

**BIO-PHYSIOLOGICAL RESPONSE OF BIOFILTER ALGAL
CANDIDATE *ULVA SP.* TO DIFFERENT NITROGEN
FORMS AND FLUXES**

**Yasser T.A. Moustafa¹; G. Bougaran²;
M. Callier³ and J.P. Blancheton^{3,4}**

¹ *Central Laboratory for Aquaculture Research, Abbassa, Abou-Hammd, Sharkia, Egypt, (ymoustafaonline@yahoo.com), Mobile: 0020 1140 888918.*

² *IFREMER, Station de Nantes, Rue de l'Île d'Yeu –B.P.21105, 44311 Nantes Cedex 3, France.*

³ *IFREMER, Station de Palavas, Chemin de Maguelone, 34250 Palavas Les Flots, France.*

⁴ *UMR ECOSYM, USTL, Place Eugène Bataillon, Montpellier, France.*

Received 8/ 10/ 2013

Accepted 24/ 11/ 2013

Abstract

The present study was carried out to study the long term effect of different nitrogen forms and concentrations (14.3, 28.6 and 57.1 μM N-NH₄ and 214, 2360, and 3700 μM N-NO₃) on the photosynthesis and relative growth rate (RGR) of *Ulva sp.* for five weeks. Nutrient-enriched seawater was supplied at an exchange rate of between 12.6 and 14.4 volumes per day. RGR was determined weekly. Photosynthetic oxygen evolution response was measured using 20 - 25 mg fresh weight seaweed incubated in a DW3 measuring chamber for 56 minutes under different irradiances with light and dark periods alternating every seven minutes. Photosynthetic light-response curves were drawn using 15 photosynthetic oxygen evolution readings normalized to dry weight. Experimental data were fitted with a Haldane model to calculate photosynthetic rate (P_{max}), saturation irradiance (I_s) and compensation irradiance (I_c). At the experiment termination, the RGR following nitrate addition were higher, but not significantly, than those with the ammonium supplied. The highest RGR was determined at the medium nitrate concentration. The photosynthetic activity of *Ulva sp.* showed a positive relationship with nitrogen concentrations from

both nitrogen forms. The highest significant P_{max} was found at the highest nitrate concentration. The lowest ammonium concentration corresponded to the lowest significant P_{max} value. No significant differences were found for I_s and I_c irrespective of treatment although, in general, ammonium treatments yielded higher I_s values than nitrate treatments. The discrepancy between the growth rate results and photosynthetic oxygen evolution is discussed in light of the reproduction activity and temperature effect.

Keywords: *Ulva sp.*, photosynthetic oxygen evolution, relative growth rate, nitrogen forms, nutrients uptake.

INTRODUCTION

As the aquaculture industry is developing worldwide, a huge amount of effluent is released into the environment. To limit the release of farm effluent into the environment and promote the sustainable development of aquaculture industry, waste water treatment needs to be optimized. Macroalgae have been successfully integrated into various-scale mariculture systems including Flow Through System (FTS), semi-recirculating and recirculating systems (Pagand *et al.*, 2000 and Schuenhoff *et al.*, 2003). The effluents of different aquaculture production systems, such as FTS and Recirculating Aquaculture System (RAS), contain various forms and concentrations of nitrogen, depending on how the systems are managed. The effluent of FTS is characterized by high flow rates and low ammonia and phosphorus concentrations whereas RAS effluent is characterized by low flow rates and high nitrate and phosphorus concentrations which may reach concentrations 10 to 100 fold those of conventional systems (Neori 1996; Pagand *et al.*, 2000 and Blancheton *et al.*, 2007). To optimize integrated *Ulva* - fish culture systems, growth rates and photosynthetic activity need to be evaluated as a function of nitrogen forms and concentrations.

Algal uptake of nitrogen depends on numerous factors including physical factors (light, temperature), environmental nutrient concentration, intracellular nutrient concentrations, and biological factors

(metabolism, age, etc.). Studies on nitrogen form preferences are conflicting. Several studies on nitrogen enrichment of *Ulva sp.* show that ammonium (NH_4^+) is physiologically preferred over nitrate (NO_3^-) given that less energy is required for its assimilation into the algal biomass (Thomas and Harrison 1985 and Fujita *et al.*, 1989). Macroalgae also show higher growth rates with NH_4^+ than with NO_3^- (DeBoer *et al.*, 1978 and Lapointe & Ryther 1979). Ale *et al.* (2011) reported ammonium is favourably assimilated by *Ulva lactuca*; giving rise to a higher RGR and biomass yield and the uptake rate of ammonium is higher than that of nitrate. However, at the end of a 10-day culture period, the biomass yield of *U. lactuca* with the nitrate treatment was higher than that with the ammonium treatment. Moreover, Luo *et al.* (2012) showed that after 180 minutes of incubation of *U. prolifera* and *U. linza* in media enriched with either nitrate or ammonium, the V_{max} (maximum uptake rate) for nitrate was higher than that for ammonium in both species. The V_{max}/K_s (K_s is the half –saturation constant for nutrient uptake) ratio was very similar for the two nitrogen forms in both *Ulva* species, which may indicate that over a longer period, both species may exhibit higher affinity for nitrate than ammonium. The presence of NH_4^+ has been shown to reduce the uptake of NO_3^- in macroalgae (Thomas and Harrison, 1987) at concentrations $>2\mu\text{M}$ NH_4^+ (D'Elia and DeBoer, 1978 and Neori, 1996). However, Ale *et al.* (2011) showed that, in the presence of NH_4NO_3 , *U. lactuca* showed a relatively higher uptake rate of NO_3^- as compared to when exposed to NaNO_3 alone.

Nitrate is usually more abundant than ammonium in coastal and estuarine water and it makes up more than 80% of dissolved inorganic nitrogen in effluents of recirculating systems containing nitrifying filters (Neori, 1996 and Pagand *et al.*, 2000). Furthermore, macroalgal proliferation has been observed in estuaries that receive nitrate-rich wastewater (Cole *et al.*, 2005). Nitrogen accumulation studies showed that nitrate is less toxic at high concentrations than ammonium

(Andersson, 1942) and that it is taken up at similar rates to ammonium (Pagand *et al.*, 2000, and more references therein). Similar growth rates with either $\text{NH}_4\text{-N}$ or $\text{NO}_3\text{-N}$ have been reported for different seaweed species (Navarro-Angulo and Robledo, 1999 and Carmona *et al.*, 2006). Additionally, the growth of *Ulva fasciata* has been found to increase with increasing NO_3^- concentrations under high lighting conditions (Lapointe and Tenore, 1981).

Most studies conducted to date have focused on the uptake rate of N because N availability often limits the growth of macroalgae (Hanisak, 1983). Moreover, these studies were conducted in the form of short-term (from hours to days) batch experiments and investigated low concentration ranges 5 - 200 μM (Fujita *et al.*, 1989; Naldi and Wheeler, 1999 & 2002; Ale *et al.*, 2011 and Luo *et al.*, 2012), which is different from the conditions of seaweeds in biofilters, fed high concentration of nutrients in a continuous flow for long periods.

Therefore, the purpose of this study was to monitor the growth and photosynthetic activity of *Ulva* sp. in the long term with different nitrogen forms and concentrations, to gain a better understanding of the effect of nitrogen form and concentration on the filtration performance of *Ulva* during the fish production cycle. This information will be useful for designing algal ponds for the purification of aquaculture waste water and will help us predict the filtration performance of *Ulva* throughout the fish production cycle.

MATERIALS AND METHODS

Plant collection and acclimation:

Vegetative fragments of *Ulva* sp. were collected from a natural pond close to the IFREMER Palavas station during May and June 2009. They were rinsed with filtered sea water and gently cleaned to remove epibiota and sediments, then cut into small 6 cm^2 pieces, to keep them

suspended and moving within the experimental bottles. Before the experiments, the plants were maintained in the laboratory for a preconditioning period of 2 weeks at 25 ± 1 °C subjected to a 12:12 hours L : D cycle at $273 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$; the same temperature and light conditions were maintained during all subsequent experiments. Irradiance was provided by four cool white fluorescent tubes (OSRAM Trade Brand 150 cm, 58 W, Germany). Light in the photosynthetically active range (PAR) was measured with a spherical sensor connected to a LiCor-1400 Data Logger radiometer (Li-Cor Inc, Lincoln, Nebraska, USA). Further details of selected temperature and light intensity levels are given in the discussion. During the preconditioning period, the plants were stocked at a density of 5 g (FW) L⁻¹. Filtered seawater was enriched with 1 mM of either NO₃-N (KNO₃) or NH₄-N (NH₄Cl) and PO₃-P (KH₂PO₄) at a N:P ratio of 20 (w:w) and renewed every 2-3 days. Aeration was provided continuously.

Growth experiment:

For five weeks, twelve 1-liter flasks received a continuous flow of seawater enriched with ammonium or nitrate at concentrations of 14.3, 28.6, 57.1 μM NH₄Cl (T1; T2 and T3) or 0.214, 2.36, 3.7 mM KNO₃ (T4; T5 and T6), respectively (two flasks for each nitrogen concentration) from storage tanks with a 40 L capacity. Phosphate (KH₂PO₄) was added to all treatments at a N:P ratio of 20 (w:w). Nutrient-enriched seawater was supplied at flow rates ranging between 12.6-14.4 volumes per day. The flow rates were adjusted manually every day. These nutrient concentrations were selected to cover the concentration range of nutrients reported in the literature for two fish production systems FTS and RAS. At the beginning of the experiment, the flasks were stocked algae at a density of 5 g Fresh weight (FW)/ flask (Fig 1).



Fig 1. The experimental system.

Growth rate determination:

The fresh weight of the plants in each flask was determined weekly and the initial density was readjusted. Relative growth rates (RGR % d⁻¹) were estimated using the following formula: $RGR = \ln(W_t/W_0) \times \Delta t^{-1} \times 100$, where W_0 is the initial biomass and W_t is the biomass on day t and Δt the time period (Evans 1972).

Photosynthetic oxygen evolution experiment:

Six flasks were used for the determination of photosynthetic oxygen evolution in response to the different nitrogen forms and concentrations. The algae were kept under similar culture conditions throughout the growth experiment, which used the same experimental system. The photosynthetic oxygen evolution rate was measured daily for 5 weeks. Measurements with each nitrogen concentration were repeated 25 times.

Measurement of photosynthetic oxygen evolution:

Samples (20 – 25 mg fresh weight or FW) were taken from the algae at each nitrogen concentration using a sharp razor blade. Photosynthetic O₂ evolution was measured with a Clark-type oxygen electrode in a DW3 measuring chamber (Hansatech Instruments, Norfolk,

UK) at 25 °C and at different irradiances (from 300 to 1000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). The algae samples were incubated in the measuring chamber containing 13 ml of filtrated natural seawater. NaHCO_3 stock solution (200 mM) was used at a rate of 0.8 ml per 13 ml seawater to provide non-limiting dissolved inorganic carbon during the incubation period. Each measuring trial consisted of a 56 minute sequence during which the algae were exposed to 7 minutes of alternating light and darkness. The photosynthesis measurements were conducted between 10 am and 6 pm.

Photosynthesis measurements were started after the first hour of illumination to avoid induction phenomena (Henley *et al.*, 1991). The treatments were alternately sampled through the experimental period. The small pieces of *Ulva* were incubated in filtered seawater, at 273 $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ and 25 °C for at least 1 hour to minimize the effect of any potential cutting damage of cells (wound respiration) on photosynthetic measurements (Zou, 2005).

Photosynthetic oxygen evolution rates were normalized to dry weight (DW). Data obtained from measurement of the photosynthetic rates used to draw net photosynthesis versus irradiance (*P-I*) curves were the means of 15 out of 25 readings. To evaluate photosynthetic activity (*P*) taking photoinhibition into account, experimental data were fitted with a Haldane model (equation 1), as modified by Papacek *et al.* (2010)

$$P = P_{\max} \times \frac{I}{K_s + I + I^2/K_i} - R_d \quad \text{equation 1}$$

Where P_{\max} is the maximal photosynthetic capacity ($\mu\text{mol O}_2 \text{ gDW}^{-1} \text{ h}^{-1}$), K_i is the inhibition constant ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$), K_s is the half-saturation constant ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and R_d is the dark respiration expressed as oxygen consumption ($\mu\text{mol O}_2 \text{ gDW}^{-1} \text{ h}^{-1}$).

Saturation irradiance (I_s) and compensation irradiance (I_c) were calculated according to equations 2 and 3.

$$I_c = \frac{P_{\max} - R_d + \sqrt{(R_d - P_{\max})^2 - (2R_d \sqrt{K_s K_i})^2}}{2R_d / K_i} \quad \text{equation 2}$$

$$I_s = \sqrt{K_s K_i} \quad \text{equation 3}$$

Statistical analysis:

The Haldane model was fitted to data using Matlab (version 6.00) software. The data were analyzed statistically using one way ANOVA (Microsoft office Excel 2007) and expressed as the means \pm SE. Differences among treatments were tested for significance with the *t-test* with the significance level set at 0.05.

RESULTS

Growth rate:

The relative growth rate (RGR) of the algae is shown in Fig (2). After one week, the RGR with the lowest ammonium concentration (T₁) was the highest and differed significantly ($P < 0.05$) from the RGR of the algae receiving nitrate treatment (T₄, 5 and 6), but not significantly from those with the other concentrations of ammonium (T₂ and 3). After two weeks, it became the significantly lowest RGR ($P < 0.05$).

At the end of the experiment, the RGR with the three nitrate treatments were higher, but not significantly, than those with ammonium treatments. The highest RGR was observed with the medium nitrate concentration (T₅, fig 2). A positive trend over time was observed for RGR with the nitrate treatments, while no particular trend was noticed for RGR in the ammonium treatments. Generally, algae receiving nitrate, particularly medium and high concentrations, showed a biweekly reproduction rhythm.

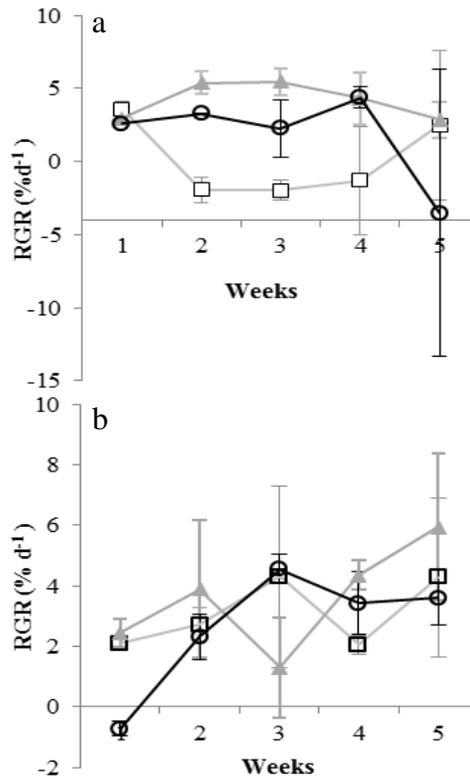


Fig. 2. Mean relative growth rate (% per day) of *Ulva sp.* with (a) different ammonium concentrations, squares (T₁), triangles (T₂), and circles (T₃), corresponding to 14.3, 28.6, 57.1 μM NH_4Cl , respectively and (b) nitrate concentrations, squares (T₄), triangles (T₅), and circles (T₆), corresponding to 0.214, 2.36, 3.7 mM KNO_3 , respectively. Error bars represent SE values (n= 2).

Photosynthetic activity:

The photosynthetic activity of *Ulva* showed a positive relationship with nitrogen concentrations for both nitrogen forms (Figure 3 & Table 1). All nitrate treatments yielded higher P_{max} values than the ammonium treatments. The highest photosynthetic rate (P_{max}) was recorded in response to the highest nitrate addition, followed by that at the medium nitrate concentration. These two latter values were

significantly ($P < 0.05$) higher than any other N treatments. The lowest ammonium concentration corresponded to the lowest P_{max} values. All the nitrate concentrations resulted in higher photosynthesis rates for a given irradiation level (Table 1).

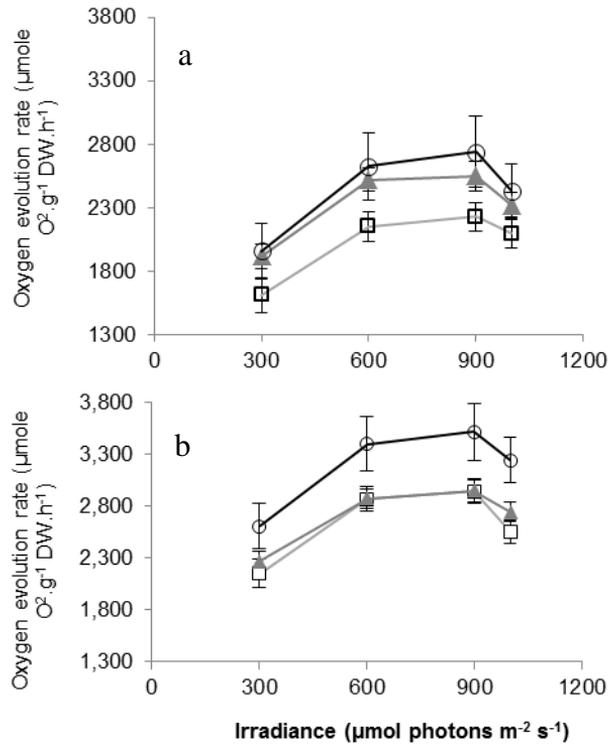


Fig. 3. Photosynthetic oxygen evolution with (a) different ammonium concentrations, (a) different ammonium levels, squares (T_1), triangles (T_2), and circles (T_3), corresponding to 14.3, 28.6, 57.1 $\mu\text{M NH}_4\text{Cl}$, respectively and (b) nitrate concentrations, squares (T_4), triangles (T_5), and circles (T_6), corresponding to 0.214, 2.36, 3.7 mM KNO_3 , respectively. Error bars represent SE values (n= 15).

Table (1). The photosynthetic parameters values (mean \pm SE) under different nitrogen sources and concentrations. Significant differences among groups as identified by pairwise contrasts are indicated by different letters. (n=15).

N source	I_c	I_s	P_{max}
NH ₄ _0.2 mg N/L	48 \pm 6	595 \pm 35	2532 \pm 112.66 ^c
NH ₄ _0.4 mg N/L	45 \pm 5	619 \pm 36	2625 \pm 97.83 ^c
NH ₄ _0.8 mg N/L	51 \pm 6	658 \pm 31	2745 \pm 262.33 ^c
NO ₃ _3 mg N/L	47 \pm 6	615 \pm 47	3013 \pm 250.41 ^c
NO ₃ _33 mg N/L	47 \pm 5	606 \pm 22	3034 \pm 160.18 ^b
NO ₃ _53 mg N/L	57 \pm 3	598 \pm 26	3644 \pm 234.27 ^a

Within each column, different letter indicates significant differences at $P < 0.05$.

In general, the ammonium treatments yielded higher saturation irradiance I_s values than the nitrate treatments. The lowest value of P_{max} ($595 \pm 35 \mu\text{mol photon m}^{-2} \text{s}^{-1}$) yielded at the lowest ammonium concentration (T₁). No significant differences were detected among the treatments, the values ranged between 595 ± 35 to $658 \pm 31 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ (Table 1).

No significant differences were found for predicted compensation point values (I_c) between the different nitrogen forms and concentrations. The lowest predicted I_c value was found at the medium ammonium concentration (T₂), i.e. $45 \pm 5 \mu\text{mol photon m}^{-2} \text{s}^{-1}$, while the highest predicted I_c value was found with the highest nitrate concentration (T₆), i.e. $57 \pm 3 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ (Table 1).

DISCUSSION

Vandermeulen and Gordin (1990) reported that increasing flow rates in culture tanks up to 8 volumes day⁻¹ significantly increased growth rates of *Ulva*. In the present study, *Ulva sp.* were maintained in a

continuous flow rate of nutrients of 12-14 volumes day⁻¹, which is comparable to the flow rates reported in several studies, i.e. 12-18.8 volumes days⁻¹ (Fujita *et al.*, 1989; Neori *et al.*, 1991 and Figueroa *et al.*, 2009). The maximum growth rate for *Ulva sp.* presented here (8.37 % d⁻¹ at the end of the growth experiment with the medium nitrate concentration) is comparable to the maximum growth rates of 7.86 % d⁻¹ for *Ulva sp.* reported by Rosenberg and Ramus (1981), of 7% d⁻¹ for *Ulva pertusa* (de-Casabianca *et al.*, 2002) and the growth rate obtained by Floreto *et al.* (1993) at a similar density (of 5g FW /L) of *Ulva pertusa* using Provasoli medium.

The higher growth rates reported in other studies for *Ulva rigida* (Lavery and McComb, 1991), *Ulva lactuca* (Neori *et al.*, 1991) may be partially attributed to the use of lower stocking density (i.e. 1.67 - 3.6 g /L, Neori *et al.*, 1991 and Figueroa *et al.*, 2009) compared to that of the present study (5 g FW/L). Some studies (e.g. Lapointe and Tenore 1981 and Mata & Santos, 2003) have reported that the optimal stocking density is around 0.8 - 1.3 Kg m⁻² (corresponding to 1.4 -2.4 g/L FW). Some authors have reported that increasing stocking density decreases the growth rate and yield of *Ulva* (DeBusk *et al.*, 1986 and Neori *et al.*, 1991) and other algal species (Neish *et al.*, 1977; Lapointe and Ryther 1978). However, these studies were carried out in the field and the algae received fish effluent. Fujita *et al.* (1989) found that growth rates of *Ulva rigida* were higher in outdoor cultures (10 % d⁻¹) than in indoor cultures (4 % d⁻¹). Additionally, Neori *et al.* (1991) found that fish pond effluents produced higher yields (by up to 38 %) than pulse nutrient addition and Floreto *et al.* (1993) reported that a high nutrient turnover in natural conditions may explain higher algal growth rates compared to indoor culture environments.

Israel *et al.* (1995) reported that the optimal mean temperature for growth of *Ulva lactuca* is about 20 °C, but higher temperatures may favour growth during spring. Many studies have reported that the optimal temperature for growth of *Ulva sp.* is 23 - 24 °C (i.e. Riccardi and Solidoro 1996). Therefore, the applied temperature (25 ± 1 °C) in the present study may be considered relatively high, particularly for the *Ulva* species collected on French shores. It could explain, to some extent, the reduced growth rate observed during this work. de-Casabianca *et al.* (2002) showed that growth in *Ulva rigida* was limited at temperatures outside the range 7 - 25 °C and that temperatures higher than the optimal values (from 12 to 23 °C) reduce the growth of *Ulva rigida* collected from the same site as that used for the present study. However, Mohsen *et al.* (1973a) found that at 25 °C, maximum amino acids and sugar contents were reached in *Ulva fasciata*, from Alexandria in Egypt.

This relatively high temperature was applied to control reproduction activity in the present study. Nordby (1977) reported that the optimal temperature for reproduction in species of *Ulva mutabilis* is 21 - 22 °C and that temperatures higher than 24 °C resulted in a drop in sporulation. Furthermore, Nordby (1977) showed that maximum sporulation was achieved at light intensities between 22.8 - 152 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Therefore, to inhibit reproduction in the present study, the temperature was maintained at 25 ± 1 °C and irradiance at a high level (273 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) since gamete formation is usually enhanced when temperatures are lowered to less than 20 °C (Mohsen *et al.*, 1973a). These conditions, however, did not inhibit reproduction, particularly with the nitrate treatments. In addition, the pH of the water in the experimental bottles ranged between 8 - 8.76, which is almost the optimal pH range for *Ulva mutabilis* sporulation according to Nordby (1977).

A strong positive correlation between light intensity and growth rate was noticed up to 35 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ while above 42 μmol

photon $\text{m}^{-2}\text{s}^{-1}$, bleaching was observed in thalli (Mohsen *et al.*, 1973b). However, according to Ramus and Venable (1987), optimal irradiance values are between 400 and 500 $\mu\text{mol photon m}^{-1}\text{s}^{-1}$ for *Ulva rigida* growth, and between 200-500 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ for *Ulva curvata* (Coutinho and Zingmark 1993).

The differences in growth rates of *Ulva* sp. among published studies may also be attributed to a combination of different culture conditions, i.e. light quality (Steffensen 1976), types of lamps, intensity (Mohsen *et al.*, 1973b; Bjornsater and Wheeler 1990) and culture management (Steffensen, 1976), or use of culture media enriched with macro and micro-nutrients as well as vitamins (Floreto *et al.*, 1993), and lower stocking densities 0.8-2.0 g FW /L (Bjornsater and Wheeler 1990; Floreto *et al.*, 1993).

Floreto *et al.* (1993) showed that form of nitrogen significantly affected growth of *Ulva pertusa* during the 6 first days of culture, with factors such as temperature becoming dominant after that. According to that, during the first week of our experiment, the lowest ammonium concentration, at a light intensity of 273 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and a temperature of 25 °C, was found to be the optimal for growth, resulted significantly higher RGR than all the nitrate treatments. However, the highest nitrate concentration under the same conditions was the more effective triggering factor for reproduction, which might have masked the real RGR under this treatment.

In a study of *Ulva lactuca* RGR response according to nitrogen form, Ale *et al.* (2011) noticed a decrease in RGR after 5 - 6 days of culture in media enriched with ammonium or nitrate, respectively. Similarly, Bjornsater and Wheeler (1990) noticed that after 5 - 7 days of maintaining *Ulva fenestrata* under 200 $\mu\text{M NO}_3\text{-N}$ and 12 $\mu\text{M PO}_4\text{-P}$, the growth rate decreased from 30 % d^{-1} to about 10 % d^{-1} , and then fluctuated between 20 % d^{-1} and less than 10 % d^{-1} during the remaining

experimental period. Similar fluctuations were also recorded in the present study which indicates that it may be a normal phenomenon in *Ulva*. Moreover, de-Casabianca *et al.* (2002) found that high fluctuations (from 0 % to 8 % d⁻¹) could be observed in RGR of *Ulva rigida* in nature.

Ulva spp. reproduction may be triggered by many factors (i.e. change in nutrient concentrations or handling of algae) and results in loss of part of the yield (Steffensen, 1976, Neori *et al.*, 1991). Mohsen (1974 a & b) reported that a rich supply of nitrogen promotes asexual reproduction in *Ulva fasciata* while sexual reproduction is enhanced by nitrogen depletion. This explains the difficulties encountered in determining the RGR in natural *Ulva* in which sporulation may easily be induced by changes in any of the culture conditions (i.e. temperature, light, nutrients, culture media etc) (Mohsen *et al.*, 1973 a&b, Thiadens and Zeuthen 1976) or by weighing procedures (Bjornsater and Wheeler 1990). The authors also reported that N tissue contents may have an effect on sporulation activity. Therefore, to avoid reproduction interference, sterile clones of *Ulva* were used (DeBusk *et al.*, 1986) or short-term, indoor experiments never exceeding 14 days (Fujita *et al.*, 1989; Naldi and Wheeler, 2002) were conducted.

The results of this study indicated that moving *Ulva* into higher nitrate concentrations (from 1 mM, during the preconditioning period to 3.8 mM) triggered their reproduction faster than moving them to lower concentrations, and this may have masked their real RGR in that treatment. Nitrate nitrogen induced a fortnight reproduction pattern, while no particular pattern was noticed with the ammonium treatments. Many *Ulva* species have been found to release swarms in a 14-15 day cycle (Rhyne 1973), which explains RGR fluctuation, particularly with the nitrate treatments.

The highest P_{max} was found for algae maintained under the highest nitrate concentration (3.8 mM), while the highest RGR achieved during this experiment was with the medium concentration of nitrate (2.36 mM). This apparent discrepancy could be explained by the intense reproductive activity observed at the highest nitrate concentration, which might have overridden the real growth rate with this treatment.

The irradiance-saturated photosynthetic rate does not necessarily reflect growth rate since it is related to electron flow between PSII and PSI (Dubinsky *et al.* 1986). However, a higher P_{max} value results from an increase in RuBPCase activity, which means that more inorganic nitrogen could be used for this purpose (Coutinho and Zingmark, 1993). In addition, P_{max} is dependent on light-independent reactions which are largely controlled enzymatically (Bannister, 1974), which may lead to higher nutrients metabolism rates or at least metabolites. Photosynthesis has been reported to be correlated with growth in *Ulva lactuca* (Israel *et al.*, 1995) and in *Gracilaria tikvahiae* (Lapointe & Duke, 1984) where carbon fixed during photosynthesis is rapidly converted into organic matter with minimum accumulation. Figueroa *et al.* (2009) reported that high nitrogen supplies resulted in higher Electron transport rates, which was reflected in higher photosynthetic activity in *Ulva lactuca*. Geider *et al.* (1993) also found that nitrate nitrogen supply affects photosynthetic performance in *Phaeodactylum tricornutum* (Bacillariophyceae). Moreover, in the present study, the lower P_{max} and I_c values observed with ammonium treatments indicated lower photosynthesis efficiency than the higher P_{max} and I_c values observed at the highest nitrate concentration, as evidenced by the slopes of the curves.

The light intensity of 320 - 360 $\mu\text{mol m}^{-2}\text{s}^{-1}$ was found to be saturating for photosynthesis of *Ulva fenestrata* (Bjornsater and Wheeler 1990), and 300 - 370 $\mu\text{mol m}^{-2}\text{s}^{-1}$ for *Ulva lactuca* (Israel *et al.*, 1995). Gordillo *et al.* (2001) reported irradiance at 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$ as saturating

for the net photosynthesis of *Ulva rigida*. In our experiments, light saturation ranged between 597.8 to 657.9 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$, which is similar to that reported in the work of Gordillo *et al.* (2001) and higher than the stated values for *Ulva sp.* in the other studies. This may be due to the high irradiance at which they were maintained during the long term experiment, which was bearable due to a high nutrient supply. Figueroa *et al.* (2009) reported that nutrient-rich conditions, particularly nitrogen supply, can help *Ulva lactuca* withstand stressful conditions such as high irradiance and temperatures as it stimulates protection, through increased production of certain protective substances and accelerates the biochemical recovery of damaged structures (Korbee *et al.*, 2004). It is worth mentioning that the highest photosynthesis rates were recorded at a light intensity higher than I_s (of 900 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$) with both nitrogen forms regardless of the concentration of nitrogen. Similarly, Henley (1993) reported that a considerable increase in photosynthesis occurs above I_s .

The significant high P_{max} at the nitrate concentration of 3.8 mM indicates that nitrogen was the limiting factor for photosynthesis at the temperature of 25 °C and irradiance of 273 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. This implies that *Ulva sp.* can use nitrate at high concentrations, that are even higher than 3.8 mM, i.e. 5 mM NO_3 which is reported as the saturating concentration for *Ulva rigida* by Gordillo *et al.* (2001), for long periods (weeks) without any toxic effects being observed. Ammonium on the other hand was found to inhibit growth of *Ulva lactuca* at concentrations above 64.3 $\mu\text{M N/L}$ (Waite and Mitchell 1972).

CONCLUSION

A key finding of this study is that nitrate can be assimilated by *Ulva sp.* and yield similar or even higher growth rates than ammonium on the long term. Moreover, nitrate at a high concentration (3.7 mM) yielded a higher photosynthetic rate and improved photosynthetic efficiency

compared to the ammonium concentrations. This would have been reflected in a high RGR if reproduction had not been triggered.

Unlike ammonium, high concentrations of nitrate (up to 3.7 mM) are not toxic for *Ulva* sp. In fact *Ulva* showed higher photosynthetic activity, which implies they may filter RAS effluent more efficiently than FTS effluent with long term exposure to high nutrient concentrations.

The P_{max} , indicates that nitrate nitrogen was the limiting factor for photosynthesis at the concentration of 3.7 mM and at a temperature of 25 °C and 273 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiance. This indicates that *Ulva* can filter nitrate even at higher concentrations than 3.7 mM, up to the saturation concentration.

Moving algae suddenly to higher nutrient concentrations triggers their intense reproduction faster than moving them to lower concentrations. Nitrate induced a fortnight pattern of reproduction, which is important for commercial-scale seaweed production.

ACKNOWLEDGEMENTS

This work was supported by a research grant attributed by the French embassy in Cairo. We would like to express our gratefulness to the French embassy in Cairo as well as to the staff at IFREMER Institute, Palavas station- Montpellier, particularly to Mr. Sebastien Triplet who set up the experimental system and Mr. Benoist de Vogüé for carrying out water chemical analysis, and other contributions to this work and to the congenial atmosphere in which it was conducted. Special thanks to Prof. Dr. Evelyne Bachere and Mr. Emmanuel Rezzouk Directors of the Palavas station. We are grateful to Prof. Dr. Azza Abd El-Rhaman and Prof. Dr. Mohsen Abd El-Tawwab for comments on an earlier version of the manuscript.

REFERENCES

- Ale, M.T.; J.D. Mikkelsen and A.S. Meyer, 2011. Differential growth response of *Ulva lactuca* to ammonium and nitrate assimilation. *J. Appl Phycol*, 23: 345–351.
- Andersson, M., 1942. Eingeiernahrungsphysiologische Versuche mit *Ulva* und *Enteromorpha*. *K Fysiogr Sallsk Lund Forh*, 12(4): 42-52.
- Bannister, T.T. 1974. Production equations in terms of chlorophyll concentration, quantum yield, and upper limit to production. *Limnol Uceanogr*, 19: 1-12.
- Bjornsater, B.R. and P.A. Wheeler, 1990. Effect of nitrogen and phosphorus supply on growth and tissue composition of *Ulva fenestrata* and *Enteromorpha intestinalis* (Ulvales: Chlorophyta). *J. Phycol.*, 26: 603-11.
- Blancheton, J.P.; R. Piedrahita; E.H. Eding; R.D. Orbcastel; G.L. emarie; A. Bergheim; S. Fivelstad, 2007. Intensification of land based aquaculture production in single pass and reuse systems. In Bergheim A. (Ed.) *Aquacultural engineering and environment*. Research Signpost publisher, ISBN 978-81-308-0199-5.
- Carmona, R., G.P. Kraemer and C. Yarish, 2006. Exploring northeast American and Asian species of *Poryphra* for use in an integrated finfish-algal aquaculture system. *Aquac.*, 252: 54-65.
- Cole, M.L.; K.D. Kroeger; J.W. McClelland; I. Valiela, 2005. Macrophytes as indicators of land-derived waste water: Application of $\delta^{15}\text{N}$ method in aquatic systems. *Water Resour Res*, 41: W01014, 10. 1029/2004WR003269.
- Coutinho, R. and R.Z. Ingmark, 1993. Interactions of light and nitrogen on photosynthesis and growth of the marine macroalga *Ulva curvata* (Kiitzing) De Toni. *J. Exp. Mar. Biol. Ecol.*, 167: 11-19.

- DeBoer, J.A.; H.J. Guigli; T.L. Israel; C.F. D'Elia, 1978. Nutritional studies of two red algae. 1. Growth rate as a function of nitrogen source and concentration. *J. Phycol.*, 14: 261-266.
- DeBusk, T.A.; M. Blakeslee; J.H. Ryther, 1986. Studies on the outdoor cultivation of *Ulva lactuca* L. *Bot. Mar.*, 29: 381-386.
- de-Casabianca, M.L.; N. Barthelemy; O. Serrano and A. Sfriso, 2002. Growth rate of *Ulva rigida* in different Mediterranean eutrophicated sites. *Bioresource Technol.*, 82: 27-31.
- D'Elia, C.F.; J.A. DeBoer, 1978. Nutritional studies of two red algae. II: Kinetics of ammonium and nitrate uptake. *J. Phycol.*, 14: 266-272.
- Dubinsky Z.; G. Falkowski; K. Wyman, 1986. Light harvesting and utilization by phytoplankton. *Plant Cell Physiol.*, 7: 1335-1349.
- Evans, G.C., 1972. The quantitative analysis of plant growth. *Studies in Ecology*. Blackwell Scient Publ, Oxford, pp 247-254.
- Figueroa, F.L.; A. Israel; A. Neori; B. Martínez; E-j. Malta; S. Inken; R. Marquardt and N. Korbee, 2009. Effects of nutrient supply on photosynthesis and pigmentation in *Ulva lactuca* (Chlorophyta): responses to short-term stress. *Aquat. Biol.*, 7: 173-183.
- Floreto, E.A.T.; H. Hirata; S. Ando; S. Yamasaki, 1993. Effects of temperature, light intensity, salinity and source of nitrogen on the growth, total lipid and fatty acid composition of *Ulva pertusa* Kjellman (Chlorophyta). *Bot. Mar.*, 36: 149-158.
- Fujita, R.M.; P.A. Wheeler and R.L. Edwards, 1989. Assessment of macroalgal nitrogen limitation in a seasonal upwelling region. *Mar. Ecol. Prog. Ser.*, 53: 293-303.
- Geider, R.J.; J. LaRoche; R.M. Greene; M. Olaizola, 1993. Response of the photosynthetic apparatus of *Phaeodactylum tricorutum*

- (Bacillariophyceae) to nitrate, phosphate, or iron starvation. *J. Phycol.*, 29: 755–766.
- Gordillo, F.J.L.; F.X. Niell; F.L. Figueroa, 2001. Non-photosynthetic enhancement of growth by high CO₂ level in the nitrophilic seaweed *Ulva rigida* C. Agardh (Chlorophyta). *Planta*, 231: 64-70.
- Hanisak, M.D. 1983. The nitrogen relationships of marine macroalgae. In: Carpenter EJ, Capone DG (Eds.) Nitrogen in the marine environment. Academic Press, New York, 699-730.
- Henley, W.J., 1993. Measurements and interpretation of photosynthetic light-response curves in algae in the context of photoinhibition and diel changes. *J. Phycol.*, 29:729-739.
- Henley W.J.; G. Levavasseur; L.A. Franklin; C.B. Osmond; J. Ramus, 1991. Photo-acclimation and photoinhibition in *Ulva rotundata* as influenced by nitrogen availability. *Planta*, 184: 235-243.
- Israel, A.A.; M. Friedlander; A. Neori, 1995. Biomass yield, photosynthesis and morphological expression of *Ulva lactuca*. *Bot. Mar.*, 38: 297-302.
- Korbee, N.P.; R.T. Abdala Díaz; F.L. Figueroa and W.E. Helbling, 2004. Ammonium and UV radiation stimulate the accumulation of mycosporine-like amino acids in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina. *J. Phycol.*, 40: 248–259.
- Lapointe, B.E. and K.R. Tenore, 1981. Experimental outdoor studies with *Ulvafasciata* Delile. I. Interaction of light and nitrogen on nutrient uptake, growth, and biochemical composition. *J. Exp. Mar. Biol. Ecol.*, 53(2/3): 135-152.

- Lapointe, B.E. and C.S. Duke, 1984. Biochemical strategies for growth of *Gracilaria tikvahiae* (Rhodophyta) in relation to light intensity and nitrogen availability. *J. Phycol.*, 20: 488-495.
- Lapointe, B.E. and J.H. Ryther, 1979. The effects of nitrogen of seawater flow rate on the growth and biochemical composition of *Gracilaria fliifera* var. *angustissima* in mass outdoor culture. *Bot. Mar.*, 22: 529-537.
- Lapointe, B.E. and J.H. Ryther, 1978. Some aspects of the growth and yield of *Gracilariatikvahiae* in culture. *Aquac.*, 15: 185-193.
- Lavery P.S. and A.J. McComb, 1991. The nutritional eco-physiology of *Chaetomorpha linum* and *Ulva rigida* in Peel Inlet, Western Australia. *Bot. Mar.*, 34: 251-260.
- Luo M.B.; F. Liub and Z.L. Xu, 2012. Growth and nutrient uptake capacity of two co-occurring species, *Ulva prolifera* and *Ulva linza*. *Aquat. Bot.*, 100: 18– 24.
- Mata L. and R. Santos, 2003. Cultivation of *Ulvarotundata* (Ulvales, Chlorophyta) in raceways, using semi-intensive fishpond effluents: yield and biofiltration. In: Chapman AR, Anderson RJ, Vreeland VJ, Davison IR (Eds), *Proceeding of the 17th Inter. Seaweed Symp, CapTown 2001*. Oxford University Press, 237-242.
- Mohsen, A.F.; A.F. Khaleafa; M.A. Hashem and A.M. Metwalli, 1974b. Effect of different nitrogen sources on growth, reproduction, amino acid, fat and sugar contents in *Ulvafasciata* Delile. *Bot. Mar.*, XVII: 218-222.
- Mohsen, A.F.; A.F. Khaleafa; M.A. Hashem and A.M. Metwalli, 1974a. Effect of Some Vitamins on Growth, Reproduction, Amino Acid, Fat and Sugar Contents in *Ulvafasciata* Delile. *Bot. Mar.*, XVII: 208-212.

- Mohsen, A.F.; A.H. Nasr and A.M. Metwalli, 1973a. Effect of Temperature variations on growth, reproduction, amino acid synthesis, fat and sugar content in *Ulvafasciata* Delile. *Plants Hydrobiol.*, 42: 451-460.
- Mohsen, A.F.; A.H. Nasr and A.M. Metwalli, 1973b. Effect of different light intensities on growth, reproduction, amino acid synthesis, fat and sugar contents in *Ulva fasciata* Delile. *Hydrobiol.*, 43 (1/2): 125-135.
- Naldi, M. and P.A. Wheeler, 1999. Changes in nitrogen pools in *Ulvafenestrata* (Chlorophyta) and *Gracilariapacifica* (Rhodophyta) under nitrate and ammonium enrichment. *J. Phycol.*, 35: 70-77.
- Naldi, M. and P.A. Wheeler, 2002. ¹⁵N measurements of ammonium and nitrate uptake by *Ulva fenestrata* (Chlorophyta) and *Gracilariapacifica* (Rhodophyta): comparison of net nutrient disappearance, release of ammonium and nitrate, and ¹⁵N accumulation in algal tissue. *J. Phycol.*, 38: 135-144.
- Navarro-Angulo, N. and D. Robbledo, 1999. Effects of nitrogen sources, N: P ratio and N-pulse concentration and frequency on the growth of *Gracilaria cornea* (GracilarialesRhodophyta) in culture. *Hydrobiol.*, 398/399: 315-320.
- Neish A.C.; P.F. Shacklock; C.H. Fox and F.J. Simpson, 1977. The cultivation of *Chondrus crispus*. Factors affecting growth under greenhouse conditions. *Can J. Bot.* 55: 2263-2271.
- Neori, A., 1996. The type of N-supply (Ammonia or nitrate) determines the performance of seaweed biofilters integrated with intensive fish culture. *Isr. J. Aquac. Bamidgeh*, 48: 19-27.

- Neori A; I. Cohen and H. Gordin, 1991. *Ulva lactuca* biofilter for marine fishpond effluents II. Growth rate, yield and C:N ratio. *Bot. Mar.*, 34: 483-489.
- Nordby, O., 1977. Optimal conditions for meiotic spore formation in *Ulva mutabilis* Foyn. *Bot. Mar.*, 20: 19-28.
- Pagand P.; J-P. Blancheton; J. Lemoalle and C. Casellas, 2000. The use of high rate algal ponds for the treatment of marine effluent from a recirculating fish rearing system. *Aquac. Res.*, 31: 729-736.
- Papáček S.; S. Celikovský; B. Reháček and D. Stys, 2010. Experimental design for parameter estimation of two time-scale model of photosynthesis and photoinhibition in microalgae. *Mathematics and Computers in Simulation*, 80: 1302–1309.
- Ramus, J. and M. Venable, 1987. Temporal ammonium patchiness and growth rate in *Codium* and *Ulva* (Ulvophyceae). *J. Phycol.*, 35: 518-523.
- Rhyne, C., 1973. Field and experimental studies of the systematics and ecology of *Ulva curvata* and *Ulva rotundata*. Sea grant publication, UNC-SG-73-09.
- Riccardi, N. and C. Solidoro, 1996. The influence of environmental variable on *Ulva rigida* C. Ag. Growth and production. *Bot. Mar.*, 39: 27-32.
- Rosenberg, G.; J. Ramus, 1981. Ecological growth strategies in the seaweed *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae): The rate and timing of growth. *Bot. Mar.*, 24: 583-589.
- Schuenhoff, A.; M. Shpigel; I. Lupatsh; A. Ashkenazi; F.E. Msuya and A. Neori, 2003. Assemi-rcirculating, integrated system for the culture of fish and seaweed. *Aquac.*, 2001: 167-181.

- Steffensen, D.A. 1976. The effect of nutrient enrichment and temperature on the growth in culture of *Ulva lactuca* L. *Aquat. Bot.*, 2: 337-351.
- Thiadens, A.J.H. and E. Zeuthen, 1976. Meiosis and sporulation induced in sporophytes of *Ulva mutabilis* (slender) with synchronous mitosis. *Planta (Berl)*, 72: 60-65.
- Thomas, T.E. and P.J. Harrison, 1985. Effect of nitrogen supply on nitrogen uptake, accumulation and assimilation in *Porphyra perforata* (Rhodophyta). *Mar. Biol.*, 85: 269-78.
- Thomas T.E. and P.J. Harrison, 1987. Rapid ammonium uptake and nitrogen interactions in five intertidal seaweeds grown under field conditions. *J. Exp. Mar. Biol. Ecol.*, 107: 1-8.
- Vandermeulen, H.; H. Gordin, 1990. Ammonium uptake using *Ulva* (Chlorophyta) in intensive fishpond systems: mass culture and treatment of effluent. *J. Appl. Phycol.*, 2: 363-374.
- Waite, T. and Mitchell, 1972. The effect of nutrient fertilization on the benthic alga *Ulva lactuca*. *Bot. Mar.*, 15: 151-156.
- Zou, D.H., 2005. Effects of elevated atmospheric CO₂ on growth, photosynthesis and nitrogen metabolism in the economic brown seaweed, *Hizikia fusiforme* (Sargassaceae, Phaeophyta). *Aquac.*, 250: 726-35.

الإستجابة البيوفسيولوجية لطحلب الأولفا - كمرشح بيولوجي - للصور وكذا التدفقات المختلفة من النيتروجين

ياسر مصطفى^{١*} ، جال بوجارو^٢ ، ميريام كلير^٣ و جون بول بلانشيتون^٤

^١المعمل المركزي لبحوث الثروة السمكية، العباسية، أبو حماد، الشرقيه، مصر.

([ymoustafaonline@yahoo.com](mailto:yroustafaonline@yahoo.com)), Mobile: 0020 1140888918

² IFREMER, Station de Nantes, Rue de l'Ile d Yeu -B.P.21105, 44311 Nantes Cedex 3, France

³ IFREMER, Station de Palavas, Chemin de Maguelone, 34250 Palavas Les Flots, France

⁴ UMR ECOSYM, USTL, Place Eugène Bataillon, Montpellier, France.

الملخص العربي

أجري البحث الحالي لدراسة التأثير على المدى الطويل للصور والتركيزات المختلفة من النيتروجين ($14.3, 28.6$ and $57.1 \mu\text{M N-NH}_4$ and $214, 2360, 3700 \mu\text{M N-NO}_3$) على كل من نشاط البناء الضوئي و معدل النمو لطحلب الأولفا لمدة خمسة أسابيع. ولقد تم الإمداد بماء البحر بعد إضافة العناصر الغذائية لها بمعدل $12.6-14.4$ حجم لكل يوم. وتم تقدير معدل النمو للطحلب أسبوعيا. أما إستجابة نشاط البناء الضوئي فقد تم تقديرها بأخذ نحو $20-25$ ملجم من الطحلب و تحضينها في جهاز القياس لمدة 56 دقيقة تحت إضاءة مختلفة الشدة تتخللها فترات إظلام كل منها لمدة 7 دقائق. ولقد تم رسم منحني البناء الضوئي بإستخدام متوسط 15 قراءة للبناء الضوئي بعد نسبها الى الوزن الجاف للطحلب- وتم تطبيق النموذج الرياضي لهالدين لحساب أقصى معدل إنتاج للإكسجين من خلال البناء الضوئي (P_{max})، إضاءة الإشباع للبناء الضوئي (I_s)، أقل إضاءة مطلوبة للبناء الضوئي (I_c). وعند نهاية التجربة الخاصة بتقدير النمو- كان معدل النمو بعد إضافة النترات أعلى - ولكن غير معنويا- من النمو بعد إضافة الأمونيوم. وسجل أعلى معدل للنمو عند التركيز المتوسط للنترات. اما بالنسبة للبناء الضوئي فلقد لوحظت علاقة إيجابية بين تركيزات النيتروجين و البناء الضوئي. وسجل أعلى معدل للبناء الضوئي (P_{max}) عند أعلى تركيز للنترات - في حين أن أقل تركيز للأمونيوم أنتج أقل معدل للبناء الضوئي. هذا و لم تلاحظ أي

إختلافات معنوية بين قيم كل من إضاءة التشبع (I_s) وأقل إضاءة مطلوبة لنشاط البناء الضوئي (I_c) - على الرغم من أنه بصفة عامة كان لمعاملات الأمونيوم قيم أعلى من (I_s) من معاملات النتترات. هذا و يمكن تفسير التناقض الظاهري بين نتائج معاملات النمو والبناء الضوئي من خلال مناقشة نشاط التكاثر وكذا تأثير درجة الحرارة.