Culture of *Spirogyra* sp. in a flat-panel airlift photobioreactor

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Abstract

Spirogyra is a green filamentous freshwater algae on which recent studies reveal several promising properties and potential application possibilities in biotechnology. However, little is known about cultivation of *Spirogyra* and even less about large-scale cultivations in closed growth systems. Therefore, the aim of the present study was to elaborate the growth kinetics of *Spirogyra* sp. in a commercially available and scalable photobioreactor. For this purpose, *Spirogyra* sp. was grown indoors in distinct flat-panel airlift photobioreactors equipped with culture-flow directing installations. Hereby, special attention was laid on light administration and specific light availability and it was found that *Spirogyra* sp., in combination with the photobioreactor in question, required high photon-flux densities ($100 \mu\text{mol m}^{-2} \text{s}^{-1} \text{g}_{\text{DW}}^{-1}$) for maximum proliferation which is in accordance with its abundance in epipelagial waters in nature. Applying photon-flux densities of up to $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$, a maximum volumetric productivity and final biomass concentration of $1.15 \text{ g}_{\text{DW}} \text{L}^{-1} \text{day}^{-1}$ and $14.28 \text{ g}_{\text{DW}} \text{L}^{-1}$ were achieved, respectively, the highest to be reported for the alga. To the knowledge of the authors, this is the first report on the growth of *Spirogyra* in a flat-panel photobioreactor.

Keywords Flat-panel airlift photobioreactor · *Spirogyra* · Filamentous · Growth · Biomass · Algae

Introduction

Algae in biotechnology

Current developments, such as the growing world population, scarcity of fossil resources, progressing climate change and an increasing demand for energy, create a need for high protein food and alternative energy sources. Algae are often considered to have a great potential as a renewable resource (Schenk et al. 2008). These photosynthetic organisms can convert sunlight into chemical energy and thereby sequester carbon dioxide (Masojídek et al. 2013). Compared to terrestrial plants, they do not compete for arable land, can grow in salt and waste water, show higher growth rates and higher

biomass yields per hectare and can be harvested nearly all year round (Schenk et al. 2008; Ramaraj et al. 2015).

For the above reasons, and because some algae accumulate large quantities of cellulose, starch, glycerine and oils or produce hydrogen (Sastre 2012), they are one of the most promising source of renewable energy (Schenk et al. 2008). In addition, algae produce valuable compounds such as fine chemicals, fatty acids, pigments used as natural colorants or antioxidants and polysaccharides that are used as gelling agents or stabilizers (Sastre 2012).

Nevertheless, only few of these possible applications are actually put into practice, most of them concerning products for the cosmetics and food industry and other high-value compounds (Priyadarshani and Rath 2012; Spolaore et al. 2006). One of the reasons therefore is that growth in reactor systems is still not very well studied and shows only moderate growth rates (Spolaore et al. 2006). More research is needed to make algal mass production more profitable, especially for the production of low cost products.

Spirogyra

Spirogyra sp. is a common and widely distributed freshwater algae, commonly referred to as pond scum. In fact, its

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filaments contain plenty of valuable and useful compounds which make it an object of research with a great potential to be used for biotechnological applications. The genus *Spirogyra* comprises some 400 species and is characterized by its unbranched, free floating filaments (Linne von Berg 2012).

Application of *Spirogyra* sp.

A main part of the research that has been done on *Spirogyra* focusses on the use of non-living biomass for removal of heavy metals from aqueous solutions, called biosorption (Davis et al. 2003). Owing to human activities, concentration of heavy metal ions in soils and water is increasing, posing a threat to the environment and human health (Ronda et al. 2007). Thus, there is a rising need for cost-efficient and environmental friendly methods to treat industrial effluents, but common procedures for the removal of metal ions entail disadvantages such as the formation of toxic waste, incomplete metal removal and high costs (Ahalya et al. 2003). Biosorption, however, proved to be a promising alternative method which does not suffer from the disadvantages mentioned above. Due to its high capacity to absorb metal ions and due to its local abundance (Gupta and Rastogi 2008), *Spirogyra* biomass was found to be suitable material for biosorption of heavy metals like chromium(VI) (Gupta et al. 2001), lead (Gupta and Rastogi 2008; Romera et al. 2003), copper (Gupta et al. 2006; Romera et al. 2003), nickel (Romera et al. 2003), cadmium (Romera et al. 2003), zinc (Romera et al. 2003) and Cr(III) (Bishnoi et al. 2007). Further studies revealed its ability to remove fluoride and reactive dyes from wastewater (Khalaf 2008; Özer et al. 2006; Venkata Mohan et al. 2002; Venkata Mohan et al. 2007).

Moreover, *Spirogyra* is a typical component of South-East Asian food (Buri 1978) and was found to have health-beneficial effects as it contains a large quantity of proteins, minerals, vitamins, fat and carbohydrate (Ontawong et al. 2013). As a result it could be used for the commercial production of health foods by the food industry (Tipnee et al. 2015).

Some species of *Spirogyra* have recently drawn attention due to their antioxidant activity in vivo and in vitro (Ontawong et al. 2013; Thumvijit et al. 2013a, b) and their antimutagenicity (Thumvijit et al. 2013a) in vitro as well as antidiabetic and renoprotective effects (Ontawong et al. 2013) in rats. Researchers also found strong antibacterial activity of methanolic extract made from *Spirogyra micro-punctata* against several multidrug resistant bacterial pathogens (Alshididi and Jawad 2015). These findings suggest, that *Spirogyra* could be used for the development of new drugs and as a source of natural antioxidants (Dash et al. 2014; Kumar et al. 2015) and antimicrobial agents (Alshididi and Jawad 2015).

Another possible application might be the use of *Spirogyra* biomass for the production of so called “third generation” biofuels (Maity et al. 2014). The term “third generation” biofuels is used for biofuels made from algae, mostly referring to microalgae. Nevertheless, there are studies considering *Spirogyra* as a potential source of renewable energy as it is available abundantly around the world, fast growing (Ramaraj et al. 2015), can accumulate a high amount of sugar (Ramaraj et al. 2015) and has a simple cell wall which does not require any chemical pre-treatment (Eshaq et al. 2010). Several investigations demonstrated that energy production from *Spirogyra* biomass is feasible and that it is suitable for the production of biogas (Ramaraj et al. 2015), biodiesel (Hossain et al. 2008), bioethanol (Eshaq et al. 2010) and a good source for hydrogen production (Ortigueira et al. 2015).

Studies on the allelopathic activity of *Spirogyra* have recently drawn attention as *Spirogyra* has been shown to enhance growth and toxin production of cyanobacterium *Oscillatoria agardhii* (Mohamed 2002). Toxic cyanobacterial blooms represent a global problem, endangering human health and the ecological system (Bláha et al. 2009). Understanding of allelochemical interactions might be useful for handling this problem (Mohamed 2002).

Apart from the above outlined application possibilities of *Spirogyra*, its natural occurrence makes it an interesting target for commercial biomass production. The use of native species is preferable as they are already adapted to the prevailing conditions and therefore do not require special technology and additional energy (Munir et al. 2015).

Cultivation of *Spirogyra*—the present state of the art

Most literature and cultivations in the field of algae focus on unicellular microalgae. Nevertheless, there are some large-scale cultivations of multicellular algae. The most prominent example therefore is the filamentous cyanobacterium *Arthrospira platensis*. Tredici and Zittelli (1998) for example investigated growth of *Arthrospira platensis* in a constantly illuminated flat chamber reactor with an optical path of 2.5 cm and 5.4 L volume and thereby obtained volumetric productivities of $1.93 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ day}^{-1}$ and a mean cell concentration of $3.2 \text{ g}_{\text{DW}} \text{ L}^{-1}$.

With regard to *Spirogyra*, however, only few cultivations have been reported. For most studies biomass has been collected from nearby rivers and ponds and was maintained or grown in flasks so that proper biotechnological cultivation was not necessary. Gallego et al. (2013) and Munir et al. (2015) for example investigated growth of *Spirogyra* in flasks using culture volumes of 100 mL.

Most large scale cultivations were carried out in open ponds (Han et al. 2009). Ramaraj et al. (2015) used an open outdoor jar with 5 L working volume to grow *Spirogyra*

ellipsoidea. Pacheco et al. (2015) reported about the growth of *Spirogyra* sp. in raceway ponds with 400 L capacity.

With respect to closed systems, hardly any literature is found. Lawton et al. (2013) successfully cultivated *Spirogyra* sp. in aerated 20 and 60 L plastic buckets and Pacheco et al. (2015) used 1 L glass bubble column reactors and 10 L bubble column photobioreactors to produce biomass for the inoculation of a 400 L pond, where the final cultivations were carried out.

When targeting commercial biomass production, it might be preferable to focus on closed photobioreactors rather than open systems. This is because closed systems offer several advantages over open culture systems and it is often assumed that they are going to replace open systems for commercial mass cultures of algae in the future (Borowitzka 1999; Chisti 2007). In closed systems, it is easier to control culture conditions and to avoid contaminations. This allows consistent product quality and the cultivation of algae that have specific requirements with respect to environmental conditions (Borowitzka 1999). Furthermore, obtainable biomass concentrations are nearly 30 times higher (Chisti 2007), resulting in higher productivities and lower harvesting costs (Borowitzka 1999). When comparing production costs of algae biomass in open ponds, horizontal tubular photobioreactors and flat-panel photobioreactors, Norsker et al. (2011) came to the conclusion that for an optimized production, lowest production costs can be achieved in flat-panel photobioreactors (Norsker et al. 2011).

The present work addresses growth of *Spirogyra* sp. in a flat-panel airlift photobioreactor. The objective of the experiment was to find out whether cultivation of *Spirogyra* sp. in the applied reactor system is feasible and to investigate growth kinetics, maximum cell density and volumetric productivity. To the authors knowledge this is the first report about cultivation of *Spirogyra* sp. in a flat panel photobioreactor.

Materials and methods

Algae and medium

Spirogyra sp. (Fig. 1) was kindly supplied by the Culture Collection of Algae (SAG) in Göttingen, Germany, and cultivated in Bold's Basal Medium (from Bischoff & Bold 1963, modified after Starr and Zeikus 1993) with triple nitrate and vitamins (3N-BBM).

The medium had the following composition: NaNO_3 : 0.69 g L^{-1} , KH_2PO_4 : 0.1 g L^{-1} , K_2HPO_4 : 0.01 g L^{-1} , Citric Acid: 0.012 g L^{-1} , CaCl_2 : 0.025 g L^{-1} , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.075 g L^{-1} , NaCl : 0.025 g L^{-1} , $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$: 0.012 mg L^{-1} , $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$: 0.25 mg L^{-1} , $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$: 0.025 mg L^{-1} ,

Fig. 1 Light microscopic image of *Spirogyra* sp. (Microscope type Axio Lab.A1, Carl Zeiss AG, Oberkochen, Germany)

ZnCl_2 : 0.03 mg L^{-1} , Biotin: 0.4 mg L^{-1} , Thiamin-HCl: 2 mg L^{-1} , Vitamin B12: 0.1 mg L^{-1} .

Light

Light was provided 24/7 by an external high pressure sodium vapour lamp (type Plantastar 600W, OSRAM GmbH, Munich, Germany) on one side of the reactor.

The intensity was measured with a light meter (type: LI-250A, LI-COR, Inc., Nebraska, USA) at 18 points throughout the illuminated surface of the reactor and averaged. With respect to varying degrees of mutual shading (Myers 1976), the illumination was adapted to the biomass concentration, finally using approximately $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1} \text{ g}_{\text{DW}}^{-1}$.

Dry weight

Growth was estimated by daily measurements of the dry cell weight. Therefore, volumes of 10 mL of the algal suspension were filtered through a pre-weighted glass fiber filter (type MN 85/70, 55 mm, MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany) and then washed with 20 mL of deionized water and dried for at least 2 h at 105°C . Dry cell weight ($\text{g}_{\text{DW}} \text{ L}^{-1}$) was then determined using a microbalance (type ABS 220-4, KERN & SOHN GmbH, Balingen, Germany).

Nutrients

During the cultivation, concentrations of phosphate and nitrate were measured twice a week and added to the algae suspension if necessary in order to obtain concentrations of $200 \text{ mg PO}_4^{3-} \text{ L}^{-1}$ and $500 \text{ mg NO}_3^- \text{ L}^{-1}$. Iron was added to a final concentration of 2.5 mg Fe L^{-1} using ferric citrate.

Prior to the measurements, the samples were centrifuged at 4816g for 5 min (centrifuge type Heraeus Megafuge, 40R, rotor 75003607, Thermo Fisher Scientific Inc., Waltham, USA). Nitrogen concentration was determined using colorimetric cuvette tests (type LCK339, Hach Lange GmbH, Berlin, Germany). The supernatant, which was used for the measurement, was diluted in order to achieve the prescribed range of 1–60 mg L⁻¹.

Phosphorous concentration was measured using QUANTOFIX® Phosphate test strips (MACH-EREY-NAGEL GmbH & Co. KG, Düren, Germany). The colorimetric cuvette test was distorted by the coloration of the supernatant.

Volumetric productivity

In order to define a sigmoidal function that describes growth characteristics of the culture, the five parameters logistic non-linear-regression model (Cardillo 2012) has been applied, using the software MATLAB (R2015a, The Math-Works, Inc., Natick, Massachusetts, USA). The resulting curves were then differentiated in order to determine the course of the volumetric productivity ($g_{DW} L^{-1} day^{-1}$).

Pre-cultures

Cell suspension for inoculum was first incubated in 100 mL Erlenmeyer flasks containing 50 mL of autoclaved 3N-BBM medium and later transferred to 250 mL Erlenmeyer flasks filled with 100 mL of autoclaved 3N-BBM medium. The flasks were shaken by an orbital shaker (Unimax 2010, Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 20 °C and under continuous illumination with an intensity of 20 $\mu mol m^{-2} s^{-1}$.

A total of 0.5 L of the culture was then transferred to an autoclaved 5 L glass bottle, containing a magnetic agitator and 1 L of autoclaved 3N-BBM medium. The culture was mixed by a magnetic stirrer (200 rpm) and provided with 60 L h⁻¹ filter-sterilized air (filter with a pore size of 0.2 μm , Midisart 2000 type 17805, Satorius AG, Goettingen, Germany) enriched with technical carbon dioxide (2% v/v). The cell suspension was continuously illuminated with fluorescent tubes (type Lumilux 18W/840, Osram GmbH, Munich, Germany) providing a light intensity of 30 $\mu mol m^{-2} s^{-1}$.

The final cultivation was carried out in a 6 L flat-panel airlift photobioreactor as described in detail below.

Culture system

The final cultivations were carried out in a flat-panel airlift photobioreactor with static mixers. The applied flat-panel airlift (FPA) photobioreactor was developed at the Fraunhofer Institute for Interfacial Engineering and Biotechnology

Fig. 2 Side view of the flat-panel airlift photobioreactor with static mixers. The arrows indicate the circulation which is generated due to the special shape of the reactor when air bubbles rise to the top

IGB, Stuttgart, Germany, and is now distributed by Subitec GmbH, Stuttgart, Germany. It is characterized by a special shape including static mixers (see Fig. 2), which generate a circulating flow within the PBRs compartments when air bubbles rise to the top. The vertical tube in the middle of the PBR leads the algal suspension back to the bottom, resulting in a vertical circulation of the reactor content (see Fig. 3). Light intensity decreases approximately exponentially (Zarmi et al. 2013) within the chamber, resulting in different photic zones. As a consequence, the cells switch constantly between the bright and the dark sections of the reactor, which enhances photosynthetic efficiency of the cells (Janssen et al. 2001; Sforza et al. 2012). In addition to optimize the light availability of the cells, good mixing also enhances gas exchange and mass transfer between the medium and the cells (Grobelaar 1994). As the reactor is available in different sizes with a capacity of 6, 28 and 180 L, laboratory experiments can easily be scaled up. The largest unit can be combined to form large production lines for industrial application and exploitation.

In this work, a FPA photobioreactor with 6 L culture volume, an illuminated area of 0.296 m² and an optical path of 3 cm was used. It was made of transparent PVC. The temperature of the reactor content was controlled by a water bath, covering the lower third of the bioreactor. For any kind of inflow and outflow (air, harvest, medium, inoculum, foam), the reactor was attached to silicone tubes, that have been autoclaved before use. Incoming air, enriched with 1.6–5% v/v technical carbon dioxide, was passed through a sterile

Fig. 3 The flat-panel airlift photobioreactor with 6 L culture volume. Due to the rising air bubbles from the bottom of the reactor, the biomass is led down through the tube in the middle of the reactor (downcomer), generating a vertical circulation of the biomass throughout the reactor

filter (pore size: 0.2 μm , type Midisart 2000 type 17805, Satorius AG, Goettingen, Germany). The amount of air was determined and adjusted by means of a variable area flowmeter (CO_2 : type DK800, KROHNE, Messtechnik GmbH, Duisburg, Germany; air: Machined Acrylic Flowmeter, Key Instruments, Trevose, USA). Exhaust air and foam were led into a 1 L glass bottle, equipped with a sterile filter of the same type as the one used for incoming air. Added medium and nutrient solution were also sterile filtered (Medium: Sartobran P MidiCap 5235307H8—00, pore size: 0.2 μm , Sartorius AG, Goettingen, Germany; nutrient solutions: Minisart NML syringe filter with 0.2 μm pore size, Sartorius AG, Goettingen, Germany).

Culture operation

The reactor was operated at 23 °C and the culture had a pH of 6.9 on average. Temperature and pH were daily monitored using permanently attached pH electrode (LZY, Hach Lange GmbH, Berlin, Germany).

Variations of the pH were compensated by adjusting the amount of carbon dioxide in order to have constant pH levels around 7. Prior to the inoculation, the illuminated reactor

was filled with hydrogen peroxide (3% v/v) for at least 24 h and flushed with deionized water twice. Biomass was taken from the pre-cultures for inoculum and transferred to the reactor under sterile conditions. The reactor was then filled with medium.

Spirogyra sp. was grown indoors at the laboratory of Subitec GmbH in Stuttgart, Germany, during a total period of 19 weeks. Cultivations were carried out at constant illumination in fed-batch mode with discontinuous feed and under autotrophic conditions. In order to compensate the loss of water by foaming and evaporation, the reactor was daily refilled with fresh medium so that the volume remained constant during the whole experiment. When the increase in biomass ceased or decreased for several days, the cultivation was terminated. The biomass was then diluted by exchanging part of the culture for fresh medium.

Results and discussion

Culture maintenance in pre-cultures

Since algae normally grow much slower than bacteria, with a common cell doubling time around 24 h and 3.5 h during exponential growth (Chisti 2007), it takes several weeks to obtain a dense algae culture from one single cell. Therefore, it is desirable to maintain actively growing cultures over a long period of time, in order to always have enough culture that can be used for inoculation.

As described above (see “Pre-cultures”), the pre-cultures were maintained in stirred and aerated 5 L flasks provided with constant illumination. Fresh autoclaved media was added twice a month and the same amount of cell suspension was removed if necessary. Under these conditions the culture was maintained for 23 weeks.

Cultivation

Spirogyra sp. was grown indoors at constant illumination in a flat-panel airlift photobioreactor with 6 L culture volume [as described in detail above (see “Culture system”)]. The experiment was repeated five times in fed batch mode. At the beginning of each cycle, the biomass was diluted with medium resulting in initial biomass concentrations between 0.1 and 4.2 $\text{g}_{\text{DW}} \text{L}^{-1}$. During the cultivation, the culture was not completely axenic. By light microscopic examinations, small amounts of bacteria were observed. However, it is assumed that algal growth was not affected.

As the density of the culture increased, the medium in the bioreactor turned dark green and the supernatant of the centrifuged samples was found to become increasingly red and viscous. This can most likely be explained by mucilage

Fig. 4 *Spirogyra* sp. in the flat-panel airlift photobioreactor at a dry cell weight concentration of $4.2 \text{ g}_{\text{DW}} \text{ L}^{-1}$. Filaments can be seen with the naked eye

production of *Spirogyra*, which is a strategy of defending itself against epiphytes (Weber and Schagerl 2007). Despite the high viscosity, the static mixers and the downcomer ensured a good mixing of the culture during the experiment. Figure 4: *Spirogyra* sp. in the flat-panel airlift photobioreactor at a dry cell weight concentration of $4.2 \text{ g}_{\text{DW}} \text{ L}^{-1}$ shows the culture in the reactor at a cell density of $4.2 \text{ g}_{\text{DW}} \text{ L}^{-1}$.

Moreover, cells did not attach to the reactor surface, thus no biofilm was formed. This is important as biofilm can greatly reduce the amount of light intensity per cell resulting in lower growth rates. Moreover, it complicates harvesting and downstream processes and is associated with a high cleaning effort.

Growth

Although many attempts have been made to identify the most suitable photobioreactor design, a standard model has not been established yet (Richmond 2013). Consequently,

most cultivations that are described in literature have been carried out in different growth systems and under different conditions, which makes it difficult to directly compare the results. Nevertheless, the following section mentions some examples from literature in order to give an overview of existing data.

As a general rule, it is desirable to achieve high cell densities in small culture volume (Richmond 2013). In this work, the growth performance was assessed by means of the volumetric productivity. It indicates how much biomass (g) has been produced per litre culture volume and per day.

Cell density

Growth of the culture is shown in Fig. 5. Five cultivation cycles have been performed during the experiment. The typical sigmoidal growth characteristics consists of a short lag phase followed by exponential growth. As cell density increases, the slope flattens because of mutual shading (Myers 1976), resulting in linear growth. Finally, a stationary phase or even death phase can be observed, which is characterized by constant or decreasing cell concentrations. In the present work, the lag phase lasted about 1–3 days. Most of the time, the cultures grew in a linear mode. Hardly any exponential increase could be observed, which is common for algal growth growth as dense cultures always face light limitation. In the first three attempts growth stopped when dry cell weight reached 3.3 , 5.5 and $5.2 \text{ g}_{\text{DW}} \text{ L}^{-1}$ respectively. The highest cell densities were monitored in the fourth cultivation, where the culture grew until reaching a maximum of $14.28 \text{ g}_{\text{DW}} \text{ L}^{-1}$ after 65 days. This is the highest cell density in large scale cultivation of *Spirogyra* sp. found in literature. Salim (2012) reported a cell density of $12.03 \text{ g}_{\text{DW}} \text{ L}^{-1}$ as a result of heterotrophic cultivation of *Spirogyra* sp. in 500 mL Erlenmeyer flasks

Fig. 5 Kinetics of *Spirogyra* sp. growth during a continuously illuminated, autotrophic and aerated (CO_2 : 1.6–5% v/v) fed-batch cultivation carried out in a flat-panel airlift photobioreactor

with a working volume of only 300 mL. Cultivations of *Spirogyra* sp. in open ponds resulted in much lower concentrations of $0.773 \text{ g}_{\text{DW}} \text{ L}^{-1}$ (Pacheco et al. 2015). High biomass concentrations are desirable as they facilitate downstream processes, and thereby reduce costs and energy consumption.

Apparently, growth has not been severely inhibited by high cell densities or any metabolic products up to the obtained density.

Volumetric productivity

Figure 6 shows the course of volumetric productivity of each cultivation cycle. The highest maximum productivity was attained in the last cycle, reaching $1.15 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ day}^{-1}$. The other cultivations showed much lower maximum volumetric productivities of $0.46 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ day}^{-1}$ in the first, $0.64 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ day}^{-1}$ in the second, $0.52 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ day}^{-1}$ in the third and $0.55 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ day}^{-1}$ in the fourth cycle. Anyhow, when considering cultivation four in parts, e.g. day 99–day 102, productivity was comparable ($1.04 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ day}^{-1}$). The increase in productivity over the course of the cultivations can be explained by multiple reasons. First, specific light availability, thus photon-flux density per g_{DW} , was successively increased starting from $76 \mu\text{mol m}^{-2} \text{ s}^{-1} \text{ g}_{\text{DW}}^{-1}$. As can be seen for cultivation one, biomass accumulation directly correlated to applied photon-flux density indicating a high light demand of *Spirogyra* sp. as other green algae, e.g. *Chlorella sorokiniana*, easily reach biomass concentrations of $10 \text{ g}_{\text{DW}} \text{ L}^{-1}$ at light intensities of $400 \mu\text{mol m}^{-2} \text{ s}^{-1}$. This in accordance with its abundance in epipelagial waters where *Spirogyra* sp. is frequently exposed to high levels of light

intensity. Productivity increased with increasing the specific light availability as can be seen for cultivation two and three. Second, cultivation four and five were started at a higher biomass concentration applying $100 \mu\text{mol m}^{-2} \text{ s}^{-1} \text{ g}_{\text{DW}}^{-1}$ from the start. Applying a similar specific light availability at a higher biomass concentration increases the proliferation of more cells, thus increases productivity. At biomass concentration greater than $14 \text{ g}_{\text{DW}} \text{ L}^{-1}$, cultures became severely light limited and a further increase in photon-flux density did not allow for further growth (see cultivation four). Growth irruption within cultivation four may be explained by an too abrupt increase in photon-flux density as these seem to be correlated, but further investigations need to be performed on that phenomenon.

Overall, the obtained productivities are very encouraging, taking into account that the conditions are yet to be optimized. Tredici and Zittelli (1998) for example report that growth of the filamentous cyanobacterium *Arthrospira platensis* in a flat chamber reactor of similar dimensions (optical path of 2.5 cm and 5.4 L volume) resulted in a volumetric productivity of $1.93 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ day}^{-1}$ and a mean cell concentration of $3.2 \text{ g}_{\text{DW}} \text{ L}^{-1}$. In view of the fact, that growth of *Arthrospira* has been much better researched, the attained results are promising. Pacheco et al. (2015) achieved a volumetric productivity of $0.013 \text{ g L}^{-1} \text{ day}^{-1}$ growing *Spirogyra* sp. in a 400 L open raceway pond. However, no comparable values for the productivity of *Spirogyra* in closed systems are found.

High productivity rates are an important objective of mass culture. In order to achieve this, the culture should be maintained only in the fast growing period of the cultivation, when biomass concentration is low and light intensity per cell is high (Tamiya et al. 1953). On the other hand, it should be considered, that high cell concentrations reduce energy costs for downstream processes (Lawton et al. 2013). The challenge is to find the right balance between high cell densities and fast growth. This could be best achieved in the last cultivation. As represented in Fig. 6, the maximum volumetric productivity of the last cultivation exceeds the productivity of the other cultivations almost twice at relatively high cell densities.

Conclusion

The results clearly demonstrate that *Spirogyra* sp. can be grown in the flat-panel airlift photobioreactor. The culture showed good growth, even though the applied growth conditions (temperature, pH, light intensity, medium composition, and aeration) have not been optimized yet. During the experiment, a maximum productivity of $1.15 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ day}^{-1}$ and an average productivity of $0.78 \text{ g}_{\text{DW}} \text{ L}^{-1} \text{ day}^{-1}$ were attained in the last cultivation (Fig. 7).

Fig. 6 Course of the volumetric productivity of *Spirogyra* sp. during a continuously illuminated, autotrophic and aerated (CO_2 : 1.6–5% v/v) fed batch cultivation carried out in a flat-panel airlift photobioreactor

Fig. 7 Course of the volumetric productivity of *Spirogyra* sp. dependent on dry cell weight. Data derives from a continuously illuminated, autotrophic and aerated (CO₂: 1.6–5% v/v) fed batch cultivation carried out in a flat-panel airlift photobioreactor

Obviously, *Spirogyra* is suitable for high cell density cultivations since a maximum dry weight concentration of 14.28 g_{DW} L⁻¹ was reached. This is the highest cell density in large scale cultivation of *Spirogyra* found in literature. Salim (2012) reported a cell density of 12.03 g_{DW} L⁻¹ as a result of heterotrophic cultivation of *Spirogyra* sp. in 500 mL Erlenmeyer flasks with a working volume of only 300 mL. Cultivations of *Spirogyra* sp. in open ponds resulted in much lower concentrations of 0.773 g_{DW} L⁻¹ (Pacheco et al. 2015). High biomass concentrations are desirable as they facilitate downstream processes, and thereby reduce costs and energy consumption.

Nevertheless, further research should be done to gain more knowledge about the optimal growth conditions for *Spirogyra* in closed culture systems, especially with regard to nutrient uptake, temperature and illumination. It has been proved many times, that culture conditions such as light (Munir et al. 2015), temperature (Sorokin and Krauss 1962) and nutrient supply (Gallego et al. 2013; Munir et al. 2015; Rhee 1978) strongly influence algal growth, thus it is to be assumed that the obtained results of growth kinetics and productivity can be increased by a multiple.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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