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BIOCHEMICAL RESPONSES OF *Chlorella vulgaris* AND *Scenedesmus* SP. UNDER NUTRIENT LIMITATION

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ABSTRACT

Increasing microalgae carbohydrate content through nutrient limitation has been regarded as a potential strategy to optimize microalgae bioethanol production. This study assessed three different media in which nitrogen, phosphorus and sulfur concentration was decreased by 10-folds with regard to the control media. The performance of two robust microalgae, namely *Chlorella vulgaris* and *Scenedesmus* sp., were evaluated in terms of growth and biochemical composition. At the stationary growth phase, sulfur and phosphorus limited media supported the highest carbohydrate accumulation on *Chlorella vulgaris* and *Scenedesmus* sp., respectively (approximately 4-fold). Under these scenarios, *Scenedesmus* biomass did not exhibit arrested growth while *Chlorella* decreased its concentration by 10%. This decrease was attributed to the decreased photosynthetic performance that concomitantly affected nutrients uptake. After 15 days cultivation, the biochemical analysis revealed that out of the total carbohydrates obtained under those limited media (30% DW for *Chlorella* and 22% DW for *Scenedesmus*), only 5% was starch and therefore the main fraction of carbohydrates was contained in the cell wall. Likewise, the carbohydrate profile presented fast changes in short periods of time and therefore the importance of an appropriate harvesting schedule to obtain carbohydrate rich biomass was highlighted.

INTRODUCTION

Algae-based fuels are the third generation of biofuels. Microalgae feedstock seems much better than terrestrial plants due to their high growth rate, their ability to grow in areas unsuitable for agricultural purposes, absence of lignin, and efficient CO_2 capture via photosynthesis according to **Chen *et al*. (2013)**. Additionally, microalgae can be used for wastewater bioremediation *via* transformation of inorganic nutrients into organic biomass, which can be subsequently employed as a feedstock for biofuel production according to **González-Fernández *et al.* (2011) and Passos *et al.* (2013)**. For all these reasons, microalgae are envisaged as a potential (clean, efficient and sustainable) feedstock for biofuel production.

Compared to lipids and proteins, microalgae carbohydrates have the lowest energy content (carbohydrates for 15.7 kJg^{-1}, proteins for 16.7 kJg^{-1}, for lipids 37.6 kJg^{-1}. However, carbohydrates are the main substrates for several biofuels such as bioethanol, biobutanol and biohydrogen. Ethanol seems to be the biofuel most viable in the near future. In this manner, efforts are devoted to enhance microalgae carbohydrate content and understand their metabolic activities changes with regard to different conditions that may take place during their cultivation. Gaining insights in this field will improve the economic feasibility of microalgal-based bioethanol production.

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Two approaches are normally followed to enhance the amount of a target molecule in microalgae cells, namely genetic modification or changes in operational conditions. This later strategy is probably more feasible compared to genetic modifications since microalgae cultivation is relatively easy to control. Photosynthetic carbon partitioning in microalgae may be driven differently by changing operational conditions. In this manner, cellular biochemical composition may shift with regard to their relative amounts of protein, lipids and carbohydrate. A recent review outlined how some operational conditions (irradiance, temperature and nutrients) affects intracellular enzymatic activities and hence carbohydrates synthesis according to **González-Fernandez and Ballesteros *et al.* (2012)**.

Within the nutrients used for microalgae cultivation, nitrogen, phosphorus and sulfur are the most frequently evaluated compounds to drive C allocation towards a target macromolecule. Generally, nitrogen limitation results in arrested proteins production while sulfur limitation causes cell division blockage and hence both strategies lead to carbohydrates accumulation according to **Taiz and Zeiger *et al.* (2010)**. In the case of phosphorus, this limitation causes triose phosphate accumulation within the chloroplast and thus proteins and chlorophyll synthesis is stopped while carbohydrates cell content increases according to **Sigee *et al.* (2007)**. These are general effects that those key-nutrients may play, however the extent of this effect is probably specie dependent. Furthermore, harsh conditions (nutrients limitation) are also linked to arrested microalgae growth. Therefore, the ideal strategy to enhance carbohydrate accumulation should avoid arrested growth and an appropriate balance of biomass productivity and carbohydrate content should be found.

Since microalgae metabolisms is strain specific, a general conclusion on how nutrient limitations affects this type of biomass cannot be withdrawn. In this study, two of the most robust microalgae were subjected to different nutrients limitation. More specifically, *Chlorella vulgaris* and *Scenedesmus* sp. were monitored in terms of carbohydrate production and biomass growth under different nitrogen, phosphorous and sulfur nutrient limitation.

MATERIALS AND METHODS

Different Mineral Media Employed for the Microalgae Cultivation

Mineral medium employed for microalgae cultivation had the following initial composition (mg/L): 560 NH_4Cl, 25 $CaCl_2$. $2H_2O$, 150 $MgSO_4.7H_2O$, 75 K_2HPO_4, 175 KH_2PO_4, 25 NaCl, 50 disodium EDTA, 31 KOH, 4.98 $FeSO_4.7H_2O$, 11.42 H_3BO_3, 17.64 $ZnSO_4.7H_2O$, 2.88 $MnCl_2.4H_2O$, 1.42 MoO_3, 3.14 $CuSO_4.5H_2O$, 0.98 $CoNO_3$. $6H_2O$ and 2.42 g acetate (as a carbon source) in distilled water. This media was used as a control while in the nutrient limited media the control medium was modified as follows: nitrogen limited medium presented NH_4Cl concentration of 56 mg/L; phosphorus limited medium presented a concentration of K_2HPO_4 and KH_2PO_4 at 15 and 35 mg/L, respectively and for the sulfur limited medium, $MgSO_4$. $7H_2O$ was replaced by 102.1 mg/L $MgCl_2$. $6H_2O$ to keep the Mg^{2+} concentration at the same level as in the control mineral medium. In this manner, the concentrations of the key-nutrients investigated in the present study were 10-folds less than the concentrations used in the control medium.

Cultivation Conditions

The microalgae were cultured in 0.5 L flasks with constant illumination (fluorescent lamps) at constant temperature (25ºC). Cultivation broth was agitated with a magnetic stirrer at 300 rpm. Sampling of the cultivation broth was carried out periodically in order to determine microalgae growth rate and carbohydrates profile along the cultivation time. Each conditions tested was run in duplicate.

Table 1. Starch content (mg/g DCW) at the end of cultivation time

	Chlorella vulgaris	*Scenedesmus* sp.
Control	38.3 ± 0.1	31.9 ± 0.1
N deprived	35.4 ± 0.1	36.8 ± 0.2
S deprived	50.0 ± 0.1	38.1 ± 0.3
P deprived	37.4 ± 0.1	46.7 ± 0.5

Analytical Procedures

The chlorophyll A and B were monitored by measuring optical density using at a wavelength of 665 and 649 nm. Total chlorophyll content (mg/L) was calculated using the following equation according to **Lichtenthaler and Wellburn (1983)**:

$$Chl = 6.63 * A665 + 18.08 * A649 \quad (1)$$

The total solids and volatile suspended solids (VSS) were determined in accordance with the Standard Methods according to **Eaton *et al.* (2005)**. Total nitrogen was determined using the Kjeldahl nitrogen method. Protein content was calculated by multiplying the total nitrogen by 5.95 according to **López *et al.* (2010)**. For the total carbohydrate determination, the phenol-sulfuric acid method was employed according to **Dubois *et al.* (1956)**. Intracellular carbohydrates content was measured as starch. Starch content was quantified based on total hydrolysis of starch by 30% perchloric acid and quantification of liberated glucose by colorimetry according to **Brányiková *et al.* (2011)**. Lipid and other compounds (nucleic acids) were determined by subtracting the percentage of protein and carbohydrate from 100%.

The culture broth of the different media was analyzed periodically in order to follow up the nutrients consumption. The supernatants were obtained by centrifuging the culture broth at 10000 rpm. Soluble chemical oxygen demand (COD) was analyzed with Merck kits. A Crison micropH 2002 (Crison Instruments, Barcelona, Spain) was used for pH determination.

RESULTS AND DISCUSSION

In order to gain insights on two robust microalgae behavior (*Chlorella vulgaris* and *Scenedesmus* sp.), growth parameters and biochemical composition were monitored under different nutrients limitation (nitrogen, phosphorus, and sulfur) and compared to the control medium.

pH Profile

Ideal microalgae growth requires optimal light intensity, temperature, and pH. These factors play a significant role in microalgae growth rate and composition. In order to ensure that nutrient limitation was the only limiting factor in the cultures, all flasks were at constant temperature and illumination. The average pH values of *Chlorella* and *Scenedesmus* culture broths are shown in Figure 1. All the flasks started close to neutrality and slightly shifted to alkaline pH (8.5-8.7) along the experimental time. The slight pH increase registered is a normal feature occurring during photosynthetic CO_2 uptake. This pH levels were in the correct range to maintain maximum growth rate. As a matter of fact, both microalgae strains were reported to maintain a maximum growth rate in a wide range of pHs (6.0-9.0). Likewise, carbohydrates accumulation takes places at relatively high pH according to **Khalil *et al.* (2010)**.

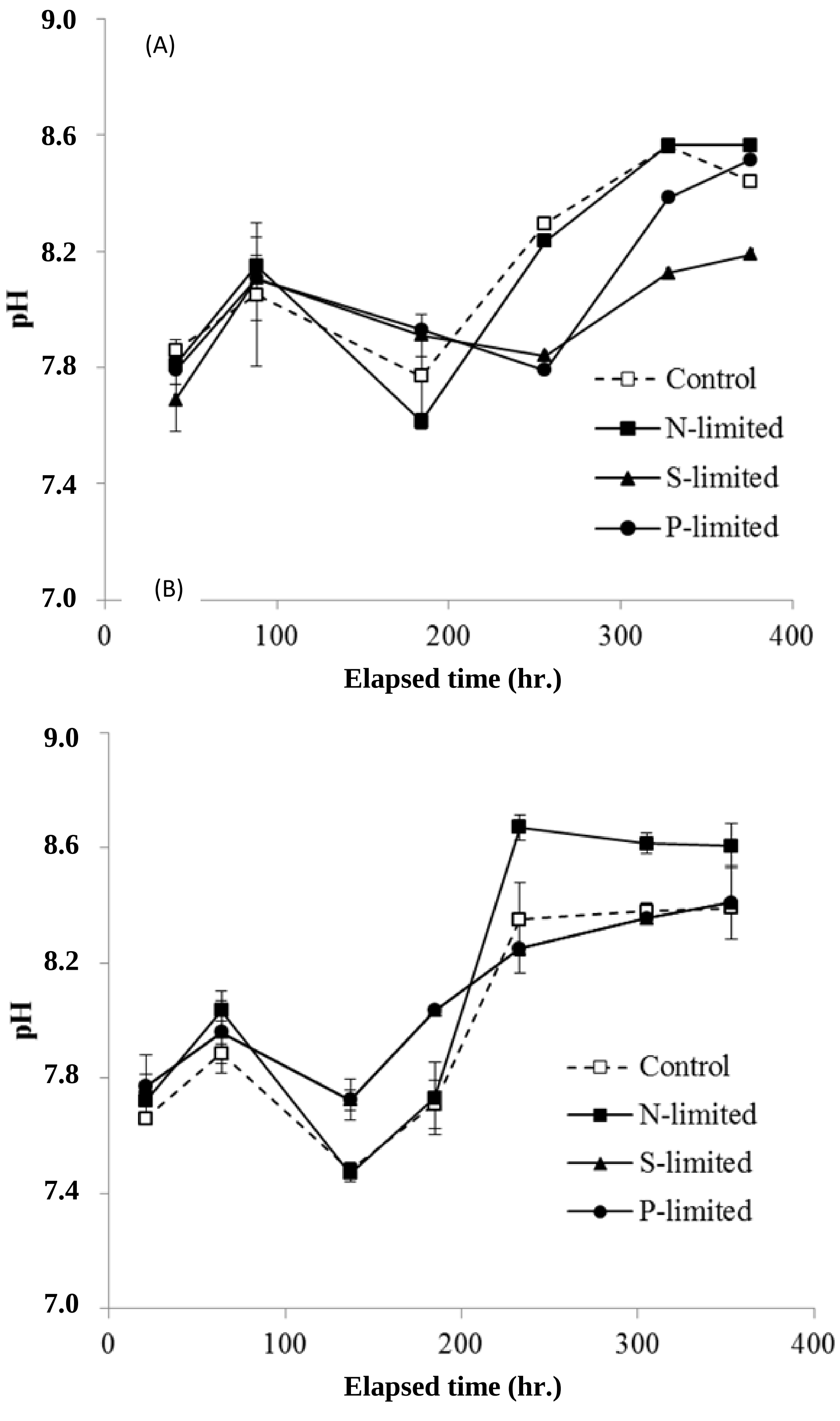


Fig. 1. pH values along cultivation time of (A) *Chlorella vulgaris* and (B) *Scenedesmus* sp. under different nutrient limited media

Effect of Nutrient Limitation on Growth Rate of Microalgae

Volatile suspende solid (VSS) and chlorophyll content were measured along the cultivation time since both parameters are sensitive indicators of arrested or failure growth. Since the experimental work was not conducted in aseptic conditions, the chlorophyll determination was crucial to confirm microalgae photosynthetic performance. On the other hand, VSS refers to the microorganism's growth in the culture medium.

Chlorella vulgaris growth rate

C. vulgaris showed slight differences among the cultivation media tested during the first hours of experiment. Nevertheless after 100 hr. cultivation, chlorophyll content pattern changed drastically (Figure 2A). *C vulgaris* cultivated in control and nitrogen limited media followed the same tendency and reached total chlorophyll steady content after 184 hr. while microbial growth kept increasing until 260 hr. At this point, biomass concentration reached steady state for all the media except the S limited. This later limited media provided the lowest chlorophyll content and thus the lowest VSS concentration (Figs. 2A and 3A). Opposite to this trend, Phosphorus limited media supported the highest cell chlorophyll content. This was an unexpected fact, since p limitation is crucial in algae cell growth and metabolism. Phosphorus is incorporated into organic compounds through phosphorylation. In general, limited Phosphorus diminishes the rate of photophosphorylation and thereby reduces the rate of carbon fixation according to **Rychter and Rao *et al.* (2005)**. It can be thus concluded that phosphorus affects the rate of photosynthesis. Unfortunately, this conclusion does not stand for all microalgae species. When comparing several microalgae species, **Qi *et al.* (2013)** observed that some specie stopped cellular division while under the same scenario of phosphorus limitation some other species-maintained cell division and concomitantly increase chlorophyll content. This later behavior was the case of *C. vulgaris* in the present study.

The highest biomass production values were attained in normal medium culture followed by nitrogen and phosphorus limited media which presented a pretty similar growth (Fig. 3A). After 10 days of *Chlorella* cultivation, the VSS production ranged 1.5 - 1.8 g/L. It seemed that chlorophyll measurements were directly correlated with VSS content. However, there were slightly discrepancies between both parameters in normal and nitrogen limited media cultures. The chlorophyll content was steadily kept at the same level until the end of the experiment while the VSS curve continued to increase. The same trend was observed by **Šoštariè *et al.* (2009)** who attributed the discrepancy to the variability in chlorophyll levels within individual cells.

Scenedesmus sp. growth rate

As it can be seen in Fig. 2B, the initial chlorophyll content of *Scenedesmus* at the beginning of the cultivation was very similar for all the evaluated media. Opposite to *Chlorella vulgaris* in which phosphorus limited enhanced the chlorophyll content along the experimental time, the nitrogen limited media promoted the highest chlorophyll content in *Scenedesmus*. Nevertheless after 240 hr. of cultivation, chlorophyll content decreased along with time. This is typical behavior of microalgae biomass growing in nitrogen deficient media according to **Forján *et al.* (2007)**. The same trend may be observed for *Chlorella*, however this effect was less marked. Likewise, another similitude among *Chlorella* and *Scenedesmus* was that sulfur limited media supported again the lowest chlorophyll content and microbial growth (Figs. 2B and 3B). At approximately 60 hr. of cultivation, s limited media resulted

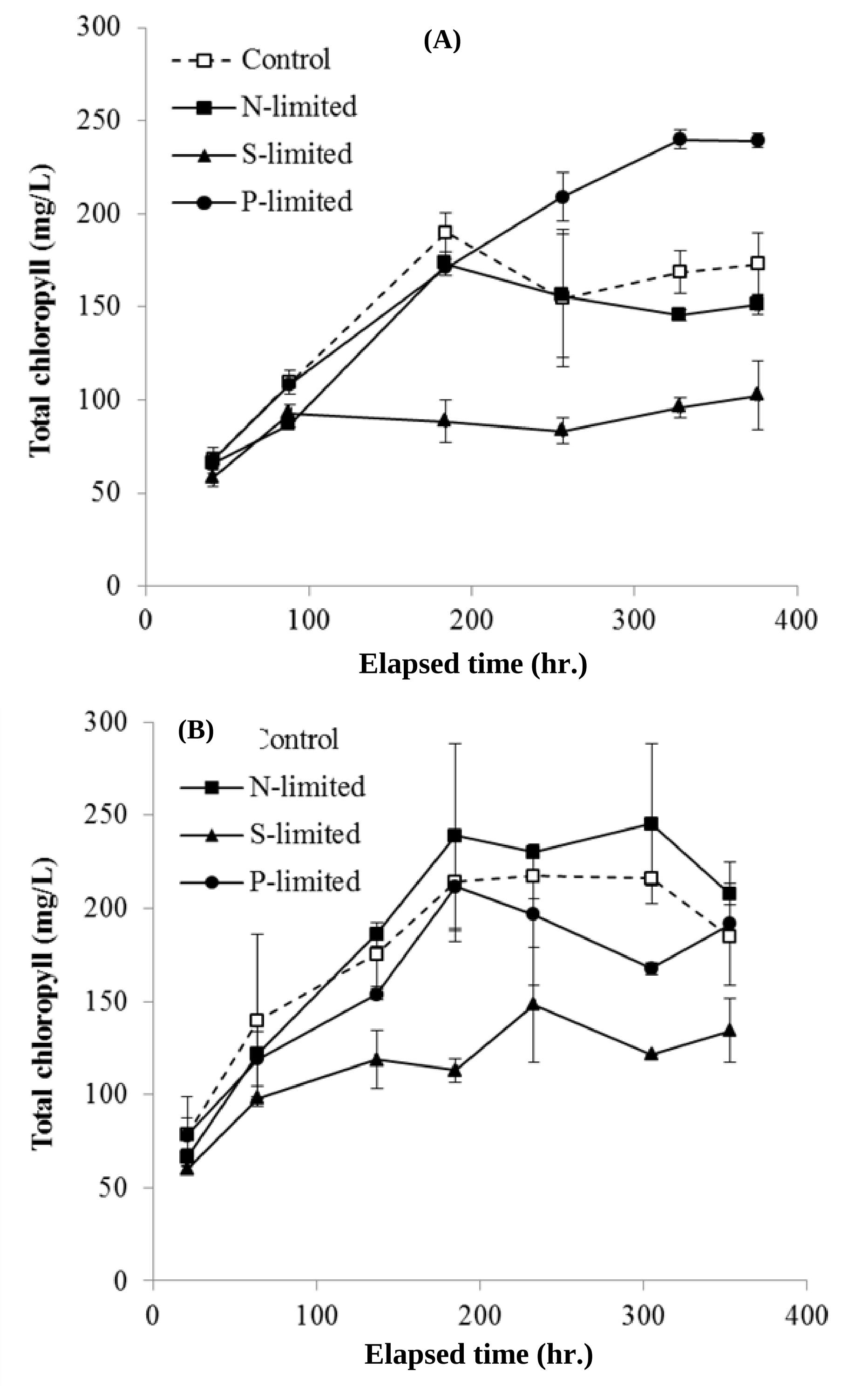
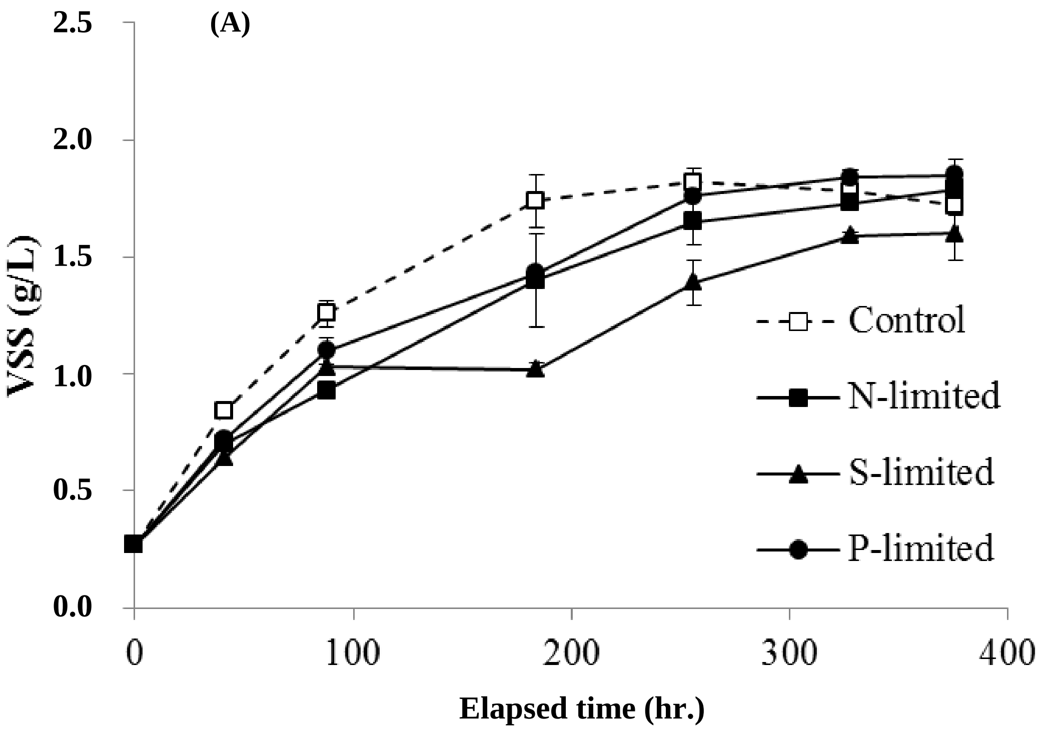


Fig. 2. Time-course profiles of total chlorophyll content during the growth of (A) *Chlorella vulgaris* and (B) *Scenedesmus* sp. under different nutrient limited media



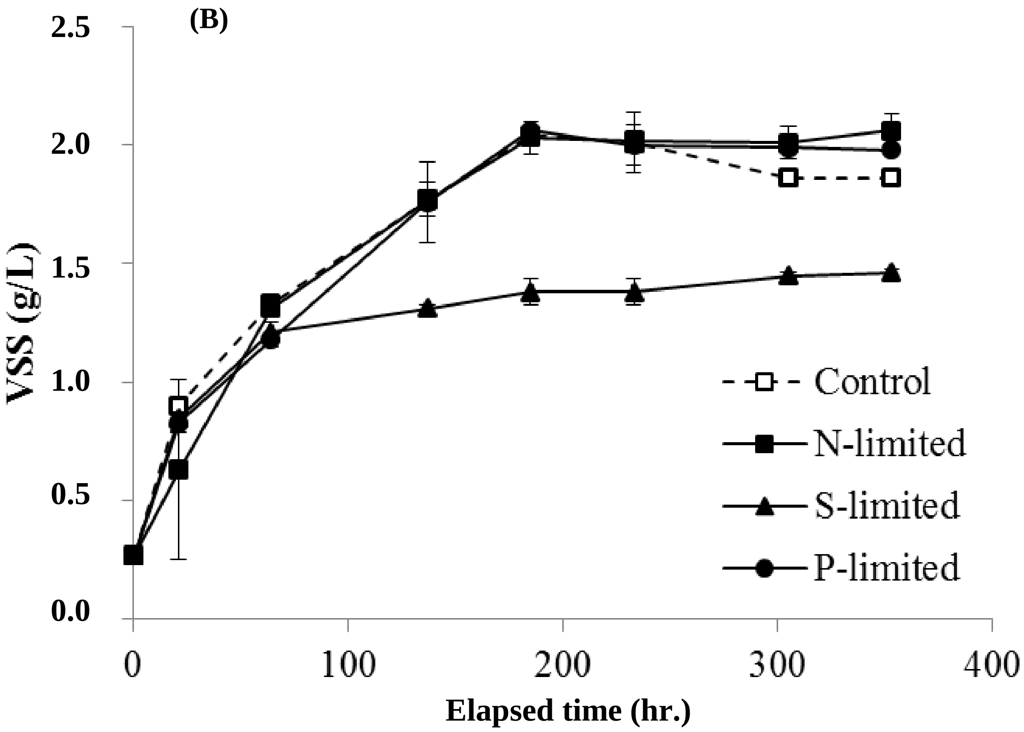


Fig. 3. Microalgae growth (A) *Chlorella vulgaris* and (B) *Scenedesmus* sp. on different nutrient limited media

in a biomass production plateau at around 1.3 g/L (biomass production decreased by 35% compared with the other studied media). **Gorbi *et al*. (2007)** suggested growth rate inhibition of *Scenedesmus acutus* at low sulfur concentration (3%) or in absence of sulfate due to the fact that those cells remained in the phase that precedes cell division. These results are consistent with previous observation showing that the rate of electron transport from photosystem II to photosystem I decrease under sulfur limitation and thus, the decreased photosynthetic performance concomitantly affects cell growth according to **Antal *et al.* (2003)**.

Despite the different profiles attained for the chlorophyll content on the other media, microalgae growth was similar for all of them (exception made for the sulfur limited media as explained above). The VSS concentration for *Scenedesmus* biomass reached 2 g/L at 195 hr of cultivation. Under the same cultivation circumstances, *Scenedesmus* biomass productivity was higher than *Chlorella* (Figure 3).

Nutrients Removal

Media chmemical oxygen demand (CODS) was analyzed along with cultivation time since this parameter can be used as a key indicator of nutrients consumption. COD removal ranged 75-84% at the end of cultivation experiment in the case of *Chlorella* (Fig. 4A). For *Scenedesmus* biomass, COD removal (nutrients uptake) was directly linked with VSS concentration. In this manner control, N and P limited media provided the highest COD removal (88%) while S media decreased significantly the COD removed. More specifically, under this limitation COD removal was 62%, which clearly affected biomass production (Fig. 3B). Likewise, it seemed that sulfur limitation had a strong effect on *Scenedesmus* cell division while this effect was affecting *Chlorella* biomass in a minor extent. When comparing both microalgae species, *Scenedesmus* sp. biomass supported higher COD removal than *Chlorella* after approximately 15 days of cultivation (Fig. 4). This fact probably led to the enhanced biomass productivity registered in *Scenedesmus* biomass (Fig. 3).

Effect of Nutrient Limitation on Carbohydrate Accumulation in *Chlorella vulgaris* and *Scenedesmus* sp.

It has been repeatedly shown that nutritional conditions and other operational factors are directly linked with microalgae composition. The possibility of increasing carbohydrate content by limitation of major components in the cultivation medium was investigated as a viable and environmental friendly option to control microalgae composition. Carbohydrate content of both microalgae species were low at the initial stage of the cultivation (*Chlorella vulgaris* contained 8% DW and *Scenedesmus* sp contained 6% DW). Similar low carbohydrate content (6-9%) was reported previously and it was attributed to unsuitable conditions that forced microalgae to change their storage compounds according to **Biller and Ross (2011).**

Effect of Nutrient Limitation on Carbohydrate Accumulation in *Chlorella vulgaris*

After culturing *Chlorella vulgaris* for 48 hr, carbohydrate content dramatically increased in nitrogen limited media (345.4 mg/g DW, Fig. 5A). As a matter of fact, nitogen limited media enhanced carbohydrate content by 5-folds while the other cultivation media only exhibited 2.5-fold increase. After this carbohydrate peak in the nitrogen limited media, the carbohydrate content decreased continuously until an average value of 235 mg/g DW. Similar values were attained for phosphorus limited and control media (236 and 220 mg/g DW, respectively). In addition to nitrogen, the

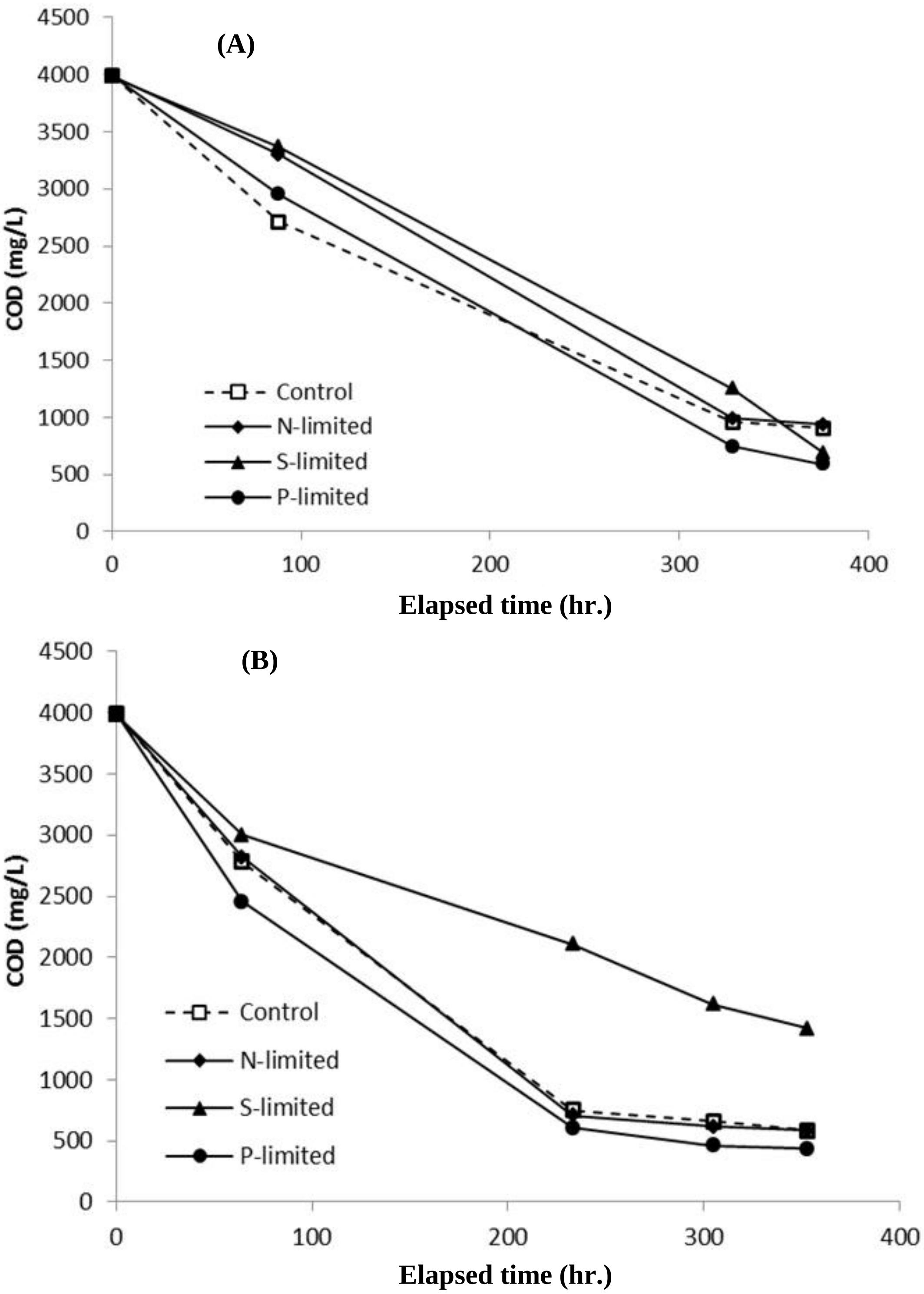


Fig. 4. Nutrients removal from the culture of (A) *Chlorella vulgaris* and (B) *Scenedesmus* sp. on different nutrient limited media

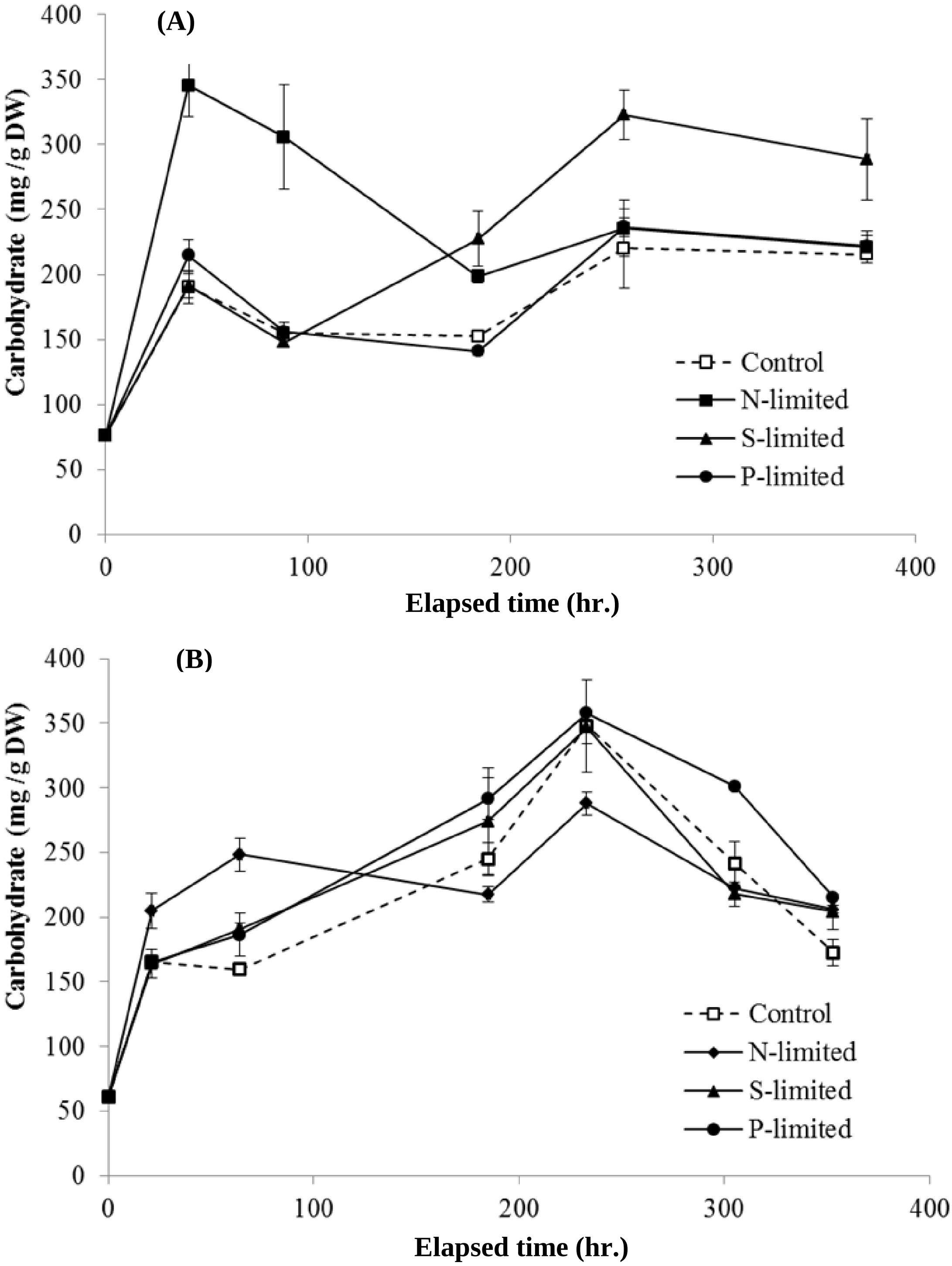


Fig. 5. Time-course profiles of carbohydrate content during the growth of (A) *Chlorella vulgaris* and (B) *Scenedesmus* sp. under different nutrient limited media

sulfur limited media also provided carbohydrate increase. However, this increase (322.8 mg/g DW) was reached after approximately 10 days of cultivation. Carbohydrates enhancement was in the same values under nitrogen and sulfur limited media, however the cultivation time elapsed at which the same values were attained presented 10 days delay. The observed enhancements were in good agreement with data regarding carbohydrate accumulation under nitrogen starvation of different *Chlorella* strains. **Ho *et al.* (2013)** reported enhancements ranging 1.4 to 4.4-fold depending on the microalgae strain.

Several studies have demonstrated that nutrients limitation among other operational factors can affect not only microalgae metabolisms but also growth rate according to **Dragone *et al.* (2011)**. As a matter of fact, the most common effect is the enhancement of a target molecule by compromising microalgae growth rate. The delayed or arrested growth will depend on the harsh conditions applied. It is worth to note that the highest carbohydrate content achieved in the N limited media was reached at very diluted biomass concentration. Microalgae harvesting is one of the most critical issues regarding microalgae biobased technology according to **Collet *et al.* (2011)**. In order to decrease costs, cells concentration technologies should be applied at high cell density. On the other hand, the S limited media that also enhanced carbohydrate content presented this peak together with high biomass concentration. In this manner, S limited media was suggested to be the most appropriate media to increase carbohydrate content in *Chlorella vulgaris* biomass.

Carbohydrates may be found either accumulated in the chloroplast (starch) or forming part of the cell wall (cellulose). In order to identify in which form those carbohydrates were, starch content was measured in the biomass harvested at the end of the experimental time (Table 1). All biomasses exhibited a low content of starch (32 to 50 mg/g DW), which indicated that the majority of the carbohydrate content determined was forming part of the cell wall. Most literature regarding carbohydrate accumulation on *Chlorella vulgaris* concluded that sulfur limitation is the most suitable nutrient limitation to enhance starch content. For instance, **Branyikova *et al.* (2011)** studied the effect of nitrogen, phosphorus and sulfur limitation on *Chlorella* biomass and they reached starch content of 60% DW after 15 hr cultivation on Sulfur limited media. This study also showed that control media achieved 45% starch at 15 hr with a culture broth that presented 1.4 g/L while the S limited exhibited 0.9 g/L. In this manner, the volumetric starch content (g starch/L) is less in the sulfur limited media than in the control and thus nutrient restriction to obtain a target macromolecule may not be justify.

Furthermore, starch content is not prolonged and within few hours its concentration may decrease dramatically. According to **Mizuno *et al.* (2013)** S-deficient medium promotes the decrease in starch granules followed by an increase of lipids drops. Overall, it seems that while structural carbohydrates (cell wall) are more stable in time, the storage carbohydrate (starch) may change within hours.

Effect of Nutrient Limitation on Carbohydrate Accumulation in *Scenedesmus* sp.

During the first hours of *Scenedesmus* cultivation, all limited media exhibited higher carbohydrate content than control media (Fig. 5.B). As observed previously for *Chlorella*, nitrogen limited media displayed the fastest response with regard to carbohydrates. Nevertheless, all media continuously increased biomass carbohydrate content along cultivation time until approximately day 10. At this point, carbohydrate content of biomasses corresponding to control and P and S limited media surpassed carbohydrate content of biomass grown in N limited media. Indeed, this later media supported quite stable carbohydrate content along the cultivation (231.1±3.2% DW). After 10 days of cultivation, all biomasses decreased carbohydrate content. Once again, the harvesting time to obtain biomass rich in carbohydrates was shown to be crucial. Opposite to *Chlorella*, slightly higher carbohydrate content was registered in *Scenedesmus* biomass grown in P limited media. The decrease on carbohydrates observed after 10 days was delayed in P limited media. Despite the fact that different nutrient limitation was identified for *Chlorella* and *Scenedesmus* to increase its carbohydrate content, the enhancement achieved was similar for both species (3.8 to 4.2-folds, Figure 5B). Additionally, the P limited media providing the highest carbohydrate content did not compromise biomass productivity (Figure 3B). Literature concerning nutrients limitation on *Scenedesmus* carbohydrates is scarce. The majority of the studies focused on lipids accumulation through nitrogen limited media according to **Mandal and Mallick (2009)**. With regard to carbohydrates accumulation, nitrogen starvation displayed 1.35-fold enhancement on *Scenedesmus* sp. after 3 days cultivation, while lipids accumulation prevailed after 5 days according to **Ho *et al.* (2012)**. As described for *Chlorella*, it seems likely that carbohydrate accumulation is limited to a short period of time after which lipids accumulation prevail.

Taking a closer look to the carbohydrate composition in the biomass obtained at the end of the experimental time, starch content was similar to the observed values for *Chlorella* (Table 1). *Scenedesmus* starch content ranged 32-38 mg/ g DW for all the studied media, except for the P limited. Biomass grown in this later media provided the highest starch content (46.7±0.5 mg/ g DW) together with the highest carbohydrate values (215.1±1.2 mg/ g DW). These results indicated that the majority of the carbohydrates were forming part of the cell wall (170 mg/g DW).

These findings led to the conclusion that nutrient starvation was species dependent. *Chlorella* showed an enhanced carbohydrate content during the early stage when grown in N limited media while S limited was most suitable during the stationary growth phase. On the other hand, *Scenedesmus* exhibited highest carbohydrate content when grown in P limited media. Noteworthy to mention that the harvesting time was identified as one of the most crucial points when biomass rich in carbohydrates was targeted.

Effect of Nutrient Limitation on Final Microalgae Biomass Composition

The biochemical characterization of the biomass obtained at the end of the experimental time was compared with the macromolecular content of the biomass used as inoculum. As it is shown in Fig. 6A, *Chlorella* increased the carbohydrates content by 2.9-3.8-folds by decreasing mainly the allocation of nutrients as proteins. Together with the highest increase on carbohydrates, the S limited media supported the highest decrease on protein content (24%). In the case of *Scenedesmus*, the carbohydrate enhancement in the cells ranged 2.8-3.6-folds at the end of the experimental time (353 hr, Fig. 6B). The highest carbohydrate achieved was again mediated by a decrease of proteins content. With regard to the proteins determined in the inoculum, all the limited media decreased the protein content by 20%.

Regarding the lipids fraction, no further discussion was attempted since this fraction included other molecules than lipids and it was not analytically measured. In this manner, the different allocation of nutrients

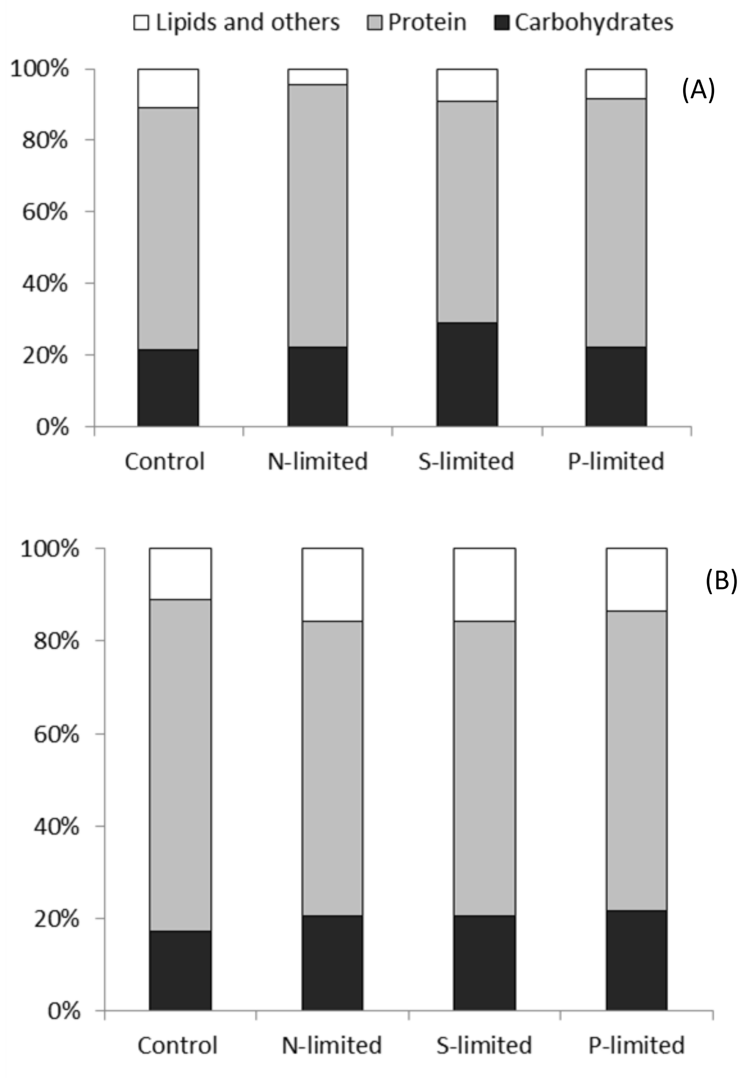


Fig. 6. The biochemical composition of (A) *Chlorella vulgaris* and (B) *Scenedesmus* sp. under different nutrient limited media at the end of the experimental time

into proteins and carbohydrates was only discussed. Furthermore, both fractions represented 85-90% of the microalgae cells and thus the contribution of lipids and other molecules in this balance was rather low.

Under stressful conditions, microalgae remobilize carbon to produce energy storage products (lipids and starch) in order to acclimate to the changed nutrient availability. The macromolecule accumulation depends not only in nutrients limitation but on many other factors such as irradiance, temperature or even microalgae growth stages. For example, in *Chlorella*, carbon allocation to proteins occurred during the lag phase of a batch culture while during stationary growth; carbon is mainly allocated as carbohydrates according to **Lu *et al.* (2010)**. Under nutrient limitation, the reported microalgae responses are quite broad and specie- and strain-specific. Many microalgal strains could transform proteins or peptides to lipids or carbohydrates under nutrients limitation. according to **Huo *et al.***

(2011) Likewise, the storage compounds (starch or lipid) exhibit a quite similar metabolic pathway and carbon allocation seems to involve more subtle relationships than a mere competition according to **Siaut *et al.* (2011)**. Overall, changes in enzymatic activities and metabolic fluxes of carbohydrates biosynthesis deserve further investigation.

Conclusion

The results indicated that nutrient limitation to enhance carbohydrate content was specie dependent. Nitrogen and sulfur limited media were the cultivation media supporting the highest carbohydrate accumulation on *Chlorella vulgaris* (more than 4-folds) while in the case of *Scenedesmus* sp., phosphorous limited media enhanced the biomass carbohydrate content. This study also showed that carbohydrates were accumulated at the expense of proteins. Carbohydrate content changed drastically within few days and thus an appropriate harvesting schedule was also highlighted as crucial step to collect a carbohydrates-rich biomass. Overall, changes in enzymatic activities and metabolic fluxes of carbohydrates biosynthesis deserve further investigation.

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الملخص العربي

الاســتجــابــات البيــوكيميــائيــة لطحلـب الكلــوريــلا وطحلـب السينديسـموس تحـت تأثير المغذيات

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تعتبر زيادة محتوى الكربوهيدرات من الطحالب الدقيقة من خلال الحد من المغذيات بمثابة استراتيجية محتملة لتحسين إنتاج الإيثانول الحيوي من الطحالب الدقيقة. قيمت هذه الدراسة ثلاث وسائط مختلفة انخفض فيها تركيز النيتروجين والفوسفور والكبريت بمقدار 10 أضعاف فيما يتعلق بوسائط التحكم. تم تقييم أداء اثنين من الطحالب الدقيقة القوية، *Chlorella vulgaris* وsp *Scenedesmus* من حيث النمو والتركيب الكيميائي الحيوي وهما في مرحلة طور الثبات، دعمت الوسائط المحدودة للكبريت والفوسفور أعلى تراكم للكربوهيدرات على التوالي *Chlorella vulgaris* وsp *Scenedesmus* (حوالي 4 أضعاف) في ظل هذه السيناريوهات، لم تظهر الكتلة الحيوية *Scenedesmus* نموًا متوقفًا بينما قللت *Chlorella* تركيزها بنسبة 10%. يعزى هذا الانخفاض إلى انخفاض أداء التمثيل الضوئي الذي أثر بشكل متزامن على امتصاص العناصر الغذائية. بعد 15 يومًا من الزراعة، أظهر التحليل البيوكيميائي أنه من إجمالي الكربوهيدرات التي تم الحصول عليها في ظل تلك الوسائط المحدودة (30٪) DW لكلوريلا و22% DW لـ *Scenedesmus*، كان 5% فقط عبارة عن نشا، وبالتالي تم احتواء الجزء الرئيسي من الكربوهيدرات في جدار الخلية. وبالمثل، قدم ملف الكربوهيدرات تغييرات سريعة في فترات زمنية قصيرة، وبالتالي تم تسليط الضوء على أهمية جدول الحصاد المناسب للحصول على الكتلة الحيوية الغنية بالكربوهيدرات.

الكلمات الاسترشادية: الكلوريلا ، السينديسموس ، المادة العضوية ، الوزن الجاف.

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