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Propagation of Inoculum for *Haematococcus pluvialis* Microalgae Scale-Up Photobioreactor Cultivation System

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Abstract: An increasing number of microalgae strains are used for commercial production of metabolites. When conducting research, the moment of the process scaling tends to be very difficult. One of the most complex issues is related to planning and designing an efficient system for propagation of appropriately high amounts of inoculum required for inoculating cultures on a semi-technical and industrial scale. The following paper aimed at designing an automated station for the preparation of microalgae inoculation material intended for inoculation of the system, comprising of six 90 dm^3 volume photobioreactors. The system, comprised of eight airlift photobioreactors of 12 dm^3 volume each, installed in mobile storage units connected to the control system in the form of a docking station. Each of the photobioreactors had a separate system used for monitoring temperature and pH, mixing, and LED lighting. The station constituted the last stage of preparing the inoculation material for inoculating technical-scale photobioreactors, used for conducting experiments with *Haematococcus pluvialis* microalgae. Achieved results, repeatability of the processes, and the ergonomics of the station increased the productivity and quality of the research and development processes.

Keywords: microalgae; inoculation; scaling-up; bioprocess control; photobioreactor

1. Introduction

Microalgae are microscopic unicellular organisms that can convert solar energy to chemical energy through photosynthesis [1]. Despite the small size, they are producers of 60% of the Earth's oxygen. To this date, 30,000 species of microalgae have been identified [2]. Few hundred species are being analyzed to determine their chemical composition and metabolite production abilities. Only a few strains are cultivated on an industrial scale to be used in the production of commercial products [3]. This group includes, among others: *Spirulina*, *Chlorella*, *Haematococcus*, *Dunaliella*, *Botryococcus*, *Phaeodactylum*, *Porphyridium*, *Chaetoceros*, *Crypthecodinium*, *Isochrysis*, *Nannochloris*, *Nitzschia*, *Schizochytrium*, *Tetraselmis*, and *Skeletonema* [4].

Microalgae are very resistant to environmental conditions. Due to their ability to utilize solar energy more efficiently, microalgae have the higher photosynthetic potential for producing valuable compounds or for energetic use when compared with higher plants [1]. In optimal cultivation conditions, they produce protein and biomass from 30 to 100 times faster than terrestrial plants [2]. Microalgae can be used to produce a wide range of metabolites, such as protein, lipids, carbohydrates, carotenoids, or vitamins [1]. These microorganisms are widely known as a valuable and renewable raw material used by various economy sectors, especially by food, feed, pharmaceutical and cosmetic industry. They are also used to produce bio-fertilizers and biofuels as well as for removing pollutants from wastewater [5,6]. One could say that microalgae are natural bioreactors that enable the production

of various metabolites, which are considered to be highly valuable products. Microalgae cultivations are an interesting alternative to the conventional synthesis of the selected chemicals [2]. Unlike most vascular plants, microalgae cultivation does not require soil, and the cultivation installations take up limited space [7]. Cultivation ponds and tanks may be used in the desert, on rocky terrain and idle agricultural land. Production of microalgae will not deplete supplies of drinking water as it may take place in seawater, brackish water, or wastewater [8].

Microalgae, used for commercial purposes, are currently being produced in two main types of cultivation systems: open and closed. The open system, which utilizes both natural and artificial ponds (raceway ponds), is the easiest microalgae cultivation method [9]. The artificial ponds are similar in structure to small ponds or shallow channels. Paddle wheels, among other measures, are used to force circulation of the growth medium [10]. In the open ponds, microalgae absorb all sunlight necessary for their growth. There is no need to use artificial lighting [11]. The essential advantage of open ponds is the fact that they are easy to build, and they enable to cultivate microalgae in their natural environment. The disadvantage of these types of tanks is the high possibility of contamination [2].

The second method of cultivating microalgae is based on using closed systems known as photobioreactors (PBR). They are specially designed transparent tanks in various sizes and shapes, intended for cultivating microalgae in the optimal conditions, automatically controlled by means of process monitoring equipment [11]. Photobioreactors solve the problem of evaporation and susceptibility to contamination, which are the biggest problems in the case of the open ponds systems [9]. Transparent walls of PBRs allow for the light to reach the inside of the photobioreactor. In comparison to the open ponds, photobioreactors allow for obtaining higher productivity of biomass as well as easier, and as a result, cheaper harvesting [2]. Unfortunately, the costs of cultivating microalgae in closed systems are high. For that reason, these systems are applied commercially mainly to produce high-value compounds [12].

Scaling-up the process to the commercial level is a complex task since it is difficult to assess which factors will have a deciding impact on the scaling-up process. As a result, most large-scale cultivations systems provide a lower yield than is expected, considering the results obtained on a laboratory scale [8]. One of the fundamental problems that needs to be solved is the problem of optimizing the process of producing a sufficient amount of inoculum for large volume open ponds or photobioreactors. The two most relevant criteria, rarely analyzed in a laboratory scale, are minimizing the required time and costs. Coming up with suitable methods and procedures is necessary for the processes conducted on a large-scale. It is closely related to cultivated microorganisms, used cultivation systems, and the location of the production plant. The efficiency of such systems and their reliability gets better with time as the system operators, who manage the cultures of cultivated microorganisms, gather knowledge, and gain experience. Commercial producers are not eager to disclose the details of industrial solutions [13].

Improvement in efficiency while scaling-up bioprocesses can be achieved by manipulating the key process parameters [8]. In the case of microalgae cultivation, the parameters that influence the biomass growth and secondary metabolites accumulation are time profile of light intensity, coupled with culture agitation time-profile, aeration rate, availability of nitrogen, and carbon dioxide source, and temperature. It is also essential to progressively adapt the abovementioned process parameters, along with the culture growth rate and its physiological state [14]. At the stage of research and development, the experiments should be conducted long enough to reveal any problems related to the aging of the system. Such issues, for example, contamination, biofouling, and degradation of material are noticeable only after a minimum of a few months of continuous work [8].

It is recommended to gradually propagate the culture of microorganisms when cultivating microalgae on a larger scale. Generally, in microalgae cultivations at the point of moving to the following stages of scaling-up, it is advised to use inoculation levels of 1% to 10% of the volume of tanks used for the next stage of cultivation. For this reason, it is necessary to scale-up cultivation in stages from a lower volume of the initial production to the final stage that is commercial-scale

production [15]. At every stage of scaling-up, it is necessary to check the immunity and viability of cells of a particular microalgae strain. The density of cells is another important parameter that should be higher than 10-million cells per mL to avoid the long lag phase after inoculation [8]. Many stages and the extended time required to carry out such process increase costs, but also the risk of contamination [13]. When conducting experiments, or producing microalgae, a pure culture of microorganisms should be cultivated in consistent and short time intervals (every week or every fortnight) in liquid culture to be able to quickly provide "healthy" inoculum ready for immediate use. In the case of a medium storage period (6 months), it is recommended to cultivate cultures on solid-state medium, preferably under relatively low light (PAR < 25 μmol m^2s^{-1}). Long-term storage of strains should be conducted by freezing the strains using cryoprotectants [15].

Conducting scientific experiments is very often closely related to the possibility of using the results in the economic environment, in large scale industrial production. Sooner or later, scientists come across issues related to moving experiments from laboratory-scale to semi-technical and industrial scale. When conducting research on a laboratory-scale, one can buy suitable devices from companies that produce them commercially. However, when it comes to larger-scale experiments, there is a necessity for either ordering appropriate prototypical solutions suited to the individual preferences and needs of the ordering party or for preparing suitable experimentation systems individually.

The aim of this paper was to design, produce, and program an automated station for the preparation of inoculation material intended for inoculation of the system comprising of six 90 dm^3 volume photobioreactors used for conducting experiments and producing microalgae biomass.

2. Materials and Methods

The *Haematococcus pluvialis* G1002 microalgae strain was used to conduct the experiment. This strain comes from the CAUP culture collection (Charles University in Prague, Czech Republic). The tests were conducted using the Bold's Basal Medium (BBM), which was sterilized at 121°C, for 15 min, in the Tuttnauer 5075 ELV-D WR laboratory autoclave (Breda, Netherlands). The microalgae cultivation was conducted over 8 days in the laboratory room, in a temperature range of 23–25°C. The pH value was maintained automatically at 7.00 (–) and with hysteresis at the level of 0.2 (–). It was regulated by introducing carbon dioxide into the photobioreactors. Over the course of the experiments, a manual setting of the intensity of the LED lighting was used at the level of 54% of power throughout the course of cultivation.

Building the workstation required using eight glass air-lift bioreactors with a total volume of 12 dm^3 each, which were designed by AlgaeLabs (Wroclaw, Poland). The photobioreactors were built out of two glass tubes. The walls of the tubes were 5 mm thick (Schott). An internal tube (riser), which is 125 mm in diameter and 400 mm in length, was placed inside an external pipe (downcomer), which is 190 mm in diameter and 500 mm in length. Thus, obtained photobioreactors have a total volume of 11.97 dm^3. The photobioreactors were covered with a top dome, which included necessary ports for connecting the temperature, and pH sensors. The bottom dome was fitted with an enclosed diffuser that allowed for the effective atomization of the introduced gases and the nozzle responsible for filling and emptying the photobioreactor. The photobioreactors were sterilized using a 4% solution of perhydrol. The photobioreactors were filled with perhydrol for a period of two hours. Subsequently, they were rinsed with deionized water. Bioreactors, which were prepared in such manner were filled with culture medium and the microalgae inoculant and supplemented with deionized water to obtain 10 dm^3 of working volume.

Photobioreactors temperature measurements were conducted using thermo-resistant sensors STPt 100 (Hydromet, Gliwice, Poland) in a borosilicate glass vessel, which is resistant to aggressive solutions such as concentrated alkalis, concentrated and diluted acids (including oxidizing acids) as well as organic-water mixtures. The sensors were capable of measuring the temperature within the 0 and 100 °C range. The sensors cooperated with temperature transmitters with the RS485 interface and Modbus RTU (AlgaeLabs, Wroclaw, Poland) communication protocol. Composite electrode ERH-13X2

(Hydromet, Gliwice, Poland) in a glass case was used to measure pH. This electrode was intended for measuring the pH in water or water solutions in industrial applications. The pH sensors cooperated with pH measurement transducer with the RS485 interface, and Modbus RTU (AlgaeLabs, Wroclaw, Poland) communication protocol.

The lighting of the photobioreactors was designed using EMPA-d (epiLED, Wroclaw, Poland) type strips with electroluminescent LED diodes, powered by 12 V DC, color temperature- warm white 2800~3200 K, with a 120° beam angle and light output of 9600 lumens. Power consumption of 96W/5 m of the LED strip with 120 diodes per 1 m of LED strip, type 2835 (REFOND Optoelectronics, Shenzhen, China) were located on 1 m of LED strip. Four light panels with 0.5 m long LED strips were prepared for each station. Diodes and strips drivers LED RGB SPL-3C (Dagon, Leszno, Poland) were used to control the LED lighting. The source of the voltage signals 0–10 V was an 8-channel analog output module SDM-8AO (SFAR) with serial interface RS-485 and Modbus RTU communication protocol.

Electrically controlled solenoid valves 322ME (AZ Pneumatica, Misinto, Italy) type 3/2 NC with 24 V DC coils were used to control the gas flow. Circumference of the connection G$\frac{1}{4}$", flow rate (6 bar, p 1 bar), operating pressure 1–10 bar, maximum temperature 60 °C. The device which was responsible for controlling the work of the electromagnetic valves was a 16-relay output module SDM-16RO (SFAR) with a 3 A maximum current relay output, serial interface RS485 and Modbus RTU communication protocol. Eight relays were used to power the coils of the electrovalves. The flow rate of dosed air and carbon dioxide was maintained at a constant of 0.5 dm^3 gas per minute.

A PLC device, with an integrated control panel -series XLe (Horner APG, Indianapolis, United States) was used to control the station for the propagation of microalgae inoculum. This device has a 2.2-inch diagonal monochromatic screen, resolution: 128 × 64 pixels, 10 fully programmable function keys, and an alphanumeric keyboard. This allowed displaying basic information about the controlled process parameters and provided access to the parameter settings for all photobioreactors. The XLe driver has two serial communication ports RS232/485. One of the ports was used to maintain cooperation with the station's measuring and control devices.

The work control system of the inoculation station was designed in a way that enabled the station to work continuously. The software allowed for starting the process in consecutive photobioreactors at any time and ending it on other stations without disrupting the work of the entire system. The PLC device, which has two serial ports RS232/485, was used as a master device. All of the measurement and control devices were attached to the PLC driver using an industrial serial communication interface RS-485 (Figure 1). The interface is resistant to interference and allows, with the help of a two-wire bus cable, to connect with 32 cooperating devices. All connected devices were communicating using the serial transmission protocol Modbus RTU.

Measurements of the temperature and pH value, separately in each of the photobioreactors, were carried out by means of sensors connected to the measurement transducers with digital to analog converter and RS-485 communication module.

The PLC device software was developed in the Cscape 9 environment (Horner APG, Indianapolis, United States), which allows for performing hardware configuration of the driver, saving the control algorithms, and designing the control screen of the user's interface.

The PLC device has a built-in monochromatic screen, programmable function keys, and numeric keyboard. These features enable to create a user interface which can be used by the station operator to carry out all the activities connected with controlling the process of cultivating the *Haematococcus pluvialis* microalgae (Figure 2). Information was displayed using 19 different screens: start screen, 8 screens responsible for monitoring the process in consecutive photobioreactors, 8 screens which allow to carry out calibration of the pH systems, the screen which enables to change hysteresis of pH regulation, and the screen which displays a hint related to the two-point method of calibrating pH sensors.

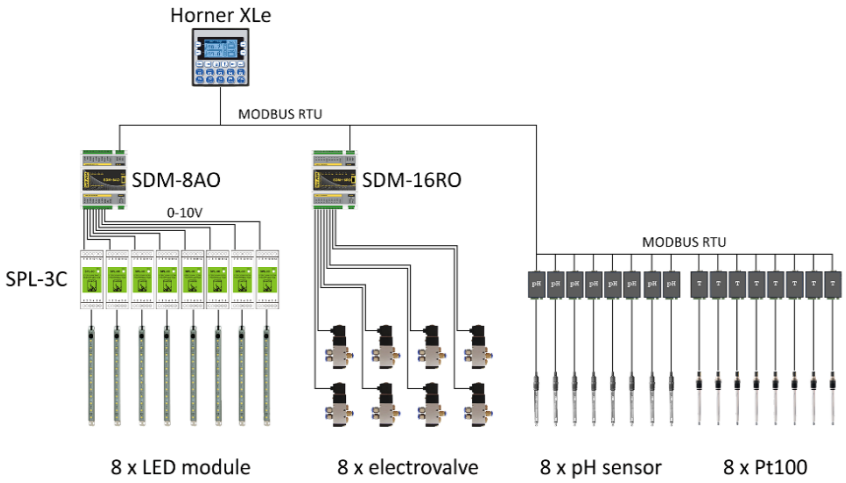

Figure 1. Diagram of the measurement and control unit.

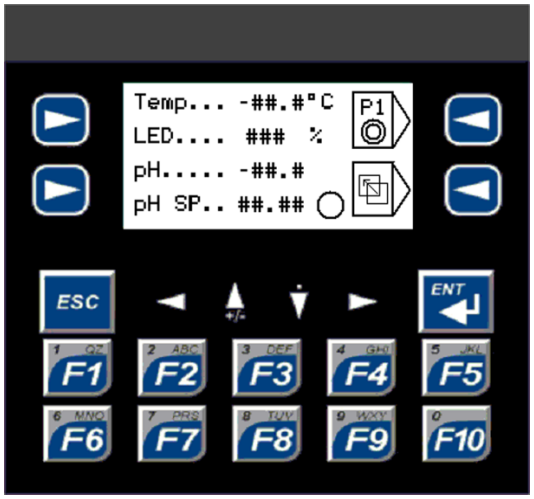

Figure 2. XLe programmable logic controller user interface.

Function keys from F1 to F8 were used to switch between screens responsible for displaying and entering information related to eight stations for the propagation of inoculum. The F9 function key permitted to switch to the screen, which enabled to set hysteresis of pH regulation, and the F10 key was used to go back to the start screen. After choosing the right photobioreactor station number, the operator received information (displayed on the screen) regarding the current temperature measurement (Temp ... -##.# °C), the light intensity of the LED diodes at this particular workstation (LED .... ### %), current pH value (pH.. .... -##.#) and set pH value (pH SP.. ##.##).

At the level of the process control screen, system the operator has the option to run the process at a given station (top right arrow P1) and bring up the screen used for calibration of the pH sensor from the particular photobioreactor (bottom right arrow). An algorithm of two-point calibration of pH electrode was used.

The on-off pH control algorithm was also saved in the software. The results of the pH measurements, conducted in photobioreactors, were compared with set-points set by the operator. It was also possible to set regulation hysteresis. As a result of exceeding the set-point, the PLC driver sent an appropriate control command to the electromagnetic valve responsible for introducing air and carbon dioxide into the photobioreactor. As a result of starting an electrovalve, the air supply was shut off and the introduction of carbon dioxide was initiated. Carbon dioxide, similarly to air, triggered the agitation of

photobioreactor's content, influenced the acidification of the culture medium (increasing pH value) and served as the source of carbon for the metabolic processes of microalgae.

Over the course of conducting the *Haematococcus pluvialis* microalgae propagation processes, one 20 cm^3 sample was collected at 24 h intervals. The sample was analyzed for the content of microalgae biomass, temperature, and pH.

The spectrophotometric method was used to measure the increase in the *Haematococcus pluvialis* microalgae biomass. Measurements were carried out using the DR5000 (Hach, Loveland, United States) spectrophotometer, at wavelength λ = 620 nm (in reference to distilled water). To determine the increase in the biomass of *Haematococcus pluvialis* microalgae, the 20 cm^3 sample was centrifuged at 13,131 g for 10 min., and then diluted with distilled water. To calculate dependence between absorption (OD620) and the dry matter content of microalgae (Cb ($g*dm^{-3}$)), the MB25 (OHAUS, Parsippany, United States) moisture balance was used to prepare a standard curve of the form of: Cb = 0.1204*OD 620 − 0.0404 (determination coefficient R^2 = 0.9923). For analysis purposes, a 5 cm^3 sample was centrifuged at 9000 g for 10 min. and subsequently diluted in 2 cm^3 of distilled water.

Over the course of cultivation, the temperature and pH were monitored using sensors placed inside the photobioreactors as well as in the samples (taken for testing) utilizing laboratory pH-meter CP-505 (Elmetron, Zabrze, Poland).

3. Results

This paper presents the system for the propagation of inoculum designed to conduct research and produce microalgae biomass. The system is composed of six 90 dm^3 photobioreactors, which together create an experimentation system with a combined volume of over 0.5 m^3. Initially, the preparation of inoculum on such scale was executed using traditional, commercial solutions used earlier over the course of experiments conducted on a laboratory scale (Figure 3). The propagation of microalgae was conducted using laboratory bottles of 5 dm^3 volume. A magnetic stirrer was used to agitate the contents of the vessels. The vessels were illuminated using typical fluorescent lamps. Carbon dioxide was dosed manually by opening the valve (by hand) and leaving it open for a set period. No measuring sensors were installed in the bottles.

Figure 3. Inoculum propagation station designed using typical laboratory equipment.

This solution had many disadvantages. Bottles were placed on a laboratory countertop to provide laboratory personnel access to all elements of the system. As a result, the entire system took up a large area of the laboratory. The use of mechanical agitation and manual dosing of carbon dioxide was

reflected in the low productivity of such cultivations. Additionally, there was an infection problem. The content of the two five-liter bottles was used to inoculate a single photobioreactor of 90 dm^3 volume.

Due to the intensification of the experiments, it became necessary to build a prototype of a station for propagating the inoculum intended for six main 90 dm^3 airlift photobioreactors. The photobioreactors were used to conduct experiments using *Haematococcus pluvialis* microalgae. The experiments lasted for approximately 4 weeks (Figure 4).

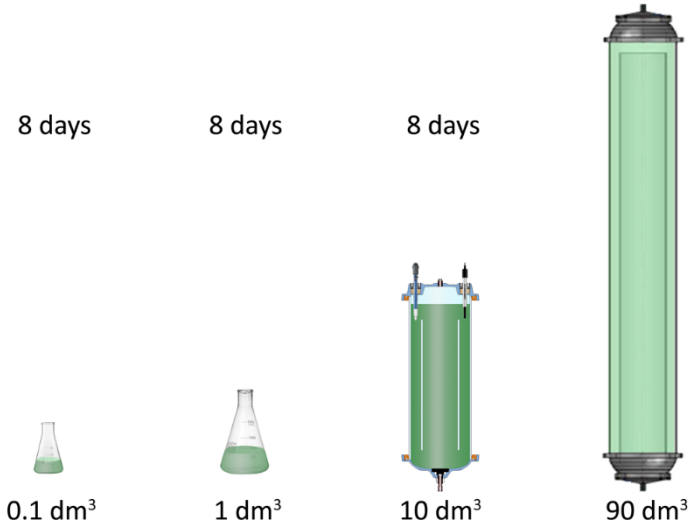

Figure 4. System for preparing inoculum for photobioreactors of 90 dm^3 volume.

The system used scaling-up of cultivation by a factor of 10 per step with eight days allocated for each stage [13]. As a result, it was possible to prepare a suitable amount of inoculum to start experiments in all six main photobioreactors (volume of 90 dm^3) within 24 days. One photobioreactor from the inoculation station was intended to be used to inoculate one 90 dm^3 photobioreactor. Using eight photobioreactors, in the propagation station, in comparison to using six main photobioreactors, allowed for the experiments to proceed smoothly and to gain a safety buffer in case of an accident, e.g., contamination of cultivation, a necessity to repeat the experiments or anomalies in the propagation process.

Eight air-lift photobioreactors constituted the primary part of the inoculum propagation station. The weight of one full photobioreactor with top and bottom domes (used for closing the photobioreactor) and the remaining equipment amounted to about 25 kg. The photobioreactors were installed in specially designed storage units fitted with wheels to facilitate the work of laboratory staff operating the station. Each storage unit was big enough for 2 photobioreactors (Figure 5).

The control board was in the form of a docking station. Storage units with photobioreactors were rolled next to the control board, where they were attached to the temperature and pH sensor cables, power cable for the LED lighting, and pneumatic cables used for dosing gases. Such a solution allowed saving laboratory space. A top dome was fitted with a connector, which made it easier to use the photobioreactor. The connector was used for filling the photobioreactor. The bottom dome was fitted with a connector that allowed for gas to be introduced. The connector has also allowed pumping inoculum to the inoculated photobioreactor. Control board and storage units with photobioreactors were made based on the modular system of MB aluminum profiles (ITEM, Solingen, Germany).

Figure 5. Automatic inoculum propagation station used to cultivate the *Haematococcus pluvialis* microalgae.

The limitation of the developed system for propagation of inoculum is the lack of a separate temperature regulation system for each of the eight photobioreactors. Being able to maintain the temperature of the process is largely dependent on the temperature in the laboratory room in which the system was installed. This may lead to problems with maintaining the stability of temperature in the photobioreactors, especially in the summer months when the temperature outside stays high for a prolonged period, or as a result of possible issues related to the efficiency of the air-conditioning system in the laboratory.

Effectiveness, repeatability of results, lack of contamination, and the ergonomics of the station, were all confirmed in the everyday laboratory work, over the course of multiple processes of preparing the inoculation material for the conducted experiments. All processes of inoculating the *Haematococcus pluvialis* microalgae, in which temperature, pH, and the concentration of the biomass were observed, proceeded similarly. Figures 6–9 present the results collected during one of the experiments, conducted in six photobioreactors (PBR 1 do PBR 6). Other than the on-line measurements of temperature and pH in one-minute intervals, additionally, temperature, pH, and the microalgae biomass concentration were measured in 24 h intervals over the course of the process. Figure 6 presents temperature changes in all six photobioreactors in eight days (Figure 6).

While monitoring the temperature changes occurring in the photobioreactors, it was noted that the temperature in photobioreactors was increasing systematically over the course of the process, from the level of 20.9 °C in PBR 5 to the maximum value of 28.5 °C, observed in PBR 3.

The results of the pH value measurements in the samples taken from photobioreactors over the course of cultivation were presented in Figure 7.

Similarly to the temperature measurements, a systematic increase in pH value was noted in the consecutive days of conducting cultivation. The pH increased from the level of 5.78 (–) in PBR 4 to the maximum level of 6.92 (–) in PBR 1.

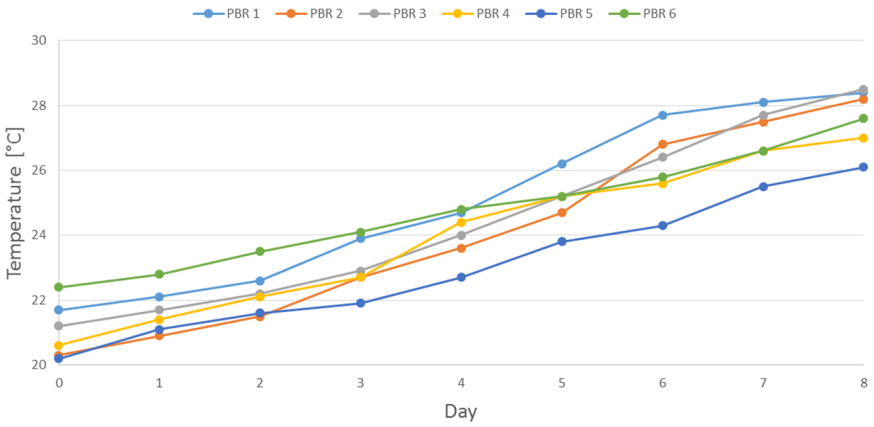

Figure 6. Changes in temperature over the course of the eight-day microalgae cultivation processes.

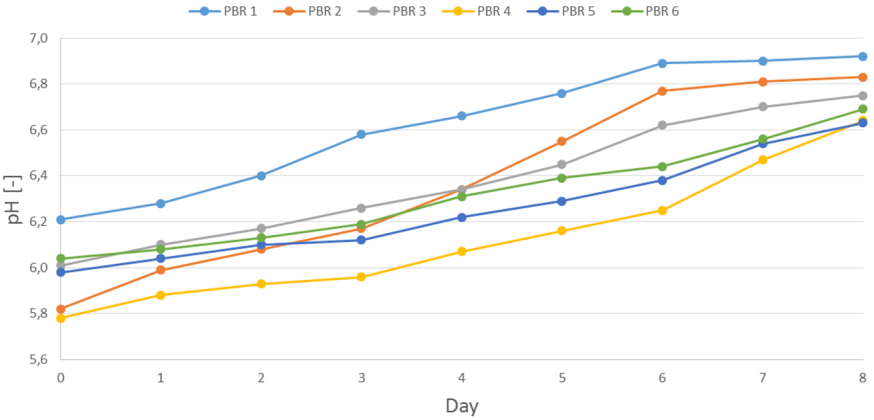

Figure 7. Changes in the pH value over the course of the eight-day microalgae cultivation processes.

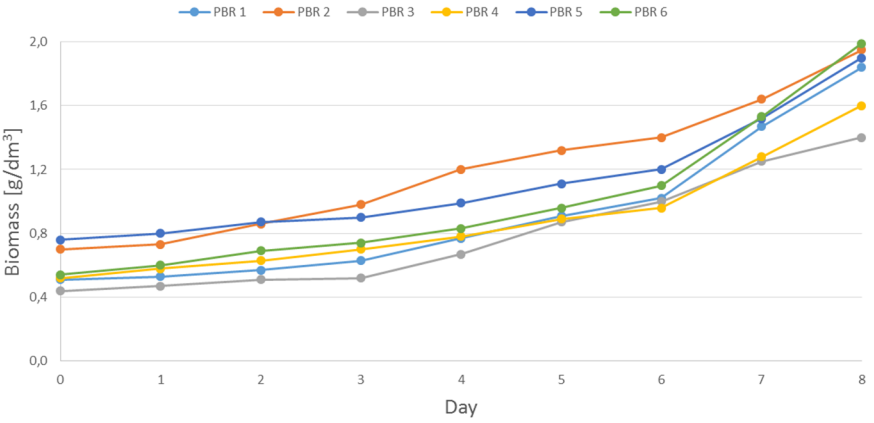

Figure 8. Changes in the biomass content over the course of the eight-day microalgae cultivation processes.

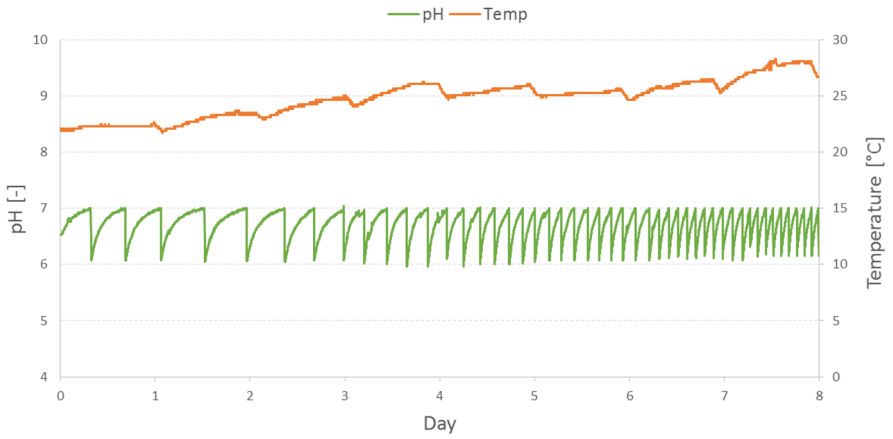

Figure 9. Changes in the temperature and pH value over the course of the eight-day microalgae cultivation processes.

Satisfying effects were achieved over the course of the experiments carried out in the developed station, regarding the productivity of the cultivation (Figure 8).

The initial biomass content after the inoculation of the photobioreactors was fluctuating between 0.47 g/dm^3 in PBR 3 and 0.76 g/dm^3 in PBR 5. The content of biomass after eight days ranged between 1.40 g/dm^3 for PBR 3 and 1.99 g/dm^3 for PBR 6.

Temperature and pH were also automatically monitored over the course of experiments. The results from the PBR 3 were presented in Figure 9.

Figure 9 presents, aside from the upward tendency, the characteristic, recurring temperature fluctuations associated with the 24-h temperature cycle in the laboratory. Whereas the oscillations visible on the pH figure present the way of functioning of the on-off pH control algorithm when the set point is at the level of pH 7.0.

4. Discussion

While conducting the research and development activities related to scaling-up the process of cultivating microalgae, it is necessary to properly evaluate and extrapolate the efficiency of the designed system. Over the course of conducting developmental activities, it is essential to use storage tanks of a similar type that work similarly to the photobioreactors intended for the full-scale cultivation [8]. All the photobioreactors used in our system were of the airlift type, as well as the photobioreactors used in the last stage of preparation of inoculate and the final photobioreactors in the semi-technical scale. They had a similar system for regulating pH and mixing. All experiments must be conducted in the same operating conditions in which the commercial installation will be operating. Large volume, commercial photobioreactors should not be designed. Using smaller storage tanks or modular constructions allows maintaining the necessary flexibility and introducing improvements using trial and error. In the case of large volume containers, every problem which arises (e.g., pollution) influences the entire culture and entire production batch [8]. For that reason, the described inoculate preparation comprises of smaller units. The units are entirely separate and work independently of each other. Even in cases when sporadic contamination or irregularities occurred over the course of conducting the cultivation, they were always related only to a single photobioreactor. That allowed to maintain continuity and reliability of the work of the entire system. Switching between automated and manual modes of cultivating microalgae is a very good solution. It is especially useful to have the separate parameters control opportunity in individual modules (photobioreactors), which enables to carry out the cultivation process using various parameters e.g., light intensity, agitation rate [15].

The main environmental factors ensuring that the process of photosynthesis in microalgae proceeds satisfactorily is light (sunlight or artificial light), temperature, and pH [16].

The best temperature range for the proper functioning of the *Haematococcus pluvialis* microalgae cultivation is the 20–27 °C temperature range [17]. Exceeding this range by 2–4 °C may have a significant influence on the course of enzymatic reactions in the microalgae cells (especially photosynthesis) and may lead to loss of the entire cultivation [18]. It was not planned for the station to use a temperature regulation system. However, the system used for monitoring temperature allowed for controlling it, which is very important for maintaining the highest possible productivity [19]. The results of measurements collected over the course of experiments confirmed that the work of the station in an air-conditioned room at a constant temperature of 22 °C prevents the upper-temperature threshold from being exceeded, as proposed by other authors, e.g., in [20,21]. The systematic increase of temperature over the course of the experiments (Figures 6 and 9) was caused by the heat released by the LED lighting installed inside each storage unit. The temperature did not exceed the acceptable upper threshold for the *H. pluvialis* microalgae. As a result, there was no need to use the temperature control system.

Among all of the environmental parameters, pH has the most influence on the rate of cell growth and synthesis of chlorophyll of the *Haematococcus pluvialis* microalgae [22]. It also determines the solubility and diffusion of carbon dioxide in the media, which in turn has an influence on the productivity of photosynthetic carbon bonds and has a direct or indirect impact on metabolism in microorganisms [23]. In the presented inoculate propagation station both, the mixed cultures as well as the control of pH were implemented by means of introducing gases into the photobioreactor. Compressed gases were used to agitate the content of the photobioreactors. Air and carbon dioxide were administered alternately to ensure continuity of agitation. The flow rate of dosed air and carbon dioxide was maintained at a constant of 0.5 dm^3 gas per minute. Due to that, the mixing of the medium remained steady, regardless of the introduced type of gas (air, carbon dioxide). The use of the air-lift system allowed for efficient mixing of gases with the culture medium in the internal tube (riser). Additionally, the microalgal agglomerate was broken-up by turbulent flow. After reaching the top of the photobioreactor, the medium would spill over the edge of the riser to the external tube (downcomer) and flow in a laminar manner.

The process of agitating the suspension of microalgae is a crucial aspect of cultivation as it prevents sedimentation of cells and has a positive influence on the distribution of carbon dioxide, oxygen, and nutrients contained in the culture media. Additionally, the mixing process provides required access of microalgae cultures to light and facilitates the exchange of heat [24]. Providing carbon dioxide ensures simultaneously access to the source of carbon and adjustment of the pH value of the culture medium. This method works exceptionally well in vertical photobioreactors with aeration [19].

The monitoring of pH over the course of cultivation enabled to control the amount of dosed carbon dioxide to maintain a neutral pH. The undisturbed photosynthesis process and the growth of microalgae take place when pH is maintained at the level of 7.0. Indirectly, this parameter provides information about the photosynthetic activity of the microalgae cultivation [15]. The on-off pH control algorithm was satisfactorily used over the course of the experiments. The control had a pH set point of 7.0 and 0.2 hysteresis. It is consistent with the research results [25] in which the highest value of chlorophyll was achieved at pH 7.0. Additionally, in Nagaraj et al. [23], the maximum increase in cell count and maximum concentration of chlorophyll a and b was noted at pH 7.0. A gradual increase in the pH of the medium, between 5.78 and 6.92, was observed during the experiments (Figure 7). This tendency is typical for microalgae cultivations, which limits the enzymatic activity of microalgae and, in consequence, inhibits their growth [26]. Over the course of the experiments, the volume of carbon dioxide was not measured. The control system, based on the on-off control algorithm with hysteresis, made the decisions related to dosing the carbon dioxide when the pH of the medium would rise above pH 7.0 (Figure 9). It was indicative of, among other things, of running out of carbon dioxide introduced into the photobioreactor in the previous control cycle. With this, the dosing of carbon dioxide took place as a response to the demand of microalgae and indicated indirectly by the change in pH value. A similar pH control system was used in research, where the microalgae were used to capture and recycle CO_2 [27]. While analyzing the operating frequency of the control algorithm, it can

be seen that as time went by the dosing of carbon dioxide happened more frequently. It serves as an indication of the increasing demand for carbon, sourced from the dosed carbon dioxide. This increase was a result of the natural course of microalgae growth and biomass growth, which accompanying it [27].

Light is one of the most vital factors in the cultivation of microalgae because it provides the energy required in the process of photosynthesis [28]. Energy captured by microalgae is used to bind carbon dioxide, release oxygen as a byproduct, and produce biomass. Optimizing the access to light is crucial for obtaining high efficiency of the cultivating system as well as for reducing the cost of its functioning [28]. In the presented system electroluminescent LED diodes were used to illuminate the photobioreactors. The diodes replaced fluorescent lights, which were used in 5 dm^3 laboratory bottles solution. The diodes not only have low power consumption, but they also guarantee the correct light spectrum, which corresponds with the spectra of photosynthetic absorption of microalgae. They also allow for smooth control over the intensity of illumination [29]. LED strips were mounted in four corners of each station and connected to the drivers equipped in PWM (pulse-width modulation) outputs. It enabled to proportionally control the LED illumination intensity in the range of 0–100% of power using a standard analog signal of 0–10 V. Thanks to that system, the operator was allowed to run the LED lighting using the power of his choosing.

The described system for propagation of inoculum was supposed to be characterized by the lowest level of complexity. Thus, only two basic types of sensors were used along with the option to dose air and carbon dioxide, and the least complicated lighting system, which uses LED diodes of one type, and color. Admittedly, the use of blue light has a positive influence on increasing the size of cells and red light improves cell division rate. However, in this case, a decision has been made to use warm white light in the inoculation module because other wavelengths of the light are needed for the correct cell metabolism and maintaining high cell activity [29]. We chose the warm white LED diodes because the wavelength of light that they generate is the closest to the natural sunlight with the predominance of red color. This compromise solution supports the intensification of the inoculate propagation without complicating the lighting system. The results of the experiments, which were the confirmation of choosing the correct type of lighting, studied the influence of the color of LED lighting on the productivity of microalgae cultivation in the airlift type photobioreactor [30]. Among the following colors of lighting: blue, orange-red, deep red, sunlight, yellow, and warm-white, the highest productivity of cultivation was achieved using yellow and warm-white light. However, in the case of yellow lighting, the authors used blue-light supplementation to stimulate typical growth and increase productivity [30].

The primary task set before the described station was the preparation of a suitable amount of inoculate needed for inoculating of the system comprising of six 90 dm^3 volume photobioreactors each. Biomass concentration in the range 1.40–1.99 g/dm^3 (Figure 8) was obtained on the eighth day of conducting experiments was decidedly higher than the one reported by Zhao et al. [31]. Depending on the used culture media after the eighth day of the cultivation, they have obtained biomass concentration within the range of about 0.73–0.93 g/dm^3. The experiments were carried out, similarly to the ones conducted in AlgaeLabs (cf. the beginning of the Results chapter), in 5 dm^3 glass tanks. However, it is necessary to take into consideration the fact that, in their research, they have used a different strain of *H. pluvialis*, and the experiments started with a constant content of microalgae biomass at the level of 0.15 g/dm^3. The volume of biomass was significantly lower than in the presented experiments where the starting volume of biomass was taken on the value within a range of 0.42–0.78 g/dm^3. When conducting experiments in a glass column photobioreactors, also the ones with *H. pluvialis* microalgae, the maximum specific growth rate was reached at the initial volume of biomass at 0.5 and 0.8 g/dm^3 [32,33].

The final value reached over the course of experiments is similar to the results obtained in Zhang et al. [34], which aimed at comparing the influence of different light intensities on the growth of the *H. pluvialis* biomass. The experiment was conducted in aerated glass photobioreactors, a working

volume of 0.7 dm^3, and an initial cell density of 0.3 g/dm^3. After eight days of biomass cultivation, the concentration increased to about 1.2–2.9 g/dm^3, with light intensity between 50 and 400 $\mu mol\ m^{-2}\ s^{-1}$. The estimated value of photosynthetic photon flux densities in the designed system for propagation of inoculum was about 190 $\mu mol\ m^{-2}\ s^{-1}$. The experiments were conducted at 50% of light intensity. At the much smaller diameter of photobioreactors (5–19 cm) the penetration and availability of light have been higher. Despite that, a similar biomass efficiency has been obtained at almost 15 times higher working volume, which may be a confirmation of the suitable choice of scaling parameters [34].

The doubling time of microalgae biomass was reached on the fifth and sixth day of the cultivation, which is comparable to the results of research [20], in which in the 12 L photobioreactor, the doubling of the number of *Haematococcus pluvialis* microalgae cells was reached on day 6.9.

The utilized way of scaling the culture by a factor of 10 per step proved itself in practice. Admittedly, many researchers use higher factors (20–30) when scaling-up cultures [35–37]. However, in that way they are breaking the practical rule used, i.e., by fermentation technology, which says that each increase of scale should not surpass a factor of 10. Ignoring this rule results in, for example, the appearance of contamination and biological degradation, which translates into a decrease in the reliability, and stability of the production process [8].

5. Conclusions

The presented inoculum propagation station included eight photobioreactors of 12 dm^3 volume. It enabled to effectively prepare the inoculation material for experiments carried out in six 90 dm^3 photobioreactors. The modular construction of the station enabled to independently turn on and turn off individual photobioreactors at any time without halting the work of an entire system. After starting the process, the station was working automatically, which released the operators from the obligation to manually conduct the process while providing an expected amount of cultivation material of a suitable activity. The station presented in this paper greatly improved the last stage of inoculation both from the standpoint of conducting cultivation as well as from the standpoint of ergonomics of the station.

6. Patents

Wiśniewski M., Grzebyk M., Zawada M., Stankiewicz M., Czop Ł., Borowiak D.: Photobioreactor for microorganism cultivation, especially microalgae. Patent PL No.233555 dated 31.10.2019.

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