Bioresource Technology 225 (2017) 165–174

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Reaction engineering analysis of *Scenedesmus ovalternus* in a flat-plate gas-lift photobioreactor

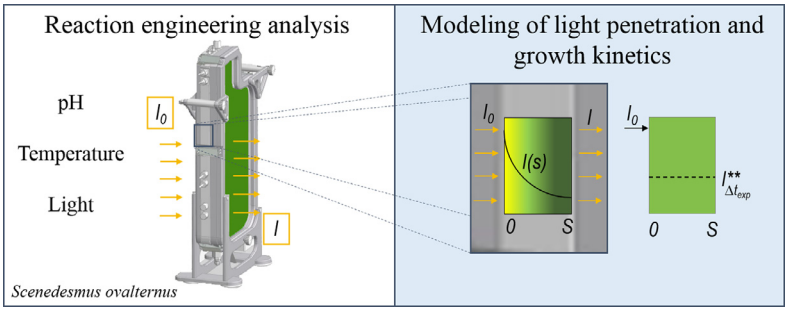
Anja Pia Koller, Lara Wolf, Dirk Weuster-Botz *

Institute of Biochemical Engineering, Technical University of Munich, Boltzmannstr. 15, 85748 Garching, Germany

HIGHLIGHTS

- pH 8, 30 °C and 1300 µmol photons m^{-2} s^{-1} are best growth conditions for *S. ovalternus*.
- 7.5 g_{CDW} L^{-1} were achieved within 6 days at a growth rate of 0.11 h^{-1}.
- Mean integral photon flux densities were estimated using Schuster's law.
- A kinetic growth model was identified using Andrews' substrate inhibition model.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 July 2016
Received in revised form 7 October 2016
Accepted 6 November 2016
Available online 11 November 2016

Keywords:
Scenedesmus ovalternus
Growth kinetics
Flat-plate gas-lift photobioreactor

ABSTRACT

Microalgal strains of the genus *Scenedesmus* are a promising resource for commercial biotechnological applications. The temperature-, pH- and light-dependent growth of *Scenedesmus ovalternus* has been investigated on a laboratory scale. Best batch process performance was obtained at 30 °C, pH 8.0 and an incident photon flux density of 1300 µmol photons m^{-2} s^{-1} using a flat-plate gas-lift photobioreactor. Highest growth rate (0.11 h^{-1}) and space-time yield (1.7 ± 0.1 g_{CDW} L^{-1} d^{-1}) were observed when applying these reaction conditions. Biomass concentrations of up to 7.5 ± 0.1 g_{CDW} L^{-1} were achieved within six days (25.0 ± 0.5 g_{CDW} m^{-2} d^{-1}). The light-dependent growth kinetics of *S. ovalternus* was identified using Schuster's light transfer model and Andrews' light inhibition model (K_S = 545 µmol photons m^{-2} s^{-1}; K_I = 2744 µmol photons m^{-2} s^{-1}; μ_{max} = 0.21 h^{-1}). The optimal mean integral photon flux density for growth of *S. ovalternus* was estimated to be 1223 µmol photons m^{-2} s^{-1}.

1. Introduction

Microalgae do not compete with conventional crop cultivation and use sunlight and carbon dioxide for their growth. They can be produced year-round in regions with lower-quality land and water supply and use solar energy more efficiently than plants do (Quinn et al., 2011). Microalgae are therefore a promising bio-economic alternative as a renewable raw material. Many microalgal strains such as *Spirulina* sp., *Chlorella* sp. or *Dunaliella* sp. are currently used in commercial biotechnological applications in the food and feed industry or for the production of ecologically valuable products (Pulz and Gross, 2004).

Scenedesmaceae is a family of green algae with great potential for industrial applications. Many *Scenedesmus* sp. are mesophile, but can also survive in extraordinary habitats and are able to endure extreme conditions such as high temperatures and irradiances (Sánchez et al., 2008). Their metabolism reacts quickly to environmental changes, even under limiting conditions (Dean et al., 2010). *Scenedesmus* produces large amounts of oils and secondary metabolites (carbohydrates and carotenoids) that can

* Corresponding author.
E-mail addresses: a.koller@lrz.tum.de (A.P. Koller), d.weuster-botz@lrz.tum.de (D. Weuster-Botz).

http://dx.doi.org/10.1016/j.biortech.2016.11.025
0960-8524/

Nomenclature

AIC_c:	corrected Akaike's information criterion
$I(s)$	photon flux density as a function of layer thickness S
CDW	cell dry weight
c_x	concentration of cell dry weight, g L^{-1}
E_a	light absorption coefficient, m^2 kg^{-1}
E_s	light scattering coefficient, m^2 kg^{-1}
f_x	correlation factor for calculation of biomass concentration during the cultivation process, –
I_0	incident photon flux density on the reactor surface, W m^{-2} or µmol photons m^{-2} s^{-1}
I	photon flux density, W m^2 or µmol photons m^{-2} s^{-1}
I^*	integral photon flux density, µmol photons m^{-2} s^{-1}
$\bar{I}$	mean integral photon flux density, µmol photons m^{-2} s^{-1}
K_i	inhibition constant; highest photon flux density at which the specific growth rate corresponds to one-half of the maximum specific growth rate
K_s	saturation constant; photon flux density at which the specific growth rate is equal to one half of the maximum specific growth rate
OD	optical density, –
S, s	thickness of cultivation chamber, m
t	time, d
$t_{exp\ beginning}$	beginning of the exponential growth phase
$t_{exp\ end}$	end of the exponential growth phase
μ_{opt}	optimal specific growth rate, h^{-1}
μ_{max}	maximum specific growth rate, h^{-1}
μ	specific growth rate, h^{-1}
β	empirical parameter from Webb (Edwards, 1970)
ε	extinction coefficient, m^2 kg^{-1}
φ, γ, κ	empirical parameters from Camacho Rubio et al. (2003)

be used commercially. The cells are able to accumulate up to 73% (w/w) lipids (Matsunaga et al., 2009) that consist mostly of saturated C16 and C18 fatty acids (Allard and Templier, 2000). These lipids can be used in the production of biofuels such as biodiesel, which makes *Scenedesmus* sp. attractive for an energetic use (Ho et al., 2010; Jena et al., 2012). In addition, Ördög et al. (2004) described strong antimicrobial activity, which allows for further application in that field.

The production of large amounts of algae biomass is certainly necessary to successfully apply microalgae as a regenerative resource. Appropriate microalgal mass-production systems have to be available in addition to fast-growing microalgal strains. Various photobioreactors with different geometric properties have been suggested for producing microalgal biomass to date (Apel and Weuster-Botz, 2015; Béchet et al., 2013; Rorrer and Mullikin, 1999; Zhang et al., 2015). However, reactor type alone doesn't affect process performance. Growth parameters such as medium composition, temperature, carbon dioxide supply, and light greatly impact microalgal production processes as well (Richmond, 2004). Each microalgal strain has thereby its own optimum temperature, pH, and light that should be known for a successful and reasonable utilization. pH depends on the relative number of inorganic carbon species present in the medium and can be regulated by gassing the culture with CO_2 (Richmond, 2004). Light is the most influential factor for microalgal growth if pH and temperature can be controlled during the process and the medium contains sufficient amounts of nutrients (Acién Fernández et al., 1997; Cornet et al., 1992; Pruvost et al., 2002; Rabe and Benoit, 1962). Growth rates and biomass productivities increase with increasing photon flux density in the form of a light response curve until a saturation point has been reached. Higher photon flux densities can cause photodamage and impair the production process (Huesemann et al., 2012). In addition, light is not homogenously distributed within a bioreactor (Rabe and Benoit, 1962) and shading effects play an influential role if the cell density is too high (Cornet et al., 1992; Sorokin and Krauss, 1958). Therefore, light distribution within a bioreactor needs to be described in more detail for better appreciation of the production process (Cornet et al., 1992; Lee et al., 1987; Yun and Park, 2003). A common application calculates an average photon flux density assumed to be available in the photoreactor to characterize light transfer in the reactor (Lee et al., 1987; Rabe and Benoit, 1962). This average photon flux density can then be used to identify kinetic parameters and thus serves to further characterize microalgal strains and improve production processes.

In this study, the pH, temperature, and light dependent growth of the freshwater microalgae *Scenedesmus ovalternus* SAG 52.80 will be investigated using a fully controlled lab-scale flat-plate gas-lift photobioreactor system. The strain is classified taxonomically via 18S rRNA analysis (Kessler et al., 1997) but is not yet well characterized in literature. Since *Scenedesmus* species have strong potential for commercial applications and Schulze et al. (2016) identified *Scenedesmus ovalternus* SAG 52.80 as a promising production strain for primary metabolites such as β-Glucan, this strain should be characterized in more detail. Moreover, light dependent growth kinetics will be identified as a function of mean integral photon flux densities on the basis of a well-known but appropriate adjusted light attenuation model. This investigation should provide the basic data to make a potential subsequent scale-up and process optimization feasible.

2. Materials and methods

2.1. Microalgal strain and process conditions

The Department of Pharmaceutical Biology of the Institute of Pharmacy at the Ernst-Moritz-Arndt-University in Greifswald, Germany, supplied *Scenedesmus ovalternus* SAG 52.80. M. Lefèvre had isolated it in Europe by 1948. It is also known under the name *Scenedesmus obtusus* Meyen (SAG, 2016). Autotrophic BG-11 (Waterbury and Stanier, 1981) was used as medium for storage in liquid medium at room temperature and laboratory light as well as for all batch processes in a flat-plate gas-lift photobioreactor: 1.5 mg L^{-1} $NaNO_3$, 0.04 mg L^{-1} K_2HPO_4, 0.75 g L^{-1} $MgSO_4 \cdot 7\ H_2O$, 0.02 g L^{-1} $Na2CO_3$, 0.036 g L^{-1} $CaCl_2 \cdot 2\ H_2O$, 0.001 g L^1 Na-EDTA, 0.006 g L^{-1} citric acid, 0.006 g L^{-1} ferric ammonium citrate. The micronutrient solution was derived from Kuhl and Lorenzen (1964): 0.061 g L^{-1} H_3BO_3, 0.223 g L^{-1} $MnSO_4 \cdot 4\ H_2O$, 0.287 g L^{-1} $ZnSO_4 \cdot 7\ H_2O$, 0.0025 g L^{-1} $CuSO_4 \cdot 5\ H_2O$, 0.0125 g L^{-1} $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$

All growth studies were performed in a highly automated pH- and temperature-controlled flat-plate gas-lift photobioreactor system (Labfors 5LUX, Infors HT, Bottmingen, Switzerland). The working volume was 1.8 L with an illuminated, 0.09 m^2 surface area and 2 cm depth. A LED-panel with 260 warm white LEDs enabled lighting from one side in the visible wavelength range (400 mm to 800 nm) at photon flux densities between 0 and 4200 µmol photons m^{-2} s^{-1} (0–100 %). The pH was varied between pH 7 and pH 10 to investigate the parameters most influencing microalgal