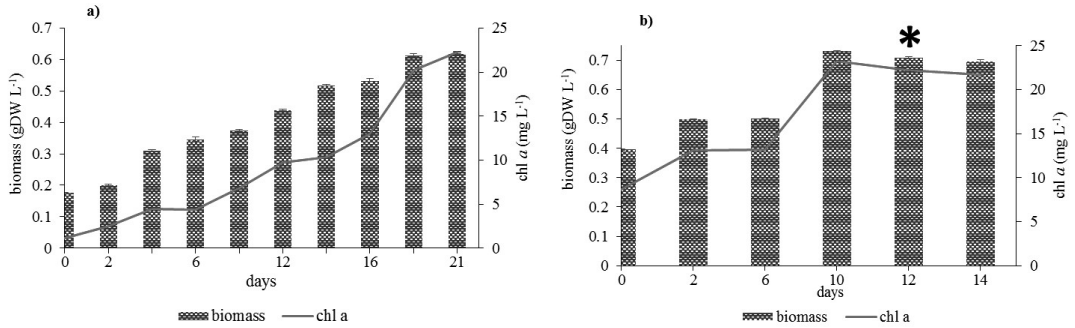

Fig.1 Biomass concentration (gDW L^{-1}) and Chl *a* concentration (mg L^{-1}) of *Desmodesmus* sp. in *Run1* (a) and *Run2* (b) were Cu and Ni solutions (*) were added after 12 days of cultivation. Data are mean values ± standard deviations.

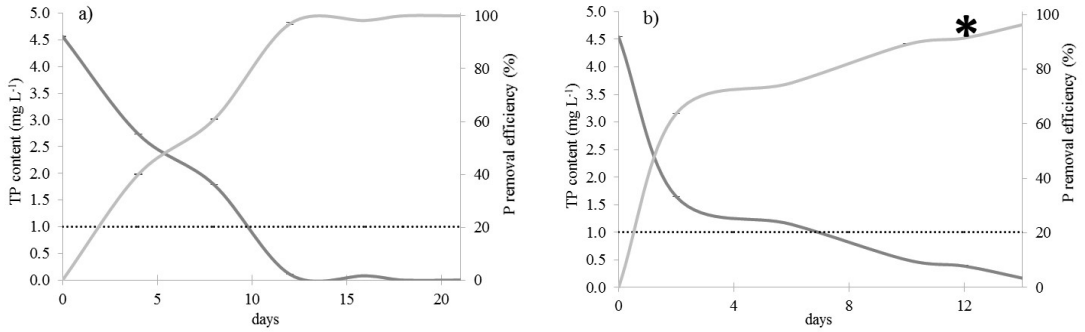

Fig.2 TP concentration (mg L^{-1} yellow line) and P removal efficiency (% green line) of *Desmodesmus* sp. in *Run1* (a) and *Run2* (b; * indicates the addition of Cu and Ni in solution). Dotted lines indicate the EU limit for P concentrations in effluent discharge in sensitive aquatic ecosystems. Data are means ± standard deviations.

Tab.1 Final concentration of Cu and Ni in liquid solution (C_f), metal removal efficiency (E) and metal uptake (q) in different types of *Desmodesmus* sp. biomass (LB2: living biomass collected at the end of *Run2*; DB1 and DB2: freeze-dried biomass from *Run1* and from *Run2,* respectively).

	LB2	**DB1**	**DB2**
Cu (9.8 mg L^{-1})			
C_f (mg L^{-1})	0.56	1.00	3.38
E (%)	94.28	89.80	65.47
q (mg gDW^{-1})	13.18	0.88	0.64
Ni (7.4 mg L^{-1})			
C_f (mg L^{-1})	1.1	4.24	5.22
E (%)	85.20	42.93	29.74
q (mg gDW^{-1})	9.04	0.32	0.22

630

631 **Tab. 2.** Fatty acid methyl ester (FAME, %) obtained from *Desmodesmus* sp. biomass grown in
632 *Run1* (without metals) and *Run2* (with Cu and Ni).

FAME	COMMON NAME	FAMILY	*RUN1* (%)	*RUN2* (%)
C14:0	Myristic acid	SATURATED	0.39	0.51
C15:0	Pentadecylic acid	SATURATED	0.09	0.37
C16:0	Palmitic acid	SATURATED	28.58	35.61
C16:1 (9)	Palmitoleic acid	MUFA	8.50	6.48
C16:2 (9,12)		PUFA	6.76	2.67
C16:3 (4,7,10)		PUFA	2.27	/
C16:4 (4,7,10,13)		PUFA	18.27	4.14
C17:1 (CIS 10)	Margaroleic acid	MUFA	0.27	0.85
C18:1 (13)	Vaccenic acid	MUFA	1.71	1.17
C18:1 (9)	Oleic acid	MUFA	2.63	34.03
C18:2 (9,12)	Linoleic acid	PUFA	27.08	10.23
C18:3	ɣ-linolenic acid	PUFA	2.76	0.69
C18:3 (9,12,15)	α-Linolenic acid	PUFA	0.32	/
C19:0		MUFA	0.24	0.57
C20:1		MUFA	/	0.42
C20:4(8,11,14,17)		PUFA		0.72
C20:4 (5,8,11,14)	Arachidonic acid	PUFA	/	0.97
C22:0	Methyl behenate	SATURATED	/	0.59
C22:1 (13)	Erucic acid	MUFA	0.13	/
LIPID YIELD (% gDW)			**17.6**	**12.9**

633

634

635

636

637

638

639

640

641

642 **Tab. 3** Biodiesel properties calculated from the FAME profile of *Desmodesmus* sp. and compared
643 with European (UN-EN14214) and American (ASTM-D6751) standards.

	SAT (%)	**MUFA (%)**	**PUFA (%) *PUFA>4 db**	**C18:3 (%)**	**CN**	**IV**	**SV**	**DU**	**LCSF**	**CFPP (°C)**
Run1	29.06	13.48	57.46 */	2.76	37.91	156.87	202.82	128.16	2.86	-7.50
Run2	37.08	43.52	19.42 * < 1%	0.69	55.07	83.32	198.34	81.76	3.56	-5.29
UN-EN14214				12.0	≥51	≤120	/	/	/	-45 /4.5
ASTM-D6751					≥47	/	/	/	/	/

644 **Note:** db: double bounds; *CN*: cetane number; *IV*: iodine valued; *SV*: saponification value; *DU*: degree of unsaturation; *LCSF*: long-chain saturated
645 factor; *CFPP*: cold filter plugging point.

646

647

648

649