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Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae

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Abstract

Engineering analyses combined with experimental observations in horizontal tubular photobioreactors and vertical bubble columns are used to demonstrate the potential of pneumatically mixed vertical devices for large-scale outdoor culture of photosynthetic microorganisms. Whereas the horizontal tubular systems have been extensively investigated, their scalability is limited. Horizontal tubular photobioreactors and vertical bubble column type units differ substantially in many ways, particularly with respect to the surface–to–volume ratio, the amount of gas in dispersion, the gas–liquid mass transfer characteristics, the nature of the fluid movement and the internal irradiance levels. As illustrated for eicosapentaenoic acid production from the microalga *Phaeodactylum tricornutum*, a realistic commercial process cannot rely on horizontal tubular photobioreactor technology. In bubble columns, presence of gas bubbles generally enhances internal irradiance when the Sun is low on the horizon. Near solar noon, the bubbles diminish the internal column irradiance relative to the ungassed state. The optimal dimensions of vertical column photobioreactors are about 0.2 m diameter and 4 m column height. Parallel east–west oriented rows of such columns located at 36.8°N latitude need an optimal inter-row spacing of about 3.5 m. In vertical columns the biomass productivity varies substantially during the year: the peak productivity during summer may be several times greater than in the winter. This seasonal variation occurs also in horizontal tubular units, but is much less pronounced. Under identical conditions, the volumetric biomass productivity in a bubble column is $\sim 60\%$ of that in a 0.06 m diameter horizontal tubular loop, but there is substantial scope for raising this value.

Keywords: Microalgae; Photobioreactors; Scale-up; Photosynthetic culture; Eicosapentaenoic acid; *Phaeodactylum tricornutum*

1. Introduction

Photobioreactors for large-scale monoculture of microalgae have conventionally been designed as devices with large surface–to–volume ratios. Various types of tubular photobioreactors are examples of this approach (Lee, 1986; Borowitzka,

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1996). These reactors occupy vast land areas: they are expensive to build; difficult to maintain; and only somewhat scaleable. Tubular photobioreactors can usefully satisfy only medium level production demands. Attempted, large–scale production in horizontal tubular loops has failed quite spectacularly in one case (Fig. 1); hence, other reactor configurations are needed for the production of larger quantities of pharmacologically active compounds that certain microalgae can potentially produce.

The areal productivity, i.e. productivity per unit land area, is low for conventional tubular photobioreactors and in large units as well as modular designs, sterile operation to the levels demanded in the pharmaceutical industry is difficult. Some low surface–to–volume, pneumatically agitated photobioreactors can potentially overcome these significant disadvantages. Examples of the latter type are bubble columns and airlift bioreactors. Large scale culture of microalgae in these systems has not been investigated as it has always been assumed that small surface–to–volume ratios of these devices would make them ineffective. This need not be so as reported in this work which deals with comparative outdoor evaluation of pilot scale bubble column photobioreactors with respect to performance in horizontal tubular loops.

Data are reported on three aspects of comparative characterization: (a) gas–liquid hydrodynamics and mass transfer; (b) internal irradiance levels as functions of Sun's location relative to the photobioreactors; and (c) performance during culture of the microalga *Phaeodactylum tricorntum*. Also reported are the effects of hydrodynamics on survival behavior of algal cells.

2. Comparison of performance

The vertical and the horizontal tubular photobioreactors differ in several significant ways including differences in light regimens, gas–liquid hydrodynamics and mass transfer behavior. Some of these factors—e.g. hydrodynamics and light regimen—are interrelated. Their impact on culture performance is discussed below.

2.1. Hydrodynamics

The hydrodynamics of flow in horizontal tubes and vertical columns are generally quite different. The necessarily gas sparged bubble columns and airlift reactors tend to have substantially greater gas holdups than do horizontal tubular solar receivers. The latter are virtually free of gas and any bubbles present are localized to a narrow zone along the upper portion of the tubes; moreover, the bubbles are relatively small. In contrast, there are many more and larger bubbles in vertical photobioreactors and the gas–liquid flow is much more chaotic than the highly directional flow in a small-diameter horizontal pipe. Differences in gas holdup and the bubble size affect light penetration, gas–liquid mass transfer, mixing and shear stress levels.

2.1.1. Gas holdup

Gas holdup measurements in a bubble column photobioreactor confirm that holdup increases with specific power input in accordance with previously published data (Chisti, 1989). Thus, the holdup data in tap water (Fig. 2) closely followed the equation

$$\varepsilon = 3.317 \times 10^{-4}\left(\frac{P_G}{V_L}\right)^{0.97}, \quad (1)$$

whereas data in sea water (Fig. 2) agreed with the correlation

$$\varepsilon = 7.958 \times 10^{-5}\left(\frac{P_G}{V_L}\right)^{1.249}. \quad (2)$$

In these equations the power input due to aeration is calculated as

$$\frac{P_G}{V_L} = \rho_L g U_G, \quad (3)$$

where ρ_L is the density of the liquid, g is the gravitational acceleration and U_G is the superficial gas velocity in the column. Eqs. (1) and (2) were established by Chisti (1989) for tap water and 0.15 M aqueous sodium chloride, respectively. The equations apply to the bubble flow regime, or U_G values less than about 0.05 m s^{-1} ($P_G/V_L \approx 500$ W m^{-3}) The obvious decline in the rate of increase of gas holdup with increasing specific

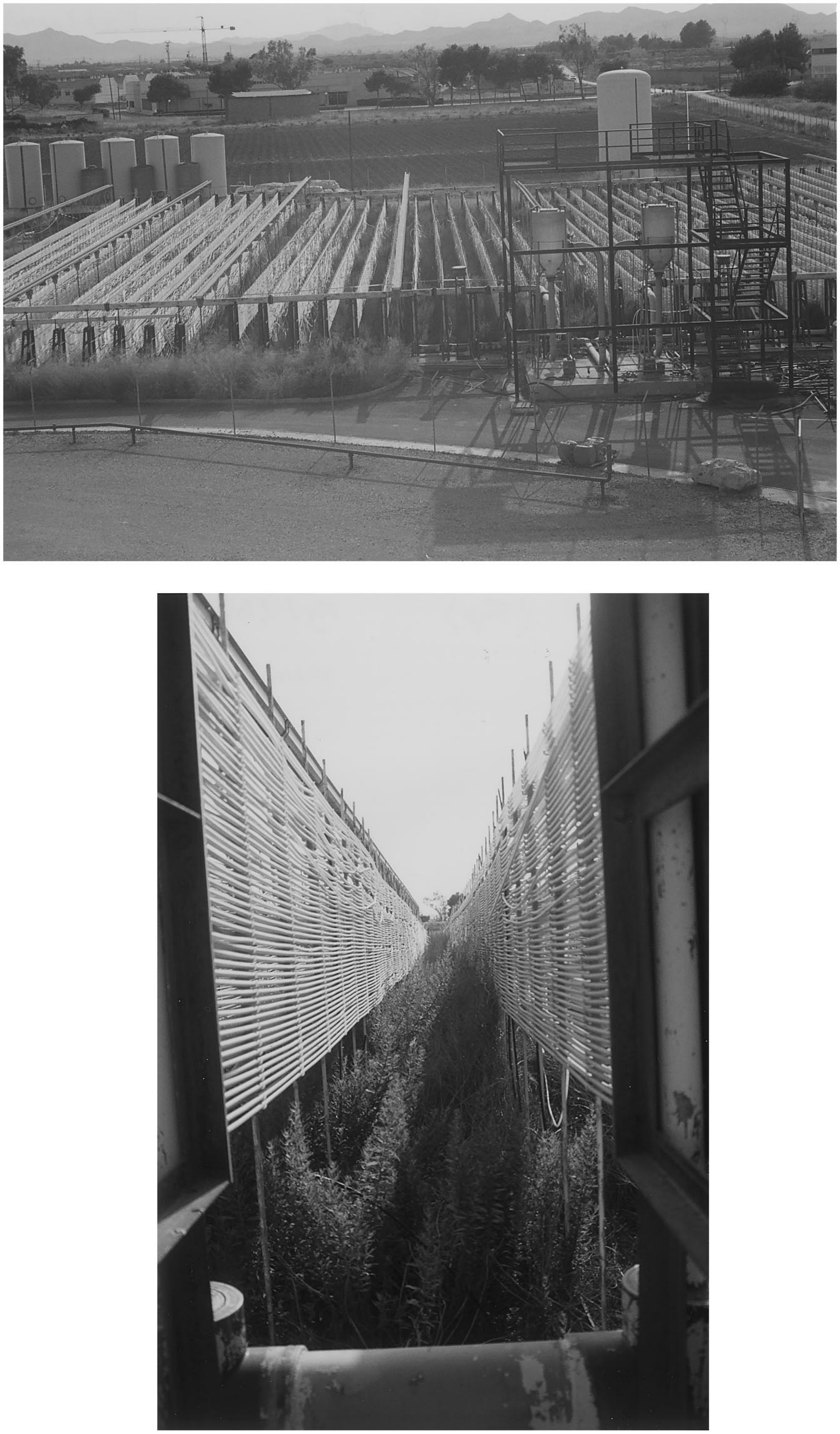

Fig. 1. A commercial horizontal tubular bioreactor facility that failed to perform to expectations and was abandoned. This facility was located in Cartagena, Spain and it was owned by Photobioreactors Ltd.

power input values above about 400 W m^{-3} (Fig. 2b) is due to a well-known change in the flow regime from bubble to churn turbulent flow (Chisti, 1989). Equations for estimating gas holdup in the latter regime have been published (Chisti, 1989), but that regime is not likely to be used in microalgal culture in vertical photobioreactors because of the cell damaging potential of intense turbulence (Silva et al., 1987; Suzuki et al., 1995; Contreras et al., 1998; Chisti, 1999a). Data in Fig. 2 reveal that the flow transition occurs earlier in tap water, around a power input of 280

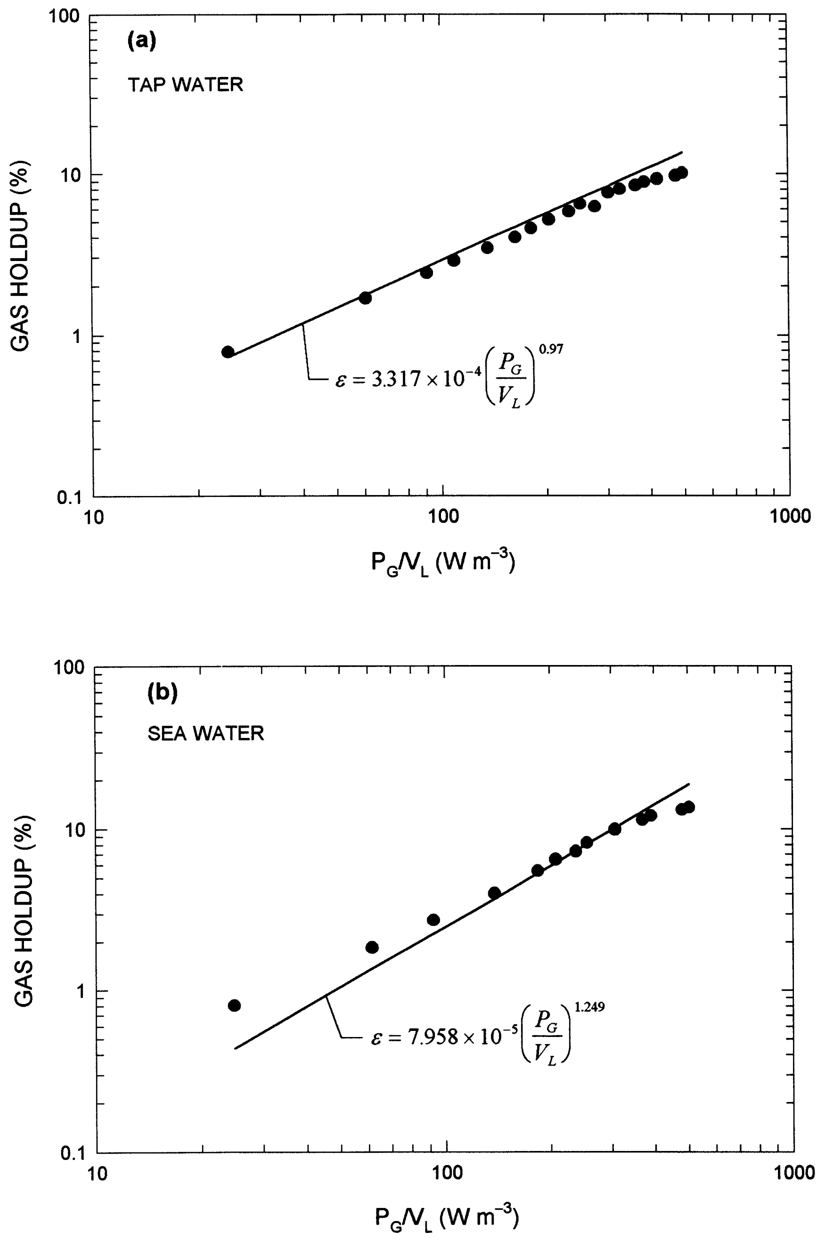

Fig. 2. Variation of fractional gas holdup with specific power input in the bubble column: (a) tap water; and (b) sea water.

Table 1
Composition of Almería bay Mediterranean sea water (Contreras et al., 1998)

Component	Amount (kg m^{-3})
Chloride	20.812
Sulfate	2.866
Bicarbonate	0.168
Na^{+}	10.552
Mg^{2+}	1.362
Ca^{2+}	0.519
K^{+}	0.413
Total dissolved solids	36.608
Total organic carbon	Negligible
Ionic strength	0.727

W m^{-3}. The coalescence inhibiting effect of salts in sea water allows bubble flow to persist longer in that medium. Similar results have been reported earlier (Chisti, 1989). The data in Fig. 2 were obtained in an outdoor bubble column with a gas-free liquid height of 1.93 m. Either tap water or Mediterranean sea water was the liquid phase. The composition of the latter is noted in Table 1. Except during culture, a small amount of hypochlorite (50 ml corresponding to 35 g l^{-1} free chlorine) was added to sea water to prevent microbial growth. Air was sparged through a perforated pipe with 17 holes of 1 mm diameter, giving a gas flow area that was 0.05% of the total column cross-sectional area. The sparger head was shaped into a cross that was located centrally, 0.076 m above the base of the column. The internal diameter of the column was 0.19 m and it was made of 3.3 mm thick poly(methyl methacrylate), except for the lower 0.25 m section that was made of stainless steel. For the power input range of relevance here, Eqs. (1) and (2) are known to apply to bubble columns irrespective of the size of the sparger holes. Agreement of the data with Eqs. (1) and (2) suggests that changes in sparger characteristics are unlikely to affect the holdup in the two fluids.

2.1.2. *Effects of holdup on irradiance*

The effect of gas holdup on the measured internal irradiance inside the column is shown in Figs. 3–6. The data in these figures were obtained on clear, cloudless days. The irradiance levels were measured using one spherical quantum sensor—either a Biospherical QSL 100 (Biospherical Instruments Inc., San Diego, CA) unit or a LI-COR LI-196SA (Li-cor, Inc., Lincoln, NE) device—located always at the centerline of the column, 0.87 m below the static surface of the liquid. Data were

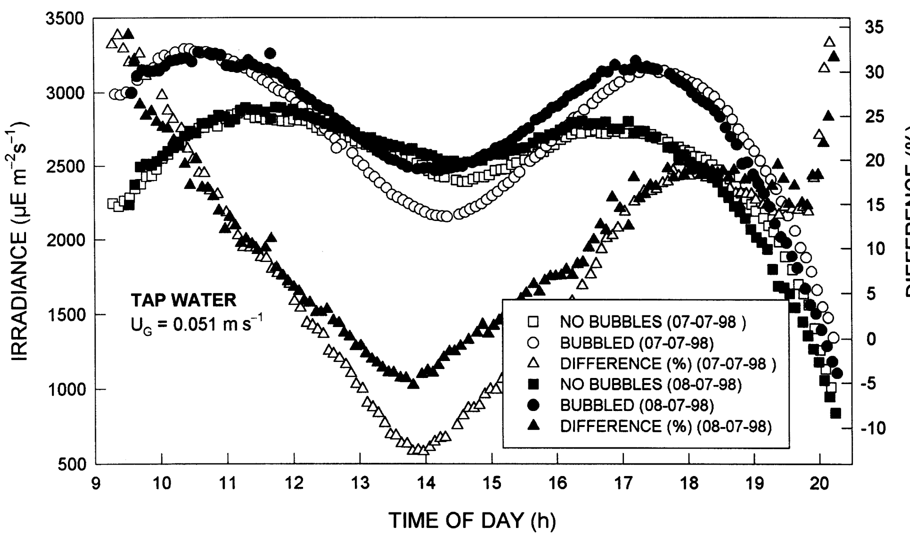

Fig. 3. Daily variation in internal irradiance in the bubble column in tap water. The gas holdup value was 0.101. The difference between the aerated and unaerated irradiance levels is also noted. The data are for 7 and 8 July, 1998.

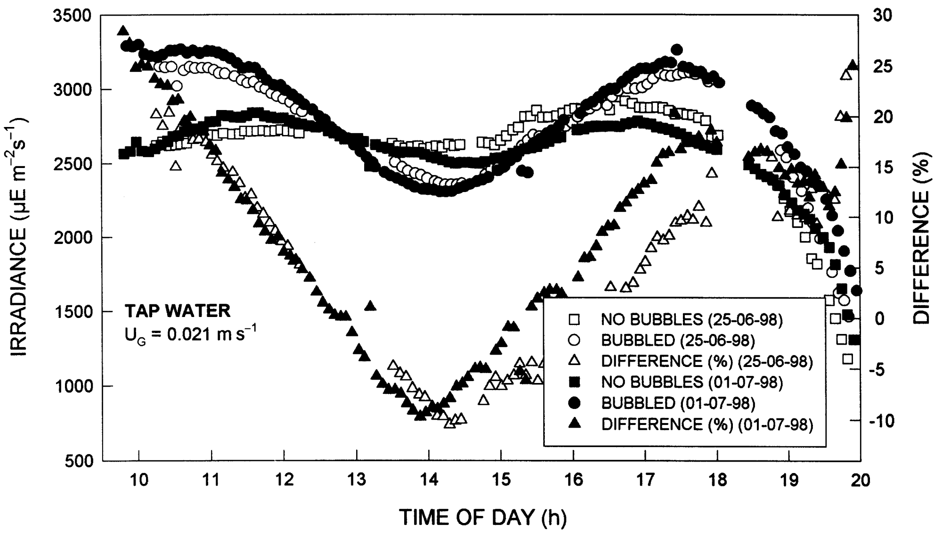

Fig. 4. Daily variation in internal irradiance in the bubble column in tap water. The gas holdup value was 0.052. The difference between the aerated and unaerated irradiance levels is also noted. The data are for 25 June and 1 July, 1998.

acquired using a DaqBook/100–901 (IOtech, Inc., Cleveland, OH) data acquisition card connected to an IBM compatible 486 computer operating at 100 MHz.

The trough in irradiance around 14 h (Figs. 3–6), or solar noon, occurs when the Sun is at its apex and most nearly aligned with the vertical axis of the bubble column (Fig. 7a). In this position, the direct sunlight flux on the column is at it's lowest, hence, the internal irradiance level is low. Also, around solar noon, because of a high angle of incidence, a greater amount of radiation is reflected off the surface of the column compared to when the angle of incidence is small (afternoon, morning). The irradiance peaks at 11:30 and 17:30 h in the absence of bubbling (Figs. 3–5) occur when the Sun is low on the horizon and most closely aligned with the normal to the bubble column's vertical axis (Fig. 7b). Typically, the internal irradiance levels at these times exceed those at solar noon. This general pattern is seen irrespective of whether the reactor is aerated or if it is gas-free. Of course, in a horizontally placed tubular loop the pattern of variation of irradiance with time will be different, the irradiance profile will have a single peak (García Camacho et al., 1999) that will coincide with the solar noon.

As shown for tap water in Fig. 3, bubbling at a constant high aeration rate ($U_G = 0.051$ m s^{-1}) corresponding to a gas holdup of 0.101, may either enhance or reduce the level of internal irradiance depending on the position of the light source, the Sun. Irradiance is enhanced during periods when the Sun is most closely aligned with the normal to the vertical axis of the column (Fig. 7b). Around solar noon, when the Sun is best aligned with the vertical axis (Fig. 7a), presence of bubbles reduces internal irradiance. Depending on time, the difference between the aerated and unaerated irradiance levels may range from -12 to $+35\%$ (Fig. 3). The effect of aeration on internal irradiance diminishes as the gas flow rate is reduced (Fig. 4); however, even at a low superficial gas velocity of 0.0025 m s^{-1}, corresponding to a fractional gas holdup of 0.008, or less than 1%, the irradiance level is affected by up to $\sim 15\%$ relative to gas-free operation in tap water (Fig. 5). Aeration at any level consistently shifts the irradiance maxima relative to the ungassed state (Figs. 3–6), the morning irradiance maximum occurs up to 1 h earlier than in the absence of bubbling. Similarly, the afternoon irradiance maximum occurs up to 1 h later than when no aeration is employed.

Irradiance enhancement and reduction by bubbles result from a combination of two factors: shading of culture by bubbles; and the phenomenon of total internal reflectance. In the bubble flow regime, the bubble shape is predominantly ellipsoidal (Fig. 8) with the major axis of the bubble roughly perpendicular to the vertical axis of the bubble column. At a given gas holdup and bubble size, the bubbles provide a greater shading effect when the light source is high on the horizon than when it is low (Fig. 8a). Furthermore, when the light source is high, i.e. closely aligned with the vertical axis of the column, the surface of the bubble reflects incident light poorly and much of the radiation penetrates into the gas phase. In contrast, when the light source is low on the horizon, it is more closely aligned with the major axis of the bubble (Fig. 8b) and the low approach angle of radiation relative to the gas–liquid interface causes total internal reflection of a large portion of the radiation (Fig. 8b). In effect, each bubble becomes a reflector that allows better dispersion of incident radiation within the liquid. This total internal reflectance more than compensates for the smaller shadows of sideways illumination, the shadow region behind a bubble is illuminated by reflectance from other nearby bubbles. Because of reflectance the total internal irradiance is greater relative to bubble-free operation when much of the incident radiation simply passes through the bubble column. Whether these effects will occur and to what extent, in optically less clear algal culture broths remains to be seen. Experiments are currently underway to establish this point.

A comparison of Figs. 3–5 reveals that both the irradiance enhancement and reduction effects in tap water are stronger at the higher aeration rate when the gas holdup and the number of bubbles are greater. With more bubbles there are more reflectors in the fluid when the light source is low on the horizon. Similarly, the shadowing effect of many more bubbles is stronger when the Sun is at its apex. The data in Figs. 3–5 are for the bubble flow regime and the irradiance patterns could be different in the churn turbulent regime and also when the gas holdup is so high that clear patches of liquid allowing through penetration of light do not exist.

Comparing Figs. 4 and 6 for tap and sea water, respectively, at identical aeration rates, the patterns of variation in irradiance are generally similar; however, the irradiance enhancement in sea water is somewhat greater than in tap water. In

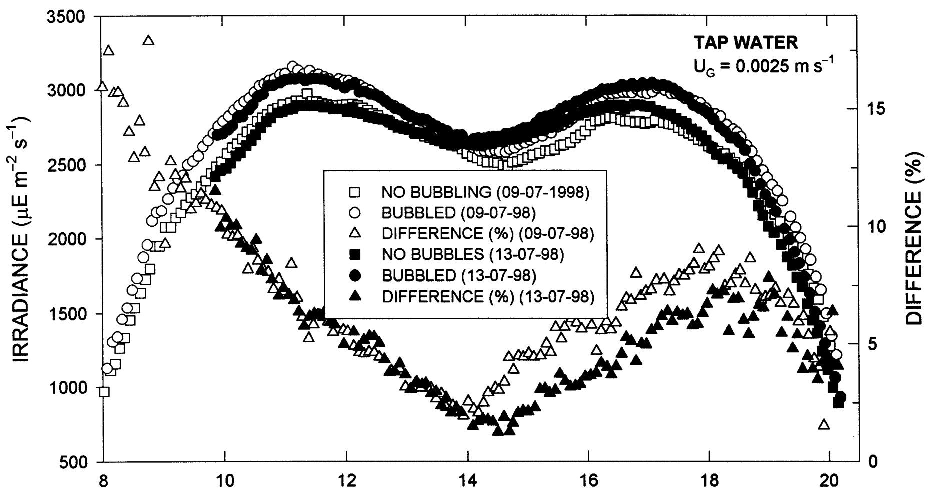

Fig. 5. Daily variation in internal irradiance in the bubble column in tap water. The gas holdup value was 0.008. The difference between the aerated and unaerated irradiance levels is also noted. The data are for 9 and 13 July, 1998.

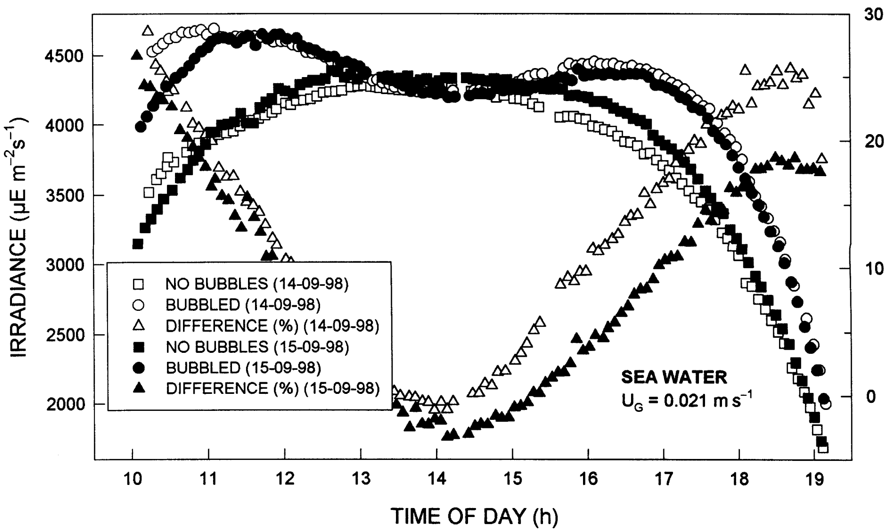

Fig. 6. Daily variation in internal irradiance in the bubble column in sea water. The gas holdup value was 0.065. The difference between the aerated and unaerated irradiance levels is also noted. The data are for 14 and 15 September, 1998.

contrast, the irradiance diminishing effect is virtually non-existent in sea water up to a gas velocity of 0.021 m s^{-1}: the bubble size is slightly smaller than in tap water, the holdup is somewhat greater, and the sphericity of the bubbles is higher. These factors combine to reduce the shadowing effect of the bubbles during midday relative to that experienced in tap water. Quantitatively, the shadowing effect of bubbles can be shown to depend on bubble diameter and the gas holdup as follows:

$$\text{Shadowing effect} \propto \frac{\varepsilon}{d_B}, \tag{4}$$

where ε is the fractional gas holdup and d_B is the mean bubble diameter. The above relationship originates from the fact that the light shadowing effect of bubbles depends on their number in a given volume (V_D) of dispersion and on the mean diameter of bubbles, i.e.

$$\text{Shadowing effect} \propto \frac{N_B \cdot d_B^2}{V_D}. \tag{5}$$

Because the number of bubbles depends on the volume of gas (V_G) in dispersion,

$$N_B \propto \frac{V_G}{d_B^3} \tag{6}$$

and, by definition, the overall gas holdup is given as

$$\varepsilon = \frac{V_G}{V_G + V_L} = \frac{V_G}{V_D}, \tag{7}$$

relationship (4) is derived readily. Expression (4) does not consider bubble sphericity effects, however, with reference to Fig. 8a it is easily shown that at constant gas holdup an increase in sphericity should reduce the size of the shadow and hence the irradiance diminishing effect at midday.

At much higher aeration rates than in Fig. 6, the situation in Fig. 9 is observed in sea water. Fig. 9 is for a highly turbulent system ($P_G/V_L \approx$ 500 W m^{-3}) when the bubble size is quite small and the gas holdup is relatively high ($>13\%$) so that the gas–liquid dispersion is cloudy (Fig. 10). Light penetration is now reduced quite a lot relative to the bubble-free case (Fig. 9). Note that in Fig. 9, the irradiance enhancement seen between 10 and 12 h is not repeated to the same extent in the afternoon around 16–18 h. This is because at commencement of aeration in the morning the fluid is not as cloudy as in the afternoon, the fine bubbles responsible for cloudiness accumulate with time and their concentration gradually increases to some steady state value from the time of commencement of aeration. The size of these fine bubbles is such that even several hours after the gas flow is stopped, the water remains cloudy; however, overnight the bubbles rise and the water becomes clear.

Note that the internal irradiance level is a function of the measurement position. The discussion here pertained to the lowest irradiance values, i.e. those at the deepest point (the centerline) from the principal illuminated surface or the walls of the vessel.

2.2. Light regimen

2.2.1. Effects of depth and fluid motion

Light regimens in vertical bubble columns or airlift reactors and the horizontal tubular loops differ. Some of the factors responsible for the differences have already been discussed, including different orientations of the bioreactors and the presence of a large number of gas bubbles in pneumatically agitated vertical vessels. Another factor is the culture depth. Depth is typically 0.02–0.06 m in horizontal tubes, but much greater, e.g. 0.2 m or more, in vertical columns. At constant external illumination, small changes in culture depth are known to generally affect productivity of microalgae cultures (Kobayashi and Fujita, 1997), including those of *P. tricornutum*.

Because of a lower depth and because the light intensity declines exponentially with depth according to Beer–Lambert law, the light level is always greater in the relatively thin horizontal tubes than in a larger diameter vertical column when the systems are compared at identical orientation of the light source relative to the reactors' surface. For otherwise fixed conditions, increase in diameter of tubes significantly reduces culture productivity (Kobayashi and Fujita, 1997). For example, Kobayashi and Fujita (1997) showed using *Chlorella* sp. cultures that growth rate and cell concentration were reduced with increasing diameter of thin vertical tubes over the range 0.016–0.050 m internal diameter. Although the volumetric productivity declined, the culture pro-

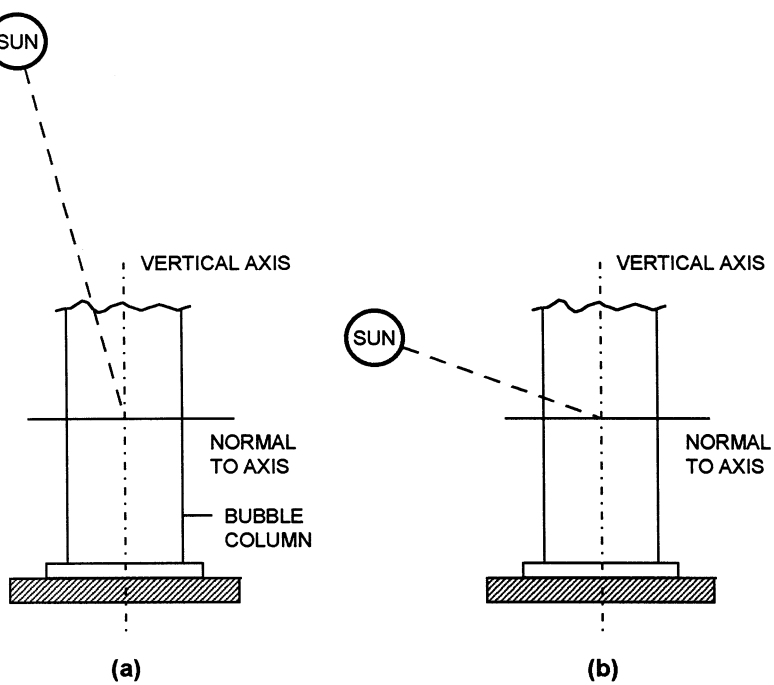

Fig. 7. Schematic representation of the Sun's position with respect to the axes of the bubble column in the northern hemisphere: (a) at solar noon; and (b) in afternoon or early morning.

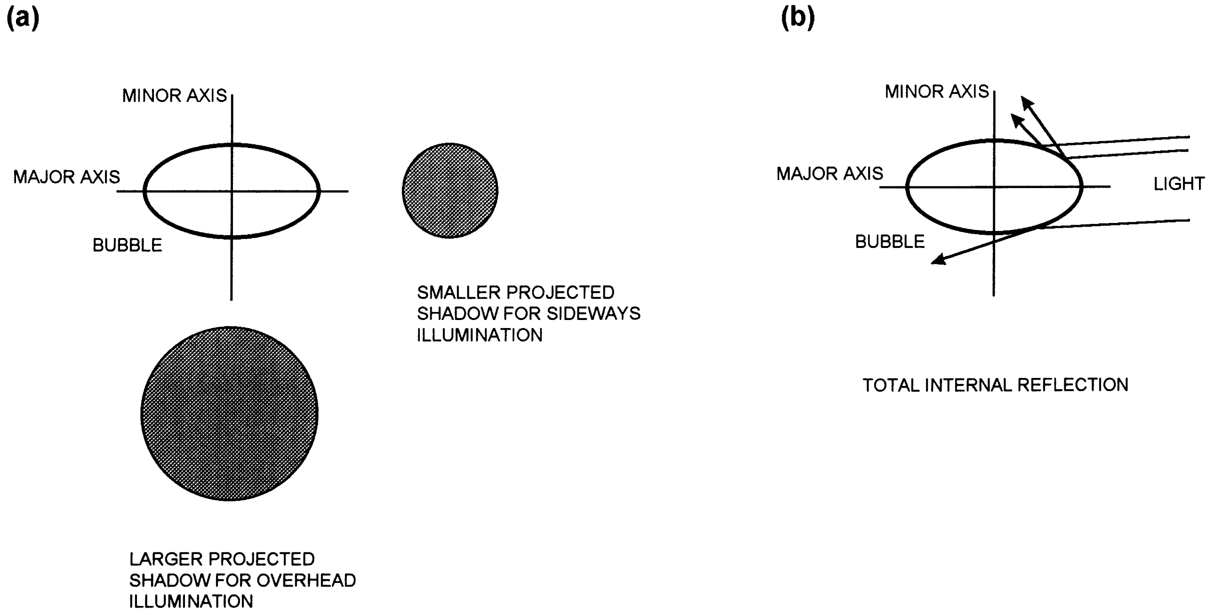

Fig. 8. Projected shadows of an ellipsoidal bubble illuminated from top or side (a). The shadow is much larger for overhead illumination compared to when sideways illumination occurs. (b) Total internal reflection at bubble–liquid interface when the incident radiation beam is closely aligned with the major axis of the bubble. The minor axis of the bubble is generally parallel to the vertical axis of the bubble column.

ductivity (kg m^{-2} h^{-1}) per unit bioreactor footprint area increased with increasing tube diameter (Kobayashi and Fujita, 1997) for a constant external level of illumination. The areal productivity more than doubled with increasing tube diameter over the noted range.

Although culture depth is an important influence on production characteristics, consideration of depth alone is unsatisfactory; the nature and magnitude of fluid movement also need taking into account. In any photobioreactor, vertical or horizontal, the depth to which light penetrates depends on the external irradiance level, the biomass concentration and the absorption characteristics of the biomass. The culture volume adjacent to the illuminated walls is relatively better lit. At a certain depth, the light intensity falls to growth limiting levels and a 'dark zone' is encountered. Because short dark periods interspersed among sufficiently long and intense light periods do not affect the growth rate, the growth reducing potential of a dark zone is minimized by ensuring that elements of fluid do not reside in this zone for long (Molina Grima et al., 1999). A dark zone must exist for at least part of the year even in a shallow photobioreactor tube that is optimally designed to make full use of the available light during summer noons. The depth of the dark zone is inevitably greater in large-diameter vertical columns than in narrow horizontal tubes; however, because of a highly chaotic flow regime and movement of bubbles, the radial movement of fluid that is necessary for improved light–dark cycling is substantially greater in bubble columns than in tubular loops. The latter have a highly directional flow in which the radial component is small compared to the axial component. Yet, the better radial flow in vertical aerated columns does not fully compensate for their larger diameter, hence, the volumetric productivity is reduced relative to narrow horizontal tubular photobioreactors. For best performance, bubble columns need to be operated at the highest feasible aeration rates consistent with the shear tolerance of the microalga; however, the aeration rate must not be so high as to produce a gas holdup level that prevents light transmission through the column. Ideally, the culture environment in the column should be manipulated to promote formation of relatively large bubbles ($d_B \geq 0.006$ m) that rise rapidly. Radial flow into and out of the central dark core can be enhanced by ensuring that quite large spherical cap bubbles ($d_B \approx 0.02$–0.04 m) rise through this zone intermittently (Fig. 11). These schemes are currently being experimented with.

In summary, the low surface–to–volume photobioreactors can potentially approach the volumetric productivity of horizontal tubular loops, however, attaining this requires that: (i) the cumulative residence time of the cells in the light zone of the reactor should be comparable to that of the tubular device; and (ii) the frequency of light–dark transition cycle in the 'deeper' vessels should be comparable to that of tubular systems. These two objectives can be substantially achieved in bubble columns and airlift bioreactors by enhancing radial movement of cells. In airlift bioreactors, but not in batch bubble columns, radial flow may be enhanced by using static mixing elements.

2.2.2. Vertical photobioreactor emplacement

The maximum number of the vertical column reactors that may be accommodated in a given area depends on the height of the column which, together with the position of the Sun, establishes the maximum extent of the column's shadow on the ground. The length of the shadow from the column's base is given by

$$L_s = \frac{h_c}{\tan\theta_i}, \tag{8}$$

where h_c is the height of the column and θ_i is the angle of incidence of the direct solar radiation. The angle of incidence—the inclination of the Sun from the normal to the vertical axis of the bubble column—depends on the geographic latitude ϕ, the day of the year N, and the solar hour $\hbar$; the angle of incidence is given as (Liu and Jordan, 1960):

$$\theta_i = 90° - \cos^{-1}(\cos\delta \cdot \cos\phi \cdot \cos\omega + \sin\delta \cdot \sin\phi) \tag{9}$$

where ϕ is the geographic latitude. The angles ω and δ are related to the solar hour and the day of the year (Liu and Jordan, 1960), respectively, as follows:

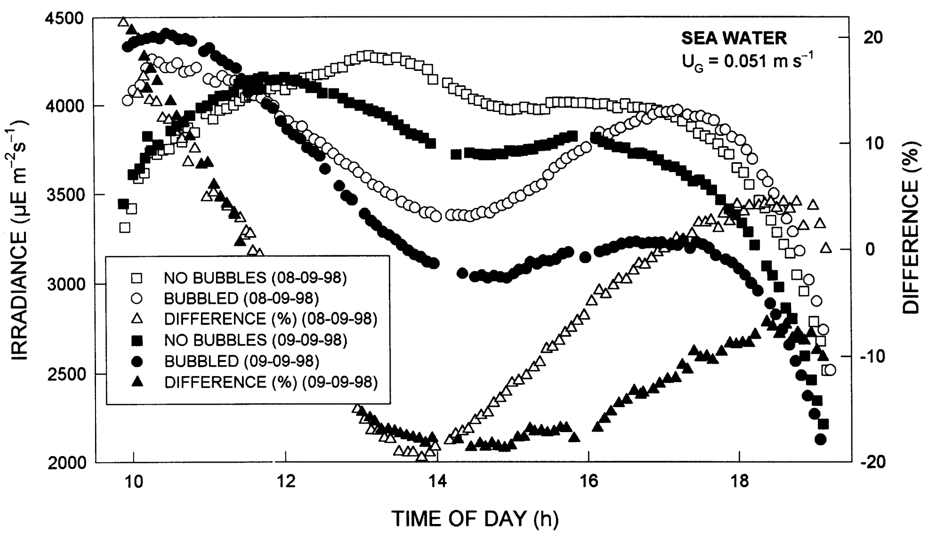

Fig. 9. Daily variation in internal irradiance in the bubble column in sea water. The gas holdup value was 0.135. The difference between the aerated and unaerated irradiance levels is also noted. The data are for 8 and 9 September, 1998.

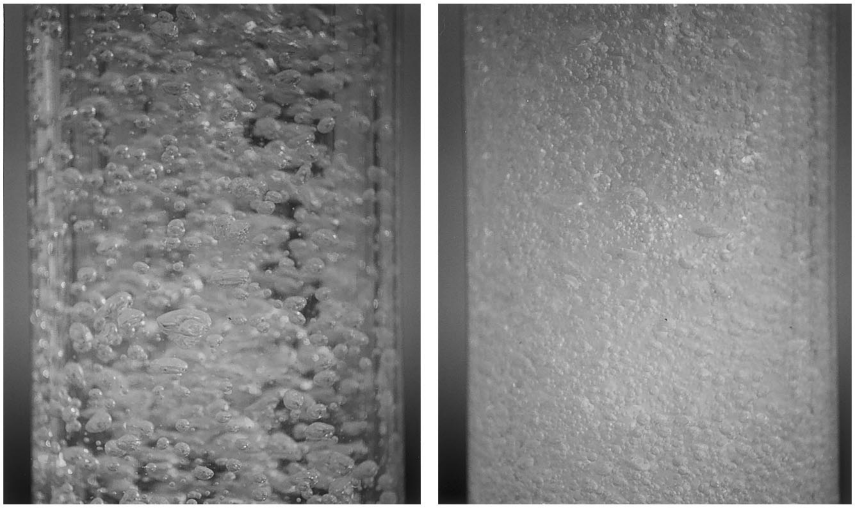

Fig. 10. The bubble dispersion on the left allows through transmission of light whereas the cloudy dispersion on the right blocks light. These pictures were taken in sea water at aeration power inputs of 154 and 518 W m^{-3}, respectively. The perforated pipe sparger hole diameter was 1 mm in both cases.

$$\omega = 15(12 - \hbar), \quad (10)$$

and

$$\delta = 23.45 \cdot \sin\left(\frac{360(284+N)}{365}\right). \quad (11)$$

The loci of the maximum extent of the shadow of a 2 m tall bubble column are plotted in Fig. 12 for representative days in winter, spring, and summer seasons at the geographic location of Almería (36.8°N, 2.9°W). The maximum extent of the shadow in January is about 7.4 m, whereas the maximum extent in July is about 1.3 m. These distances are measured north–south between parallel east–west lines passing through the base of the vertical column and the tip of the column's shadow. Ideally, parallel east–west rows of bubble columns should be spaced by at least the maximum length of the shadow in winter. This would assure that the reactors are never mutually shaded, however, a more optimal setup would place the rows of reactors closer, about midway between the high extremes of the shadow length in the summer and the winter. Consequently, there will be no mutual shading in the summer but some shading would occur during the winter. In a single east–west row of columns the columns could be spaced quite close together; e.g. a spacing of 0.35 m between centers of adjacent columns of 0.2 m diameter. Close spacing within east–west rows has no impact on illumination, but it improves efficiency of land use.

2.3. Shear effects

Shear stress has been implicated as an important factor in culture of several microalgae (Silva et al., 1987; Suzuki et al., 1995; Contreras et al., 1998; Chisti, 1999a). A cell damaging hydrodynamic environment is easily attained in bubble columns and airlift reactors (Silva et al., 1987; Suzuki et al., 1995; Contreras et al., 1998; Chisti, 1998a), but damage to algal cells has never been documented within tubes of tubular photobioreactors. This may suggest that the damage is somehow linked with the presence of bubbles in pneumatically agitated devices, but extensive studies prove otherwise. With most microalgae, increasing aeration rate up to quite high values improves culture productivity (Silva et al., 1987; Contreras et al., 1998), but damage occurs when the turbulence is so intense that the fluid microeddy size approaches cellular dimension. Only in one case—that of the commercially important but unusually fragile marine alga *Dunaliella*—has damage been associated directly with the bubbles

(Silva et al., 1987). Survival of *Dunaliella* in aerated systems is improved by supplementing the culture with viscosity enhancers such as carboxymethyl cellulose and agar (Silva et al., 1987), but this approach may not be applicable universally (Chisti, 1999a).

In one study with *D. tertiolecta*, culture in a bubble column was quite successful, but when the bubble column was converted to an airlift device by inserting a vertical baffle, the productivity declined (Suzuki et al., 1995). Under conditions that were earlier identified as optimal, no growth was observed in the airlift reactor whereas good growth occurred in the bubble column. Microscopic examination showed significant disruption of the cells in the airlift device (Suzuki et al., 1995). This was associated with the hydrodynamic stresses generated as the culture flowed over the upper edge of the baffle into the downcomer (Suzuki et al., 1995). This effect could have been avoided, or at least minimized, by hydrodynamic smoothing of the upper and lower parts of the baffle to prevent flow separation (Chisti, 1998a). In the bubble column, the growth was sensitive to aeration rate: growth rate increased with increasing superficial gas velocity until a velocity of about 0.6 m min^{-1}, or a specific power input of ~ 98 W m^{-3}. Further increase in aeration rate reduced growth, apparently because of hydrodynamic stresses in the fluid (Suzuki et al., 1995). Under non-growth conditions (no light), the specific death rate in the bubble column was shown to increase with superficial gas velocity for velocities exceeding 0.6 m min^{-1} (Suzuki et al., 1995). At a fixed aeration velocity ($U_G = 1$ m min^{-1}), the specific death rate decreased with increasing height of the culture fluid in the column (Suzuki et al., 1995), probably because the specific power input and, hence, the turbulence intensity declined (Chisti, 1998a). Similar behavior has been reported with animal cells in bubble columns (Emery et al., 1987; Tramper et al., 1987a,b).

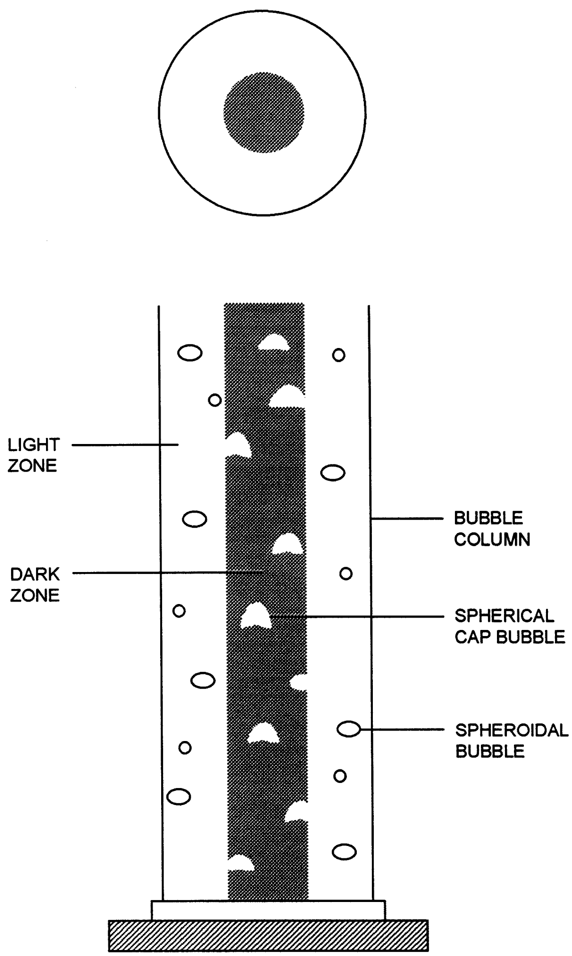

Fig. 11. Use of spherical cap bubbles in the central dark core to enhance radial light–dark interchange of fluid in a bubble column. The bubbles in the peripheral light zone are predominantly ellipsoidal with a diameter of about 0.006 m.

Improved growth with increasing aeration up to a limit has been documented for several algae including *Dunaliella* (Silva et al., 1987), *P. tricornutum* (Contreras et al., 1998) and others (Chisti, 1999a). This effect has been observed invariably under light limited conditions and it is best explained by improved light–dark cycling due to improved agitation. Indeed, the effect occurs even in the absence of gas when mechanical agitation is used to improve light–dark interchange of fluid in photobioreactors that contain a light limited zone. In indoor batch cultures of *P. tricornutum* carried out in a draft-tube sparged concentric tube internal-loop airlift photobioreactor at a constant illumination of 1200 μE m^{-2} s^{-1} on the surface of the reactor, Contreras et al. (1998) showed that the maximum specific growth rate increased with increasing aeration velocity in the riser zone. The increase occurred until a velocity of ~ 0.055 m

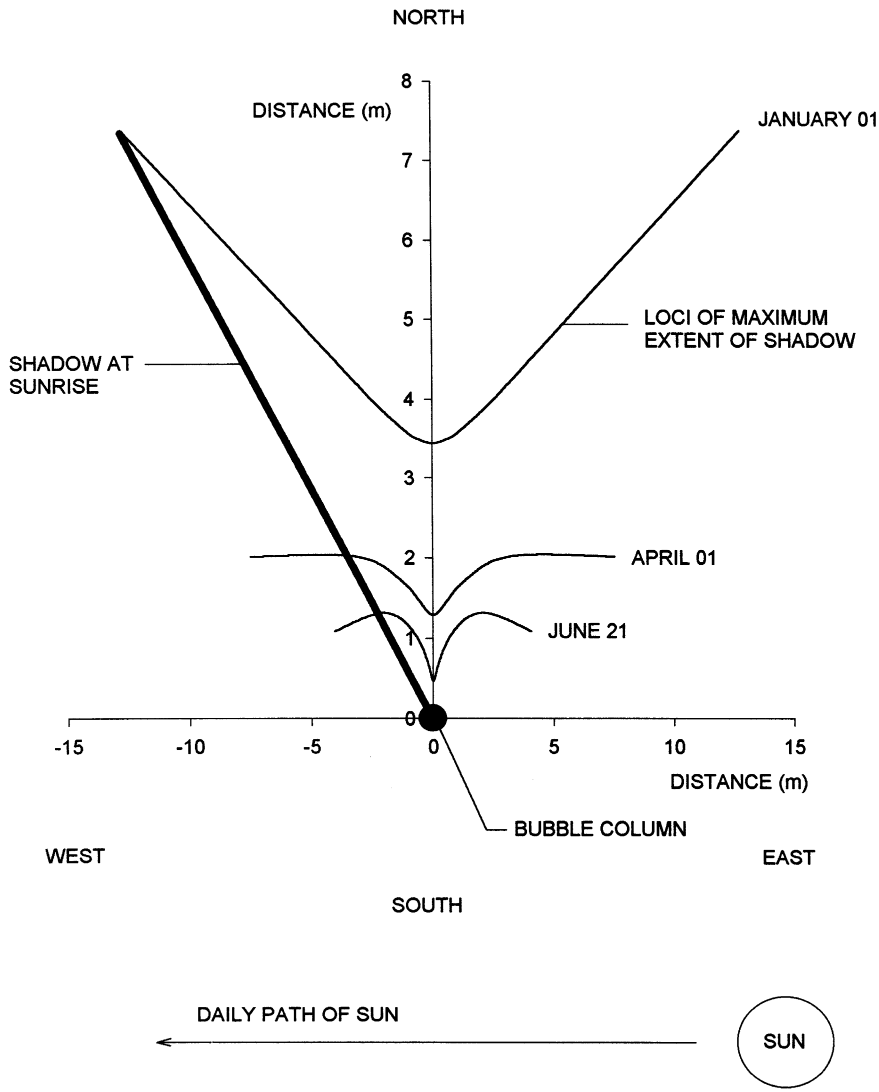

Fig. 12. Locus of the shadow of a 2 m tall bubble column during the day. The loci are shown for three representative seasons for 8 h (January) or 10 h (April, June) days, centered at midday.

s^{-1}, corresponding to a specific power input of ~ 270 W m^{-3}. Whether improved growth was due to enhanced fluid interchange between the light and dark zones was unclear and other factors may have contributed. The cultures were clearly light limited whenever the biomass concentration exceeded about 1 kg m^{-3}.

Increase in aeration rate beyond 0.055 m s^{-1} substantially reduced the specific growth rate of *P. tricornutum* cultures (Contreras et al., 1998). At the critical aeration rate value of 0.055 m s^{-1}, the calculated Kolmogoroff microeddy scale was 45 μm, or comparable to the dimensions of the algal cells (up to 35 μm long and 3 μm wide (Lewin et al., 1958)). Except for robust microorganisms, damage to cells has generally been observed when the dimensions of the isotropic turbulence microeddies approach those of the cells (Chisti, 1999a), however, the assumption of isotropic turbulence is almost never justifiable under typical operation of bubble columns and airlift systems (Chisti, 1998a).

Why has cell damage not been observed in horizontal tubular photobioreactor pipes is readily explained. For a 200 m straight run smooth horizontal tube of 0.06 m diameter, the culture velocity would have to exceed 1.14 m s^{-1} for the microscale of turbulence to approach 45 μm—the value that damaged the *P. tricornutum* cells in the vertical airlift device. This velocity value is easily estimated from the relationship between the Kolmogoroff microeddy length scale (ℓ) and the specific energy dissipation rate (Chisti, 1999a):

$$\ell = \left(\frac{\mu_L}{\rho_L}\right)^{3/4} E^{-1/4}. \quad (12)$$

The specific energy dissipation rate E in (Eq. (12)) is related with the pressured drop (ΔP), i.e.

$$E = \frac{U_L \Delta P}{\rho_L L}, \quad (13)$$

where L is the length of the tube and U_L is the culture velocity. The pressure drop in (Eq. (13)) is obtained using the expression:

$$\Delta P = 2C_f \frac{L}{d} \rho_L U_L^2 . \quad (14)$$

In (Eq. (14)), the Fanning friction factor (C_f) is obtained from the Blasius equation:

$$C_f = 0.0792\left(\frac{\rho_L U_L d}{\mu_L}\right)^{-0.25}, \quad (15)$$

where d is the diameter of the tubing. For the above calculations, the measured viscosity of the algal culture was virtually the same as that of water.

In practice, because of low pressure ratings of typically used transparent materials of construction, a culture velocity as high as 1.14 m s^{-1} cannot be attained in long-run continuous tubings. Typically, the culture velocity in tubular loops does not exceed 0.5 m s^{-1}, or less than half the damaging threshold value. In theory, high culture velocities should benefit productivity by increasing the allowable length of a continuous run tube before oxygen concentration builds up to high inhibitory levels. In reality, pumping becomes difficult as the tube length increases. The cell damaging potential of pumps is another consideration that is especially relevant to tubular photobioreactor culture. Damage in pumps is treated elsewhere (Chisti, 1999a).

2.4. Gas–liquid mass transfer

Microalgae generate oxygen during photosynthesis, hence, dissolved oxygen levels equivalent to several times air saturation are easily reached in closed cultures. Oxygen concentrations above air saturation generally inhibit photosynthesis in microalgae (Aiba, 1982). In studies with *Chlorella vulgaris*, Märkl and Mather (1985) noted that the rate of photosynthesis increased by 14% when there was almost no dissolved oxygen. Saturation of the medium with pure oxygen reduced the photosynthesis rate by 35%. Accumulation of oxygen to inhibitory levels is a major problem in horizontal tubular photobioreactors and this problem becomes more severe as the length of continuous run tubing increases. With *Spirulina platensis* under intense artificial illumination, oxygen production rates have been estimated at 0.35–0.5 g l^{-1} h^{-1} for radiation intensity levels of 1500–2600 μE m^{-2} s^{-1} (Tredici et al., 1991). In one case, a tubular photobioreactor designed as a commercial production unit with several kilometer long tube runs (Fig. 1) failed to produce partly because of oxygen accumulation. This device was apparently the largest horizontal tube photobioreactor ever constructed and it's failure is clear evidence of limited scalability of this type of bioreactor. Existing functioning tubular photobioreactors are typically a mere fraction of the size of the device shown in Fig. 1.

Oxygen removal into the exhaust gas-phase is substantially greater in pneumatically agitated vertical reactors than in horizontal tubes. For example, for a power input value of 24 W m^{-3}—the lowest value in Fig. 2a—the overall volumetric gas–liquid mass transfer coefficient ($k_L a_L$) in the bubble column was estimated at 0.0037 s^{-1} using the equation

$$k_L a_L = 2.39 \times 10^{-4}\left(\frac{P_G}{V_L}\right)^{0.86}. \quad (16)$$

Eq. (16) applies to air–water systems (Chisti, 1989). The estimated k_La_L in the column was about 4-fold the estimated value for a horizontal tube with a reported 3% maximum gas holdup (Camacho Rubio et al., 1999). In view of these results, vertical tubular photobioreactors such as bubble columns should easily maintain a dissolved oxygen level only a little higher than the air saturation value. Consequently, vertical reactors will experience little oxygen inhibition. Indeed, in one case the dissolved oxygen level did not exceed the air saturation value during outdoor culture of *P. tricornutum* in a draft-tube airlift reactor (Contreras, 1996). Because of a reduced level of dissolved oxygen, the photooxidation-associated loss of biomass and product metabolite will be lower in vertical reactors. Photooxidation occurs especially when high levels of dissolved oxygen combine with an intensely irradiated environment.

2.5. Overall productivity: the case of EPA

Eicosapentaenoic acid (EPA) is a polyunsaturated fatty acid that is potentially medically significant in treatment of certain cancers and heart disease (Rambjor et al., 1996; Simonsen et al., 1998). EPA is produced by several microalgae and some other microorganisms. EPA occurs also in fish oil. Current annual demand of EPA is approximately 125 tonnes in Japan alone and a world-wide demand has been estimated at 300 tonnes per annum (Corden, personal communication). A facility producing just 20 tonnes EPA per annum from microalgal sources would need to generate 24 tonnes of wet biomass daily, operating continuously for 95% of the calendar year. This figure assumes an EPA content of about 2% (w/w, dry weight basis) in *P. tricorntum*, an optimistic 80% recovery of the EPA and 85% moisture in the biomass. For this production capacity, an optimally operated horizontal tubular photobioreactor (0.06 m tube diameter) would have a volume of 2350 m^3. This volume is based on an experimentally observed dry biomass productivity of 2.02 kg m^{-3} d^{-1} in June, or an average annual productivity of 1.535 kg m^{-3} d^{-1}. The latter value accounts for the experimentally documented 8% per month decline in productivity going from mid summer to mid winter (Acién Fernández et al., 1998). Such a reactor will occupy a surface area of about 14.1 ha. This estimation of surface area assumes an optimal distance of 0.11 m between adjacent parallel horizontal tube runs. In theory, this distance could be reduced by half although some loss of productivity would occur due to mutual shading.

A bubble column tank farm for the same 20 tonne annual EPA production would have a total volume of 8220 m^3 based on experimentally measured dry biomass productivity of 0.64 kg m^{-3} d^{-1} in June, or a mean annual productivity of 0.486 kg m^{-3} d^{-1}. Such a tank farm will occupy an area 16.3 ha. Each bubble column would be 0.2 m in diameter, 2.1 m tall, with a culture volume of about 0.06 m^3. East–west oriented rows, 995 m long, will have a spacing of 0.35 m between column centers, inter-row spacing will be 3.4 m measured between column centers. Because a 3.4 m inter-row spacing results in mutual shading of columns for part of the year, the mean annual volumetric productivity of biomass declines to 0.439 kg m^{-3} d^{-1}, or about 90% of the unshaded productivity. The land use efficiency may seem lower for the bubble column tank farm, however, the column height used was only 2.1 m and efficiency improves with increasing column height as shown in Fig. 13. Thus, for a more realistic column height of 4 m, the total volume needed to for 20 tonnes per annum EPA capacity will be about 10 120 m^3. The mean annual volumetric productivity of biomass will decline further to 0.356 kg m^{-3} d^{-1} because of increased mutual shading of the taller columns, but the area demand will reduce to ∼40% of that with 2.1 m columns. Because of the larger volume per column, fewer columns will be needed. The effect of bubble column height on mean annual volumetric biomass and EPA productivities is shown in Fig. 14 for a facility with 46 columns arranged in four east–west oriented rows with 3.4 m inter-row spacing. The volumetric productivity declines with increasing column height because of increased duration of the mutually shaded period. The increased production with increasing column height (Fig. 13) occurs because the culture volume depends directly on height and more volume is accommodated on a given land surface when taller columns are employed. Because taller columns have reduced volumetric productivity as a result of longer periods of mutual shading, increasing height does not increase the annual production linearly (Fig. 13). Beyond a height of 5 m, the annual production from a given area is no longer sensitive to column height (Fig. 13) because the decline in volumetric productivity exactly balances the effect of increased volume.

The biomass productivity numbers used in these estimations were obtained in a 0.2 m^3 horizontal tubular reactor that occupied an area of 12 m^2. The reactor was located on a reflective surface with an albedo of 2 and it produced *P. tricorntum* biomass. The culture was carried out in Mann and Myers (1968) medium (Table 2) formulated in sea water (Table 1) at 3-fold concentration than recommended by Mann and Myers (1968). The optimal dilution rate during the summer (June) was $\sim 0.05\ h^{-1}$, giving a biomass productivity of 2.02 kg m^{-3} d^{-1} with 4 kg m^{-3} biomass in the effluent. The maximum a real productivity of the reactor was a mere 0.034 kg m^{-2} d^{-1}.

As noted earlier, the experimental productivity in a single 2 m tall column, 0.2 m in diameter, was 0.64 kg m^{-3} d^{-1} in June. The maximum areal productivity of the single reactor was 0.093 kg m^{-2} d^{-1}, accounting for the 1.3 m shadow length and 0.3 m effective width of the column. The culture medium was a modified Ukeles (1961) medium (García Sánchez, 1994) made in sea water (Table 1) at three times the components concentrations noted in Table 2. Differences in media had no effect on culture behavior.

Comparing superficially, the mean annual volumetric productivity of the vertical column was only 30% of that of the horizontal tubular loop, however, it needs emphasizing that the horizontal device was located on a reflective surface that enhanced the total incident radiation on it's surface by a factor of two. If the vertical column is placed on a similarly reflective background its productivity will increase minimally by a factor of 1.8; hence, comparing on an equal basis, the volumetric productivity of the vertical unit is about 57% of that in the horizontal device.

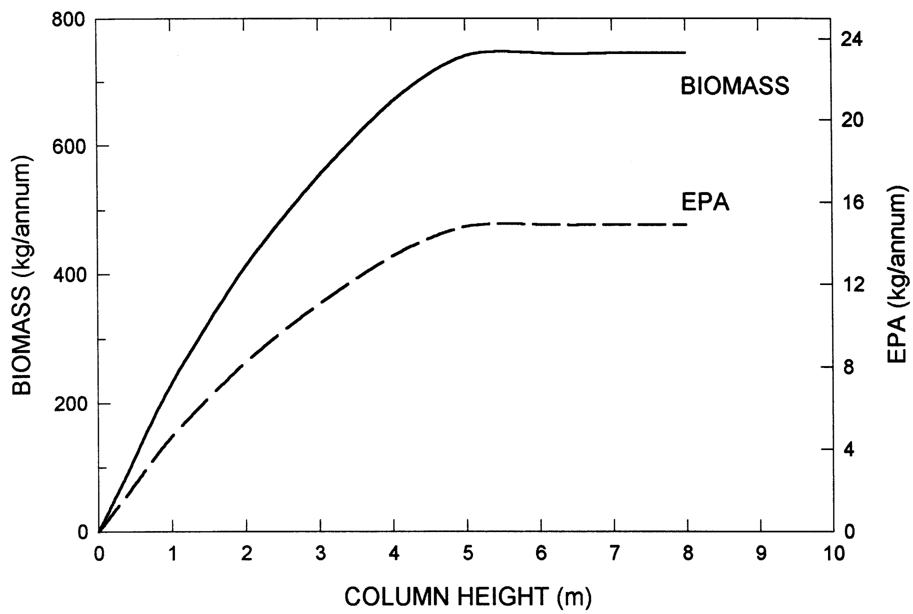

Fig. 13. Annual biomass and EPA production of a multiple bubble column facility as a function of column height. Column diameter was 0.2 m. Forty-five columns were accommodated on a 13.9 × 5.9 m surface with 3.4 m center-to-center between east–west oriented rows.

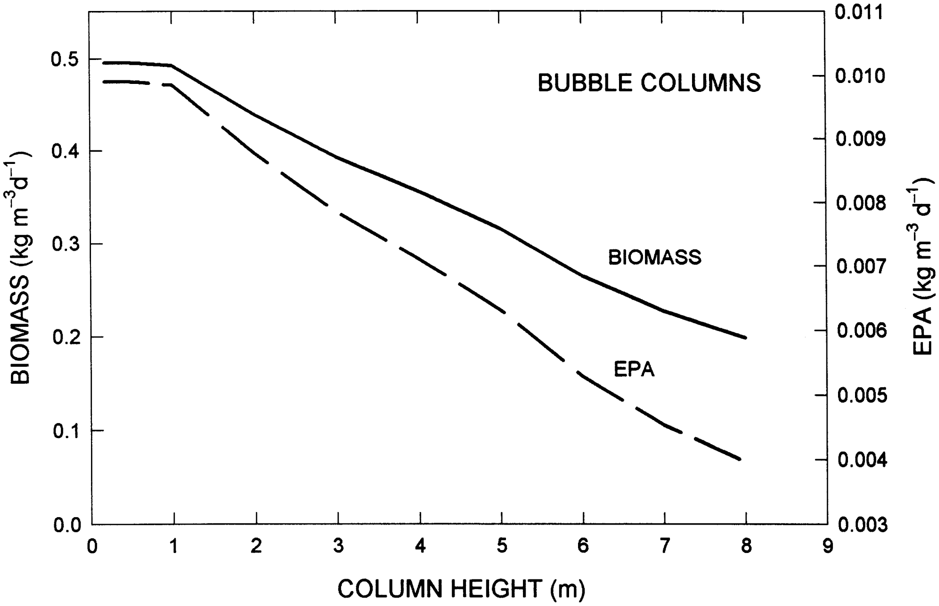

Fig. 14. Effect of bubble column height on mean annual volumetric productivity of biomass and EPA. Forty-five columns were placed on a 13.9 × 5.9 m surface with 3.4 m center-to-center between east–west oriented rows.

In any outdoor culture, the mean monthly productivity varies during the year because of cyclic changes in external irradiance: in horizontally oriented photobioreactors productivity peaks during summer when the Sun is highest on the horizon. Productivity is lower during winter. As noted earlier, the experimentally measured decline in productivity is about 8% per month going from mid summer to mid winter for horizontally placed tubes (Acién Fernández et al., 1998). Whether the same level of decline would occur in vertical tubes remains to be confirmed. Annual variation in productivity in a vertical photobioreactor may follow a different cyclic pattern than observed with horizontal devices. During summer when the Sun is highest on the horizon, a vertical bubble column should experience *lower* average irradiance than in winter when the direct radiation from the Sun affects a greater part of the reactor's surface. This variation is similar to that seen in the gas-free state in Figs. 3–6, except that the cycle is annual. This annual cyclic pattern has important advantages for the vertical oriented photobioreactors. Higher irradiance levels in the winter should reduce any heating demand whereas lower irradiance in the summer should reduce the need for cooling. Furthermore, the low surface–to–volume ratio should help reduce heat losses during the night.

The expected variation in biomass and EPA production for a bubble column farm and a horizontal tubular loop photobioreactor occupying the same land area (82 m^2) is shown in Fig. 15. The production rate values in Fig. 15 are for a system in which the adjacent horizontal tubes of 0.06 m diameter are spaced optimally at 0.11 m, whereas the bubble columns are spaced 0.3 m wall-to-wall within an east–west row and the inter-row spacing is 3.4 m. Again, the assumed mean annual culture biomass productivity is 0.438 kg m^{-3} d^{-1} based on experimentally observed productivity of 0.64 kg m^{-3} d^{-1} in a single bubble column for cultures of *P. tricornutum* during June. For the tubular loop system, the assumed average annual productivity is 1.535 kg m^{-3} d^{-1}. The bubble column diameter used is 0.2 m and the column height is 2 m. This diameter is close to the maximum practicable in view of the light penetration needs.

For a given land area and 0.2 m diameter columns, the annual biomass production increases with culture height as shown in Fig. 13 even though the volumetric productivity declines (Fig. 14). The optimal column height is about 5 m (Fig. 13), but because of considerations such as wind speed and strength of optically transparent materials such as glass and thermoplastics, the maximum permissible height is 4 m. The effect of inter-row spacing of 2 m tall columns (0.2 m diameter) on annual production from a fixed land area of 82 m^2 is shown in Fig. 16. Clearly, to attain maximum yield while keeping the number of columns to a minimum implies an optimal inter-row spacing of between 2.5 and 3.5 m.

Use of multiple vertical columns has other important advantages: (i) a more uniform and better controlled pH than could ever be possible in a plug flow tubular loop; (ii) improved culture homogeneity and, therefore, a consistent metabolic state of the cells; (iii) greater operational flexibility in view of the ability to easily vary the number of columns in production at a given time; (iv) ability to culture several different algae at the same time in separate units; and (v) substantially reduced need for pumping the culture as there would be no recirculation. Flow management among the individual column bioreactors as well as sampling can be easily automated (Chisti, 1998b). Similarly, rapid and automatic clean–in–place and chemical sterilization of individual photobioreactor units becomes feasible (Chisti and Moo-Young, 1994; Chisti, 1999b) while a part of the facility remains in production.

Table 2
Composition of the media

Component	Concentration (kg m^{-3})	
	Mann and Myers (1968)	Modified Ukeles' medium
$MgSO_4 \cdot 7H_2O$	1.20	–
$NaNO_3$	1.00	1.00
$CaCl_2$	0.30	–
K_2HPO_4	0.10	–
$NaH_2PO_4 \cdot 2H_2O$	–	0.016
Na_2EDTA	0.03	0.01
H_3BO_3	6×10^{-3}	–
$FeSO_4 \cdot 7H_2O$	2×10^{-3}	–
Fe(III) citrate	–	4.9×10^{-3}
$MnCl_2$	1.4×10^{-3}	–
$MnCl_2 \cdot 4H_2O$	–	9.9×10^{-4}
$ZnSO_4 \cdot 7H_2O$	3.3×10^{-4}	–
$ZnCl_2$	–	1.36×10^{-4}
$Co(NO_3)_2 \cdot 6H_2O$	7.0×10^{-6}	–
$CoCl_2 \cdot 6H_2O$	–	2.4×10^{-5}
$CuSO_4 \cdot 5H_2O$	2.0×10^{-6}	2.5×10^{-5}
$Na_2MoO_4 \cdot 2H_2O$	–	2.42×10^{-4}

Among factors that could significantly improve the EPA–from–microalgae process feasibility is improved yield. Raising EPA content of the biomass from the current 2% of dry weight to 3% under consistent outdoor production will reduce the production facility size by about 30%. Similarly, a 50% improvement in bubble column bioreactor productivity from the current 0.486–0.729 kg m^{-3} d^{-1} (no shading) will reduce the facility size further. Overall, with these two improvements, a 20 tonne per annum bubble column based EPA facility will require a volume of 3653 m^3, or less than 45% of the volume needed when the yield and illumination are not enhanced. Both the noted improvements are realistic. EPA content of biomass may be improved by genetic manipulation and selection of better producing strains of *P. tricornutum*. Accomplishing this will affect the bubble column and the tubular loop based facilities equally. Improved illumination and consequent biomass productivity enhancement is feasible only for the bubble column system as the tubular loop productivity numbers are already the maximum attainable in an outdoor system with the external irradiance level enhanced to twice the direct radiation from the Sun. The bubble column volumetric productivity can be further enhanced by employing artificial illumination at night, technology exists for placing a low power vertical light source at the axis of the column. This kind of efficient internal illumination is not practicable in horizontal tubular loops. Of course, artificial illumination may add to capital cost and it will certainly add to the operating expense, but for sufficiently high-value products the improved productivity may more than compensate for the greater expense.

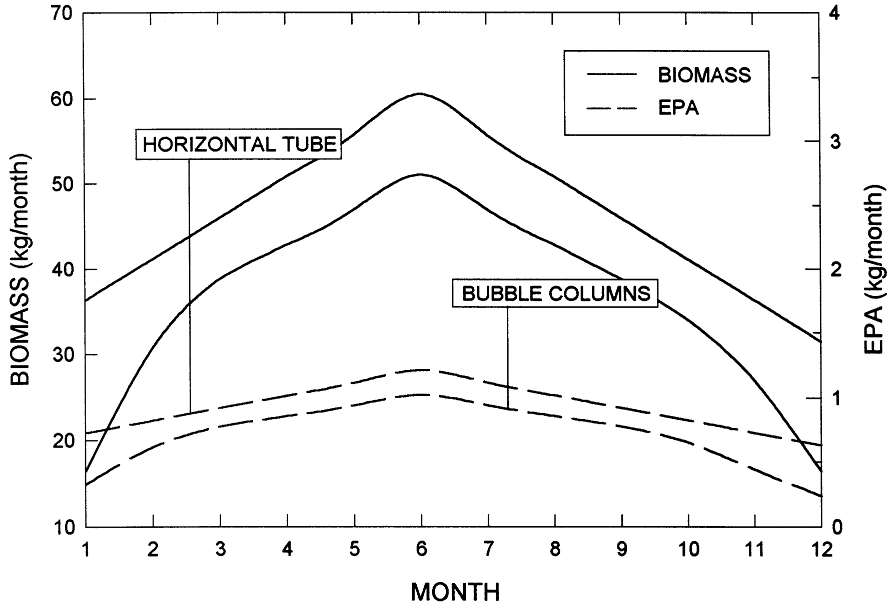

Fig. 15. Mean monthly biomass and EPA production rates of a bubble column (0.2 m diameter, 2 m tall, 3.4 m center-to-center between east–west oriented rows) farm and a horizontal tubular loop photobioreactor (0.06 m tube diameter) occupying equal land areas (82 m^2). The column farm accommodated 45 columns.

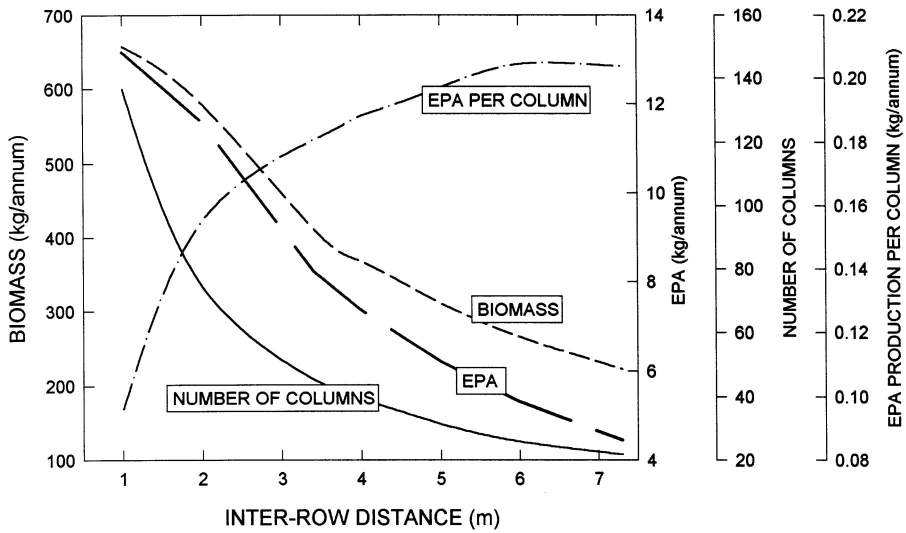

Fig. 16. Effect of inter-row spacing on annual production from 82 m^2 land area accommodating a maximum number of bubble columns (2 m tall, 0.2 m diameter) in 13.9 m long east–west oriented rows.

3. Conclusions

Horizontal tubular photobioreactors are generally believed to be the most practicable culture system for fully contained large-scale monoculture of microalgae, nevertheless, as discussed here, detailed analyses reveal severe limitations of tubular photobioreactors. Unless the concentration of the desired microalgal metabolite in the biomass is unusually high and the market size for the product is exceedingly small, the use of horizontal tubular photobioreactors would be impossible in commercial production. Unlike horizontal tubular devices, vertical reactors such as bubble columns and airlift vessels appear to be the only ones that can be effectively used in large-scale culture of microalgae. As demonstrated here, overall, the vertical bubble columns perform better than the horizontal loops. Bubble columns are more scaleable and provide a relatively homogeneous culture environment. In addition, the low surface–to–volume bubble columns demand less cooling. Vertical columns experience less photoinhibition during periods of high light intensity; during low light seasons such as winter, the vertical reactors still receive substantial total radiation because they receive more reflected light than do horizontal tubes. So far, bubble columns and airlift bioreactors have been ignored for large-scale culture of photosynthetic organisms, an obvious low surface–to–volume ratio of these devices relative to horizontal tubular loops has in the past been automatically perceived as a barrier to their use, but such perceptions have never been substantiated.

4. Nomenclature

A_d cross-sectional area of downcomer zone (m^2)
A_r cross-sectional area of riser zone (m^2)
C_f Fanning friction factor
d diameter (m)
d_B mean bubble diameter (m)
E energy dissipation rate per unit mass (W kg^{-1})
EPA eicosapentaenoic acid
g gravitational acceleration (m s^{-2})
h_c height of the column (m)
$\hbar$ solar hour (h)
$k_L a_L$ overall volumetric gas–liquid mass transfer coefficient for oxygen (s^{-1})
L length of tubing (m)
L_s length of the shadow from the column's base (m)
ℓ length scale of microeddies (m)
N day of the year
N_B number of bubbles in dispersion
ΔP pressure drop (Pa)
P_G power input due to aeration (W)
U_L superficial liquid velocity in the tube (m s^{-1})
V_D volume of gas–liquid dispersion in bioreactor (m^3)
V_G volume of gas in liquid (m^3)
V_L volume of liquid in bioreactor (m^3)

Greek symbols

δ declination the angular position of the Sun at solar noon with respect to the plane of the equator, north positive ($-23.45° \leq \delta \leq 23.45°$), defined by Eq. (11)
ε fractional gas holdup
θ_i angle of incidence defined by Eq. (9)
μ_L viscosity of liquid (Pa s)
ρ_L density of liquid (kg m^{-3})
ϕ geographic latitude
ω angle corresponding to the solar hour, defined by (Eq. (10)

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