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PHOTOSYNTHESIS OF PERIPHYTON: RELATIONSHIPS BETWEEN LIGHT AND AGE OF ALGAL MAT

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ABSTRAK

*Mengamati sebuah perbandingan laju proses fotosintesis perifiton yang diukur pada berbagai akumulasi biomassa (umur 7 hingga 35 hari) dan intensitas cahaya yang berbeda. Sampel perifiton diperoleh dari hasil kultur massal pada sebuah foto-reactor di bawah kondisi periode terang/gelap 15/9 jam, intensitas cahaya sekitar 50 µmol m^{-2} s^{-1}, dan suhu sekitar 20 °C. Substrat berupa keramik dengan ukuran sekitar 65 cm² ditempatkan pada box flaxy dengan volume sekitar 1000 ml dan diekspos dengan berbagai intensitas cahaya (dari 30 hingga 900 µmol m^{-2} s^{-1}). Laju fotosintesis dan respirasi dikuantifikasi berdasarkan perubahan konsentrasi oksigen sesaat yang berlangsung selama pengukuran. Tidak kelihatan efek inhibisi yang ditimbulkan oleh peningkatan intensitas cahaya. Pada penelitian ini model hubungan fotosintesis-intensitas cahaya memilki persamaan saturasi [P = Pmax * I/(I +KI)]. Parameter Pmax dan KI secara gradual meningkat dengan bertambahnya umur komunitas perifiton. Laju produksi net diekspresikan sebagai perbedaan antara nilai fotosintesis dan respirasi dan secara linear miningkat hingga umur 21 hari (±1.75 g O_2.m^{-2}.d^{-1}) dan cenderung menurun pada umur lebih tua. Jika diekspresikan per satuan klorofil, laju produksi net sekitar 9 mg O_2.mg chl. a^{-1}. h^{-1}, sementara laju respirasi sekitar 1 mg O_2.mg chl. a^{-1}. h^{-1}.*

Kata kunci : perifiton, fotosintesis, respirasi, algal, produksi net

ABSTRACT

*A comparison of the photosynthesis rate have been measured between various periphytic bio-films of increasing biomass (7 to 35 days old). The samples of periphyton were provided by cultures carried out under the following conditions: 15/9 dark-light cycle and 50 µmol m^{-2} s^{-1} , and 20 °C. The tile (about 65 cm²) with bio-film was placed in a closed volume (about 1 liter) of chamber and exposed to increasing light intensities (30 to 900 µmol m^{-2} s^{-1}). The photosynthesis and respiration rates were deduced from the instantaneous variations of the dissolved oxygen concentration. No inhibition effect was observed for the higher light intensities. The photosynthesis corresponded to an equation of saturation [P = Pmax * I/(I +KI)]. Pmax and KI Parameters as well as the respiration rate gradually increased with the age of bio-films. The net production rate expressed as the difference between the photosynthesis and the respiration rates linearly increased up to 21 days (±1.75 g O_2.m^{-2}.d^{-1}) and then slightly decreased for older bio-films. Expressed versus chlorophyll unit, the net production rate was about 9 mg O_2.mg chl. a^{-1}. h^{-1}, while the respiration rate was about 1 mg O_2.mg chl. a^{-1}. h^{-1}.*

Key words : periphyton, photosynthesis, respiration, algal mat, net production,

INTRODUCTION

Periphyton is an important component of many lotic systems. It can produce high biomass (Moss, 1968; Hansson, 1988a) influencing nutrient, carbon cycling, and invertebrate composition (Lock *et al*., 1984; Meyer *et al*., 1988; and Lamberti *et al*., 1989); it may also store a part of the newly mineralized nutrients at the sediment surface, thereby having a competitive impact on phytoplankton (Carlton & Wetzel, 1988; Hansson, 1989, 1990).

Photosynthetic and respiratory activities of periphytic algal communities change from shallower to deeper zones in

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response to changes in physical variables, including light intensity, radiation spectra, and water temperature at the bottom of the littoral zone (Hill, 1996; DeNicola, 1996). The changes in these physical conditions also could largely explain differences in the dominant species, biomass of periphytic algal communities (Kairesalo, 1980; Loeb & Reuter, 1981; Stevenson & Stoermer, 1981; Kingston *et al*., 1983; Jonsson 1987, 1992; Hawes & Schwarz, 1996).

Relatively few papers have been published on light effects on benthic algae and research on algal-light interactions in freshwater benthos is poor compared to that in the water column. Fewer than 20 photosynthesis irradiance (P-I) have been published for benthic algae, whereas hundreds of P-I phytoplankton responses are in the literature (Richardson *et al*., 1983). Photosynthesis-irradiance relationships are measured for two basic reasons : i) to evaluate ecophysiological responses to light and ii) to predict *in situ* photosynthesis. The present study was performed to determine the pattern of photosynthesis activity during the algal mat development in the stream laboratory.

MATERIAL AND METHODS

Culture of the periphyton

Algal suspension obtained by scraping stones from the Garonne river was cultivated on ceramic tiles in a laboratory-stream, with a medium using ground water medium enriched with nutrients (0.115 NH_4Cl mg.l^{-1}, 0.022 mg KH_2PO_4 mg.l^{-1} and 0.018 mg O_3Na_2Si, $5H_2O$ mg.l^{-1}) and adjusted to a final pH of 7. The medium was necessarily changed every two days to keep away from deficiency of nutrient, and the pilots were placed under light/dark cycle (15/9 hours, at ±50 µmol. $m^{-2}.s^{-1}$), and at ±20 °C and at least three replicates were done for the photosynthesis rate measurements.

Design of Photosynthetic Chamber

Photosynthesis measurement has been conducted by using a rectangular chamber (fig. 1) of plexi-glass having length = 25 cm, width = 18 cm and height = 7 cm. This device was first described by Duff *et al.* (1984), modified by Benmoussa (1995) and was improved for this study. The volume of the incubation chamber is approximately of 0.8 liter, and enclosed in a box connected with a cryostat to maintain constant the water temperature. A magnetic stirrer was used to ensure a water circulation at the surface of the mat sample (about 75 rpm). The chamber was installed under a lighting system, using a halogen lamp Phyto Claude (400 watt).

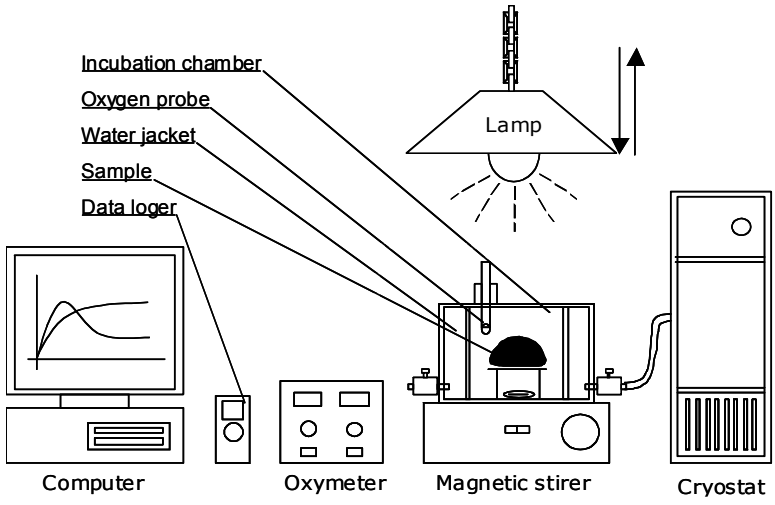

Figure 1. Primary Productivity and Respiration Measurements : Experimental device.

Photosynthesis and respiration measurements

Every week, from 7 days to 35 days old culture, the oxygen evolution was measured under five light intensities (30, 100, 300, 600 and 900 $\mu mol.m^{-2}.s^{-1}$). Dissolved oxygen was measured using an oxygen electrode YSI 5300 model inserted in the chamber.

The tile was immersed in the chamber which was filled up with water avoiding gas bubbles and closed : the photosynthesis and respiration measurements were carried out immediately. The oxygen slope was measured with a 15 minutes interval for different light intensities, from the lower one to the highest values.

The oxygen quantity being consumed or produced by the periphytic algal sample was calculated using the relation:

$$Y\ (mg\ O_2.surface\ area^{-1}\ h^{-1}) = [\{(X^*\ (O_2)t\ *\ V)100^{-1}\}]\ (T)^{-1}(A)^{-1} \quad (1)$$

with:

X = Oxygen variation in % during incubation period

$(O_2)t$ = Dissolved oxygen value in water related to temperature function ($mg\ l^{-1}$)

V = Volume of incubation chamber (L)

T = Incubation time (h)

A = Substrate surface (m^2)

The decreasing oxygen concentration during dark conditions was assumed as the respiration rate and the increasing oxygen concentration in light conditions was calculated as gross photosynthesis of periphytic biofilms. Photosynthesis of samples, as a nonlinear function of light were fitted to the following equation:

$$P = Pmax * I\ [(KI + I)]^{-1} \quad (2)$$

where P is the photosynthesis rate, Pmax the maximal production rate, I the light intensity and KI the half-saturation constant for light.

Biomass analysis

The sampling was carried out every week during five weeks to estimate chlorophyll *a*, ash-free dry mass (AFDM), and the final composition of species. The chlorophyll *a* and ash-free dry weight were analyzed by scraping a tile and suspending the matter in 50 ml of distilled water. This suspension was immediately homogenized by using a mixer (Ultra-Thurax) and concentrated (10 mL) using a refrigerator centrifuge (1200 rpm at 4 °C during 20 minutes). After removing the supernatant solution, chlorophyll was extracted with acetone 90 % (10 mL), placed in a flask and stored in the freezer approximately 24 hours. The pigment content was estimated by using the spectrophotometer methods proposed by Scor-Unesco (1966) and modified by Jeffrey & Humphrey (1975). The algal biomass was dried at 80 °C during 12 hours to estimate the dry weight and then burned (550 °C during 1 hour) to calculate ash-free dry weight and to deduce organic matter (loss on the ignition) from the weight without ashes; the results are expressed in $g\ m^{-2}$.

RESULTS

Photosynthesis rates were measured for biomass accumulation ranging respectively from 34 to 58 mg Chl.*a* m^{-2}, 13 to 32 mg Pheopigment. m^{-2} and 3 to 21 g AFDM. m^{-2} . The photosynthesis rate linearly increased with biomass development until 21 days (age) and next decreased with higher biomass (Fig.2). The communities were dominated by *Stigeoclonium, Diatoms* and *Lyngbya* genera's (about 45%, 35%, and 20% respectively).

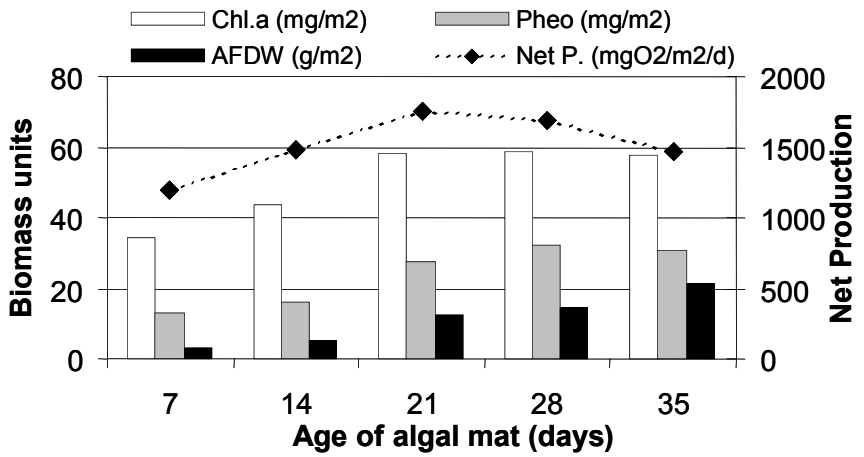

Figure 2. Biomass and Net Production Values Versus Age of Algal Mat.

The photosynthesis - light relationships (represented by saturation model, equation 2) show that the photosynthesis rates increased nonlinearly during the algal development (from 7 to 35 days) (Fig.3). The variation of photosynthesis-light relationships versus algal mat developments showed that :

- the value of Pmax increased from 310 to 500 mg $O_2.m^{-2}.h^{-1}$, but appeared to be quite constant if expressed *per* chlorophyll *a* unit (about 9 mg O_2.mgChl.$a^{-1}.h^{-1}$),
- the value of KI increased gradually from 70 to 110 µmol.$m^{-2}.s^{-1}$ with periphyton age (Fig.5)

and the respiration rate increased with both chlorophyll *a* and surface units, from 0.7 to 1.4 mg O_2.mg Chl.$a^{-1}.h^{-1}$, and from 26 to 80 mg $O_2.m^{-2}.h^{-1}$ respectively.

According to the age of the periphyton sample (Fig 3 and 4), the highest production rate occurred at 21 days (about 400 mg $O_2.m^{-2}.\ h^{-1}$) and the lowest one was measured for a one-week old sample (about 259 mg $O_2.m^{-2}.\ h^{-1}$) while the respiration rate increased gradually with age and biomass. Taking into account the age of the periphyton sample, the respiration rate represents 9 % of gross photosynthesis rate for a young community compared to 21 % for a 35 day old periphyton sample.

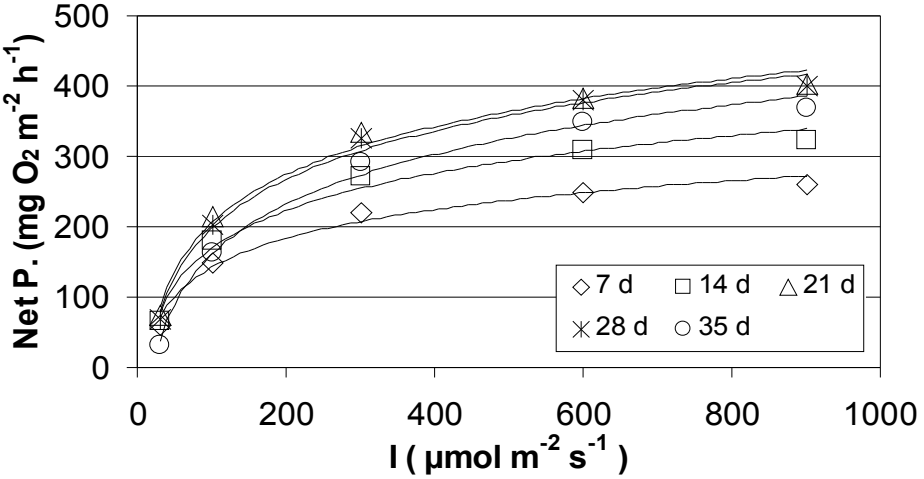

Figure 3. Photosynthesis Curves for Algal Mats Aged from 7 to 35 days.

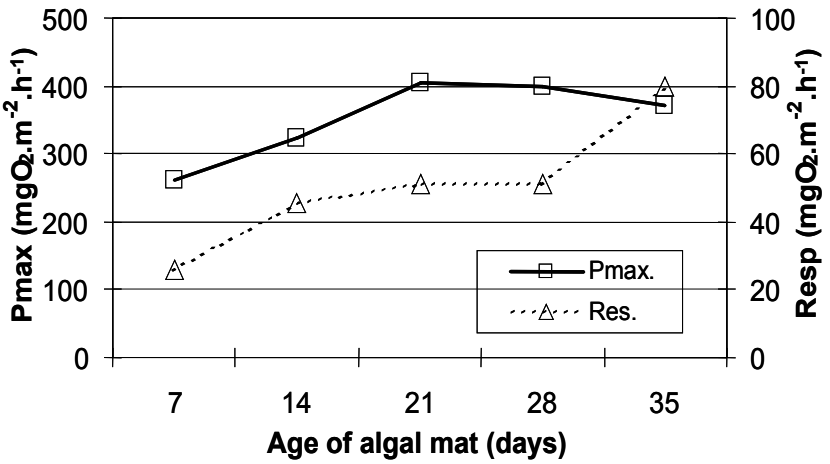

Figure 4. Maximal Photosynthesis (Pmax) and Respiration (Res) Evolution Versus Age of Algal Mat.

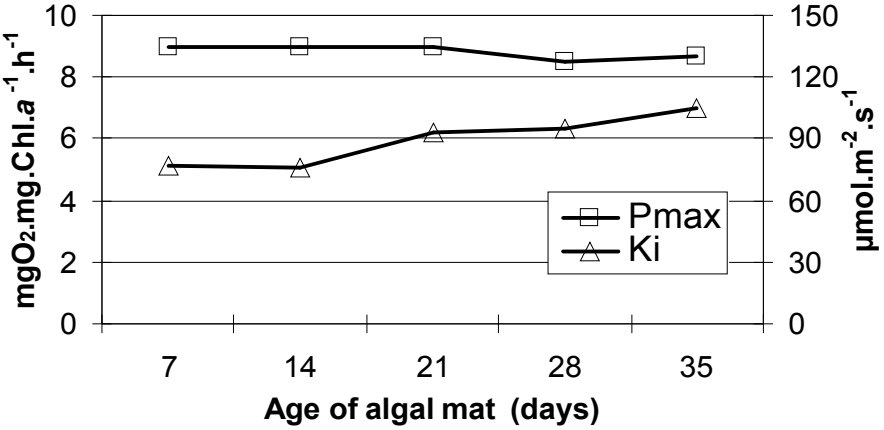

Figure 5. Variation of Pmax and Ki Versus the Age of Algal Mat.

DISCUSSION

Because photosynthesis responds quantitatively to changes in light, environmental variation in its quantity and quality potentially accounts for much of variation in the physiology, population growth and community structure of benthic algae (Hill, 1996).

Several authors (Sand-Jensen & Revsbech, 1987; Boston & Hill, 1991; Dodds, 1991; Graham *et al*., 1995; Hill, 1996) have reported the changes of photosynthesis rate of algal periphyton versus the variation of light intensities. However, the relationships between biomass, photosynthesis and respiration rates during the development of the periphyton mat is largely unexplored. This study points out that the pattern of photosynthesis rate is closely related to the variation of thickness of the mat, with some border values for biomass (up to 58 $mg.m^{-2}$ for chlorophyll a, 27 $mg.m^{-2}$ for phaeopigment, and 12.5 $g.m^{-2}$ for AFDW) after the which there is no increase of gross photosynthesis while the respiration is clearly related to the age.

This means that there is a self-shading effect induced by a dense matrix so

that a stronger photosynthesis reduction may be expected for biomass values upper than in these experiments. In natural habitats, this phenomenon, linked to the growth of the mat, is combined to other aspects as the consequence of dislodging or the grazing effects (McCormick & Stevenson, 1991; Feminella & Hawkins, 1995; Steinman, 1996). Moreover, high water turbidity level or inorganic sedimentation in the river has also an effect on photosynthesis (Yamada & Nakamura, 2002).

In this study, all of algal growth stages showed a good relation between photosynthesis and light intensity and a photo-inhibition effect was not observed on both younger and older algal substrates. Hill & Boston (1991) indicate that a strong inhibition would occur when light intensities exceeded 1,100 μmol m^{-2} s^{-1} for 2 h at least for early stages of development. In addition, Hill & Boston (1991) produced (C^{14} uptake) photosynthesis-irradiance curves for four developmental stages from three stream locations that differed in canopy cover, and found that the photo-inhibition in the periphyton from the two shaded sites declined with development, indicating that self-shading significantly influenced photosynthesis in older communities. Self-shading caused by the vertical accumulation of biomass could protect underlying cells from photo inhibition and may increase the amount of light required for saturation of community photosynthesis. Conversely, the self-shading within the periphyton matrix may induce phenotypic or genotypic photo adaptation to low light. The photo adaptation to low light commonly induces a decrease in saturation intensities and an increase of quantum efficiencies (Prézelin, 1981; Falkowski, 1981).

In this study, Pmax (maximum photosynthesis rate) linearly increased with biomass up to 21 day old, dark respiration rate strongly increased at 35 days, and KI slightly increased up to 35 days (Fig.4). The change of those parameters with the age of substrate shows that the biomass and community structure are two important components that determine the net primary production of the periphyton community in a lotic system (Stevenson *et al*., 1996). Richardson *et al*., (1983), reported that the compensation point (*Ic*) and the irradiance maximum (I max) can change depending on the species composition of the periphyton and also change the light requirements for photosynthesis (Sheath, 1984).

As attached microorganism, periphyton vertically grows on the substrate as a complex matrix community. Thus, the change of physical structure of periphyton during their growth alters the environmental conditions within the matrix and influences the growth of cells and their rates of primary production and nutrient uptake. The light is attenuated by the vertical matrix of algal cells and inorganic particules. Self-shading and other aspects of development in a matrix seem to be important determinants of periphyton P-I relationships (Boston & Hill, 1991), and taking into account the results obtained in this study, explain the gradual increase of KI as the algal mat grew.

Other cumulated effects on the photosynthesis parameters have been reported: self-shading would reduce KI and Ic twofold for *Potamogeton* epiphytes (Sand-Jensen & Revsbech, 1987), and it appears to have dramatic effects on the slope biomass/Photosynthesis curve of *Phragmites* epiphytes (Meulemans, 1988), to cause a strong decline in α Chlorophyll (chlorophyll-specific alpha) and biomass-specific Pmax (Hill & Boston, 1991). Paul and Duthie (1989) reported that development lowered saturation intensities for both individual cells and communities. Other studies have suggested functional adaptation to light within the periphyton matrix based on comparison of photosynthetic pigment ratios in overstory and understory cells (Hudon *et al*., 1987).

The set of data of this study is consistent with these previous results: the

variations of the parameters related to the photosynthesis model obviously indicate that a high density bio-film requires higher light intensity for saturation production than a lower density community, with a loss due to respiration increasing gradually with age. The respiration rate (from 9 to 20 % of Pmax) is of the same range of those of phytoplankton. However, abundant heterotrophs that are usually associated with benthic algae cause values to be higher than those measured in communities with fewer heterotrophs (McIntire & Phinney, 1965). Even it can be stated that the heterotrophs are increasing with algal mat age, in this study, because medium is mineral, the part of heterotrophs must remain low ; this is not the case of natural communities which are submitted to nutrient conditions including organic compounds able to enhance the growth of heterotrophs.

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