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Lipid productivity of microalgae *Chlorella vulgaris* and *Nannochloropsis oculata* in externally illuminated lab scale photobioreactor

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Abstract: Lipid productivity of *Chlorella vulgaris* and *Nannochloropsis oculata* was evaluated in a 5 L lab scale externally illuminated photobioreactor. The effect of operating conditions such as pH, temperature, light intensity and photoperiod on biomass productivity, lipid content and lipid productivity was evaluated. At optimum conditions, a maximum lipid productivity of 69.46 and 192.3 mg lipid. L^{-1} .day-^{1} was observed for *C.vulgaris* and *N.oculata* respectively.
Keywords: Lipid productivity, microalgae, *Chlorella vulgaris, Nannochloropsis oculata,* Photobioreactor.

1. Introduction

Recently, microalgae are emerging as one of the most promising sources of biodiesel because of their high photosynthetic efficiency and faster growth as compared to any energy crop [1]. A few microalgal species, including some *Chlorella* species [2], *Dunaliella* species [3], *Nannochloris* sp.[4] , *Parietochloris incise*[5], *Neochloris oleoabundans* [6], and *Botryococcus braunii* [7] have been reported to accumulate large quantities of lipids. However, the growth characteristics and lipid % of microalgae are known to significantly depend on the cultivation conditions. The most important parameters are: pH, temperature, light intensity and photoperiod. These four parameters can be accurately controlled and optimized in a photobioreactor to achieve maximum lipid and biomass productivities.

In this regard, the present study focuses on optimization of parameters (pH, temperature, light intensity and photoperiod) affecting the cultivation of two algae species viz., *Chlorella vulgaris* and *Nannochloropsis oculata* in a lab scale Photobioreactor.

2. Materials and methods

2.1 Batch cultivation of algae species

Two algae strains (*Chlorella vulgaris*-SAG 211-11b, and *Nannochloropsis oculata*-SAG 38.85) were procured from EPSAG (Experimentelle Phykologie und Sammulung von Algenkulturen), Gottingen, Germany and maintained on Basal media [8].Cells from the original culture were transferred aseptically to 300 ml sterilized media in a laminar air flow chamber (ALPHA LINEAR, Model No. 1222-H). During exponential phase, the cultures were transferred to 5 L photobioreactor containing 3 L media.

2.2 Construction and working of Photobioreactor

Schematic and PID diagrams of Photobioreactor used in the present study are shown in Fig 1 and 2 respectively. The set up consists of a 5 L borosilicate vessel connected to a panel of straight glass tubes through

a diaphragm pump (Longer pump). The panel of straight glass tubes consists of 6 glass tubes (1 inch diameter, 80 cm long) connected to each other through bends, flexible couplings and O-rings. The media from 5 L vessel is circulated to straight glass tubes by diaphragm pump. The reactor is equipped with pH, temperature and light intensity controllers. pH electrode (Mettler Tolerdo) and Pt-100 sensor have been used to measure pH and temperature of media in reactor. LED panels are arranged around 5 L vessel and straight glass tubes.

The algae culture from batch cultivation was transferred to 5 L vessel containing 3 L media. Operating conditions for a given run viz., pH, light intensity, and temperature were set in controller panel. The range of parameters under which the reactor was operated is given in Table 1. Diaphragm pump was operated at 100 rpm. At any given time, 2 L of culture was maintained in 5 L vessel and 2 L was in recirculation. Samples were drawn through sample port and analyzed for biomass (by gravimetric method) and lipid content (Bligh and Dyer method) [9]. Biomass productivity and lipid productivity were calculated as,

Biomass productivity = (Xn-Xo)/n …………………………….. (1)

Lipid Productivity = Lipid % × Biomass productivity ×10 .... (2)

where Xo and Xn are biomass (in g/l) zeroth and n^{th} day respectively.

Table 1- Ranges of parameters for the operation of photobioreactor

Parameter	Range
pH	6-8
Temperature (^oC)	24-30
Light intensity (klux)	5-8
Light: dark cycle(h)	16:8-8:16

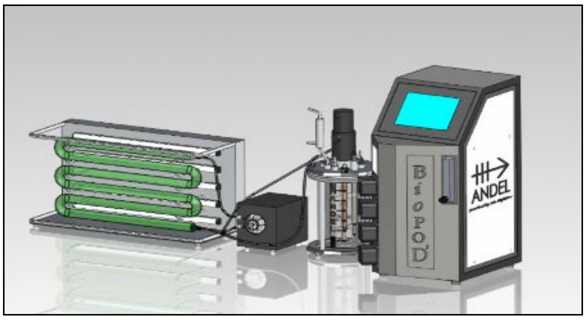

Fig.1- Schematic diagram Photobioreactor

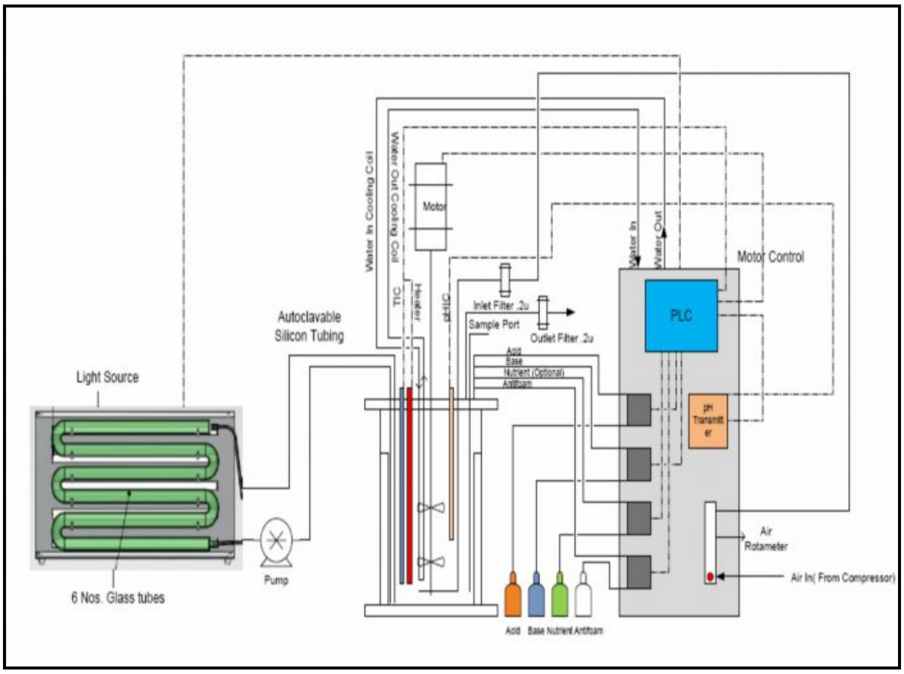

Fig.2- PID diagram of Photobioreactor

3. Results and discussion

Various experiments were conducted to optimize the growth conditions of each alga. Parameters were optimized sequentially in the order: pH, Temperature, light intensity and light to dark cycle ratio. For a given run, biomass productivity, lipid content and lipid productivities were calculated each day. It was observed that lipid productivity increased with time and approached a maximum value at the end of exponential phase. These values are depicted in Table 2 and 3 for *C.vulgaris* and *N.oculata* respectively.

3.1 Effect of pH

Optimum pH was found to be 7.2 and 6.8 for *C.vulgaris* and *N.oculata* respectively. However at pH 8, lipid productivities of both species were very low. This is because, growth under higher pH requires more light energy to be available per cell, but also that a large fraction of that light is not efficiently used by the microalgae. Such inefficiency could partly be caused by a lower light-saturated photosynthesis at increased pH.

Table 2- Maximum lipid productivity obtained for various runs for *Chlorella vulgaris*

pH	Temp(^{o}C)	Light to dark cycle ratio (h)	Light intensity (klux)	Biomass productivityg dry biomass/(L.day)	Lipid content (%)	Lipid productivity (mg lipid.L^{-1}.day^{-1})
6.2	25	12.12	4	0.145	15..62	22.64
6.4	,,	,,	,,	0.15	16.2	24.3
6.6	,,	,,	,,	0.174	16.48	28.6
6.8	,,	,,	,,	0.185	17.22	3185
7	,,	,,	,,	0.214	18.94	40.53
7.2	,,	,,	,,	0.225	19.92	44.82
7.4	,,	,,	,,	0.216	19.45	42.01
7.6	,,	,,	,,	0.198	19.22	38.05
7.8	,,	,,	,,	0.18	18.67	33.61
8.0	,,	,,	,,	0.14	16.2	22.68
7.2	24	12:12	4	0.21	19.5	40.95
,,	26	,,	,,	0.236	20.77	49.01
,,	27	,,	,,	0.248	21.54	53.41
,,	28	,,	,,	0.24	20.81	49.94
,,	29	,,	,,	0.224	20.11	45.04
,,	30	,,	,,	0.216	19.42	41.94
7.2	27	16:8	,,	0.204	18.85	38.45
,,	,,	14:10	,,	0.227	20.2	45.85
,,	,,	10:14	,,	0.268	22.3	59.76
,,	,,	8:16	,,	0.214	21.2	45.36
7.4	27	10:14	5	0.271	23.53	63.76
,,	,,	,,	6	0.284	24.46	69.46
,,	,,	,,	7	0.263	23.21	61.04
,,	,,	,,	8	0.253	22.89	57.91

Table 3- Maximum lipid productivity obtained for various runs for *N.oculata*

pH	Temp (^oC)	Light to dark cycle ratio	Light intensity (klux)	Biomass productivityg dry biomass/(L.day)	Lipid content (%)	Lipid productivity (mg lipid.L^{-1}.day^{-1})
6.2	27	12.12	4	0.344	24.65	84.79
6.4	,,	,,	,,	0.368	25.64	94.35
6.6	,,	,,	,,	0.396	26.98	106.8
6.8	,,	,,	,,	0.425	28.15	119.63
7	,,	,,	,,	0.422	27.47	115.92
7.2	,,	,,	,,	0.417	26.84	111.922
7.4	,,	,,	,,	0.364	24.22	88.16
7.6	,,	,,	,,	0.343	21.74	74.56
7.8	,,	,,	,,	0.292	20.19	58.95
8.0	,,	,,	,,	0.252	18.96	47.77
6.8	20	12.12	4	0.387	24.26	93.88
,,	22	,,	,,	0.396	25.17	99.67
,,	24	,,	,,	0.452	30.54	138.04
,,	26	,,	,,	0.434	29.47	127.89
,,	28	,,	,,	0.412	28.12	115.85
,,	30	,,	,,	0.39	27.46	107.1
6.8	24	16:8	,,	0.418	26.22	109.5
,,	,,	14:10	,,	0.425	28.74	122.14
,,	,,	10:12	,,	0.404	25.47	102.89
,,	,,	8:12	,,	0.369	25.95	95.75
6.8	24	12:12	5	0.474	31.59	149.736
,,	,,	,,	6	0.482	32.74	157.8
,,	,,	,,	7	0.512	33.83	173.21
,,	,,	,,	8	0.528	34.79	183.69
,,	,,	,,	9	0.546	35.22	192.3
,,	,,	,,	10	0.514	34.85	179.1

3.2 Effect of temperature

As can be observed from Table 2 and 3, lipid productivity decreased from was obtained at 27^{o}C and 24^{o}C for *C.vulgaris* and *N.oculata* respectively. Attilio et al [10] reported that optimum temperature for growth of *N.oculata* was 20^{o}C, but its lipid productivity did not change appreciably between 15-25^{o}C. For *C.vulgaris*, lipid productivity decreased from 25-30^{o}C while optimum temperature was 25^{o}C.The disagreement in results of our values with literature may be due to difference in the origin of strains.

3.3 Effect of light intensity

At low light intensities, lipid productivity will be low due to limitation of the energy required for the sequence of photosynthetic reactions. At high irradiance, photoinhibition affects the growth rate of algal species. Hence, an adequate supply of light energy is most critical parameter in the operation of photobioreactor. In the present studies, 6 klux and 9 klux were determined as optimum light intensities for *C.vulgaris* and *N.oculata* respectively.

3.4 Effect of photoperiod

During light period, microalgae store the energy through exergonic reactions. During dark period, this energy will be used for endergonic reactions. Hence, the ability of alga to exhibit high lipid productivity depends on its capacity to store energy during light period and utilize it during dark periods. Further, it has been shown in the literature that microalgae could show preferences with respect to the duration of the light periods, resulting from the environmental conditions in which they were isolated in nature. In the present study, 10 h: 12 h and 12 h :12 h (light: dark) were found to be the optimum photoperiods for *C.vulgaris* and *N.oculata* respectively.

Conclusions

The performance of a lab scale externally illuminated PBR under various operations conditions was evaluated in terms of lipid productivity of two algae species *C.vulgaris* and *N.oculata*. The effect of pH, temperature, light intensity and photoperiods was evaluated. The optimum parameters were: pH-7.2, Temperature=27^{o}C, light intensity=6 klux and photoperiod=10:12 for *C.vulgaris* and pH-6.8, Temperature=24^{o}C, light intensity=9 klux and photoperiod=12:12 for *N.oculata* respectively.

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