Evaluation of Growth Yield of *Spirulina maxima* in Photobioreactors

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The paper deals with the evaluation of the parameters for the cultivation of *Spirulina maxima* in two reactors (large-laboratory scale (LL) and semi-technical scale (ST)), whose illuminated areas in respect of the illuminated volume are different, and with the operating costs. We evaluated the growth yield coefficients for *Spirulina maxima* cultures. In the LL, the following factors were identified: $Y_{O_2/X}$ – 65.5; Y_{X/CO_2} – 0.0806; Y_{X/P_2O_5} – 0.0082, while in the ST: $Y_{O_2/X}$ – 583; Y_{X/CO_2} – 0.017; Y_{X/P_2O_5} – 0.0023. Although the reactor in the ST was equipped with many devices that should have improved the efficiency of cultivation, the obtained result was lower compared to the culture conducted in the LL. It was proved that it was possible to perform the cultivation of *Spirulina maxima* under temperate climate conditions in simply constructed, low cost reactors.

Key words:

Spirulina maxima, large-laboratory scale, semi-technical scale, cultivation, nutrient utilization

Introduction

Microalgae biomass has become a valuable material, the attractiveness of which is familiar to a wider group of customers, not only as a biomass (a prepared product), but also as a raw material for the production of a number of valuable substances like ethanol, hydrogen, hormones, biostimulants, and many other. Human nutrition, animal feeding, biofuel production, and sustainable agriculture – these are the branches of industry that are interested in microalgae biomass[1,2].

The literature on the subject presents various studies on the productivity of different microalgal culture systems[3–7]. The highest productivity was obtained for *Chlorella* and *Spirulina*: 7.70 g L^{-1} day^{-1} and 130 g m^{-2} day^{-1} for *Chlorella*, and 4.3 g m^{-2} day^{-1} and 51 g L^{-1} day^{-1} for *Spirulina* respectively[8]. Commercial production of *Spirulina*, that in the strict sense is not a microalga, but a cyanobacterium[1], has gained worldwide attention for its use in human food supplements, animal feed, and pharmaceuticals. The production of *Spirulina* with reduced costs is necessary when considering a large-scale cultivation for industrial purposes.

For microbial cultures, the growth rate and growth yield are equally important. Although there are many papers that deal with the effect of culture conditions on the growth rate[9–12], there is little information concerning their influence on the growth yield, and thus the efficiency of the conversion of substrates into biomass. Such knowledge is not only necessary for the efficient cultivation of cells, but also allows for assessment of the economic aspects of the process. The greatest disadvantage incurring the highest cost in microalgae cultivation is the reactor/photobioreactor construction. Many advanced and complicated solutions for the cultivation of microalgae have been proposed[12–15]. If cheaper construction of a reactor for the cultivation of photoautotrophic organisms with a better utilization of nutrients could be used instead of technically advanced solutions, greater economic sustainability could be achieved[16–19].

Two reactors that differ in their construction, ratio of illuminated surface (A) to volume (V), and operating costs were used. The aim of this work was to present whether the more advanced construction would bring about better conditions for growth of cells, and at same time, higher nutrients utilization effectiveness. By using two sets of reactors on a large-laboratory and semi-technical scale, the present work aimed at evaluating the growth yield of *S. maxima*, and estimating the photosynthetic, carbon, and phosphorus utilization efficiencies.

Material and methods

Microorganism

Microalga *Spirulina maxima* was obtained from the Culture Collection of Algal Laboratory (CCALA) Institute of Botany, Academy of Sciences of the Czech Republic.

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Culture media

The microalga was cultivated in the Schlösser[20] medium, prepared for *S. maxima* with technical grade reagents.

Reactors for cultivation of microalgae

Two photobioreactors/open ponds for the cultivation of microalgae were applied, first on a large-laboratory scale with a capacity of 250 L, and second on a semi-technical scale with a capacity of $10 \cdot 10^3$ L. Table 1 and Fig. 1 present and compare the design parameters of the reactors. The first reactor was a tank reactor (dimensions 0.187 m × 1.32 m) of 250 L capacity, covered by a glasshouse, but not equipped with additional machines or additional source of light (only natural) or mixing; the second reactor was a stirred tank reactor (dimensions 1.12 m × 3.6 m) of $10 \cdot 10^3$ L capacity, covered also by a glasshouse, equipped with a biomass separation system (six bag filters, average pore size 6 μm, Desjoyaux Co., Ltd.), a mixing system (pumps), and six lamps 300W Astral Pool, Poland.

Table 1 – *Comparison of reactors for the cultivation of microalgae*

Parameter	Capacity	
	large-laboratory scale (LL) 250 L	semi-technical scale (ST) $10 \cdot 10^3$ L
h, m	0.187	1.12
r, m	0.66	1.8
V, m^3	0.256	11.4
A, m^2	1.37	10.2
A/V	5.34	0.895
Mixing	No	Yes
Illumination	only sun	Yes (natural+artificial)
Heating	only sun	Yes (thermostated heater)

Analysis

In order to compare the effect of the parameters of reactors for the cultivation of microalgae on the utilization of substrates, the cultures were cultivated in the two reactors shown in Fig. 1 on a large-laboratory scale, and a semi-technical scale, both having the same shape but different parameters (A/V ratio) of design of the reactors, which is important in the culture of the photoautotrophic organisms[21]. An important parameter of the design of the reactors for the growth of photosynthetic organisms is the ratio of the illuminated surface to the volume of culture solution. By appropriate selection of the volume (V) and the irradiated surface (A) ratio, it is possible to reduce undesirable effects of self-shading, or limited access to light where some cells act as screens for others. The high value of the A/V ratio is the desired parameter for photobioreactors (Becker, 2007 and Watanabe and Hall, 1995). The inoculation of the photobioreactor of ST scale was performed at 10 % of its capacity, 1000 L of inoculum was carried out in three laboratory-scale systems of 40 L presented elsewhere[22]. The culture solution in the large-laboratory scale reactor with a capacity of 250 L was obtained from an ongoing culture in a pilot-scale reactor of $10 \cdot 10^3$ L, in order to provide the same initial conditions. Samples of microorganism suspension from both cultures were collected at the same time from both cultures cultivated on the LL as well as on the ST scale. Nutrient levels and pH-value were monitored to maintain stable conditions during growth, and provide a high level of biomass production. The conditions during culturing on the large-laboratory scale and semi-technical scale are showed in Table 2.

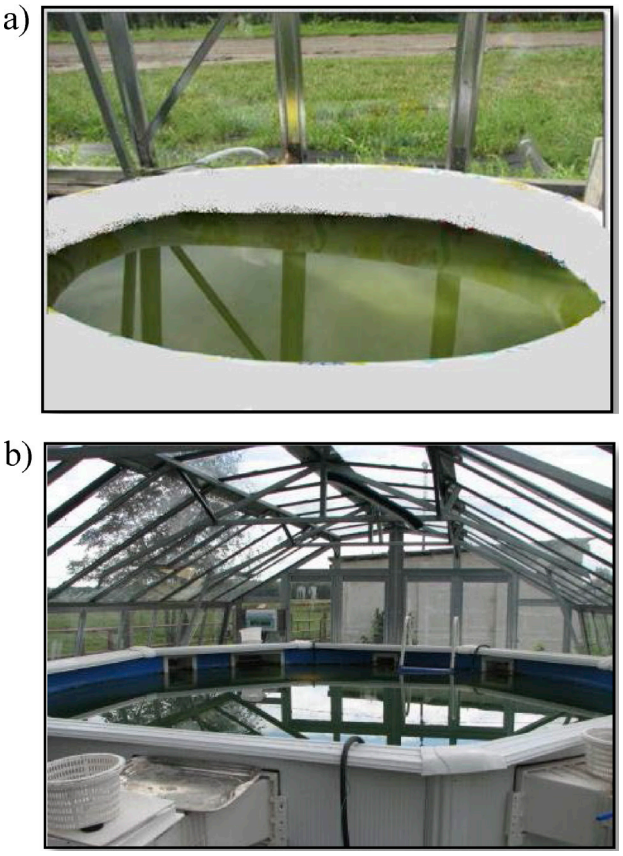

Fig. 1 – *Two reactors tested for cultivation of Spirulina maxima in the a) large-laboratory scale, and b) semi-technical scale*

Table 2 – *Conditions prevailing during cultivation of Spirulina maxima, the same for the culture of the large-laboratory scale (250 L) and the semi-technical scale ($10 \cdot 10^3$ L)*

Day	Forecast			Reading inside the glasshouse			
	T, °C	sunlight, h	humidity, %	T_{air}, °C	T_{water}, °C	light intensity, W m^{-2}	humidity, %
1	29	11.3	35	20.5	32	51.8	74
2	26	8.3	50	16.9	29	304.5	90
3	19	6.3	80	15.0	26	6.7	94
4	22	9.2	70	41.0	30	5.6	96
5	23	7.5	55	38.0	29	7.8	–
6	23	6.3	60	48.5	31	4.9	32
7	23	7.8	72	34.6	30	5.7	30
8	21	5.6	78	36.8	30	5.9	44
9	20	5	72	29.8	30	6.8	54
10	13	1.1	85	17.9	29	6.8	45
11	21	4.3	70	20.0	25	226.4	84
12	24	5.6	72	28.6	26	76.0	86

Cell growth

The biomass concentration of microalgae was measured spectrophotometrically. Samples from each culture were taken daily to determine optical density. The optical density was the absorbance of samples at 560 nm (OD560) in a UV/Visible spectrophotometer (Varian Cary 50 Cone). Each sample was diluted to make the absorbance less than 1.0 if the optical density was greater than 1.0. The concentration of *Spirulina maxima* was determined by equation (1) describing the relationship between the absorbance A_{560} and the concentration of dry mass:

$$X = 0.739 \cdot A_{560} - 0.058,\ R^2 = 0.995 \qquad (1)$$

pH measurements were conducted with pH-meter Mettler-Toledo (Seven Multi, Switzerland) equipped with an electrode InLab413 (Seven Multi, Switzerland) with the compensation of temperature. Dry weight was measured after the microalgae biomass had been dried. The biomass was dried at 60 °C for three days (Manufacturing of medical and laboratory equipment, WAMED; Warsaw, Poland).

The specific growth rate of the microalgae was calculated using equation (2):

$$\mu = \frac{\ln X_t - \ln X_0}{t} \qquad (2)$$

where: t – time period (in days), after which the culture concentration was measured (assuming $t^0 = 0$), X_t – the culture concentration after time t (mg L^{-1}), X_0 – the initial concentration of the culture (mg L^{-1}). Relative growth rate was determined from the graphically depicted correlation $\ln X = f(t)$. The linear regression for logarithmic phase of the growth was described by equation (3):

$$\ln X_t = \mu \cdot t + \ln X_0 \qquad (3)$$

where parameter μ, day^{-1} is the slope.

Determination of dissolved oxygen O_2

The determination of the amount of dissolved oxygen in thepermeate of the microalgae broth was made by the Oxygen meter SevenGoproTM (Mettler Toledo) with Inlab® 650 dissolved oxygen sensor. The oxygen sensor was calibrated at one point – 100 %.

Determination of dissolved carbon dioxide CO_2 concentration

Carbon dioxide concentration measurements were conducted with the Mettler-Toledo (Seven Multi) meter equipped with a module for measuring pH-value, CO_2, conductivity, and ionic concentration. A two-point calibration was conducted at 100 mg L^{-1} and 1000 mg L^{-1}. To prepare the calibration, solutions $NaHCO_3$ (from POCh S.A., Gliwice) were used. An amount of 18 mL of calibration solution was mixed with 72 mL of distilled water and 9 mL of conditioning solution. The conditioning solution was prepared as follows: 249 g of $Na_3C_6H_5O_7$ (from POCh S.A., Gliwice) was dissolved in 500 mL of distilled water and 100 mL of 32 % HCl in a 1-L measuring flask that was then filled to its full capacity. A carbon dioxide measuring electrode was immersed in an aqueous solution

of sodium chloride (8.5 g NaCl (from POCh S.A., Gliwice) in 1 L).

Colorimetric determination of phosphorus concentration

The soluble P_2O_5 concentration in the culture medium was measured by the colorimetric vanadomolybdophosphoric acid colorimetric method with a Varian Cary 50 Cone UV-Visible Spectrophotometer at 420 nm. The method is based on the formation of yellow vanadomolybdophosphoric acid upon the addition of ammonium molybdate and vanadium to an ortho-phosphate solution. Ammonium molybdate reacts under acid conditions to form a heteropolyacid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed, the intensity of which indicates the amount of orthophosphate. The concentration of soluble P_2O_5 ($C_{P_2O_5}$, mg L^{-1}) was determined by means of equation (4) describing the relationship between the absorbance A_{420} and the concentration of P_2O_5.

$$C_{P_2O_5} = 0.0436 + 0.0227 \cdot A_{420}, \ (R^2 = 0.999) \quad (4)$$

Calculations

Model parameters of equations describing growth of biomass cells were determined using a nonlinear estimation and multiple regression modules of *Statistica* software *ver. 8.0*. The correlation was considered statistically significant at $\alpha < 0.05$.

Results and discussion

The 12-day growth of *S. maxima* was tested in two different reactors. The cultivation on a semi-technical scale (ST) and the cultivation on a large-laboratory scale (LL) was carried out in parallel. The cultivation was carried out under the same conditions with the exception of the reactor construction parameters. The construction of both reactors was based on solutions available on the market. The first reactor was a simple inflatable pool made of PVC material, while the second reactor was also a portable swimming pool made of steel, covered with foil and equipped with a new solution taken from the swimming pool industry – a filter unit that played the role of a mixing module when the filter material was removed. By application of market-available solutions used in the swimming pool construction industry, the costs were limited. The cost of construction was 16 000 Euro and 6 Euro for STS and LLS, respectively. If specific reactors for microalgae had been used, the costs would have been several times higher. The construction parameters are compared in Table 1. Both reactors had the same shape. Cultivation in the LL scale of 250 L capacity, was performed without stirring or additional source of light, apart from sunlight, but with a better/higher A/V ratio. The culture conducted on the LL scale of 250 L capacity of was used to compare the consumption of substrates with the effectiveness of cultivation performed on the ST scale of $10 \cdot 10^3$ L capacity, as shown in Fig. 1.

The value of the specific growth rate μ, [day^{-1}] as seen in Fig. 2a, was twice as high for the culture conducted in the LL in comparison with the culture conducted in the ST. A similar result for the cultivation of *Spirulina platensis* (μ = 0.23 1 day^{-1}) was obtained by Binaghi *et al.*[23] Fig. 2b shows the change in the rate of increase in biomass/shows how the increase rate of biomass changes (productivity) in both cultures. An average growth rate of biomass was calculated, $3.96 \cdot 10^{-3}$ g L^{-1} day^{-1} for the LL that was approximately 18 times higher compared with the average growth rate for the culture carried out in ST, which was $0.216 \cdot 10^{-3}$ g L^{-1} day^{-1}.

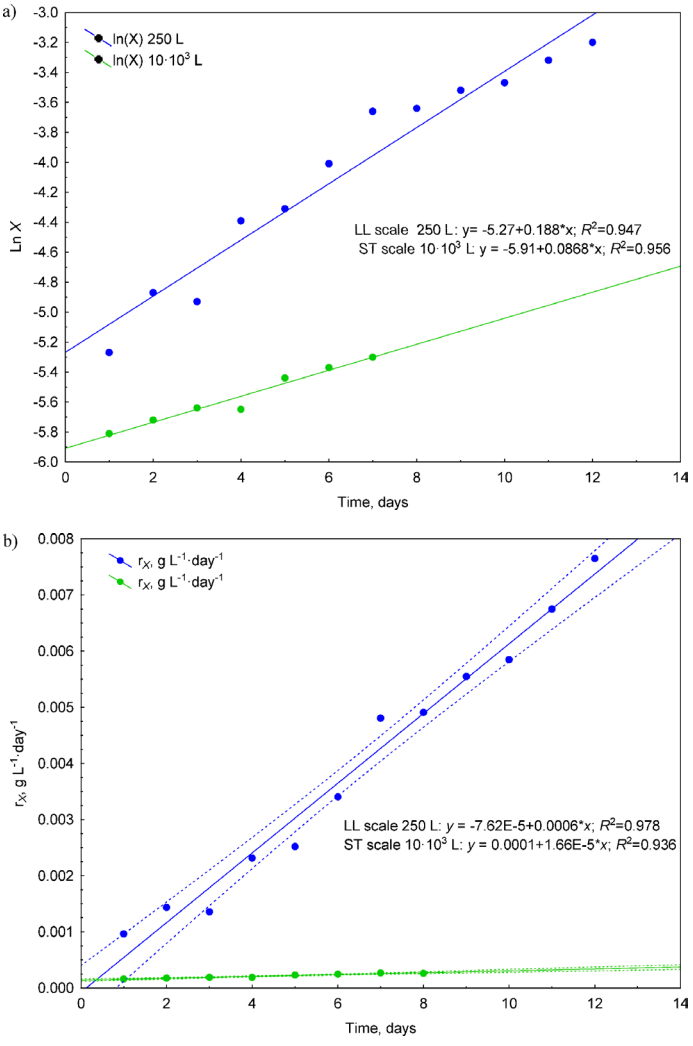

Fig. 2 – *a) Growth curve of Spirulina maxima in the reactor of 250 L capacity and $10 \cdot 10^3$ L, and b) changes in growth rate with time in the reactors of 250 L and $10 \cdot 10^3$ L capacity*

Productivity related to the dissolved O_2

High levels of dissolved oxygen, above 35 mg L^{-1}, are toxic to most algae, which coupled with strong sunlight could cause photooxidation, and as a result lead to the death of the cells. The productivity of a microalgae culture is strongly correlated with the production of O_2 during photosynthesis; the accumulation of O_2 is, however, a major problem in closed algae farming systems. The stoichiometric equation for the autotrophic growth of *Spirulina maxima* is presented by equation 5.

$$0.17\,NO_3^- + 0.91\,H_2O + HCO_3^- \xrightarrow{h\theta} \xrightarrow{h\theta} CH_{1.65}O_{0.531}N_{0.17} + 1.36\,O_2 + 1.17\,OH^- \quad (5)$$

Fig. 3 presents the biomass concentration and oxygen concentration during the growth of *Spirulina maxima* in the LL and the ST. The level of dissolved oxygen concentration depends on many factors: light intensity, air temperature, efficiency of the process of photosynthesis, as well as on the accumulation of the produced oxygen. Because the cultivation process was performed in a closed room, the concentration of dissolved oxygen in the two cultures was about 20 mg L^{-1}. This level of oxygen concentration leads to a constant photosynthesis activity.

Equation (6) was used to determine the yield coefficient of biomass relative to the dissolved O_2 (as product) Y_{O_2/C_S} for both cultures:

$$Y_{P/X} \overset{\text{def.}}{=} -\frac{dP}{dX} = \frac{r_P}{r_X} \quad (6)$$

The high value of Y_{O_2/C_S} equal to 583 mg g^{-1} for the culture in the ST, and small changes in the cell biomass concentration, could have resulted due to the mixing system not overcoming the inappropriate A/V ratio, thus leading to growth inhibition, and inefficient removal of the reaction product, which is O_2[25] and consequently, the occurrence of product inhibition.

This value, which is close to those reported in the literature for various reactor configurations, points out that the ability of light to enter the deepest zones of the bulk could become the factor limiting the growth at high biomass levels[23].

Productivity related to the dissolved CO_2

The concentration of dissolved CO_2 is related to the pH-value of the broth culture. Fig. 4 shows the relationship between pH-value and the concentration of dissolved CO_2 in the broth for both LL and ST cultures. During autotrophic growth, the environment became more alkaline, mainly due to the utilization of carbon dioxide by the cells ($HCO_3^- \rightleftharpoons CO_2 + OH^-$)[24],

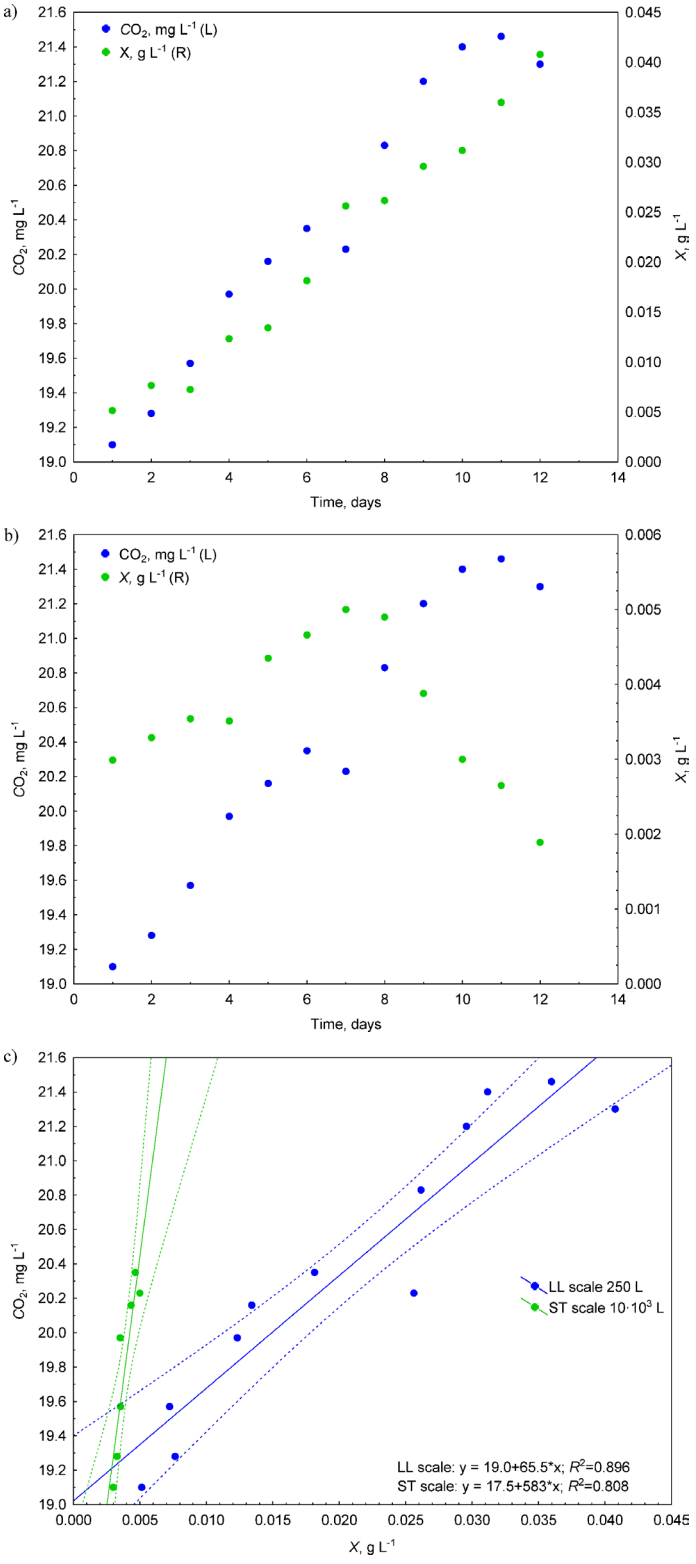

Fig. 3 – *Changes in the concentration of O_2, mg L^{-1} and X, g L^{-1} a) large-laboratory scale 250 L, and b) semi-technical scale $10 \cdot 10^3$ L, c) The dependence between O_2 concentration and biomass concentration X, g L^{-1}, dashed lines – 95 % confidence intervals*

which is shown in Fig. 4, both for the LL (250 L) and ST ($10 \cdot 10^3$ L). It can be concluded that the increase in pH was an effect of biomass production via photosynthesis, CO_2 served, in this case, as a

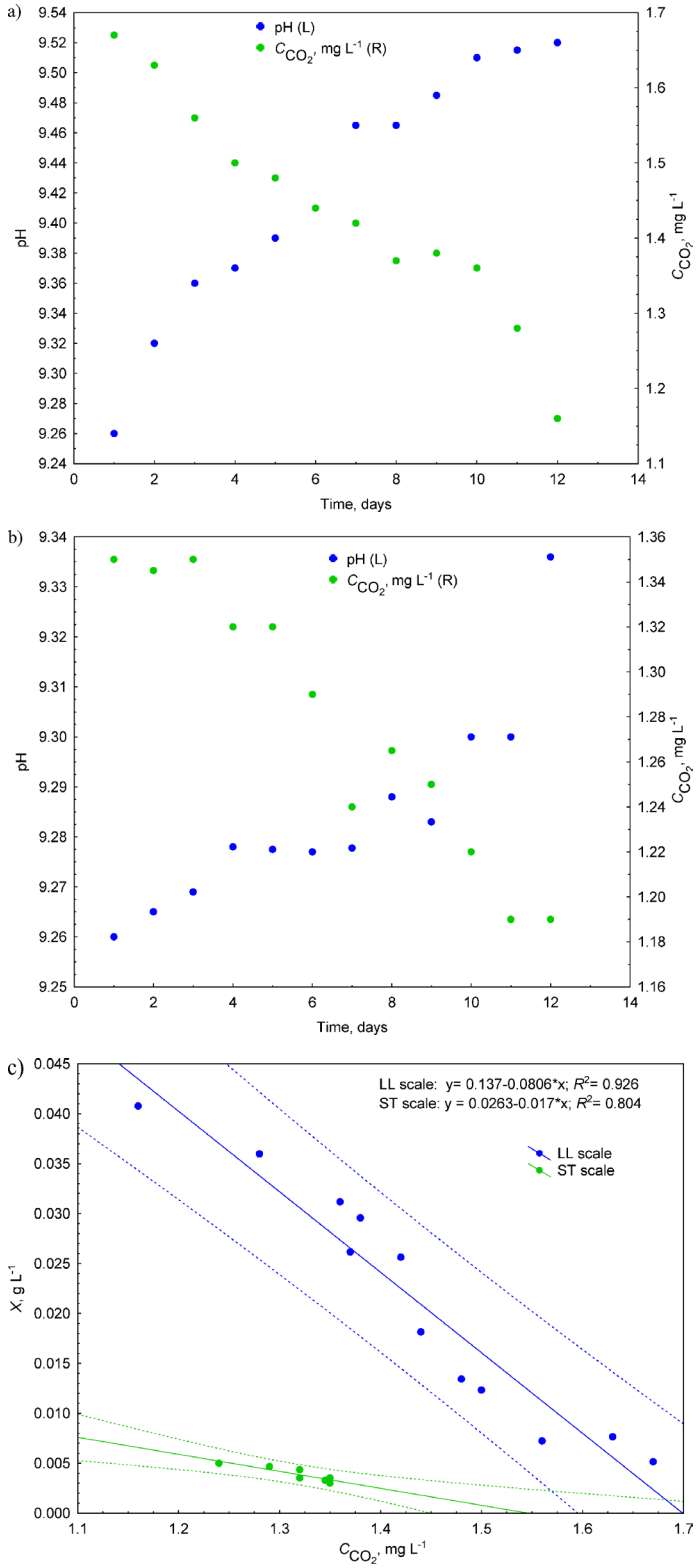

Fig. 4 – *Changes in the concentration of CO_2, mg L^{-1} and pH with time in a) large-laboratory scale 250 L, and b) semi-technical scale $10 \cdot 10^3$ L, c) biomass yield coefficients from CO_2 – Y_{X/CO_2} with time in large-laboratory (250 L), and semi-technical scale ($10 \cdot 10^3$ L), dashed lines – 95 % confidence intervals*

substrate for the production of biomass and its depletion was observed. During the photosynthesis, the concentration of dissolved CO_2 decreased because it was used in the photosynthesis, and the pH increased as an effect of products formed during the growth (pH ↑, concentration of dissolved CO_2 ↓ and X↑). Fig. 4c shows the relationship between biomass concentration and CO_2 concentration $\frac{dX}{dC_{CO_2}}$, from slope $f(C_{CO_2}) = X$ the coefficient of biomass productivity related to the dissolved CO_2 $Y_{X/C_{CO_2}}$ was obtained (equation 7) for both cultures. Biomass productivity related to the dissolved CO_2 in the LL was approximately 4.7 times higher as compared with the culture in the ST. Changes in rate of CO_2 consumption in both cultures at the time is shown in Fig. 5b.

$$Y_X \overset{\text{def.}}{=} -\frac{dX}{dS} = \frac{r_X}{r_S} \tag{7}$$

On the basis of the rate of CO_2 consumption at a time, an average rate of CO_2 consumption for both the LL and ST cultures was calculated as 0.0667 mg L^{-1} day^{-1}, and 0.0186 mg L^{-1} day^{-1} respectively.

Productivity related to the P_2O_5

The current concentration of nutrients depends on the concentration of biomass in the culture, and the losses are caused by precipitation. In the case of a high concentration of Ca(II), the precipitation of $Ca_3(PO_4)_2$ can occur. Moreover, usually about 25 % of the phosphorus present in the medium precipitated in the form of $FePO_4$; a high pH and high concentration of dissolved oxygen can reverse this negative/unwanted process[26]. This phenomena was not observed at the LL scale or the ST scale. Fig. 5 presents changes in the phosphate concentration in both cultures.

Fig. 5 shows that a higher consumption of phosphate is observed in the case of the culture conducted on the LL scale when compared to that of the ST. This is related to a 3 times higher growth rate of biomass r_X, g L^{-1} day^{-1} in the culture conducted in LL. On the basis of the utilization rates of P_2O_5, the average rate of P_2O_5 consumption was 0.656 mg L^{-1} day^{-1} for cells in the LL culture (Fig. 5). For the cultivation in the ST, the average rate of P_2O_5 consumption was about 4.3 times lower and amounted to 0.137 mg L^{-1} day^{-1}.

Biomass yields related to the P_2O_5, $Y_{X/C_{P_2O_5}}$ for both cultures were determined (Fig. 5). For the culture in the LL, it was 0.0082 g mg^{-1}, and for that in the ST – 0.0023 g mg^{-1}; biomass yields related to the P_2O_5 in the LL were approximately 3.5 times higher compared to the ST, indicating a more efficient synthesis of biomass.

Comparisons and correlations

Table 3 presents the parameters of cell growth in the culture of *Spirulina maxima* in LL and ST. An

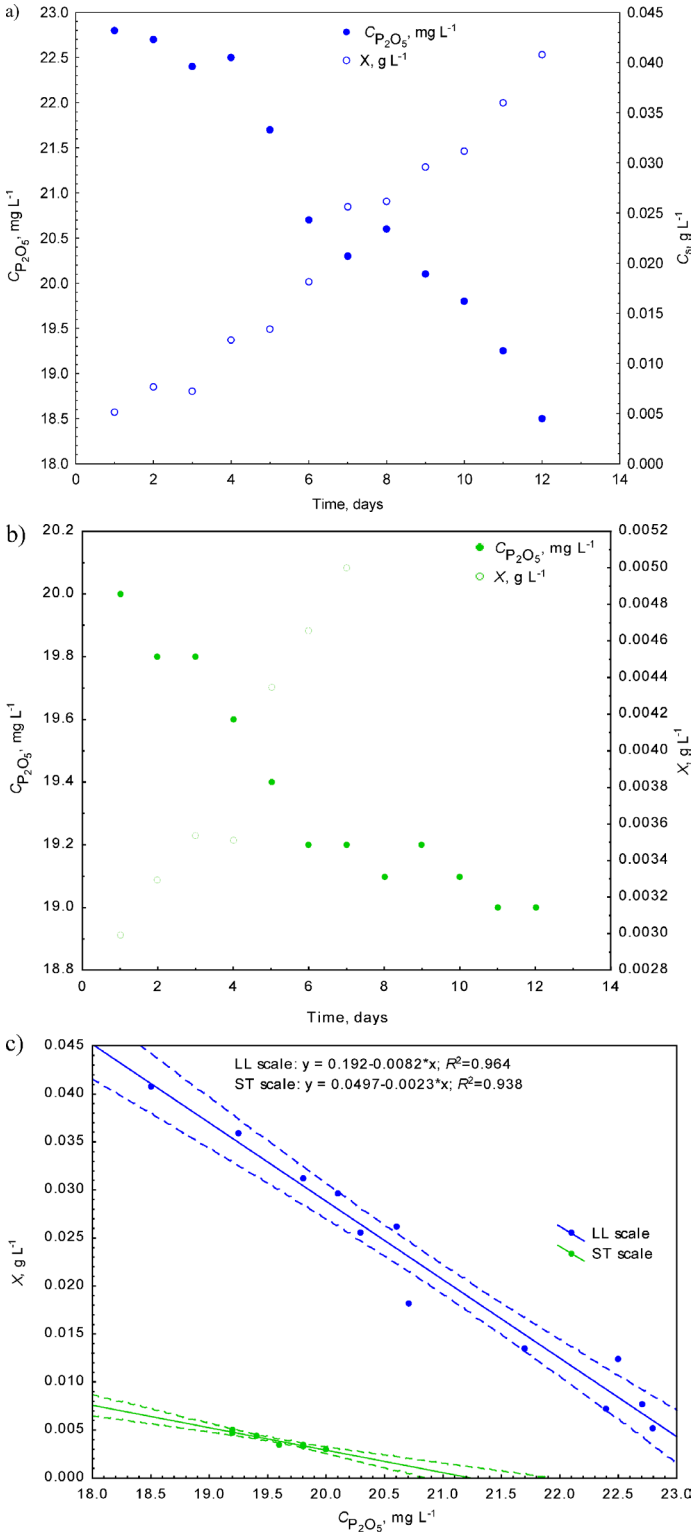

Fig. 5 – *Changes in the concentration of P_2O_5, mg L^{-1} and X, g L^{-1} a) large-laboratory scale 250 L, and b) semi-technical scale $10 \cdot 10^3$ L, c) biomass yield coefficients from P_2O_5-Y_{X/P_2O_5} with time in large-laboratory (250 L), and semi-technical scale ($10 \cdot 10^3$ L), dashed lines – 95 % confidence intervals*

increased consumption of the substrate and product formation, and thus a faster growth of cells in the LL was observed. Commercial production of *Spirulina* is usually performed in open ponds 15–18 cm deep with

Table 3 – *Equilibrium parameters of growth of Spirulina maxima in two reactors: large-laboratory scale (250 L) and semi-technical scale ($10 \cdot 10^3$ L)*

Parameter	Capacity	
	large-laboratory scale (LL) 250 L	semi-technical scale (ST) $10 \cdot 10^3$ L
μ, day^{-1}	0.188	0.0868
r_x	0.0006	$1.66 \cdot 10^{-5}$
C_{O_2}	↑	↑
Y_{O_2/C_S} (g g^{-1})	65.5	583
pH	↑	↑
C_{CO_2}	↓	↓
Y_{C_S/CO_2} (g g^{-1})	0.0806	0.017
$C_{P_2O_5}$	↓	↓
Y_{C_S/P_2O_5} (g g^{-1})	0.0082	0.0032

an area from several to tens of hectares[24]. The main reason for poor growth in the culture in ST was probably the too low irradiation that influences photosynthesis of microalgae cells and stimulates the CO_2 fixation rate of the cell, thereby improving microalgae growth. Inefficient irradiation in comparison with the volume of the culture was expressed as the ratio A/V = 0.893, which was smaller when compared with open ponds in LL. When comparing the size of the irradiated surface of both cultures, it can be concluded that the cells in the culture conducted on a semi-technical scale have access to a 6 times lower light intensity relative to the cells in the culture conducted on a large-laboratory scale. The high value of $Y_{O_2/X}$ may indicate an inefficient mixing, which, combined with a low A/V ratio, explains the low utilization rates of substrates in the culture conducted in the ST.

Table 4 presents the results of the correlation analysis. Strong correlations ($p < 0.05$) were observed between the biomass concentration and all the parameters considered: the concentration of O_2, CO_2, P_2O_5, and pH-value.

Table 4 – *Correlation factors between the concentration of biomass, O_2, CO_2, P_2O_5 and pH in the growth of Spirulina maxima*

	C_S, g L^{-1}	C_{O_2}, mg L^{-1}	C_{CO_2}, mg L^{-1}	$C_{P_2O_5}$, mg L^{-1}	pH
C_s, g L^{-1}	1.00	–	–	–	–
C_{O_2}, mg L^{-1}	0.862	1.00	–	–	–
C_{CO_2}, mg L^{-1}	–0.831	–0.725	1.00	–	–
$C_{P_2O_5}$, mg L^{-1}	–0.742	–0.621	0.963	1.00	–
pH	0.972	0.840	–0.867	–0.800	1.00

An attempt to describe the biomass concentration as a function of O_2 concentration, CO_2, as well as the P_2O_5 concentration and pH-value was made. The choice of independent parameters was dictated by an earlier analysis of the correlation, which showed the effect of O_2, CO_2, P_2O_5 and pH-value on the biomass concentration. Model parameters were estimated using multiple regression, and are presented in Table 5.

Table 5 – *Parameters of elaborated model (multiple regression) of C_S as a function f(C_{O_2}, C_{CO_2}, $C_{P_2O_5}$ and pH)*

	β_x	Level of p
Constant	–1.099	<0.0001
C_{O_2}, mg L^{-1}	0.112	0.318
C_{CO_2}, mg L^{-1}	–0.23	0.408
$C_{P_2O_5}$, mg L^{-1}	0.256	0.278
pH	0.883	<0.001
R	0.978	
R^2	0.957	
$R^2_{corrected}$	0.945	
Se	0.00299	
χ^2	0.0965	
n	19	

Using the following model: $X(g\ L^{-1}) = -1.1 + 0.112 \cdot C_{O_2}(g\ L^{-1}) - 0.23 \cdot C_{CO_2}(g\ L^{-1}) + 0.256 \cdot C_{P_2O_5}(g\ L^{-1}) + 0.883 \cdot pH$, it is possible to describe the amount of obtained biomass in 96 ± 11.2 % with 96.5 % accuracy. Fig. 6 presents graphs of the relationship between the predicted values (the model) and observed values, with a 95 % confidence interval, which confirms that the model obtained for X describes the experimental points accurately (Fig. 6).

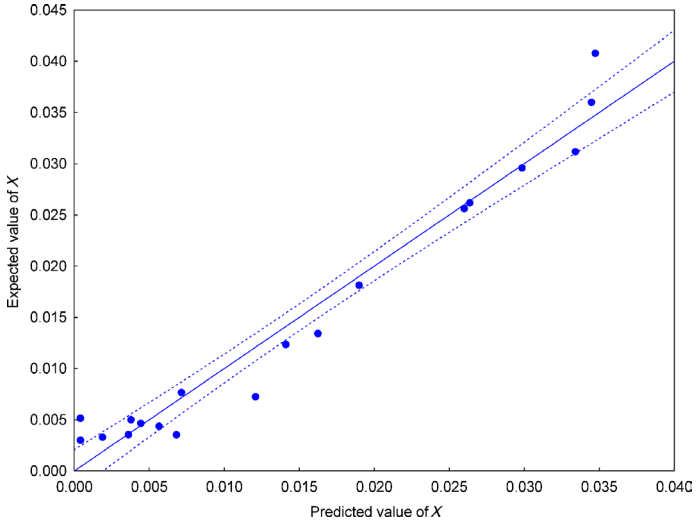

Fig. 6 – *Dependence between the expected values (from model) and observed values related to the biomass concentration*

Nowadays, the commercial production of *Spirulina* is mainly performed in open ponds, which are cheap and easy to operate since they use solar irradiance as a free source of energy. However, several studies list many disadvantages of open ponds, for example: they do not allow reaching high biomass productivity due to the difficulty of maintaining the optimum temperature, and so they are restricted to tropical and sub-tropical regions[27]. The presented work proves that it is possible to obtain the biomass of *Spirulina* in open-pond type reactors under a temperate climate.

The mixotrophic condition became one of the most important parameters in terms of biomass productivity[27,28]. An increase in risk of contamination requires that the process be run under aseptic conditions, even for *Spirulina*, which grows under an alkaline pH-value that is unfavorable for many microbial contaminations, cannot be ignored. Although the use of an organic carbon source can provide energy promptly utilizable, and allow attainment of a high final biomass concentration, the investment cost for the construction of closed reactors that operate under aseptic conditions renders the decision-makers reluctant to establish a culture plant under a temperate climate, which is not very beneficial/lucrative for the growth of (a) photoautotrophic organism(s). The air temperature in Central Europe (for example in Poland) in 2010 was around 7.5 °C (CSO, 2012). During that year, from April to September (5 months), the average temperature was above 10 °C, from May to August it was above 15 °C. The insolation in 2010 was 1800 hours[29]. The wide range of products that could be obtained from microalgae biomass has interested many investors in establishing cultivation plants in Central Europe. To prolong the time suitable for cultivation, placing the reactors/open ponds under a glasshouse should be applied. Then, even when the temperature is lower than 15 °C, the cultivation of microalgae could be possible from April to September. Additionally, to encourage investors, the cost of investment would be limited by applying simple and low-cost solutions.

The recommendation for cultivating microalgae, in view of the presented research, is to improve the A/V ratio for higher biomass productivity, which would be the cheapest solution; of course, many other solutions are available, such as addition of another module like pumps or light source, but they would result in higher costs, which, in the final calculation of effectiveness, would probably not improve profitability.

Conclusions

Two cultures of *Spirulina maxima* in open-pond type photobioreactors that had a different *A*/*V* ratio, were conducted. The open pond on a semi-technical scale was equipped with a mixing pump and a thermostat, while that on a large-laboratory scale was used without a mixing or heating system. The work presented here aimed to further examine the improvement of culture conditions in order to gain more biomass of good quality, and to attain efficient nutrient removal/utilization. This study suggests that it might be possible to cultivate *Spirulina maxima* in temperate climate conditions in low-cost open ponds.

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