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Real-time monitoring and automatic density control of large-scale microalgal cultures using near infrared (NIR) optical density sensors

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Abstract

Signals from near infrared (NIR) light transmittance sensors were used for both real-time monitoring of algal biomass density in growing mass cultures (200 l tubular biofences), and also as feedback in a system that controlled the density of the culture by automatic injection of fresh growth medium. When operated in a semi-continuous production mode between predefined density values, diurnal growth patterns were recorded on-line that provided information on the dynamics of the microalgal cultures with respect to environmental conditions. The bioreactor system was also programmed to operate in constant biomass density mode, thereby maintaining the culture at the optimal population density (OPD), and sustaining high biomass production levels. The system has potential for operating a dynamic density set point for microalgal cultures where the optimal population density varies as a function of ambient growing conditions.
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Keywords: Photobioreactor; Biomass; Optical density sensors; Near infrared (NIR); Microalgae; *Nannochloropsis oceanica*; Biofence

1. Introduction

Marine microalgae are exploited industrially as a source of long-chain polyunsaturated fatty acids (PUFAs), polysaccharides, vitamins, β-carotene and pigments (Abalde et al., 1991; Molina Grima et al., 1994; Chini Zittelli et al., 1999; Arad and Richmond, 2004). Although microalgae biotechnology has focused on the use of algae in the production of high value fine chemicals, therapeutics and health food; marine microalgae are now increasingly used as feed for marine organisms, constituting both a source of energy as well as the essential vitamins and PUFAs.

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Nannochloropsis, a marine unicellular algae belonging to the Eustigmatophyceae, is one such microalgal group that is commonly cultivated in fish hatcheries as feed for rotifers due to its high content of an essential PUFA, eicosapentaenoic acid (EPA, C20:5n3). Despite the widespread use of *Nannochloropsis* as aquaculture feed, its industrial exploitation as a source of EPA has been unable to compete with fish oil due to high production costs (Zhang et al., 2001).

Large-scale production facilities provide the possibility of delivering a continuous supply of high quality and contaminant-free microalgae for aquaculture. Research from the past decade has focused on new reactor designs as a progression from open pond and raceway designs that are synonymous with relatively low productivity rates. However, operational costs are generally higher in the 'new-generation' closed production designs and it is therefore important to maintain the culture at close to optimal culturing conditions in order to maximise output, thereby reducing production costs. Control over the growing culture is necessary on a time-frame relevant to production rates and in the case of algaculture, important decisions with respect to fertilising, harvesting, lighting and temperature may need to be taken on an hourly basis to prevent economical losses. As such, the development of an integrated system for monitoring growth parameters is important for commercial viability, providing the grower with valuable information with which to optimise production processes and reduce costs (Gitelson et al., 2000).

To implement a culture control strategy, an accurate method for automatic biomass estimation is required. The last few years have seen the development of methods for in situ growth monitoring of unicellular cultures in laboratory scale bioreactors, from flow injection analysis systems based on turbidimetric measurements (Meireles et al., 2002), to techniques based on monitoring O_2 production from the increase in pressure inside a closed reactor (Cogne et al., 2001). The use of optical density (OD) as a turbidimetric measure of biomass is the most usual and widespread method of non-invasive biomass estimation and the optical density is usually measured manually on a spectrophotometer (Meireles et al., 2002).

The goal of this study was to investigate the application of light transmittance sensors for real-time monitoring and density control of microalgae biomass production in large-scale tubular reactors.

2. Cultivation conditions

2.1. Bioreactor system

Three tubular photobioreactor biofence systems, each of 200 l volume capacity, (Cellpharm, UK) were mounted in climate-regulated greenhouses at UMB as a research tool for the production of large-scale microalgae (Fig. 1a). Fig. 1b shows a schematic diagram of the microalgal culture system. The fence serves to increase surface area for light absorption, and consists of 48 PVC tubes of length 2.44 m and 30 mm internal diameter. The growing microalgal culture is circulated through the biofence system by a centrifugal pump via a buffer tank. The circulation velocity in the transpar-

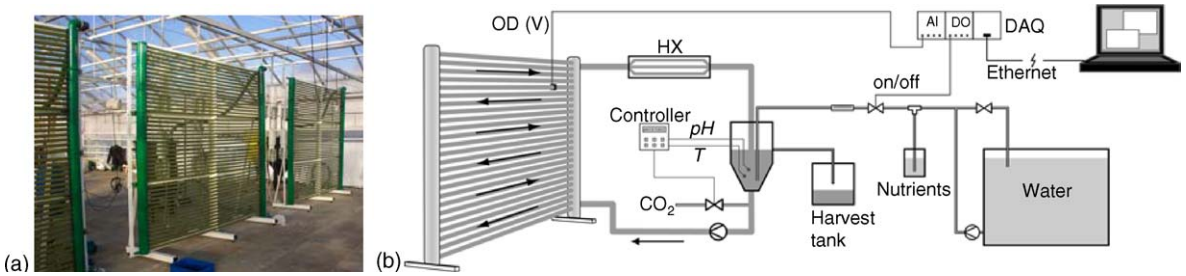

Fig. 1. (a) The biofence systems mounted in climate-regulated greenhouses. (b) Schematic representation of the biofence system. The 200 l algal culture is continuously circulated from a buffer tank through the fence of transparent tubes where incident light is absorbed by the algae. A heat exchanger (HX) coupled to a cooling machine facilitates temperature regulation. A controller unit records culture temperature and pH, and regulates CO_2 dosing. The right side of the diagram shows the density control system where measured optical density (OD) values are used to control the injection of a water/nutrient mix into the buffer tank. The overflow of algae suspension is harvested in separate tanks.

ent tubes is approximately 1 m s^{-1}, which corresponds to a volume flow rate of 340 l min^{-1}. The inclusion of buoyant, polymer beads (3 mm in diameter, supplied by Cellpharm, UK, now under development by Varicon Aqua, UK) in the culture medium prevented sedimentation and accumulation of algae on the plastic tubing.

A controller unit continuously records culture temperature and pH measured by sensors submersed in the buffer tank. The pH is regulated by CO_2 dosing into the outlet tube from the buffer tank to maintain the culture within set limits, preset in the current studies between pH 7.3 and 7.8 for *Nannochloropsis* production. This enables CO_2 to be dosed on demand, i.e. during growth periods and disabled during night respiration in the culture. Specially constructed cooling systems have been developed as integrated system components on each of the three biofence systems.

The biofence system is operational in climate-regulated greenhouses that give further possibilities for controlling day and night-time temperatures. Data for culture temperature and pH are recorded every 15 s and averaged over 5 min; similarly solar radiation measurements from a pyranometer (Kipp & Sonen CM6B) is averaged and recorded at 5 min intervals. For studies involving artificial lighting, high-pressure sodium lamps (Power Osram HQI-BT 400 W/D), commonly used in greenhouse production, were installed in front of the biofences.

2.2. Medium composition

A nutrient feed consisting of a combination of agricultural fertilisers and urea represented a cost effective and time-saving alternative to Guillard's *f*/2 medium (Guillard, 1975). The nutrient combination used in the case studies described in the following section was: 1 g l^{-1} Red Superba (Norsk Hydro, 7% N, 4% P, 21% K), 1 g l^{-1} Urea and Guillard's *f*/2 vitamins and trace metals (1 and 0.5 ml l^{-1}, respectively). In dilute batch cultures in this medium, growth rates were shown to be equal to, or higher than, standard Guillard's *f*/2 nutrients at salinities between 20 and 35 g l^{-1} and the higher phosphate concentrations in the fertiliser feed supported a high density culture. Filtered seawater (200 μm) that was treated to avoid contamination (acidified and subsequently neutralised) was used in all investigations.

2.3. Microalgal culture

Nannochloropsis oceanica was isolated from an operational hatchery in western Norway. The species was genetically determined by sequencing of 18S rDNA by CCAP, Scotland, UK and recorded as a new species in their culture collection by CCAP 849/10 (Hart et al., 2005). The light absorption spectra of *N. oceanica* (CCAP 849/10), includes several distinguishing features due to the characteristic lack of chlorophyll b and c pigments in this algal group and shows clear similarities to published absorption spectra of other *Nannochloropsis* species (Owens et al., 1987; Gitelson et al., 2000). Light absorption decreases sharply to a minimum value within the NIR range and it is the transmittance of light in the NIR region of the spectrum (880 nm) that was used for estimating the dry cell mass in growing cultures of *N. oceanica* in this study.

3. Culture control system

3.1. Construction and calibration of optical density sensors

An optical density sensor was constructed and mounted externally on one of the transparent tubes of the biofence system, and consisted of an array of near infrared (NIR) light emitting diodes (LEDs) of wavelength 880 nm. The transparent tube was reduced locally to approximately 10 mm as illustrated in Fig. 2, to increase the total transmitted light, which was measured by a photodiode detector positioned on the opposite side of the tube. The number of diodes (five) in the LED array was selected in order to give high

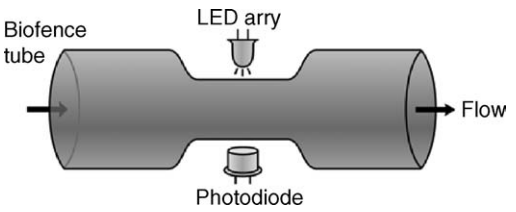

Fig. 2. Sketch of the optical density sensor mounted on a section of the transparent tube with reduced diameter. The sensor consists of five LEDs and a single photodiode detector opposite measuring the intensity of the light transmitted through the culture. The wavelength of the emitted light is 880 nm.

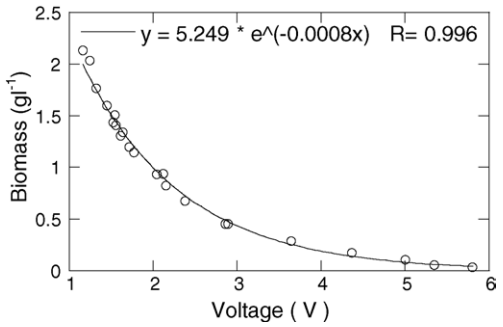

Fig. 3. Calibration curve and calculated regression equation describing the relationship between the instrument output voltage and culture biomass for one instrument and biofence. Each sensor/system/species combination must be individually calibrated.

instrument sensitivity over a culture density range suitable for biomass production studies in the system ($\sim$0.5–2 g l^{-1}).

The light intensity output from the LEDs varies with temperature, and therefore self-heating of the LEDs and varying ambient temperatures can cause drift in the output signal from the optical sensor. However, with a constant, high drive current the equilibrium temperature of the LEDs is kept high (around 70 °C) and is fairly stable. In the current studies, the biofence systems are mounted in climate-regulated greenhouses and the culture temperature is controlled and varied over a relatively narrow range. The effect of temperature on the optical sensor output has therefore in practice been minimal and is here disregarded.

The optical sensors were calibrated to both algal dry weight and cell number counts for the biomass range required for culture experiments. Dry weight algal samples were determined by centrifuging, resuspending in ammonium formate for salt removal and drying at 100 °C overnight. Cell numbers were determined using a Coulter Multisizer particle counter. An example of a calibration curve for one of the optical density sensors is shown in Fig. 3 and the given regression equation describes the relationship between culture biomass and the instrument voltage. The accuracy of the instrument was determined and showed a maximum error of approximately 8% of the total biomass.

3.2. Biomass density control system

The optical density sensors described above were mounted one for each of three biofence systems. The output voltages from the optical sensors are logged using a distributed data acquisition (DAQ) module (Fieldpoint, National Instruments). The data recorded by the DAQ module are continuously transferred through the local area network (LAN) to a computer where the data are treated, displayed and filed using the LabView software (National Instruments). This system thus provides the means for on-line monitoring of the biomass density and rate of growth in the culture, and the data can be downloaded from any location with access to the network.

An algal density control system has been developed in order to obtain real-time control of the biomass density in the culture based on the continuously measured optical density values (see Fig. 1b). In order to maintain a constant culture density, a water/nutrient mix is pumped into the buffer tank when the biomass density measured by the optical sensors exceeds a preset value. The water/nutrient inlet is positioned close to the tank outlet such that the fresh medium is mixed with the culture in the circulation loop. As the culture volume expands, the higher density culture in the buffer tank is harvested through an overflow tube positioned at a certain height in the tank. The overflow culture volume is collected in designated harvest tanks.

The pumping in of the water/nutrient mix is controlled using the same DAQ module and software program that measures the optical density values in the culture. Every 10 min, the controller program compares the optical density sensor output measured on the analogue input (AI) channels of the DAQ module to preset values corresponding to a maximum biomass density set point for the cultures. When the density exceeds the set point, an electronically controlled valve is opened such that the water/nutrient mix can be pumped into the buffer tank. The switching of the valve is achieved by setting a digital output (DO) channel on the DAQ module high as illustrated in Fig. 1b. The valve is then open for a set, predefined period, which can range from 0 to 10 min (corresponding to 0–100% duty cycle for the pumping system). The water/nutrient injection procedure is, if necessary, repeated until the output from the optical sensor reads below the density set point.

When the culture system is in constant density mode, it is obviously not possible to measure biomass productivity directly by the density increase as previously described. Instead, the time periods Δt that the valve is open are recorded, and the water/nutrient mix

input volume is given by $\Delta V = q\Delta t$, where q is the constant volume flow rate measured on manual flow meters. Assuming negligible evaporation losses, the overflow harvest volume is equal to the volume input, and the harvested biomass is thus $m = X\Delta V$, where X is the (constant) biomass density in the culture.

Although only constant density mode is demonstrated in this study, it is possible to use this system to run a dynamic density set point for the culture. This could be of interest when algal species are cultured that show significant differences in growth responses under varying ambient conditions (light, temperature, etc.), and where increased productivity could be achieved by varying the density accordingly. The complexity of such a control strategy is only limited by the number of ambient parameters one measures.

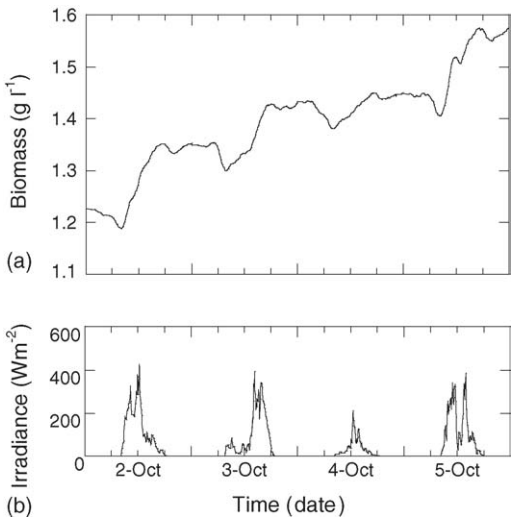

Fig. 4. (a) Diurnal growth patterns of the microalgal culture from data recorded using the optical density sensor. This can be directly related to ambient irradiance levels as illustrated in (b). The major tick marks on the *x*-axis represent midnight for each day.

4. Applications

4.1. On-line growth monitoring

Understanding the immediate and delayed responses of mass culture dynamics to changes in environmental conditions, such as ambient light and temperature, is necessary for optimisation of large-scale microalgal production. As such, continuous, real-time measurement of culture biomass has applications for both laboratory research purposes and industrial scale production systems.

Fig. 4a shows a typical biomass production curve over a 4-day period measured by an optical density sensor mounted on one of the biofences. During this period, the biomass in the batch culture increased from, approximately, 1.23 to 1.57 g l^{-1} in response to the varying autumn irradiance as plotted in Fig. 4b. From these diurnal growth curves, it is possible to identify and quantify different phases of growth, such as daily algal biomass increase in response to incident light during the day, constant night biomass and night loss in biomass. Culture dynamics can be related to ambient growing conditions such as light, temperature, nutrients and pH, and in this way, direct 'cause and effect' responses can be established and quantified.

In a study investigating the effects of light durations on biomass production, the biofence systems were operated with a constant artificial light source in front of the biofence. An array of high-pressure sodium lamps produced an average light intensity of 397 μmol m^{-2} s^{-1} incident on the fence. Interference from ambient daylight was minimised using shading curtains around the fences. The optical density sensors were used to monitor diurnal patterns of microalgal growth and an example is shown in Fig. 5 of the consistent responses of microalgal biomass to the light source. The graph shows two experimental periods where the biofences operated in a semi-continuous production mode, with the culture growing from 1 to 2 g l^{-1}, at which point the cultures were automatically diluted for

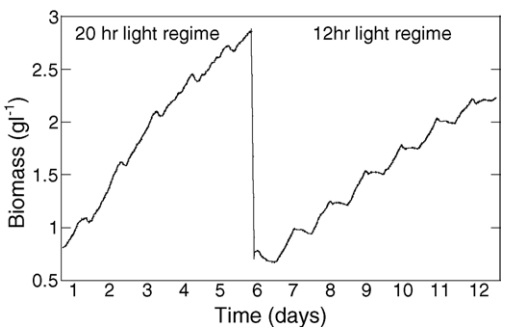

Fig. 5. Application of optical sensors in monitoring diurnal patterns of microalgal growth in a study investigating light regimes (20 and 12 h light durations).

the next light treatment. In the first experimental period, the algae were cultured under a 20 h light and 4 h dark cycle, and the second period shows biomass production with a 12:12 light to dark cycle. The daily cycle of microalgal growth in Fig. 5 is clearly seen by a daily increase in production (a function of the light duration), followed by a period of night loss in the system.

4.2. Maintaining optimal population density (OPD) in for continuous production

Microalgal density represents an important parameter affecting the production of photoautotrophic mass, influencing both the productivity rates or total yield and the cellular composition of the microalgae. The cell density at which the cell mass or concentration in continuous cultures reaches its highest output rate of biomass is defined as the 'optimal population density (OPD)' and it is at this cell density that the culture is most stable (Richmond, 2004). The aim for management of mass cultures is to sustain this optimal state in order to maximise yields and reduce costs.

Fig. 6a shows microalgal production volume output in a 200 l biofence culture maintained at constant biomass density of 1.5 g l^{-1} using the optical density control system. In this experiment, the culture was subjected to 16:8 h light:dark cycles using artificial lights. Interference from ambient daylight was minimised using shading curtains. During the 24 h period shown in the figure, 29.8 l of algal biomass was produced at a culture density of 1.5 g l^{-1} as shown by the cumulative plot in Fig. 6b. This yields a total microalgal dry weight biomass of approximately 44 g day^{-1} from this particular 200 l biofence system operational under the experimental 16:8 light:dark regime.

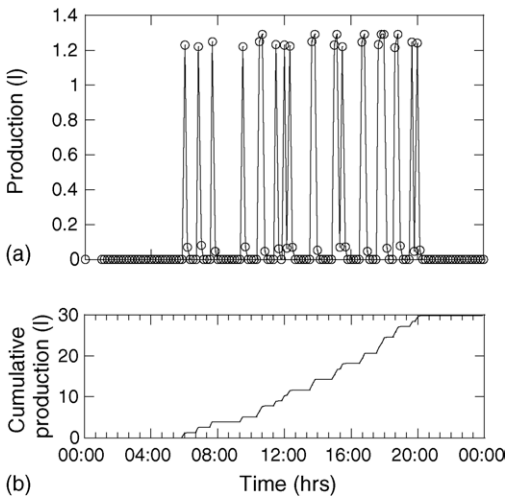

Fig. 6. (a) Production (l) of *N. oceanica* in a biofence during a 24 h cycle under a 16:8 light:dark regime. The graph shows the volume of *Nannochloropsis* produced when the biofences were maintained at a constant operational biomass of 1.5 g l^{-1} in the system. The corresponding cumulative production of microalgae is shown in (b).

5. Conclusions

A NIR optical density sensor has been developed that provides fast and automatic biomass determination of growing mass cultures. Optical density measurements from the sensor were used to monitor diurnal growth patterns of *N. oceanica* in 200 l tubular bioreactors, and also as a feedback signal in a system that controlled the density of the culture by automatic dilution. The density control system has potential for maximising biomass production since the culture is automatically maintained at optimal population density.

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