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Analysis of Microalgae Growth in Residual Light: a Diagnostics Tool for Low-Cost Alternative Cultural Media

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Chapter 1 Most microalgae grow in photoautotrophy and some benefit from mixotrophy and their growth essentially depends on the effective available light intensity. Non transparent media are frequently obtained as the byproduct of bioprocessing but laboratory testing on the suitability of such media may provide misleading results even in laboratory scale photobioreactors.
Chapter 2 Cicci et al. (2013) introduced the procedure for calculating a semi-empirical normalised growth rate accounting for a time-varying light limitation inside symmetric photobioreactors and permitting to cancel out the effects of unknown quantities in the photosynthetic response of the microalgal biomass and help diagnose the nutritional suitability of the composite medium.
This paper presents the results of applying the semi-empirical normalised growth rate to microalgal growth experiments carried out on media obtained from an anaerobic cattle digestate and from an olive oil mill wastewater (OOMW) and discusses use, inherent opportunities of the proposed diagnostic tool.

1. Introduction

Chapter 3 When it comes to growing microalgae for low-valued applications, the impact of every cost item should be minimised and any growth promotion potential should be exploited. Byproducts from agro-food processing are prime candidate for reuse both because they convey nutrients and sometimes organic carbon which are required (or may be used) by the microalgal biomass and because they need to be treated before discharge anyway.
Chapter 4 Most microalgae grow in photoautotrophy and some benefit from mixotrophy. Therefore their growth essentially depends on the effective available light intensity. Within a photobioreactor, photosynthetically active light is maximum near the liquid boundary facing light supply and reduces at a distance from it. The reduction of useful irradiance is due to absorption from the microalgae capture itself, but non-transparent media (both due to dissolved and suspended material) add up to the absorbance contribution of the former to a non negligible degree.
Chapter 5 Non transparent media are frequently obtained as the byproduct of processing in the agro-food and in ancillary domains, and they are frequently assayed for their suitability as growth media for various microbial biomasses, including microalgae. However, the suitability of such media, judged by the specific growth rate obtained therein may be misleading due to the low transparency of the byproduct itself and to the length of the light path even in laboratory scale photobioreactors.
Chapter 6 Cornet et al. (1995) introduced the concept of working illuminated volume, based on a semi-rigorous estimation of both scattering/absorption effects of microalgal biomass in an illuminated volume in an otherwise clear growth medium. However, their approach lacks easy mathematical tractability and does not easily lend itself to considering the simultaneous absorption effects determined by the suspended biomass and by a coloured culturing medium.
Chapter 7 Cicci et al. (2013) introduced the procedure for calculating a semi-empirical normalised growth rate accounting for a time-varying light limitation inside symmetric photobioreactors. Key points of the

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proposed approach are: (1) accounting for the calculated volume distribution of residual light obtained from absorbance measurements carried out during the culturing time and (2) normalising the specific growth rate to that measured in conditions of infinite (or very high) dilution of the medium. Together, (1) and (2) permit cancelling out the effects of unknown quantities in the photosynthetic response of the microalgal biomass and help diagnose the nutritional suitability of the composite medium.
This paper presents some significant test cases where this procedure is applied on biomass growths carried out on media obtained from an anaerobic cattle digestate (Cicci et al., 2014) and from olive mill wastewater (Cicci et al., 2013) for analysing nutritional scarcity and toxic effects.

2. Modelling of Specific Growth Rate in Varying Light, Nutritional Shortage and Toxic Effects

In order to separate the nutritional/toxic effects and the energetic effect (deriving from different illumination) of media composition, residual light intensity in a cylindrical photobioreactor, containing a suspension of microalgae in an assigned culture medium with a specific absorbance spectrum, was expressed as a function of the radial position. Then, the instantaneous specific growth rate in the reactor volume was calculated by integration. The average specific growth rate during the whole run was calculated by substituting the instantaneous specific growth rate calculated at the average absorbance of the culture (medium + microalgae) to the integral over the culture lifetime of the instantaneous specific growth rate at the prevailing optical density. The synthetic culture medium was considered transparent. The reference wavelength for the calculation of absorbance and local specific growth rate was assumed to be 690 nm, which is very close to the absorbance peak of chlorophyll, and the wavelength at which experimental determinations are usually carried out. in order to cancel out unknown constants in the Monod form of specific growth rate (μ_{max} e K_I) the calculated average specific growth rate was normalised by dividing it by the value of the average specific growth rate calculated for the culture developed on the synthetic medium.

As a consequence of the Lambert-Beer law, the light intensity at depth *x* in a suspension is:

$$I = I_o \cdot e^{-A \cdot x} \tag{1}$$

Within a cylindrical photobioreactor light intensity decreases by effect of depth and increases by effect of its distribution on an area which is *r*/R times smaller than peripheral surface, so that the following applies:

$$I(r,t) = I_o \cdot \frac{R}{r} e^{-A(t)\cdot(R-r)} \tag{2}$$

where *r* is the generic radial position and R is the internal radius of the cylindrical photobioreactor.
The instantaneous specific growth rate can be written as:

$$\mu(r,t) = \mu_{max} \frac{I(r,t)}{K_I + I(r,t)} \tag{3}$$

where:

$$\mu^* = \mu_{max} \cdot \frac{S_N}{K_{S_N} + S_N} \cdot \left(1 - \frac{S_t}{S_{t,max}}\right) \tag{4}$$

where N refers to the limiting nutrient compound and t to the most toxic compound which is present in the raw wastewater. For a wastewater which contains an excess of nutrients and no toxic compounds, which we will assume initially, μ^* equals μ_{max}. For an illumination intensity below the saturation value, Eq. (4) may thus be approximated by:

$$\mu(r,t) \sim \mu_{max} \frac{I(r,t)}{K_I} \tag{5}$$

The volume-averaged instantaneous specific growth rate can be calculated by integration:

$$\bar{\mu}(t) = \frac{\int_0^R \mu(r,t)\cdot 2\pi r\cdot dr}{\int_o^R 2\pi r\cdot dr} = \frac{2\cdot\mu_{max}\cdot I_o}{\pi R^2\cdot K_I}\cdot\int_0^R \frac{R}{r} e^{-A(t)\cdot(R-r)}\cdot 2\pi r\cdot dr \quad (6)$$

which symplifies into:

$$\bar{\mu}(t) = \frac{2\cdot\mu_{max}\cdot I_o}{A(t)\cdot R\ \cdot K_I}\cdot\left[1-e^{-A(t)\cdot R}\right] \quad (7)$$

Assuming the cultural medium absorbance to be constant along the culture lifetime, and denoting:

$$\bar{A} = A_{CM} + \frac{A_B(t=t_o)+A_B(t=t_f)}{2} \quad (9)$$

as the average absorbance of the culture during the experimental run, the time-averaged specific growth rate during the experimental run can be expressed as:

$$\bar{\bar{\mu}} = \frac{2\cdot\mu_{max}\cdot I_o}{\bar{A}\cdot R\ \cdot K_I}\cdot\left(1-e^{-\bar{A}\cdot R}\right) \quad (8)$$

The estimated growth rate estimate relevant to the generic medium mxxx can be normalised by dividing it by the estimated value at infinite dilution in the same medium, i.e., that in the synthetic medium (Ctr); in the absence of any limitation and toxic effect coming from the wastewater, μ_{max} can be simplified and a (dimensionless) Semi-Theoretical Normalised Specific Growth Rate (STNSGR) can be defined:

$$\mu_n(mxxx) = \frac{\bar{A}(Ctr)}{\bar{A}(mxxx)}\cdot\frac{\left[1-e^{-\bar{A}(mxxx)\cdot R}\right]}{\left[1-e^{-\bar{A}(Ctr)\cdot R}\right]} \quad (10)$$

It should be noted that the only experimental quantity in this expression is the average absorbance defined by Eq. (8). In analogy, we can calculate a ratio of the measured specific growth rates at the various dilutions to the specific growth rates at the maximum dilution ratio, that is, the Experimental Normalised Specific Growth Rate (ENSGR in the following). We should pay attention to the fact that, in general, $\mu^* \neq \mu_{max}$. Therefore, the ratio of the measured specific growth rate to the specific growth rates at the maximum dilution ratio equals:

$$\mu_n^e(mxxx) = \frac{\frac{S_N\cdot D}{K_{S_N}+S_N\cdot D}\cdot\left(1-\frac{S_t\cdot D}{S_{t,max}}\right)}{\frac{S_N\cdot D_{max}}{K_{S_N}+S_N\cdot D_{max}}\cdot\left(1-\frac{S_t\cdot D_{max}}{S_{t,max}}\right)}\frac{\bar{A}(Ctr)}{\bar{A}(mxxx)}\cdot\frac{\left[1-e^{-\bar{A}(mxxx)\cdot R}\right]}{\left[1-e^{-\bar{A}(Ctr)\cdot R}\right]} \quad (11)$$

where D the volume fraction of the wastewater in the medium employed for the culture, S_t is the actual concentration of toxic component and $S_{t,max}$ the concentration of the toxic component that annihilates growth. By taking the ratio of ENSGR to STNSGR and denoting it by the symbol μ_R, we can write:

$$\mu_R = \frac{\frac{S_N\cdot D}{K_{S_N}+S_N\cdot D}\cdot\left(1-\frac{S_t\cdot D}{S_{t,max}}\right)}{\frac{S_N\cdot D_{max}}{K_{S_N}+S_N\cdot D_{max}}\left(1-\frac{S_t\cdot D_{max}}{S_{t,max}}\right)} \quad (12)$$

by definition μ_R equals unity for $D\rightarrow D_{min}$. When plotting μ_R as a function of D, it is useful to expand the abscissa near the maximum dilution by taking the natural logarithm of D.

3. Results and Discussion

The meaning and use of the introduced quantities is illustrated in Figure 1. The data reported by Cicci et al. (this conference) were used to calculate the quantities themselves. The semi-theoretical growth rates reflect the expected growth rate, normalised by the value at the maximum dilution considered. In the case of the balanced medium, which is transparent, the ratio is unity.

For any non-transparent wastewater fraction, the medium offers an opacity to light transmission which depends upon its dilution (assumed with a transparent medium). Biomass absorbance adds up to medium absorbance to yield total absorbance which dictates effective growth-enabling irradiance. When medium NSTSGR exhibits a maximum at an intermediate dilution, this means that the maximum irradiance is

observed at an intermediate dilution because digestate absorption is the controlling factor at the minimum dilution and absorbance by the (rapidly developed therein) biomass is controlling factor at the minimum dilution. μ_R values calculated from growth tests carried out at different dilutions of the same medium can be plotted versus the logarithm of dilution and the obtained plot can be inspected for trends or maxima to derive information regarding the medium compatibility with the cultivated biomass. If light availability were the only parameter having a growth rate-related effect, μ_R would be constant across the dilution range. Conversely, if μ_R exhibits deviations across the dilution range, a signal is provided that other demotion or promotion effects add up to the bare light energy-growth rate balance. For instance, if a decreasing trend is observed, it is suggestive of an additional concentration-dependent growth demotion effect, e.g. a toxic effect.

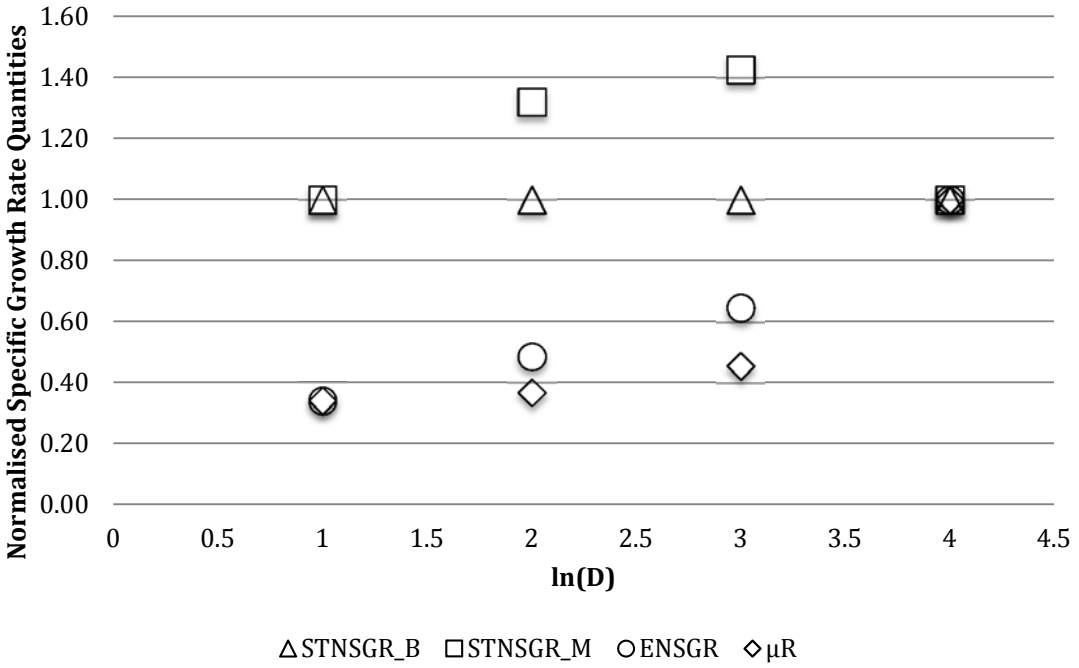

Figure 1: Illustration of the various normalised specific growth rate quantities involved in the analysis as a function of culture medium dilution. STNSGR_B and STNSGR_M refer to the semitheoretical growth rate calculated separately using the biomass or the coltural medium absorbance, respectively.

The μ_R's were calculated as a function of the dilution and compared to the experimental values for both *Scenedesmus dimorphus* 1237 and *Arthrospira platensis* (Figure 2).

For *A. platensis*, n digestate with balanced medium μ_R exhibites a maximum (Figure 2a). This hints that specific growth rate might benefit from adding (a small amount of) digestate to the culture medium, despite of its lower transparency. Further additions result in a collapse of growth but, at high concentration, the effect of diluted digestate seems to "stabilise": once the effect of reduced transparency has been cancelled out, it appairs to behave similarly to a balanced medium. If digestate dilution is carried out with tap water, and referring μ_R to the maximum dilution used, growth rate drops very fast (Figure 2a). By comparing the two rightmost points both in Figure 2a and Figure 2b, one may conclude that while digestate is supporting growth (likely owing to its organic carbon supply) as much as it hinders it, it is by no means a complete medium and at higher concentration it results in increasingly unbalanced formulation.

It can be observed that *S. dimorphus* 1237 μ_R exhibites a sharp decrease of growth rate mainly at high dilutions. Again, concentrated digestate exhibits a smoothing effect arguably due to the increased biomass growth support awarded by higher organic compounds supply.The same can be observed when dilution is carried out with tap water instead of balanced medium but, surprisingly, with a lower growth demotion.

OOMW always causes a larger growth rate reduction compared to the limiting light effect (Figures 2E and 2F). The reduction is more consistent for *A. platensis* than for *S. dimorphus*. For this latter, a large part of this reduction effect seems to occur over the lower reduction range and seems to somewhat stabilise at higher concentrations. This owes, possibly, to increasingly concentrated additional nutrient sources given that *S. dimorphus* is not a mixotroph, and thus cannot benefit from organic substrates.

An important effect that may offer room for practical exploitation is growth promotion, which does come from the fact that experimental growth rate decreased, with respect to the control culture in transparent medium, less than it would be expected from the calculated average light decrease. Promotion, here, denotes therefore an opportunity for enhancing growth and productivity by tailoring culture illumination, e.g. resorting to short light-path photobioreactors.

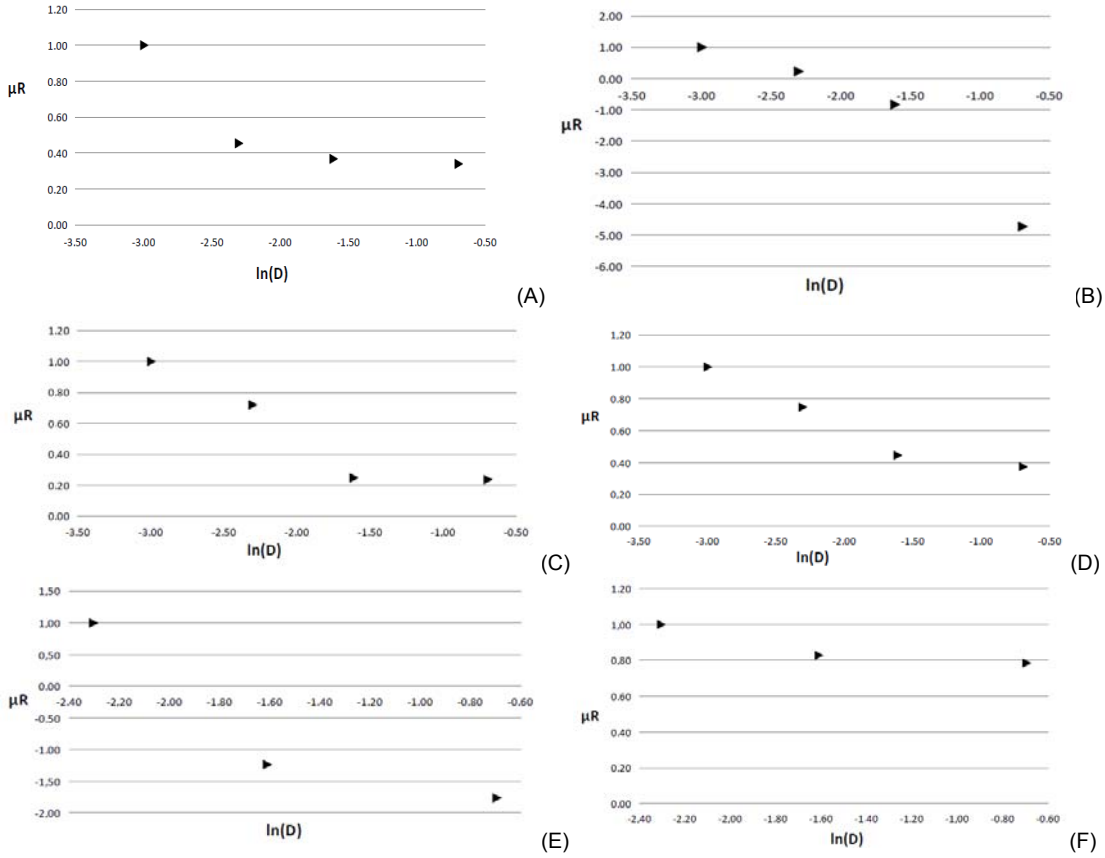

Figure 2: Normalised specific growth rates in digestate surnatant diluted with balanced medium and tap water for A. platensis (A and B, respectively) and S. dimorphus (C and D, respectively) and in OOMW diluted with balanced medium for A. platensis (E) and S. dimorphus (F).

As a first approximation, from Eq. (9) the gain expected from lowering the light path length in linear, low-light regime is in the order of the ratio of light path lengths for equivalent geometries: R_1/R_2 for uniformly illuminated cylindrical photobioreactors, s_1/s_2 for layered photobioreactors illuminated from one side only (s meaning optical thickness).

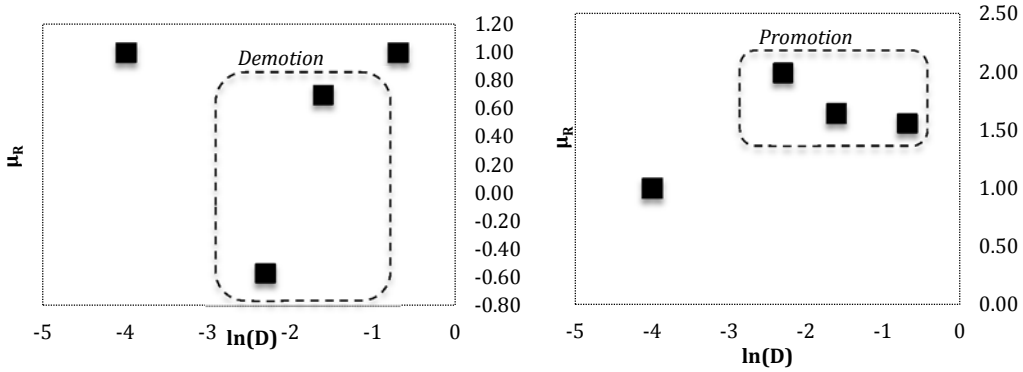

Figure 3: Demotion and promotion phenomena for S. dimorphus and Arthrospira platensis cultures in OOMW at various dilutions with the respective balanced medium.

Figure 3 shows that growth is demoted in concentrated OOMW for *A. platensis* (Figure 3a) but is promoted for *S. dimorphus* (Figure 3b).
The present analysis should be taken as an example of how the characteristics of a wastewater can be experimentally analysed for the purpose of microalgae production. However, the results should be taken with care because aging significantly changes the characteristics of a raw wastewater. Light path tailoring can be a useful opportunity, but actual gains should also take high-light effects (e.g., photoinhibition) into account. Most of these latter effects could be offset with a smart choice of the photobioreactor, e.g. by resorting to thin layer hydrodynamically flashed units such as the local recirculation photobioreactor (Torzillo et al., 2010 and Moroni et al., 2013).

4. Conclusions

Testing optically thick wastewaters as a substitute medium for the growth of microalgae generally shows some impairment with respect to a balanced culture in a transparent medium. This work showed that by taking into account the lower light availability, the variation of the growth rate at different dilution ratios can offer some insight as to the reasons (carence/toxicity) for such reduction, signal productivity gains opportunities and suggest the geometry to adopt to exploit it.

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