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Scaling-up sustainable *Chlorella vulgaris* microalgal biomass cultivation from laboratory to pilot-plant photobioreactor, towards biofuel

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Abstract

Unicellular microalgal culture represents a new opportunity for producing significant biofuel quantities in the future along with other specialty products, due to several major advantages microalgae species present when compared to conventional crops, including much faster growth rates, cultivation in a variety of environments and photobioreactor systems, and almost 100% recycling of nutrients. In the current research, the scaling-up of the cultivation of Chlorella vulgaris microalgae to a 4 m^3 pilot-plant photobioreactor is examined, compared to the performance of a 25 L automated laboratory bioreactor. Beyond the size and configuration, the main differences of the two bioreactors are the mode of operation, the illumination nature and depth, the temperature, and pH. Specifically, temperature and illumination are naturally varying from day to day and season to season into the pilot-plant photobioreactor that is set inside a greenhouse. The specific growth factor appears to be higher for microalgal cultivation in the laboratory bioreactor. It is also found that the growth kinetics is severely slowed down during the winter months. This is primarily due to the low temperatures and the poor illumination observed during winter.

Keywords: Scale-up, pilot-plant, photobioreactor, microalgae, *Chlorella vulgaris*, biomass, growth kinetics, sustainable, biofuel.

1. Introduction

Large scale microalgae cultivation represents a potential new source of sustainable energy in the form of biofuel. Microalgae are individual plant cells which have the ability to photosynthesize and, therefore, utilize CO_2, micronutrients and solar light to multiply and produce carbohydrates, lipids and proteins. Major advantages of microalgae over land grown crops are a much faster growth rate and the potential for cultivation in large scale in either closed photobioreactors or open ponds. As microalgae culturing removes atmospheric CO_2, their cultivation can be potentially scaled up in such a way as to absorb CO_2 emissions from fossil fuel burning factories. Additionally, the spent algal mass, after the oil extraction, can be used either as an animal feed, as it contains substantial quantities of proteins, carbohydrates, some remaining oil and various micronutrients or as an agricultural soil additive-nutrient enhancer. Also, opportunities arise for the exploitation of their micronutrient content.

In the last years, several studies, both on fundamental and on application-oriented basis, regarding microalgae cultivation for sustainable biofuel production have been reported in literature (Kothari *et al.*, 2017; Chen *et al.*, 2015; Matos *et al.*, 2014; Mata *et al.*, 2010). This constitutes a priority for many policy makers, especially in places where conditions are favourable for cultivation of microalgae. Oil prices, limited resources, and environmental pollution, including greenhouse gas effect caused by fossil fuel burning, are pressing for alternative recycled fuel forms. Particularly, current research effort on biofuel production from algae mainly focuses on cultivating microalgae due to its less complex structure, fast growth rate, and high oil content. Several studies report that microalgae utilization as feedstock for biofuel production represents a great challenge nowadays and that it has a lot of advantages compared to conventional terrestrial crops (Simas-Rodrigues *et al.*, 2015; Singh and Gu, 2010; Lardon *et al.*, 2009). Further noticeable advantages of microalgae that reveal their potential to serve as "solar-powered factories" compared to plant-based production systems are also highlighted in other works (Panagiotidou *et al.*, 2014; Hempel *et al.*, 2011). Moreover, spent microalgal biomass has been proved to act as an accelerator agent in biodegradation of soil organic matter, without imparting negative effects on soil chemical properties (Gougoulias *et al.*, 2018).

The cultivation of microalgae can take place in a variety of photobioreactors: both open and closed systems as well as several bioreactors having unique characteristics have

been proposed (Arun *et al.*, 2017; Catarina Guedes and Xavier Malcata, 2011; López *et al.*, 2006; Choi *et al.*, 2003; Sánchez Mirón *et al.*, 2002; Molina *et al.*, 2001). Major configurations of photobioreactors used are the open raceway bioreactor (open system), and from the closed systems, the major representatives are various configurations of tubular bioreactors, the flat plate bioreactors and the air lift and bubble bioreactors. Each has distinct advantages and disadvantages. Open systems are simpler and cheaper to construct, while closed systems incur higher construction costs. However, biomass productivity is higher in closed bioreactors, and also culture in closed bioreactors is not likely to be contaminated by other species.

The culture conditions (system variables) significantly affect kinetics and biomass yield. The major variables of a production system are: 1) the type of microalgae, 2) the temperature, 3) the light flux, 4) the solution pH, 5) the composition of the culture media, 6) the aeration rate, and generally the hydrodynamics of the reactor, 7) the CO_2 feed and 8) the critical cell density. Most of these variables not only affect the kinetics and biomass yield but also the macronutrient content and the lipid profile of the biomass (Yusof *et al.*, 2011; Liang *et al.*, 2009). Adopting an optimal nutrient limitation strategy can enhance lipid productivity, and also result in both cost savings by avoiding unnecessary nutrient additions and sustainable use of resources (Sakarika and Kornaros, 2017). Changing the population of symbiotic bacteria can also have positive effects on the microalgal biomass growth (Kim *et al.*, 2015). Thus, in such a complex system, it is important to define the specific product that is to be produced, whether it is the lipids or the protein content or something else, so that the system parameters can be tailored towards production optimization.

In the current research, the scaling-up of the cultivation of the microalgal species *Chlorella vulgaris* to a 4 m^3 pilot-plant photobioreactor is examined, compared to the performance of a 25 L automated laboratory bioreactor. The performance of the pilot-plant bioreactor, which is an open pond system operated in a greenhouse, is first evaluated and compared during the winter and summer periods in order to determine variability in biomass production, which is mainly related to differences in light levels and temperatures during these two periods. Then, the kinetics and biomass productivities during culture in the pilot-plant photobioreactor and a flat-plate 25 L closed laboratory bioreactor are compared. Beyond the size, main differences of the two bioreactors are the mode of operation, the nature and the depth of illumination, the temperature and pH. In the laboratory bioreactor, all parameters are controllable, and set near optimum values, while in the pilot-plant bioreactor, except the pH, all other parameters are not controlled. Specifically, the pilot-plant bioreactor is set inside a greenhouse, and temperature and illumination are naturally varying from day to day and also season to season. The parameters that are compared are the reactor critical cell density, the biomass growth kinetics, the maximum bioreactor biomass concentration attained and the biomass and oil productivity.

2. Materials and methods

The microalgae *Chlorella vulgaris* has been obtained from Experimental Phycology and Culture Collection of Algae at the University of Goettingen Germany (EPSAG). It has been kept at 4°C and each vial was used within 3 months. The growth medium was of the following constitution suggested by Andersen *et al.* (1991): Each 50 L of the growth medium contains: 10 g KNO_3, 1 g K_2HPO_4, 1 g $MgSO_4 \cdot 7H_2O$, 1500 mL of soil extract and 250 mL of solution containing the following micronutrients: (1 mg $ZnSO_4 \cdot 7H_2O$, 2 mg $MnSO_4 \cdot 4H_2O$, 10 mg H_3BO_3, 1 mg $Co(NO_3)_2 \cdot 6H_2O$, 1 mg $MoO_4 \cdot 2H_2O$, 0.005 mg $CuSO_4 \cdot 5H_2O$, 700 mg $FeSO_4 \cdot 7H_2O$ and 800 mg EDTA)/L. Also, traces of vitamin B12 were added into the growth media.

The three circular pilot-plant bioreactors (2.10 m in diameter and 40 cm in height) were set in the greenhouse in series. The overflow of each reactor can be directed as feed to the next one. Therefore, the three reactors can be operated either as one in a continuous mode or separately in a batch or semi-batch mode. In the current experiments, they were operated in a batch mode.

The laboratory bioreactor is a 25 L flat-plate photobioreactor (Photon Systems Instruments). The back cover is illuminated with two sets of LED light, white and red, whose intensity can be independently set. The LED illumination can be operated either continuously or in pre-set frequencies. The bioreactor is fully aerated from a bottom. Its temperature is controlled and the concentration of dissolved CO_2 and the pH are continuously monitored and shown during the cultivation period. The illumination intensities used in the experiments were: White LED: 520 μmol(photon)/(m^2-sec) and Red LED: 120 μmol(photon)/(m^2-sec).

Figure 1. The 25 L laboratory flat plate photobioreactor

All bioreactors set in the greenhouse were subjected to exactly the same growing conditions. Temperature was allowed to vary naturally but, in one of the three bioreactors, the temperature was set at 30°C, to compare its performance with a second bioreactor where temperature during the winter months was much lower. The growth media pH and the temperature were monitored daily. Biomass concentrations were measured indirectly by measuring the absorbance (665 nm) using a UV/Vis spectroscopy instrument. Mass concentrations on a dry basis (mg dry algal mass/L of growth medium) were

determined from the volume of medium and the weight of algal mass after evaporation of the water and drying of the sludge, and calibrated with the absorbance readings. Absorbance readings of 0.1, 0.3, 0.5, and 0.7 correspond approximately to a mean algal biomass concentration of 110, 280, 700, and 1300 mg/L respectively. Above absorbance values of 0.3, the relation between absorbance and biomass concentration becomes progressively non-linear.

3. Results and discussion

3.1. The biomass growth kinetics

The microalgal biomass growth kinetics is presented in Figures 2 and 3.

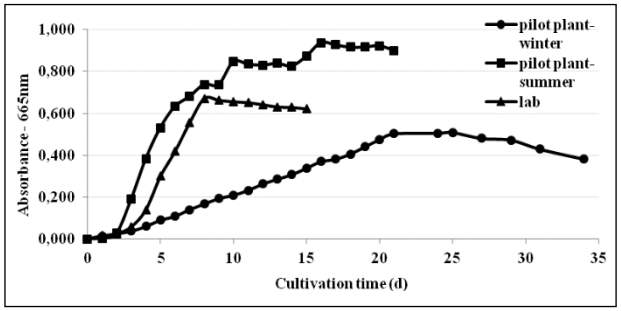


Figure 2. Comparison of biomass growth kinetics during winter and summer in the pilot-plant photobioreactor and also in the laboratory bioreactor

3.2. The specific growth rate

The instantaneous *specific growth rate* is given by Equation 1:

$$\mu = (1/X)(dX/dt) \quad (1)$$

where (in Eq. 1) X is the concentration of biomass in mg/L and t is the cultivation time in days (d). By integrating Equation 1, Equation 2 is obtained:

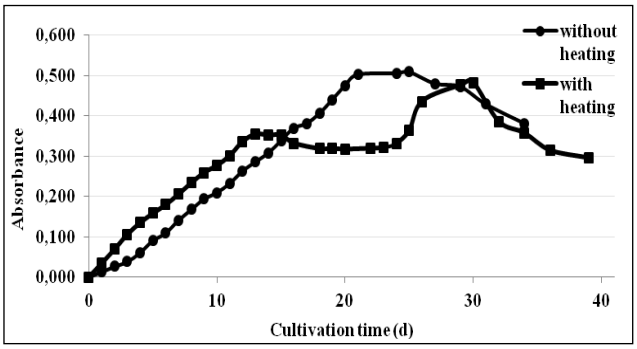
$$\mu(t) = [\ln(X(t)/X_o)]/t \quad (2)$$

However, by observing Figure 2, it becomes evident that two different specific growth rates should be used for cultivation in a batch reactor. The average growth rate μ_{av} (1/d), which is obtained from time 0 (inoculation time) until the time where the stationary phase is achieved and harvesting of the cells can begin, as well as μ_{exp} (1/d), which is the specific growth rate during the exponential growth phase, and is obtained by integrating between the time the exponential growth begins and the time it ends (beginning of the stationary phase), are are given by the following two equations (Equations 3 and 4):

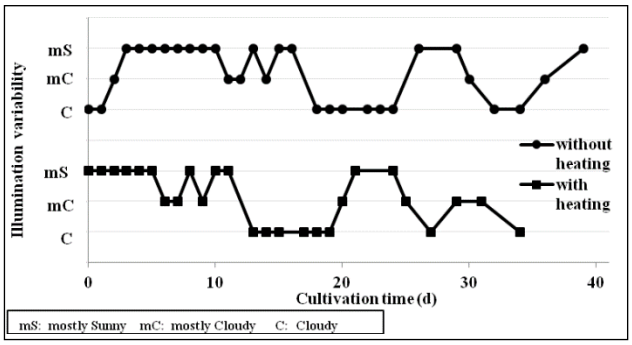
$$\mu_{av} = [\ln(X_{max}/X_o)]/t_T \quad (3)$$

$$\mu_{exp} = \ln[X_2/X_1]/t_{exp} \quad (4)$$

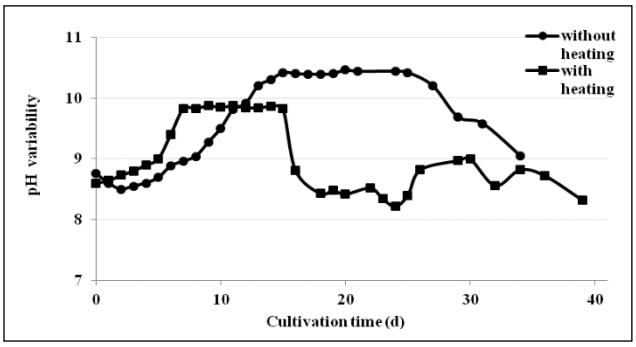
where X_{max}, X_o, X_2 and X_1 are the concentrations (or absorbances) of the biomass in the stationary phase, just after inoculation, in the beginning of the exponential phase, and at the end of the exponential phase respectively. The times t_T and t_{exp} are the times to reach the stationary phase from inoculation and the time of the exponential growth rate respectively.



(a)



(b)



(c)

Figure 3. Comparison of biomass growth kinetics (a); natural variation of illumination (b) as well as of pH (c), upon cultivation of *Chlorella vulgaris* in the pilot-plant photobioreactor during winter, heating (T held constant at 30°C) and without heating

From the results in Figure 2 and the Equations 3 and 4, the following data are obtained and shown in Table 1, for the average specific growth rate and for the exponential specific growth rate for the biomass growth kinetics: in the pilot-plant bioreactor during a) winter and b) summer, as well as c) in the laboratory reactor.

It can be noted (Table 1) that the average specific rate is highest for the laboratory bioreactor when compared to the pilot-plant one during the summer, although in the pilot-plant reactor during summer a higher biomass concentration is attained. This is due to the fact that the stationary phase is attained in a shorter time in the laboratory bioreactor. However both rates in the exponential growth period are similar for cultivation in the pilot-plant during summer and in the laboratory

bioreactor. Average summer temperatures in the growth media in the pilot-plant bioreactor are 28-29ºC, comparable to 30ºC in the laboratory bioreactor. Main differences between the two types are: a) the reactor configuration, b) the better aeration achieved in the laboratory bioreactor, c) differences between artificial LED (white and red) illumination and natural illumination between the laboratory bioreactor and the pilot-plant bioreactor respectively, and d) pH values during the growth period. The natural average illumination flux in the visible spectrum inside the greenhouse during the summer and winter growth period is about 440 and 55 W/m^2 which, corresponds to 2010 and 250 μmol(photon)/(m^2-sec) respectively. It is worth noting here that, at high light flux values, inhibition effects may be present.

Table 1. The specific growth rates μ_{av} and μ_{exp} for the cultivation of *Chlorella vulgaris* in the pilot-plant photobioreactor during winter and during summer, and in the laboratory bioreactors

Growth kinetics	Pilot-plant reactor (winter)	Pilot-plant reactor (summer)	Laboratory reactor
μav (1/d)	0.10	0.12	0.14
μexp (1/d)	0.17	0.42	0.39
tT (d)	20	12	11
texp (d)	10	3	4
Xmax/Xo	0.43/0.06	0.83/0.19	0.63/0.14
X2/X1	0.35/0.06	0.68/0.19	0.67/0.14
I μmol(photon)/ (m^2-sec)	250	2010	640

Both specific rates are substantially lower in the pilot-plant bioreactor due to lower temperatures and much poorer illumination. However between these two, it appears that the illumination intensity is the rate limiting factor when temperatures are adequate. This is inferred by comparing the two biomass curves in Figure 3a for the biomass kinetics in the pilot-plant reactor during winter. One curve is obtained by heating the bioreactor to a steady 30ºC and the other when the bioreactor is not heated (average temperature of 18ºC, minimum and maximum temperatures 15ºC and 23ºC respectively). Although, these data were obtained during a different period within the same month and the cloudiness of the sky differed as shown in Figure 3b, no significant differences are noted for the two cases, both with concern to the maximum biomass concentration attained and the kinetics during the exponential growth. Slopes in both curves during the exponential growth are similar, indicating that the aforementioned temperature difference between the two bioreactors did not affect growth. Maximum absorbances are approximately 0.48 (with heating) and 0.52 (without heating) separated by about 7 days, apparently due the extended cloudiness during cultivation early in the exponential growth phase in the heated bioreactor. The dip during the exponential growth rate for the pilot-plant bioreactor observed at the 12^{th} day is probably due to an extremely low natural illumination recorded during this time period (Figure 3b). Therefore it appears that the illumination intensity is the rate limiting step for microalgae growth. Microalgae naturally are exposed to changing light conditions. Indeed, the quality and the quantity of light are actually very important factors for microalgae growth, as also highlighted in other study of the operating conditions for the autotrophic microalgae *Chlorococcum sp.* growth and lipid production (Aravantinou and Manariotis, 2016). Moreover, to promote a faster microalgae growth rate associated with a higher light intensity, while also avoiding potential photodamage that can lead to a decline in growth rate to a certain degree a model of photosynthetic growth including photoacclimation was proposed (Straka and Rittmann, 2018).

Furthermore, it can be noted from Figures 3b and 3c, that, when the illumination intensity drops so does the pH of the growth media. Increased photosynthetic activity during high natural illumination causes an increase in the utilization of dissolved CO_2, and thus a decrease in its value, which correspondingly leads to an increase in pH value.

3.3. The critical mass density

The relationship between the illumination intensity (I) and the thickness (y) of penetration is given by Equation 5:

$$dI(y) = -I(y)\varepsilon X dy \quad (5)$$

By integrating Eq. 5 from y = 0 where I = Io to y = y_ϕ where the intensity drops to 10% of its initial value, the illuminated zone is obtained that, by this definition, is the thickness of the bioreactor where the light intensity is between its maximum value and 10% of its maximum value:

$$y_\phi = -\ln(0.1)/\varepsilon X \quad (6)$$

ε can be calculated from the absorbance data and is found equal to: ε = 1,5 x 10^{-3} L/(mg-cm). This value is valid for an absorbance up to about 1.2, where Beer's law is valid. Therefore the thickness of the illumination zone is given by:

$$y_\varphi \text{ (cm)} = 1.53 \times 10^3/C \quad (7)$$

The values of x_φ (cm) calculated for the three cases of Figure 3 are given in Table 2. It can be seen (Table 2) that, at the stationary phase, the illumination thickness is quite small, in the order of 1-2.5 cm. Therefore, mixing becomes of great importance, and bioreactor depth of flow in open raceway flow bioreactors should be carefully designed to take advantage of maximum illumination intensity.

The retention of effective biomass may eventually be enhanced by the use of supporting materials to facilitate the formation of biological concentrates containing several levels of organization, acting as biofilm matrices (Papadimitriou *et al.*, 2018).

Table 2. The illumination thickness or depth x_φ (cm), the critical cell density (absorbance), and the corresponding biomass concentration (mg/L) for the cultivation of *Chlorella vulgaris* in the pilot-plant bioreactor during winter, spring and summer, as well as in the laboratory bioreactor

Parameter	Laboratory bioreactor	Pilot-plant reactor Summer	Pilot-plant reactor Spring	Pilot-plant reactor Winter
Illumination thickness x_φ (cm)	1.3-1.1	1.3-1.0	1.7-1.5	2.4-2.0
Critical cell density (dC)	0.8-0.9	0.8-1.0	0.6-0.7	0.4-0.5
Biomass (dry) (mg/L)	1200-1350	1200-500	900-050	650-750

3.4. Biomass and lipid productivity

The lipid productivity is defined as the yield of the biomass productivity and the lipid content of the biomass. Therefore, indicative lipid productivities can be obtained from the data of Figure 2 and the average lipid content of *Chlorella vulgaris,* which is about 25% on a dry mass basis. The comparative lipid productivities, for operation in a batch mode, are 8 mg/L-d and 22 mg/L-d for operation in the pilot plant (open pond) in the winter and summer respectively, compared to as much as 40 mg/L-d in the laboratory flat-plate photobioreactor, also operated in a batch mode. Certainly, at higher (summer) temperatures plants use more water and sunshine producing greater biomass, compared to a poorer biomass productivity at lower (winter) temperatures (Kalavrouziotis *et al.*, 2018).

4. Conclusions

Chlorella vulgaris microalgal biomass cultivation was successfully scaled-up from a 25 L flat-plate photobioreactor to a 4 m^3 pilot-plant composed of three circular pilot-plant bioreactors set in a greenhouse in series and operated in a batch mode.

The average specific growth rate is highest for the laboratory bioreactor when compared to that of the pilot-plant one during the summer. However, specific rates in the exponential growth period are the same for cultivation of *Chlorella vulgaris* in the pilot-plant during summer and in the laboratory bioreactor.

The microalgal biomass productivities vary considerably in the pilot-plant photobioreactor during winter and summer, and are about 32 mg/L-d and 90 mg/L-d respectively. Biomass yield is highest in the laboratory flat plate bioreactor, of about 160 mg/L-d. The main cause of the low productivity in the pilot-plant photobioreactor during winter is primarily due to very low illumination intensities and secondarily to lower temperatures.

The illumination depth in the bioreactors during the stationary phase is quite short, in the range of 1-2.5 cm. In the light of the current paper findings, potential full scale applications, such as in open channel raceway bioreactors of about 40-50 cm depth, should be carefully designed and operated so that both vertical mixing and aeration be adequate to increase photon utilization and CO_2 availability during flow and as absorbances increase. This requires further study and is the scope of research endeavor underway.

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Conflict of interest

The authors declare no conflict of interest.

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