Biochemical Engineering Journal 103 (2015) 93–102

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Design and parallelisation of a miniature photobioreactor platform for microalgal culture evaluation and optimisation

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ARTICLE INFO

Article history:
Received 13 October 2014
Received in revised form 26 May 2015
Accepted 6 July 2015
Available online 10 July 2015

Keywords:
Miniature photobioreactor
Fatty acid methyl esters
Oxygen transfer
Microalgae
Optimisation
Mixing

ABSTRACT

Miniature photobioreactors (mPBr) represent a potential platform technology for the high-throughput, phototrophic cultivation of microalgae. This work describes the development and characterisation of a novel orbitally shaken twin-well mPBr, and its scale-out to a 24-well microplate format, suitable for optimisation of microalgae culture conditions. Fluid hydrodynamics, oxygen mass transfer coefficient (k_La) and light intensity distribution in the mPBr were first investigated as a function of orbital shaking frequency. High speed video analysis of the shaken wells indicated rapid fluid flow and good mixing while measured k_La values varied between 20 and 80 h^{-1}. Light intensity variation across the scaled-out platform was in the range ±20 μmol m^{-2} s^{-1}. The use of the mPBr platform was demonstrated for optimisation of conditions for the batch cultivation of *Chlorella sorokiniana*. Using a modified tris-base phosphate (TBP) medium, the highest biomass concentration and productivity achieved were 9.2 g L^{-1} and 2.5 ± 0.2 g L^{-1} d^{-1} respectively at 5% CO_2 with a light intensity of 380 μmol m^{-2} s^{-1}. In general, cell growth rate and yield increased with increasing shaking frequency (up to 300 rpm) while culture conditions had limited impact on pigment production. Overall, these results demonstrate the application of the mPBr for rapid optimisation of phototrophic culture conditions and establishment of high cell density cultures.

1. Introduction

Microalgae are one of the most ubiquitous groups of organisms on the planet and are being increasingly investigated as a 'cell factory' for use in the bioenergy, bioremediation and biotechnology sectors [1,2]. Important applications include the production of high value compounds for the pharmaceutical and neutraceutical markets [3]. Evaluation of the growth of microalgae involves investigation of numerous parameters including strain selection, media design, feeding strategies, light intensity and photo-period (light:dark cycles). Culture performance can be optimised for biomass, lipid, pigment or protein production depending on the particular application. Such experiments are currently performed in illuminated shake flasks and other laboratory scale photobioreactors (PBRs) [4–7] which places limitations on the number of experimental variables that can be investigated in parallel.

Microwell based culture devices now find widespread use for rapid and early stage assessment of culture conditions for microbial and mammalian cells. A number of these high-throughput systems have been characterised and reported in the literature [8–13]. Characterisation of the engineering environment within orbitally shaken microwell systems has shown the importance of shaking frequency, culture volume and well geometry on the overall performance [14,15] while progressive improvements have been made in terms of aeration and control of environmental parameters [16–19]. Recently the use of a 24-well microplate for heterotrophic cultivation of microalgae was reported [20]. There remains, however, the need for a small scale, high-throughput platform for the phototrophic culture of microalgae if the full range of their biological diversity is to be explored and their commercial potential evaluated.

In this work we report on the design and initial engineering characterisation of a novel, shaken twin-well miniature photobioreactor system and its subsequent scale-out to a 24-well microplate format. The impact of fluid hydrodynamics, shaking frequency, oxygen mass transfer coefficient, k_La, light intensity, CO_2 concentration and media composition were examined to establish optimal conditions for phototrophic culture of *Chlorella sorokiniana*. *C. sorokiniana* was investigated due to its high specific growth rate and tolerance to high irradiance and CO_2 concentrations [21,22].

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http://dx.doi.org/10.1016/j.bej.2015.07.006

Nomenclature

A_{pbr}	Bioreactor illuminated surface area (m^2)
BBM	Bold basal medium
C.	*Chlorella*
$C_{chl\text{-}a,b}$	Chlorophyll concentration (a and b) ($mg\,L^{-1}$)
C_{ppc}	Carotenoid concentration ($mg\,L^{-1}$)
d_o	Orbital shaking diameter (mm)
FAME	Fatty acid methyl ester
HSM	High salt medium
k_La	Oxygen mass transfer coefficient (h^{-1})
LED	Light emitting diodes
MTP	Microtitre plate
mPBr	Miniature photobioreactor
MUFA	Monounsaturated fatty acids
OD	Optical density
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
S/V	Surface area to volume ratio (m^{-1})
TMSH	Trimethyl sulphuric hydroxide
UFA	Unsaturated fatty acids
X_1	Initial dry cell weight ($g\,L^{-1}$)
X_2	Final dry cell weight ($g\,L^{-1}$)
$Y_{x,E}$	Biomass yield on photon energy ($g\,mol^{-1}$)

Culture performance was assessed in terms of growth rate, pigment concentration and fatty acid production in batch cultures and the results compared to data from conventional, laboratory scale PBRs [9]. The results show the utility of the microwell platform for high-throughput strain selection and subsequent optimisation of culture conditions.

2. Materials and methods

2.1. Chemicals and microorganisms

The microalgae *C. sorokiniana UTEX 1230* was kindly provided by Dr. Saul Purton, (Institute of Structural and Molecular Biology, University College London). This was maintained on nutrient agar slants stored at 4 °C. The growth media consisted of various inorganic salts in different proportions as described in Table 1 for high salt medium (HSM), bold basal medium (BBM), tris-base phosphate medium (TBP) and tris-acetate phosphate medium (TAP). All chemicals were of the highest grade.

2.2. Design and characterisation of mPBr

2.2.1. Twin-well mPBr prototype

The mPBr prototype was designed to be geometrically similar to a single well from a conventional, pyramid base 24-well microtitre plate. A transparent Perspex was chosen for construction due to its favourable optical and mechanical properties: light transmittance of >92%, minimal light diffraction and intensity loss, refractive index of 1.92, tensile strength of >62 MPa, softening temperature of >110 °C. The light path across the well is 16.5 mm and the wall thickness is 2 mm. With a working volume of 4 mL, the maximum liquid height was 17 mm as shown in Fig. 1a. Each well was illuminated by a cool white light emitting diode (LED) from the side. The total surface area available for light absorption was 272.3 mm^2. Light intensity from the LED was 160 $\mu mol\,m^{-2}\,s^{-1}$ and was constant for all cultures.

Mixing was achieved using an incubator shaker (Infors HT, Switzerland) equipped with temperature, humidity and CO_2 sensors coupled to a control unit. CO_2 levels were controlled by blending air with 100% CO_2 from a cylinder. The mPBr was mounted on the shaking platform using a sticky mat (Infors HT, Switzerland). The orbital shaking diameter was 25 mm for all experiments with shaking frequency varied between 250 and 400 rpm.

2.2.2. Novel shaking platform for 24-well parallel mPBr

The novel shaker platform was designed to house six, 24-well parallel mPBr plates as shown in Fig. 1b. The high power warm white LEDs used was composed of wavelengths between 450 and 620 nm and also provided variable light intensity of up to 2400 $\mu mol\,m^{-2}\,s^{-1}$ at 5 cm distance from the platform. The LED unit supplied by Infors HT (UK) was suspended in the incubator below the Perspex shaker platform on which the 24-well mPBr plates sat. Excess heat generated by the LED panel was removed by cooling water circulated around a refrigerated circulating water bath (Grant Instruments, Cambridge, UK). A Quantum Li-Cor light meter (Li-Cor Bioscience, Cambridge, UK) was used to monitor the light intensity throughout all experiments. Control experiments showed that there was uniform light distribution between multiple plates on the shaker platform and between the 24 wells on an individual plate [23].

In order to translate the engineering conditions in the twin-well mPBr to a parallel, 24-well mPBr, the light path-length and total surface area available for light absorption were kept constant. Three geometries of 24-well mPBr were employed having a pyramid base, a round base and a square base as shown in Supplementary Fig. A1. The square-based plates had opaque walls, preventing well-to-well light diffraction, while the two other plate designs had translucent walls.

2.2.3. Visualisation of fluid hydrodynamics

Investigation of fluid hydrodynamics employed a DVR Fastcam high speed video camera (Photron, California, USA). This was mounted perpendicular to the 24-well plate on the shaking platform and the resolution was set at 640 × 480 pixels for all experiments. Two halogen red lamps (National Instruments, UK) were used to provide additional light for improved brightness and clearer focus. The camera was set to capture images at 125 fps over a period of 5 min for each of the experimental runs. The images captured were stored for analysis using ImageJ software (http://rsbweb.nih.gov/ij/). Each experimental run was carried out using reverse osmosis (RO) water.

2.2.4. Quantification of oxygen mass transfer coefficient (k_La)

k_La, values in the orbitally shaken 24-mPBr were determined using the dynamic gassing out technique [16]. Prior to each experiment, a fibre-optic oxygen micro-sensor probe (PreSens, Germany) was calibrated between 0% dissolved oxygen (using 1% v/v sodium thiosulphate dissolved in RO water) and 100% (in humidified atmospheric air). All experiments were carried out at ambient temperature and varying shaking frequency. For a well-mixed liquid in the mPBr, the volumetric oxygen mass transfer coefficient, k_La, was determined from the measured dissolved oxygen-time profiles. The Micro TX3 software supplied with the sensor contains an algorithm for averaging percentage dissolved oxygen readings over four repeat samplings. The percent oxygen saturation plotted against time was linearised and the gradient is equal to k_La. The measured probe response time was <1 s in all cases and therefore it was not necessary to account for this in calculating the k_La values [24]. However, this could be accounted for as discussed in Dang et al. [25].

2.2.5. Quantification of evaporation rates

The average evaporation rate across the parallel, 24-well SUPBr was determined by two methods: the first was by direct changes in mass and the second by optical density (OD) measurement of