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Diurnal variation of various culture and biochemical parameters of *Arthrospira platensis* in large-scale outdoor raceway ponds

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ABSTRACT

The use of microalgae for nutraceuticals, food, and feed has recently gained great interest. Having a good understanding of the trends, including the diurnal variations, of the physiological parameters and biochemical composition of the biomass in commercial scale systems is of high importance as it may lead to finding potential ways to optimize productivity and product quality. Not much is known about the diurnal variation of the physiological parameters and biochemical composition of microalgae grown in large-scale outdoor raceway ponds in general and *Arthrospira* in particular, even though a lot is known about such diurnal variations in natural aquatic and laboratory environments. *Arthrospira* (*Spirulina*) *platensis* is a filamentous cyanobacterium widely used as dietary supplement, functional food, food, and animal feed. It has been grown commercially for about 40 years. In the present experiments the diurnal variation of some photosynthetic parameters and of the product quality was studied in *Arthrospira platensis* grown outdoors in a 5000 m^2 raceway pond over a 24-hour period at three different months of the year. Specific trends for *Arthrospira* in commercial scale setup were described for the first time. Significant variation was observed in the biomass composition throughout the course of the day. The photosynthetic pigment content of *Arthrospira* (chlorophyll *a*, total carotenoids, and total phycocyanin) declined during the light hours, and recovered during the night. The same pattern was seen in the case of the crude protein content of the biomass. The total carbohydrate content however showed an opposite trend to that of the protein. Selected parameters were simulated in the lab in benchtop photobioreactors and the trends were compared with the large-scale system. The results of the experiments are discussed in relation to diurnal changes in some physical and chemical factors.

1. Introduction

Recently there has been an increased interest in the use of microalgae for food, nutraceuticals and feed. From an industrial point of view a comprehensive knowledge of the trends in the biochemical composition of the biomass in production scale systems is of high importance. Understanding the mechanisms that lie behind the diurnal variation of the physiological parameters and biochemical composition of the algae may shed light on potential ways of optimizing productivity and product quality.

There have been numerous studies published on the diurnal evolution of photosynthesis and photosynthetic pigment content (chlorophyll and carotenoids) of phytoplankton populations in natural water bodies, principally in marine and estuarine environments [1–6], including primary production and diel oxygen rhythm [4,7]. In some of the more recent works pulse amplitude modulated (PAM) fluorometry was used to get a deeper understanding of the photosynthetic processes [5,6]. Defined patterns were observed over the course of 24 h. Some authors proposed the diurnal change in light intensity [1–3,5] temperature, and nutrient concentration [2] as possible reasons for the periodicity.

A number of laboratory studies published focus on the diel patterns of photosynthesis, photosynthetic pigment content (chlorophyll, phycocyanin, and carotenoids), growth, and cell division cycle, including the relationship of these parameters to each other, related to individual algae species rather than phytoplankton populations [8–14]. In these laboratory experiments the algae were normally exposed to different light and temperature trends (light–dark cycles with constant light intensity in the light phase using artificial illumination, constant temperature) than those prevailing outdoors. Therefore these studies did

Abbreviations: DIC, Dainippon Ink and Chemicals; Fo, minimal fluorescence yield; Fm, maximal fluorescence yield; Fv/Fm, maximum quantum yield of photosystem II; DO, dissolved oxygen; PAM, pulse amplitude modulated; PAR, photosynthetically active radiation; PBR, photobioreactor; PC, phycocyanin; *PSII*, photosystem II; ROS, reactive oxygen species; SLL, simulation at low light

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not take into account the potential effect of the typical diurnal fluctuation of light and temperature on the studied parameters. Nevertheless a laboratory setup makes it possible to evaluate whether only external resources control the diel variation of certain metabolic processes and cell components, or internal regulatory mechanisms (circadian clock) are as well involved. Circadian rhythms are endogenous biorhythms driven by a circadian clock. They possibly arose to give an evolutionary advantage to primitive organisms by either making it possible for certain physiological processes (i.e. DNA synthesis) to take place at night to avoid the potential damages caused by strong solar radiation reaching the early Earth, or by enabling to separate incompatible reactions in time (i.e. nitrogen fixation and photosynthesis). Circadian rhythms are characterized by: having a free-running period of approximately 24 h in the absence of external time cues, being entrainable (especially by light-dark and temperature cycles), and exhibiting temperature compensation. Cyanobacteria are the simplest organisms recognized to have circadian oscillators [15,16]. The usual way to examine whether the diel pattern of certain process or cell component is controlled solely by exogenous factors or endogenous factors are as well involved in the regulation is to perform studies using light and dark cycles, then examine the variations of the parameter in question at continuous light [11–13]. If the rhythm persists at continuous light with a period of about 24 h it can be said that internal mechanisms are involved in its regulation [15,16]. Ragni, M. & D'Alcala, M. R. (2007) found that external resources such as the light availability, internal regulatory mechanisms, and the cell cycle all affect certain parameters, more specifically the synthesis of photosynthetic and photoprotective pigments studied in their experiment in a diatom species [11].

Diurnal studies of indigenous phytoplankton communities in natural water bodies, and diel experiments in laboratory conditions with select algae species may give an indication on what might happen in large-scale biomass cultures, nevertheless they possibly cannot fully describe the trends and the extent of the variation in the composition and physiological parameters of the cultivated alga. There are many interdependent biotic and abiotic factors (evolution of the light availability, temperature, nutrient availability, dissolved oxygen concentration, presence of other organisms etc.) influencing the behavior of the alga in commercial scale cultures that may be hardly reproducible in laboratory conditions, and the effect of which may be difficult to infer from studies of phytoplankton populations in their natural environment.

There were several experiments carried out with *Spirulina platensis* in small-scale raceway ponds (the work performed by Richmond, A. & Vonshak, A. (1978) [17] being the first one) studying the diel evolution, especially during the light hours, of physiological processes such as photoinhibition, growth (estimated by oxygen production rate) and of parameters such as the temperature and dissolved oxygen concentration [17–22], however the extent of these variations may not translate to large-scale systems. In commercial scale raceway ponds the mixing conditions might be different than in small scale systems, affecting the morphology, dissolved gas concentrations, light and nutrient availability to the algae, and therefore potentially the biomass composition. In large ponds there is usually insufficient turbulence attained by paddlewheel mixing, especially in *Arthrospira platensis* cultures where too vigorous mixing would prevent to maintain an optimal trichome shape and might lead to filament breakage [23]. The heat transfer is also different in small-scale and large-scale ponds.

The present studies were carried out with *Arthrospira (Spirulina) platensis* in a large 5000 m^2 area experimental outdoor raceway pond. *Arthrospira platensis* is a multicellular filamentous planktonic cyanobacterium. It has a long history of human use, and has gained a lot of attention due to its potential health benefits including antioxidant/anti-inflammatory and immune regulation effects. It is widely used as dietary supplement, functional food, food, and animal feed, and has been grown commercially for about 40 years [24].

The importance and objective of the present diurnal studies lies in establishing a baseline for further growth and quality enhancement research in large-scale *Spirulina* cultivation, as no relevant study was found in the literature on the diel pattern of the culture parameters and biochemical composition of *Arthrospira platensis* in commercial scale biomass cultures. The experiments aimed to describe the diurnal trends at different months of the year.

Besides the large-scale experiments we also carried out a laboratory simulation, monitoring selected parameters, and compared the large-scale and the small-scale systems' behavior. Our intention was to highlight some parameters that may be important in designing simulation experiments in the future.

2. Materials and methods

2.1. Diurnal studies in a large outdoor raceway pond

The experiments were carried out at Earthrise Nutritionals LLC's Calipatria, CA location. The large-scale diurnal studies were performed in an experimental 5000 m^2 area outdoor raceway pond. In the experimental pond the initial culture depth was approximately 21 ± 1 cm, and the estimated average culture flow velocity was 29 cm/s. The strain used was *Arthrospira* (*Spirulina*) *platensis*. Modified Zarrouk's culture medium was used [25]. The initial pH was adjusted to 10.1 ± 0.1 prior to the experiments. No CO_2 was supplied for pH adjustment during the studies.

Three experiments were performed at three different months of the year: March (3/16/16–3/17/16), April (4/12/16–4/13/16), May (5/10/16–5/11/16). 10 samples were collected during each experiment from 5:00 am the starting day until 8:00 am the following day. The sampling took place every 3 h. The samples were taken from the well-mixed zone after the paddlewheel, to ensure representative sampling. Two types of samples were collected at each sampling time: 500 ml liquid sample for immediate analysis; 70–120 l filtered biomass sample to be analyzed later. Regarding the latter sample type: The filtration took place on site. A 1 mm opening size sieve was used as a first step to take out any possible debris or foreign material from the culture to be filtered. The biomass was then filtered using 20 μm opening size sieves. After this, the biomass was further concentrated in the laboratory via a vacuum filtration apparatus, and was put in the freezer at − 20 °C. The frozen biomass was freeze-dried by a Virtis Benchtop 6 K ES freeze-dryer. Within 24 h after having taken the samples off the freeze-dryer each dry sample was separated in small bags (one bag for each analysis to be performed), and the small bags were individually vacuum-sealed in DIC oxygen barrier bags (i.e. bags resistant to oxygen diffusion). The vacuum-sealed bags were opened right before the analysis. The purpose of this process was to maintain the integrity of the sample during the storage time, and minimize any biomass quality degradation by oxidation.

The following parameters were measured from the 500 ml liquid samples: ash-free dry weight, optical density at 540 nm, chlorophyll fluorescence and pH. The measurements were done in triplicates except for the pH.

The ash-free dry weight was measured by collecting a determined volume of culture on a pre-rinsed filter, drying it to a constant weight at 105 °C ± 5 °C, and incinerating it in a muffle furnace for 1 h at 550 °C ± 10 °C. The filter + dish weight was measured at each step (empty filter + dish, filter + dish after oven-drying, filter + dish after incineration), and the ash-free dry weight was calculated based on the measured weights according to the following simplified formula: $[mg/l] = \frac{A-B}{V} \times 1000$. Where A = filter + dish weight after oven-drying [mg], B = filter + dish weight after incineration [mg], V = sample volume filtered [ml].

The optical density was determined spectroscopically at 540 nm, using deionized water as a blank.

The chlorophyll fluorescence was measured by pulse amplitude modulated (PAM) fluorometry using a Walz MINI-PAM photosynthesis