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

Ebenezer O. Ojo, Hadiza Auta, Frank Baganz, Gary J. Lye*

The Advanced Centre for Biochemical Engineering, Department of Biochemical Engineering, University College London, Torrington Place, London WC1E 7JE, UK

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].

* Corresponding author.
E-mail address: g.lye@ucl.ac.uk (G.J. Lye).

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

Table 1
Media compositions used for batch cultivation of *C. sorokiniana*.

Stock solutions	Medium components	TAP media concentration[a] ($g\,L^{-1}$)	Volume (mL) per litre of medium	TBP media concentration ($g\,L^{-1}$)	Volume (mL) per litre of medium	HSM media concentration ($g\,L^{-1}$)	Volume (mL) per litre of medium
5x Beijerinck's solution	NH_4Cl	40.0	10.0	40.0	10.0	100.0	5
	$CaCl_2{\cdot}2H_2O$	5.0		5.0		2.0	
	$MgSO_4{\cdot}2H_2O$	10.0		10.0		4.0	
Phosphate solution	K_2HPO_4 (anhydrous)	14.3	8.3	14.3	8.3	288.0	5
	KH_2PO_4 (anhydrous)	7.3		7.3		144.0	
	Tris-acetate	Tris-base	242.0	10.0	242.0	100.0	–
	Glacial acetic acid (mL)	100.0		–		–	
Trace elements solution	EDTA-Na_2	50.0	1.0	50.0	1.0	50.0	1
	H_3BO_3 (boric acid)	11.1		11.1		11.1	
	$ZnSO_4{\cdot}7H_2O$	22.0		22.0		22.0	
	$MnCl_2{\cdot}4H_2O$	5.1		5.1		5.1	
	$FeSO_4{\cdot}7H_2O$	5.0		5.0		5.0	
	$CoCl_2{\cdot}6H_2O$	1.6		1.6		1.6	
	$CuSO_4{\cdot}5H_2O$	1.6		1.6		1.6	
	$(NH_4)_6Mo7O_{24}{\cdot}4H_2O$	1.1		1.1		1.1	
RO water			up to 1 L		up to 1 L		up to 1 L

RO—Reverse osmosis, TAP—Tris-acetate phosphate, TBP—Tris-base phosphate, HSM—High salt media.
[a] Stock solution concentration.

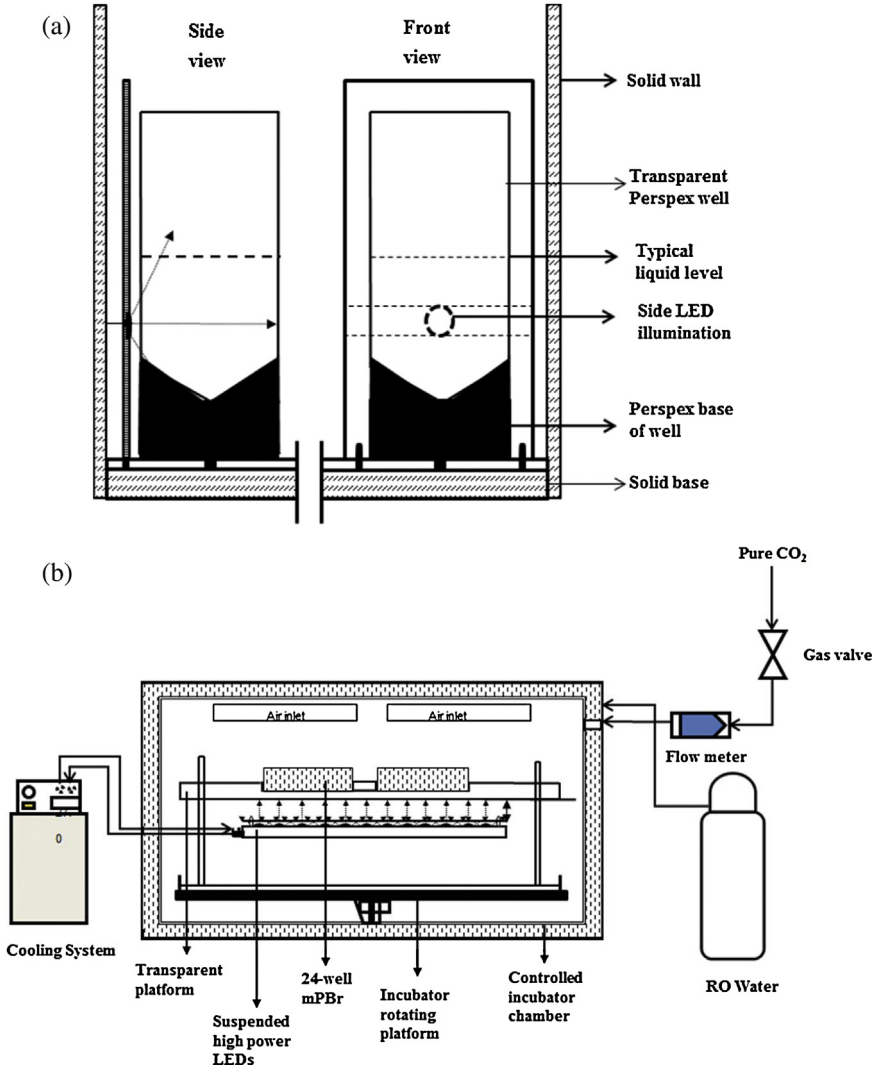

Fig. 1. Schematic diagram of experimental set-up: (a) prototype twin-well mPBr with pyramid-shaped base, illuminated from the side and (b) photo-incubator shaker housing scale-out 24-well mPBr illuminated from the base.

a 0.002% v/v blue dye solution at 630 nm (Super Cook, Leeds, UK). An individual well was filled with dye stock solution (4 mL fill volume), sealed with semi-permeable membrane (breathe easy sterile adhesive seal, VWR International, Leicestershire, UK) and shaken in the photo-incubator system at 32 °C, relative humidity 85% and 300 rpm with an orbital shaking diameter of 25 mm for 5 days. Light intensity conditions used for actual culture conditions were mimicked. The OD at 750 nm was measured using a spectrophotometer (Ultrospec 1100, Amersham Biosciences, UK). Likewise, the weight of the plate was measured over time and evaporation rate calculated as shown in Eq. (1),

$$\text{Percentage evaporation}\,(\%) = \frac{W_{\text{initial}} - W_{\text{final}}}{W_{\text{initial}}} \times 100$$

where W_{initial} and W_{final} are the total mass of the fluid in all the wells before and after 5 days of incubation. The weight of the 24-well plate was 78.5 ± 0.40 g. Values presented here are based on triplicate measurements.

2.3. Parallel phototrophic cultivation of C. sorokiniana

2.3.1. Inoculum preparation

The seed culture was inoculated from a *C. sorokiniana* nutrient agar slant into 50 mL TBP medium using 250 mL Erlenmeyer flasks. A Kuhner incubator shaker (Kuhner AG, Switzerland) operated at 180 rpm, 28 °C, and with a light intensity of 55 μmol m^{-2} s^{-1} for 6–8 days was used for the cultivation. This culture was then repeated, using 10% v/v inoculum into a second flask, and allowed to grow for 4 days before being used for mPBr inoculation.

2.3.2. Cultivation in mPBr

The mPBr (prototype and 24-well) wells were aseptically filled with 4 mL working volume of the desired medium and inoculated with ~5% v/v inoculum prepared as described in Section 2.3.1. Media formulations are summarised in Table 1. Light intensity levels for the 24-well mPBr were varied while for the twin-well mPBr they were kept constant. The CO_2 level was maintained between 2 and 20% in air. All experiments were carried out in batch mode with three replicates. Samples (400 μL) were withdrawn at 8–12 h interval and stored at −20 °C for analysis.

2.4. Analytical techniques

2.4.1. Biomass and pigment quantification

Biomass dry cell weight was determined using 15 mm diameter Whatman fibre glass filter paper (GE healthcare, UK). A calibration curve, for each medium formulation, was generated and used for the conversion of optical density readings (OD 750 nm) to dry cell weight concentrations. Biomass concentration and growth rate were calculated as described previously [26]. Similarly, total green pigment concentrations were determined by the spectrophotometric method as described previously [25–27].

2.4.2. Freeze drying

Broth samples collected at the end of the cultures were centrifuged at 10,000 rpm, 4 °C for 10 min using a Heraeus Fresco 17 centrifuge (Thermo Scientific, Leicestershire, UK). The supernatants were then removed and the pellets washed twice with distilled water. The pellets were then prepared for freeze drying by dipping into liquid nitrogen for 2 min or stored in −80 °C overnight and immediately transferred into an Edwards high vacuum freeze dryer (Crawley, UK) for lyophilisation. The weights of the lyophilised cells were measured prior to further lipid analysis.

2.4.3. Total lipids determination

Lipids were extracted using the modified Bligh and Dyer method [28]. A mixture of chloroform–methanol (2:1, v/v) was added into the dried cell pellets and left overnight for 16–18 h in sealed vials to prevent evaporation and also kept in a fume cupboard for safety. These were further separated into chloroform and aqueous methanol layers by addition of methanol and water to give a final solvent ratio of chloroform:methanol:water of 1:1:0.9. The mix was then centrifuged at 4000 × *g* for 20 min and the organic phase separated and evaporated to dryness under nitrogen. Total lipids were measured gravimetrically and stored at −20 °C under nitrogen gas to prevent lipid oxidation or were used directly for subsequent analysis.

2.4.4. Fatty acid methyl ester (FAME) analysis

FAME was prepared by direct trans-methylation of lipid extracts in dichloromethane with trimethyl sulfonium hydroxide (TMSH). The FAME were analysed using an XL capillary gas chromatography system (Perkin Elmer Inc., Massachusetts, USA) equipped with a flame ionization detector (FID) and an omegawax 250 capillary column (30 m^3, 0.25 mm) (Sigma–Aldrich, Dorset, UK). Nitrogen was used as carrier gas. Initial column temperature was set at 50 °C (2 min), which was subsequently raised to 230 °C at 4 °C min^{-1}. The injector was kept as 250 °C with an injection volume of 2 μL under split less mode. The FID temperature was set at 260 °C. Individual FAMEs were identified and quantified by comparing their retention times and peak areas against calibration curves for each FAME.

3. Results and discussion

3.1. Miniature photobioreactor design and operation

A mPBr design for phototrophic microalgae cultivation has not been described in the literature to date despite previous miniature bioreactor studies with bacterial and mammalian cells and for the heterotrophic cultivation of microalgae [16,20,28,29]. Consequently, a novel prototype twin-well mPBr was designed to mimic a conventional 24-well microwell bioreactor as shown in Fig. 1a. Supplementary Fig. A1 gives additional detail on different well geometries and dimensions.

In order to facilitate subsequent scale-out of the twin-well mPBr, maintenance of key design parameters such as light transmittance and mixing efficiency were considered. Fluid mixing was achieved in the twin-well mPBr by orbital shaking up to a maximum frequency of 400 rpm at 25 mm shaking diameter. The maximum working volume in each well was limited to 4 mL to prevent splashing. The light path-length across the well diameter was kept below 2 cm to ensure adequate light penetration into the medium. Orbital shaking was chosen due to existing knowledge of the fluid hydrodynamics under different shaking conditions [30]. In order to ensure efficient light dispersion and scale-out to the 24-well mPBr design, the positions of LEDs on the illuminated side were decided based on the solid angles of reflection, maximum path-length and the liquid height in the wells as shown in Fig. 1a.

In a rapidly growing culture of *C. sorokiniana* in the mPBr, the light exposure of the cells will depend on the cell density (concentration) and also the fluid mixing time i.e. the frequency and duration with which individual cells are brought close to the illuminated surface of each well. At the shaking frequencies employed here (>250 rpm), mixing times in the wells were experimentally determined to be 10 s or less [23]. The mixing time represents the time taken to achieve >95% fluid homogeneity thus circulation times, the time taken for fluid (and suspended cells) to circulate around the reactor, could be expected to be of the order of 1 s. During each circulation, cells are exposed to periods of reduced

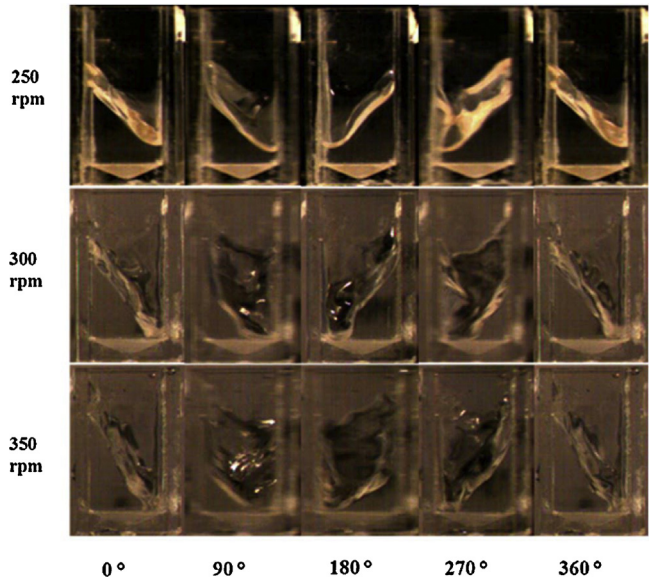

Fig. 2. Visualisation of fluid hydrodynamics in the pyramid base 24-well mPBr with angle of rotation at various shaking frequencies. Experimental conditions: d_o = 25 mm; H_L = 4 mL.

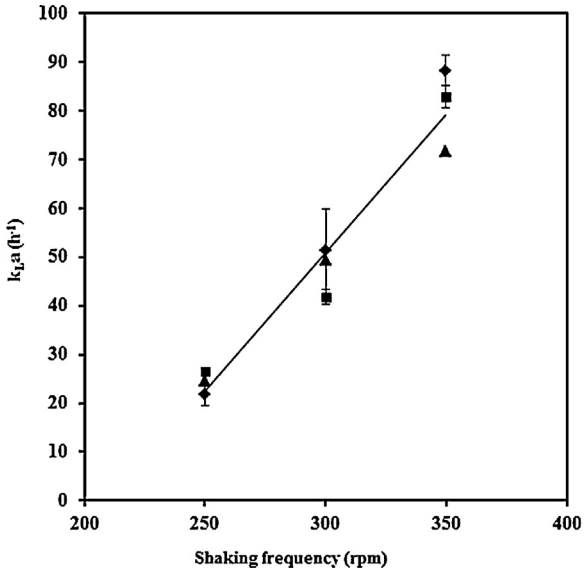

Fig. 3. Characterisation of oxygen mass transfer coefficient (k_La) in different well geometries with varying shaking frequency; (▲) square (♦) pyramid and (■) round base. Experimental conditions: d_o = 25 mm; 32 °C; RO water. Solid lines fitted by linear regression. Error bars represent one standard deviation about the mean (n = 3).

light intensity (due to light attenuation in the centre of the culture) and higher light intensity (near the illuminated walls). Overall, however, the mPBr design is considered to provide adequate illumination for the cells to grow as described in Section 3.4. In the literature there have been reports of the beneficial effects of alternating light/dark cycles on microalgal cultivation but these occur at cycling frequencies of the order of 100 Hz so are unlikely to have a significant effect on culture performance in the mPBr [31].

To facilitate mPBr scale-out and parallel operation using conventional transparent and translucent 24-well plates, a novel shaker incubator with a Perspex shaking platform (on which the 24-well plates were mounted) was designed as shown in Fig. 1b. Initial light diffusivity tests performed during orbital shaking at a 5 cm distance from the fixed LED light source indicated no significant variation across the platform surface [23]. Light intensities quoted were measured directly at the base of the wells for all subsequent experiments.

Shaking frequency and well geometry are known to influence energy dissipation, fluid motion and mixing in shaken microwells [12]. Here, fluid motion in the wells was visualised using a high speed camera as shown in Fig. 2. In general, shaking induced deformation of the fluid surface and created a sloshing motion [32] in which the medium moved around the walls of the well in-phase [30] with the orbital motion of the platform. The height of the moving surface and hence the gas–liquid surface area available for gas mass transfer increased with increasing shaking frequency. At the highest shaking frequency studied the rotating fluid surface also reached the base of the well. These observations are similar to the fluid flow predicted in a 96-well plate using computational fluid dynamics, CFD [14].

3.2. Quantification of k_La

Understanding how k_La in the 24-well mPBr varies with shaking frequency and well geometry is necessary for comparing the different bioreactor designs and informs options for mPBr scale-out or scale-up [33]. As shown in Fig. 3, k_La values were calculated, and measured with the gas-permeable seals in place, are plotted as a function of shaking frequency. It can be seen that k_La values increased linearly with an increase in shaking frequency over the range investigated. This increase in k_La correlates directly with the increase in gas–liquid surface area for mass transfer noted previously. In contrast, the well geometry had no significant impact on k_La values at the liquid fill volume and over the range of shaking frequencies investigated. The k_La values obtained were found to be in the range reported for a similar 24-well bioreactor for experimental conditions of 300 rpm, shaking diameter of 25 mm and 2.5 mL working volume [10,34,35].

3.3. Evaporation studies

The extended culture times and elevated temperatures required with many microalgae species, pose a challenge of liquid evaporation in small scale photobioreactor systems. Minimization of evaporation effects in microtitre plates has been studied previously [19] and some of the impacts of evaporation on culture performances are associated with increase in broth osmolality and inhibitory metabolite concentrations [16,36]. In general the measured rate of evaporation from the mPBr is constant under given conditions (Supplementary Fig. A2) and increased with increases in temperature. However, use of a gas permeable seal on the wells and an increased relative humidity (RH) in the shaker incubator kept evaporation rates to a minimum. For example, under typical culture conditions at 90% RH, 32 °C and light intensity of 380 μmol m^{-2} s^{-1}, only 8–10% w/w of liquid in the well was lost over 3.5 days of cultivation. This level of evaporation is considered to have minimal impact on the growth of *C. sorokiniana* and in fed-batch cultures fluid additions would off-set any evaporative losses as shown previously [36].

3.4. mPBr microalgae culture kinetics

3.4.1. Batch cultivation in the twin-well mPBr

Initial studies in the twin-well mPBr for batch cultivation of *C. sorokiniana* focused on optimisation of TBP media composition and environmental conditions as shown in Table 2. The first cultures at low tris-base concentrations of 20–60 mM yielded low biomass concentrations of approximately 1 g L^{-1}. Similar results were reported using standard TAP medium without acetate by Kumar and Das. The progressive decline in pH measured during

Table 2
Optimisation of TBP medium composition using the prototype miniature photobioreactor.

Temp. (°C)	Shaking frequency (rpm)	Tris-base concentration (mM)	Photo-period (L/D) hr	CO_2 concentration (%)	Biomass concentration ($g\,L^{-1}$)
28	250	20	24:0	Atm.	0.9
28	250	60	24:0	Atm.	1.0
28	250	200	24:0	Atm.	1.3
28	250	200	18:6	2	1.0
35	250	200	24:0	2	2.9
35	300	200	24:0	2	5.9

Atm.—atmospheric air (2% CO_2); L/D—light/dark cycles.

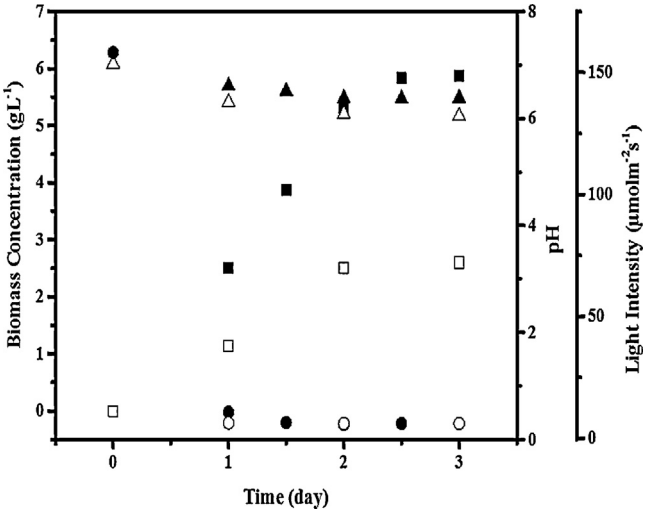

Fig. 4. Effect of shaking frequency on batch culture kinetics of *C. sorokiniana* in the twin-well mPBr; (■,□) biomass concentration, (▲,△) pH and (●,○) light intensity, where closed and opened symbols represent 300 and 250 rpm respectively. Experimental conditions: V_f = 4 mL; LI = 160 μmol m^{-2} s^{-1}; 32 °C; 85% RH.

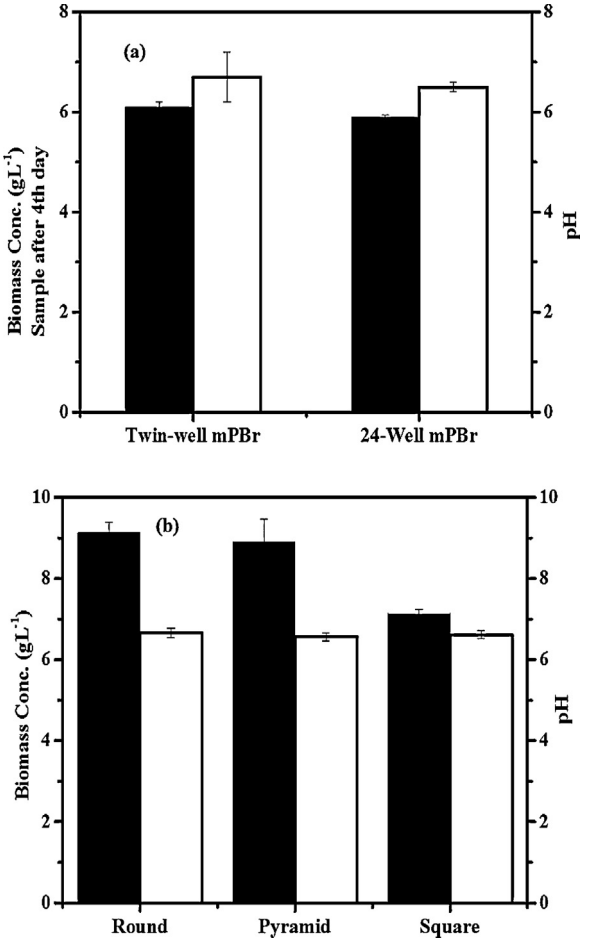

Fig. 5. Comparison of batch culture kinetics of *C. sorokiniana* in different mPBr configurations: (a) Twin-well mPBr and 24-well mPBr under identical conditions. Experimental conditions: V_f = 4 mL; LI = 160 μmol m^{-2} s^{-1}; 32 °C; 2% CO_2; 90% RH and N_f = 300 rpm. (b) Different geometries of 24-well mPBr at LI = 240 μmol m^{-2} s^{-1} (■) biomass concentration and (□) pH. Error bars represent one standard deviation about the mean (n = 24 for 24-well mPBr and n = 2 for twin well mPBr).

the cultures resulted in decreased biomass growth and also caused chlorophyll bleaching due to broth acidification [37]. Media recipes and buffer concentrations were subsequently investigated, modified and optimised as indicated in Table 2. A self-buffering medium was formulated with 0.2 M tris-base that kept the pH range during cultures between 6.0 and 7.2ee this also eliminated the need for the tris-acetate thus removing a potential source of inorganic carbon from the media. Further variation of the culture conditions such as an increase in temperature to 35 °C resulted in an almost two-fold increase in final biomass concentration (Table 2).

Variation of the shaking frequency led to further increases in biomass concentration up to nearly 6.0 $g\,L^{-1}$ (Fig. 4) representing a 6-fold improvement over the initial conditions. The approximate doubling in the growth rate and biomass yield seen when increasing the shaking frequency from 250 to 300 rpm matches the increase seen in the corresponding k_La values (Fig. 3). As increases in k_La will lead to an increased rate of CO_2 transfer, this suggests that cultures could have been partly CO_2 limited at the lower shaking frequency. Using the optimised medium formulation the pH was also maintained relatively constant throughout both cultures between pH 6–7. The highest biomass concentration achieved here is comparable with the data of Cuaresma et al. for culture of *C. sorokiniana* in a flat plate photobioreactor [39]. A similar system has also been used for the continuous culture of *Neochloris oleoabundans* [38].

3.4.2. Scale-out from prototype mPBr to 24-well mPBr

Scale-out of the twin-well mPBr format into conventional multiwell plate designs was next assessed in order to facilitate greater parallelisation and hence increased experimental throughput. Such considerations are important for use of the mPBr in early stage strain selection and media optimisation applications. Important parameters impacting on scale-out operation include light intensity, fluid hydrodynamics and mixing and the surface areas available for light absorption and gas–liquid mass transfer. For the 24-well mPBr, the light path-length measured from the platform surface was taken as the reference light source and was matched to the light path length in the twin-well mPBr. As shown in Fig. 5a, by maintaining the light path length and illuminated surface area approximately constant in wells of the same design (pyramid-shaped base) then similar rates [23] and extents of cell growth were obtained and similar pH values recorded.

For the scaled-out mPBr various 24-well plate geometries are available as shown in Supplementary Fig. A1. The dimensions of

all the square edges in the upper parts of the wells are practically the same, yet the total surface area available for light absorption at the base in the wells differed depending on the base geometry (round, pyramid or flat/square). Fig. 5b shows the impact of base geometry on culture performance at a fixed light intensity of 240 μmol m^{-2} s^{-1}. These results show that polypropylene, translucent-walled round and pyramid-shaped base wells yielded similar biomass concentration of ~9.2 g L^{-1}. In contrast, the square flat-base mPBr, which had opaque walls, exhibited an approximately, 22% reduction in final biomass concentration. Data on chlorophyll concentration in each of the cultures [23] indicated an approximately 56% higher chlorophyll concentration in the flat-based mPBr wells. These results suggest that in cases when screening for increased pigment production is required, the opaque-walled, square flat-based plates are the most suitable design while when investigated algal growth kinetics and biomass yields the round and pyramid-based mPBr designs are most appropriate. These differences are most likely due to the actual light intensities experienced by the cultures in the different plate designs. Further evaluation of the different media formulations described in Table 1 using the round base 24-well mPBr shows TBP to have achieved the highest biomass concentration [23]. As this was also void of any acetate additions TBP was consequently chosen for further experiment.

3.4.3. *Reproducibility of parallel microalgae cultivation*

An essential requirement of any parallel, multiwell platform is reproducible culture performance across individual wells under identical operating conditions. Fig. 6a shows biomass and chlorophyll concentrations for parallel *C. sorokiniana* cultivation in the 24-well mPBr. The experiments were performed at two different set of conditions of shaking frequency and light intensity. Comparison of overall biomass concentrations shows a two-fold increase at higher shaking frequency and light intensity (270 rpm, 180 μmol m^{-2} s^{-1}). Generally, well-to-well evaluation of biomass concentration at the tested conditions showed good reproducibility across the 24-wells. Only in the outer wells of the mPBr plate, closest to the incubator door, were any edge effects observed so results from these wells are excluded from this analysis. Quantitative assessment of chlorophyll production in the wells on each row also yielded similar concentrations for chlorophylls a, b and C_{ppc} (carotenoids concentration) as shown in Fig. 6b. In essence, use of the mPBr for parallel microalgae cultivation shows consistent and reproducible results.

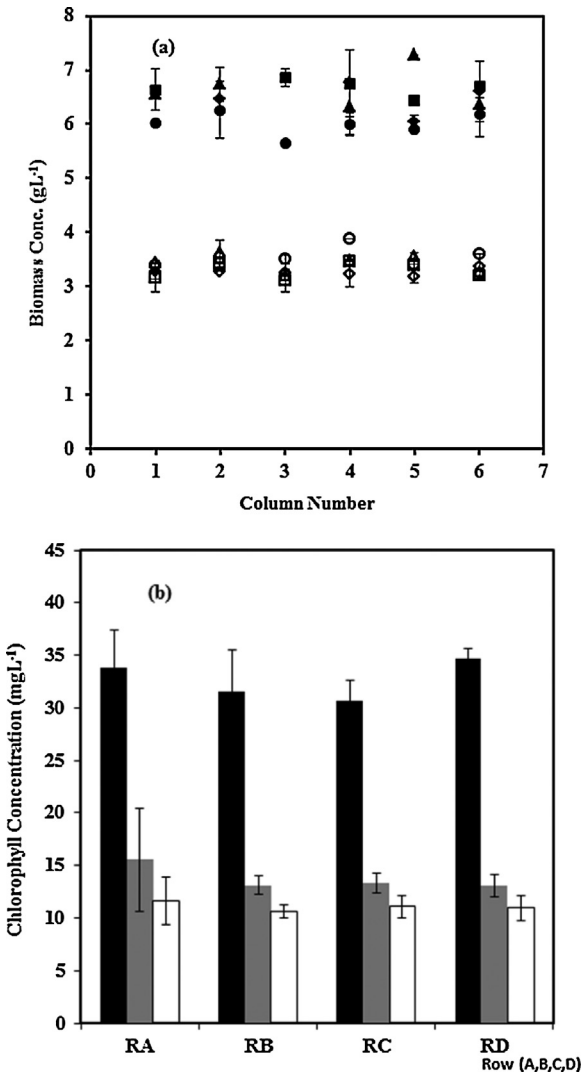

Fig. 6. Evaluation of well-to-well performance during batch cultivation of *C. sorokiniana* in the 24-well mPBr. (a) Biomass concentration at 4 days under two different illumination conditions, condition 1: N_f = 270 rpm, LI = 180 μmol m^{-2} s^{-1}, temperature 32 °C, 2% CO_2 (♦,■,▲,●); condition 2: N_f = 250 rpm, LI = 160 μmol m^{-2} s^{-1}, temperature 30 °C, 2% CO_2 and (◊,□,Δ,○) for rows A, B, C and D of the plate respectively. (b) Measured chlorophyll concentrations for experiments at LI = 180 μmol m^{-2} s^{-1}. (■) chlorophyll a, (■) chlorophyll b and (□) C_{ppc}. Error bars represent one standard deviation about the mean (n = 24).

3.5. *Optimisation studies using the mPBr*

3.5.1. *Effect of light intensity on culture kinetics and chlorophyll*

Phototrophic cultivation of *C. sorokiniana* depends largely on the intensity of light supplied and the rate of photosynthesis occurring in the cell [39]. Assessment of the effect of increasing light intensity on biomass productivity showed increases in biomass productivity up until 380 μmol m^{-2} s^{-1} after which no significant different was observed. In general, as light intensity increases, specific growth rate increases slightly, while doubling time reduces as shown in Fig. 7a. Optimal biomass productivities of 2.5 ± 0.2 g L^{-1} d^{-1} were achieved at 380 μmol m^{-2} s^{-1} above which an inhibitory effect due to excessive light saturation above the required threshold caused a reduction in cell productivity. Understanding the light thresholds for optimal biomass productivity becomes very important for implementing mPBr systems [40].

An assessment of the effect of light intensity on chlorophyll production by *C. sorokiniana* in the mPBr shows reduction in Chl 'a' concentrations at higher light intensities. While no significant difference was observed in the Chl 'b' and C_{ppc} except at the highest light intensity tested where significant reduction in pigment productivity was observed as shown in Fig 7b. In the published literature it is generally believed that pigment bleaching is caused by two phenomena; light saturation above the threshold intensity and acidification of growth media due to uncontrolled pH. The highest concentration of Chl 'a' observed at the lowest light intensity tested was thought to be due to the limited rate of photon capture leading to reduced rate of biomass formation (Fig. 7b).

3.5.2. *Effect of CO_2 concentrations on culture kinetics and total lipids*

The biomass productivity in photosynthetic culture not only depends on light availability but also on the amount of CO_2 present in the culture broth for microalgae uptake. The effect of CO_2 concentrations on growth kinetics was thus also evaluated and the results shown in Fig. 8a. Aeration with 5% CO_2 was found to be optimal with both biomass productivity and specific growth rate being highest. Similar observation was reported by Olivieri et al. [41] for the culture of *Stichococcus bacillaris* at 5% CO_2 and pH maintained at 7. Cultures sparged only with atmospheric air showed extended

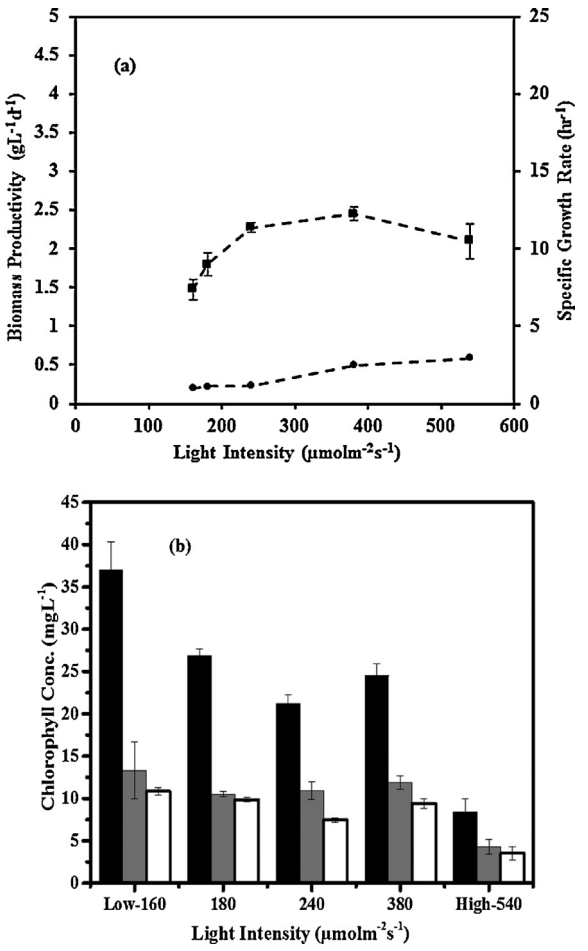

Fig. 7. Effect of light intensity on culture kinetics of *C. sorokiniana* in the 24 well mPBr. (a) (●) specific growth rate and (■) biomass productivity. (b) Chlorophyll concentration, (■) chlorophyll a (■) chlorophyll b (□) C_{ppc}. Error bars represent one standard deviation about the mean ($n = 3$).

doubling time due to the low levels of CO_2. Pigment concentrations showed no significant variation across all CO_2 levels tested as shown in Fig. 8b. Comparison of these results with those in Fig. 7b further affirms light intensity as the most critical factor in production of different pigments from photosynthetic *C. sorokiniana*. In terms of total lipid production, this was highest between conditions of atmospheric air and 5% CO_2 (50–62% w/w) while above this range the level of lipid dropped to around 30% w/w as shown in Supplementary Fig. A3.

3.5.3. *Effect of CO_2 levels on fatty acid composition*

Analysis of the FAME derived from *C. sorokiniana* lipid shows a high potential for biodiesel production. The unsaturated, especially the polyunsaturated FAMEs have lower melting temperatures which improve the low-temperature utilisation of biodiesel [42]. Monounsaturated and saturated fatty acids with carbon chain length in the range C16–C20 were considered for comparison, since these groups have been identified previously as best suited for biofuel production [43,44]. For the different esters evaluated, results at 10% CO_2 enrichment showed the highest FAME concentrations for the most prevalent polyunsaturated fatty acids (PUFA). For all the other CO_2 concentrations tested the FAME concentrations were not significantly different. FAME production under atmospheric air (0.02% CO_2 concentration) was lowest in all the tested fatty acid methyl esters except heptadecanoic acid (Fig. 8c). For the accumulation of high amounts of FAME, 5–20% CO_2 appears to be the best

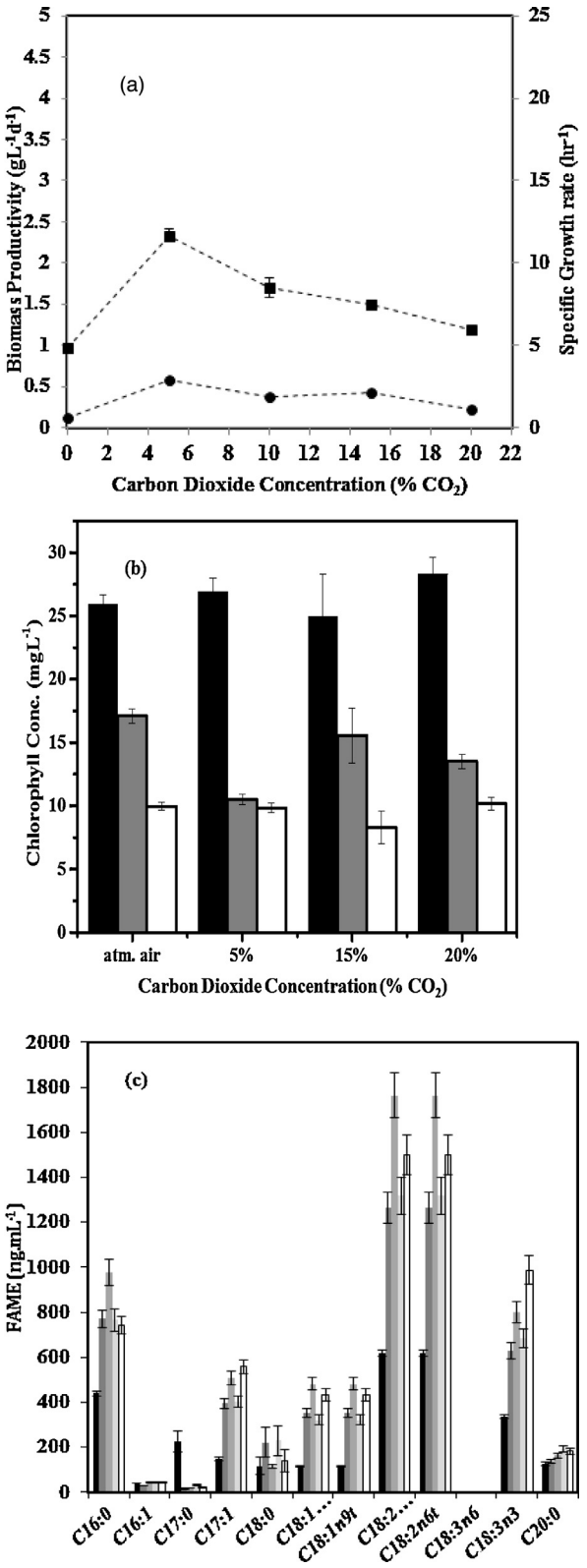

Fig. 8. Effect of CO_2 concentration on culture kinetics of *C. sorokiniana*. (a) (●) specific growth rate and (■) biomass productivity. (b) Chlorophyll concentration, (■) chlorophyll a (■) chlorophyll b (□) C_{ppc} (c) FAME ester derivatives; (■) 0, (■) 5, (■) 10 (■) 15 and (□) 20% CO_2 respectively. Error bars represent one standard deviation about the mean ($n = 3$).

range although biomass productivity decreases at the higher CO_2 levels.

4. Conclusions

This work has described a novel, orbitally shaken miniature photobioreactor platform suitable for early stage and parallel evaluation of algal cultivation conditions. In terms of the engineering environment within the mPBr, orbital shaking provides rapid mixing, effective gas–liquid mass transfer and a relatively constant light intensity throughout each well. The shaker incubator platform provides uniform control of light intensity, CO_2 levels, temperature and liquid evaporation across multiple plates. The utility of the system was illustrated for investigation and optimisation of the culture conditions of *C. sorokiniana*, In terms of practical application the mPBr shaker platform enables higher throughput evaluation of microalgae growth kinetics than current shake flask systems with an approximately 20-fold reduction in material requirements. Current work is addressing scale-translation and the ability to mimic culture conditions in larger scale photobioreactor designs.

Acknowledgements

EOO would like to acknowledge the Nigerian Petroleum Technology Development Fund (PTDF) for award of a doctoral studentship. The authors would also like to thank Infors HT and specifically Daniel Bruecher and Nick Musgrove for their advice and modifications to the incubator shaker. Use of the high speed video camera from the UK Engineering and Physical Sciences Research Council (EPSRC) Equipment Loan Pool is also acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bej.2015.07.006

References

[1] J. Rupprecht, From systems biology to fuel—*Chlamydomonas reinhardtii* as a model for a systems biology approach to improve biohydrogen production, J. Biotechnol. 142 (2009) 10–20, http://dx.doi.org/10.1016/j.jbiotec.2009.02.008
[2] M. Muthuraj, V. Kumar, B. Palabhanvi, D. Das, Evaluation of indigenous microalgal isolate *Chlorella* sp. FC2 IITG as a cell factory for biodiesel production and scale up in outdoor conditions, J. Ind. Microbiol. Biotechnol. 41 (2014) 499–511, http://dx.doi.org/10.1007/s10295-013-1397-9
[3] R. Harun, M. Singh, G.M. Forde, M.K. Danquah, Bioprocess engineering of microalgae to produce a variety of consumer products, Renew. Sustain. Energy Rev. 14 (2010) 1037–1047, http://dx.doi.org/10.1016/j.rser.2009.11.004
[4] G.O. James, C.H. Hocart, W. Hillier, H. Chen, F. Kordbacheh, G.D. Price, et al., Fatty acid profiling of *Chlamydomonas reinhardtii* under nitrogen deprivation, Bioresour. Technol. 102 (2011) 3343–3351, http://dx.doi.org/10.1016/j.biortech.2010.11.051
[5] K. Pradhan, T. Pant, M. Gadgil, In situ pH maintenance for mammalian cell cultures in shake flasks and tissue culture flasks, Biotechnol. Prog. 28 (2012) 1605–1610, http://dx.doi.org/10.1002/btpr.1614
[6] L. Rodolfi, G.C. Zittelli, N. Bassi, G. Padovani, N. Biondi, G. Bonini, et al., Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor, Biotechnol. Bioeng. 102 (2009) 100–112, http://dx.doi.org/10.1002/bit.22033
[7] J.M. Seletzky, U. Noak, J. Fricke, E. Welk, W. Eberhard, Scale-up from shake flasks to fermenters in batch and continuous mode with *Corynebacterium glutamicum* on lactic acid based on oxygen transfer and pH, Biotechnol. Bioeng. 98 (2007) 800–811, http://dx.doi.org/10.1002/bit
[8] T.A. Barrett, A. Wu, H. Zhang, M.S. Levy, G.J. Lye, Microwell engineering characterization for mammalian cell culture process development, Biotechnol. Bioeng. 105 (2010) 260–275, http://dx.doi.org/10.1002/bit.22531
[9] J.I. Betts, F. Baganz, Miniature bioreactors: current practices and future opportunities, Microb. Cell Fact. 5 (2006) 21, http://dx.doi.org/10.1186/1475-2859-5-21
[10] R. Hermann, M. Lehmann, J. Büchs, Characterization of gas–liquid mass transfer phenomena in microtiter plates, Biotechnol. Bioeng. 81 (2003) 178–186, http://dx.doi.org/10.1002/bit.10456
[11] S. Kumar, C. Wittmann, E. Heinzle, Minibioreactor, Biotechnol. Lett. 26 (2004) 1–10.
[12] M. Micheletti, T. Barrett, S. Doig, F. Baganz, M. Levy, J. Woodley, et al., Fluid mixing in shaken bioreactors: implications for scale-up predictions from microlitre-scale microbial and mammalian cell cultures, Chem. Eng. Sci. 61 (2006) 2939–2949, http://dx.doi.org/10.1016/j.ces.2005.11.028
[13] M. Micheletti, G.J. Lye, Microscale bioprocess optimisation, Curr. Opin. Biotechnol. 17 (2006) 611–618, http://dx.doi.org/10.1016/j.copbio.2006.10.006
[14] H. Zhang, S. Lamping, S. Pickering, G. Lye, P. Shamlou, Engineering characterisation of a single well from 24-well and 96-well microtitre plates, Biochem. Eng. J. 40 (2008) 138–149, http://dx.doi.org/10.1016/j.bej.2007.12.005
[15] M.P.C. Marques, J.M.S. Cabral, P. Fernandes, Bioprocess scale-up: quest for the parameters to be used as criterion to move from microreactors to lab-scale, J. Chem. Technol. Biotechnol. 85 (2010) 1184–1198, http://dx.doi.org/10.1002/jctb.2387
[16] J.P.J. Betts, S.R.C. Warr, G.B. Finka, M. Uden, M. Town, J.M. Janda, et al., Impact of aeration strategies on fed-batch cell culture kinetics in a single-use 24-well miniature bioreactor, Biochem. Eng. J. 82 (2014) 105–116, http://dx.doi.org/10.1016/j.bej.2013.11.010
[17] G.J. Lye, P. Ayazi-Shamlou, F. Baganz, P.A. Dalby, J.M. Woodley, Accelerated design of bioconversion processes using automated microscale processing techniques, Trends Biotechnol. 21 (2003) 29–37 http://www.ncbi.nlm.nih.gov/pubmed/12480348
[18] H. Zhou, J. Purdie, T. Wang, A. Ouyang, pH measurement and a rational and practical pH control strategy for high throughput cell culture system, Biotechnol. Prog. 26 (2009) 872–880, http://dx.doi.org/10.1002/btpr.369
[19] H.F. Zimmermann, G.T. John, H. Trauthwein, U. Dingerdissen, K. Huthmacher, Rapid evaluation of oxygen and water permeation through microplate sealing tapes, Biotechnol. Prog. 19 (2003) 1061–1063, http://dx.doi.org/10.1021/bp025774t
[20] F. Hillig, S. Annemüller, M. Chmielewska, M. Pilarek, S. Junne, P. Neubauer, Bioprocess development in single-use systems for heterotrophic marine microalgae, Chem. Ing. Tech. 85 (2013) 153–161, http://dx.doi.org/10.1002/cite.201200143
[21] R. Matsukawa, M. Hotta, Y. Masuda, M. Chihara, I. Karube, Antioxidants from carbon dioxide fixing *Chlorella sorokiniana*, J. Appl. Phycol. 12 (2000) 263–267.
[22] C. Sorokin, R.W. Krauss, Effects of temperature & illuminance on chlorella growth uncoupled from cell division, Plant Physiol. 37 (1962) 37–42, http://dx.doi.org/10.1104/pp.37.1.37
[23] E.O. Ojo, Photobioreactor Technologies for High-Throughput Microalgae Cultivation, University College London, 2015, Unpublished Doctoral Thesis.
[24] F. Garcia-Ochoa, E. Gomez, Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview, Biotechnol. Adv. 27 (2009) 153–176, http://dx.doi.org/10.1016/j.biotechadv.2008.10.006
[25] N.D.P. Dang, D.A. Karrer, I.J. Dunn, Oxygen transfer coefficients by dynamic model moment analysis, Biotechnol. Bioeng. 19 (1977) 853–865, http://dx.doi.org/10.1002/bit.260190606
[26] E.O. Ojo, H. Auta, F. Baganz, G.J. Lye, Engineering characterisation of a shaken, single-use photobioreactor for early stage microalgae cultivation using *Chlorella sorokiniana*, Bioresour. Technol. 173 (2014) 367–375, http://dx.doi.org/10.1016/j.biortech.2014.09.060
[27] J. Liu, J. Huang, Z. Sun, Y. Zhong, Y. Jiang, F. Chen, Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic *Chlorella zofingiensis*: assessment of algal oils for biodiesel production, Bioresour. Technol. (2011), http://dx.doi.org/10.1016/j.biortech.2010.06.017
[28] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959).
[29] K. Isett, H. George, W. Herber, A. Amanullah, Twenty-four-well plate miniature bioreactor high-throughput system: assessment for microbial cultivations, Biotechnol. Bioeng. 98 (2007) 1017–1028, http://dx.doi.org/10.1002/bit
[30] S.D. Doig, S.C.R. Pickering, G.J. Lye, F. Baganz, Modelling surface aeration rates in shaken microtitre plates using dimensionless groups, Chem. Eng. Sci. 60 (2005) 2741–2750, http://dx.doi.org/10.1016/j.ces.2004.12.025
[31] Q. Liao, L. Li, R. Chen, X. Zhu, A novel photobioreactor generating the light/dark cycle to improve microalgae cultivation, Bioresour. Technol. 161 (2014) 186–191, http://dx.doi.org/10.1016/j.biortech.2014.02.119
[32] B. Bouscasse, M. Antuono, A. Colagrossi, C. Lugni, Numerical and experimental investigation of nonlinear shallow water sloshing, Int. J. Nonlinear Sci. Numer. Simul. 14 (2013) 123–138, http://dx.doi.org/10.1515/ijnsns-2012-0100
[33] N.K. Gill, M. Appleton, F. Baganz, G.J. Lye, Design and characterisation of a miniature stirred bioreactor system for parallel microbial fermentations, Biochem. Eng. J. 39 (2008) 164–176, http://dx.doi.org/10.1016/j.bej.2007.09.001
[34] W.A. Duetz, Microtiter plates as mini-bioreactors: miniaturization of fermentation methods, Trends Microbiol. 15 (2007) 469–475, http://dx.doi.org/10.1016/j.tim.2007.09.004
[35] W.A. Duetz, B. Witholt, Oxygen transfer by orbital shaking of square vessels and deepwell microtiter plates of various dimensions, Biochem. Eng. J. 17 (2004) 181–185, http://dx.doi.org/10.1016/S1369-703X(03)00177-3
[36] N.J. Silk, S. Denby, G. Lewis, M. Kuiper, D. Hatton, R. Field, et al., Fed-batch operation of an industrial cell culture process in shaken microwells, Biotechnol. Lett. 32 (2010) 73–78, http://dx.doi.org/10.1007/s10529-009-0124-0

[37] K. Kumar, D. Das, Growth characteristics of *Chlorella sorokiniana* in airlift and bubble column photobioreactors, Bioresour. Technol. 116 (2012) 307–313, http://dx.doi.org/10.1016/j.biortech.2012.03.074
[38] A.J. Klok, J.A. Verbaanderd, P.P. Lamers, D.E. Martens, A. Rinzema, R.H. Wijffels, A model for customising biomass composition in continuous microalgae production, Bioresour. Technol. 146 (2013) 89–100, http://dx.doi.org/10.1016/j.biortech.2013.07.039
[39] M. Cuaresma, M. Janssen, C. Vílchez, R.H. Wijffels, Productivity of *Chlorella sorokiniana* in a short light-path (SLP) panel photobioreactor under high irradiance, Biotechnol. Bioeng. 104 (2009) 352–359, http://dx.doi.org/10.1002/bit.22394
[40] Q. Béchet, A. Shilton, B. Guieysse, Modeling the effects of light and temperature on algae growth: state of the art and critical assessment for productivity prediction during outdoor cultivation, Biotechnol. Adv. 31 (2013) 1648–1663, http://dx.doi.org/10.1016/j.biotechadv.2013.08.014
[41] G. Olivieri, I. Gargano, R. Andreozzi, R. Marotta, A. Marzocchella, G. Pinto, et al., Effects of photobioreactors design and operating conditions on *Stichococcus bacillaris* biomass and biodiesel production, Biochem. Eng. J. 74 (2013) 8–14, http://dx.doi.org/10.1016/j.bej.2013.02.006
[42] D. Tang, W. Han, P. Li, X. Miao, J. Zhong, CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels, Bioresour. Technol. 102 (2011) 3071–3076, http://dx.doi.org/10.1016/j.biortech.2010.10.047
[43] T. Li, Y. Zheng, L. Yu, S. Chen, High productivity cultivation of a heat-resistant microalga *Chlorella sorokiniana* for biofuel production, Bioresour. Technol. 131 (2013) 60–67, http://dx.doi.org/10.1016/j.biortech.2012.11.121
[44] S. Chader, B. Mahmah, K. Chetehouna, E. Mignolet, Biodiesel production using *Chlorella sorokiniana* a green microalga, Rev. Des Energies Renouvelables 14 (2011) 21–26.