J Appl Phycol (2014) 26:1639–1648
DOI 10.1007/s10811-013-0224-z

Screening of two terrestrial cyanobacteria for biotechnological production processes in shaking flasks, bubble columns, and stirred tank reactors

S. Kuhne · D. Strieth · A. Weber · K. Muffler · M. Lakatos · R. Ulber

Received: 17 July 2013 / Revised and accepted: 2 December 2013 / Published online: 22 December 2013

Abstract Terrestrial cyanobacteria are rarely used for biotechnological processes, although they show great potential in terms of value-added substances. Cyanobacteria from arid habitats are of particular interest because they tolerate higher temperatures and feature a different product spectrum compared with their aquatic counterparts. In addition, terrestrial cyanobacteria may represent an interesting source of pharmaceutical products. To investigate the future use of these organisms in biotechnological processes, the growth rates of *Trichocoleus sociatus* (formerly *Microcoleus sociatus*) and *Nostoc muscorum* were examined using three different cultivation systems: shaking flasks, bubble columns, and stirred tank reactors. Parameters including pH, temperature, CO_2 level, and power dissipation were investigated quantitatively in the three systems for their impact on growth rate. The highest growth rate of the terrestrial cyanobacteria could be achieved in a stirred tank reactor under enriched CO_2 concentration. In this system, the growth rate was 1.15 day^{-1}±0.08 (2 % vol.) for *T. sociatus* and 0.72 day^{-1}±0.22 (5 % vol.) for *N. muscorum*, based on dry weight. Furthermore, a basic mathematical model was created as an add-on to predict growth rates of terrestrial cyanobacteria based on their dependency on temperature, pH, and substrate concentration, in general. This model was used to estimate growth of *N. muscorum* in stirred tank reactor experiments with an accuracy of 98.8 % and with 75 % accuracy for *T. sociatus*.

S. Kuhne · D. Strieth · A. Weber · K. Muffler · R. Ulber (✉)
Institute of Bioprocess Engineering, University of Kaiserslautern, 67663 Kaiserslautern, Germany
e-mail: ulber@mv.uni-kl.de

M. Lakatos
Experimental Ecology, University of Kaiserslautern, 67663 Kaiserslautern, Germany

Keywords Terrestrial cyanobacteria · Growth rate · Stirred tank reactor · Shaking flask · Bubble column · Mathematical modeling

Introduction

Cyanobacteria produce numerous value-added substances that have steadily growing economic potential in the field of biotechnologically produced pharmaceutical compounds, comestible goods, and renewable resources (Mata and Martins 2010; Wijffels and Barbosa 2010). Furthermore, they represent a rich source of polysaccharides (exopolysaccharides), lipids, amino acids (cyanophycin), vitamins (pantothenates, B12), steroles, enzymes, pharmaceuticals (cyto-, hepato-, neuro-, and endotoxins), bioactive substances (Singh et al. 2005), and fine chemicals (carotenoids, chlorophylls, phycobilisomes; Prasanna et al. 2010). They also have high rates of production of renewable energy sources, including lipids (Hu et al. 2008), ethanol (Dexter and Fu 2009; Deng and Coleman 1999), and bio-hydrogen (Bandyopadhyay et al. 2010; Min and Sherman 2010), making cyanobacteria the focus of a great deal of research in recent years (Rittmann 2008; Mata et al. 2010; Wijffels and Barbosa 2010). Although the biotechnological utilization of cyanobacteria for the production of valuable compounds has been reported regularly, aquatic cyanobacterial species have mainly been used because the available cultivation technology is solely submerged (Pulz 2001; Pulz and Gross 2004).

Recently, terrestrial cyanobacteria have attracted more attention because of their important ecological impact in nutrient-poor arid and xeric habitats (Belnap and Lange 2003). Terrestrial cyanobacteria adapt well to abiotic environmental conditions and can withstand temperature fluctuations and low water availability (Rascher et al. 2003). They are also capable of producing numerous biologically active and unique

pharmaceutically important compounds such as cryptophycin, the antiviral cyanovirin-N, scytovirin, and scytonemin (Boyd et al. 1997; Bewley et al. 1998; Muller-Feuga 2000). Furthermore, terrestrial cyanobacteria produce extracellular polymeric substances (EPS), which often feature biological activity and photoprotective agents (carotenoids; Kuhne et al. 2013; Lakatos et al. 2001; Palmqvist et al. 1995) at higher rates than their aquatic counterparts because of their genetic pre-adaptation to survival in arid habitats (Lakatos et al. 2001). Carotenoids are pharmaceutically important compounds that act as antioxidants in lipophilic systems to prevent photoinhibition of photosynthesis and photooxidation (Krinsky 1979; Palozza and Krinsky 1992). In this context, several studies have shown that elevated intracellular amounts of carotenoids can prevent damage to algal cells caused by solar irradiation and that carotenoids can also be used to prevent human tissue from damage in the same way (Heinrich and Tronnier 2010). The pigment chlorophyll *a* is associated with carotenoids in the photosystems of cyanobacteria and plays a key role in oxygenic photosynthesis. Both the carotenoid and the chlorophyll *a* contents of terrestrial cyanobacteria depend on the amount of exposure to solar irradiation (Lakatos et al. 2001). Both pigments are also used as colorants for food and textiles (International Congress on Pigments in Food. 5 2008; Spolaore et al. 2006).

Because of these advantages of terrestrial cyanobacteria, the current work focused on the determination and optimization of cyanobacterial growth rates in relation to parameters such as CO_2 concentration, pH level, and the type of power dissipation. To achieve this, different bioreactor systems (shaking flasks, bubble columns, stirred tank reactor) that are considered to be standard cultivation devices in biotechnological processes were examined. Therefore it is important to investigate which kind of set-up matches the requirements for growth and productivity of terrestrial cyanobacteria properly. In addition, a simplified growth model (describing the influence of CO_2, temperature, and pH on the individual growth rate) was derived from the experimental data to further enhance the potential use of terrestrial cyanobacteria in biotechnological production processes.

Materials and methods

The terrestrial cyanobacteria *Nostoc muscorum* (PCC 7906) and *Trichocoleus sociatus* (formerly *Microcoleus sociatus*; Culture Collection Burkhard Büdel, TU Kaiserslautern, Germany; BB 92.2), isolated from xeric soil habitats in Columbia (USA) and Nizzana (Israel), respectively, were used in all experiments. Standard BG 11 medium (Rippka et al. 1979) was used for cultivation of terrestrial cyanobacteria. The pH level of BG 11 medium was adjusted aseptically in a laminar flow hood following heat sterilization for the shaking flask experiments investigating different pH values.

Shaking flask experiments

To determine the growth rates of *N. muscorum* and *T. sociatus* under different pH conditions and power dissipation, shaking flask experiments were carried out. A lit shaker was used (Multitron2, ATM 00, Infors, Switzerland) with 500 mL Erlenmeyer shake flasks and an eccentricity of 5 cm. The temperature was set to 24 °C and the light intensity to 100 μmol photons m^{-2} s^{-1} (using continuous white lighting). The light intensity was checked with a quantum sensor (Licor 190A, Licor-Biosciences USA). The power dissipation was varied using different volumes of medium (50 and 150 mL) in combination with different shaking rates (90 and 120 rpm) and calculated as previously described (Peter et al. 2006). The influence of pH on growth was tested within a pH range of 4–11 at fixed settings of a 50 mL filling volume and shaking at 120 rpm. pH optimization was carried out using one shake flask per pH value with six repetitions in total. Growth rates for each pH value were determined during the exponential phase and statistically evaluated. Furthermore, strain-specific data regarding the pH values were also used for the model developed in the current study.

Bubble column experiments

To determine the growth rates of *N. muscorum* and *T. sociatus* under different kinds of power dissipation and aeration, bubble column experiments were carried out. A "Kniese" bubble column (Edwards, Kniese & Co., Marburg, Germany) was used (see Fig. 1). Columns had a working volume of 1 L, a height h of 40 cm, a diameter d of 6.5 cm, and a filling height h_f of 27 cm. The temperature was set to 24 °C and the light intensity to 100 μmol photons m^{-2} s^{-1} (continuous white lighting) using a water bath and neon lighting tubes (L36W, OSRAM, Germany), respectively. The light intensity was checked with a quantum sensor (Licor 190A, Licor-Biosciences USA). The power dissipation was varied by using different aeration rates (0.3, 0.6, and 1.6 vvm) and calculated according to Storhas (2012), assuming the bubble size to be constant (Eq. 1). The results of the bubble column experiments are not part of the model.

$$\left(\frac{P}{V}\right) = \frac{V_G \times \rho_L \times g \times H_f}{V_L} \quad (1)$$

where V_G = gas volumetric flow, ρ_L = liquid density, g = acceleration of gravity, H_f = filling height, and V_L = liquid volume.

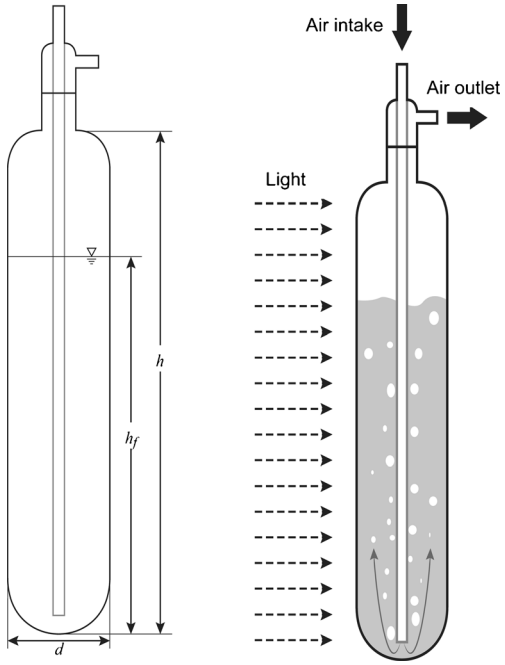

Fig. 1 Schematic of the "Kniese" bubble column. Columns have a working volume of 1 L, a height (h) of 40 cm, a diameter (d) of 6.5 cm, and a filling height (h_f) of 27 cm. The temperature was maintained using a water bath set to 24 °C, and the columns were lit with neon lighting tubes at an intensity of 100 μmol photons m^{-2} s^{-1} (*continuous white light*)

Stirred tank reactor experiments

Growth rates of *N. muscorum* and *T. sociatus* were determined as a function of power dissipation and different CO_2 values in stirred tank reactor experiments. A standard stirred tank reactor was used (Minifors, Infors) with a maximum working volume of 2.5 L and two blade agitators. The temperature was set to 24 °C and the light intensity to 100 μmol photons m^{-2} s^{-1} using continuous white lighting from external LEDs (5 W, white, 3×; Paulmann Licht, Germany). The light was measured inside the tank. The light intensity was checked with a quantum sensor (Licor 190A). Different CO_2 concentrations were achieved using a volume-controlled mixture of ambient air and 99.9 % CO_2. The mixing quality was checked using exhaust gas analysis prior to connection to the reactor system (BlueSense gas sensor, Germany). The filling volume was set to 1 L, the aeration to 1 vvm, and agitation rate to 150 rpm throughout all experiments. No pH control was used. The power dissipation was calculated at the end of each experiment (Storhas 2012).

Culture preparation and biomass determination

Starter cultures of *N. muscorum* and *T. sociatus* were cultivated for 7 days in an illuminated shaker (Multitron2, ATM 00, Infors) using 500 mL Erlenmeyer shake flasks. The dry weight was determined twice using two 1.5 mL reaction vessels per sample. Each sample was dried for 24 h and then stored in an exsiccator. A culture volume of 1 mL (fresh weight) was taken from each vessel, centrifuged at 10,600×g, and then dried at 60 °C for at least 24 h. The dry weight was then calculated from the weight difference between the filled and the empty vessel. The growth rates were determined during exponential growth phase.

Simplified growth model

Based on the data from the growth experiments of *N. muscorum* and *T. sociatus* in relation to temperature (data not shown), pH, and CO_2 concentration, we developed a mathematical model to predict further cultivations and experiments. For simplification, the parameters were assumed to be constant over time (except for the substrate concentration) and to not affect one another.

The basic approach is described through Eq. 2:

$$\mu = \mu_{max} \times f_{substrate} \times f_{temperature} \times f_{pH} \times f_{CO_2} \quad (2)$$

The factors f_i describe the individual influence of these parameters on the growth rate in the range 0–1. If the respective parameter is optimal, the associated factor f_i is equal to one.

Hence, the maximal growth rate depends on temperature, and the corresponding factor can be described by a bell-shaped curve (Gerber and Span 2008):

$$f_{temperature} = e^{-\left(\frac{Tx-Topt}{A}\right)^2} \quad (3)$$

where T_x = temperature (°C), T_{opt} = organism specific optimal temperature (°C), A = peak width.

The growth rate of any organisms is further dependent on the pH value. Presser and colleagues (1997) predicted that the growth rate is proportional to the difference between the actual H^+ ion concentration and the H^+ ion concentration, where growth is no longer possible.

$$\mu = c \times \left([H^+_{max}] - [H^+]\right) \quad (4)$$

where c = proportionality constant (empiric factor), $[H^+_{max}]$ = H^+ ion concentration where growth is no longer possible, and $[H^+]$ = current H^+ ion concentration.

It is further assumed that $[H^+_{max}]$ equals pH_{min}. Incorporation of this into Eq. 4 results in Eq. 5 (Presser et al. 1997):

$$\mu = \left(c \times 10^{-pHmin}\right) \times \left(\frac{10^{-pHmin} - 10^{-pH}}{10^{-pHmin}}\right) \quad (5)$$

Addition of the equation $c \times 10^{-pHmin}$ with μ_{max} and further transformation of the formula results in (Presser et al. 1997):

$$\mu = \mu_{max} \times \left(1 - \frac{10^{pHmin}}{10^{pH}}\right) \quad (6)$$

To cover the entire pH range (0–14), the final formula regarding pH influence on the growth is:

$$f_{pH} = \left(1 - \frac{10^{pHmin}}{10^{pH}}\right) \times \left(1 - \frac{10^{pH}}{10^{pHmax}}\right) \quad (7)$$

The influence of CO_2 was only tested at three different values (see Chapters 2 and 3). Thus, it is not possible to derive a reasonable mathematical equation. Gas exchange measurements indicate a logarithmic curve, but until further experiments are carried out, the influence of CO_2 is not included in the model. The growth dependency on the main substrate is usually described via a Monod approach:

$$\mu = \frac{\mu_{max} \times S}{K_S + S} \quad (8)$$

where μ_{max} = maximum growth rate, S = concentration of limiting substrate (g l^{-1}), and K_S = saturation constant.

Experiments to examine the exact effects of substrate limitation have not yet been carried out. Therefore, we assumed that CO_2 is a substrate but remains constant throughout cultivation. Hence, other substrates, such as phosphate or nitrogen, have to be the bottle neck because growth curves indicate a clear substrate limitation of some kind. The final formula derived from our experimental data is shown below:

$$\mu = \mu_{max} \times \frac{S}{K_S + S} \times e^{-1\left(\frac{T_x - T_{opt}}{A}\right)^2} \times \left(1 - \frac{10^{pH_{min}}}{10^{pH}}\right) \times \left(1 - \frac{10^{pH}}{10^{pH_{max}}}\right) \quad (9)$$

Results

To investigate which cultivation system and combination of parameters are optimal for achieving maximum growth rates of terrestrial cyanobacteria, different cultivation systems were tested. To ensure the comparability of the results shown in this work, each of the three different systems used are relatively common, and more or less standard in every laboratory.

Shaking flask experiments were conducted to determine the influence of power dissipation and pH level. Different power dissipations were achieved by varying the filling volume and the shaking frequency (rpm). High filling volumes and low shaking frequencies resulted in low power dissipation, whereas low filling volumes and high shaking frequencies resulted in high power dissipation. Three different volume/shaking combinations were chosen using BG 11 medium and a standard pH of 7: 50 ml and 90 rpm, 150 ml and 120 rpm, and 50 ml and 120 rpm, resulting in power dissipations of 358, 429, and 858 W m^{-3}, respectively. Strain-specific optimal pH values were studied with settings of 50 ml and 120 rpm. The results of all pH optimization experiments are shown in Fig. 2 and Table 2.

The growth rate of *T. sociatus* varied significantly from 0.02 day^{-1}±0.002 (150 mL/120 rpm) at maximum to 0.006 day^{-1}±0.0003 (50 mL/90 rpm; $p \leq 0.05$). A pH shift from 7 (standard BG 11) to 8 increased growth rate by 30 %, from 0.01±0.001 to 0.013 day^{-1}±0.001. The growth rate of *N. muscorum* varied from 0.036 day^{-1}±0.0017 (150 mL/120 rpm) at maximum to 0.0046 day^{-1}±0.003 at minimum. An optimization of the pH value from 7 to 6 increased growth rate by 47 %, from 0.0057±0.0008 to 0.0084 day^{-1}±0.001. All measured growth rates at different pH values are shown in Table 2. While the growth rate at the second lowest power dissipation differed significantly from all others ($p \leq 0.05$), only results of those experiments with an optimized pH level were significantly different to the experiments carried out at standard pH 7.

To investigate the influence of power dissipation and CO_2 concentration, bubble column experiments with varying aeration rates (vvm) were conducted. High aeration rates result in an increased power dissipation and high dissolved CO_2 concentration. This is because the dissolved CO_2 concentration depends on the k_La value, which increases with the size of the surface enabling the gas transfer. That could be achieved by higher stirring rates (in case of the stirred tank reactor) or by a

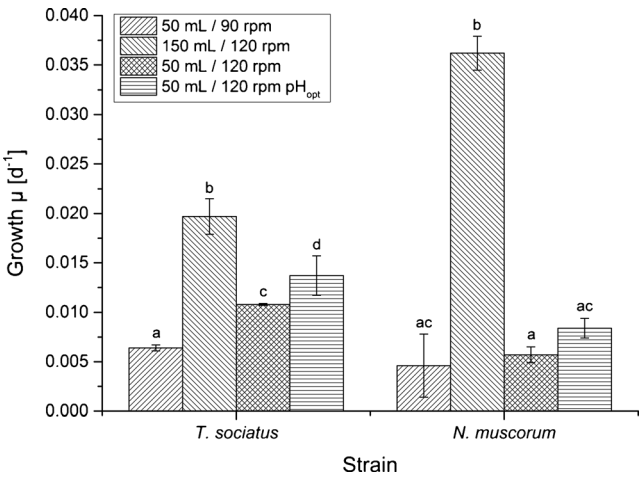

Fig. 2 Comparison of different growth rates in shaking flask experiments. The *y*-axis shows the growth rate (μ) per day, while the *x*-axis shows the different organisms used for the experiments. The different pattern indicates the filling volume used as well as the revolutions per minute (mL/rpm). The parameter of power dissipation was varied by using different filling volumes (50 and 150 mL) in combination with different shaking rates (90 and 120 rpm). Statistically significant differences between different settings are indicated by *lowercase letters. Bars with the same letter* do not differ significantly, while *bars with different letters* are significantly different ($n = 6$, $p \leq 0.05$) according to a two-sided *t* test. All results are based on dry weight (gDW)

higher aeration rate, resulting in more gas bubbles and therefore a larger area for gas transfer. Three different settings were chosen 0.3 vvm (14.7 W m^{-3}), 0.7 vvm (29.4 W m^{-3}), and 1.6 vvm (70.6 W m^{-3}), sorted from lowest to highest power dissipation and CO_2 concentration. The results of all experiments are shown in Fig. 3. The growth rate of *T. sociatus* varied from 0.08 day^{-1}±0.01 (0.7 vvm) at maximum to 0.028 day^{-1}±0.008 (0.3 vvm), whereas the growth rate of *N. muscorum* had a maximum of 0.12 day^{-1}±0.014 (1.6 vvm) and a minimum of 0.035 day^{-1}±0.01 (0.3 vvm). The difference between the highest and lowest growth rates of *N. muscorum* as a result of aeration were significantly different ($p \leq 0.05$), while there was no significant difference in growth rate at the lowest and highest aeration rates for *T. sociatus*.

To examine the influence of defined CO_2 concentration on growth, stirred tank reactor experiments were conducted. Differences in CO_2 concentration were achieved by varying the CO_2 concentration (% vol.) in the gas input. Three different settings were chosen: 0.03 % vol., 2.0 % vol., and 5.0 % vol. The power dissipation was calculated to be 1.5 kW m^{-3} for all experiments. The results of all experiments are shown in Fig. 4. The growth rate of *T. sociatus* varied from 1.15 day^{-1}±0.08 (2 % vol.) at maximum to 0.11 day^{-1}± 0.01 (0.03 % vol.) at minimum, whereas the growth rate of *N. muscorum* had a maximum of 0.72 day^{-1}±0.22 (5 % vol.) and a minimum of 0.06 day^{-1}±0.01 (0.03 % vol.). Statistically there was no difference between 2 % vol. and 5 % vol. CO_2 concentration in regard to the growth rates of both organisms, while a

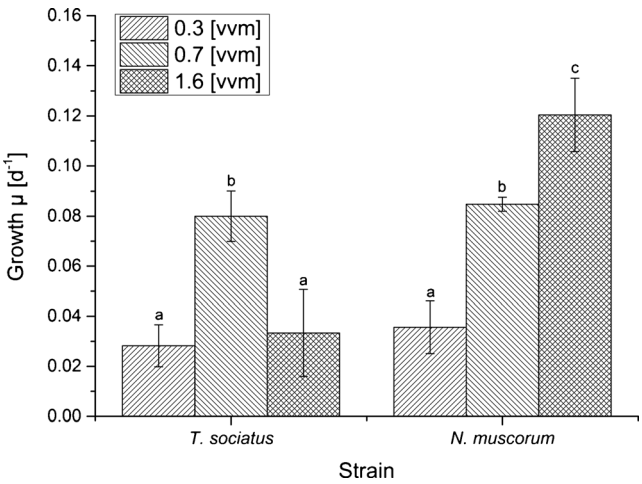

Fig. 3 Comparison of different growth rates in bubble column experiments. The *y*-axis shows the growth rate (μ) per day, while the *x*-axis shows the different organisms used for the experiments. The different patterns indicate the aeration rate in volume per volume and time (vvm). The parameters of power dissipation and aeration were varied by using different aeration rates (0.3, 0.6, and 1.6 vvm). Statistically significant differences between different settings are indicated by *lowercase letters. Bars with the same letter* do not differ significantly, while *bars with different letters* are significantly different ($n=6$, $p \leq 0.05$) according to a two-sided *t* test. All results are based on dry weight (gDW)

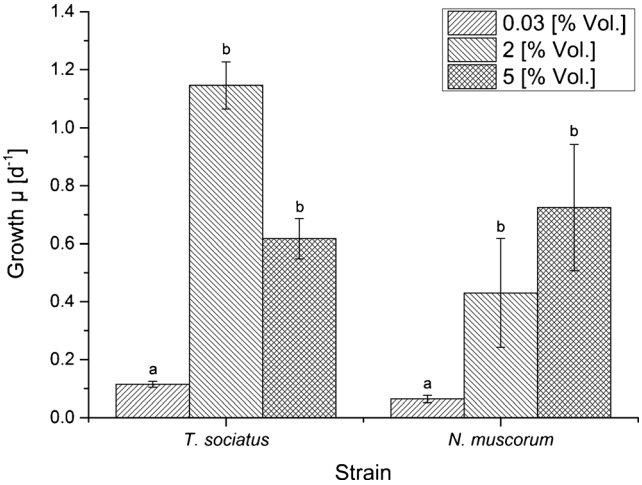

Fig. 4 Comparison of different growth rates in stirred tank reactor experiments. The *y*-axis shows the growth rate (μ) per day, while the *x*-axis shows the different organisms used for the experiments. The different patterns indicate the CO_2 concentration used for aeration (% vol.). A standard stirred tank reactor was used with a maximum working volume of 2.5 L and two blade agitators. The temperature was set to 24 °C and the light intensity to 100 μmol photons m^{-2} s^{-1} (using continuous white lighting). Different CO_2 concentrations in the aeration were maintained using a volume-controlled mixture of ambient air and 99.9 % CO_2. The filling volume was set to 1 L, the aeration to 1 vvm, and the agitation rate to 150 rpm throughout all experiments. No pH control was used. Statistically significant differences between different settings are indicated by *lowercase letters. Bars with the same letter* do not differ significantly, while *bars with different letters* are significantly different ($n=6$, $p \leq 0.05$) according to a two-sided *t* test. All results are based on dry weight (gDW)

significant difference ($p \leq 0.05$) was observed between CO_2 concentrations of 0.03 % vol. and 2.0 or 5.0 % vol.

To predict the outcome of further cultivations, a mathematical model based on the experimental data was developed. Ideally, a model should predict changes in biomass (growth rate μ) dependent on common cultivation parameters such as substrate concentration, temperature, pH, and CO_2. For simplification, the parameters were assumed to be constant over time (except for the substrate concentration) and not to affect one another. The experimental data and parameters presented in Table 1 for the stirred tank reactor were used in the mathematical formula (Eq. 9) to compare the outcomes of the mathematical model with actual experimental data.

The determination of the optimal growth temperature for each of the organisms was carried out previously (data not shown). Experiments concerning the optimal pH value are shown in Table 2. Values for S and K_S were estimated according to previously published data. Because the limiting substrate was not determined yet, S was assumed to be 2 g L^{-1}, which is derived from the soluble substances in medium BG 11 (Rippka et al. 1979). Data from the microalgae *Porphyridium* sp. was used to estimate the saturation constant K_S (0.7 g L^{-1}) because it was cultivated using similar conditions (Popova and Boyadjiev 2008). Although CO_2 was the main substrate during the cultivations, no exact formula could have been derived

Table 1 Parameters used for mathematical model

	μ_{max} (day^{-1})	S ($g\ L^{-1}$)	K_S ($g\ L^{-1}$)	T_{opt} (°C)	A	pH_{opt}	pH_{min}	pH_{max}
Nostoc muscorum	0.72	2	0.7	24	8.19	6	3	12
Trichocoleus sociatus	1.15	2	0.7	24	8.19	8	3	12

The parameters used for the simulation originate from experiments examining the pH level, temperature, and growth rate, as well as from data from the literature (marked with *asterisks*)

because only three different concentrations (0.03, 2.0, and 5.0 % vol.) were examined so far. The effect of different CO_2 concentrations on the growth rate of terrestrial cyanobacteria is therefore not included in Eq. 9. The Berkeley Madonna program was used to simulate the growth of *N. muscorum* and *T. sociatus* from the data in Table 1. A comparison between the simulation and the experiment is shown in Fig. 5. The model had a match of 98.8 % (R^2= 0.988) to the experimental data for *N. muscorum* and 75.0 % (R^2=0.75) in the case of *T. sociatus*.

Discussion

Green algae, such as *Chlorella vulgaris* and *Chlamydomonas reinhardtii*, are commonly used in biotechnological applications (Heredia-Arroyo et al. 2011; Ong et al. 2010; Chen and Melis 2013). Terrestrial cyanobacteria, however, are highly under studied and seldom utilized in biotechnological processes. This is because of the more challenging cultivation techniques and more complex determination of standard reference parameters, such as cell size and number, for terrestrial cyanobacteria (Andersen 2005). The latter is easy to perform in unicellular microalgae, but highly challenging for filamentous strains. Whereas green algae are robust in terms of power dissipation, terrestrial cyanobacteria tolerate higher temperatures and desiccation and feature a broader product spectrum (Sand-Jensen and Jespersen 2012; Pulz and Gross 2004). The aim of the current work was therefore to develop a suitable cultivation methodology using a basic research approach to evaluate important cultivation parameters.

Growth rates of microalgae depend on many factors, which occasionally influence each other. Therefore, we tested pH, temperature, CO_2 concentration as the main carbon source, and power dissipation. Power dissipation is especially important to compare different cultivation systems commonly used for cultivation of microalgae (Buchs et al. 2000), as it allows an assessment of mixing quality. Because nutrients are mainly transported to the cells by mixing a culture, this directly affects the growth rate. In addition, because the type of power input is not the same in each cultivation system, the power dissipation is not directly comparable. Nevertheless, the results give a good indication of whether two different growth rates could be compared.

Shaking flasks are commonly used to screen factors influencing a particular target parameter because they are easy to use and multiple experiments can be performed simultaneously. This system was therefore used to determine the strain-specific pH and temperature optimums (data not shown) and to investigate the optimization potential regarding the application of a strain-specific, optimal pH. It was expected that each of the two strains tested would have an individual pH optimum where the specific growth rate was higher than at pH 7, as it is in the BG11 medium used worldwide as standard. *T. sociatus* originates from an arid habitat in the north of Israel, where the soil pH ranges from 6.8 to 9.7 (Breckle 2008). Therefore, a corresponding optimum in this region was expected and was confirmed to be pH 8. The optimum pH for *N. muscorum* was previously found to be between 7 and 8.5 (Allison et al. 1936; Sand-Jensen and Jespersen 2012). This could not be confirmed in our current experiments (pH 6); however, *N. muscorum* is a cosmopolitan species that

Table 2 Growth rates of *Nostoc muscorum* and *Trichocoleus sociatus* in relation to different pH levels

pH value	4	5	6	7	8	9	10	11
T. sociatus growth rates (day^{-1})	0.0052± 0.0007	0.0066± 0.0007	0.0119± 0.0003	0.0126± 0.0002	0.0137± 0.0004	0.0125± 0.0002	0.0118± 0.0002	0.0115± 0.0002
Statistical difference	a	a	bc	bc	b	bc	c	c
N. muscorum growth rates (day^{-1})	0.0056± 0.0001	0.0061± 0.0001	0.0084± 0.0003	0.0078± 0.0002	0.0061± 0.0005	0.0060± 0.0001	0.0059± 0.0001	0.0059± 0.0001
Statistical difference	ade	a	b	cd	ace	cd	e	ade

The influence of pH levels ranging from 4 to 11 was examined at fixed settings of a 50 mL filling volume in a 500 ml flask with shaking at 120 rpm. pH level optimization was examined using one shaking flasks per pH value and six repetitions. Growth rates for each pH value were determined during the exponential phase and statistically evaluated

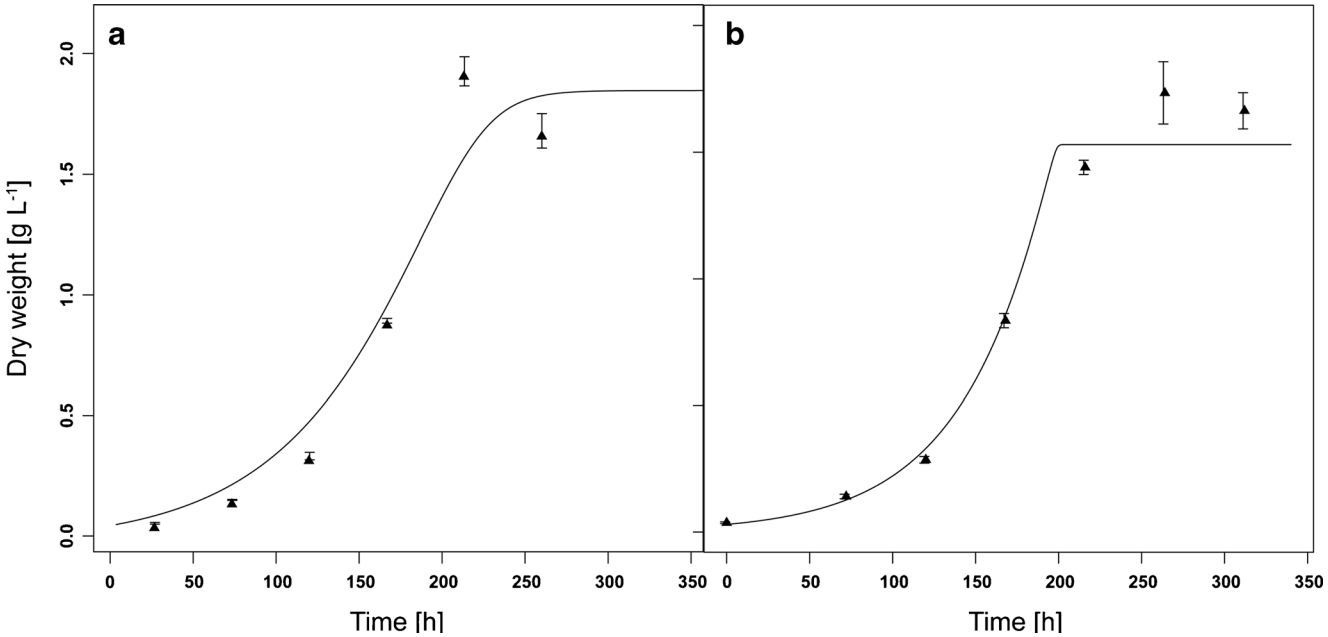

Fig. 5 Simulation model of *Nostoc muscorum* (a) and *T. sociatus* (b). ▲ indicates the experimental data for dry weight over time in stirred tank reactor experiments. — shows simulated growth using the same parameters as were used for the experiments. Experimental data are taken from stirred tank reactor experiments investigating the growth rate and from shaking flasks experiments investigating temperature and pH level. Growth simulation was carried out using Berkeley Madonna software

is very adaptable (Allison et al. 1936; Sand-Jensen and Jespersen 2012), which may lead to strain-specific differences. On average, the use of a medium with an optimum pH value for each of the organisms increased the growth rates by about 37 %. The greatest effect was seen in the *N. muscorum* optimization experiments (47 %). This example shows the potential of an optimum pH level for optimizing growth rate.

The concentration of CO_2 is decisive in determining whether the growth rate increases or inhibition occurs. To examine if the growth of the terrestrial cyanobacteria could be increased, increasing concentrations of CO_2 in inlet air compared with ambient air were examined in a stirred tank reactor. Using this system, the organisms were specifically examined in relation to the effect of elevated CO_2 concentrations under the same power dissipation conditions (regarding the stirrer) and using an exhaust gas analysis. It was expected that an increase in CO_2 concentration would increase the growth rate, as the concentration of CO_2 in ambient air is not optimal for growth (Richter 1998). Increased growth rates were previously observed at a CO_2 concentration of 1 % by volume (Harris 1989; Sultemeyer et al. 1995). An increase in carbon supply generally leads to a steady state in green algae both with and without CCM (Palmqvist et al. 1995), and more than 3 % CO_2 resulted in no further improvement in the growth rate of *C. reinhardtii* (Geier 2011). Our results agreed with the hypothesis, with a significant increase in the growth rate in the presence of 2 % CO_2 compared with ambient air, which did not significantly differ from the growth rate at 5 % CO_2. The data even suggested that 5 % CO_2 may have a negative effect compared with 2 % CO_2 for *T. sociatus*. Further tests may provide additional information, particularly in the range of 1–3 % CO_2, to determine the exact growth optimum in terms of CO_2.

Mechanical stress and power dissipation during culturing can fundamentally alter the growth of cyanobacteria and green algae. Depending on the strain, different optima for the power dissipation are expected, as resistance to mechanical stress is different for each organism. A high power input is correlated with a high rate of gas exchange, and therefore an improved substrate supply can be achieved. We hypothesize that through the interaction of mechanical stress and gas exchange, there will be an optimal power dissipation for each bacterial strain, wherein the maximum growth rate is attained. If this optimum is exceeded, mechanical damages are expected, while in falling below the optimum, it is assumed that gas exchange is the limiting factor. These relations have been described for the green algae *Phaeodactylum tricornutum* and *Porphyridium cruentum* (Sobczuk et al. 2006). These expectations were confirmed by our experimental results; however, significant differences between different cultivation systems were observed. Although the maximum growth rates were obtained in the system with the highest power dissipation (stirred tank reactor), the system with the lowest power dissipation (bubble column) showed the second highest growth rates, although the power input in the shaking flasks was, on average, 10 times higher. However, the liquid volume to gas surface ratios in the two systems are quite different. Using the shaking flasks, gas

transport between the air–water interfaces can only occur at the surface of the rotation paraboloid. Thus, only small k_La values can be achieved. The situation is much improved in bubble columns, where high gas hold-ups are possible, according to the superficial gas velocities. In addition, depending on the diameter of the bubbles, the k_La can reach values from 108 to 720 h^{-1}. In small bubble columns (100–200 mL), k_La values of 576 h^{-1} have been described in the literature (Weuster-Botz et al. 2001), while typical values for shaking flasks range from only 8 to 200 h^{-1}. The stirred tank reactor is very similar to the bubble column in terms of the type of aeration, because the medium and the organisms are aerated from the bottom up. Furthermore, the gas is dispersed by a stirrer so that the mixing is improved and the surface of the gas bubbles is increased. Thus, the diffusive gas exchange and the supply of the organisms with CO_2 can be improved. Although the mechanical stress on the cells is higher than in other culturing systems, this is outweighed by the beneficial effect of the improved gas exchange, even for sensitive cyanobacteria. Different agitators in stirred tank reactors still represent unused potential for further improvement of the cyanobacterial growth rates.

A comparison of the growth rates obtained in the current study with results of previous work is challenging because many different systems are used in the cultivation of phototrophic organisms, all with different operating parameters. The comparison is furthermore hampered because many strains are not yet described or not classified consistently and clearly. A growth rate of 0.24 day^{-1} has previously been described for *N. muscorum* in shaking flasks (Abouwaly and Shabana 1993). While the observed growth rates of the shake flask experiments are approximately 10 times lower, the measured growth rates from *N. muscorum* cultivated in the bubble column are quite similar in comparison to the reference. However, the determined growth rates in the stirred tank reactor were significantly higher (0.72 day^{-1}). This is mainly due to the aeration with elevated CO_2 concentrations and, because of the high power dissipation, improved mixing. Growth rates of 0.22–0.45 day^{-1} on a fresh weight basis were described for *Microcoleus chthonoplastes*, which is a related to *T. sociatus* (Karsten et al. 1996). The actual measured growth rates in shaking flasks as well as in the bubble column were significantly lower than the values above. Because of the higher power dissipation and the use of elevated CO_2 concentrations, the growth rates were increased in the stirred tank reactor (1.15 day^{-1}).

We hypothesized that growth rates of microalgae could be predicted by a basic model based only on three parameters (temperature, pH, and substrates from the medium). This model fit our data with an accuracy of 98.8 % for *N. muscorum* and 75.0 % for *T. sociatus* in a stirred tank reactor. Nevertheless, mathematical models and simulations describing the growth of microorganisms are only as good as the data on which they are based. It is particularly important to note that these specifications can differ significantly between different organisms. For example, organisms have different temperature optima, whereby the function that describes this data can vary greatly. The number of experimentally determined values is important, as the more data that is available, the more the derived functions will fit. For a description of the bell-shaped curve as a function of the growth of each organism in regard to temperature, four data points could be used. This may be improved by further experiments. The same applies to other parameters such as pH level and CO_2 concentration. It was also assumed that the growth rates within the pH range of 4–11 are almost identical and that the pH value remains constant throughout the cultivation. In reality, this is not the case. Moreover, the environment is alkalized through carbon assimilation, and other cells can actively adjust the pH by the release of compatible solutes into the medium (Lustigman et al. 1995; Brock 1973). For this reason, change in pH over time must be incorporated in the model. Therefore, the relationship between the change in the alkalization of the medium and the change in biomass over time should be examined. In addition, the pH level affects the availability of nutrients and shifts the dissociation of CO_2. These relationships need to be studied for better modeling of growth as a function of pH value.

The deviation of the calculated growth curve from the experimentally determined curve is because data pertaining to function in submerged systems is incomplete (regarding parameters S and K_S). Even though *Porphyridium* sp. is taxonomically distant, its K_S value was chosen because it was determined using the same cultivation parameters. In addition, interactions between the parameters (temperature, pH, CO_2 aeration) were not taken into account. Nevertheless, Eq. 9 provides a relatively precise description of the growth of *N. muscorum* and is acceptable for *T. sociatus* in a stirred tank reactor. Thus, the model provides a good basis for further improvement.

In summary, we showed that growth rates of terrestrial cyanobacteria can be improved to achieve levels that are comparable with common microbial strains. When systems are used at low power dissipation, the growth may be even better. However, when a system is used wherein the power dissipation is high, the growth rate cannot be increased proportionally because of shear stress. In these cases, there is the possibility that other types of stirrers could contribute to the optimization. Moreover, we demonstrated the importance of adapting parameters such as temperature, pH level, and CO_2 concentration to each strain to achieve optimal growth rates. Based on these optimization data, a basic model was created to describe the growth of terrestrial cyanobacteria. Although the model has not yet been fully optimized, it at least fits the

experimental data from a cultivation of *N. muscorum* with a precision of about 99 %. Future experiments will show if this basic model can be applied to other cyanobacteria. The use of a totally different cultivation system, which is specifically designed for terrestrial organisms (Kuhne et al. 2013), could be even more beneficial for improving the growth rates of cyanobacteria, as well as the production particularly of desiccation-induced products.

Acknowledgment This research was supported by the Deutsche Forschungsgemeinschaft (UL 170/7-1, LA 1026/9-1).

References

Abouwaly H, Shabana EF (1993) Recovery of *Nostoc muscorum* previously exposed to some triazine and phenylurea herbicides. Bull Environ Contam Toxicol 50:665–673

Allison FE, Hoover SR, Morris HJ (1936) Physiological studies with the nitrogen fixing alga, *Nostoc muscorum*. Bot Gaz 98:433–463

Andersen RA (ed) (2005) Algal culturing techniques. Elsevier/Academic Press, Amsterdam

Bandyopadhyay A, Stöckel J, Min H, Sherman LA, Pakrasi HB (2010) High rates of photobiological H_2 production by a cyanobacterium under aerobic conditions. Nat Commun 1:139

Belnap J, Lange OL (2003) Biological soil crusts: structure, function, and management. Ecological studies, vol 150, 2nd edn. Springer, New York

Bewley CA, Gustafson KR, Boyd MR, Covell DG, Bax A, Clore GM, Gronenborn AM (1998) Solution structure of cyanovirin-N, a potent HIV-inactivating protein. Nat Struct Biol 5:571–578

Boyd MR, Gustafson KR, McMahon JB, Shoemaker RH, OKeefe BR, Mori T, Gulakowski RJ, Wu L, Rivera MI, Laurencot CM, Currens MJ, Cardellina JH, Buckheit RW, Nara PL, Pannell LK, Sowder RC, Henderson LE (1997) Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. Antimicrob Agents Chemother 41:1521–1530

Breckle SW (2008) Arid dune ecosystems: the Nizzana Sands in the Negev Desert. Ecological Studies, vol 200. Springer, New York

Brock TD (1973) Lower pH limit for existence of blue-green-algae—evolutionary and ecological implications. Science 179:480–483

Buchs J, Maier U, Milbradt C, Zoels B (2000) Power consumption in shaking flasks on rotary shaking machines: I. Power consumption measurement in unbaffled flasks at low liquid viscosity. Biotechnol Bioeng 68:589–593

Chen HC, Melis A (2013) Marker-free genetic engineering of the chloroplast in the green microalga *Chlamydomonas reinhardtii*. Plant Biotechnol J 11:818–828

Deng M-D, Coleman JR (1999) Ethanol synthesis by genetic engineering in cyanobacteria. Appl Environ Microbiol 65:523–528

Dexter J, Fu P (2009) Metabolic engineering of cyanobacteria for ethanol production. Energy Environ Sci 2:857–864

Geier S (2011) Photobiologische Wasserstoffproduktion mit der einzelligen Grünalge *Chlamydomonas reinhardtii* unter verfahrenstechnischen Aspekten. Dissertation, Erlangen-Nürnberg

Gerber M, Span R (2008) An analysis of available mathematical models for anaerobic digestion of organic substances for production of biogas. Int Gas Union Res Conf p 230

Harris EH (1989) The *Chlamydomonas* sourcebook: a comprehensive guide to biology and laboratory use. Academic, San Diego

Heinrich U, Tronnier H (2010) Systemische Fotoprotektion durch Carotinoide. Z Phytother 31:185–187

Heredia-Arroyo T, Wei W, Ruan R, Hu B (2011) Mixotrophic cultivation of *Chlorella vulgaris* and its potential application for the oil accumulation from non-sugar materials. Biomass Bioenergy 35:2245–2253

Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 54:621–639

International Congress on Pigments in Food. 5, Helsinki (2008) Pigments in food—for quality and health. 5th International Congress on Pigments in Food, Helsinki, 14–16 August, 2008. Univ.

Karsten U, Klimant I, Holst G (1996) A new in vivo fluorimetric technique to measure growth of adhering phototrophic microorganisms. Appl Environ Microbiol 62:237–243

Krinsky NI (1979) Carotenoid protection against oxidation. Pure Appl Chem 51:649–660

Kuhne S, Lakatos M, Foltz S, Muffler K, Ulber R (2013) Characterization of terrestrial cyanobacteria to increase process efficiency in low energy consuming production processes. Sustain Chem Process 1:6

Lakatos M, Bilger W, Budel B (2001) Carotenoid composition of terrestrial cyanobacteria: response to natural light conditions in open rock habitats in Venezuela. Eur J Phycol 36:367–375

Lustigman B, Lee LH, Weissmagasic C (1995) Effects of cobalt and pH on the growth of *Chlamydomonas-reinhardtii*. Bull Environ Contam Toxicol 55:65–72

Mata TM, Martins AA (2010) Biodiesel production processes. In: Delgado JMPQ (ed) Current trends in chemical engineering. Studium Press LLC, pp 313–342

Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev 14:217–232

Min H, Sherman LA (2010) Hydrogen production by the unicellular, diazotrophic cyanobacterium *Cyanothece* sp. strain ATCC 51142 under conditions of continuous light. Appl Environ Microbiol 76:4293–4301

Muller-Feuga A (2000) The role of microalgae in aquaculture: situation and trends. J Appl Phycol 12:527–534

Ong SC, Kao CY, Chiu SY, Tsai MT, Lin CS (2010) Characterization of the thermal-tolerant mutants of *Chlorella* sp with high growth rate and application in outdoor photobioreactor cultivation. Bioresour Technol 101:2880–2883

Palmqvist K, Sultemeyer D, Baldet P, Andrews TJ, Badger MR (1995) Characterization of inorganic carbon fluxes, carbonic anhydrase(s) and ribulose-1,5-biphosphate carboxylase-oxygenase in the green unicellular alga *Coccomyxa*—comparisons with low-CO_2 cells of *Chlamydomonas reinhardtii*. Planta 197:352–361

Palozza P, Krinsky NI (1992) Antioxidant effects of carotenoids in vivo and in vitro—an overview. Methods Enzymol 213:403–420

Peter CP, Suzuki Y, Rachinskiy K, Lotter S, Buchs J (2006) Volumetric power consumption in baffled shake flasks. Chem Eng Sci 61:3771–3779

Popova P, Boyadjiev C (2008) About microalgae growth kinetics modeling. Chem Biochem Eng Q 22:491–497

Prasanna R, Sood A, Jaiswal P, Nayak S, Gupta V, Chaudhary V, Joshi M, Natarajan C (2010) Rediscovering cyanobacteria as valuable sources of bioactive compounds. Appl Biochem Microbiol 46:119–134

Presser KA, Ratkowsky DA, Ross T (1997) Modelling the growth rate of *Escherichia coli* as a function of pH and lactic acid concentration. Appl Environ Microbiol 63:2355–2360

Pulz O (2001) Photobioreactors: production systems for phototrophic microorganisms. Appl Microbiol Biotechnol 57:287–293

Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 65:635–648

Rascher U, Lakatos M, Budel B, Luttge U (2003) Photosynthetic field capacity of cyanobacteria of a tropical inselberg of the Guiana Highlands. Eur J Phycol 38:247–256

Richter G (1998) Stoffwechselphysiologie der Pflanzen: Physiologie und Biochemie des Primär- und Sekundärstoffwechsels. Thieme, Stuttgart

Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY (1979) Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111:1–61

Rittmann BE (2008) Opportunities for renewable bioenergy using microorganisms. Biotechnol Bioeng 100:203–212

Sand-Jensen K, Jespersen TS (2012) Tolerance of the widespread cyanobacterium *Nostoc commune* to extreme temperature variations (-269 to 105°C), pH and salt stress. Oecologia 169:331–339

Singh S, Kate BN, Banerjee UC (2005) Bioactive compounds from cyanobacteria and microalgae: an overview. Crit Rev Biotechnol 25:73–95

Sobczuk TM, Camacho FG, Grima EM, Chisti Y (2006) Effects of agitation on the microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum*. Bioprocess Biosyst Eng 28:243–250

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101: 87–96

Storhas W (2012) Bioverfahrensentwicklung. Wiley

Sultemeyer D, Price GD, Yu JW, Badger MR (1995) Characterization of carbon-dioxide and bicarbonate transport during steady-state photosynthesis in the marine cyanobacterium *Synechococcus* Strain PCC7002. Planta 197:597–607

Weuster-Botz D, Altenbach-Rehm J, Hawrylenko A (2001) Process-engineering characterization of small-scale bubble columns for microbial process development. Bioprocess Biosyst Eng 24: 3–11

Wijffels RH, Barbosa MJ (2010) An outlook on microalgal biofuels. Science 329:796–799