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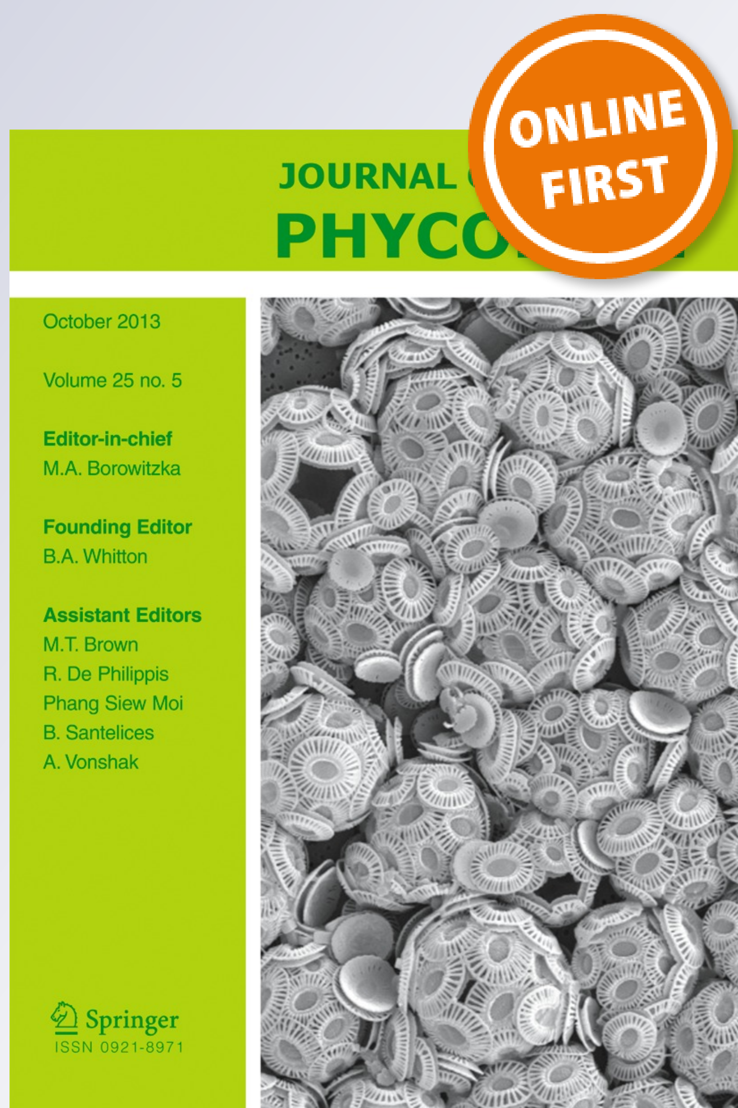

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Triacylglycerol content, productivity and fatty acid profile in *Scenedesmus acutus* PVUW12

M. Cecilia Damiani · Cecilia A. Popovich · Diana Constenla · Ana María Martínez · Enrico Doria · Paolo Longoni · Rino Cella · Erik Nielsen · Patricia I. Leonardi

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Abstract A detailed lipid characterization of *Scenedesmus acutus* PVUW12, with emphasis on the evaluation of triacylglycerols (TAGs) as a biodiesel feedstock, is presented. When algal cells were grown in nitrogen-free medium (N stress), a lipid increase was detected that was mainly due to TAG accumulation. In situ fluorescence measurements allowed the kinetics and extent of neutral lipid accumulation to be followed. Under N stress, the productivity of total lipids and TAGs increased significantly (80.99 and 63.74 mg L^{-1} day^{-1}, respectively) compared with controls (29.51 and 16.23 mg L^{-1} day^{-1}, respectively). Monounsaturated fatty acids were the major fraction and increased further (49.74 %) in stressed cells, with oleic acid as the most abundant compound (46.97 %). The polyunsaturated fatty acid composition of this algal oil appears to meet the European Standard EN 14214. These results indicate that *S. acutus* oil meets the requirements for its use as a biodiesel feedstock. Since this strain was also proposed for wastewater bioremediation, this opens up the possibility of its use in an integrated system combined with biofuel production.

Keywords *Scenedesmus acutus* · Nitrogen limitation · Triacylglycerol · FAMEs composition · Biodiesel · Feedstock

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Introduction

Algae, particularly green microalgae, have been proposed for a long time as a potential renewable biofuel source (Benemann et al. 1977), especially during the last few years when they have received special attention. However, biodiesel obtained from microalgae cannot be commercially profitable at today's fossil fuel prices (Park et al. 2011). Among the several strategies taken into account, the coupling of microalgal biomass production with concurrent wastewater phycodepuration has been considered a promising approach to meet the economical requirements for biodiesel production (Mallick 2002).

Some *Scenedesmus* species show a remarkable ability to grow in wastewaters (Ruiz-Marin et al. 2010; Tang et al. 2011), thus being exploited simultaneously for bioremediation and energy production (McGinn et al. 2012). Recent characterization studies carried out on *Scenedesmus acutus* PVUW12 showed that this strain can be used effectively for bioremediation of urban wastewater (Doria et al. 2012).

Diverse strains of *Scenedesmus obliquus* and *Scenedesmus* sp. have been proposed as a potential source of feedstock for biodiesel production due to their high growth rate, high lipid

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content, and fatty acid profile (Abou-Shanab et al. 2011; Gouveia and Oliveira 2009; Griffiths et al. 2012; Ho et al. 2010; Mandal and Mallick 2009). Doria et al. (2012) determined biomass and total lipid productivity of *S. acutus* PVUW12 grown in wastewater, while Chaichalerm et al. (2012) evaluated biomass yield, lipid content, and lipid productivity of a *S. acutus* strain grown in four different enriched culture media (3NBBM, N-8, Kuhl, and BG-11). Recently, Ördög et al. (2013) analyzed lipid productivity and fatty acid composition in several *Scenedesmus* strains grown under intermediate levels of nitrogen. However, a detailed characterization of TAGs was not carried out for *S. acutus* grown in a nitrogen-free medium. In the present work, *S. acutus* PVUW12 was cultivated under optimal conditions as well as extreme stress conditions (N-free medium) in order to obtain basic information about its: (1) biomass accumulation, (2) total lipid and TAG content and productivities, (3) fatty acid methyl ester (FAME) composition, and (4) oil quality for biodiesel production. Furthermore, the utility of a staining method for evaluating the kinetics of TAG accumulation was carried out under stress conditions in order to define the timing of TAG accumulation. This information was used to estimate lipid productivity and to find the best harvesting time.

Materials and methods

Algal strain and culture conditions

Scenedesmus acutus (strain PVUW12), from the CCALA (Culture Collection Autotrophic Organisms from the Institute of Botany, Academy of Sciences of the Czech Republic), was collected from the Pavia (Italy) urban wastewater treatment plant. Flasks with 3 L of culture (15 cm diameter and 12.5 cm height) were used for the experiments. The cells were cultured in Bold's basal medium (BBM) (Stein 1973). The medium was autoclaved, and the pH was adjusted to pH 7.0. Cells were grown at 25± 1 °C with continuous bubbling of air (500–700 cm^3 min^{-1}). An enriched air stream containing 1 % CO_2 was supplied for 4 h every day. Light was supplied by cool white fluorescent lamps providing an average irradiance of 170 μmol photons m^{-2} s^{-1}, with a 16:8-h light/dark photoperiod. The following experiments were carried out: (a) 3 L of complete BBM was inoculated at a cell concentration of 4.55×10^6 cells mL^{-1} for 12 days (control condition) and harvested for biochemical analysis, and (b) an inoculum of 4.55×10^6 cells mL^{-1} was resuspended in 3 L of complete BBM, harvested by centrifugation (3,600× g) at the stationary phase, and transferred to 3 L of nitrogen-free BBM for 15 days (N stress condition). Finally, the biomass was harvested for biochemical analyses.

Growth rate and biomass measurements

Cell concentration was determined by counting three replicate samples by means of Sedgwick–Rafter chamber. Growth rate (k) and doubling time (t_d) were calculated according to Damiani et al. (2010). For dry biomass determination, duplicated samples (20 mL) were filtered through pre-dried and pre-weighed glass fiber filters (Whatman GF/C) which were dried for 12 h at 100 °C, cooled in a desiccator, and weighed.

Neutral lipid analysis with Nile Red staining method

Duplicate samples of *S. acutus* PVUW12 from N-stressed cultures were taken daily and frozen at −20 °C. Five μL of Nile Red (9-diethylamino-5*H*-benzo[*a*]phenoxazine-5-one, Sigma CAS number: 7385-67) in acetone (1 mg mL^{-1}) was added to a 5-mL cell suspension (Priscu et al. 1990). For in situ fluorescence measurements, various solvents, such as methanol, isopropanol, acetone, and dimethyl sulfoxide (DMSO), were applied prior to Nile Red staining, getting the best fluorescence signal with DMSO ($p<0.05$). Cell suspension (250 μL) was pretreated with 125 μL of DMSO for 10 min at 30 °C and stained with 5 μL of Nile Red solution. Then, 4.75 mL of pure water was added, and this mixture was gently vortexed and incubated for 10 min at 30 °C in the darkness. Excitation wavelength was set at 480 nm, and emission wavelength was scanned from 500 to 750 nm using a spectrofluorometer (Shimadzu RF-5301PC, spectrum mode with excitation and emission slits set at 5 nm). The relative fluorescence intensity (RFI, in arbitrary units, au) was obtained upon subtraction of both the autofluorescence of microalgal cells and the fluorescence intensity of Nile Red itself. For neutral lipid detection at cell level, *S. acutus* samples from N-stressed cultures were stained with NR and analyzed by means of the Leica DMIRE2 confocal TCS SP2 SE microscope with a 475-nm band excitation filter and a 580-nm band emission filter.

Pigment extraction and quantification

Chlorophyll *a* and carotenoid concentrations from control and N-stressed cultures were estimated by means of Ritchie's and Strickland and Parsons' equations (Pruvost et al. 2011), respectively, using the Shimadzu UV–Vis 1603 spectrophotometer. Triplicate samples were extracted with 6 mL of methanol (99.9 %) and sonicated three times for 15 min in an ultrasonic bath (40 KHz, 160 W) thermostated by cool water circulation at 4–5 °C. After this disruptive step, samples were kept overnight in the darkness at −18 °C. Then, they were clarified by GF-F filtration and absorbance values at 652, 665, and 750 nm measured.

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Biochemical analysis

For biochemical analysis, microalgal cells were concentrated by centrifugation (3,600× *g*) at the end of both control and N-stressed cultures, and cell pellets were lyophilized for the analyses described below.

Protein and carbohydrate content For protein and carbohydrate quantification, triplicate samples of 10 mg of lyophilized biomass were immersed in 5 mL ultrapure water. The samples were placed in an ultrasonic bath (40 KHz, 160 W) and sonicated for 30 min. Then, protein and carbohydrates were quantified using the techniques of Bradford (1976) and Dubois et al. (1956), respectively, according to the detailed methodology explained in Popovich et al. (2012).

Lipid content, lipid fractionation, and fatty acid profile Lipid content (percentage of dry weight (% dw)), lipid fractionation into neutral lipids, glycolipids and phospholipids (% dw), and FAMEs (%) were performed as described previously by Popovich et al. (2012). All chemicals used were analytically graded.

Lipid productivities

Total lipid and TAG productivities were estimated according to the following equations:

$$P_{\mathrm{TL}}\left(\mathrm{g\ L^{-1}\ day^{-1}}\right) = \frac{\mathrm{DCW}\left(\mathrm{g\ L^{-1}}\right) \times C_{\mathrm{TL}}\left(\mathrm{g\ g^{-1}}\right)}{\mathrm{At\ (day)}} \quad (1)$$

$$P_{\mathrm{TAG}}\left(\mathrm{g\ L^{-1}\ day^{-1}}\right) = \frac{\mathrm{DCW}\left(\mathrm{g\ L^{-1}}\right) \times C_{\mathrm{TAG}}\left(\mathrm{g\ g^{-1}}\right)}{\mathrm{At\ (day)}} \quad (2)$$

where P_{TL} and P_{TAG} are total lipid and TAG productivities, respectively; DCW is dry cell weight; C_{TL} and C_{TAG} are total and TAG cell lipid contents, respectively; and At is the accumulation time estimated from the kinetics of neutral lipid increase obtained by using Nile red fluorescence intensities.

Iodine value

The iodine value was calculated according to AOCS recommended practice Cd 1c-85 (Firestone 2010). This method estimates the gram of halogen absorbed by 100 g of the oil.

Data analysis

The differences in the mean values of the chlorophyll *a*, carotenoid, protein, carbohydrate, and lipid contents ($n=2$ or 3) as well as in the mean values of the percentages ($n=4$) of the different fatty acid contents were assessed with ANOVA and Student's *t* test, being statistically different at a significance level of 5 %.

Results

Growth and biochemical composition

The growth rate and doubling time (t_d) of *S. acutus* PVUW12 under control conditions were 0.98±0.26 div day^{-1} and 0.70±0.18 days, respectively (Fig. 1). The culture showed no signs of growth from days 9 to 12, when it was harvested. The biomass concentration reached a value of 2.53 g L^{-1}. Under N stress culture conditions, an inoculum of approximately 2.61 g L^{-1} resuspended in N-free BBM failed to show any biomass increase being the biomass of 2.49 g L^{-1} at the end of the test (15 days).

The biochemical composition (% dw) of *S. acutus* PVUW12 under control and N stress culture conditions is shown in Table 1. The carbohydrate content showed a significant increase ($p<0.05$) under N stress (45.70 % dw) compared with control conditions (40.30 % dw). In contrast, under these conditions, the protein level decreased significantly ($p<0.05$) (16.63 % dw). The total lipid content of control was 14.05 % dw, but it increased significantly ($p<0.05$) up to 32.53 % dw, in cells grown under N stress conditions. In addition to lipid accumulation, a significant decrease ($p<0.05$) in chlorophyll *a* content was observed (0.32 % dw) as compared to the control (1.50 % dw). However, no significant difference in carotenoid

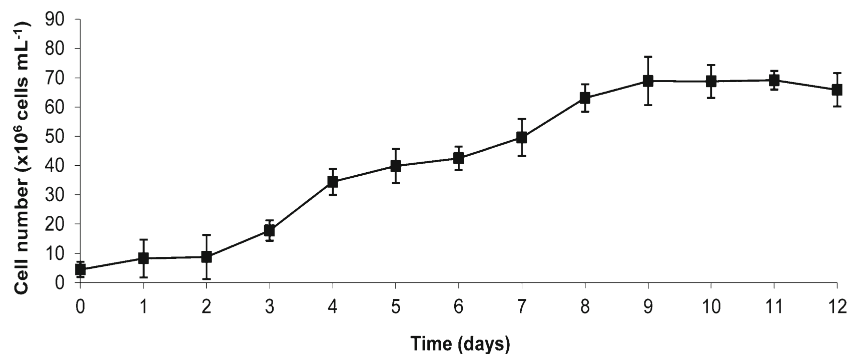

Fig. 1 Growth curve of *S. acutus* in control culture. *Error bars* denote standard deviations among replicates

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Table 1 Contents of carbohydrates, proteins, chlorophyll *a*, carotenoids, lipids, and lipid fractions—neutral, glycolipid, and phospholipid fractions—(in percentage of dry weight biomass (% dw) of *S. acutus* grown under different culture conditions

Conditions	Carbohydrates (% dw)	Proteins (% dw)	Chlorophyll *a* (% dw)	Carotenoids (% dw)	Total lipids (% dw)	Neutral lipids (% dw)	Glycolipids (% dw)	Phospholipids (% dw)
Control	$40.30^{a}\pm0.75$	$36.40^{c}\pm0.34$	$1.50^{e}\pm0.06$	$0.65^{g}\pm0.25$	$14.05^{a}\pm0.25$	$7.67^{c}\pm0.24$	$4.47^{e}\pm0.10$	$1.98^{f}\pm0.03$
N stress	$45.70^{b}\pm0.45$	$16.63^{d}\pm0.38$	$0.32^{f}\pm0.02$	$0.43^{g}\pm0.02$	$32.53^{b}\pm1.75$	$25.60^{d}\pm1.86$	$5.05^{e}\pm0.80$	$2.05^{f}\pm0.68$

Values are means ± standard deviations of two or three replicates. Differences were not significant ($p>0.05$) for groups with the same superscript

content was observed between N stress (0.43 % dw) and control conditions (0.65 % dw).

Neutral lipid fluorescence measurements

The content of neutral lipids was evidenced by the observation of yellow-gold fluorescence droplets, the number of which was found to increase under N stress conditions (Fig. 2). Furthermore, neutral lipids intracellular accumulation in *S. acutus* under N stress conditions was analyzed by RFI kinetics (Fig. 3). These results show that nitrogen deprivation caused up to a 12-fold increase in RFI. In addition, these in situ measurements allowed the determination of the harvesting time at day 15. Under control conditions, RFI ranged between 5 and 25 au.

Lipid fractions and productivities

Neutral lipid content, as compared to control (54.52 % of total lipids), increased significantly under N stress conditions (76.95 % of total lipids) (Fig. 4a), with TAGs the only source of fatty acids. Neither diacylglycerols nor monoacylglycerols were detected. These results clearly indicate that lipid accumulation influenced TAG content, which increased up to 25.6 % dw (Table 1). Conversely, the glycolipid and phospholipid contents showed no significant changes in N stress conditions, as compared to controls (Table 1).

In addition, a higher total lipid productivity (80.99 mg L^{-1} day^{-1}) was obtained under N stress conditions. Likewise, lipid fractionation allowed to estimate a higher TAG productivity (63.74 mg L^{-1} day^{-1}) in N-stressed cultures with respect to controls ($P_{TL\ control}$=29.51 mg L^{-1} day^{-1}; $P_{TAG\ control}$=16.17 mg L^{-1} day^{-1}).

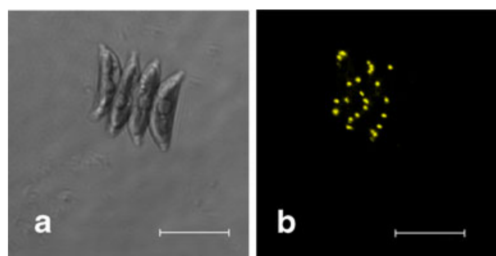

Fig. 2 Light micrographs of *S. acutus* under N stress conditions after 15 days. **a** Phase contrast microscopy. **b** Epifluorescent microscopy. Neutral lipid droplets are shown. *Scale bars*=11 μm

Lipid classes and fatty acid composition analysis (FAMEs)

Regarding FAME composition of the *S. acutus* lipids under control and N stress conditions, the most abundant ones were the following: palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9*cis*), linoleic (C18:2 n-6*cis*), and linolenic (C18:3 n-3) acids (Table 2, additional information).

In particular, in the neutral lipid fraction, the percentage of saturated fatty acids (SFAs) showed a significant decrease (α=0.05) in N stress conditions (25.87 %), with respect to control (29.04 %) along with a reduced content of both palmitic and stearic acids (Fig. 4b, Table 2, additional information). The monounsaturated fatty acids (MUFAs) represented the most abundant class of neutral lipids, and their percentage was significantly higher in cultures grown under N stress conditions, 49.74 vs. 47.22 % of control. The most abundant one was oleic acid (44.67 and 46.97 % under control and N stress conditions, respectively) (Fig. 4b). The content of polyunsaturated fatty acids (PUFAs) showed a significant decrease under N deprivation conditions (Table 2, additional information), along with a lower proportion of linolenic acid (10.12 %) compared to the value detected under control conditions (Fig. 4b). In contrast, the glycolipid and

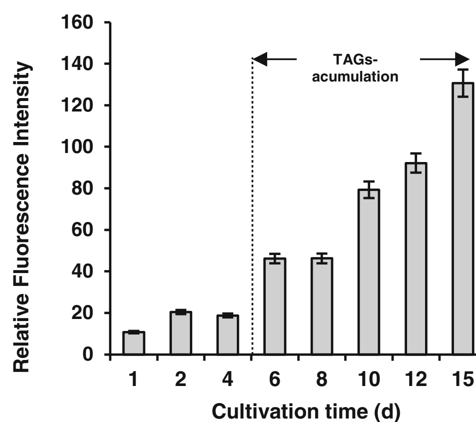

Fig. 3 Relative fluorescence intensity kinetics of cell suspensions of *S. acutus* (diluted 1:20) under N stress conditions from days 1 to 15. Data are expressed as mean and standard deviation values of three replicates

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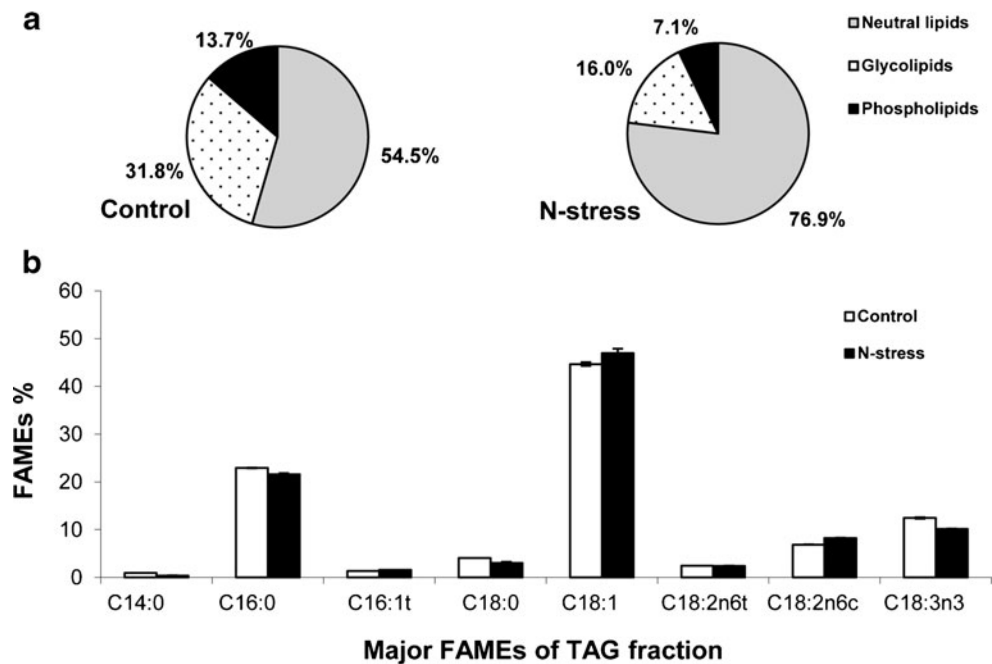

Fig. 4 **a** Relative proportions of lipid fractions (in percentage (%) of total lipids) under control and N stress culture conditions. **b** Percentage of major fatty acid methyl esters (*FAMEs*) of triacylglycerols (*TAG*) fraction under control and N stress culture conditions

phospholipid fractions showed a higher percentage of PUFAs with respect to the neutral lipid fraction.

Discussion

With regard to biodiesel production, microalgae show the following advantageous characteristics: sustained production of a biomass rich in lipids (Rodolfi et al. 2009), high proportion of total lipids made up by TAGs (Hu et al. 2008), TAG fatty acid profile rich in MUFAs (Knothe 2005, 2008), and high lipid productivity (Griffiths et al. 2012). Many *Scenedesmus* strains have been studied, and many of them were considered to be suitable for biodiesel production (Griffiths et al. 2012; Ho et al. 2010; Mandal and Mallick 2009). However, additional information on TAG accumulation kinetics and lipid composition is still needed for a better strain selection.

It has been reported that different strains of *Scenedesmus* grown in diverse synthetic freshwater media reach biomass values ranging from 0.82 to 5.24 g L^{-1} (Ho et al. 2010; Yoo et al. 2010; Abou-Shanab et al. 2011; Tang et al. 2011; Li et al. 2011; Chaichalerm et al. 2012; Ördög et al. 2013). In these studies, the way different environmental conditions (e.g., temperature, CO_2 levels, light intensity, and cycles) affect microalgal growth rate, lipid content, lipid productivity, and feedstock quality was evaluated.

We report here that *S. acutus* PVUW12 reaches a biomass level of 2.53 g L^{-1} in control cultures grown in optimal medium, while the same amount of algal biomass inoculated under N stress conditions shows no relevant variations during 15 days. This observation indicates that this N starvation period does not reduce the biomass stock significantly. On the other hand, the lack of biomass increment during N limitation in *S. acutus* could be related to light limitation. As was indicated by Sánchez et al. (2008), other species of *Scenedesmus* such as *Scenedesmus almeriensis* are tolerant to high irradiance. Therefore, the light conditions used in this study may lead to the underestimation of the potential of this strain regarding lipid accumulation.

Carbohydrates, proteins, lipids, and pigments are the major constituents of microalgal biomass, and their relative amounts vary depending on algal species (Brown et al. 1997) and culture conditions. For example, an increase of lipid content in N-stressed cultures can mainly occur only at the expense of other cellular compounds, such as proteins (Rodolfi et al. 2009). It is well known that N starvation is one of the major chemical stimuli needed to trigger lipid accumulation in oleaginous microalgae (Hu et al. 2008). In *S. acutus* PVUW12 grown under N stress conditions, both lipid and carbohydrate contents showed a significant increase, while protein content significantly decreased. This is considered a common response in microalgae growing in N-deficient media (Griffiths and Harrison 2009). In the present study, a total lipid accumulation level of up to 32.5 % of dry weight was obtained in N-stressed cultures. A lipid content as high as up to 43 % was also reported in *S. obliquus* grown under N deficiency (Mandal and Mallick 2009) and *Scenedesmus* sp. (Griffiths et al. 2012). A different behavior was observed in *Scenedesmus* sp. DM, where the lipid content was practically unaffected by N deprivation (Rodolfi et al. 2009). On the other hand, Ördög et al. (2013) reported a high variation in the increase of total lipid contents among different strains of *Scenedesmus* grown under intermediate N levels. These results emphasize the concept that the intrinsic

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ability to produce large quantities of lipids is species-specific (Hu et al. 2008).

In addition to total lipid accumulation, N stress conditions have been associated to important physiological responses of microalgae as, for example, a modification in the level of photosynthetic pigments (Pruvost et al. 2011; Solovchenko et al. 2011). In *S. acutus* PVUW12 cultured under N stress conditions, a 10-fold decrease (from 1.5 to 0.32 % of the biomass dry weight) in chlorophyll *a* content was observed. Even though, in this study, nutrient depletion kinetics were not measured, when nitrate deprivation was applied at the onset of the culture, the essential role of nitrate in sustaining the growth of *S. acutus* PVUW12 is revealed by growth cessation as well as by the decrease of chlorophyll content. In parallel, it is always possible to observe a relevant lipid accumulation as described above.

Scenedesmus acutus PVUW12, as many other green microalgae, possesses a thick, rigid cell wall that may prevent Nile Red dye penetration into the cell and binding with neutral lipids, thus avoiding the observation of fluorescence (Chen et al. 2009). In this study, Nile Red staining was complemented with a DMSO pretreatment which allowed evaluation of TAG accumulation kinetics. Low RFIs recorded during the 1st day of N stress experiments suggested the absence of TAG accumulation. This may be due to continuing culture growth in spite of the lack of external nitrate at the expense of internal stores, as reported to occur when microalgae face environmental conditions in which nitrogen sources are depleted (Reynolds 2006). After this gap period, around at day 5 of culture, increasing RFIs indicate that neutral lipid accumulation began to increase. Then, it accelerated further from day 10 reaching the maximum at day 15, and then eventually decreasing. The information about TAG kinetics is useful both for evaluating the ability of a microalgal strain to accumulate lipids and to determine the optimal harvesting time. The latter depends on the accumulation time rather than cultivation time, and is an important parameter for a large-scale culture of microalgae for biodiesel production, where wrong decisions may lead to a significant reduction in productivity or to a culture loss (Gitelson et al. 2000).

Under favorable growth conditions, microalgae synthesize fatty acids, mainly polar lipids (e.g., glycolipids and phospholipids), which are the major constituents of intracellular membranes. However, under stress conditions, many microalgae alter their lipid biosynthetic pathways starting to accumulate neutral lipids, mainly in the form of triacylglycerol (Harwood and Jones 1989; López Alonso et al. 2000; Hu et al. 2008). Lipid accumulation in *S. acutus* PVUW12 was noticeable in N-stressed cultures and was mainly due to TAG accumulation, which reached a 77 % of total lipids and a twofold increase with an average value of 25.6 % dw. On the contrary, glycolipid and phospholipid contents showed no significant changes in N stress conditions with respect to control.

The total lipid productivity rose up to 80.99 mg L^{-1} day^{-1} (2.7-fold) after 10 days of cultivation under N stress conditions. In a Thai *S. acutus* strain, a total lipid productivity of 33 mg L^{-1} day^{-1} in BG-11 medium with 1.5 g L^{-1} $NaNO_3$ was reported (Chaichalerm et al. 2012), while several *S. acutus* strains showed lipid productivities ranging between 32.4 and 58.5 mg L^{-1} day^{-1} at day 14 under moderate N stress conditions (Ördög et al. 2013). The higher lipid content observed in *S. acutus* PVUW12 can be explained by the more severe N stress condition used in the present study. Besides, in this species, TAG productivities increased in N stress conditions to 63.74 mg (3.9-fold). With respect to biodiesel production, TAG productivity represents a better index of the potential of a strain as feedstock.

The oil fatty acid composition significantly affects biodiesel features and performances (Knothe 2005). For example, the most important fatty acid properties influencing biodiesel quality are the length of the carbon chain and the number of double bonds (Knothe 2005). In *S. acutus* PVUW12, the length of major fatty acids is intermediate with a maximum of 18 carbons and a maximum degree of chain unsaturation of three double bonds. In addition, FAMEs with longer and more saturated chains have higher melting points than FAMEs with shorter and more unsaturated chains. Biodiesel with a high concentration of SFAs can have an unsuitable viscosity as well as poor cold flow properties (Stansell et al. 2011). In particular, in *S. acutus* PVUW12, SFAs content did not increase under N stress conditions. Moreover, MUFA occurrence was prevailing under both conditions and increased under N stress conditions, being oleic acid the most abundant. Ramos et al. (2009) suggested that a high concentration of this fatty acid satisfy the limits imposed by the European Standard UNE EN 14214 for critical parameters like cetane number, iodine value, and cold filter plugging point (CFPP). Thus, it is noteworthy that *S. acutus* PVUW12 contained 47 % of oleic acid in the TAG fraction. In addition, monounsaturated FAMEs, such as methyl oleate, are considered to be better than polyunsaturated ones, such as methyl linoleate and methyl linolenate for cetane number and iodine value without any adverse effect on biodiesel cold properties (Imahara et al. 2006; Knothe 2005). Regarding PUFAs, the European Standard EN 14214 limits the amount of the methyl ester of linolenic acid for vehicular use to up to 12 % and the quantity of methyl esters with four or more double bonds to a maximum of 1 % (CEN 2008). The oils extracted from *S. acutus* PVUW12 meet these specifications. Likewise, the calculated iodine values (g I_2 $(100\ g)^{-1}$) for oils were 88.18 and 86.34 g I_2 $(100\ g)^{-1}$ under control and N stress conditions, respectively, thus meeting the biodiesel quality specifications (<120 g I_2 $(100\ g)^{-1}$). We attribute this value to the low amount of PUFAs. Similar value (82 g g I_2 $(100\ g)^{-1}$) was reported in *Scenedesmus* sp. grown under N-limited conditions (Griffiths et al. 2012).

In summary, in this work, a rapid technique to evaluate in situ TAG accumulation kinetics in *S. acutus* was set up in

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order to estimate the actual production rate of oil useful for transesterification. Both total lipid and TAG productivities as well as MUFA content (in particular, oleic acid) were shown to be significantly increased under N stress conditions, indicating that *S. acutus* PVUW12 is able to produce a relevant amount of good quality oil. Taking into account the results obtained by Doria et al. (2012) concerning its use for wastewater bioremediation, *S. acutus* PVUW12 appears to be a promising strain to be used for combining wastewater treatment and biodiesel feedstock production. In fact, the use of a combined strategy may be crucial for cost-effective biofuel production characterized by a reduced environmental impact.

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