Heterotrophy compared to phototrophy for growth characteristics and pigment compositions in batch cultures of four green microalga

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Introduction

Small-scale batch culture trials are frequently used to compare growth of microalgal species in different conditions. The accuracy of this approach depends on proper analysis of growth curves, which is facilitated by automated data acquisition. Here we show growth characteristics for four green microalga cultivated under photoautotrophy, heterotrophy and dim-light-assisted heterotrophy. Because pigments are valuable microalgal products [1], we evaluated the effect of trophic status on pigment contents of the four species.

Results

1. A valuable correction method for growth curves obtained with Multi-cultivator MC1000

Two local strains (*Scenedesmus acutus* and *Chlorella vulgaris)* and two strains from collections *(Scenedesmus vacuolatus SAG 211.11n* and *Chlamydomonas reinhardtii CC-1690)* were cultivated on Bold-3N medium in Multi-Cultivator MC1000 (Photon System Instruments), which provides on-line monitoring of optical density at 720 nm (OD_{720}). However, this apparent optical density is not linearly related to true optical density (measured at 750 nm in a spectrophotometer, OD_{750}). A general equation was found suitable to correct the OD signal :

$OD_{750}=OD_{720}*(A*EXP(B*OD_{720}))$, where 'A' and 'B' are species-dependent constants.

Corrected growth curve of phototrophically grown (**Fig. 1**) showed the expected succession of exponential and deceleration (linear) phases ([2]), from which **specific growth rate (*μmax*)** and **maximum growth velocity (*Vmax*)** could be calculated, respectively. OD/biomass relationships were then established in order to express *Vmax* as **biomass volumetric productivity** ($g.l^{-1}.day^{-1}$).

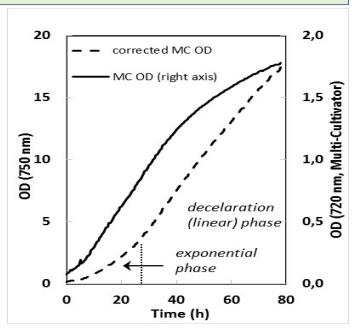

Fig. 1. Growth curve correction for *C. vulgaris* grown photoautotrophically at 700 $\mu mol.m^{-2}.s^{-1}$ PAR (note different Y scales).

2. Under photoautotrophy, *μmax* and *Vmax* are differently affected by light intensity

Table 1

μMax in photoautotrophy (h^{-1})	100 PAR	700 PAR
S. acutus	0,05	0,07
C. vulgaris	0,09	0,15
C. reinhardtii	0,05	0,15
S. vacuolatus	0,07	0,12

Vmax in photoautotrophy ($g.l^{-1}.d^{-1}$)	100 PAR	700 PAR
S. acutus	0,53	1,74
C. vulgaris	0,46	1,49
C. reinhardtii	0,28	1,20
S. vacuolatus	0,54	1,40

In photoautotrophically-grown cells (high CO_2 condition), *μmax* (exponential phase) showed limitation at around 300-500 $\mu mol.m^{-2}.s^{-1}$ (reflecting photosynthetic saturation when biomass density is low). In contrast, *Vmax* (deceleration phase phase, high biomass density) showed less light saturation (**Fig. 2**). The transition from exponential to deceleration phase corresponds to the appearance of a light-limited state as the biomass density becomes sufficient to absorb all incident light. This behaviour was general for the four species. *μmax* and *Vmax* values for the four species at moderate and high light are shown in **Table 1**. *μmax* and *Vmax* values are not correlated when different species are compared,

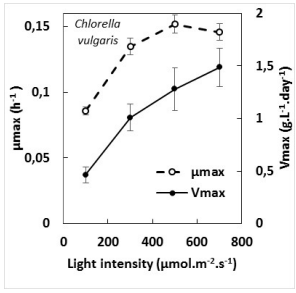

Fig. 2. Different light-dependence of μmax (exponential phase) and Vmax (linear phase) for photoautotrophic cultivation of *C. vulgaris*.

3. Heterotrophic versus photoautotrophic growth

Table 2

μMax in heterotrophy	Glucose	Acetate
S. acutus	0,025	0,024
C. vulgaris	0,132	0,109
C. reinhardtii		0,036
S. vacuolatus	0,084	0,080

Table 2 shows *μmax* values on acetate (2 $g.L^{-1}$) and glucose (15 $g.L^{-1}$) for the four species. *μmax* values were strongly species-dependent but not or poorly dependent on the carbon source. Only for *C. vulgaris* and *S. vacuolatus* were μmax values in the same ranges as for photoautotrophic growth.

We compared growth characteristics of the four strains grown either photoautotrophically or heterotrophically using glucose or acetate as carbon substrate. The effect of weak light (5 $\mu mol.m^{-2}.s^{-1}$ PAR) on heterotrophic growth ([3]) was also investigated, but was found to be weak or non-significant.

Under glucose (15 $g.L^{-1}$), fast and sustained exponential growth was found for *C. vulgaris* and *S. vacuolatus*. In these strains, higher growth rates could be reached compared to photoautotrophy under high light (700 $\mu mol.m^{-2}.s^{-1}$ PAR) due to the extended exponential phase (**Fig. 3**).

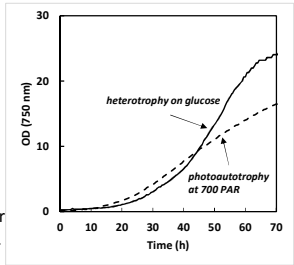

Fig. 3. Growth curves of *C. vulgaris* grown either heterotrophically on glucose or photoautotrophically at 700 PAR.

4. Pigment content in autotrophy and heterotrophy

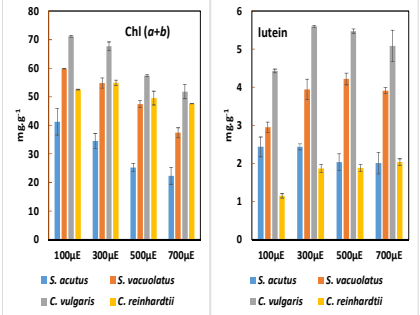

Fig. 4. Chl and lutein biomass contents of photoautotrophically grown cells (deceleration phase).

Pigment biomass contents (as determined by HPLC) were analyzed in relation to trophic status. Examples are shown for Chl (*a+b*) and lutein.

Under photoautotrophy, light adaptation of Chl content was found during deceleration phase for all species except *C. reinhardtii* (**Fig. 4**). Lutein and Chl showed distinct light-dependence profiles. This shows that even at high biomass densities reached during deceleration phase, high light is sensed, most likely as a result of flashing effects.

Heterotrophy in total darkness resulted in lower pigment contents than under weak light (5 $\mu mol.m^{-2}.s^{-1}$ PAR), which was efficient to promote pigment accumulation on both substrates, except for *C. vulgaris* (**Fig. 5**). Noteworthy, in *S. acutus* the pigment content on glucose under weak light was as high as in photoautotrophically-grown cells. Pigment content was also dependent on the carbon source (glucose or acetate), in a species-dependent manner.

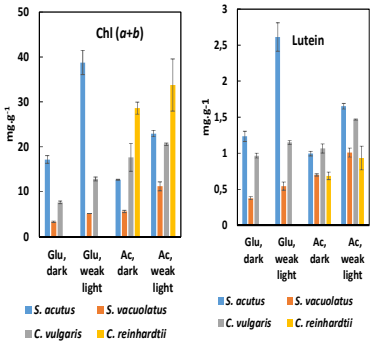

Fig. 5. Chl and lutein biomass contents of heterotrophically grown cells (exponential phase) and effect of weak light (5 $\mu mol.m^{-2}.s^{-1}$ PAR).

Conclusions

While the specific growth rate is an indication of growth capabilities, it is of little interest for evaluating the potential of a microalga for photoautotrophic biomass production, which is performed at high biomass densities. We showed for that the more relevant biomass productivity parameter (based on Vmax) can be estimated after proper correction of growth curves obtained with multicultivators. Heterotrophic cultures, which are not limited by light penetration, can be characterized by their specific growth rates (μmax). In some species, weak light can be used to promote pigment accumulation under heterotrophy on glucose or acetate.

References

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3. Killam A & Myers . (1956) A special effect of light on the growth of Chlorella vulgaris. American journal of Botany. Vol 34, No.8, pp. 569-572.