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Additional Information

Behavior of mixed Chlorophyceae cultures under prolonged dark exposure. Respiration rate modeling.

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ABSTRACT

The behavior of three different microalgal cultures, when exposed for a long period (>48 h) to dark conditions, was studied with a methodology based on respirometry. The cultures were transferred to darkness and the oxygen evolution in the reactors was monitored after successive air injections. Several sequential oxygen uptake rates were thus calculated and a respiration constant, assuming a first order decay of a fraction of the biomass, was obtained by calibration. Initial specific oxygen uptake rates were in the range of 0.9-5.1 mg $O_2 \cdot g\ TSS^{-1} \cdot h^{-1}$ and dark respiration constants in the range of 0.005-0.018 h^{-1}.

Keywords

Decay; microalgae; modeling; respiration.

1. INTRODUCTION

Interest in microalgae has risen in the last decade due to the potential these microorganisms have on fighting climate change (Kumar et al., 2011), producing renewable biofuels (Parmar et al., 2011) and removing pollutants from wastewater (Rawat and Kumar, 2010).

Predicting the behavior of microalgal cultures is a very complex task, especially when outdoor conditions (variable light and temperature) and operational conditions (light attenuation, photobioreactor dimensions, hydrodynamics, etc.) should be taken into account. However, the wider the knowledge about microalgal behavior and the ability to predict it, the easier it will be to optimize their cultivation and to fully develop their potential.

Different approaches have been taken to model algal growth. Many models were developed in order to represent the behavior of microalgae in rivers and lakes (Buzzelli et al., 2014; Reichert et al., 2001; Muylaert et al., 2005). Other models have been developed in order to predict metabolites production (lipids, carbohydrates, substances of interest, etc.) (Adesanya et al., 2014; Mairet et al., 2011; Packer et al., 2011). Some authors have also taken into account physical phenomena such as variable light and temperature, mixing, or gas transfer. (García-Camacho et al., 2012; Pegallapati and Nirmalakhandan, 2012; Vunjak-Novakovic et al., 2005).

When microalgae are cultivated in closed photobioreactors, microalgal biomass density increases and, due to selfshading, a fraction of the culture cannot receive the amount of light required for photosynthesis. This fact makes it necessary to take respiration losses into account, since they will be the dominant metabolic activity in the dark regions. This will also be the case for the whole culture during dark periods (nights) and inside dark

plant elements. In these cases, microalgal growth in the reactor will cease and therefore biomass loss will take place. Under optimal conditions, respiration rates are about 20-30 % of growth rates, but the ratio of respiration to growth increases under suboptimal conditions (Geider and Osborne, 1989). Several authors report respiration losses of 2-10% of biomass in outdoor cultivation systems (Kethesaan and Nirmalakhandan, 2013), although losses of up to 35% of the biomass have been reported during nighttime (Vonshak, 1987, for *Spirulina*).

When modeling microalgal growth there is usually a hidden term in the “net” growth rate which accounts for some dark respiration losses. Indeed, a fraction of respiration losses is proportional to microalgal growth. This fraction corresponds to the cost of biosynthesis: ion uptake, transformation of ions into intermediates, synthesis of cell structural and functional metabolites, etc. The other term which contributes to respiration losses is the maintenance metabolic costs (also called basal respiration: motility, volume regulation, turnover of macromolecules). These costs are, by definition, independent of growth and proportional to biomass (Geider et al., 1998).

Knowing the rate of microalgal respiration in the dark is important for microalgal growth modeling under outdoor conditions, since biomass production and net carbon balance are affected by day/night cycles and by the volume fraction of the photoreactors which is in the dark (Bernard, 2011). This affects the economic and environmental balance of the system. Moreover, gaining knowledge on the behavior of microalgae under prolonged exposure to dark will help designing other wastewater treatment systems with opaque modules such as those for microalgae storage, filtration, etc.

The aim of this work was to study the behavior of different microalgal cultures during prolonged exposure to darkness (>48 h). To our knowledge, no such long exposures

have been previously studied. Based on dissolved oxygen measurements (respirometry), a methodology was developed to obtain the associated respiration rate. A comparison of the behavior of the cultures and a possible explanation for the differences among them is also given.

2. MATERIALS AND METHODS

2.1. Microorganisms

Three different types of microalgae cultures were studied. Microalgae had previously been isolated from the walls of the secondary clarifier in the "Cuenca del Carraixet" WWTP (Valencia, Spain) and maintained as a mixed autochthonous culture in the laboratory in 7 l semicontinuous reactors (for a more detailed explanation of the reactors setup see Ruiz-Martinez et al., 2012). The effluent of a submerged anaerobic membrane bioreactor (SAnMBR, described in Giménez et al., 2011) was used as growth medium.

The cultures used in the experiments were the following:

- Dark kept culture (DC): this culture was stored in a dark fridge (8 ºC) for 15 days previous to the respirometric study. The culture was allowed to increase temperature until reaching 20ºC during 12 hours prior to the respirometry. It consisted of a mixed culture of microalgae dominated by the class Chlorophyceae.
- Nutrient limited culture (LC): this culture was the accumulated purge of a semicontinuous reactor kept under laboratory conditions as explained above. The purge was accumulated in a translucent container with no agitation nor CO_2 addition. pH did not increase higher than 8. The respirometric study started 48 h

after nutrient depletion, a total of 96 h after being purged from the reactor. The microalgae present were Chlorophyceae, of which >99% from the Chlorococcales order.

- Replete culture (RC): this culture was directly taken from a running semicontinuous reactor and placed under dark conditions immediately. The microalgae present were Chlorophyceae, of which >99% from the Chlorococcales order.

2.2. Experimental setup and operation

The experiments were carried out in three photobioreactors (PBRs). Each PBR consisted of a cylindrical, transparent methacrylate tank (20 cm internal diameter) with total and working volumes of 10 and 7 l, respectively. The PBRs were (not hermetically) sealed and they were kept in the dark in a climatic chamber with a temperature set point of 20 ºC. A pH probe connected to a multiparametric analyzer (CONSORT C832, Belgium) recorded the pH in the reactors, which ranged in all cases between 7 and 8.4.

Under the above mentioned conditions photosynthesis could not take place and only respiration (oxygen consumption for maintenance) occurred. Dissolved oxygen concentration (DO) was measured using a Cellox 325 electrode (WTW, Germany) connected to an oximeter (Oxi 320, SET WTW, Germany). The measured dissolved oxygen values were logged in a PC using data acquisition software. Ambient air was injected at a flow rate of 0.8 – 1.0 $l \cdot min^{-1}$ through four fine bubble diffusers mounted at the bottom whenever DO concentration was below 1 mg $O_2 \cdot l^{-1}$ until it reached again a concentration of 6 mg $O_2 \cdot l^{-1}$.

The experiments were programmed to last 50 h. However, in view of the results (see section 3) in the case of LC it was prolonged until 200 h and in the case of RC until 600 h.

2.3. Analytical Methods

Ammonium and phosphate were determined according to Standard Methods (APHA 2005) (4500-NH3-G and 4500-P-F, respectively) in a Smartchem 200 automatic analyzer (Westco Scientific Instruments, Westco). Total suspended solids (TSS) were determined according to Standard Methods (APHA 2005). All analyses were conducted in duplicate.

3. RESULTS AND DISCUSSION

3.1. Dissolved Oxygen Measurements and Respiration Rates

The time evolution of dissolved oxygen concentrations was obtained for the three experiments (figures 1a, b and c). Due to microalgal consumption in the dark, oxygen concentration decreased linearly after each air addition until it reached 1 mg $O_2 \cdot l^{-1}$ and the aeration was switched on again. Successive Oxygen Uptake Rates (OUR, mg $O_2 \cdot l^{-1} \cdot h^{-1}$) were calculated as linear regressions ($R^2 > 0.95$ for all) of these decreasing stretches (figure 1a). Due to the aeration, the DO concentration increased up to 6 mg $O_2 \cdot l^{-1}$, and then the blower was switched off. A significant decrease was observed in most cases immediately afterwards (first 10 minutes). This corresponded to the re-establishing of the equilibrium and had been previously observed in blank reactors with only tap water (data not shown).

Successive OURs became smaller with time, which means that endogenous respiration declined in all cases. This showed that, due to the adverse environmental conditions (darkness, absence of nutrients) and the consumption of microalgal reserves, the metabolic activity decreased. The calculated OURs (mg $O_2 \cdot l^{-1} \cdot h^{-1}$) during each experiment are represented in figure 2 versus time. It can be appreciated that the OUR values did not decrease linearly with time. Instead, they decreased exponentially, approaching a stable value in the long term (hence the prolonged experiment time for LC and RC).

At the beginning of the experiments, the OURs were 0.75 mg $O_2 \cdot l^{-1} \cdot h^{-1}$for DC and RC, and 1 mg $O_2 \cdot l^{-1} \cdot h^{-1}$ for LC. These values are however not directly comparable, due to the different TSS in the cultures. For comparison, TSS were measured in each reactor at t = 0 of each experiment, allowing the calculation of the specific respiration rate. Results are shown in table I: specific respiration rate was highest for LC and similar values were obtained for DC and RC.

Respiration rates depend on the physiological state of the culture (growth state, age of the culture) and environmental conditions (nutrients, cell content, light intensity, temperature) (Geider, 1989). For instance, Grobbelaar and Soeder (1985) reported lower respiration rates after growth at optimal temperature than at other temperatures, and after growth under weaker illumination than under stronger irradiations. Ogbonna and Tanaka (1996) also found that microalgae with higher cell carbohydrate content showed higher biomass loss during the night.

The values obtained in this study are in accordance with the range obtained by Grobbelar and Soeder (1985). The lowest value corresponded to RC, the replete or *“healthiest”* culture, whereas the highest value corresponded to LC, the culture which

was subject to nutrient stress and higher light (lower biomass concentration and therefore less selfshading results in higher light). A similar value was obtained for DC, the culture which, although it had been exposed to very dim light, was in the worst state regarding nutrient levels at the beginning of the respirometry.

Oxygen consumption of the microalgal cultures during the first hours after dark exposure (for instance, 12 hours of darkness in outdoor nights) can be estimated from the results of this study. Assuming that there is no microalgal growth during a long dark period, the COD balance indicates that the oxygen consumption rate is equal to the biomass COD loss. Therefore, the culture RC would present the smaller night COD loss: 11 mg COD·g TSS^{-1}, the culture DC would present a loss of 16 mg COD·g TSS^{-1}, and cultures under nutrient limiting conditions would loose around 61 mg COD·g TSS^{-1}, which could represent between 3 and 4% of the biomass. This depends on the biomass composition, since lipids, proteins and carbohydrates have different COD/TSS ratios and therefore their degradation results in different TSS losses for the same COD loss.

The resistance stage in which the culture entered allowed the microalgae to keep their photosynthetic capacity until the end of the respirometric experiment: a rapid increase in dissolved oxygen was observed when the light was switched on at the end of the three experiments (data not shown). This described survival strategy is in accordance with previous observations (Myers and Cramer, 1947; Geider et al., 1998) although not such long exposures to dark had been reported up to date.

3.2. Data fit and calibration

During endogenous respiration, microalgae catabolize accumulated metabolites, usually carbohydrates (Geider and Osborne, 1989; Ogbonna and Tanaka, 1996). Due to the

observed OURs (figure 2), and in agreement with literature (Buehner et al., 2009), in this study an exponential decay of biomass was assumed:

$$\frac{dx}{dt} = -k_r \cdot x \qquad (1)$$

where x represents the biomass, expressed in mg COD·l^{-1} and k_r (h^{-1}) is the respiration constant.

And since biomass decay equals the oxygen consumption rate:

$$\frac{dx}{dt} = -k_r \cdot x = \frac{dO_2}{dt} \qquad (2)$$

The combination and integration of equations 1 and 2 renders:

$$\exp(-k_r \cdot t) = \frac{\left[\frac{dO_2}{dt}\right]_t}{\left[\frac{dO_2}{dt}\right]_0} \qquad (3)$$

which enabled the reproduction of the obtained data (figures 3a, b and c) and determine with a good fit ($R^2 > 0.9$ in all cases) the respiration constant k_r for each culture (table I). An ANOVA analysis (carried out using SPSS 16.1) of the three sets of data represented in figures 3a, b and c confirmed the difference (P-value < 0.05) among the cultures (2 degrees of freedom, F value 3.33).

The obtained k_r values were, as expected, different for each culture. Other authors have determined smaller (0.006 h^{-1} for *Nannochloropsis* by Hueseman et al., 2013; 0.0004 h^{-1} for *Chlorella* by Decostere et al., 2013), similar (0.015 h^{-1} for *Nannochloropsis* and *Scenedesmus* by Pegallapati and Nirmalakhandan, 2012) and higher (Sciandra, 1985 obtained a range of 0.006 – 0.03 h^{-1} for *Phaeodactylum*) respiration constants.

A higher respiration rate (DC) means a faster decrease in the OUR values consecutively measured, and a lower respiration rate achieved after a set amount of time. A smaller

respiration rate (RC) means a more stable value of the consecutive OURs measured, which become smaller with time but at a much slower speed.

4. CONCLUSIONS

In this study specific dark respiration rates were obtained for three different microalgal cultures by measuring dissolved oxygen evolution after being transferred to darkness. The smallest specific respiration rate (0.9 mg $O_2 \cdot g\ TSS^{-1} \cdot h^{-1}$) was obtained for a fresh and nutrient replete Chlorophyceae culture, whereas the highest specific respiration rate (5.1 mg $O_2 \cdot g\ TSS^{-1} \cdot h^{-1}$) corresponded to a nutrient limited culture previously grown under continuous illumination. The methodology used proved useful for the calibration of an exponential decay equation and obtention of the respiration constants for each case, which ranged from 0.005 h^{-1} to 0.018 h^{-1}. The accuracy of current basic models which otherwise do not take respiration into account could be improved by including this decay equation and determining the corresponding respiration constant.

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TABLE I

Table I: Initial specific respiration rates and obtained respiration constants for the three cultures

	Dark Culture	Limited Culture	Replete culture
Initial TSS ($mgTSS \cdot l^{-1}$)	588	206	833
Initial dark respiration rate ($mg\ O_2 \cdot g\ TSS^{-1} \cdot h^{-1}$)	1.3	5.1	0.9
k_r (h^{-1})	0.018	0.014	0.005

FIGURE LEGENDS

Fig 1: Dissolved Oxygen concentration during the experiments. DC (a), LC (b) and RC (c)

Fig 2: OURs (mg $O_2 \cdot l^{-1} \cdot h^{-1}$) measured over time in DC, LC and RC systems.

Fig 3: Data fit to equation 3 for DC (a), LC (b) and RC (c)