## Global population Estimates and Projections

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UN, 1996.
Appendix - 2

Key To The Genera Of Chlorophyceae

Volvocales

1. Unicellular ................................................................................. 2
2. Colonies, aggregates or coenobia ............................................. 5
   2. Cells with thick cell wall or lorica, not closely appressed to...
      protoplast ........................................................................ Phacotus
   2. Cells with delicate cell wall .................................................. 3
3. Cells pear shaped with cup shaped chloroplasts, bi or quadriflagellate
   ........................................................................................... 4
3. Cells elongated elliptical ............................................. Chlorogonium
4. Cells biflagellate ................................................................. Chlamydomonas
4. Cells quadriflagellate .......................................................... Carteria
5. Cells aggregated not enclosed in a mucilaginous matrix .......... 6
5. Cells occurring as irregular definite colonies or coenobia ........ 9
6. Cells arranged in tiers .......................................................... 7
6. Cells arranged otherwise ......................................................... 8
7. Cells biflagellate ................................................................. Pyrobotrys
7. Cells quadriflagellate .......................................................... Spondylochormor
8. Cells in irregular colonies or dendroid colony ....................... 9
8. Cells arranged as coenobia ..................................................... 11
9. Cells arranged irregularly in sheets of mucilage............. Tetrasporidium
9. Cells arranged inside tubular mucilaginous or dendroid colonies .... 10
10. Cells arranged in tubular colonies .................................... Tetraspora
10. Cells arranged in dendroid manner ................................. Ecballocystis
11. Coenobia flat, 4-32 cells .................................................... Gonium
11. Coenobia spheroid or ellipsoid ............................................. 12
12. Cells of coenobia of uniform size ........................................ 13
12. Cells of half or one fourth of coenobia are of smaller size ......... 14
13. Cells pyriform contiguous at center of coenobia .......... Pandorina
13. Cells spherical arranged in alternating tiers ..................... Eudorina
14. Coenobia with upto 50% non reproductive anterior cells... Pleodorina
14. Coenobia with large reproductive cells in the posterior side,
    made of several thousand cells ....................................... Volvox
Chlorococcales

1. Unicellular, loosely aggregated, cells of varying shapes, chloroplasts one or many ......................................................... 2

2. Colonial forms with cells of varying shapes chloroplasts one or many ............................................... 20

2. Unicells free living, or held in common mucilage ...................... 3

2. Unicells endophytic or associated with other organisms attached to substratum .................................................. 16

3. Unicells simple spherical or elongated without any appendages .......... 4

3. Unicells spherical, elongated, tetrahedric with bristles, setae. Ridges or spines ................................................................. 7

4. Cells spherical, chloroplast cup shaped or laminate .................. Chlorella
   (C. vulgaris Beji.)

4. Cells spherical to ellipsoidal, chloroplast parietal, hollow sphere or irregular peripheral lobes with or without pyrenoids ............. 5

5. Chloroplast parietal, hollow sphere with one-many pyrenoids .......................................................... Chlorococcum

5. Chloroplast axial with irregular peripheral lobes and one central pyrenoid ............................................................. Trebouxia

6. Cells spherical with radiating bristles, spines, warts and setae with or without tubercles ............................................ 7

6. Cells of varied shapes with appendages .................................. 10

7. Cells with radiating bristles .................................................. Golenkinia

7. Cells with small spines, warts or setae .................................. 8

8. Cells with spines or warts .................................................. Trochiscia

8. Cells with setae with or without tubercles ................................ 9

9. Cells with tubercles ............................................................. Lagerheimia

9. Cells without tubercles ....................................................... Chodatella

10. Cells spherical to ellipsoidal, fusiform ................................ 11

10. Cells cylindrical, tetrahedral. Polygonal or pyramidal, appendages from angles .................................................. 12

11. Cells in spindle shaped envelope ....................................... Desmatractum

11. Cells spherical without any envelope with fine sheath appendages having blunt or bifurcate ends ................................ Pachycladon

12. Triangular to pyramidal angular ends extended as setae .............................................................. Trebauria
12. Cells cylindrical to polygonal with rounded angular pointed arms with or without short spine ..................................................... 13
13. Cells polygonal with or without short spines .......................................................... 15
13. Cells cylindrical to crescent shaped with one spine from each end .................................................. 14
14. Cells cylindrical ...................................................... Closteridium
14. Cells crescent shaped ................................................ Schroederia
15. Cells with rounded angles ................................................ Tetraedron
15. Cells tetrahedral with one to several bristle from each angle .............................................................. Polyedriopsis
16. Cells endophytic ...................................................... Chlorochytrium
16. Cells associated with other organisms or attached to substratum .................................................. 17
17. Free floating intermingled with gelatinous envelope of other organisms .................................................. Kentosphaera
17. Attached cells elongated with parietal chloroplast .......................................................... 18
18. Attached with stalk which is expanded at base, epiphytic .......................................................... Characium
18. Cells with bifurcate stalk, always epizoic .......................................................... Korshikoviella
19. Cells forming compact colonies .......................................................... 20
19. Cells enclosed in mucilage envelope or forming loose colonies .................................................. 30
20. Cells arranged forming radiating flat spherical or cylindrical colonies .................................................. 21
20. Cells arranged otherwise .......................................................... 23
21. Colony macroscopic forming a net formed by cylindrical or cylindrical cells .................................................. Hydrodictyon
21. Colonies microscopic .......................................................... 22
22. Colony plate like with 4-64 cells, cells multinucleate .......................................................... Pediastrum
22. Colony spherical radiating from center 4-64 stalked Cells .......................................................... Sorastrum
23. Colonies spherical indefinite forming net like compound colonies or with globose cells connected by mucilaginous pads .................................................. 24
23. Colonies flat plate like, cells in groups of variable shapes .................................................. 25
24. Cells coccoid to ellipsoidal connected by thick mucilagenous bands .......................................................... Botryococcus
24. Cells connected by mucilaginous pads .......................................................... Coelastrum
25. Colonies flat irregular, cells in two planes oblong, ellipsoidal, fusiform cells arranged in longitudinal axis ................................... 26
25. Colonies in groups of four cells angular, ellipsoidal with or without Marginal spines .......................................................... 28
26. Colonies 2-8 (32) cells arranged in two rows with longitudinal axes, cells parallel ................................................... Scenedesmus
26 Colonies of 4 cells crescent shaped or luneate 2 each in different planes ............................................................................... 27
27. Colonies in mucilaginous matrix ........................................ Tetrallantos
27. Colonies without any mucilage, free ....................................... Dicloster
28. Cells with marginal spines ................................................... Tetrastrum
28. Cells without marginal spines ............................................... 29
29. Cells with remains of mother cell wall outside ...................... Hoffmannia
29. Mother cell wall absent ....................................................... Crucigenia
30. Cells of the colony with appendages ..................................... 31
30. Cells of the colony without appendages ................................ 32
31. Cells with conical extension from one side ......................... Conococcus
31. Cells with many long hyaline setae ....................................... Micractinium
32. Cells enclosed in mucilage envelope as loose colonies ........... 33
32. Cells enclosed in mucilage colony interconnected with each other by mucilage or attached together .................................. 36
33. Cells enclosed in mucilage, free from each other .................. 34
33. Cells separated by dark bands of mother wall ...................... Gloeotaenium
34. Cells spherical, ovoid, ellipsoidal colonies, cells flattened angular ................................................................. Dispora
34. Cells of varying shapes ....................................................... 35
35. Cells ovoid to ellipsoidal ..................................................... Oocystis
35. Cells curved, oblong, reniform ........................................... Nephrocytium
36. Cells connected by mucilage threads .................................... 37
36. Cells attached together by mucilage .................................... 39
37. Cells of the colony of one shape ......................................... 38
37. Cells of the colony of two different shapes in groups of fours in irregular colonies .......................................................... Dimorphococcus
38. Cells spherical to ovoid connected by distinct dichotomous threads ............................................................... Dictyosphaerium
38. Cells spherical in group of four held together by mother wall ............................................................... Westella
| 39. | Cells elongated | 40 |
| 39. | Cells lunate or ovate cuneate | 44 |
| 40. | Cells fusiform to acicular in loose aggregates | 44 |
| 40. | Cells attenuated and connected at apices or ovoid, club shaped joined by their ends to form radiating colonies | 43 |
| 41. | Cells of moderate length, with or without mucilage envelope | 42 |
| 41. | Cells very long remain solitary with an axial row of several pyrenoid |

- **Closteriopsis**
- **Ankistrodesmus**
- **Quadrigula**
- **Dactylococcus**
- **Actinastrum**
- **Gloeoactenium**

42. Cells without mucilage envelope

42. 2-8 or more cells in mucilage envelope

43. Cells attenuated form loose colonies

43. Cells forming radiating colonies

44. Cells ovate-cuneate arranged in radiating groups at the periphery of envelope

45. Cells within mucilage envelope or mother cell wall

45. Cells fairly regularly arranged back to back without mucilage envelope

46. Cells small in number 1-4 within mother cell wall

46. Cells 4-many within mucilage envelope

**Ulotrichales**

1. Filaments uniseriate unbranched, filaments joined end to end, cells uninucleate

2. Filaments uniseriate cells multinucleate

2. Cells with parietal plate or band like or reticulate chloroplasts with one or more pyrenoids or without pyrenoids

3. Cells with one or more pyrenoids

4. Cells with net like chloroplasts without pyrenoids cell wall made of H-pieces

4. Filaments enclosed in a broad mucilaginous sheath

5. Cells spherical, lenticular cell walls overlapping

5. Cells cylindrical of uniform width

- **Sphaeroplea**
- **Cylindrocapsa**
- **Microspora**
- **Radiofilum**
- **Geminella**
6. Filaments short, of uniform width fragment into short celled
   filaments .............................................................................. 7
6. Filaments differentiated into base and apex .......................... 8
7. Filaments of indefinite length, more than 16 cells .......... *Hormidium*
7. Filaments of limited length, chloroplast single .......... *Gloeotilopsis*
8. Filaments with cells grouped in pairs .................. *Binuclearia*
8. Filaments otherwise .......................................................... 9
9. Filaments with a prominent apical cell .................. *Uronema*
9. Filaments attached by modified basal cell ............. *Ulothrix*

**Cladophorales**

1. Filaments unbranched cell cylindrical producing
   rhizoids .................................................................................. *Rhizoclonium*
1. Filaments branched ................................................................. 2
2. Filaments with intercalary and terminal akinetes .......... *Pithophora*
2. Filaments without akinetes ............................................... *Cladophora*

**Chaetophorales**

1. Basal system prominent erect system rudimentary or absent .......... 2
1. With simple or well developed erect system .................. 4
2. Epiphytic on aquatic angiosperms, some cells with elongated
   unicellular hairs ........................................................................... 3
2. Cells with cytoplasmic extensions ............................... *Coleochaete*
3. Filaments epiphytic creeping with setae ..................... *Aphanochaete*
3. Erect system made of sparsely branched filaments
   ....................................................................................... *Thamniochaete*
4. Erect system well developed ................................................. 5
4. Erect system poorly developed, thallus prostrate and attached
   .......................................................................................... 10
5. Filaments with elongated basal rhizoids, partly
   parenchymatous .................................................................. *Fritschiella*
5. Filaments not multiseriate or parenchymatous ..................... 6
6. Filaments enclosed in a gelatinous sheath .......... *Chaetophora*
6. Filaments free without gelatinous sheath ...................... 7
7. Main branches with broader cells and lateral filaments diminishing in
diameter, not dimorphic ...................................................... 8
7. Main axis larger, lateral branches tuft-like ........................................ 9
8. Basal system well developed ........................................... Stigeoclonium
8. Filaments attached by single holdfast .................. Microthamnion
9. Main axis of uniform sized cells ........................................... Draparnaldia
9. Main axis consisting of alternating longer and shorter cells

.................................................................................. Draparnaldiopsis
10. Plants entirely prostrate ........................................... Protoderma
10. Plants with few erect branches ............................................. 11
11. Erect filaments straight with calcium encrustation ................. Gongrosira
11. Erect filaments torulose ................................................ Leptosira

Oedogoniales

1. Filaments unbranched, cells long and cylindrical ............ Oedogonium
1. Filaments branched .............................................................. 2
2. Cells with long bulbous setae ................................... Bulbochaete
2. Cells without setae ................................................ Oedocladium

Zygnematales

1. Thallus simple filamentous .......................................................... 2
1. Thallus unicells, semicells occurring individually or joined to form chains appearing like filaments ................................................. 10
2. Cells cylindrical filaments unbranched chloroplasts single .......... 3
2. Chloroplasts two or many ........................................................ 5
3. Chloroplasts axile plate like with pyrenoids showing gametangial cells

.................................................................................. Temnogametum
3. Chloroplasts axile plate like with gametangial cells ................. 4
4. Sporangia filled with pectic cellulose, without any cytoplasmic residue in gametangia ........................................... Debarya
4. Cytoplasmic residue left in gametangia .......................... Mougeotia
5. Chloroplasts two ................................................................. 6
5. Chloroplasts one to many .................................................... 9
6. Chloroplasts discoid, globose or stellate .................................. 7
6. Chloroplasts flat, straight parallel plates or ribbons .... Sirocladium
7. Chloroplasts stellate.................................................... Zygnema
7. Chloroplasts otherwise .......................................................... 8
8. Chloroplasts globose, gametangia filled with pectic cellulose.................................................. *Zygnemopsis*

8. Chloroplasts ovoid cytoplasmic residue left in gametangium...................................................... *Zygogonium*

9. Chloroplasts spirally arranged and ribbon like with conjugation tubes ............................................ *Spirogyra*

9. Chloroplasts spirally arranged without conjugation tubes .......................................................... *Sitogonium*

10. Cell wall unsegmented without pores ............................................................................................ 11

10. Cells wall segmented with constriction or forming semi-cells........................................................ 15

11. Cells elongated, cylindrical, unconstircted forming loose filaments cell wall ornamented .................. *Gonatozygon*

11. Cells short unconstricted cell wall smooth ................................................................................... 12

12. Each cell with single chloroplast .................................................................................................. 13

12. Each cell with two chloroplast ...................................................................................................... 14

13. Chloroplasts spirally twisted ........................................................................................................ *Spirotaenia*

13. Chloroplasts plane axile ................................................................................................................. *Mesotaenium*

14. Chloroplasts stellate radiating from central pyrenoid......................................................................

14. Chloroplasts with longitudinal ridges ............................................................................................ *Netrium*

15. Cells cylindrical elongated with central constriction ........................................................................ 16

15. Cells showing diversity of forms, cells with two halves called semicells ........................................ 17

16. Cells straight cylindrical ............................................................................................................... *Penium*

16. Cells elongate curved attenuated towards each end with two chloroplasts ........................................ *Closterium*

17. Cells solitary or in colonies isthmus plane ...................................................................................... 18

17. Thread like colonies with girdle like thicking or isthmus ................................................................ 21

18. Cells remain as solitary individuals .............................................................................................. 19

18. Cells remain attached to form colonies .......................................................................................... 29

19. Cells elongated and cylindrical with slight constriction .................................................................... 20

19. Cells short compressed or radiating with deep constrictions .......................................................... 23

20. Apices of cells truncate or rounded ............................................................................................... 21

20. Apices of cells cleft, incision widely open or narrow ..................................................................... 22

21. Base of semi cells plicate ............................................................................................................. *Docidium*

21. Base of semi cells plane ................................................................................................................... *Pleurotaenium*

22. Cell wall with rings of furcate processes ....................................................................................... *Triploceras*

22. Cell wall plane apical cell rounded ............................................................................................... *Tetmemoros*
23. Cells compressed vertical view fusiform or elliptical ...................... 24
23. Cells in vertical view radiating, triangular, quadrangular or upto 11 radiate ........................................................................... Staurastrum
24. Cells with apical incision and lobed margin ........................................... 25
24. Cells with more or less entire margin with spines or warts .............. 26
25. Apical incision and moderately lobed margin ........................................... Euastrum
25. Cells compressed with deeply lobed margin ........................................... Micrasterias
26. Cells with a central protuberance ....................................................... 27
26. Cells without any protuberance .......................................................... Arthrodnesmus
27. Cell wall smooth granulated or verrucose ........................................... 28
27. Cell wall with regularly arranged spines usually in pairs .....................
........................................................................................................ Xanthidium
28. Semi cells showing clear sthmus ....................................................... Cosmarium
28. Semi cells elongate without clear sthmus ........................................... Actinotaenium
29. Colonies thread like cells attached by apices into long filaments or cells
attached by spherical apical processes .............................................. 30
29. Cells apices plane and flat ................................................................. 31
30. Apical processes very short ......................................................... Sphaerozosma
30. Apical processes long and overlapping the apex of adjoining cells
........................................................................................................ Onychonema

31. Cells deeply constricted or not ......................................................... 32
31. Cells triangular, fusiform, elongate or cylindrical ................................... 33
32. Cells deeply constricted ..................................................................... Spondylosium
32. Cells slightly constricted ..................................................................... Hyalotheca
33. Cells short triangular or fusiform ....................................................... Desmidium
33. Cells cylindrical elongated ................................................................. Gymnozyga
Bioengineering design:

The major issue in the commercial cultivation of photoautotrophic algae is the sustained trapping of solar energy at as high an efficiency as possible throughout the year; the more efficiently, this can be accomplished, the sounder the economic basis for this promising biotechnology would become. Converting solar irradiance to chemical energy at 2% efficiency on a year-round basis which is definitely theoretically possible (Hall et al., 1987), implies annual net yields averaging between 30–40 g dry matter m\(^{-2}\) d\(^{-1}\) or some some 140 t dry matter ha\(^{-1}\) yr\(^{-1}\). From an industrial standpoint, it is very important to achieve significant increase in production rates because, this alone will reduce production costs to an extent.

With increased irradiance, up to light saturation, the rate of photosynthesis increases. But the efficiency by which light is converted into chemical bond increases (Richmond 1992). Viewing microalgaculture from this angle sharpens the focus on the scientific challenge to develop the species, the culturing devices and the management protocol which would permit maximal exploitation of the super saturating photon flux densities (PFD) existing outdoors for a significant part of the day. To produce photosynthetic chemical from microalgae at competitive prices, efficient large scale photobioreactor must be designed two prototype photobioreactors have been designed and constituted for the large scale cultivation of photosynthetic cells of *Chlorella* in which easy scale up is the primary design criterion- Bioheliotron and Bioroof photosystems.

Conventional old-age large scale culture systems:

Examining the engineering design of algal cultivation systems of a size sufficient to produce tones of algae or algal products daily involves consideration of not only the application desired but also a host of factors, many of which are uncontrollable or not as
controllable in the natural environment as in laboratory cultures, and some of which are probably not yet known.

Some known factors requiring consideration in design are:

(1) The specific application.

(2) (a) Media requirements of the species of algae to be grown to produce a desired biomass, product, products or process.

(b) Various media inputs as a function of quality and availability for economical production.

(3) Local climatological conditions, including geographical and seasonal variations in illumination, temperature, precipitation, evaporation, relative humidity and other factors.

(4) Physical properties of the design area including soil type, slopes, drainage, water quantity and quality.

(5) Specific physical requirements for cultures: mixing, depth, residence time and power inputs to attain needed productivity of algae or products under a given set of conditions.

(6) Attainable efficiencies and productivities.

(7) Harvesting and processing.

(8) Costs.

Many of the above items are more or less interrelated and few can be dealt with independently. Nevertheless, in the following we shall deal first with existing and proposed applications of algae and then with each factor in the order mentioned, pointing out relevant interactions and problems as a case arises.

Species control:

Because of the wide range of tolerance for different media compositions by most micro-algae, it is extremely difficult, if not impossible, to control the species of algae being produced through media changes alone. Higher salinity, high pH or absence of fixed nitrogen have been noted above, but it is well known that other factors such as cell residence time, temperature and predation are often as important as nutrients in determining species dominance in a given culture. Mass inocula and physical selection
and retention (or recycling) of cells have also been demonstrated to be effective in species retention once selection has been made (Weissman & Benemann, 1979) but microscopic examination inevitably proves that there are no sustained axenic cultures out of doors in the real world.

**Climatological factors:**

Climate is a most important factor in the design of micro-algal cultures because it is virtually uncontrollable in multi-hectare systems and yet is one of the primary determinants for technical feasibility. Without appropriate temperatures and sufficient sunlight, economical applications of massive micro-algal photosynthetic systems cannot occur. Artificial illumination and heating of large-scale cultures could only be produced and a low cost source of heat such as geothermal water could be applied (Bedell, 1986). Even with geothermal water, because of the cost of heat exchangers and pond covers it would probably be more economical to locate the production at a site where natural light and ambient temperature would suffice. Ideally, large-scale cultures should be sited where both light and temperature permit growth outdoors during a major portion of a year. Although the tropics may be ideal for temperature, one problem that often occurs there is sustained cloud cover, which attenuates available light while temperature remains high. In this case nocturnal algal cell respiration at a rate proportional to the temperature can be almost equal to cell synthesis, and the net production of biomass per unit of area and time is diminished. For this reason ideal locations may be temperate and semi-tropical deserts, where cloud cover is minimal and rare, and nights are generally cooler than days. However, tropical areas without frequent cloud cover can have very high productivities. Kitto et al., 1999 reported 60 g m$^{-3}$ d$^{-1}$ from *Skeletonema* in arid regions from a Biothermostat cultivar with a total solar collection area of 560 m$^2$ at Nellore, India.

Each algal species is expected to have an optimum temperature for its growth but the optimum is known for only a few species. A few of the known optima are approximately 20° C for *Chlorella* spp., 30° C for *Spirulina* spp. and 32° C for halophilic *Dunaliella* spp.
One difficulty with algal growth in desert areas is the high rate of evaporation from open water surfaces. This may pose a problem both from the standpoint of increasing concentrations of salts in production ponds and in the acquisition of sufficient water to make up for evaporation. For this reason any desert site that is selected should have an abundant source of low salt content make-up water and a location where excess salty water can be disposed of (blown down) without causing pollution problems. A source of solar-distilled water would be of great value but is very costly. Wet tropical areas, on the other hand, may become severely diluted. Because production ponds rarely exceed 10 to 30 cm in depth and since 24 h rainfalls of 200mm or more are not uncommon in the wet tropics, severe culture dilution and loss of nutrients may occur during heavy rains. In areas where such floods may occur, ponds should be equipped with overflow spillways to protect equipment and avoid levee washouts. Also in such areas, a covered, deep storage pond into which cultures can be pumped temporarily before predicted heavy rains may be justified economically by the amount of culture and media it can save.

Relative humidity is important with regard to both evaporation losses and pond cooling. Where humidity is low, high rates of evaporation occur, particularly during windy periods. Where humidity is high and there is little or no wind, and sunlight is abundant, the water in shallow cultures (10 to 20 cm deep) may heat up to as much as 40° C each day and become lethal to all but the hardies algae. Accordingly, sites considered should have average relative humidities below 50% to 60% most of the year.

Wind is an important factor in site selection, because surface mixing, cooling and evaporation are enhanced by gentle wind. On the other hand, strong winds may damage levees and pond liners and are likely to introduce foreign materials, such as dust, pollen and weeds into the ponds. Dust, of course, interferes with light transmission and may contaminate cultures with unwanted algae and contaminate the product with soil and bacteria. Pollen also is sometimes sufficiently thick to interfere with light. Weeds are troublesome, since they foul screens, mixing systems, and liquid transfer structures and may harbour mosquito larvae. Wave height is not usually a problem in shallow ponds, since waves cannot exceed the depth of water in amplitude, and the velocity of waves, being directly proportional to depth, is small for shallow ponds.
Available solar energy as a function of latitude and month is shown in Appendix. This table should be viewed as highly approximate, and is certainly not a substitute for local data or on-site measurements. It is particularly useful for theoretical designs where no other information is readily available.

**Carbonation** (Oswald, 1988a)

Carbonation (CO₂ supply) is one of the most difficult processes in micro-algae cultivation. Although CO₂ can be added to cultures in the form of sodium bicarbonate and similar salts, such salts usually cost more than three times as much as gaseous carbon dioxide per unit of carbon. On the other hand, the transfer of CO₂ through a neutral gas liquid interface is so slow that special methods must be devised to provide the lengthy time and wide surface area required to maximize transfer. Packed-column carbonation often used in industry is discouraged because its low pH frequently harms or disrupts algal cells. The most effective compatible method currently available to transfer CO₂ to algal cultures is counter current carbonation in which the gas in injected as minute bubbles into a column of water. The water velocity is adjusted so that the small bubbles of CO₂ rising against the current essentially hang suspended in the water until fully absorbed. Using this technique, Laws (1984) has reported a 70% efficiency in CO₂ transfer.

**The need for mixing**:

All shallow outdoor cultures of micro-algae must be mixed continuously to avoid cells settling and sticking to the bottom and to avoid thermal stratification of the water. In the author's experience at Richmond, California, and elsewhere, unmixed ponds as shallow as 30 cm were found to have a temperature difference of as much as 8° C from top to bottom on warm days. Such drastic temperature differences arise when the top few centimeters of a dense culture of algae absorb and convert most of the sunlight energy to heat. This generally occurs with a 10-fold higher efficiency than the conversion of light to chemical energy in the cells. The warmed surface water tends to remain at the surface because it is lighter than the water in lower strata. Algae in this layer quickly deplete it of available bicarbonate ion and the pH value rises rapidly to as much as 11.
Under those conditions CaCO₃ and essential nutrients such as Fe and P tend to precipitate and settle, often carrying the surface algae with them toward the bottom. With clearing of the surface layer, a second layer is exposed to the sunlight and the process repeats itself until an entire unmixed shallow pond may become precipitated. Once algae have reached the pond bottom they tend to form large agglomerations held together by extracellular polymers and bacterial, fungal and blue-green algal filaments. If light then reaches these layers, they tend to flake off the bottom and rise to the pond surface in mats of varying size buoyed up by adhering bubbles of photo-synthetically released oxygen. These floating mats in turn are swept to the pond edges by wind, such mats thickly wind-rowed at a pond's edge, quickly become anoxic and odorous. They then serve as breeding places for flying insects and may ultimately host large concentrations of Clostridium botulinum, with attendant likelihood of wildfowl poisoning and death.

**Channelized mixing** (Oswald, 1988a)

The objectionable chain of events described above is entirely avoided by continuous flow mixing in shallow channelized ponds. A simplified channel layout, through which water is forced to move essentially like a shallow stream confined by outside walls and channel dividers. Flow mixing is easily induced in channelized ponds by propeller or screw pumps, by airlift pumps and by paddle wheels. Properly designed paddle wheels are by far the most efficient and durable pond mixers because they involve mainly low speed moving parts and can be made of corrosion proof materials. Air lift pumps, while effective involve air compressors or blowers that tend to wear out in a few years and are less efficient than paddle wheels of the type. These wheels are designed like vane pumps and consequently discharge virtually all of the water entering them and are thus highly efficient. Propeller and screw pumps are no longer considered efficient for algal growth systems.

**Limitations on productivity** (Oswald, 1980)

If depth, cell residence time, and culture concentration are accurately known, productivity can be determined. One might assume then that to increase productivity one should increase depth or decrease cell residence time. The reality, unfortunately, is that equilibrium cell concentration decreases with depth (usually more rapidly than
depth is increased, due to extraneous turbidity), so increases in productivity resulting from increased depth are very limited (although mixing may be facilitated). By the same token, if cell residence time is shortened by decreasing the hydraulic residence time of the culture liquid, the cell concentration tends also to decline because of dilution, so again an increase in productivity may not occur.

**Closed Photobioreactors - Design Considerations:**

Over the last 20 years the main emphasis of large-scale microalgal culture has been culture in outdoor raceway ponds. These ponds, originally developed for wastewater treatment, have proven to be relatively cheap to construct, easy and cost-effective to operate, and are generally quite efficient (Oswald 1988a). Other systems that are used for commercial algal culture at present include the central-pivot system used in Taiwan for *Chlorella* culture and the very extensive, unmixed, shallow ponds used for the culture of *Dunaliella salina* in Australia (L.J. Borowitzka 1991; Schlipalius 1991).

These open culture systems have proven to be adequate for the culture of algae such as *Spirulina, Chlorella*, and *Dunliella*, i.e., species that grow in very selective environments such as high pH, high nutrients, or high salinity. However, the development of new algae and new algal products such as astaxanthin from *Haematococcus*, fatty acids from several marine algae, as well as antibiotics and pharmaceuticals, have highlighted the limitations of the existing culture systems (Chaumont 1993; Radmer and Parker 1994; M.A. Borowitzka 1995) and has led to increased emphasis on the development of suitable closed algal culture systems. Closed systems that have been and are being studied include: (1) bag culture, which is widely used for the culture of algae for aquaculture (Daintith 1993); (2) alveolar panels and other flat plate reactors with internal illumination (Tredici *et al.*, 1991; Torzillo *et al.*, 1993; Silva and Cortinas 1994); (3) stirred tank reactors with internal illumination (Pohl *et al.*, 1988; Wohlgenschaffen *et al.*, 1992; Hilaly *et al.*, 1994); (4) tower reactors with internal fiberoptic illumination (Mori *et al.*, 1987; Burgess *et al.*, 1993); (5) suspended narrow bags or tubes (Groeneweg 1978; Cohen and Arad 1989); and (6) tubular reactors (Pirt *et al.*, 1983; Torzillo *et al.*, 1986; Chaumont *et al.*, 1993; Richmond *et al.*, 1993; Chrismadha and Borowitzka 1994b). None of these reactor designs is really new, and the classic papers by Davis *et al.*, (1953) and by Arthur D. Little, Inc. (1953) on the culture of
*Chlorella* described the predecessors of many of these systems. Literatures published in the last decade receive little or no mention of closed algal systems (e.g., Richmond 1986; M.A. Borowitzka and Borowitzka 1988; Cressewell et al., 1989).

For efficient and reliable large-scale culture of microalgae, several criteria need to be considered and these are summarized in Table 32.

**Light:**

When considering commercial-scale microalgal culture, the source of light is usually sunlight since artificial light is too expensive for all but the most valuable products. The amount of light available to the algal cells is the critical factor affecting the productivity of microalgal cultures. In general, shallower or thinner cultures can reach a greater cell density and, ultimately, a greater productivity since the effects of self-shading are minimized (Grobbelaar 1981). However, almost all open pond systems must be operated at a depth of >15 cm due to hydraulic limitations in large ponds and the difficulty of controlling salinity changes due to evaporation (Oswald 1988a). The only real exceptions to this are the sloping cascade systems developed at Trebon in Czechoslovakia (Setlik et al., 1970: Balloni et al., 1981) and also used by Microalgae Research Farms Pty Ltd in Western Australia. These systems can operate with a culture depth of several millimeters and are very well suited for the cultivation of *Chlorella* or *Scenedesmus*, but are not suitable for marine algae since evaporation results in large salinity changes.

Closed algal systems can be designed so that the light path to the algae is very short, thus optimizing the light utilization efficiency. This has been achieved mainly by using flat transparent panels (e.g., Tredici *et al.*1991), Ratchford and Fallowfield 1992: Tredici and Materassi 1992), tubes in various configurations (e.g., Bocci *et al.* 1988; Chaumont *et al.* 1988; Robinson *et al.* 1988), introducing light via fiberoptics (e.g., Mori *et al.* 1987; Burgess *et al.* 1993), and other means (Broneske *et al.* 1995; Pulz *et al.* 1995).

It is interesting to note that most early tubular reactors used tubes 10-30 cm in diameters (e.g., Torzillo *et al.* 1986), but almost all tubular reactors in use now have a tube diameter less than 40 cm. The narrower tube diameter not only improves the light utilization efficiency, but also provides more turbulence which enhances growth. For
example, Boussiba (1993) found that reducing the tube diameter of his reactor from 5 cm to 3 cm increased the productivity of *Anabaena siamensis* from 310 to 370 mg l⁻¹ d⁻¹.

The orientation of the reactors with respect to the sun is also important so that the algae receive the maximum amount of light throughout the day (Lee and Low 1991). The flat inclined plate reactors can be mounted so that their orientation can be changed during the day; however, tubular reactor orientation is fixed. Most of the tubular reactor designs consist of serpentine tubes lying on the ground so that the lower part of the tubes receive less light than the upper parts. Torzillo *et al.* (1993) have attempted to optimize light availability by arranging the tubes in two planes with the tubes in the upper plane placed in the vacant space between the ones in the lower plane. An alternative arrangement is to arrange the tubes vertically and the most efficient way to do this is to have the tubes wrapped around a vertical tower. If the diameter of the lower is sufficiently great, then little shading takes place and this also makes the most efficient use of the available land area. This is the Biocoil design used by Biotechna Ltd (L.J. Borowitzka and Borowitzka 1989a). Recently, Lee *et al.* (1995) in Singapore used a tubular reactor with the tubes in an Ω configuration in which the culture is lifted by air to a receiver tank and then flows down parallel tubes placed at an angle of 25° to the horizontal to another set of air riser tubes, which again lift the culture to another receiver tank. From this tank they flow down another set of tubes to the base of the first set of air riser tubes. This design also makes good use of the available sunlight. Compared to the helical tubular reactor, however, the Ω reactor is more difficult to construct and requires more expensive materials, especially for the tubing.

Although all of these systems are quite effective on the laboratory scale, several of these systems cannot be scaled up easily or cost-effectively. Thus they may be adequate for the production of very high value products such as pharmaceuticals, but would be too expensive for lower value products such as carotenoids or even algae for aquaculture feeds. Three basic designs, however, are promising for large-scale production; these are (1) the arrays of hanging bags developed by Cohen and Arad (1989), (2) the horizontal tubular photobioreactors (Chaumont *et al.* 1988; Richmond *et al.* 1993; Torzillo *et al.* 1993), and (3) the helical tubular photobioreactor (Robinson *et al.* 1988; L.J. Borowitzka
and Borowitzka 1989a). The flat panel reactors, although very efficient, are probably too expensive for very large-scale commercial operations.

An alternative way of avoiding the difficulties of supplying adequate light is to grow the algae heterotrophically in the dark, which allows more conventional fermenter designs to be used (Day et al. 1991; Barclay et al. 1994; Running et al. 1994). Such a system is used by Martek Inc. for the production of long-chain polyunsaturated fatty acids (Radmer and Parker 1994). Unfortunately the range of algal species able to grow heterotrophically is limited as is the range of products available from these species.

Turbulence:

In order to achieve high productivity all algal cultures have to be mixed. The method of providing adequate mixing (turbulence) is critical, however, since many algae are quite shear-sensitive (Silva et al. 1987; Gudin and Chaumont 1991). In tubular reactors higher flow rates generally enhance productivity (Chrismadha and Borowitzka 1994b) and flow rates of 30-50 cm/s are generally used. Although for some algae such as *Spirulina* and *Phaeodactylum* this flow can be achieved by using peristaltic, diaphragm, lobe, or centrifugal pumps, airlights are generally the least damaging to cells and the most effective (Richmond et al. 1993). Narrower tube diameters also have the advantage of allowing faster flow rates that not only increase the algal productivity but also reduce fouling on the inside of the tubes. The helical tubular reactors also have the advantage that there are no sudden reversals in the direction of flow as occurs in other tubular reactor designs. Not only in there a significant energy loss in such 180° bends (Pirt et al. 1983), but in narrow diameter tubes algal cells may accumulate at these bends. In most systems the flow is continuous, however, Trozillio et al. (1986) and Tredici and Materassi (1992) found that pulsed mixing gave better results for the culture of *Spirulina* in a tubular reactor.

Temperature control:

Many studies in both open ponds and in closed reactors have shown that temperature is an important factor affecting productivity (e.g. Payer et al. 1980; Richmond 1992). Compared to open ponds, closed reactors generally warm up faster in the morning, thus reaching the optimum temperature for algal photosynthesis earlier in
the day (Tredici and Materassi 1992). Closed reactors, however, can also overheat and thus require some sort of temperature control system. In closed systems this control is achieved by either incorporating a heat exchanger into the design and/or by evaporative cooling by spraying water onto the surface of the reactor (Ortega and Roux 1986; Lee and Low 1992; Torzillo et al. 1993; Chrismadha and Borowitzka 1994b). The tubular reactor operated at Cadarache (France) used another method, which involved floating the reactor tubes in a large water-filled tank. In order to heat the culture the tubes were floated on the surface of the tank and if cooling was required the tubes were submerged (Chaumont et al. 1988).

Economic considerations generally favour evaporative cooling, and heat exchangers are used mainly to heat the culture in colder climates. Algal strains with a higher temperature optimum should also be considered, whenever possible, to reduce cooling costs.

Gas transfer:

As in all fermenter systems, gas transfer presents a significant problem to the designer of the reactor. In algal systems excess oxygen must be removed and carbon dioxide introduced.

In the flat panel reactors or the hanging tubes, photosynthetically produced oxygen is removed directly from the culture by the degassing action of the air used to circulate the culture. However, in tubular reactors the excess oxygen is removed in a degasser or in the gas riser located at the end of the tubes. This means that the oxygen concentration in the culture will build up during the passage of the algae through the tubular part of the reactor, rapidly reaching supersaturation. If an airlift is used to circulate the culture, this problem is exacerbated since the airlift virtually ensures that the medium always will be at least at oxygen saturation. Little work has been done so far to determine how much the high oxygen concentration reduces the productivity (Torzillo et al. 1986). At this time there seem to be few solutions open to the reactor designer, the only one being to keep the tube length as short as economically possible so that the degree of oxygen build-up is minimized.
Closed systems also require CO₂ addition and this must be optimized since CO₂ is expensive. The CO₂ is usually mixed with the air used to mix the culture, and the amount of CO₂ required can be minimized by monitoring culture pH and only adding CO₂ as required. In tubular reactors CO₂ can also be injected at the beginning of the tube so as to maximize the time the CO₂ is in contact with the medium. It may also be possible to maximize the CO₂ transfer rate by using hollow-fiber membranes to give high bubble densities (Matsuoka et al. 1992).

**Operational considerations:**

Any reactor design must also take into account operational considerations such as the ease of maintenance, ease of cleaning (and sterilization if necessary), and reliability. For example, the large diameter bags generally used in the culture of microalgae for aquaculture feeds are relatively easy to maintain, they are supplied clean and sterile, but the cultures are often unreliable due to very inadequate mixing, thus greatly increasing the cost of the final algal product (Fulks and Main 1991). Most other closed reactor designs, on the other hand, are much more reliable but are also more difficult to clean and generally difficult to sterilize. At this time the requirement for most large-scale algal cultures is not for axenic culture, but just for unialgal culture, thus sterilization is not a major issue. Where axenic culture is required, then conventional fermenters with internal lighting are still the best systems. It is also likely that the solar pane type reactors constructed of glass could be adapted to axenic culture without too much difficulty, whereas large tubular reactors would be difficult to construct so that an axenic culture could be maintained.

Closed reactors also may have a problem with fouling of the walls of the reactors by the algae. In general, narrower tubes and/or higher turbulence (Reynolds number) reduce fouling. Several methods for cleaning the inside of the reactor using either small balls or scouring pad "pigs" to scour the inside of the reactor tube during operation have been developed. Using such systems, fouling has not been a major problem in the operation of these reactors.

For commercial systems reliability is also an important consideration. Algal cultures grown in the helical tubular reactors have proven to be especially stable and we
have cultured several species including the marine algae, *Phaeodactylum tricornutum*, *Tetraselmis chuii*, and *Isochrysis* sp. for periods in excess of three months (Chrismadha and Borowitzka 1994a and unpublished results). These systems also are particularly suited for continuous or semi-continuous culture, and this permits much better control over the culture conditions and provides a consistent product quality, something that is very difficult to achieve in open systems (e.g., Belay et al. 1994).

An additional operational advantage of most closed systems is that the cell densities reached are much greater than those achieved in open systems (see Table 2). This not only enhances the areal productivity but reduces harvest photoinhibited, whereas at very high cell densities self-shading can reduce productivity as individual algal cells do not receive the optimum level of light (Vonshak and Guy 1992; Qiang and Richmond 1994).

One particular advantage of a closed reactor over open systems is that the operation of the reactor can be easily automated, thus greatly reducing operating costs. Finally, closed reactor systems have a significantly smaller land area requirement compared to open systems. This can be important in countries where land is expensive. The helical tubular photobioreactors also have a much smaller requirement than most other tubular systems and the overall construction of the reactor is much simpler (and therefore cheaper) than alternative systems.

**Materials:**

The choice of materials in reactor design requires consideration of the compatibility of the materials with the algae, their effect on final product quality due to leaching of unacceptable chemical residues or heavy metals, ease of maintenance and cost. The photoreactor part of closed systems with external illumination has been constructed of glass, methyl polymethacrylate, polycarbonate, polyethylene, silicon, Teflon, or PVC, whereas reactors with internal illumination have been constructed of stainless steel or polypropylene (Chaumont 1993). Other components of the reactors are usually made of similar materials. Our experience has been that all of these materials are suitable, except that stainless steel is usually not recommended for marine or
halophilic algal culture due to corrosion problems and some leaching of heavy metals and accumulation of these by the algal biomass.

Glass is an excellent material; however, for large commercial-scale tubular reactors it is too expensive and too fragile, although some large pilot-scale reactors have been constructed (Pulz 1994). Silicone tubing also has many desirable properties; however, it is also rather costly. Our experience has been that PVC tubing is the most economical. Although PVC becomes more opaque with time under the influence of UV light, thus reducing the amount of light reaching the culture and this reduction in transmitted light reaching the culture and this reduction the amount of light reduces productivity slightly, commercial considerations show that the use of this material is still more cost effective than using a more expensive material such as glass or polycarbonate.

Scale-up:

Most of the closed reactor systems developed so far have been less than 200 liters in volume. Commercial-scale production systems will have to have volumes of at least 1000-10,000 liters. The only systems operated successfully at this scale are the arrays of narrow hanging bags used in Israel, the helical tubular photobioreactors (the BiocoiP) of Biotechna Plc, in Melbourne and Perth in Australia and in Luton and Dorking in the UK, and the tubular reactor operated in Cadarache, France (Chaumont et al. 1988). An attempt at using a meandering tubular system for the culture Dunaliella salina was also made in Spain; however, this failed due to scale-up problems that included low Beta-carotene productivities and fouling problems in the bends at the ends of the tubes.

In general scale-up of any reactor system should proceed gradually with each successive stage being not greater than about 10 times the previous stage (L.J. Borowitzka and Borowitzka 1989a). Scale-up of the flat plate reactors is more likely to proceeded by just increasing the numbers of reactors rather than significantly increasing the size of individual reactors. Whether this is economical remains to be determined. The helical tubular photobioreactors are somewhat easier to scale-up. This is because larger reactors consist of several parallel layer of tubes linked by common manifolds so that the hydraulic head of the system remains fairly constant and the residence time of an individual algal cell in the tubular portion of the reactor also remains the same.
irrespective of the total reactor volume. Reactors of several tens of thousand of litres can therefore be constructed.

Comparing the main algal culture systems and their properties. It is clear that the existing open systems are deficient in several aspects as are some of the closed culture systems. Despite this, several algae such as *Dunaliella salina*, *Chlorella*, and *Spirulina* are being grown commercially in such systems. Algae such as *Haematococcus*, *Isochrysis*, *Porphyridium*, and *Phaeodactylum* have also been grown on open systems, but it is generally difficult to manage the cultures.

Algae that have been grown successfully in large (> 100 litre) closed systems such as tubular photobioreactors, narrow hanging tubes, and flat plate reactors include *Haematococcus*, *Porphyridium*, *Phaeodactylum*, *Tetraselmis*, *Pavlova*, *Isochrysis*, *Chroomonas*, *Anabaena*, *Skeletonema*, *Chaetoceros*, *Dunaliella*, etc. Considering that the designs of closed systems are still relatively new, some of the existing deficiencies are still relatively new, some of the existing deficiencies are very likely to be remedied, but it is also apparent that the flat panel reactors and the tubular reactors are the best designs available at present. Ultimately the choice as to which system to use for the culture of a particular alga will be based on economic criteria.

**Modern closed culture systems:**

Vertical tubular reactors were among the first real closed systems described in the literature (Cook, 1950). This design has since been developed by several authors. James and Al-Khars (1987) studied the growth and the productivity of *Chlorella* and *Nannochloropsis* in a translucent vertical air-lift photobioreactor and obtained maximum productivities of 20 to 26 g m⁻² d⁻¹ on a dry weight basis. Miyamoto et al. (1988) used vertical glass tube (5 cm diameter, 2.3 m high) and obtained, with *Monoraphidium*, productivities of 23 g m⁻² d⁻¹. This concept of ‘bubble column reactor’ has as its main advantage good light penetration, although its scale up potential seem difficult. Polyethylene bags (transparent polyethylene sleeves sealed at the bottom in a cone shape to prevent cell settling) are also widely used, mainly in aquaculture operations. Using 50 liters polyethylene bag cultures operated as turbidostats, Trotta (1981) obtained yields of 20 to 30 g m⁻² d⁻¹ for *Tetraselmis*.
During the last ten years, great attention has focused on tubular closed bioreactors. Several tubular photobioreactors have been studied and developed since the pioneering work of Tamiya et al. (1953). These reactors are generally a serpentine in form, made of glass or plastic as the solar receptor, a gas exchange vessel where air, CO\textsubscript{2} and nutrients are added and O\textsubscript{2} removed, and a recirculation of the culture between these two parts by the use of a pump (Gudin & Chaumont, 1983) or an air-lift (Pirt et al., 1983). The most sophisticated is certainly the culture system developed at Batelle (Anderson & Eakin, 1985) for the production of polysaccharides by Porphyridium cruentum. The system was a modular design, resembling a solar collector with a photodetector for angular adjustment of the glass surface. The polysaccharide productivities published by the authors ranged from 20 to 25 g m\textsuperscript{-2} d\textsuperscript{-1}.

Biologically active substances for medicine and the cosmetic industry were obtained from dry Chlorella and Spirulina biomass (Albitskaya and Semionov, 1990; Zadorin, 1990). An industrial-scale cultivator was made from glass tubes 6.4 cm in diameter, equipped with centrifugal and dosing nutrient pumps, heat and gas exchanger apparatus, tanks for nutrient solution preparation, a tank for biomass washing, separators and dryer, indoor inoculators, and instrumentation and automation. Industrial gaseous atmospheric emission with a CO\textsubscript{2} concentration of 6-10\% were used instead of CO\textsubscript{2} from cylinders. The suspension portion (freshwater Chlorella or Spirulina) was drained off daily. The biomass was isolated from culture liquid in separators, then it was washed and directed to a spray dryer. Production is practically wasteless: the main metabolite – oxygen – is isolated in a gas exchange apparatus. After the algal biomass is separated, the culture liquid is piped back into the photoreactor. Cell concentration in suspension is 2-3 g/L. Production of 1 Kg of dry biomass takes about 25 kwt/h (Karpov, 1990).

An industrial-scale (over 100 m\textsuperscript{3}) tubular photobioreactor for growing Chlorella consists of some modules and is working at Turkmenistan, in the former USSR. Another industrial scale cultvator, constructed on the roof of a yeast production facility in Uzbekistan utilizing 280 m\textsuperscript{2} of solar receptors (volume 4m\textsuperscript{3}) yielded 12-15 kilogram d\textsuperscript{-1} of dry biomass (2 metric tons yr\textsuperscript{-1}). 10 m\textsuperscript{3} volume was illuminated out of 14 ms volume. The tube diameter (cm) / length (m) was 5.8 / 4600; Ratio surface / volume
= 28.0 and the cultured species was *Chlorella vulgaris*. Maximum production (gm⁻²d⁻¹) of 43 was achieved..

Chaumont *et al.* (1988) experimented from 1986 to 1989 with a 100 m² culture unit (7 m³ culture) made of a double layer of flexible polyethylene tubes (6 cm diameter) the upper containing a variable volume of air. Each section of the culture serpentine incorporated a flexible tube oxygen degassing. Temperature control was achieved by floating or submerging the tubular reactor containing the culture on or in a pool of water; the advantage of this technology was to respond not only to daily but also to seasonal air temperature variations. With *Porphyridium cruentum*, these authors achieved steady state continuous culture for about 2 months and obtained productivities of 20 to 25 g m⁻² d⁻¹. Gudin and Chaumont (1991) have pointed out the drastic influence of pumping on microalgae physiology: biomass productivity may increase up to 75% when the pump is replaced by air-lift for culture circulation. They then set up a new rigid tubular prototype made of methyl-polymetacrylate (Chaumont *et al.*, 1991).

A new company named Heliosynthese SA has been recently established to develop this tubular technology in the South of France. A similar culture equipment is under study in Israel (Richmond *et al.*, 1993). The tubes of the solar captor are made either of polyvinylchloride (50 mm in diameter) or of polycarbonate (32 mm in diameter). The rate of flow in the tubes ranges between 30 to 50 cm s⁻¹. These authors conclude that, when the tube diameter is reduced from 50 mm to 32 mm, the optimum population density of *Spirulina platensis* and *Anabaena siamensis* increases, resulting in a higher productivity per culture volume.

Torzillo *et al.*, (1986) also studied a comparable closed serpentine bioreactor (100 m², 10 m³ of culture) initially made of flexible polyethylene tubes (14 cm in diameter) and later of methylpolymetacrylate tubes (13 cm in diameter). Culture pumping was continuous, but the culture circulation was intermittent in order to maintain an adequate culture flow rate inside the tubes: the culture fell continuously into a receiving tank and then was raised to a feeding tank by a siphon that allowed an intermittent discharge in to the bioreactor. They obtained maximum productivities of 25 g m⁻² d⁻¹ with *Spirulina*. 
Photobioreactor Ltd (PBL), in collaboration with Reading University, set up a vertical tubular serpentine (4 m$^3$ of culture) made of flexible 12 mm diameter polyethylene tubes in Murcia (Spain). The circulation is by an air-lift and the temperature is maintained at the optimal level by water spray. This company cultured *Dunaliella* but no results are available in the literature. PBL was in operation until the beginning of 1992.

A pilot plant called 'Biocoil' has been set up in the UK and in Australia (Robinson, 1987). The solar collector is arranged as a coil of polyethylene tubes (30 mm diameter) arranged around an open circular framework with a high surface/volume ratio (100 m$^2$ for 1.2 m$^3$ of culture). A heat exchanger between the solar collector and the pump allows control of the culture temperature. A large pilot unit operated in Australia for several years (*Spirulina* and *Tetraselmis* cultures). However, the Australia company operating this unfortunately went bankrupt, but operations are continuing in the U.K. by Biotechna-Graesser A.P. Ltd. Small tubular photobioreactors of the Biocoil design (40-1001) are also used experimentally at Murdoch University (Australia) to study lipid formation and carotenoid production (M.A. Borowitzka, pers. comm.). Biotechna-graesser A.P. Ltd proposes several biocoil plant sizes (from bench up to 10 000 litres), with internal light for *Chlorella* culture. Their biocoil systems are claimed to be used in conjunction with bacterial or algal growth to break down toxic wastes, to extract metals from liquid streams or to remove nitrates from potable water.

Triple wall panels in translucent PVC have been studied by Ortega and Roux (1986) to culture *Chlorella*. This double layer panel (8 m$^2$) is laid on the ground, one of the layers is used for the circulation of the culture, the other for circulation of the temperature-controlled water. Tredici et al. (1991) and Tredici and Matarassi (1992) have recently developed a vertical alveolar panel (2.2 m$^2$) based on the same type of material. This panel has a variable orientation with respect to the sun’s ray and mixing and deoxygenation of the culture suspension are effected by continuously bubbling air at the bottom of the reactor. Comparable designs but with pump circulation (Muller-Feuga et al., 1992) or with air-lift circulation (Ratchford & Fallowfield, 1992) are also under development. In all cases, high productivities were obtained because of a high
surface/volume ratio but biomass output could be limited by photo inhibition and problems with temperature control.

Several authors have suggested production of microalgae autotrophically in conventional type aerobic fermenters modified by the insertion of internal light sources such as fiber optics. Such systems could have controllable illumination for up to 24 hours a day rather than variable duration sunlight exposure. They can utilize artificial light only (Pohl et al., 1988; Javanmardian & Palsson, 1991; Wohlgenschaffen et al., 1992), or combined sunlight and artificial light (Mori, 1985), or light source enriched in specific wavelengths (Junter et al., 1990). Several companies are now selling such equipment: e.g. Doka Company (Russia), Yamatake and Co., Ltd (Japan), Apparate und Behärtetechnik Harrislee (Germany). Fully instrumented, the culture could be computer driven. An increased duration of illumination and a much tighter control of growth conditions results in much faster growth rates. About ten patents have been applied for during the last two years on the possibilities of introducing light inside culture systems (Hoeksema, 1991; Fallowfield & Martin, 1991; Meyer et al., 1990). Nevertheless, this culture technology is still at the laboratory scale: the use of artificial light is very expensive and for capacities over 2501, the control of culture temperature becomes a technical problem. Such a technology is suitable for productions of labeled compounds.

Dialysis culture systems have been proposed in particular for the growth of fragile algae such as Dinoflagellates. In these systems, the algal culture is confined inside a dialysis tube which is maintained in the culture medium. Nutrients and extracellular metabolites can freely diffuse through the dialysis membrane, whereas the algae cell remain inside the tube (Marsot et al., 1981). This could be an attractive system to culture algae when the metabolite produced could be growth limiting.

When high value products are to be produced, a heterotrophic or mixotrophic growth mode in enclosed bioreactors could be an interesting process (Lee, 1986). Algal strains able to sustain a heterotrophic growth have been listed by Droop (1974). The first commercial venture using heterotrophic cultures to be reported was by the Grain Processing Corp. (Hanson, 1967) who used the green alga Spongiococcum as poultry feed. Heterotrophic production of Tetraselmis suecica has been developed more recently
to produce algae for the early developmental stages of moluscs and crustaceans, using a
prototype production system scaled up from 5 to 50,000 l via a number of intermediate-
sized vessels (Day et al., 1991).

Another application of algal culture is its use to sustain human life in long period
space flight. This has been investigated particularly in the USA, USSR and recently in
Japan (Sogokenkyusho, 1991; Fujita Kogyo, 1991). In such a closed environment, the
problem to solve is the production of oxygen using CO₂ and human wastes. The most
convenient system to feed CO₂ to algae in weightless conditions seems to be by diffusion
through gas-permeable membranes (Lee & Hing, 1989).

Little information is available on the research conducted on algae mass culture in
eastern European Countries. The best known are those of the Rupite team (Bulgaria)
and of the researchers in the Czechoslovak Academy of Sciences (Dilov et al., 1987).
Setlik et al. (1970) developed a shallow turbulent and sloped cultivation unit including a
series of parallel troughs. After cascading on this step-like arrangement, the culture was
recycled to the top of the cascade by pumping. Systems of this design were constructed in
Trebon with surfaces up to 900 m². Based on this design, Vendlova (1969) experimented
in Rupite with a culture system where baffles were suspended in the culture in such way
as to create a rapid mixing of the culture. The Peruvian-German algal project at Sausal
in Peru was a modified version of this principle of sloped algal ponds (Heussler et al.,
1978). Large units made of glass tubes are used in the Casastan region to produce algal
suspension for animal husbandry (Dilov et al., 1987).

Although algae mass culture is commercially attractive because of the great
biological potential of these photosynthetic microorganisms, little industrial and
economic success exists. Microalgal cultivation systems. At the present time, they are
used for cultivation of few species of algae: Chlorella, Scenedesmus, Spirulina for single
cell protein and health food and Dunaliella for Beta-carotene. The two principal
advantages of open culture systems are a small capital investment for production of the
biomass and the use of a free source of energy. They are the simplest method of algae
cultivation and are only intended for algae species growing in selective environments.
The productivities obtained are far from the theoretical maximum.
Several authors have concluded that the open race-way systems which are actually used in most commercial plants are not suitable for obtaining high photosynthetic efficiencies. Closed photobioreactors which allow better control of growth parameters may be more suited to reach this biological goal. Photobioreactors have also an essential role to play in increasing the diversity of algae species for culture and the quality of biomass harvested. This technology is a more capital intensive one, but depending on the commercial target, this additional capital cost can be justified. At the present time, closed photobioreactors are limited to high value products such as phycobiliproteins, pharmaceutical or cosmetic products. It is not possible to say that one culture system is better than another: the commercial target geographic location and metabolite to be produced will determine the choice: axenic or non-axenic, continuous or batch culture, intensive or extensive culture, open ponds or closed photobioreactors.
Appendix - 4

AVERAGE RAINFALL DETAILS – PAST 10 YEARS
(Kanyakumari District)

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Average for 10 Years 1678.53

(Raj, 2002)
# Appendix - 5

Solar radiation\(^a\) probable average values of total insolation direct and diffuse on a horizontal surface at sea level in Langley's\(^b\) day\(^{-1}\)

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\(^a\) Northern hemisphere: calculated from data published by US Weather Bureau; Southern hemisphere, based on northern hemisphere data corrected for solar constant.

\(^b\) Langley = 1 g-cal cm\(^{-2}\)

Corrections: for elevation TOT \((1 + 0.0185 \text{ EL})\) where EL is elevation in thousands of feet. For cloudiness \(\text{MIN} + (\text{Max} - \text{Min}) \times 0.1\) where clarification of time weather is clear.
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Reprint

J. Aqua. Trop. 16 (2) (2001)
GROWTH PERFORMANCE, SALINITY TOLERANCE AND NUTRITIVE VALUES OF MARINE MICRO-ALGA, SKELETONEMA COSTATUM IN THE BRACKISH WATERS OF BUCKINGHAM CANAL, NELLORE, INDIA

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ABSTRACT

The chain diatom Skeletonema costatum in the brackish waters of Buckingham canal at Ananthapuram, Nellore, Andhra Pradesh, was monitored for its growth performance, salinity tolerance and nutritive value to understand its degree of tolerance to the wide range of salinities. The maximum cell density attained was 0.082 × 10^6 cells/ml at 28 ppt showed the highest natural growth rate amounting to 52.2 µg/cell dry weight, 200 µ long chains and 3 cell divisions in 24 hours per cell with the maximum of 20.5 (10^-6 µg) carbon assimilation per cell. S. costatum had 22.3% protein at optimal temperatures; 10.5% lipid (d.w.) and 4.6% carbohydrate. The growth performance improved on sunny days with 28 ppt salinity <30°C conditions. The carbon assimilation increased with diffused sunlight. The sustainability of the bloom as a dominant species in the canal is by algal succession and death of Platymonas, Monochrysis lutheri.

Key Words: Skeletonema costatum; carbon assimilation, chlorophyll-a, cell doubling, total lipid, salinity, shrimp hatchery, B12.

Because of increasing demands for algal foods for rearing larval fish and shell fish, there is increasing interest in production of mass culture facilities, (Salser and Mock, 1973), Skeletonema costatum finds application in the aquaculture of five feed for penaeid shrimp larvae, bivalve mollusc larvae and post larvae (De Pauw and Persoone, 1988). Skeletonema costatum is the best food organism with high nutritional value with regard to the content of (20:0-3) fatty acid (Huei Meei Su et al, 1988).

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*For correspondence
Native *S. costatum* bloom were originally observed in India in Chennai during June, 1982 and in Mangalore during February, 1983 (Mathew et al., 1988). Later, the boom of hatcheries in India with more than 100 functional production centres, led to the mass culture of *S. costatum* in hatcheries outdoor to meet early larval feeding requirement.

The *S. costatum* strain in the Buckingham canal is the first exotic strain from Kaoshiung Harbour, Taiwan, to reach Indian waters by December, 1992 especially the Buckingham canal brackish waters near the Thapar Waterbase Limited hatchery, Nellore, South India. Regular draining of the culture residues from the Waterbase outdoors mass algal culture facilities to the canal sustained the temperature diatom there in Indian tropical conditions well adapted to proliferate in local environmental conditions.

*S. costatum* is generally chain-forming, has siliceous cell walls, lacks cellulose, predominant pigments being carotenoids and diatomin, colour of cultures dark golden to brown (Millamena et al., 1990). The growth performance, salinity tolerance and proximate composition of the algae were qualified by taking samples with natural conditions.

**MATERIALS AND METHODS**

*Skeletonema costatum* was isolated by serial dilution technique described by Robert and James (1979). The isolated chains were placed in sterilized sea water similar to the salinity of its source (28 ppt) in room temperature (30 ± 2°C). The microalgal biomass were centrifuged and adhering sodium chloride crystals were removed by washing cells with an isotonic solution of ammonium formate. Ammonium formate does not leave any residues as it decomposes to volatile compounds, ammonia and carbon dioxide during the drying process (2 hours at 100°C). The results are expressed as dry weight per volume and since combined with determination of cell concentration per algal cell. Dry weight per cell was done as described by Coutteau (1992). Chlorophyll-a determination was according to the procedure laid down by Suzuki and Fujita (1986). Cell doubling per day (*X*) was calculated according to the formula 

\[ X = \frac{1}{N_0} N_n - 1 \times N_ch^-1 2 (t_n - t_i) \]

where *N_n* is the final cell count and *N_i* is the initial cell count, *t_n* is the final time in days and *t_i* is the initial time in days.

Total protein content was determined following the procedure modified by Lowry *et al.* (1951); total carbohydrate content by the technique of Nelson (1944) and Somogi (1945) and total lipid by the method described by Bligh and Dyer (1959), *S. costatum* density in the brackish water canal was assessed according to the method suggested by Guimaraes (1977) using a haemocytometer (Neubauer ruling). Carbon assimilation in
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*S. costatum* cells was done according to the procedures mentioned in Geider (1987). B\textsubscript{12} concentration in the canal was determined according to the method suggested by Droop (1955) and Daisley (1958).

**RESULTS**

The *S. costatum* cell density in the canal, chain length and number of chains per ml and the corresponding dry weight per cell under natural conditions with varying salinities (see Table 1) show that all the above mentioned parameters are maximum at 28 ppt salinity only. Dry weight of cell is also maximum at 28 ppt, salinity being 52.2 pg/cell. Cell density peaked at 28 ppt salinity with \(0.082 \times 10^6\) cells/ml and a chain length of 200 \(\mu\) and having 103.68 cells in a single chain.

Proximate biochemical composition of *S. costatum* (shown in Table 2) showed maximum % dry weight values of protein, lipid and carbohydrate being 22.3%, 10.55% and 4.6% respectively at 28 ppt salinity. 39 ppt salinity had a drastic negative effect on the proximate biochemical values of *S. costatum* showing least values of 10.67%, 3.655% and 1.92% for protein, lipid and carbohydrate.

Table 3 describes *S. costatum* cell division rate and carbon assimilation per cell with respect to varying salinity factors in response to sunshine and diffused sunlight. Cell division rate was the maximum at noon shine at 32 ppt being 3.25 divisions per cell in 24 hours, but with a low carbon assimilation of 10.1 \((10^{-6} \text{ pg})\). At 28 ppt, the cell divisions were good, both in noon sunshine and diffused sunlight showing 3 and 2.4 divisions respectively and a healthy amount of carbon assimilation per cell also showing 21.7 \((10^{-6} \text{ pg})\) and 21.4 \((10^{-6} \text{ pg})\) for sunshine and diffused light respectively. All the above values at 39 ppt, were negligibly low.

Percentage occurrence of *S. costatum* out of the total phytoplankton was just 10% at 12 ppt; 40% at 22 ppt; 70% at 28 ppt; 60% at 32 ppt and 25% at 39 ppt.

**DISCUSSION**

*S. costatum* chain length is coupled with light illumination but limiting at noon levels. Intensive light induced cell divisions resulting in very high population levels which led to drastic shortening of *S. costatum* chains. With respect to Nellore weather, the sea water salinity was 26 ppt by December, 29 ppt by January, 30 ppt by February, 32 ppt by March, 33 ppt by April, 36–41 ppt during May, 38 ppt by June, 35 ppt by July, 34 ppt by August, 33 ppt by September, 32 ppt by October and 30 ppt by November.
Table 1. *Skeletonema costatum* cell density and cellular dry weight at different salinities

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<th>Canal salinity (ppt)</th>
<th>Max. temp. °C</th>
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<th>Dry weight, pg/cell</th>
<th>Cell density, cells/ml</th>
<th>Canal depth, in cm</th>
<th>Chain count, per ml</th>
<th>Chain length (μ)</th>
<th>Mean cell nos./ chain</th>
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Table 2. Biochemical composition of *Skeletonema costatum*

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<th>Max. temp. °C</th>
<th>% protein (d.w.)</th>
<th>% lipid (d.w.)</th>
<th>% carbohydrate (d.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>12.31</td>
<td>December</td>
<td>28.00</td>
<td>12.39 ± 1.61</td>
<td>05.71 ± 0.10</td>
<td>2.34 ± 1.10</td>
</tr>
<tr>
<td>1.21</td>
<td>22.20</td>
<td>March</td>
<td>28.56</td>
<td>20.33 ± 1.16</td>
<td>07.23 ± 0.04</td>
<td>4.00 ± 0.22</td>
</tr>
<tr>
<td>1.25</td>
<td>28.17</td>
<td>July</td>
<td>29.74</td>
<td>22.30 ± 2.26</td>
<td>10.55 ± 1.52</td>
<td>4.61 ± 0.09</td>
</tr>
<tr>
<td>1.12</td>
<td>32.52</td>
<td>June</td>
<td>30.10</td>
<td>19.00 ± 0.29</td>
<td>09.37 ± 0.09</td>
<td>4.40 ± 0.14</td>
</tr>
<tr>
<td>0.80</td>
<td>39.14</td>
<td>May</td>
<td>30.62</td>
<td>10.67 ± 3.15</td>
<td>03.65 ± 0.11</td>
<td>1.92 ± 0.68</td>
</tr>
</tbody>
</table>

Note: All values are the means of four replicates.
Table 3. *Skeletonema* cell division rate and carbon assimilation per cell in diffused and noon sunshine

<table>
<thead>
<tr>
<th>Incident sunlight</th>
<th>Diffused</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell division per</td>
<td></td>
<td>Amount of</td>
<td>Amount of</td>
</tr>
<tr>
<td>24 hrs/cell</td>
<td></td>
<td>carbon</td>
<td>carbon</td>
</tr>
<tr>
<td></td>
<td></td>
<td>assimilated</td>
<td>assimilated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>in one cell</td>
<td>in one cell</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10⁻⁶ µg)</td>
<td>(10⁻⁶ µg)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Max. temp. °C</th>
<th>Cell division per 24 hrs/cell</th>
<th>Amount of carbon assimilated in one cell (10⁻⁶ µg)</th>
<th>Cell division per 24 hrs/cell</th>
<th>Amount of carbon assimilated in one cell (10⁻⁶ µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.61</td>
<td>1.90</td>
<td>12.60</td>
<td>1.50</td>
<td>11.80</td>
</tr>
<tr>
<td>22.23</td>
<td>2.25</td>
<td>19.90</td>
<td>1.75</td>
<td>20.50</td>
</tr>
<tr>
<td>28.41</td>
<td>3.00</td>
<td>21.70</td>
<td>2.40</td>
<td>21.40</td>
</tr>
<tr>
<td>32.24</td>
<td>3.25</td>
<td>10.10</td>
<td>2.50</td>
<td>13.30</td>
</tr>
<tr>
<td>39.30</td>
<td>1.25</td>
<td>05.20</td>
<td>1.40</td>
<td>06.20</td>
</tr>
</tbody>
</table>

Salinity of the Buckingham canal was 12 ppt by December, 22 ppt by March, 28 ppt by July, August and September, 32 ppt by June and 39 ppt by May. Migita (1969) has described the seasonal variations of *S. costatum* cell size along the coast of Nagasaki, Japan. He has suggested the temperature of 20°C as advantageous to the enlargement of cells. Table 1 shows that 28 ppt salinity and 29.7°C canal water temperature to be the best water condition for high cell density, chain length, number of chains per ml and number of cells per chain.

Huang et al. (1986) have also stated that *S. costatum* blooms seasonally in the Kaoshiung and Keelung harbours of Taiwan, particularly during spring time. In the present study, salinity increase led to shrunk in cell diameter and increase in intercellular spaces. Paasche et al. (1975) has studied *S. subsalum* (Cleve) in 1 ppt, 3 ppt, 5 ppt, 7 ppt and 10 ppt salinities observing different morphotypes.

From Table 1, it can be inferred that *S. costatum* growth was found to be effective at sub-saturating light intensities characteristic of July, August, September and October with optimal salinities and temperatures. Phytoplankton succession is indicative of a sequential process and supposedly correlating with the B₁₂ concentration of the canal water with the *S. costatum* stocks continually existing with a feeble occurrence during adverse factors and a dominating outburst during favourable conditions. This unseeded, independent existence is also attributed to the B₁₂ requirement of *Skeletonema costatum* and other B₁₂ requirement being *Platymonas, Monochrysis, Thalassiosira, Nitzchia, Cyclotella, Melosira* and *Gymnodinium* (Droop 1957, 1961; Ericson and Lewis, 1953). They appeared and died in sequence and possibly releasing necessary organic compounds especially B₁₂ for the sustenance of the latter succeeding species.

As from Table 1, the maximum B₁₂ concentration during July could be due to the eutrophied effluents from the 115 hectare shrimp ponds of the Waterbase Limited discharging water into the canal during the June crop
harvest. Burkholder and Burkholder (1956) have also supported the presence of B\textsubscript{12} in suspended solids and marsh muds. The bacterial levels peaking during the June month. Shrimp pond harvest may sustain the peaking \textit{S. costatum} blooms during July. Starr \textit{et al.} (1957) as well as Cowey (1956) stood on this view earlier on the production of B\textsubscript{12} active substances by marine bacteria. \textit{S. costatum} as specificity towards some vitamin B\textsubscript{12} like factors like B\textsubscript{12} (5,6 dimethyl benziminiazole); B\textsubscript{12} 111 (Factor I); Factor A (2-methyladenine); Factor II (2-methyl hypoxanthine); Pseudo B\textsubscript{12} (adenine) and Factor B (no nucleotide) according to Droop (1957).

Table 2 shows high % protein and chlorophyll-a by dry weight are the conductive factors of salinity and temperature. The amount of organic matter assimilated increases with decreasing temperatures. The same is true for protein, Jorgensen (1968) demonstrated that the protein concentration was twice as high in cells adapted to 7°C than cells adapted to 20°C. Despite the presence of pond harvest discharge during June, only month of July witnessed healthy cells with more protein, lipid and carbohydrate compositions. The fast flow of shrimp pond residues could have created a violent movement of canal flow and breaking-up of chains. The division rate of \textit{S. costatum} is slower in turbulent waters than in quiet waters according to Karsen (Hustedt, 1930). Too vigorous mixing leads to hydrodynamic stress influencing the development and the morphology of algae as stated by Bronnenmeier and Markl (1980).

Culture depths of 140–150 cm accelerated the photosynthetic rate coupling with other conductive factors yielded 1.25% d.w. of chlorophyll-a during July. According to Kitto \textit{et al.} (1999), areal productivity for \textit{S. costatum} in arid regions with performance in a Biothermostat cultivator ranged between 1.5 and 2 metres. Comparing the chlorophyll data and cell division rate (see Table 3), it can be corroborated that the rate of chlorophyll synthesis decreased relative to the rate of cell division.

Geider (1987) had stated about higher carbon to chlorophyll-a ration for \textit{S. costatum} lower temperatures. Table 3 shows less carbon assimilation by noon shine and at higher temperatures and higher canal salinities. Hellebust (1965) has confirmed the excessive organic carbon excretion of the total assimilation levels in \textit{S. costatum} cells at higher salinities.

It was also found that decreased rate of cell division is evident when \textit{S. costatum} cell suffers higher light intensities. Summer season (May) is characteristic of constant adverse factors like longer day length, increased solar radiation input, thermal stratification and high surface water temperatures. The competition for available nutrients and grazing of microalgae and bacteria by zooplanktons is also obvious. Nutritional deficiencies are always bound to cause structural malaise. Depletion of nutrient led to thin walled cells with dominance of other subdominant
species in the biological system of the same canal during repeated observations every year. *S. costatum* managed to thrive round the year in the same canal during 1993, 1995 and 1996 until today except for 21 May 1994 when atmospheric temperature reached 46°C in Nellore. The canal temperature 33°C was strong enough to bleach the cells to death. Periodic draining from the nearby hatchery is a strong factor for regular seeding effect.

This paper highlights the exotic *S. costatum* species, it originates from the Kaoshiung harbour and is an ecotypically different strain. The occurrence of this species in the semi-tropics of India in varying light intensities makes it a successful candidate for commercial cultures in shrimp hatcheries. Such ecotypic differentiation had also been confirmed by Gallagher et al. (1984). Further studies on the Photophysiology in response to environmental conditions can reveal more interesting details.

**REFERENCES**


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A cradle aeration system for hatching *Artemia*

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Abstract

The hatching performance of three types of aeration system was compared. For commercial applications, the cradle aeration system was superior to the single air stone system (90.7 vs 96.5% hatchability). The use of this system resulted in marginal Instar 1 production, lower E-1-arrest, high cyst water hydration activity, minimal nauplii injury, and convenience in harvest. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Cradle aeration; Artemia; Cyst; Hatching

1. Introduction

The real cost of *Artemia* nauplii to the commercial fish farmer is seldom calculated with any precision so that the price per million of nauplii fed to the larvae may vary by a factor up to 100 (Prescott, 1980). The costs of *Artemia* are significant in commercial hatcheries. Dissolved oxygen, light intensity and active mixing influence hatching (Sorgeloos et al., 1986). The purpose of this work is to develop hatching systems for commercial shrimp hatcheries.

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E-mail address: kasthuri@md5.vsnl.net.in (M.M. Michael Babu).
2. Materials and methods

The cradle aeration consists of 0.6 mm plastic tube articulated to each other to form a cradle frame work (Fig. 1) using Y and T shaped connectors. The over head air blower pipe line leads to two distal arms (with separate control valves) and which are interlinked by a surface air ring. The distal arm still extends down as a suspension collar to hold the cradle components.

The experiment was conducted in 500 l capacity black Fibre Reinforced Plastic (FRP) cylindro conical tank. The out let of the bottom tank provides regulatory screw type gate valve, which facilitates selective harvest of *Artemia* nauplii. The light
transparent area found near the bottom outlet helps to school all *Artemia* nauplii by phototactic attraction.

The filtered sea water (35 ppt) was used for hatching. The room temperature had a mean of 30°C with the range between 32–28°C. Mean temperature of hatching media was 30°C. The hatching tank was illuminated with fluorescent tubes and the light level at the water surface was 1950–2050 lux.

Disinfection of cyst was carried out by immersing cysts for 30 min in 150 ppm liquid bleach (NaOCl) and then it was washed thoroughly using filtered sea water until the cyst had no smell of chlorine. The disinfected cysts (1.68 g/l) were allowed into hatching tank and aerated by cradle type aeration. Water hydration was measured by spreading 1 g of 1 h hydrated cyst on blotting paper for 10 min. After inbibing all external water found on the hydrated cyst by the paper, the cysts were weighed. The increased weight (g) is represented as water hydration activity. Observation of E-1, E-2, Free nauplii and E-1 arrest have been observed by light microscope. First harvest was done 16 h after incubation. First harvest involved three sub harvests done within 5 min intervals. The aeration level was reduced to 50% of the initial rate after 12 h of incubation. The second harvest and final harvest was done 24 h after incubation. Water was splashed every 3 h on the side-surfacing cysts at the top. The aeration rate was 15–20 l per min. Aeration rate of the system was determined by the method of Sorgeloos et al. (1986).

The air lines were perforated with 500 μm twin hole on 2cm centre into two planes 180° apart. The harvested nauplii were strained out using a 215 μm mesh and stocked in the nauplii storage FRP cylindro conical tank (250 l capacity) with calibrations for water volume. Rinsing was performed using a nylon filter aided *Artemia* rinser, to remove the filterable microparticulates in the hatch-out debris.

Counting was done by sampling 10 times, a 1 l suspension of stored nauplii and each time diluting it 10 times (by adding 9 l sea water) and sampling 10 times about 25 ml of the naupliar suspension media and counting them by placing in a petri dish alive and after removing the possible presence of dead nauplii, the five nauplii are spread on to a dry broad filterpaper. The counted nauplii from the 25 ml sample is taken as, X, the mean being X. The X value is multiplied by 40 and the product value multiplied by 250 to get the total number of hatched live *Artemia* nauplii from the hatching tank.

Single airstone system consist of two components. One is airtube and the other is airstone. In this system, the airtubes were connected to the main air supply pipe using regulator valve, which helps to regulate air supply in the hatching systems. The air stone hangs 2 cm above the bottom surface of the hatching tank. Air flow rate was reduced after confirming full hatching. Imhoff cone, the second system compared was made up of glass or transparent plastic in different capacities comprised of one air tube in the centre of the cone that helps good aeration and quick cyst hydration.
Table 1
Hatching performance of *Artemia* cysts in different hatching systems

<table>
<thead>
<tr>
<th>Hatching system</th>
<th>Dropline aeration (Single air stone)</th>
<th>Cradle aeration (Single air stone)</th>
<th>Imhoff cone (Single air stone)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hatching system performance values</strong></td>
<td>500 l</td>
<td>500 l</td>
<td>1 l</td>
</tr>
<tr>
<td>Number of nauplii harvested in first harvest/g cyst (16 h after incubation)</td>
<td>114 612</td>
<td>143 532</td>
<td>166 713</td>
</tr>
<tr>
<td>Number of nauplii harvested in second harvest</td>
<td>101 638</td>
<td>83218</td>
<td>61037</td>
</tr>
<tr>
<td>Hatching percentage</td>
<td>86.5</td>
<td>90.7</td>
<td>91.1</td>
</tr>
<tr>
<td>Time taken for attaining E-1 stage (hours)</td>
<td>11.0–12.0</td>
<td>9.0</td>
<td>8.5</td>
</tr>
<tr>
<td>% E-1 occurrence (after 11 h)</td>
<td>45%</td>
<td>58%</td>
<td>69%</td>
</tr>
<tr>
<td>E-2 stage appearance (hours after incubation)</td>
<td>12.0</td>
<td>10.0</td>
<td>9.7</td>
</tr>
<tr>
<td>Free nauplii appearance (hours after incubation)</td>
<td>13.0–14.0</td>
<td>12.0</td>
<td>11.5</td>
</tr>
<tr>
<td>% E-1 arrest (24 h after incubation)</td>
<td>3%</td>
<td>0.5%</td>
<td>0.2%</td>
</tr>
<tr>
<td>Water hydration activity $a_w$ (g/g cysts w/w) 1 h after hydration</td>
<td>1.39</td>
<td>1.45</td>
<td>1.51</td>
</tr>
</tbody>
</table>

3. Results and Discussion

The influence of a cradle aeration aided homogeneous suspension giving higher hatching percentage and maximum naupliar output per first harvest shows critical variations from similar parameters with a single air stone. The percentage increase in hatching with cradle aeration system was 4.2%. Percentage increase in instar 1 output during the first harvest was 16.3% with the cradle aeration and is the highest recorded factor comparing all other hatching parameters. Cradle aeration efficiently triggers the cysts to the E-1 (umbrella stage) within 9 h of incubation. In 11 h after incubation, 58% of the total cysts reached E-1 stage in the cradle aeration-driven air system against the 45% E-1 in a single air stone-driven hatching media. Imhoff cone hatching results were far better than cradle aeration aided hatching. The imhoff hatching data showed 91.1% total hatching and 73.2% instar 1 harvest. Water hydration activity (g/g cysts w/w) of cysts was 1.39, 1.45 and 1.51 with single air-stone driven, cradle aeration driven and imhoff cone systems, respectively. Percentage E-1 arrest was 3% in single air-stone driven system, 0.5% in cradle aeration system and 0.2% in Imhoff cone system. All the above results have been given in Table 1.

The advantages of the present system is due to the adoption of the following functions. The eggs ought to be basically in motion (Terramoto and Kinoshita,
1961). Tube surface perforations release air to thermo regulate the hatching system without a variable temperature gradient. The breaking up of molecular air into small bubbles decrease the partial pressure of oxygen per unit area and increase its retention time in media. Vigorous aeration in the hatching column assures equi-distribution of oxygen in every unit pocket of the whole vessel. The bottom air stone helps during harvest time to rescue the crowding nauplii assuring a meagre air circulation for the bottom schooling nauplii. Unlike concentrated split-fractioning of air from a source in an air stone or diffuser, cradle aeration module has three functions. First, propelling force by the bottom air flush on the gravity settling cysts. Second, uplifting force by the air from the cradle seat, cradle suspension and medial arms on the suspended cysts. Upthrust cum immersion force of the surface tube air ring on the floating cysts. Before nauplii harvest, the cessation of surface ring aeration first helps in the broken cyst shell uprise.

Lavens and Sorgeloos (1987), state that adequate molecular oxygen with sufficient partial pressures is an essential requirement for the aerobic resumption of metabolic development and pre-nauplii emergence from the cysts. However, the present study confirms that to avoid oxygen gradients during hatching it is obvious that a good homogenous mixing of the cysts in the incubation medium is required. As had been pointed out by the authors in the present study, violent air bubbling can have a deleterious effect on the Artemia nauplii Greve (1968).

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