

Cultivation of *Spirulina* in Gas Induced Photobioreactor and Isolation of Phycobiliproteins

Harshal H Kshirsagar, Madhavi S Revankar, Madhusudan Y Kamat and Smita S Lele*

Food and Fermentation Technology Department, University Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai 400 019, India

Received 26 September 2001; accepted 4 April 2002

A novel design of Gas Induced Mechanically Agitated Photobioreactor (GIMAP) using the principle of hollow shaft impeller to sparge air is tested with *Spirulina* as model system. Gas induced impeller is a lesser-explored type of mechanically agitated contactor. It possesses unique characteristics like no separate power requirement for the sparging of the gas, moderate values of mass transfer coefficient and relatively low values of shear stress. These characteristics make it suitable as a photobioreactor although it may not be the best choice for the classical fermentations. GIMAP exhibited good performance giving biomass concentration and high specific growth rate of 4.44 g/l and 1.33 d⁻¹, respectively. Hydrodynamic stress developed in GIMAP was found to have favourable effect that resulted in production of biomass with more permeable cell walls. As a result it was possible to extract phycobiliproteins without cell rupture by simply changing the pH of the buffer. Further it was possible to recover 23% of phycobiliproteins (92% yield) from the GIMAP culture along with a by-product namely exhaust cell mass with 25% proteins. Simpler downstream processing, additional byproduct coupled and higher growth rates of *Spirulina* than the conventional cultivation methods indicate the commercial potential of the proposed GIMAP design as photobioreactor.

Keywords: *Spirulina*, phycobiliproteins, microalgae, photobioreactor, gas-inducing impellers

Introduction

Spirulina, one of the important and the oldest known microalgal species, has gained attention as a food source because of the high protein and vitamin content and due to its ability to produce large quantities of high value products like phycobiliproteins (Sarada *et al*, 1999). Traditionally, micro-algae are cultivated in open ponds. This mode of cultivation has a number of drawbacks including low cell densities, potential to contamination by other microorganisms and the inability to control environmental factors such as light, irradiation and temperature (Weismann *et al*, 1988; Chen & Zhang, 1997). Though these systems are being improved with respect to temperature control, supply of appropriate nutrients, optimization of pond depth, carbon dioxide injection, etc. still productivity remains fairly low (~100 g dry algal biomass/m²/day), hence are not commercially viable due to cost implications. Several alternatives, proposed to the open pond culture technique, recommend the use of photobioreactors

(tubular, column type with circulation, with recycle, etc.). The growth rate increases by 100% in indoor systems with internal illumination arrangements (Pulz & Scheibenbogen, 1998). Sophisticated photobioreactors such as photobioreactor with efficient light emitting diodes (Lee & Palsson, 1994), fibre optic based photobioreactor (Javanmardian & Palsson, 1991), tubular photobioreactor for outdoor cultivation (Torzillo *et al*, 1993), etc. tried at laboratory scale are not cost-effective. Hence, there is a need to explore newer designs which are compact, have lower power consumption and can achieve high growth rates without much initial investments. In the present work, authors report a novel design of photobioreactor for cultivation of *Spirulina* and recovery of phycobiliproteins, called Gas Induced Mechanically Agitated Photobioreactor (GIMAP).

GIMAP is a less explored design for fermentation in general, and as a photobioreactor in particular. It offers several advantages over conventional stirred vessel in terms of lower power consumption and lower shear levels. However, it may not be a good choice for biological systems having fast growth kinetics and oxygen mass transfer limitations. GIMAP as a photobioreactor was thought to be worth

*Author for correspondence:

Tel: +91-022-4145616; Fax: +91-022-4145614

E-mail: sslele@foodbio.udet.ernet.in

exploring since intrinsic kinetics of algal cultures are slow, almost by an order of magnitude as compared to bacteria. Secondly, significantly higher solubility of CO_2 compared to O_2 (Perry & Green, 1997) results into high CO_2 dissolution rates in GIMAP, although the same construction may not be as effective as an O_2 supply system. The power consumption is also low as the gas inducing impeller eliminates separate air sparging line and associated power requirement because of its inherent hollow shaft design (Joshi & Sharma, 1977). Thus, GIMAP may be a good choice for algal cultivation, especially at initial stage to produce a large number of cells in a short time span.

The present study also demonstrates the advantageous role of GIMAP in developing a simpler method for isolation of phycobiliproteins without cell lysis, whereby a byproduct namely exhaust cell mass having 25% proteins is also obtained. The direct leaching of phycobiliproteins from the GIMAP cultivated *Spirulina* and the availability of exhaust mass are thus an important steps in the reduction of downstream processing and separation cost. The fact that conventional downstream processing of *Spirulina* to recover phycobiliproteins involves cell lysis and no exhaust cell mass is available, the present work using GIMAP cultivation resulted in exhaust cell mass, will improve the economics of the overall process.

Materials and Methods

Design of GIMAP

Gas inducing impeller (Fig. 1) is an agitator having hollow shaft with gas-port that simultaneously produces mixing and self-induction of gas. When the impeller rotates in the fluid, the acceleration of the fluid element causes reduction in static pressure in the hollow shaft. Since the reduced pressure area is connected to the gas space, air is sucked in by induction when the reduction in pressure is sufficient to overcome all the resistance in the path of the gas. Gas induction in the liquid occurs at a speed equal to or greater than critical impeller speed (N_{critical}). This condition is represented by the following equation of force balance (Joshi & Sharma, 1977).

$$N_{\text{critical}} = \sqrt{[2g \times H_L / K_1 \times \Pi^2 \times D^2]}$$

Where, g = acceleration due to gravity, H_L = height of liquid column from the tip of the impeller (stator) to the top of the liquid, K_1 = a constant having a value of 0.9 to 1.3 and D = impeller diameter.

This equation suggests that gas induction is directly proportional to the square root of the liquid height and inversely proportional to the impeller diameter.

GIMAP used in this work consisted of 2 l BOROSIL reactor (13.5 cm diam with four glass baffles of 0.5 cm width) with a flat bottom (Fig. 2). It was fitted with a gas inducing impeller (7 cm diam) having 4 blades (2-mm diam hollow tube). Impeller to vessel diameter ratio of 1:2 was maintained in the design as per the recommendations of Joshi and Sharma (1977). The assembly was fitted with overhead motor with variable speed drive. Air (filtered through sterile cotton wool placed in the hollow shaft) was used as a source of CO_2 . All the experiments were conducted at the speed of 10-rev s^{-1} , which did not change significantly over the cultivation period of 15 days. Two circular fluorescent tube lights (Philips 32 W, 1500 Lux) were mounted around the GIMAP. 1.5 l of medium were used that resulted in a height:diam (H/D) ratio of 1:2, which was kept constant in all the experiments.

Organism, Medium and Cultivation Conditions

Spirulina platensis ARM 740 was obtained from CFTRI, Mysore, India. For all cultivations, medium employed was modified Zarrouks medium (Sarada *et al*, 1999) with initial pH 9.2 that was found to increase during cultivation and was kept at 10.5 throughout the experiment. For comparison with GIMAP, an experiment was carried out in 0.25 l flasks with 0.1 l culture medium on Gyrotory shaker (2 rev s^{-1} , 25°C).

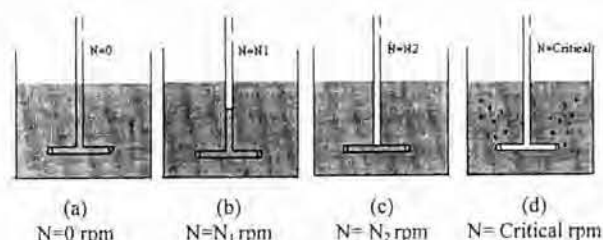


Fig. 1—Induction of Air Through Hollow Shaft

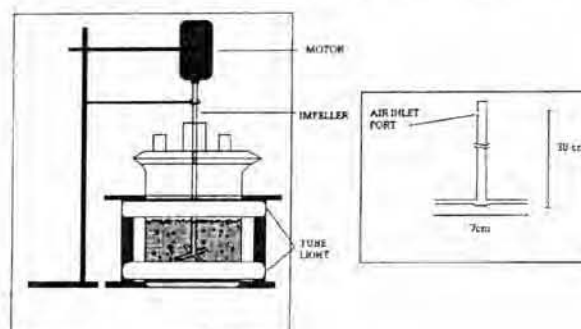


Fig. 2—GIMAP Assembly

Samples were withdrawn at suitable time intervals and dry biomass weight was estimated to plot the growth curves of *Spirulina* for GIMAP and shake flask cultivation. The results are based on the average values from five experiments with similar environmental conditions. Phycocyanin estimation was carried out by spectrophotometric method (Sarada *et al.*, 1999).

Results and Discussion

To achieve a high biomass yield, two governing factors required are, a high illuminated surface area to culture volume ratio (A/V ratio), which ensures sufficient light to carry out photosynthesis, and a high mass transfer coefficient that ensures proper supply of carbon source (generally atmospheric CO₂) and removal of undesirable O₂ by counter desorption. The light is main requirement for photosynthesis and essential to increase the biomass. Thus, in an well-illuminated system, algal growth is primarily determined by the CO₂ mass transfer limitation in the liquid phase (Contreras *et al.*, 1998). One would expect that CO₂ mass transfer would be higher in conventional stirred vessel bioreactors than the proposed GIMAP. However, the power consumption in conventional stirred type would work out to be quite high due to cultivation period of several days or weeks in the case of algal cultures. Further, the usual stirred bioreactors are not suitable for slow growing algal cultures which are shear sensitive cells due to high levels of shear stresses developed during mechanical agitation. The paddle/turbine impellers used in stirred tanks develop both cutting and shearing action on the algal cells, which leads to the disruption of the cell. GIMAP impeller would not produce such cutting action due to the cushioning action of the bubbles produced at the tip due to gas induction. Further, the moderate speed produces lower level of turbulence and lesser hydrodynamic shear than the conventional mechanically agitated types.

Cultivation in GIMAP

The main objective of this work was to investigate the application of GIMAP for micro-algal cultivation and compare it with other laboratory and commercial designs including conventional methods of large-scale cultivation in open ponds. The concentration of cells obtained in shake flask was 3.56 g l⁻¹ in 25 days. The growth rates of *S. platensis* in shake flask were comparable with those obtained in other photobioreactors (Table 1). These values are significantly higher than those for pond cultivation. Thus, it may be safely assumed that shake flask results were mimicking typical photobioreactor and are better than pond cultivation. On the other hand, during GIMAP cultivation of *S. platensis* in 15 days, the values of both productivity (4.44 g l⁻¹) and highest specific growth rate (1.33 d⁻¹) were higher when compared with other novel photobioreactors.

The higher values of cell concentration and specific growth rate obtained in GIMAP suggest that the algal cultivation system has severe CO₂ diffusion limitation during pond cultivation. The growth rate of *S. platensis* in GIMAP is much higher than that of cultivated in shake flask during the entire batch time (Fig. 3).

In classical processes involving organic carbon substrate, the role of aeration is to supply O₂. In the case of photobioreactor, air (0.03% CO₂) is used to provide the carbon source. The partial pressure of CO₂ as against that of O₂ in air is lower by a factor of 700, and in spite of higher solubility of, the overall mass transfer rate for CO₂ dissolution in a given bioreactor design is expected to be lower than that of O₂ dissolution by several orders. This suggests a possibility of contribution of gas side mass transfer resistance (Bailey & Ollis, 1977). In order to check this, *Spirulina* was cultivated using air supplemented with CO₂, generated chemically by treating sodium bicarbonate with hydrochloric acid. Since the inherent design of GIMAP does not permit direct sparging of gas, the assembly was kept in an enclosure (0.11-m³ volume) and the air in the surroundings was

Table 1—Productivity and growth rates of *Spirulina platensis* in different reactor configurations

Micro-algal species	Cell concentration	Highest specific growth rate	Reactor type	References
<i>S. platensis</i>	1.17 g l ⁻¹	Very low	Ponds	Pulz and Scheibenbogen, 1998
<i>S. platensis</i>	2.66 g l ⁻¹	0.62 d ⁻¹	Fermentor	Chen and Zhang, 1996
<i>S. platensis</i>	3.93 g l ⁻¹	0.566 d ⁻¹	Novel photobioreactor	Xiang <i>et al.</i> , 1999
<i>S. platensis</i>	3.56 g l ⁻¹	0.61 d ⁻¹	Shaker	Present study
<i>S. platensis</i>	4.66 g l ⁻¹	1.33 d ⁻¹	GIMAP	Present study

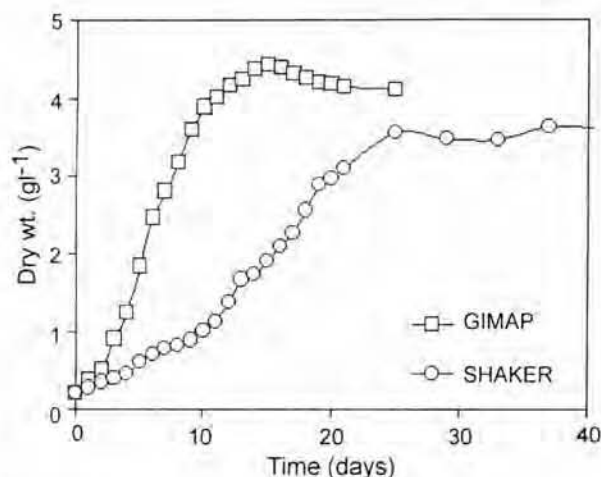


Fig. 3—Growth Curve of *Spirulina* in GIMAP and Shake Flask

supplemented with chemically generated CO_2 . This experiment showed higher growth rates (Fig. 4). This hypothesis was further checked by conducting experiments with glucose as a supplementary carbon source. *Spirulina*, when cultivated using glucose along with atmospheric CO_2 , under shake flask cultivation condition, gave 1.24 times higher biomass. Thus, the algal cultivation in shake flask suffers from carbon limitation, and reduction in diffusional resistance should improve the growth rates. Chen *et al* (1996) have also reported enhancement in *Spirulina* biomass by mixotrophic cultivation with glucose supplementation. The usual method of eliminating diffusion limitation in stirred reactors involve increasing agitation speed till no further change in the reaction (growth) rate is observed. In the case of stirred type photobioreactor, higher speed of agitation leads to higher shear stress, which is likely to have adverse effect on the trichome length of *Spirulina*. At times, the cells may get mechanically damaged leading to adverse effect. Bronnenmeier & Märkl (1982) demonstrated decreased growth rates and a shorter trichome length of *Spirulina* with increasing speed of stirring. This parameter was studied by conducting a few experiments at speeds greater than the critical speed of GIMAP. Figure 4 depicts the growth rate at 13 rev s^{-1} instead of the critical speed of 10 rev s^{-1} . Cell separation by centrifuging showed signs of cell damage (green coloured supernatant) at higher speed of agitation.

To evaluate the proposed design of GIMAP, its performance was compared with the reported results for various other designs of photobioreactor (Table 2). It is observed that GIMAP showed reasonably good

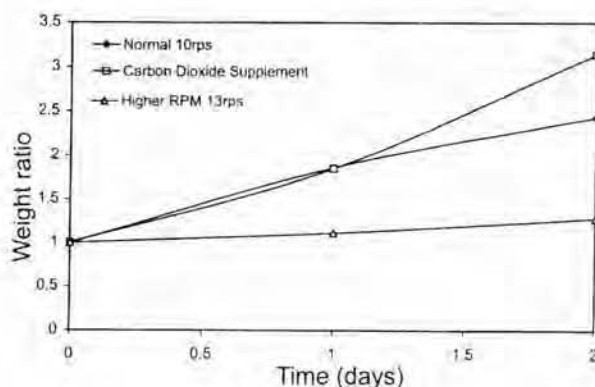


Fig. 4—Effect of Higher Speed of Agitation and Carbon Dioxide Supplementation on Cultivation of *Spirulina* in GIMAP (Weight Ratio = weight at time t /weight at $t=0$)

mass transfer characteristics and the growth rates are quite attractive in spite of lower illumination area to volume ratio. Further, GIMAP is advantageous in terms of low power consumption than other stirred types and low land requirements compared to pond cultivation. GIMAP performance is comparable to other commercially feasible designs such as stirred and column type. Very high growth rates have been reported in sophisticated methods like fibre optics, reactors with cell recycle by membranes, which result in very high cell density, etc. and may be quite expensive at large-scale. GIMAP competes with other types in terms of low power requirement since no separate power is required for air sparging.

Isolation of Phycobiliproteins

Spirulina contains phycobiliproteins (25%). Conventionally, the separation and purification of phycobiliproteins involves cell lysis by homogenization, separation of the cell debris by centrifugation and extraction of the supernatant. The supernatant needs further purification by column chromatography. The present work involves a simpler method for isolation of phycobiliprotein without cell lysis. To assess suitability of GIMAP culture for the downstream processing to recover phycobiliproteins, GIMAP and shake flask cultivated biomass were compared. The shake flask cultivated biomass was subjected to different methods of extraction. Of the total extractable phycobiliproteins, 13.4% was released using high-pressure homogenizer, while recovery of 12.5 and 10.5% was obtained using mortar and pestle homogenizer, and sonicator, respectively, with the total yield in the range of 40–53% only. Further, the spent biomass had to be

Table 2—Comparison of various photobioreactors

Micro-alga	Type of photobio-reactor	Illuminated area to reactor volume ratio cm^2/cm^3	Illumination type and intensity	Volume cm^3	Productivity g/l.d	Mass transfer coefficient $k_{La} \text{ d}^{-1}$	Reference
<i>Chlorella vulgaris</i>	Ponds	0.05	Sunlight	2×10^7	0.1	0.012	Weismann <i>et al.</i> 1988
<i>Phaeodactylum tricornutum</i>	Hollow fibre Annular with fibre optic	5-10	Light transmitted by fibre optics 0.17 W/m^2	600	7	—	Javanmardian & Palsson 1991
<i>C. vulgaris</i>	Bubble column	1.25-1.92	Upto 5 W/m^2 With light emitting diodes	80 or 52	5-6	2400-4800	Lee and Palsson 1994
<i>P. tricornutum</i>	Airlift	0.3	Fluorescent tubes 90 W/m^2	12,000	0.36	1900-2400	Contreras <i>et al.</i> 1998
<i>C. vulgaris</i>	Stirred tank	0.5-4.4	Fluorescent lamps 4 W	3000	1.25	1441 to 3480	Obgonna & Tanaka 1997
<i>Spirulina platensis</i>	GIMAP	0.38	Fluorescent tubes 2.244 W/m^2	1500	0.31	1296 *	This work

* Estimated value from experimentally measured fractional gas hold-up to obtain specific interfacial area (a), assuming true mass transfer coefficient $k_L = 1 \times 10^{-2} \text{ cm/s}$

discarded as waste. One of the objectives of the present work was reduction in downstream processing cost. GIMAP culture showed interesting results leading to direct extraction without cell rupture.

Advantageous Effect of Hydrodynamic Stress in GIMAP Cultivation

The adverse effects of hydrodynamic stress generated by an increased speed of agitation of the impeller on microbial fermentation have been well-documented. Bronnenmeier & Märkl (1982) demonstrated decreased growth rates and a shorter trichome length of *Spirulina* with increasing speed of stirring. Märkl (1991) also reported that specific growth rate of *Spirulina* decreased with increased speed of agitation. Similar observations were also noted in the present work when the speed of stirring was increased from 10 rev s^{-1} to 13 rev s^{-1} . Increased speed of agitation caused reduction in coiling and trichome length (Fig. 5). However, the hydrodynamic stress developed at the critical speed was found to have an advantageous role in the isolation of phycobiliproteins. In *Chlamydomonas*, the reduction in the growth rate due to hydrodynamic stress was coupled with the release of intracellular contents (Märkl, 1991). However, no work till date mentions such release of intracellular contents for *Spirulina* under shear stress. In the present work,

phycobiliproteins were released from the biomass without cell disruption at optimum pH conditions. Interestingly, no phycobiliproteins were released in the medium (pH 10.5) during cultivation in GIMAP. Whereas, when the biomass was removed from the medium and redispersed in 0.05 M phosphate buffer (pH 6.7) phycobiliproteins were leached in the buffer completely within 30 min. *Spirulina* contains 25% phycobiliproteins and 92% of this was released in 0.05 M phosphate buffer (6.7 pH) when cultivated in GIMAP. To compare phycobiliprotein release without cell rupture, *Spirulina* cultivated in shake flask was suspended in 0.05 M Phosphate buffer (6.7 pH). The biomass did not show any release of phycobiliproteins in the buffer. This difference in the behaviour of otherwise similar culture was attributed to the role of shear stress during the growth phase. Inherent design of shake flask allows the air to get dissolved in the media, which is moving in a gyratory motion with little back mixing. Although, it is difficult to estimate the exact level of shear stress in a shaker, it is expected to be significantly smaller than any other agitated type of fermentor. Shear stress in a mechanically agitated vessel is attributed to the eddy currents, especially at the tip of the blade of the impeller. As discussed earlier, GIMAP develops much lesser shear stress as compared to conventional stirred vessel although shear stress in GIMAP will be

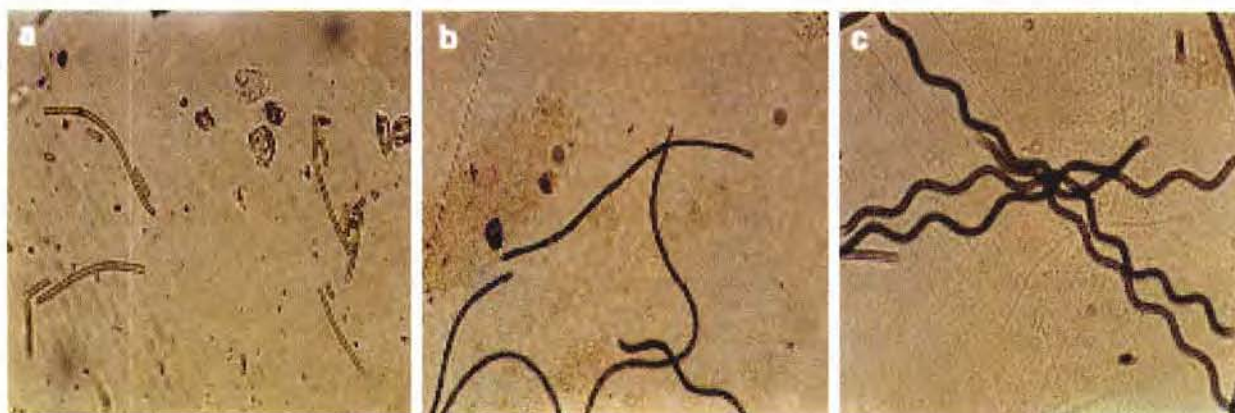


Fig. 5—Microphotographs showing characteristics of cells cultivated ($\times 10$ magnification). a. in GIMAP; b. in GIMAP & Shake flask combination; c. in Shake flask

much higher than that in shake flask. The shear stress developed in GIMAP at the critical speed (10 rev s^{-1}) used in this work was estimated to be 1.3 N/m^2 using procedure reported by Mohanty (1998). This value is lower by a factor of three than the level of shear stress existing in a typical laboratory scale fermentor using turbine impeller at 5 rev s^{-1} .

It is hypothesized that shear developed in GIMAP does not allow toughening of the cell walls as a result of which, softer and more permeable cell mass is grown (Fig. 5). Interestingly, the viability of cell was unaffected. To check the reversibility of this process (if cell wall softening/toughening was purely hydrodynamic and physical phenomenon without involving biochemical alterations, this should be reversible), *Spirulina* was cultivated in the combination of GIMAP and shake flask. The partially grown GIMAP culture was then subjected to shake flask conditions for further growth. GIMAP cells were not only shorter in length but were also translucent and the cell walls become thick and dark when the same culture was subjected to shaker conditions (Fig. 5).

When the biomass was cultivated in combination GIMAP followed by shake flask, no phycobiliproteins were released in the phosphate buffer ($\text{pH } 6.7$) without cell rupture. These cells have intermediate size and cell wall nature as compared to GIMAP or Shake flask (Fig. 5). In this case the pigment had to be recovered by the traditional method involving cell lysis. Thus, the pigment release phenomenon in GIMAP culture may be attributed to the reversible effect of hydrodynamic stress developed in GIMAP and subsequent lowering of the pH . As discussed

earlier, the shear stress induced change seems to be restricted only to the cell wall as the growth rate and biomass generation in GIMAP was in fact higher. Location of phycobilisomes in the thylakoid membrane can be a reason for the release. The phycocyanin content of the resultant extract was found to be 23% out of the total 25% of phycocyanin. The residual cell mass was found to have a protein content of 25%. In the case of pigments recovery by cell disruption, no cell mass byproduct is available. Thus, the recovery of value added metabolite from GIMAP cultivated biomass could improve economic feasibility of the overall process in two ways: simpler separation process and additional byproduct namely, exhaust cell mass.

The cultivation time is high for algal strains. As a result, it is necessary to have large hold-up capacities of the photobioreactor. If biomass production is the objective (no phycobiliprotein recovery), it may be cheaper to use combination of GIMAP and pond (shaker) for economic reasons. In principle, if a large number of small viable cells are formed in the GIMAP in a few days time, these cells may be allowed to grow into larger trichomes in the ponds. This will reduce the size (and subsequent power) requirements of GIMAP.

Combination of GIMAP and Shake Flask

For optimum growth rates using combination of GIMAP and open pond, the data from batch cultivation in GIMAP and shake flask (Fig. 3) were interpreted differently. The daily increment in biomass when cultivated in GIMAP increased up to 6 days to reach a value of 0.62 g/day , and then decreased gradually. Hence in the experiments using

combination, *Spirulina* was cultivated in GIMAP for first 6 days (at this point of time, total biomass achieved in GIMAP is 2.32 times higher than that in shake flask) and transferred to shake flask for the next 9 days. Combination cultivation resulted in only 3.05 g l⁻¹ in 15 days whereas use of GIMAP only had produced 4.44 g l⁻¹ of biomass in 15 days. However, combination resulted in higher biomass production than that in shake flask, which had produced only 2.97 g l⁻¹ in 20 days. This suggested that net growth rates achieved in GIMAP were always higher than those in shake flask even after 6 days in the subsequent cultivation period where daily incremental rate starts falling (Fig. 3). Thus, it may be concluded that carbon dioxide limitation existed even after 6 days cultivation in GIMAP. It may be noted that the total batch time varied from 10 to 20 days in the three methods of cultivation (GIMAP, Combination and Shake flask). One way to normalize the data for comparison is defining dimensionless time. This was done by expressing the actual time as a fraction of the total batch time of the respective experiment. Figure 6 shows the plot of biomass as a function of the dimensionless time in GIMAP and shaker conditions. It was evident that the growth rate was always higher in the GIMAP. The results with combination (not shown in the Fig. 6) match with the GIMAP for the first part and fall in between at the later stage. In spite of the fact that combination gives lower growth rates than that using GIMAP for the entire cultivation, one may recommend the combination depending upon the economics.

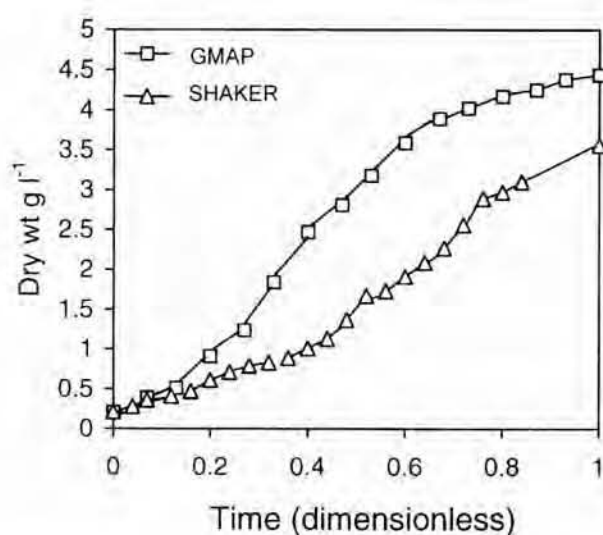


Fig. 6—Comparison of Biomass Yield on Dimensionless Time Scale

Conclusion

The novel design GIMAP using gas inducing hollow impeller was found to give 2.3 times higher growth rates for the model cultivation system using *Spirulina*. The design offers several advantages like low power consumption, low land requirement, ease of scale-up criteria (constant H/D ratio, $D_{\text{vessel}}:D_{\text{impeller}}$ ratio etc.), easy construction and operation. Further, the performance parameters of GIMAP were found to be in line with the recommendations of typical large-scale photobioreactors. In the case of *Spirulina*, it also exhibited a specific advantage for recovery of phycobiliproteins without cell lysis. This design of photobioreactor seems very promising and can be effectively used.

Acknowledgement

This work was supported by the grant given by the Department of Biotechnology, Government of India, vide letter no. BT/PR2200/PID/23/72/2000.

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