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## Toward production of microalgae in photobioreactors under temperate climate

Agnieszka Saeid\*, Katarzyna Chojnacka

Institute of Inorganic Technology, Mineral Fertilizers, Wrocław University of Technology, ul. Smoluchowskiego 25, 50-372, Wrocław, Poland

### ABSTRACT

The issue of presented paper is the cultivation of microalgae in temperate climate where the problems are: too low temperature and short vegetation season. The solution is to increase the efficiency of utilization of nutrients under unfavorable climate conditions. The cultivation could be performed in the closed systems, with the application of better source of light to lengthen the growing season and greenhouse to keep high temperature even during the night. The aim of the work was to choose the best method for growth, as well as harvesting the biomass to obtain microalgae that can be used in the human diet, animal feed or for industrial applications. The description of available photobioreactors was provided. Different aspects of photobioreactors engineering were discussed: construction, mixing, separation, as well as the overall technology of microalgal biomass cultivation. Two novel photobioreactors were also proposed: column in large-laboratory scale with the capacity of 0.5 m<sup>3</sup> and stirred tank in semi-technical scale with the capacity of 10 m<sup>3</sup> located under the glasshouse. The strain of *Spirulina maxima* was cultivated in the new photobioreactors, because of the simplicity of separation as well as the possibility to maintain the monoculture conditions. A higher growth rate was obtained in pilot-scale reactor (0.430 1/day) at a rate comparing to a column reactor (0.299 1/day), although the ratio A/V of 10 m<sup>3</sup> reactor was 22 times smaller than the capacity of the reactor with the capacity 0.5 m<sup>3</sup>. After 45 days of culture in the column photobioreactor, about 0.3 kg of dry mass, and in the reactor with the capacity 10 m<sup>3</sup> about 3 kg dry mass, was obtained.

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**Keywords:** Photobioreactors; Cultivation; Microalgae; *Spirulina*; Column reactor; Stirred tank reactor

### 1. Introduction

Mass production of microalgae started over 50 years ago. The biomass was found to be rich in protein and was expected to fill so called 'protein gap' related with the expansion of world population (Spolaore et al., 2006; Demirbas, 2009; Lee, 2001; Marquez-Rocha et al., 1995). In the 70s during the energy crisis in the U.S., the possibility of using microalgae capable of producing biofuels as a source of energy was considered (Li et al., 2008; Feinberg, 1984; Miao and Wu, 2006; Minowa et al., 1995; Peng et al., 2001; Yanqun, 2008; Sforza et al., 2012; Demirbas and Demirbas, 2011). Many other useful applications of valuable biomass of microalgae have also been developed, (Christenson and Sims, 2011; Rispoli et al., 2011). Commercial cultivation of microalgae on a large scale began in the early

60s in Japan and it was *Chlorella* culture. Shortly afterwards, in the 70s, *Arthrospira* (*Spirulina*) cultivation began in Lake Texcoco in Mexico (Spolaore et al., 2006; Borowitzka, 1999). In 1980 there were already 46 production plants, producing monthly more than 1000 kg of biomass (mainly *Chlorella*) (Spolaore et al., 2006). Within about 30 years there has been considerable development of production of microalgae (Borowitzka, 1999; Becker, 2007), mainly *Chlorella* and *Spirulina* as a healthy food source, *Dunaliella salina* (rich in  $\beta$ -carotene) and *Haematococcus pluvialis* (astaxanthin source) (Borowitzka, 1999). Currently, the annual biomass production is estimated as 5000 tons dry mass, which gives a value of US\$ 500 million.

Production of microalgae became so popular that it has already started selling ready-made concentrated medium for *Spirulina* sp. The concentrated medium, costs US\$ 12 L<sup>-1</sup> and

\* Corresponding author. Tel.: +48 71 3203902; fax: +48 71 3280475.

E-mail address: [agnieszka.saeid@pwr.wroc.pl](mailto:agnieszka.saeid@pwr.wroc.pl) (A. Saeid).

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is suitable to prepare 5 m<sup>3</sup> of the solution. In the literature comparison of the productivity of different microalgal culture systems can be found (Chisti, 2007; Feinberg, 1984; Gouveia and Oliveira, 2009; Illman et al., 2000; Li et al., 2008a; Miao and Wu, 2006; Minowa et al., 1995; Peng et al., 2001; Renaud et al., 1999; Spolaore et al., 2006; Yanqun, 2008). The highest productivity was obtained for *Chlorella* and *Spirulina*: 7.70 g/L/day and 130 g/m<sup>2</sup>/day for *Chlorella* and 4.3 g/m<sup>2</sup>/day and 51 g/L/day for *Spirulina*, respectively.

Despite the increased productivity in the laboratory conditions for *Chlorella*, commercially available biomass (average price per kg – data direct from producers) as powder is twice as expensive in comparison with *Spirulina* biomass. The reason of the difference in the prices, is probably more expensive manufacturing process. Therefore, a further part of this chapter is devoted to present data from mass cultivation of microalgae, in order to compare and choose the best.

## 2. The role of light in the microalgae cultivation

The role of light intensity as photosynthetic active radiation (photon/m<sup>2</sup> s), gas flow rates and maximum cell concentration in terms of growth rate are important operating parameters that need to be covered in the design of photobioreactors. Photosynthetic and growth rates are related to environmental variables light as well as temperature and inorganic carbon availability.

Dependent on the light supply, the driving force of photosynthesis, various volume elements of the photobioreactor can be grouped into productive light zones with sufficient and unproductive dark zones with insufficient light intensities for photosynthetic metabolism. The light penetration depth which is decisive for defining light and dark zones depends both on the culture density of the light absorbing microorganism and the geometry of the reactor (Kwon et al., 2012).

Photosynthetically active radiation (PAR) is the amount of light available for photosynthesis, which is light in the 400–700 nanometer wavelength range. PAR changes seasonally and varies depending on the latitude and time of day.

Growth rate is small, when light levels are too low. Growth phase limited by light is observed when the intensity of light is low (so-called photo-limitation). If it is too high, photoinhibition occurs. Between these two values – here is an area in which photosynthesis is not enhanced, the specific growth rate does not depend on light intensity and it is called light saturated region (Andrade and Costa, 2007; Sancho et al., 1999). Studies have shown that for *Spirulina* with increasing light intensity from 8 to 30 W/m<sup>2</sup>, linear increase in specific growth rate was observed ( $\mu$ , h<sup>-1</sup>). However, in the range of 30–50 W/m<sup>2</sup> in the specific growth rate of autotrophic culture ( $\mu$ , h<sup>-1</sup>) reaches a plateau. At higher light intensities (>50 W/m<sup>2</sup>) photoinhibition occurs in culture (Vonshak, 1997; Watanabe and Hall, 1995; Carvalho et al., 2011).

Photoinhibition is observed when photon flux absorbed by chloroplasts is too large to be endowed with high energy electrons which can be used in the Calvin cycle. These electrons react with water to form hydrogen peroxide, which destroys the structure of the intracellular compartments and the cells themselves (Vonshak, 1997; Watanabe and Hall, 1995).

## 3. Industrial production of microalgae

Fig. 1 shows the geographic global distribution of producers of *Spirulina* biomass. Considering the biomass production of *Spirulina*, United States is a major producer. Cultivation of *Spirulina* was also developed in Europe (France, Spain, Italy), but it represents a small fraction of the world production (Tomaselli, 2000). Table 5 presents the main producers of microalgae in the world. The largest *Spirulina* plant in the world is Earthrise (Earthrise Nutritional, LLC Headquarters, 2151 Michelson Drive, Suite 258, Irvine, CA 92612 USA; <http://www.earthrise.com>) farms on a 440,000 m<sup>2</sup> site in the Californian desert near Calipatria City (California, USA), with 30 cultivation ponds of 5000 m<sup>2</sup> (total 150,000 m<sup>2</sup>) each of which uses filtered mineral-rich Colorado River water supplemented with salts (Spolaore et al., 2006). Shimamatsu (2004) reports that the total industrial production of *Spirulina* is about 3000 tones a year. It was reported that production in China of 19,080 tones in 2003 rising sharply to 41,570 in 2004, worth around US\$ 7.6 million and US\$ 16.6 million, respectively (Ahsan et al., 2008).

The production of *Spirulina* biomass by Grand Basin Company, the Sevas in India consists of initial pools, where the inoculum is prepared. The culture is heated by solar cells, which are also a source of energy. The installation is equipped with a filtration system, sterilization and drying. One kg of *Spirulina* powder obtained from such an installation costs US\$ 50.

## 4. Reactors

Several techniques for microalgae culture were proposed by researchers and commercial producers. Several reviews list the technological solutions (Chaumont, 1993; Koller et al., 2012; Ugwu et al., 2008; Wang et al., 2012; Chen et al., 2011; Kumar et al., 2011; Eriksen, 2008; Grobbelaar, 2010). These methods are used until present (Olaizola, 2003): pools/tanks, large open ponds, round ponds with movable arm, raceway-type ponds (tracks), cascade systems with baffles, large bags, fermenters (for heterotrophic and mixotrophic cultures), two-stage systems (cultivation in the reactor in an internal system, the system of outside pond with paddle wheel, which enforces growth medium movement and simultaneously aerates the culture).

An important parameter of the design of reactors for growth of photosynthetic organisms is the ratio of the illuminated surface to volume of culture solution. By appropriate selection of the volume (V) and irradiated surface (A) ratio, it is possible to reduce undesirable effects of self-shading or limiting access to light by the cell to other cells. The high value of the ratio A/V is the desired parameter of photobioreactors (Becker, 2007; Watanabe and Hall, 1995).

Internal (closed) cultures allow the cultivation in the controlled conditions of light, temperature, concentration of nutrients. Cultivation in external systems (open), creates problems with maintaining the purity of strain, due to the higher probability (compared to closed systems) of infection of the culture. Therefore, commercial cultures limit the use of open fish ponds in cultivation of only those strains of microalgae, where growing conditions are so unusual that it reduces the risk of infection. The use of open ponds is limited by the large area required and the difficulty of maintaining sterility. Another problem of this solution is the impact of climatic conditions. Other less frequently used systems for microalgae cultivation in internal systems, provides the opportunities to

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