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Closed photobioreactor for microalgae biomass production under indoor growth conditions

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Abstract

As the production of energy and valuable byproducts from microalge requires large quantities of biomass, so it was important to select a cultivation system suitable for large-scale biomass production. Compared with other culturing systems, the tubular closed photobioreactor was the most suitable for good biomass productivity. It appears to hold the greatest promise for monoculture for producing tonnage quantities of the biomass. Three microalgal isolates *Chlamydomonas variabilis, Spirulina platensis* and *Microcystis aeruginosa* were cultivated using BG11 media inside 24 tubular vertical glass vessel photobiorector. The biomass productivity of *Chlamydomonas variabilis* (growth conditions, temperature 25±2ºC, pH 7.3-9 and light intensity 1500-3000 lux) reached maximum level in 48hrs (0.03 g dry/L per day) and incase of *Microcystis aeruginosa* (growth conditions, temperature 25±2ºC, pH 7.3-8 and light intensity 1500-2000 lux) the maximum growth rate reached after 72 hrs (0.7 g dry/L per day) but it took 5 days (0.26 g dry/L per day) with *Spirulina platensis* (growth conditions, temperature 28±2ºC, pH 7.3-8 and light intensity 1500-2000 lux).

Key words: Microalgae biomass, closed photobioreactor, *Chlamydomonas variabilis, Spirulina platensis*, *Microcystis aeruginosa.*

Introduction:

Microalgae are sunlight driven cell factories that exploit their CO_2 sequestration capability to produce various bioactive compounds of commercial interest **(Singh and Sharma, 2012)**. Microalgal biomass is also widely used for energy generation, as biodiesel, bioethanol, biohydrogen, and photosynthetic microbial fuel cell **(ElMekawy *et al*, 2014)**.The current world wide microalgal biomass production is about 9000 ton/year with high cost ranges from $20 to $200/kg **(Brennan and Owende 2010)**. Several cultivation techniques currently employed for the large scale production of microalgae biomass, they can be normally cultivated in the open-culture systems (lakes or ponds) and also in the closed-culture systems called photobioreactors (PBRs) **(Ramanathan *et al*., 2011)**. Although open ponds are cheap and easier to build and operate than photobioreactors, the risks of contamination always appear. Moreover, since open ponds haveproblems such as waterevaporation, low mixing rate, and poor temperature control **(Chisti 2007; Ugwu *et al*. 2008)**, open pond cultivation is not suitable for pharmaceutical or food ingredients production **(Chisti, 2007)**. To overcome the disadvantages of using open ponds, closed PBRs of various designs have been developed. The major aim of any PBR is to reduce the biomass production costs. This can be achieved by improving the design and shape of the PBR, controlling environmental parameters, and favoring minimal contamination risk. Overcoming these limitations makes monocultures and pharmaceutical and food products possible.PBRs can be categorized as flat plate **(Sierra *et al*. 2008; Slegers *et al*. 2011)**, tubular **(Molina *et al*. 2001)**, and column **(Eriksen, 2008)** according to their illuminated surface, PBRs are also grouped as stirred type, bubble column and airlift reactor bases on their mode of liquid flow. The most suitable PBRs for biomass cultivation should have highly transparent surface, minimal non illuminated part. Moreover, PBR design should be attain high biomass growth for various microalgal species universally and prevent fouling of the reactor.

Mixing the algal culture keeps algal cells in suspension, prevents thermal stratification, allows nutrient distribution, and improves gas–liquid mass transfer to prevent O_2 accumulation, especially in tubular PBRs **(Ugwu *et al*. 2008)**. Another important role of culture mixing is shuttling algal cells between the light zone near illumination surfaces and the dark-interior regions, resulting in mixing-induced periodic light/dark (L/D) cycles, which are beneficial to algal growth **(Molina *et al*., 2001; Ugwu *et al,*. 2008)**. However, excess mixing can damage the microalgal cells and should be avoided **(Barbosa *et al,*. 2003; Ramanathan *et al*., 2011)**.

Microalgae cultures are especially promising and ideal, but the cell harvest from cultivation broth has always been one of the major obstacles for the algae topharmaceuticals approach. Microalgae cells have specific characteristics such as low density (typically in the range of 0.3–5g/L) and small size (typically in the range of 2–20μm), they are technically challenging to harvest.The choice of harvesting technique depends on the characteristics of cyanobacteria and microalgae, e.g. size, density, value of the target products **(Olaizola, 2003)** and often the culture conditions **(Carlsson *et al*., 2007)**.The conventional processes used to harvest cyanobacteria and microalgae include concentration by centrifugation **(Haesman *et al*., 2000)**, foam fractionation **(Csordas and Wang, 2004)**, flocculation

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(a gravity separation process in which the microalgal cells attach to air or gas bubbles and accumulate as float, whichcan be skimmed off) **(Poelman *et al*., 1997; Knuckey *et al*., 2006)**, membrane filtration **(Rossignol *et al*., 2000)** and ultrasonic separation **(Bosma *et al*., 2003)**. Harvesting costs may contribute from 20 to 30% to the total cost of algal biomass **(Molina-Grima *et al*., 2003)**. Microalgae are typically small with a diameter of 3-30µm, and the culture broths may be quite diluted at less than 0.5g/L. Thus, large volumes must be handled.

The aim of this work is to establish indoor closed photobioreactor and to optimize the growth conditions for maximum biomass productivity of *Chlamydomonas variabilis, Spirulina platensis* and *Microcystis aeruginosa.*

Material and methods:

Microalgae Source and Maintenance:

The green alga *Chlamydomonas variabilis* and two blue green microalgae *Spirulina platensis* and *Microcystis aeruginosa* [Plate-1] were isolated from River Nile phytoplankton community. Algal strains were isolated by streaking 0.1ml of water samples on agar plates of different nitrate concentration of the BG11 media **(Stanier *et al.,* 1971).** Algal identification has been done according to the keys of identification **(Komárek and Fott, 1983; Komárek, and Anagnostidis, 1989).** Single colonies of algae were then recultivated in the specified liquid media for each strain as non-axenic batch cultures (50ml) at 25±2°C and 24hr with continuous white fluorescent lamp intensity ≈2500Lux.

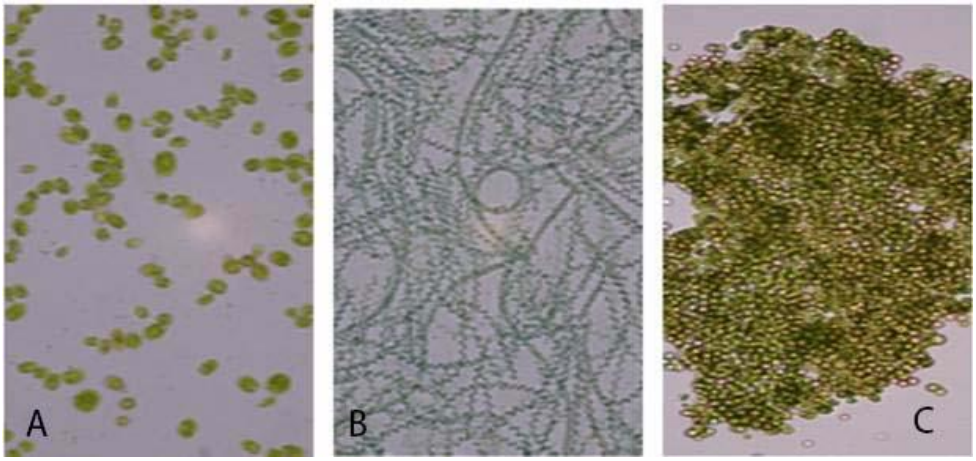

PLATE-1. *A. Chlamydomonas variabilis B. Spirulina platensis. C. Microcystis aeruginosa*

Media Compositions (Stanier et al., 1971):

Nutrient Composition of BG11 consists of macronutrient (mg/L):$NaNO_3$, 1500; K_2HPO_4, 40; MgSO4, 75($7H_2O$); $CaCl_2$, 36($7H_2O$); Citric Acid, 6.00; Na_2CO_2, 20; Na_2EDTA,1.00; Ferric Ammonium Citrate, 6.00; Micronutrient (g/l): H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; ZnSO4.$7H_2O$,0.222; Na_2 $MoO_4.2H_2O$, 0.39; $CuSO_4.5H_2O$, 0.079; Co(NO_3)$2.6H_2O$, 0.0494. Add 1ml/L into the culture medium from the micronutrient. After autoclaving and cooling pH of medium are about 7.1.

Design of the photobioreactor:

The photobioreactor (PBr) has been designed especially for long term cultivation and mass production microalgal species under controlled condition in the indoor laboratories built up to be geometrically similar to a tubular vertical closed photobioreator. A transparent glass was chosen for construction 24 vessels, each of them have a volume of 294.4cm^3. Mixing was achieved using air injection parts and CO_2 levels were controlled by regulator and copper connectors from the cylinder. The PBr glass vessels were mounted on wooden boxes with clamps. Each wooden box was illuminated by white fluorescent lamps attached to one of the sides. Algal biomass harvesting carried out via release valve and filtration piece at the base of each vessel. All the components used were purchased from the Egyptian market mainly manufactured in Egypt except for few componentswhich produced or modified to fit on the present design [Figure 1].

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FIGURE-1: Principal components of the experimental Photobioreactor prototype. 1-Air generator, 2 - CO_2source, 3-Glass column 2m height, 4 -Tap for algal growth collection, 5-Air inlet, 6-Air flow adjustment and 7-Thermometer.

Operation of photobioreactor:

For production of microalgal biomass in the photobioreactor before starting, the reactor should be flushed with warm tap water. It is important for the first start to sterilize the water, so that no foreign plankton organisms may enter thereactor, especially zooplankton (rotifers) which can destroy the whole system. The operation conditions involve temperature, pH, light intensity, growth rate of the microalgae inside the photobioreactor, the rate of media addition for optimum microalgal growth, mixing and the harvesting method and the rate of algal mass produced from the reactor.

Temperature, pH have been continuously monitored by a thermometer and a pH- meter probe. The light intensity has been measured by Lux meter-probe. Algal biomass productivity (g/L) was calculated by measuring dry weight. For dry weight measurement, the harvested microalgae cells were placed in pre-weighed beaker and dried on water bath at 40°C. The dried biomass was cooled and weighed. The difference between the initial and final weight were taken and thebiomass weight was calculated. The dry weights were expressed in g/L.

Results and discussion:

The microalgae biomass production requires an appropriate culture system with consistent cultivation parameters which will be suitable for producing potential algal biomass. The large scale cultivation of microalgae showed success with open ponds system. Major limitations in open system include contamination by predators and other fast growing heterotrophs, changes in local climatic changes and requirement of large areas of land and water. Furthermore the mass culture of microalgae will require closed system as the algae must be grown without any potential contaminants like heavy metals and microorganisms. In the present investigation a semi pilot scale tubular photobioreactor with capacity of 48L

was constructed using Egyptian, environment friendly low cost material and has been used for the mass cultivation of microalgae.

In the present study, the preliminary experiment to establish efficient operation conditions of the photobioreactor were conducted by using the green alga, *Chlamydomonas variabilis.* Where *C. variabilis* has long served as an excellent model system with which to study the assembly, maintenance and function of asymmetric cytoskeletal structure that allow the cell to respond to environmental conditions and so it considered as an ideal species that fits well within the design criteria for the photobioreactor. In addition, *Chlamydomonas* widely distributed worldwide in soil and freshwater and is primarily used as a model organism in biology in a wide range of subfield. Meanwhile *Chlamydomonas* can grow in media lacking organic carbon and chemical energy sources, and can also grow in the dark. Also, it is of interest in the biopharmaceuticals and biofuel fields **(Harris, 2001; Merchant *et al*., 2007; Lagali *et al*., 2008).**

The maximal biomass concentration of *Chlamydomonas* (0.03g/L per day) has been obtained after 72hrs at 25±2ºC, pH ranges from 7.3- 8 and under 2500 to 3000 lux illumination in a 24 hrs photoperiod and the harvesting carried out using filter presses operating under vacuum through phytoplankton net 5µm mesh size.. Although *C. debaryana* was grown in a 30L indoor photobioreactor to study the mass cultivation prospect,it showed a higher biomass yield (1.58 ± 0.02 g /L per day dry weight) **(Mishra *et al*., 2015).**

During the cultivation of *Spirulina platensis*, the growth inside the reactor took place and reached maximum rate after 5 days, pH ranges from 7.3 – 9 and under 1500 to 2000 lux illumination in a 24 hrs photoperiod. In addition, *Spirulina* tend to line and attach to the inner side of the glass vessel, so the mutual shading increases and causing photo-limitation which decreases the available lightenergy per cells and affect the alga growth rate. To overcome this problem a pair of magnets was used to remove the attached algal cells. The specific growth rate of *Spirulina* decreases with higher biomass concentrations, due to the shadowing effect **(Reichert *et al*., 2006)**.

Also, it was observed that during *Spirulina* production the photosynthetic efficiency of the microalgal cells decreased for biomass concentrations between 0.4-1.0 g/L as these cells deprived ofthe light by neighboring cells. At biomass concentration close to 0.5g/L **(Reichert *et al*., 2006).**

Travieso *et al*. (2001) studied the semi continuous cultivation of *S. platensis* in a tubular reactor for seven days with the daily removal of a portion of the old medium and addition of fresh medium, and observed a remarkable increase in biomass productivity as the renewal rate increased from 5 to 20% (v/v) although the productivity decline began at 25%.

Furthermore, *Microcystis aeruginosa* showed semi-continuous cultivation cycle for 14 days and it was carried out by withdrawing about 500ml from the system and left it in a glass cylinder for settling, the bulk was then collected and the supernatant reintroduced to the system after adding of the new culture media and completing to 500ml with distilled water. The collected biomass was dried at 60ºC.

Ashokkumar *et al*. (2014) reported that the semi-continuous cultivation of *Microcystis aeruginosa*in 20 m^2 open raceway pond yields high biomass up to 28 g /L per day, in contrast, **Da Rós *et al.* (2012)** recorded only 0.083 g/L from the same alga can be produced per day. Nevertheless, in the present investigation the biomass productivity of *M. aeruginosa* cultivated in the closed photobiorector didn't exceed 1 g/L per day (growth conditions, temperature 25±2ºC, pH 7.3-8 and light intensity 1500-2000 lux).

In order to harvest biomass, a suitable harvesting method include one or more steps and be achieved in several physical or chemical ways. Filter presses operating undervacuum was used with *Chlamydomonas variabilis* through phytoplankton net 5µm meshsize while *Spirulina platensis* and *Microcystis aeruginosa* were harvested through settling then centrifugation.

During the cultivation of *Chlamydomonas variabilis, Spirulina platensis* and *Microcystis aeruginosa,* the preculture was used to inoculate the tubular photobioreactor. Aeration and mixing were achieved by sparing air through plastic tube which was inserted to the bottom of the photobioreactor. The presence of sodium carbonate in the growth media (BG11) was enough to support the alga growth and no need to sparing more free CO_2.

TABLE -1: Summary of growth conditions of the three algal species

Growth requirement	*Chlamydomonas variabilis*	*Spirulina platensis*	*Microcystis aeruginosa*
Temperature	25±2ºC	28±2ºC	25±2ºC
Ph range	7.3-8	7.3-9	7.3-8
Light intensity	2500-3000 lux	1500-2000 lux	1500-2000 lux
Media required	BG11	BG11	BG11
Time of harvesting	72hrs	5 days	48hrs
Addition of new media	No need	Every 48 hrs	Every 48 hrs
Inoculum for new culture	Part from the harvesting culture	Pre-stock culture	Supernatant of the harvesting culture
Growth attachment	No growth was attached to the inner wall of the reactor	Microalgal growth was attached to the inner wallof the reactor and need to be removed continuously	No growth was attached to the inner wall of the reactor
Addition of CO_2	Not needed	Not needed	Not needed
Air flow	$50m^3$/day	$63m^3$/day	$50m^3$/day
Biomass productivity	0.03g dry/L per day	0.26 g dry/L per day	0.7g dry/L per day
Harvesting method	Filter presses operating under vacuum through phytoplankton net 5µm mesh size.	Settling then centrifugation	Settling then centrifugation

Conclusion

Closed photobioreactors which allow better control of growth parameters may be more suited to reachthis biological goal.Compared with other culturing system the tubular closed photobioreactor is the most suitable for good biomass productivity. It appears to hold the greatest promise for monoculture for producing tonnage quantities of the biomass.Major advantage of tubular photobioreactor would be acclimated to high light and therefore the negative effect of photoinhibition would be minimal in such system. In future the optimization of the operating parameters and intrinsic properties of algae, biology of algae and the engineering requirements is till place left for further technological advances and improvement for growth performance and higher biomass quantity cultivation inclosed photobioreactor which will enable the commercialization of new algae and algal products infuture.

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