Microalgae Technologies & Processes For Biofuels / bioenergy production in British Columbia:

by

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LIST of APPENDICES

Appendix A

Appendix A. Factors Increasing Uncertainty About Estimates of the Market Potential of Biofuels

The following factors increase the uncertainty with respect to the estimates of biofuels market potential in Chapter 1:

- The projections were made in 2005/2006, when the cost of a barrel of oil ranged from $60.32 to $70.02 per barrel, compared to an average of $119.20 per barrel for the first half of 2008 [141].
- A strong backlash against first-generation biofuel feedstocks may lead to reductions in ethanol mandates, especially in Europe. The additional potential for biofuels from currently unused farmland is estimated at 27 GL per year, in addition to existing production [75].
- Importing countries concerned about the environmental impacts of biofuel production are poised to introduce environmental certification schemes
- A current worldwide economic slow down may reduce fuel consumption compared to current scenarios used.
- Different, competing technologies are being investigated in the design of fuel efficient automobiles. The dominance of one technology over the others will in turn influence liquid fuel consumption.
- The economics and capacity for fuel production and incorporation into existing infrastructure may also influence the development of transportation technologies. For example, the availability of lignocellulosic ethanol is mainly placed in the decade of 2020-2030, which may be too pessimistic in light of recent developments.
- At present, the lower ethanol demand scenario seems more likely to occur, whereas biodiesel production may well be higher in 2030 than estimated above, in the authors' opinion. The consumption of liquid fuels for the heating, marine and aviation sectors is expected to remain smaller than that for automotive fuels, but is also likely to increase the overall demand for biofuels.

APPENDIX B

Appendix B. Further Options For Monetizing Microalgae By-Products

Hydrogen

Two main avenues are being pursued to turn biomass into hydrogen: gasification and biological hydrogen production. Both processes are still at the development stage. Gasification is feasible with substrates that contain no more than 60% water. Algae are, therefore, not an ideal feedstock for gasification because they need energy-intensive drying before they can be processed. The subsequent steps to reform the syngas to a gas with very high hydrogen content are not commercial yet.

Some varieties of algae contain hydrogenase; - an enzyme that can create small amounts of hydrogen gas, and related research is on going at several facilities. A research group at Argonne National Laboratory (Illinois) is trying to find a way to take the part of the enzyme that creates the gas and introduce it into the photosynthesis process [67]. The result would be a large amount of hydrogen gas, possibly on par with the amount of oxygen created. Obstacles to overcome in this process include the sensitivity to oxygen of hydrogenase and that the overall reaction to produce hydrogen from glucose is only slightly energy positive (hydrogen must be continually withdrawn to maintain its production) [92]. High conversion efficiencies can only be maintained for transient periods, and this must be improved upon, for economic viability to be achieved.

An alternative way to produce hydrogen from algae may be anaerobic digestion, which produces a biogas containing methane and hydrogen [60].

Butanol and Synthetic Gasoline

Butanol is an alternative to ethanol as a biofuel, and is made from the same feedstocks, using cellulosic plant material. Its longer alkyl chain results in an easier solvent extraction process, higher energy content per weight, and lower vapor pressure. Unlike ethanol, butanol is not corrosive and has very similar properties to gasoline, i.e. it can be added to gasoline in higher proportions without engine modification. The key research challenge that must be resolved is that butanol production inhibits microbial growth even at low concentrations. The result is that the product of the fermentation is less than 2% butanol. The overwhelming majority of the fermentation broth is water, so an energy-intensive distillation step is required for purification [248]. Among other companies, BP and DuPont have partnered up to commercialize biobutanol for the automotive sector [33].

A completely different way of producing a non-diesel fuel is being explored by US company Diversified Energy Corporation. It uses the Centia Process to turn oils into a gasoline-like fuel (not an alcohol), with a mass conversion efficiency of up to 90%. This process appears to lend more flexibility to biofuel production by allowing the use of a portion of the algae oil to make gasoline instead of biodiesel. The process is being demonstrated and optimized in cooperation with North Carolina State University [77]. The three steps of this process are:

- Hydrolytic conversion. The feedstock is heated under pressure to separate free fatty acids from glycerol in the triglycerides in the feedstock. Centia accommodates any lipidic compound without modification to the production process.

- Decarboxylation. The free fatty acids and solvent are heated, pressurized, and passed through a catalyst in a reactor to produce n-alkanes, the building blocks of fuels.
- Reforming long-chain alkanes. The resulting alkanes—straight-chain hydrocarbons of 15-17 carbon atoms—are reformed into branched alkanes and ring structures. The process is optimized to maximize C10 through C14 iso-alkanes. The alkanes can be reformed differently to create a variety of fuel types. By varying the catalyst, temperature, pressure, and kinetics of this third step, Centia can produce a wide range of biofuels that mimic their petroleum-derived counterparts.

Pyrolysis Products

Pyrolysis is the thermal degradation of biomass at temperatures between 400 and 600˚C. It results in different amounts of char, non-condensable gases and pyrolysis oil, depending on the feedstock and the processing temperature. The gases can be used within the process, e.g. as a heat source. Likewise, char can be burned to generate process heat, or be sold to other users. Some manufacturers, such as Canadian company ABRI, suggest mixing the char with the bio-oil, which can then be combusted as a slurry. The bio-oil itself is a mixture of condensable gases and water. The water content reduces the heat value of the oil, which can be used in industrial heat boilers, such as for the pulp & paper industry. Specialized equipment is required to use bio-oil for power generation, since its properties are very different from conventional fuels. Keeping the water content in bio-oil low requires drying the feedstock. Since algae are grown in water, extensive drying would be necessary, which will impact negatively on the energy balance. Pyrolysis for energy is still a pre-commercial process, and with their high moisture content, algae do not appear to be a suitable feedstock for this technology.

On the other hand, pyrolysis to produce an “agro-char” has been proposed as a waste elimination and carbon sequestration strategy [205]. Instead of concentrating on the oil, this process would therefore mainly produce char from dried algae. This char could be used as an agricultural soil improver, and would bear the advantage that genetically modified algae would be biologically inactivated before leaving the algae production plant. Char can improve soil quality and microbial activity, but may also lead to the release of more carbon dioxide from soil through the very same process, such that the possibility of carbon sequestration (or potential net soil carbon losses) through this route would require further investigation.

Compost

If algae or algae residue is digested, the next step is usually some post-composting of the bioreactor sludge to consolidate it, stop the anaerobic process, and produce compost that can be used in agriculture or for horticultural purposes. The value of this compost is likely to be low. Compost must also be transported and its ultimate value will depend on local demand, which may be saturated soon with industrial-size algae production. Compost can then become a cost factor in case it needs to be landfilled. Note that in case seawater is used to cultivate algae, the compost is unlikely to be of any value due to its salt content. Another method of disposing of bioreactor sludge may be to use it as a fertilizer for algae cultivation, as discussed in the main report.

Appendix C

Appendix C. Showing fatty acid composition of some normally growing phototrophically cultured microalgae (expressed as % of total lipid)

Class	*Prasinophyceae*	*Prymnesiphyceae*	*Cryptophyceae*		*Bacillariophyceae*		*Chlorophyceae*	*Cyanophyceae*
Species	*Pyramimonas virginica*[3]	*I. galbana*[4]	*Rhodomonas lens*[5]	*Oocystis sp.*[6]	*C. muelleri*[4]	*P. tricornutum*[4]	*T. suecica*[4]	*Spirulina sp.*[7]
Fatty acid								
C12:0	4.54							
C13:0	1.09							
C14:0	3.46	11.5	4	0.2	5.9	5.9	0.2	0.3
C15:0	0.59	0.1			0.3	0.2	0.1	
C16:0	29.51	4.7	13.7	3.8	7.1	11.8	13.7	46.8
C17:0	2.33						0.1	
C18:0	3.71	0.3	0.7		1.5	0.3	1.4	3.1
c22:0					0.2	0.1		
C24:0				0.1	0.1	1.1		
Total saturated	**45.23**	**16.6**	**18.4**	**4.1**	**15.1**	**19.4**	**15.5**	**50.2**
C14:1	3.7	0.5						

C16:1w7	3.21	3.6	0.4	1.5	13.9	16.1	0.2	
C16:1w5					0.7	0.8	0.9	
c16:1w9		0.1				0.1	1.2	4.4
C17:1		0.1						
C18:1w7	6.45	0.6	2.5		0.7	1.3	1.4	
C18:1w9		8.7	0.3	3.9	1	3.1	7.8	12.2
C20:1	-		0.2				1.1	
C22:1	0.13							
Total monounsaturated	**13.36**	**13.6**	**3.4**	**5.4**	**16.3**	**21.4**	**12.6**	**16.6**
C16:2w4		0.6			5.6	3.6		
C16:2w6		0.3			2.8	0.3	0.6	
c18:2w6	2.18	5.9	1.1	6.4	0.5	2.1	10.3	
C16:3	8.47				14.4	11.1	1.1	
C16:3w6							4.1	
C16:4			0.1		0.4	0.5	17.7	

C18:2w9								18.8
C18:3w3	5.28	7.9	28.1	8.1	0.8	0.4	15.4	
C18:3w6		1			0.8	0.8	1.2	14.3
C18:4w3	5.1	18.8	15.3	0.7	0.6	0.6	8.5	
C18:4		3.8					1.9	
C20:2w6	2.24							
C20:3	0.2					1.3		
C20:3w6						0.7		
C20:4w3						0.2	0.6	
C20:4w6	3.4		0.1	0.5	3	0.9	2.1	
C20:5w3	2.03	0.9	10.9	1.1	36.8	35	8.4	
C22:4w3								
C22:5w6		3.8	9					
C22:5w3	0.78							
C22:6w3	2.45	26.8				1.5		
Unidentified								

Total poly unsaturated	**32.13**	**69.8**	**64.6**	**16.8**	**65.7**	**59**	**71.9**	**33.1**

Key to sources:

1=Pratoomyot et. al. [175]; 2=Ben-Amotz et. al. [18]; 3=Webb & Chu [241]; 4=Rivero-Rodriguez et. al. [197]; 5=Milke et. al. [148]; 6= Patil et. al. [169]; 7=Tedesco and Duerr [219].

Appendix D

Appendix D. TransCanada Pipeline Specifications for Natural Gas (TCP [218])

Parameter	Specification
Hydrogen sulfide	23 $mg.m^{-3}$
Total sulfur	230 $mg.m^{-3}$
Carbon dioxide	2% by volume
Oxygen	0.4% per volume
Nitrogen	Not specified
Temperature	43.3˚C
Heating value	36.94 $MJ.m^{-3}$
Water	65 $mg.m^{-3}$
Hydrocarbon dewpoint	-10˚C (100-1000 psia)

Appendix E

Appendix E. Organizations Exploring Algae Cultivation for Energy

Organization	*Country*	*Type*	*Algae species*	*Main product*	*Method*	*Comments*
CARS	Canada	Research group	tbd	Methane	Housed pond	Canadian research project; envisage using flue gas from coal plants; also looking at biodiesel and ethanol
Chevron CRADA	USA	Research group	tbd	Transp. fuel	tbd	Cooperation between NREL and Chevron, since Oct. 2007
DARPA	USA	Military research	tbd	Algae oil	tbd	Research program for low-cost algal oil production and conversion to JP-8, the fuel used by all military aircraft, tanks and non-nuclear ships
LiveFuels	USA	Alliance of labs and scientists	tbd	Biocrude oil	Open ponds	
A2BE Carbon Capture	USA	Company	microalgae	Biofuels	Enclosed raceway	US patent application 20070048848
AlgaeLink	NL	Company	Unknown	Algae oil	Tubular bioreactor	Intend to produce jet fuel from algae oil
Algenol	USA	Company	"Metabolically enhanced" blue-green algae	Ethanol	Bioreactor	Algae produce and secrete ethanol permanently, which is constantly removed from the process. Uses CO_2 from power

Organization	*Country*	*Type*	*Algae species*	*Main product*	*Method*	*Comments*
						plant.
Aurora BioFuels	USA	Company	Microalgae	Algae oil	Bioreactor	
Aquaflow Bionomics Corp.	NZ	Company	Existing species in wastewater	Algae oil	Wastewater treatment ponds	
Cellana	USA	Company	Indigenous microalgae	Algae oil	Seawater ponds	Joint venture of BH Biopetroleum and Shell in Hawaii
De Beers Fuel	ZA	Company	Microalgae	Algae oil	Bioreactor	Technology from GreenFuel Tech.
Enhanced Biofuels & Technologies	UK	Company	Microalgae	Oil and ethanol	Bioreactor & open pond	CO_2 from coal plants; uses GreenFuel technology
GreenFuel Technologies	USA	Company	Microalgae	Algae oil	Bioreactor	Also produce delipidated algal meal and dried whole algae; are investigating wastewater streams
GreenShift	USA	Company	Blue-green algae	Unknown	Membrane bioreactor	Uses a fibre-optic system to distribute light to algae in reactor. Information no longer available on company website.
IGV	Germany	Private R&D Institute	Microalgae	Biofuels	Bioreactor	Also work on hydrogen from microorganisms
Infinfuel	USA	Company	unknown	Algae oil	Open ponds	Heated at night with geothermal energy
International	Canada	Company	Microalgae	Algae oil,	Bioreactor	Claim to have a technology that can extract

Organization	*Country*	*Type*	*Algae species*	*Main product*	*Method*	*Comments*
Energy inc.				biocrude		oil from algae without killing them
Menova	Canada	Company	Microalgae	Algae oil	Bioreactor	Uses solar concentrators and heating to maintain high temperatures during winter
Oil Fox	Argentina	Company	unknown	Algae oil	Bioreactor	
PetroAlgae	USA	Company	Patented microalgae	Algae oil	Bioreactor	
PetroSun	USA, AU	Company	Microalgae	Algae oil	Open ponds	Also use wastewater
Sapphire Energy	USA	Company	Genetically modified algae	Biocrude	Bioreactor	Crude oil that can be refined to gasoline, diesel, jet fuel
Seambiotic	Israel	Company	Nannochloropsis Dunaliella (Microalgae) Skeletonema (Diatom)	Algae oil and ethanol	Open raceway	Seawater; intend to use flue gas from coal plants
Solazyme	USA	Company	Heterotrophic algae	Algae oil	Steel containers	Genetically engineered algae
Solix Biofuels	USA	Company	Microalgae	Algae oil	Bioreactor	Uses flue gas for CO_2
Valcent Products	USA	Company	unknown	Algae oil	Vertical bioreactor	Plastic bags inside a greenhouse; use flue gas for CO_2

Appendix F

Appendix F. Algae Culture Techniques

Open pond culture:

The large-scale outdoor culture of microalgae and cyanobacteria in open ponds is well-established [12; 19; 28]. Open pond systems are usually shallow systems in which the microalgae are grown. The ponds can be excavated and used unlined, or lined with impermeable materials, or built up with walls of concrete or other materials.

Currently, the successful culture of microalgae in outdoor ponds is limited to a small number of algae species, which can tolerate extreme environmental conditions to the exclusion of most other species. These include fast growers such as *Chlorella* and species that require highly selective environments such as *Spirulina* and *Dunaliella* which thrive in highly alkaline or saline selective environments.

There are four main types of open ponds:

Unmixed open ponds

These are open ponds that are unmixed other than by wind and convection [19]. They have depths ranging from 20-100 cm. Unmixed ponds can be very large, such as pond systems used in Australia for the culture of *Dunaliella* salina which range from 1 to 200 ha (Figure 1). They are unsuitable for the culture of most other species of algae [28; 30] and have low productivities (<1 $g.m^{-2}.d^{-1}$) [19].

Raceway ponds:

Raceway ponds are the most common type of open pond currently in use. They are widely used for the commercial cultivation of *Spirulina*, *Haematococcus* and *Dunaliella* [19; 28]. These ponds utilize paddle wheels for agitation and mixing of the cultures, with reported flow rates ranging from10-30 $cm.s^{-1}$ (Figure 2, Figure 3) [19; 28; 30; 113; 243], with depths ranging from 10-30 cm and individual ponds being up to 1 ha in area. Much higher productivities have been reported from raceway ponds compared to unmixed ponds. Reported productivities have ranged from 14 $g.m^{-2}.d^{-1}$ (0.07 $g.L^{-1}.d^{-1}$) in northern California, to 50 $g.m^{-2}.d^{-1}$ (0.42 $g.L^{-1}.d^{-1}$) in Hawaii [116; 117; 208; 243].

Circular ponds:

Circular ponds are used mainly in Japan, Indonesia and Taiwan for the production of *Chlorella*. For mixing, they have a centrally located rotating arm, similar to those used in wastewater treatment (Figure 4). These ponds have depths ranging from 5 to about 30 cm [28; 126], and individual ponds can be up to 0.5 acres [125]. One of the main disadvantages of this system is the size limitation imposed on the ponds by the complexities involved with the use of the circular rotating arms. Annual average productivities achieved by commercial plants range from 8.5 $g.m^{-2}.d^{-1}$ to 21 $g.m^{-2}.d^{-1}$ [19]. These higher productivities may be attributed in part to mixotrophic growth as organic carbon was also added to the tanks [125].

Thin layer, inclined ponds:

Inclined systems were developed in the Czech Republic [207], with several pilot scale systems currently in use there [135; 136]. The initial design consisted of a shallow tray inclined at 3°, over which a 5 cm thick culture of algae flowed at 8 $cm.s^{-1}$ against slotted baffles which were

arranged perpendicular to the culture flow for mixing. At the bottom of the incline, the culture water was collected and returned to the top again (**Figure 5**). The cultures are kept in an aerated tank overnight. Improvements to the system were made in the 1990s and 2000s, which included removing the baffles, arranging the culture area into multiple cascades, changing the incline to 1.7° and reducing the depth of the cultures to 0.6-0.8 cm [68-70]. These very shallow systems are well mixed and very high productivities of up to 3.1 $g.L^{-1}.d^{-1}$ (31 $g.m^{-2}.d^{-1}$) have been reported in these systems [70].

Figure 1: Unmixed open ponds used for the production of *Dunaliella salina* at the Hutt Lagoon in Australia (Borowitzka [29])

Figure 2: Raceway ponds used for the production of *Spirulina platensis* in California (Borowitzka [29])

Figure 3: Raceway pond used for the production of *Dunaliella salina* in Israel (Wijfels [245])

Figure 4: Circular ponds used for the production of *Chlorella sp.* in Japan (Borowitzka [29])

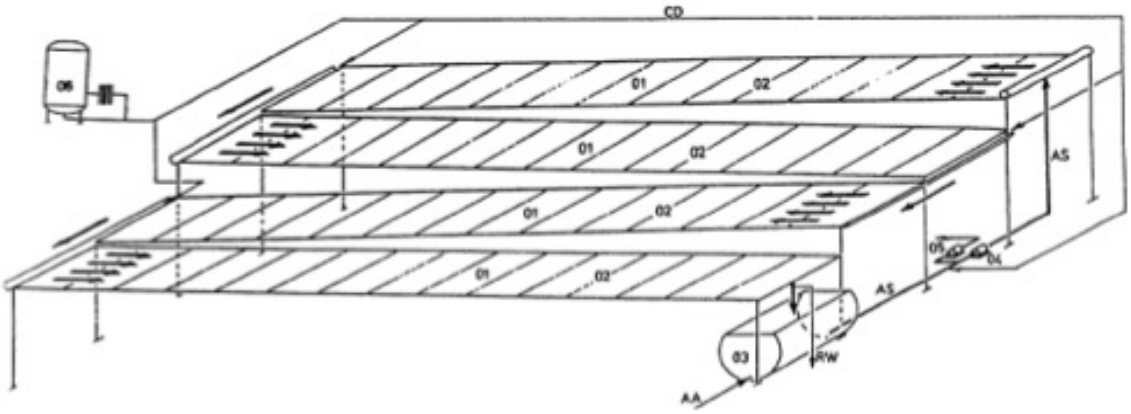

Figure 5: Schematic view of thin layer inclined culture system.1-cultivation area, 2-transverse baffles, 3-storage tank, 4-circulation pump, 5–harvesting pump, 6–CO_2 tank, AA–air, AS–algal suspension, CD-carbon dioxide, RW-rain water (Zeisler et al. [252]).

Photobioreactors (PBRs):

The main constraints related to the operation of open systems are the inability to control contamination, environmental factors, and the high cost of land. In addition to these, the lower volumetric productivities mean that very large volumes have to be processed to obtain the final product.

Closed (or mostly closed) photobioreactors (PBRs), were designed to overcome these problems with improved volumetric productivities, the reduction in risk of contamination, the ability to grow algae species that require controlled environments and the prevention of loss of water through evaporation.

A wide variety of PBRs have been described and built ranging from tubular and cylindrical systems [42; 171; 173; 180], conical systems [156; 240] flat sided systems [102; 104; 226], to flexible tubing coiled around a cylindrical framework [30; 235].

Photobioreactors can be located indoors utilizing light collection systems or outdoors using sunlight as the light source. Light collection and distribution systems are very complex and costly and for commercial bioenergy applications, natural illumination is the only feasible option [201]. As a result, even though the following sections describe systems using both natural and artificial lighting, the discussions and comparisons will be limited to systems utilizing natural illumination.

Tubular PBRs

Several tubular photobioreactors have been described and built since the 1950s [3; 153; 167; 171; 193; 233]. The tubes may be serpentine, arranged vertically as a fence, or horizontally on the ground. They are usually made of glass or plastic as the light receiving surfaces have a gas exchange vessel for the addition of CO_2 and the outgassing of O_2, and a recirculation pump (using air or water) between these two parts, facilitates mixing (see Figure 6, Figure 7, Figure 8). Maximum productivities reported in horizontal tubular PBR growing *Phaedactylum tricornutum* are: 34 $g.m^{-2}$(land area).d^{-1} and 2.2 $g.L^{-1}.d^{-1}$ [200]. When expressed as a function of the irradiated surface area of the tube for the purposes of this study, this value corresponded to 30.7 $g.m^{-2}$(tube surface area).d^{-1}. Olaizola [167] reported sustained average

productivities of 13 $g.m^{-2}$(occupied land area).d^{-1} (0.05 $g.L^{-1}.d^{-1}$) for commercial-scale cultivation of *Haematococcus pluvialis* in 25,000 L horizontal PBRs (see Table 4 also for a comparison of productivities).

Inclined Tubular PBRs

A disadvantage of horizontal tubular reactors arranged horizontally on the ground is that the total energy received by such tubes may be significantly lower than the energy received by the same tubes when they are inclined at an angle to the ground [123; 127], thereby ensuring full exposure of the entire reactor surface to irradiance through out the day. Inclined tubular PBRs are usually mixed with a combination of aeration and static mixers [232]. These authors reported a productivity of 20.89 $g.m^{-2}.d^{-1}$ (0.67 $g.L^{-1}.d^{-1}$) from an inclined reactor growing *Chlorella sorokiniana*.

Vertical bubble columns and airlift reactors:

These are cylindrical PBRs where gas bubbles are introduced at the bottom of the container. Vertical PBRs may be simple bubble column PBRs, split cylinder airlifts or draft tube airlifts (Figure 9). Sanchez Miron et al. [201] investigated the growth of *Phaeodactylum tricornutum* in the three types of bubble column PBRs shown in Figure 9, and found the same average specific growth rate for all three. In the bubble column PBR, gas is sparged into the bottom of the vessel for mixing, off-gassing of oxygen and addition of CO_2. Draft tube airlift PBRs are vertically oriented concentric tubes in which the aeration is supplied into the bottom of the inner tube. This creates a pressure gradient and an annular flow of the algae culture upwards to overflow downwards in the outer tube. Similarly, split cylinder airlift PBRs have gas sparged into the riser section, creating an annular an overflow into the downcomer section.

Sanchez-Miron et al. [200] compared bubble columns with horizontal tubular PBRs and reported that the presence of gas bubbles enhances internal irradiance, increasing irradiance when the sun was low on the horizon and reducing it when the sun was vertically overhead. The bubble columns have longer light paths than the thinner PBRs. Hence, they exhibit lower productivities. Sanchez-Miron et al. [200] reported that the volumetric biomass productivity obtained in a vertical bubble column was only 60% of that obtained in a horizontal PBR.

Sanchez-Miron et al. [200], reported an areal productivity of 93 $g.m^{-2}.d^{-1}$ (corresponding to a volumetric productivity of 0.64 $g.L^{-1}.d^{-1}$), when *P. tricornutum* was grown in a bubble column PBR (taking into consideration, the area occupied by the vertical PBR and the 1.3 m shadow cast). This was a higher areal productivity than obtained in the comparable tubular reactor which, while exhibiting higher volumetric productivity of 2.2 $g.L^{-1}.d^{-1}$, exhibited an areal productivity of 34 $g.m^{-2}.d^{-1}$. However, when the productivities obtained in both systems are expressed as a function of the exposed surface area in both cases, similar values of 34 $g.m^{-2}.d^{-1}$ and 30.7 $g.m^{-2}.d^{-1}$ are obtained for the vertical bubble column and the horizontal tube (see also Table 4).

Combined bubble column and inclined tubular PBR

A patent application submitted by Berzin [21] for a right angled triangular design for a PBR combines the principle of a bubble column with mixing by built-in static mixers in an external, inclined “downcomer” which also has a counter current of gas to increase mass transfer. Gas exchange occurs at a gas exchange vessel located at the apex of the triangle

(Figures 10a and b). A pilot plant is currently in operation at MIT, utilizing flue gas. It is comprised of a parallel array sharing combined gas spargers and common liquid media headers at the bottom of the PBRs. Schenk [205], citing press releases from MIT, claimed average productivities of 98 $g.m^{-2}.d^{-1}$ over a period of 19 days even under sub-optimal lighting conditions. The published patent application claims the capacity to produce 188.7 $g.m^{-2}.d^{-1}$ (31,000 tons per 0.45 $km^{-2}.y^{-1}$ [21]. These figures have been called into question [65; 66], as they exceed the theoretical limits of productivity.

Helical PBRs

Helical PBRs [30; 54; 235] (Figure 11), are composed of parallel sets of flexible translucent tubes (with inner diameters of between 2.4 to 5.0 cm) coiled helically around a cylindrical mesh frame. Gas exchange is accomplished via an incorporated gas exchange system at the top of the unit and a heat exchange system is generally included for temperature control. The culture is pumped upwards using either an airlift or centrifugal pump, to the gas exchange device where oxygen and air (if used), are released to the atmosphere. From the degassing device, the culture medium is gravity fed into a closed reservoir where CO_2 is added before the culture returns to the light collecting tubes again. Although the angles towards the sunlight are inflexible, Carvalho et al. [39] suggested that placing a light source inside the coil and then providing a reasonable control of light intensity may compensate for the large angle toward sunlight.

High productivities of up to 113.7 $g.m^{-2}.d^{-1}$ (0.9 $g.L^{-1}.d^{-1}$) have been obtained from helical PBRs [228]. However, it should be noted that the areal productivity appears high, being based only upon the occupied ground area. When the productivity was re-calculated based on the irradiated surface area of the tubes[1], the areal productivity obtained was 19.5 $g.m^{-2}.d^{-1}$. Similarly, the values of 29.73 $g.m^{-2}.d^{-1}$ (1.01 $g.L^{-1}.d^{-1}$) given by Uribe et al. [235] were reduced upon recalculation[2] to 10.5 $g.m^{-1}.d^{-1}$ and 0.06 $g.L^{-1}.d^{-1}$.

Flat Plate Reactors

The initial flat plate reactors (FPR) were made of two layers of translucent, rigid plastic sheets which had ribs (alveolae) running vertically up from bottom to top (**Figure 12**). The plastic sheets were sealed on three sides with rectangular pieces of plexiglass to form a very thin box and the top opening is capped. In the top and bottom part of the panel, the inner ribs (forming the alveoli) were removed for a length of about 15 cm to ensure free communication of the culture suspension from the entire panel at the top and bottom of the FPR. A perforated tube is placed running along the entire bottom of the FPR and mixing and deoxygenation of the cultures were obtained by bubbling air through this tube [226]. The FPR is cooled by running cooler water over it when the temperature rises above optimal for the culture.

A flow-through modification was described by Tredici and Materassi [227] (Figure 13) where every other wall of the alveolar sheet is shortened by about 2 cm at the bottom or 10 cm at the top of the PBR. By bubbling air into alternate channels through a pipe placed 2.5-3 cm

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[1] Illuminated surface area recalculations for helical PBRs assumed the use of both direct light striking the front of the PBR as well as diffuse light impinging the rear. Hence, calculations of illuminated surface area were calculated as total illuminated surface area divided by 2.

[2] Volume calculations included the capacity of the reservoir .

above the bottom, rapid circulation of the culture suspension through the panel is achieved when the two external channels are connected.

In another modification of the flat plate reactor, the alveoli which interfere with turbulent streaming were eliminated and mixing was accomplished by using two air tubes: one lying along the bottom of the PBR and the other at half the height of the reactor [102].

Tredici et al. [226] and Tredici and Materassi [227] achieved cooling by using triple walled panels made of translucent PVC. This double layer panel is laid on the ground and the top layer is used for circulation of the algae culture while the bottom layer has thermostated water running through it.

Productivities of 1.09 $g.L^{-1}.d^{-1}$ have been reported from flat plate reactors with *Spirulina platensis* as the test alga [228]. This corresponded to 15.3 $g.m^{-2}$ (exposed surface area).d^{-1}.

Figure 6: Fence –arranged tubular photobioreactors (Wilson [247]).

Figure 7: Horizontal, tubular photobioreactor at Algatechnology, Israel (Wijfels [245])

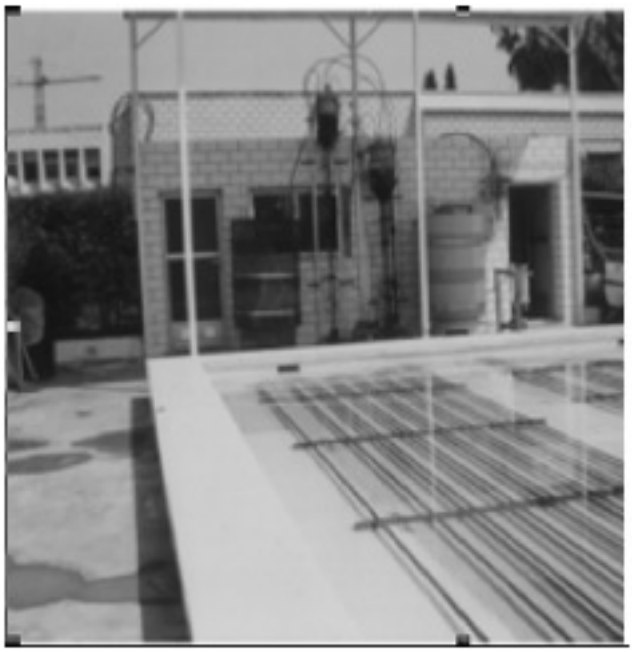


Figure 8: Airlift driven tubular, serpentine photobioreactors.

Two reactors are shown with the tubular loops immersed in a pond of cooling water. The two vertical columns at the far end are the airlift devices for circulating the culture through the horizontal loops (Molina Grima et al. [150]).

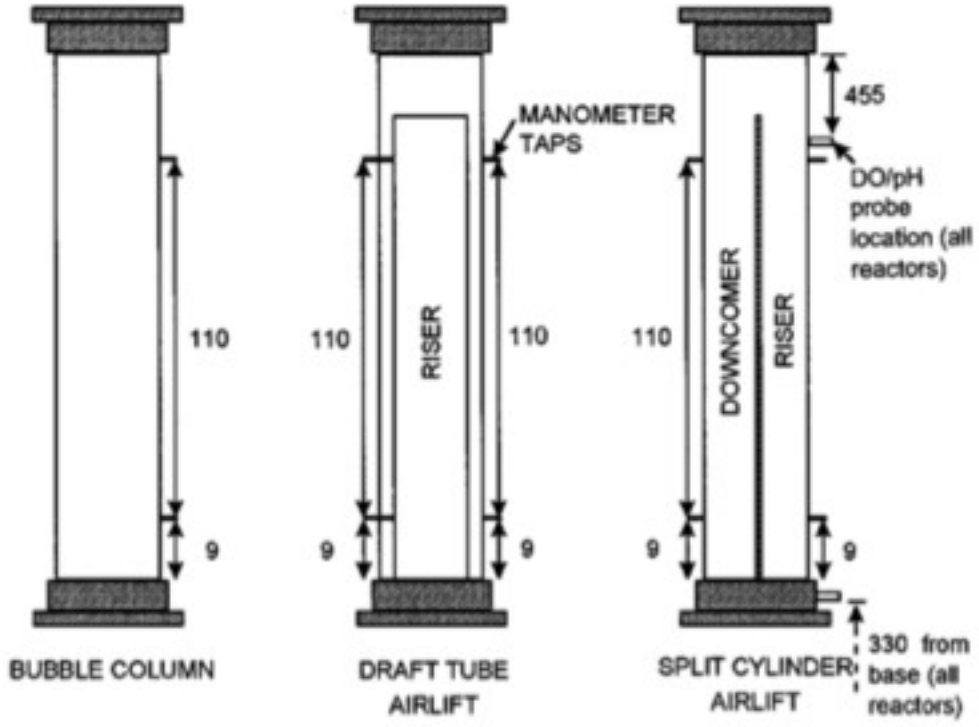
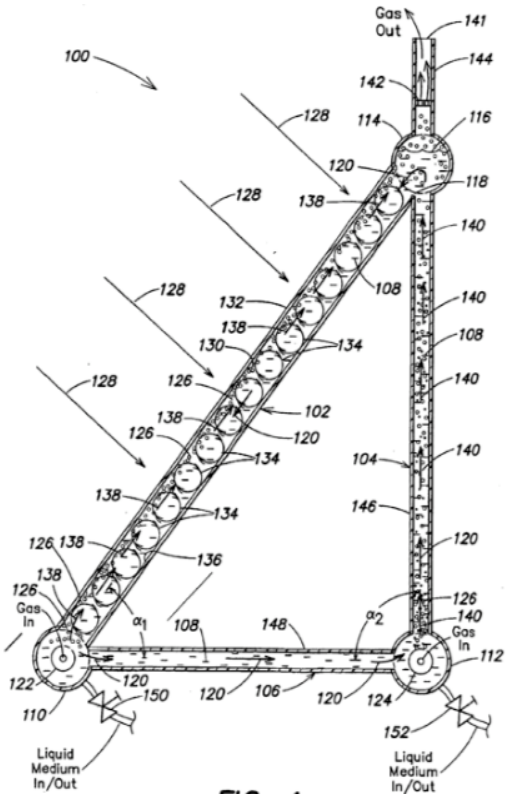
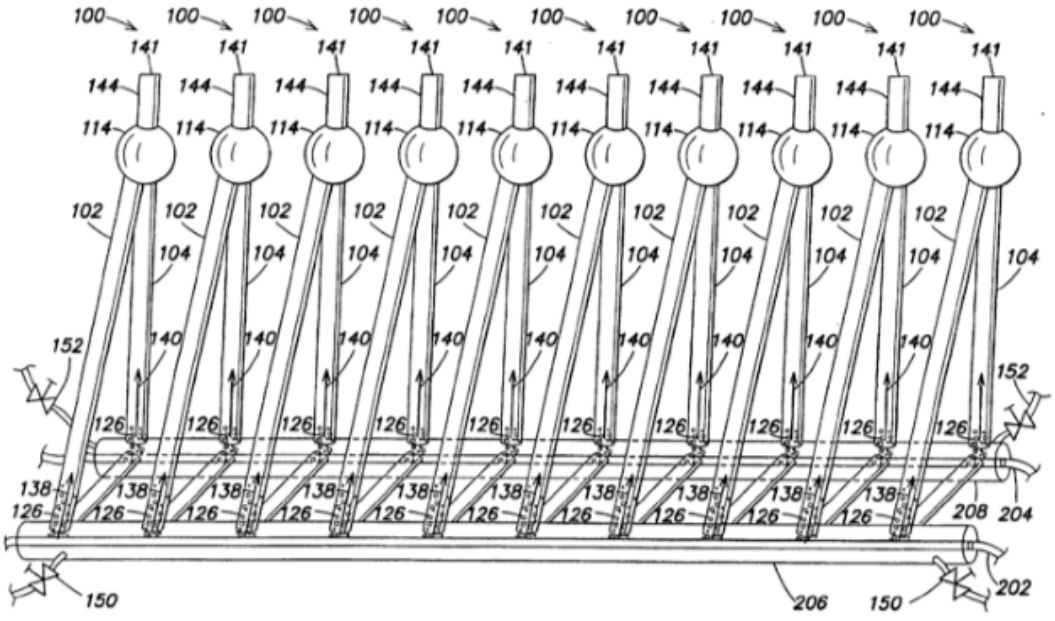


Figure 9: Different types of vertical bubble columns. (Sanchez Miron et al. [201])





Figures 10a and b: Arrangements of triangular PBR design of Green Fuels Inc. (Berzin [21])

Key:102, 106, 106-tubular conduits; 108-liquid medium; 116-Fill level; 118-Connecting joint; 120-Flow direction of liquid; 122,124-gas spargers; 126-Bubbles; 128-sunlight; 134-Recirculation vortices; 144-Gas outlet tube; 150, 152-Media inflow/outlet tube.

Figure 11: A one thousand liter, pilot scale helical photobioreactor in Australia (BEAM [11]).

Figure 12: Vertically oriented flat panel photobioreactor at Ben Gurion University of the Negev, Israel (Wijffels)[245]

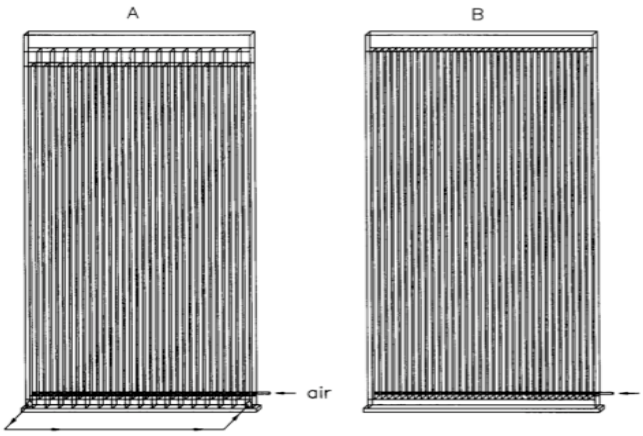


Figure 13: Scheme of a flow through (A) and a bubble column (B) FPR (Tredici and Materassi, 1992) [227])

α–type tubular photobioreactor

Lee et al. [127] designed and tested an α–type tubular photobioreactor which consisted of two sets of tubes placed at an angle of 25° with the horizontal, thus being efficiently exposed to the sunlight. The upper end of each tube was connected to a receiver and the lower end

was connected to a vertical air-riser tube (see Figure 14). The algae culture was lifted up in the air riser tubes to the receiver tank and then flowed down the light harvesting tubes to the opposite set of air riser tubes. A production of 72 $g.m^{-2}$(land occupied).d^{-1} (re-calculated as 17.6 $g.m^{-2}$ tube surface area.d^{-1}) or 2.9 $g.L^{-1}.d^{-1}$ was obtained using *Chlorella pyrenoidosa* as the algae cultured.

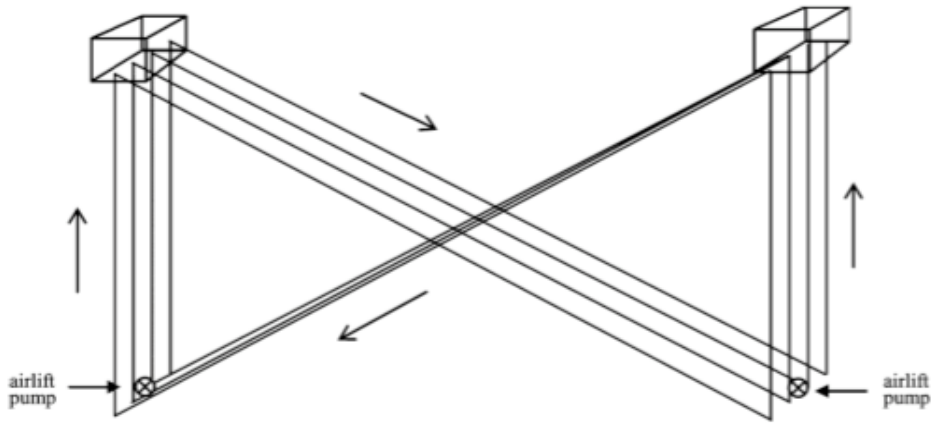


Figure 14: Diagrammatic representation of the ∝-photobioreactor system (Lee et al. [127]; Carvalho et al. [39]).

L-shaped reactor

Chae et al. [41] designed and tested an innovative, 1000 L, L-shaped PBR. This PBR was operated as an inverted L, separated into a lower, 500 L dark region and an upper, illuminated, 500 L capacity light region (Figure 15). The height of the light region was fixed at 20 cm to avoid light attenuation and a scraper in the light region was used to circulate the medium from the light to dark regions of the PBR. This PBR was used in a pilot scale culture of *Euglena gracilis*, giving a daily productivity of 9.5 $g.m^{-2}.d^{-1}$ (Illuminated surface area) or 0.11 $g.L^{-1}.d^{-1}$.

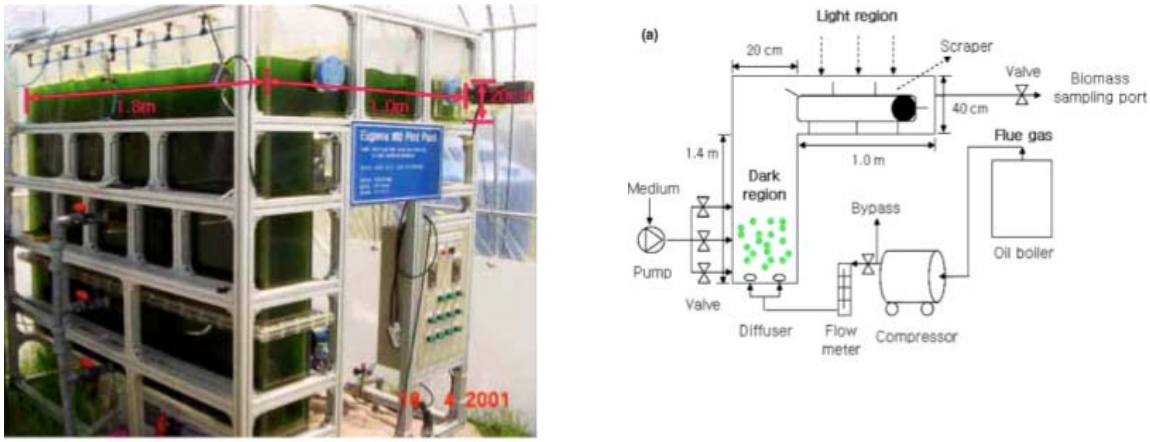


Figure 15: *L –shaped reactor Chae et al* **(41)**

Pipe reactor:

Sato et al. [203] invented a pipe type PBR, which is similar to a tubular PBR, but utilizes aeration for mixing. The air is injected along the longitudinal axis of the pipe and optimizes the flashing light effect and the light reception [203] (Figure 16).

These authors obtained productivities of 37.3 $g.m^{-2}.d^{-1}$ (0.266 $g.L^{-1}.d^{-1}$) during the cultivation of *Chaetoceros calcitrans* outdoors in winter (November and December) in Japan.

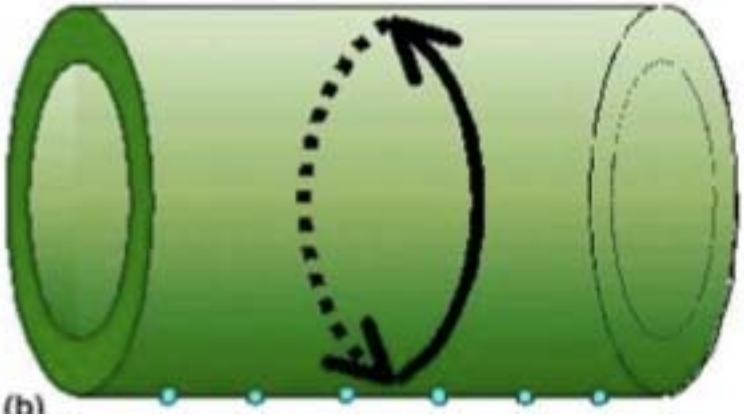


Figure 16: Aerated pipe design PBR. Air/CO_2 are injected through the lateral injection ports (Sato et al. [203])

Artificially Illuminated Cultures

Bag culture

Polythene PBRs are frequently used to grow algae for aquaculture use. These bags, which can be obtained in rolls, are heat sealed at one end and are usually placed upright on a plastic or foam base, supported within a plastic coated wire mesh (**Figure 17**). These cultures are illuminated externally by artificial light (banks of fluorescent lights) and housed in a temperature controlled building. Aeration is supplied through glass rods inserted close to the bottoms of the bags. These bags are cheap and are disposed of after each use, eliminating the need for cleaning and disinfection. Horizontally laid polythene bags with natural illumination are also used for large scale production in hatcheries (Figure 18).

Figure 17: Algae culture bags in mesh cages in a hatchery in BC.

Figure 18: Horizontal "lay flat" algae culture at a commercial hatchery in Washington State

Cylinder culture:

Algae are also cultured in translucent fibre glass sheets which are formed into a cylindrical shape with a solid base. These systems are also used to grow algae for aquaculture use. Illumination is similarly provided by an external bank of artificial lights and aeration is supplied through flexible air lines which are inserted into the columns (Figure 19).

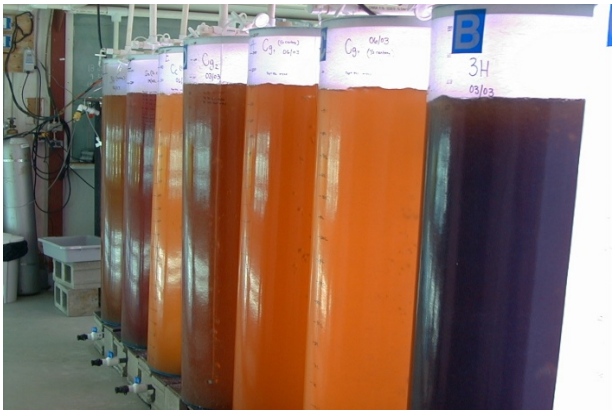


Figure 19: Algae culture cylinders in a hatchery in BC.

Turbidostat cultures:

Liang and Jones [130] described an operating turbidostat system which was designed to provide high concentrations of microalgae for aquaculture use. This was an internally illuminated system consisting of two cylinders made of translucent acrylic and contained a light unit of six fluorescent lights. Cooling was obtained by running cooling water through cooling tubes moulded to the outer fibre glass jacket (Figure 20) [97]. The system was run as a continuous culture and daily yields of 14.8 to 21.4 $g.m^{-2}$ (exposed surface area).d^{-1} corresponding to 0.13 – 0.19 $g.L^{-1}.d^{-1}$ of *Isochrysis galbana* in 80 L volume were obtained. When *Tetraselmis suecica* was the algae grown, the areal and volumetric productivities obtained were 22.8 to 37.6 $g.m^{-2}.d^{-1}$ and 0.2 to 0.33 $g.L^{-1}.d^{-1}$, respectively.

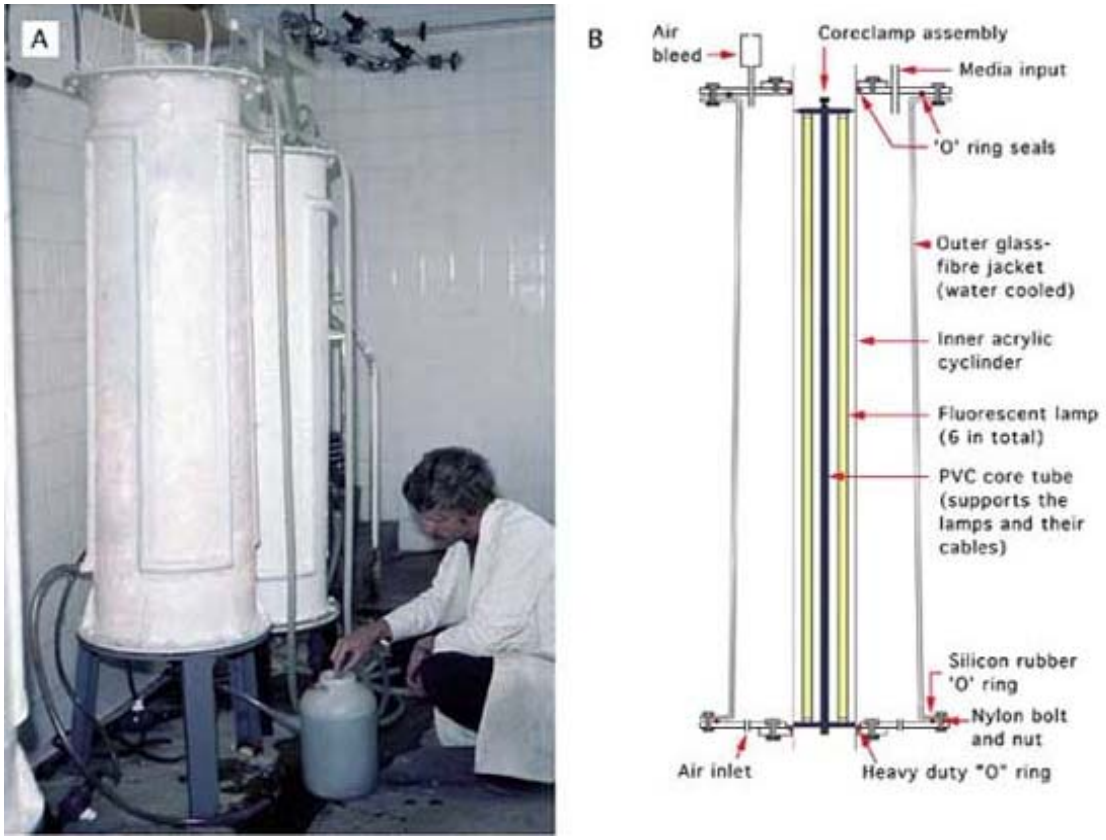


Figure 20: Turbidostat system of Liang and Jones [130]. (Helm and Bourne [97])

Annular reactors

An annular reactor which could be operated with either artificial light, natural illumination, or both was described by Chinni-Zittelli et al. [49]. This differed from the turbidostat model of Liang and Jones [130] by having the outer cylinder also made of translucent material and the cooling was accomplished via a cooling coil inside the algae cultures. A ten unit pilot scale plant (total volume of 1,200 L), was run over most of the year (except for summer), to produce *Nannochloropsis* oculata for fish hatcheries. Maximum productivities obtained when these units were run without temperature control in a greenhouse were 4.64 $g.m^{-2}.d^{-1}$ (0.21 $g.L^{-1}.d^{-1}$). This increased to 6.4 $g.m^{-2}.d^{-1}$ (0.29 $g.L^{-1}.d^{-1}$) when temperature control was added.

Modular Flat Panel PBR

Chinni Zittelli et al. [47] designed and tested a modular flat panel PBR specifically meant for aquaculture use. This system consisted of six removable alveolar panels placed inside a thermo regulated white chamber. Artificial light was supplied in the form of banks of fluorescent tubes placed between the panels. The productivities obtained ranged from 3.6 $g.m^{-2}.day^{-1}$ (0.6 $g.L^{-1}.d^{-1}$), when the culture was illuminated only from one side at 115 $\mu m.m^{-2}.s^{-1}$, to 8.74 $g.m^{-1}.d^{-1}$ (1.45 $g.L^{-1}.d^{-1}$) when it was illuminated from both sides at 230 $\mu mol.m^{-2}.d^{-1}$.

Artificially illuminated helical reactor

Hall and Watanabe [91] tested a small (12 L capacity) helical reactor using artificial illumination from within the centre of the coil. Using *S. platensis*, they obtained production rates of 30 $g.m^{-2}$ (basal area).d^{-1} (equivalent to 2.5 $g.m^{-2}$ (total surface area).day^{-1}) or 0.62 $g.L^{-1}.day^{-1}$. These authors further improved on this idea by constructing a conical helical PBR (**Figure 21**), which had the advantage of increasing the illuminated area while maintaining the same basal area. Using artificial light at low irradiance, these authors obtained productivities of 15.9 $g.m^{-2}$ base area.d^{-1} (2.6 $g.m^{-2}$ total surface area.day^{-1}) or 0.65 $g^{-1}.day^{-1}$.

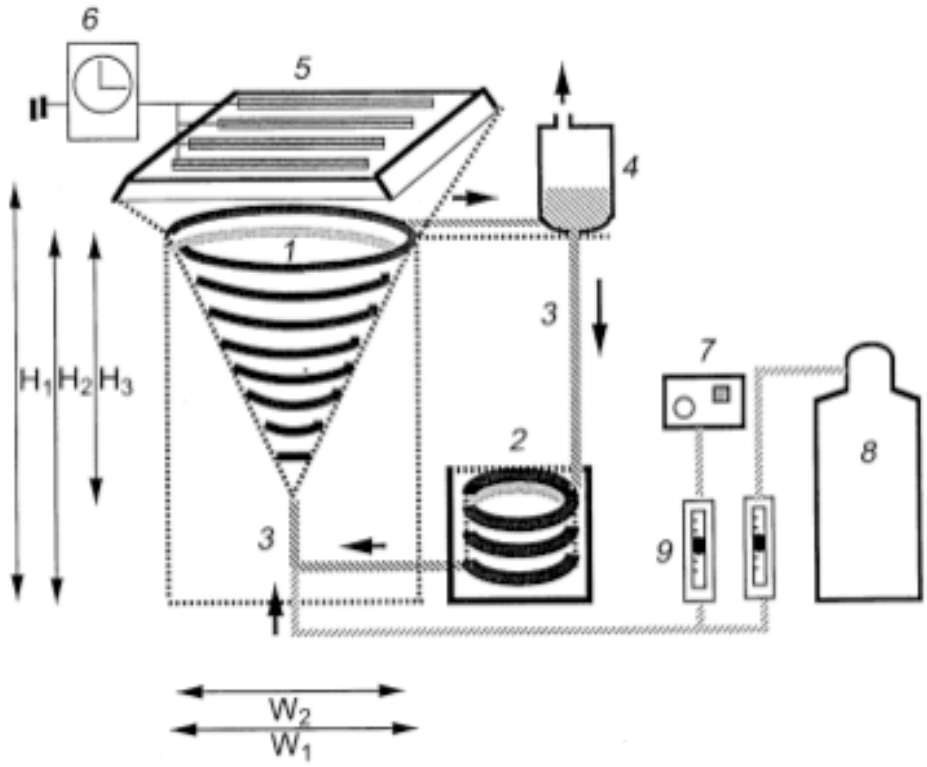


Figure 21: Schematic diagram of the cone shaped helical tubular PBR. 1=Helical photostage; 2=Helical heat exchanger; 3=Downflow and upflow pipes; 4=Degasser; 5=Fluorescent lamps; 6=Timer switch; 7=Air pump; 8=CO_2 gas cylinder; 9=Flow meter (Watanabe and Hall [240])

Glass Column Photobioreactor

A glass column photobioreactor was described by Hu and Richmond [99], which consisted of thirty-two glass tubes with dimensions of 2.6 cm x 150 cm (diameter x height), each. These tubes were fixed on three parallel plastic panels and aligned at an angle of 60° to the horizon (Figure 26). This PBR was used to culture *Isochrysis galbana* with productivity rates of 1.6 $g.L^{-1}.d^{-1}$ (1.04 $g.m^{-2}.d^{-1}$).

Artificial Light Photobioreactor (ALP)

Muller Fuega et al. [158] described an innovative, internally illuminated PBR for production for aquaculture and pharmaceutical operations. The ALP consisted of interconnected cylindrical light chambers, each containing a fluorescent tube housed inside a transparent, concentrically placed, inner tube (Figure 22). The inlet and outlet ports for the culture are placed at the low and high extremes of the chamber and the culture medium is pumped upwards through the interconnected light chambers to the outflow at the top from where it flows downwards again through an oblique tube to the bottom of an airlift. From here, the culture is moved to a transparent degasser for the outgassing of oxygen and then back into the inlet port.

Despite requiring artificial illumination, the authors extrapolated that the cost was competitive when compared with that obtained in a tubular PBR utilizing solar light. When *Porphyridium cruentum* was grown in a 101 L unit, a productivity of 25 $g.m^{-2}.d^{-1}$ (0.37 $g.L^{-1}.d^{-1}$) was obtained.

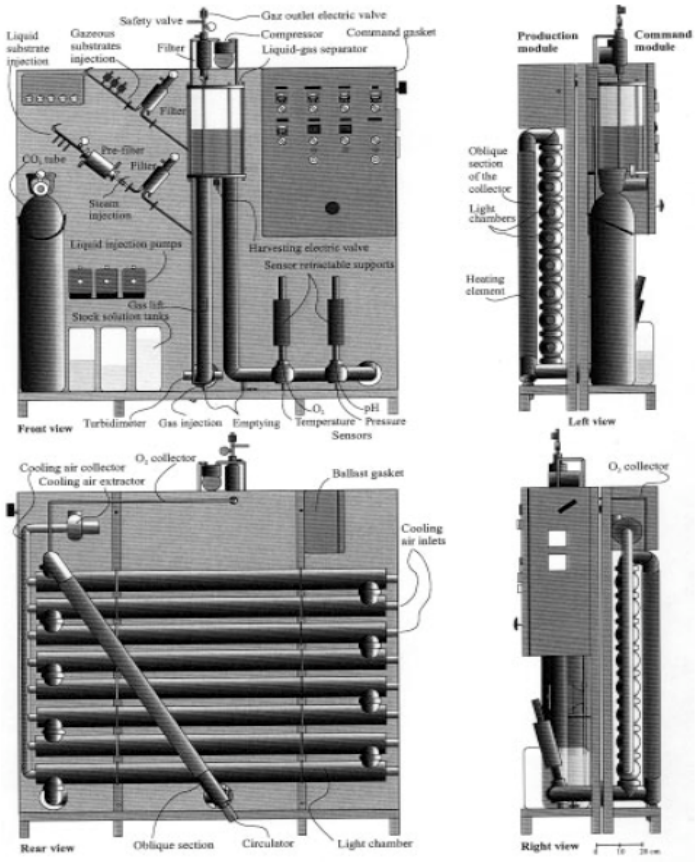


Figure 22: Four views of the artificial light photobioreactor of Muller-Fuega et al. [158].

Bright box system

The bright box PBR (Figure 23), developed by the National Research Council of Canada consists of a fibre glass box with dimensions of 1m x1m x 1m. Internal illumination is provided by a series of 32 fluorescent tubes passed through the culture. Mixing is obtained by sparging air into the bottom of the bright box and pH is controlled by the addition of CO_2 and temperature control is achieved by use o a heat exchanger. A productivity of 0.6 $g.L^{-1}.d^{-1}$ has been reported from the system with *Isochrysis galbana* when run as a semi-continuous system [25].

Bright cylinder system

The bright cylinder system is a 2500 L plastic tank [56], which is similar to the bright box system in having illumination provided by multiple fluorescent tubes enclosed in protective transparent tubes and immersed in the culture (Figure 24). Mixing and O_2 degassing is by sparging air into the bottom of the cultures and pH control is by the addition of CO_2. Productivity obtained from this system was 0.06 $g.L^{-1}.d^{-1}$. A shortcoming of both of these internally illuminated systems (the bright box and the bright cylinder systems) was the considerable amount of time and manpower required to clean them.

Figure 23: Bright box algae photobioreactor

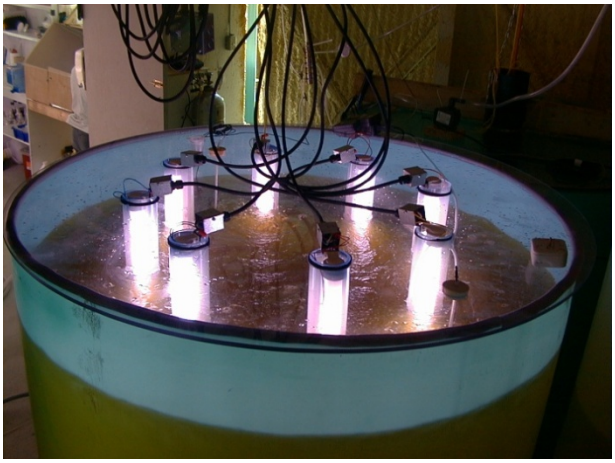


Figure 24: Bright cylinder photobioreactor (Courtesy Bruce Clapp)

Fermenter type illuminated cultures

The phototrophic production of microalgae in conventional type aerobic fermenters modified by the insertion of internal light sources such as fibre optics have been demonstrated by several authors. These systems feature controllable illumination and much better control of growth parameters.

Mori [155] focused solar light into optical fibres inserted into the algae culture using fresnel lenses to filter out the IR and UV wavelengths. This method was later modified by passing the fibre optic cables into transparent rods in acrylic tubes, which were inserted into the cultures. Cooling was not necessary as the infrared portion of the light had been split off and the wavelengths obtained were heat free.

Another very high density reactor was designed by Javanmardian and Palsson [107], inside which light radiators were arranged as concentric cylinders providing 1 m^2 of radiative surface per 3.3 L of culture. An automated ultrafiltration device was used to dialyze the culture medium at a high flow rate, exchanging secreted wastes and /or inhibitors with fresh medium.

Lee and Palsson [119] used light emitting diodes (LED) to deliver up to 50 $mW.cm^{-2}$ of light into very small reactors (53 cm^3 and 80 cm^3). Gas transfer was by internal sparging and nutritional and biological limitations were controlled by continuous perfusion. When the reactor was operated at a dilution rate of 6 reactor volumes per day, the densities attained were 4 x $10^9.ml^{-1}$ (about 25 $g.L^{-1}$), which corresponds to 3.15 $g.L^{-1}.d^{-1}$ (44 $g.m^{-2}.d^{-1}$). A modification of this system designed for space travel was tested by Lee and Palsson [120] in which internal gas sparging was replaced by a hollow fibre gas exchanger with comparable results.

Promising Technologies

Mixed systems

Mixed systems, which would combine the advantages of both open culture systems and closed PBRs, have been considered as a means to improve the productivity of algae cultures. Pushparaj et al. [179] designed a mixed system combining open ponds with closed reactors to overcome the temperature problems unique to both, i.e. sub-optimal temperatures in open ponds and overheating in closed PBRs with high surface-to-volume ratios. The principle was to expect a rapid increase of the morning culture temperatures in the open ponds and a reduction of the maximum temperatures in the PBR, eliminating the need for costly cooling systems. The mixed systems used consisted of a flat panel PBR oriented either to the east to receive the morning illumination, or to the south. These authors reported that the volumetric productivities in the integrated systems were higher than the productivity in the open pond. In addition to which, depending on the orientation of the panel, the productivities obtained in the mixed systems were at least equal to the sum of the individual productivities of the flat panel and the raceway pond operating separately (east facing PBR), or were superior to the sum of the individual units by 15% (south facing PBR) (Table 1). These authors reasoned that this better performance could be attributed to many factors such as better mixing, improved temperature conditions and better exposure of the culture to solar irradiance. The east facing unit, being more exposed to the sun, suffered from photoinhibition, which explained why it did not perform as well as the south facing unit.

These authors emphasized the importance of properly sizing such units in order to maintain adequate temperatures. To maintain the temperature around 35°C in the 25 m^{-2} tubular PBR in summer, it was necessary to reduce the ratio between PBR and pond parts to below 1. This was likely the reason for the failure of an earlier attempt at creating a mixed system between a tubular PBR and raceway ponds [179].

Sequential mixed system

Similar to the mixed system described above, this system also integrates both closed photobioreactors and open ponds into a single production system. Huntley and Redalje [103] described a two-stage process of maximizing production of oil and astaxanthin from *Haematococcus pluvialis* in which the first stage consisted of maintaining constant conditions favouring continuous cell division and preventing contamination in a closed PBR,

followed by a second stage during which the cells are exposed to nutrient starvation and other environmental stresses which lead to the increased synthesis of astaxanthin (or oil), in open ponds. The effect of nutrient manipulation on oil content is described more fully in Appendix I

Table 1: Volumetric and total productivity and quantum yield of *Arthrospira platensis* grown outdoors in a raceway pond, in a panel, and in two integrated culture systems at optimal population densities. (Data are means of 10 day determinations ± standard deviation). (Pushparaj et al. [179]).

Culture system	Optimal pop. density ($g.L^{-1}$)	Volumetric productivity ($g.L^{-1}.day^{-1}$)	Total productivity ($g.reactor^{-1}.day^{-1}$)	Quantum yield ($g.mol^{-1}$ photon)
Pond	0.75	0.18 ±0.02	55.00 ±0.62	0.25 ±0.01
Panel	4.00	2.10 ± 0.17	29.00 ± 0.63	0.35 ±0.01
SO unit	1.60	0.32 ± 0.01	97.00 ± 0.55	0.32 ± 0.01
EO unit	1.60	0.29 ± 0.01	86.00 ± 0.35	0.29 ± 0.01

Stacked PBR

Grobbelaar and Kurano [87] designed and tested a stacked PBR designed to take advantage of the acclimation of algae to different light intensities and thereby culture algae that are acclimated to high, medium and low light intensities within different chambers of the same PBR (**Figure 25**). Using this design, the panel closest to the light source would consist of algae acclimated to high light intensities and at the same time, while providing shade for the cells in the lower compartments, they would also contribute to the overall productivity.

When *Synechocystis aquatilis* SI-2 was cultured in this PBR, an areal productivity of 49.19 $g.m^{-2}.d^{-1}$ was obtained compared to a control made of a single layer PBR with an areal productivity of 36.07 $g.m^{-2}.d^{-1}$ in the same time period.

This PBR design achieves high productivities due to its ability to avoid or minimize photoinhibition whereby dense cultures of algae under bright light are in fact each acclimated to low light regimes per cell and therefore susceptible to photoinhibition at high light intensities and light limitation at lower light intensities. A disadvantage of the design is the requirement to run (at least the upper chamber) only as a continuous culture to prevent light limitation occurring in the lower chambers of the PBR. Further work is needed to define the light path of each chamber of the PBR (which will depend on the test alga), as well as the optimal dilution rates (which will depend on growth rates) and the design scale-up models.

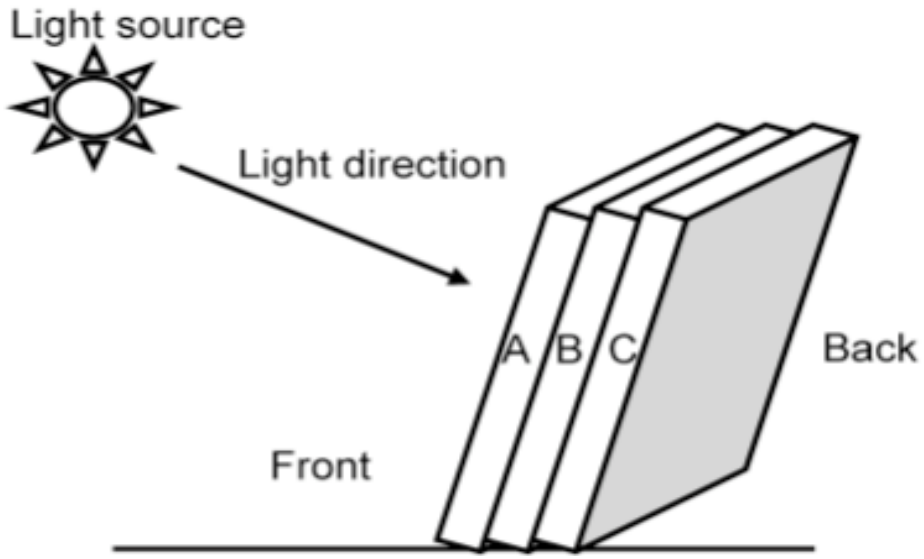


Figure 25: Configuration of the compartments of the stacked PBR of Grobbelaar and Kurano. A, B and C correspond to the photo-acclimated state of the algae. (Grobbelaar and Kurano [87]).

Flat Panel Airlift Reactor

The flat panel airlift reactor (Figure 26, Figure 27) is a rectangular PBR with an illuminated front area and a small downcomer zone on one side of the reactor. The front riser zone was divided into interconnected chambers by means of horizontal baffles attached alternately to the front and back of the larger faces of the reactor. When air is injected through the gas spargers at the bottom of the riser section, the fluid in the chambers circulated so that the cells regularly moved between the narrow, illuminated zone and the deeper, dark zone. High productivities of 23.6 $g.m^{-2}.d^{-1}$ (2.67 $g.L^{-1}.d^{-1}$) were reported when *Chlorella vulgaris* was grown in this system [64], and 25.18 $g.m^{-2}.d^{-1}$ (2.35 $g.L^{-1}.d^{-1}$) when *Phaeodactylum tricornutum* was grown in the system [144].

A large scale pilot plant utilizing this technology is in operation in Germany, producing *Haematococcus pluvialis* (Figure 27). Schenk et al. [205] reported that the high energy required for mechanical mixing is the major drawback of this system but no productivity figures are available.

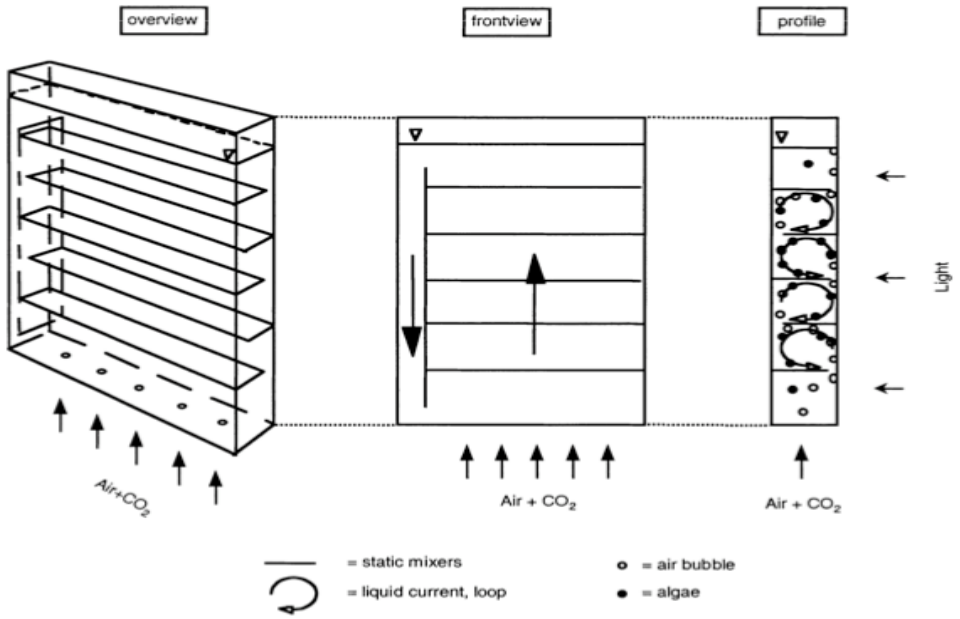


Figure 26: Scheme of flat panel airlift (FPA) photobioreactor (Degen et al. [64])

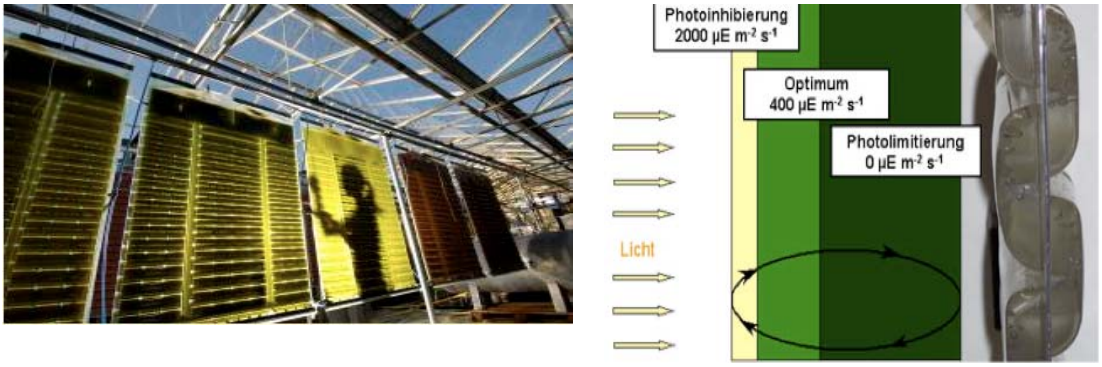


Figure 27: Flat panel airlift PBR pilot plant used for astaxanthin production in Germany (Bio-Pro, [24]; Subitec [214])

Co-culture of algae and bacteria

Beliaev [17] described a novel mixed culture approach consisting of co-culture of heterotrophic and oxygenic photosynthetic microorganisms in closed PBRs. The growth organisms were *Shewanella sp* and a cyanobacterium, *Cyanothece sp* (**Figure 28**). By careful control of the fluxes to balance generating and consuming fluxes of particular metabolites, the bacterium was able to utilize the oxygen produced by the cyanobacterium, which in turn was able to utilize the CO_2 produced by the bacterium, leading to an increase in biomass of both organisms (Figure 29). Apart from the obvious savings in CO_2 costs and the reduction or elimination of O_2 buildup that this method offers, it also has the advantage of reducing the input energy as the authors reported no requirement for external agitation or aeration once the two cultures were combined.

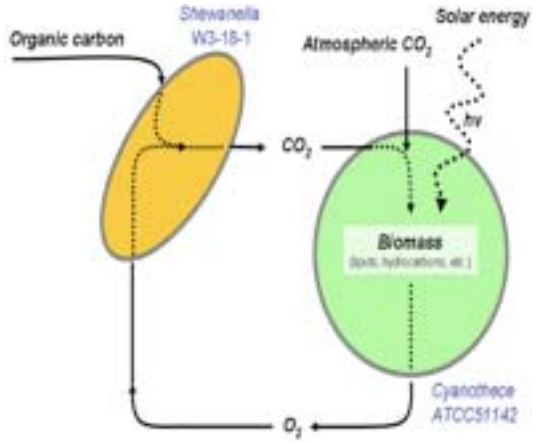


Figure 28: Scheme of mixed culture approach (Belieav [17])

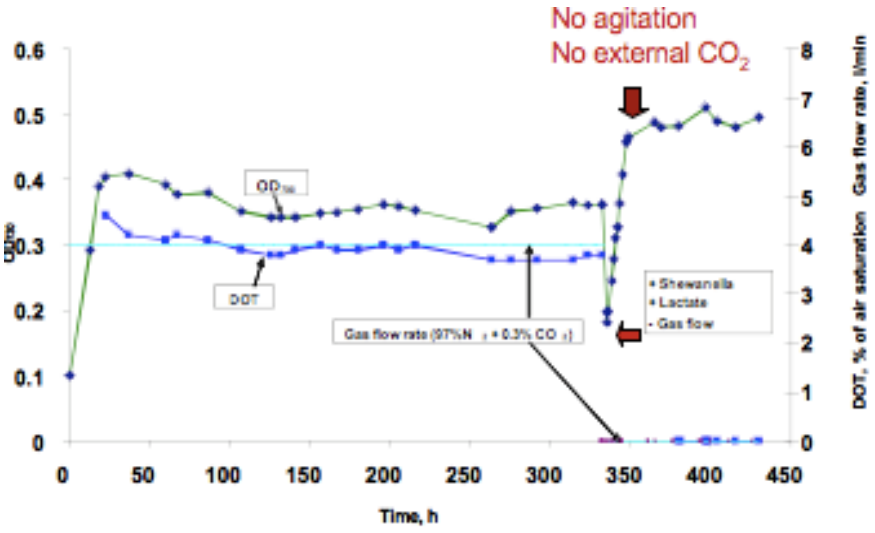


Figure 29: Controlled binary culture of *Cyanothece sp*. ATCC 51142 and *Shewanella sp.* strain W3-18-1 under light conditions in the presence of lactate (5mM) (Belieav [17])

Discussion

A quick introduction to the light response curve is appropriate here to form a background to the discussion that will follow:

Introduction Light

As light is the base source of energy for phototrophic microalgae, the availability and intensity of light available to the culture is one of the key parameters affecting the success or failure of algae cultures.

In a dilute culture of microalgae where all the cells are exposed to light and no other conditions are limiting, the response of the algae to illumination follows the light response curve described by Goldman [79]: At a very low light intensity, the low growth rate of the algae is balanced by cell decay giving a net growth of zero (called the compensation point) [125]. As the illumination increases above the compensation point, the rate of photosynthesis increases with the light intensity until a point is reached where the growth rate is the maximum attainable (the light saturation point) [79; 118; 191]. Increasing the illumination beyond this point does not increase the growth rate and actually stimulates a process of photooxidation, which damages the photosynthetic receptor system, decreasing the photosynthetic rate and leading to a decrease in productivity of the culture (also called photoinhibition, Figure 30).

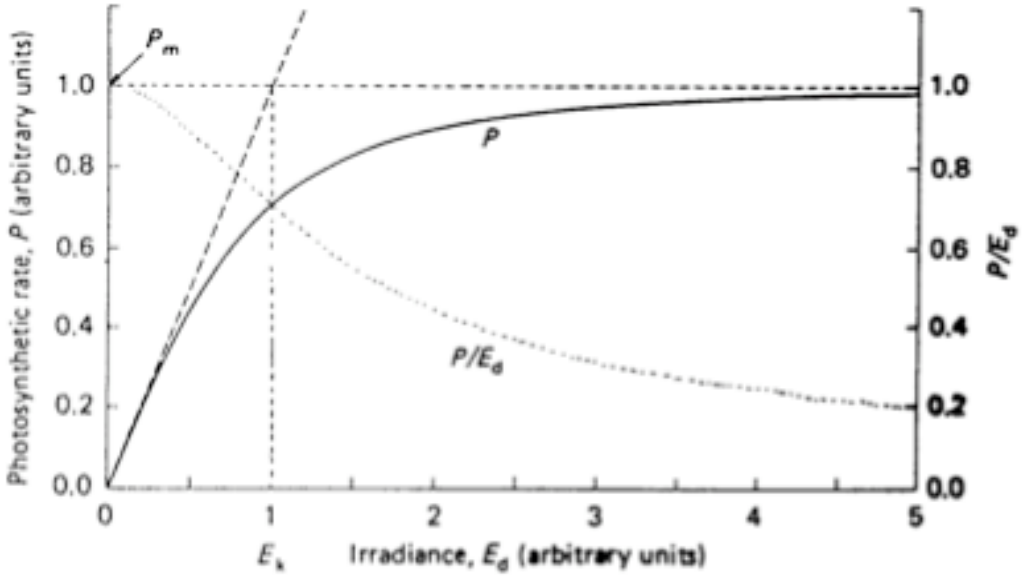


Figure 30: Idealized curve of specific photosynthetic rate (P) as a function of irradiance (Ed), illustrating the maximum photosynthetic rate, Pm, and the saturation onset parameter, Ek. The variation of P/Ed is a measure of the efficiency of utilization of incident light (Richmond [191]).

As the cell concentrations of the algae in the culture increase, the light intensity transmitted through the culture drops very quickly with distance from the light source because the antenna structures of the algae are so efficient that they will absorb all the photons that hit them even though they cannot all be used in photosynthesis. Thus, at high cell concentrations, nearly all the available light is absorbed within a small layer of cells leaving the cells in the deeper regions in the dark (mutual shading). As a result, algae culture units generally have short light path lengths to be able to effectively utilize the available light. At a certain depth in such a culture (which depends on cell concentration), the light intensity is the saturation intensity and all the light that penetrates beyond this point is utilized with maximum efficiency. In contrast, the cells higher up in the water column cannot utilize all the photons of light hitting them. Only a fraction of the incident light is utilized and their efficiency of light utilization is lower than at lower light intensities.

The intensity of solar light (about 1700-2000 $\mu E.m^{-2}.s^{-1}$) is much greater than the photosynthetic light saturating intensity of most microalgae of about 200 $\mu E.m^{-2}.s^{-1}$ [79; 142; 177; 223; 228]. Thus mass cultures of algae relying on solar illumination stand the risk of light

inhibition. The most practical way to deal with this is to increase the cell densities of the algae to a point at which mutual shading causes each cell (or the majority of cells) to receive only intermittent light, thereby minimizing the period of exposure of each cell to sunlight. This approach followed the finding that high frequencies (>10Hz) of brief flashes of high intensity light followed by a longer dark period does did not reduce productivity compared to continuously illuminated cultures [114; 164]. This is because once the required photons have been absorbed by the chlorophyll antennae, additional photons cannot be used as the cell has to undergo a brief dark period to complete the conversion of the absorbed light to chemical energy [105].

In each culture, an optimal cell density exists which results in the maximum output per unit volume or unit area, given the available light and mixing if all other conditions are not limiting [190; 195]. The importance of maintaining cell density at its optimum in mass cultures of algae is clear: If the cell density maintained is too low in relation to the available light, photoinhibition would occur. On the other hand, if the cell density is too high for the light available, the culture would get light limited. Since the individual cells are illuminated intermittently, light availability is described in terms of the Light-Dark (L-D) cycle, which is the duration of the exposure of the cells to light and dark regions of the culture. It is possible to shift the optimal cell concentration of the culture higher by increasing turbulent mixing, which also increases the frequency of the exposure of the cells to light and hence, the productivities of the cultures [12; 83; 88; 101; 150; 192; 231].

The depth (light path) of the culture is also a determinant of the frequencies of the L-D cycles and hence, the productivity. Richmond and Grobbelaar [194] showed that decreasing the culture depth increased the cell concentration of algae cultures. This is also illustrated in

Table 2, which shows significant improvements in both volumetric and areal yield when the light path was reduced from 10.4 to 1.3 cm, reflecting the improved utilization of light by the increased frequency of the L-D cycles [102].

In open culture systems, the higher productivities obtained in the inclined systems as opposed to the other open systems (see Table 8) may be explained in part by their shorter light path length (about 0.8 mm) as opposed to the longer light path lengths (15-30 cm) in the other open systems.

A key goal for outdoor cultures must be to increase the utilization of sunlight by the algae cultures while minimizing the negative effects of the sunlight on the cultures. Thus, effective outdoor cultures of microalgae must balance the often opposing goals of avoiding light saturation and photoinhibition on the one hand and ensuring that the light intensity is enough to avoid (or minimize) the dark zones in the culture.

Productivities generally increase with light intensities [54; 100; 144; 190], and to maximize light harvesting capacities, most PBRs have large surface areas (flat plate PBRs, tubular PBRs and helical PBRs). However, one of the reasons for the still low productivities obtained in PBRs is the inefficient conversion of the available light into biomass [228], with a major disadvantage being caused by the reduction in light intensity caused by the materials used in the PBRs not being fully transparent. In addition, during culture, some algae will adhere to the internal walls of the PBR, further reducing light penetration. This may be further aggravated by the accumulation of dust and the condensation of water on the surfaces of the PBRs. Solutions exist for these, however, they will increase the costs of the PBRs relative to the open systems: Adhering algae may be removed by circulating closely fitting transparent balls, neutrally buoyant particles or suspended sand particles to clean the internal surfaces and abrade adhering biomass using highly turbulent flows. The outside surface of the PBR

may be cleaned by cooling water if it is used for cooling. When light goes from a medium of one refractive index to another, it is reflected and refracted except for light that enters the second medium at a perpendicular angle. Thus, PBRs with curved surfaces, such as tubular and helical PBRs, will have less light available than the flat plate reactors. In addition, vertically oriented PBRs will also not receive direct sunlight for much of the day.

Inclined PBRs and flat plate PBRs can be inclined to maximize sun exposure. However, these are exposed to the risk of light inhibition at very high solar light intensities. Tredici and Zitelli [228] showed that the reduction in photosynthetic efficiencies (PE) (reduced by 30 to 55%), obtained in a horizontal flat panel PBR at mid day compared to tubular PBRs was directly attributable to the effects of light saturation and photoinhibition in the flat-plate PBR.

Similarly, Pulz [178] obtained record PEs (areal productivity of 175 $g.m^{-2}.d^{-1}$) when he arranged flat plate modules only 20 cm apart from each other, thus mutually shading each other and being exposed mainly to the lower energy diffuse radiation as opposed to the direct light. Indeed, several authors have shown that the photosynthetic efficiencies of microalgae are higher at lower light intensities than at higher light intensities [150; 223], and significant efforts have been put into strategies to obtain spatial dilution of the light in order to increase PEs.

However, the processes of photoinhibition and increased productivity caused by the deeper penetration of higher-intensity light occur simultaneously in outdoor mass cultures and the resulting net productivity reflects the dominant process. Hence, many researchers have obtained increased productivities in higher light intensities even while also obtaining lower PEs. For instance, Tredici and Materassi [227] obtained mean net productivities of 18 $g.m^{-2}.d^{-1}$ and 24 $g.m^{-2}.d^{-1}$ in vertical and sun oriented flat plate PBRs respectively, demonstrating the higher productivities that could be achieved by improving the absorption of sunlight in the sun oriented PBR. The photosynthetic efficiencies of the sun-aligned PBR was, however, lower than obtained in the vertical PBR which did not receive direct sunlight [227]. This is attributed to light saturation and inhibition obtained when the plates are aligned towards the sun in contrast to vertical flat panels where the sunlight received is diffuse.

Thus, while the dilution of light energy increases the utilization of the available light, it also imposes a maximum limit upon the productivity that can be obtained in the system as an increase in PE does not necessarily involve an increase in productivity which is influenced by the amount of light penetrating into the culture (and high PEs are often obtained at low light intensities). In fact, a significant limitation to the utilization of high light intensities in algae cultures arises when mixing and other factors are not optimal. This is illustrated by referring to

Table 3, where it can be seen that at 500 $\mu mol.m^{-2}.s^{-1}$, the productivity obtained increased from 70 to 100 $mg.L^{-1}.hr^{-1}$ when aeration was increased from 0.6 to 2.0 $L(air).l^{-1}.min^{-1}$. A further increase in the aeration rate did not lead to a further increase in productivity. However, when the illumination was increased to 1800 $\mu mol.m^{-2}.s^{-1}$, the productivity obtained was higher at the lowest rate of 0.6 L $(air).L^{-1}.min^{-1}$ at 200 $mg.L^{-1}.hr^{-1}$ compared to all the values at the lower illumination. This can be attributed to better penetration and utilization of the higher-intensity light. In addition, increasing the aeration resulted in a continued increase in productivity.

The limitation of the productivity observed at 1800 μmol.m^{-2}.s^{-1} and 0.6 to 200 mg.L^{-1}.hr^{-1} can be attributed to photoinhibition at that low mixing rate. As it can be seen that when the mixing rates were increased, the productivities also increased.

The importance of adequate mixing in the ability of the algae to utilize high light intensities has been confirmed by others [98; 100; 102; 190] who have shown that at optimal rates of mixing and if optimal cell densities are maintained, increasing light intensities to almost full daylight (1800 μmol.m^{-2}.s^{-1}) was accompanied by a continuous increase in productivities and PE in contrast to observations by other researchers. This confirms that high-intensity solar energy can indeed be utilized without dilution if mixing and other factors are optimal.

The strategy described above relies on the dilution of the light energy among the cells of the culture so that optimal cell densities would depend on the light intensity received by the culture. Even at high light intensities, each cell in such a dense culture would only be exposed to low light and thus, be acclimated to a low light regime. In such a dense culture, cells continually at the surface would be exposed to too much light and be light inhibited. As described earlier, the processes of light inhibition and increased productivity caused by deeper light penetration occur simultaneously and considerable effort has been devoted to avoiding light inhibition in algae cultures.

In the wild, microalgae cells generally adapted for low light have developed strategies to cope with fluctuations in light intensity with the light harvesting antennae complexes (LHC) being arranged for optimal light capture. The cells respond to increases and decreases in light intensities by either down-regulating or increasing the transcription of the LHC proteins and their isoform compositions to adjust to the changing irradiance [4; 159]. Chlorophyll has a high extinction co-efficient and under high light intensities, when the rate of photon absorption exceeds the rate of photosynthetic turnover, the excess light energy is dissipated as heat or fluorescence, resulting in a low per chlorophyll productivity [73; 174; 204].

It has been speculated that a reduction in the sizes of the antenna complexes of the microalgae would reduce or eliminate this waste of light and enable the algae to efficiently utilize the available light and this has been proven by some researchers who have demonstrated increased photosynthetic capacities when genetically modified microalgae were produced with reduced light harvesting complexes [145; 159-161; 174]. The reduction in the size of the LHCs also improved light transmission and reduced photoinhibition (Figure 31) [159].

However, such genetically modified organisms are likely to be applicable only to PBRs as in open systems, they would be rapidly outcompeted by native contaminant algae having the conventional high antennae-to-reaction centre ratios [19].

Another promising approach to tackling the problem of high light intensity using natural light is the stacked PBR design of Grobbelaar and Kurano [87]. By constructing the PBR in layers, the algae can be grown in separate layers at different light intensities to which they are acclimated. This promising approach can reduce or eliminate photoinhibition while also contributing to increased productivities. While this design is promising in terms of the increased productivity it promises (an increase of 37% over unstacked flat plate PBRs was reported by the authors), it is unlikely that the increased costs caused by essentially stacking two or three reactors on top of each other will be justified by the increased productivity expected. Additional research into ways to reduce the costs of this PBR design is required.

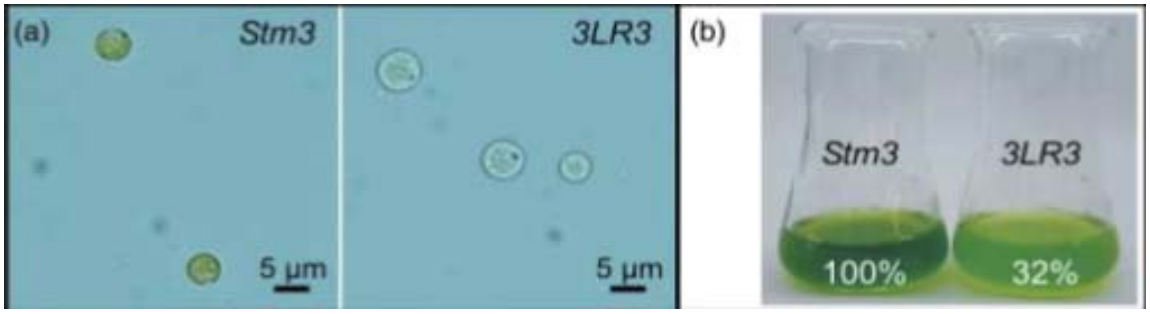


Figure 31: Microscopic images (a) of *Chlamydomonas sp.* cells with normal (Stm3) and reduced (3LR3) antennae pigments. Picture (b) shows equal concentrations of the two strains (6 x 106 cells/ml) after heterotrophic growth). Relative chlorophyll concentrations are indicated on the flasks (Mussgnug et al. [159]).

Table 2: The effect of the light path on basic parameters related to growth and productivity of cell mass of *Spirulina platensis* (Richmond [190]).

	Light path (cm)		
Parameter	10.4	1.3	Effect
Culture volume ($L.m^{-1}$)	104	13.0	8-fold decrease
Optimal culture density ($g.L^{-1}$)	1.7	15.8	9 fold increase
Specific growth rate ($u.h.^{-1}$)	0.014	0.021	50% increase
Estimated L-D frequency ($m.sec^{-1}$)	<700	<100	7 fold decrease
Volume yield ($g.L^{-1}.day^{-1}$)	0.3	4.3	14-fold increase
Areal yield ($g.m^{-2}.day^{-1}$)	33	51	50% increase

Temperature

The temperature requirements of microalgae vary widely depending upon the species and strains cultured. Generally, the growth of microalgae increases exponentially with increasing temperatures until the optimum temperature is reached. Once the optimum is passed, most algae cultures exhibit a steep decline with increasing temperatures.

In open systems, there is generally no control over the culture temperature, which is determined by the atmospheric temperature, the extent and duration of solar irradiance, and humidity (which determines the extent of evaporative cooling). This lack of temperature control is a major disadvantage in many ways:

- In many algae producing regions, the differences in diurnal pond temperatures may be close to 20°C [28; 167; 188], and large bodies of water such as in raceway and unmixed ponds will have a long response time to air temperature such that, even when the

air temperature is optimal, the temperature of the culture could still be 10-15˚C below the optimal for photosynthesis and growth [28]. As a result, the optimal temperatures are achieved for only a fraction of the total light period [188; 194].

- More seriously, while the optimal culture temperatures may not be achieved until after mid-day, the intensity of solar light increases very rapidly in the morning [109; 212; 238]. This lack of synchronization between the two most important environmental factors affecting photosynthesis creates a unique stress situation under which photo inhibition of the cultures may occur at low levels of light intensity at sub-optimal temperatures [137; 238] (Figure 32).

Approaches to dealing with this are limited in open ponds and raceways. In small to medium-sized ponds, such approaches have included covering the ponds with transparent sheeting [194]. However, even in such cases, culture temperature still lags behind air temperature by 6-8˚C. This problem is much reduced in the inclined, thin layer system as the thin cultures rapidly gained solar heat in the morning [70; 109], thus maintaining a closer synchronization between temperature and irradiance.

Microalgae lose biomass during the night due to respiratory metabolism and at high irradiance or sub-optimal temperatures, the rate of biomass loss is enhanced. In such situations, an excess of carbohydrates is produced the following day, resulting in respiratory losses the following night [224].

Generally, problems with high temperatures are less prevalent in open ponds as they have the benefit of evaporative cooling. Despite this, when mid-day temperatures are high in warmer climates, overheating of the cultures may occur. Such has been found by Weissmann and Goebel [242], who reported increasing clumps of cellular debris as pond temperatures increased over 25˚C and rapid culture collapse of *Scenedesmus quadricauda* at temperatures over 35˚C. These authors found that a 50% decrease in photosynthetic efficiency obtained in the afternoons correlated with higher pond temperatures.

Higher temperatures during the dark period have been shown to increase biomass losses [242]. Thus, an important goal in algae cultures should be to get the culture to optimal temperatures quickly during the morning and to rapidly decrease the temperature after darkness thereby maintaining high productivity during the day and minimizing biomass loss at night.

The importance of temperature in algae culture also extends to the selective pressures that the temperature regime exerts in open systems which are open to colonization by different species, the effect of culture temperatures on determining the species composition is illustrated by the works of Goldman and Ryther [80], and Weissmann and Goebel [242], who both found that the dominant species of algae in open cultures was highly dependent on the prevailing temperature.

Photobioreactors lack the advantage of evaporative cooling that open systems have and function in effect as solar collectors since any light absorbed by the algae and not used in photosynthesis is converted into thermal energy and fluorescence [79; 118; 159]. The temperatures in a closed PBR can reach 10-30˚C higher than ambient temperatures for several hours a day [193; 227], adversely affecting the growth of mesophilic microalgae. While the problem may be partly alleviated by the use of thermotolerant strains of microalgae, most outdoor PBRs need to be cooled in some fashion. Temperature control has been achieved by various means including spraying cool water over the systems [170; 193; 227; 228], using a heat exchange system [228], immersion in cooling ponds [3; 150; 151; 153], flooding with

cool oceanic water [103; 167] and placement of the reactors in temperature controlled greenhouses [177]. All of which add to the costs and complexities of operating a PBR.

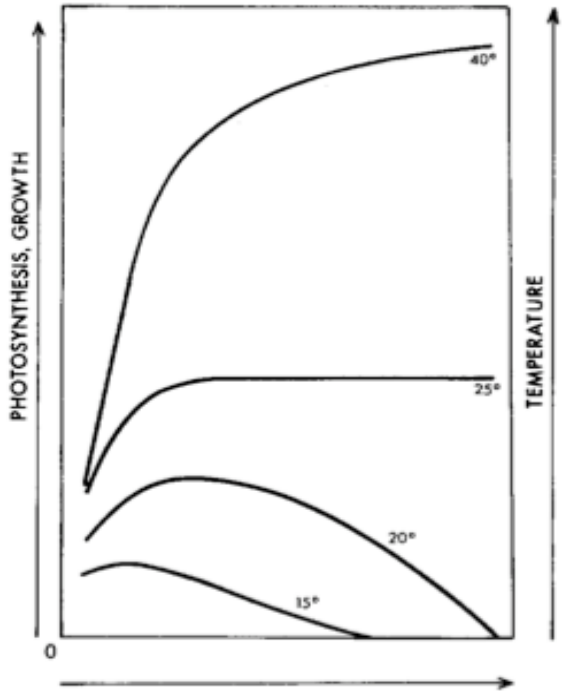


Figure 32: Interrelationships between irradiance and temperature as expressed in rate of growth and photosynthesis. Data for *Chlorella pyrenoidosa* (Richmond [189])

If the overheating can be controlled, the tendency of PBRs to retain heat represents a significant advantage over open pond systems (other than the inclined, thin layer systems). Similarly to open ponds, PBRs also suffer from a dis-synchronisation between the temperature and light regimes [212], although this difference is generally smaller than obtained in open ponds (Figure 33). This is because the cultures in the PBRs will attain the optimal culture temperatures earlier in the day, thus maintaining better temperature regimes which will encourage better productivities compared to open ponds. For instance, Richmond et al. [193] reported that cultures of *Spirulina sp.* achieved optimal temperatures (35-37˚C) earlier and maintained it for 8 hours (between 10:00 hours to 18:00 hours) in tubular reactors in contrast to open raceways, which only attained and maintained their optimal temperatures for 2 hours (between 14:00 to 16:00 hours). These authors also reported a faster rate of heating up of tubular cultures in the morning from between 6.5˚C - 7˚C.hr^{-1} in contrast to open ponds, which warmed up at 2˚C.hr^{-1} in the open and 3˚C.hr^{-1} in a greenhouse (see Figure 33). Hence, the PBRs are less affected by photoinhibition caused by the combination of low temperature and high irradiance compared to open systems. In addition, the ability of the PBRs to retain heat can lead to an extension of the cultivation period in temperate regions when the temperatures are too low to grow significant amounts in ponds.

Mixing

As discussed above, in dense cultures, the zones where the microalgae can receive enough light for photosynthesis can be quite shallow, so adequate mixing is essential to provide all the cells with a uniform, average exposure to light. Mixing also decreases the boundary layer around cells facilitating the increased uptake and exudation of metabolic products [82; 83; 242].

A major criticism of raceway ponds has been that much of the production potential is not realized because the cultures are maintained at too high a density relative to the extent of turbulent mixing and the depths of the ponds [188; 223]. In this aspect, the turbulent streaming reported in the thin layer cultures gives them an advantage over the other types of open culture systems. Some researchers have improved on the mixing in raceway ponds by utilizing drag boards [236], or mixing foils [116; 117]. These were shaped like aircraft wings, and when fixed in the tanks, gave improved mixing in the range of 0.5 – 1 Hz, and improved productivities. Declines in photosynthetic efficiencies and lowered productivities were obtained when the foils were removed and the flow pattern become laminar instead of turbulent. Other researchers have also reported increased productivities following turbulent mixing [12; 88; 100; 190; 192; 195]. However, the exact mode by which proper mixing improves algae productivities still remains unresolved as it is unlikely that the very high frequency fluctuations required for proper L-D cycling (>10 Hz) [82; 114; 221], can be achieved in open ponds [19; 82; 106; 150; 151; 153; 192; 200; 208].

Regardless of whether or not the required frequencies can be obtained by mixing, it is obvious that the continual movement of algae cells between light and dark periods ensures that individual cells are not stationed exclusively in the light or dark regions of the culture and will contribute to an increase in productivity (

Table 3. This was supported by Grobbelaar [83] who demonstrated that the effects of mixing (L-D cycling and beneficial effects of turbulence) worked synergistically to improve the productivity of microalgae cultures.

Several methods are used for mixing in PBRs: In inclined tubular PBRs, mixing is accomplished by the use of static mixers and gas spargers [21; 232-234]. Bubble columns inject gas into the culture through gas spargers or air stones. In both helical and flat plate reactors, mixing is provided by aeration, while in horizontal or near horizontal tubular reactors, it is accomplished by having an elevated riser section (where the degassers and heat exchange systems are situated), from where the vertical drop back into the solar tubes generates the required turbulence (Figure 8) [3; 150; 151; 153; 227].

Horizontal PBRs are necessarily limited in run length by the increasing resistance and the force needed to push the culture through long distances of very thin pipe [191; 223].

While increases in algae productivities by turbulent mixing have been well documented [98; 100; 190], it now appears that the random motion currently obtained by turbulent mixing in most PBRs does not enhance productivity as much as ordered mixing with a regular light-dark cycle of about 1Hz with a light vs. dark cycle frequency of about 1:10 [64; 229]. These authors demonstrated this in an airlift, flat plate PBR with baffles to generate ordered mixing, and an ordinary flat plate reactor without baffles. These authors reported that the regular, defined mixing pattern in the airlift FPR effectively simulated a regular flashing light and gave a higher productivity compared to the turbulent mixing in a regular flat panel PBR where the mixing was chaotic, without a well-defined frequency. The productivity obtained was 1.08 $g.L^{-1}.d^{-1}$ (17.9 $g.m^{-2}.d^{-1}$) and 0.65 $g.L^{-1}.d^{-1}$ (11.55 $g.m^{-2}.d^{-1}$) when *Chlorella*

vulgaris was grown under the same conditions, in two 30 mm path length flat panel PBRs - with and without the inclusion of mixing baffles. It appears that in addition to the ordered mixing, another reason for this increase may be explained by the L:D cycles used by these workers as other workers [85; 86], have demonstrated a 6.7 fold increase in photosynthetic rates when the L:D cycles were increased from equal 1D:1L to 2D:1L. Although these increases were also accompanied by an increase in the cycles from 10s to 10ms (which are difficult to achieve with current technology), it is still likely to lead to increased productivities as Grobbelaar [88] demonstrated that the stimulatory effect of intermittent light becomes pronounced with cycles shorter than 10s, which has been achieved by other researchers [64; 229].

While the use of high velocity flows for turbulent mixing is beneficial for the cultures, care must be taken to avoid shear damage to the microalgae which will occur when the turbulence is such that the size of the fluid micro eddies approach the cellular dimensions of the algae [51; 202]. The sensitivities of microalgae to shear differ greatly and this, as well as the shearing stress imposed by the mixing device, will determine the species to be cultured. This may be a problem in some PBRs where the configuration precludes the culture of some types of microalgae, especially the helical and thin tubular PBRs.

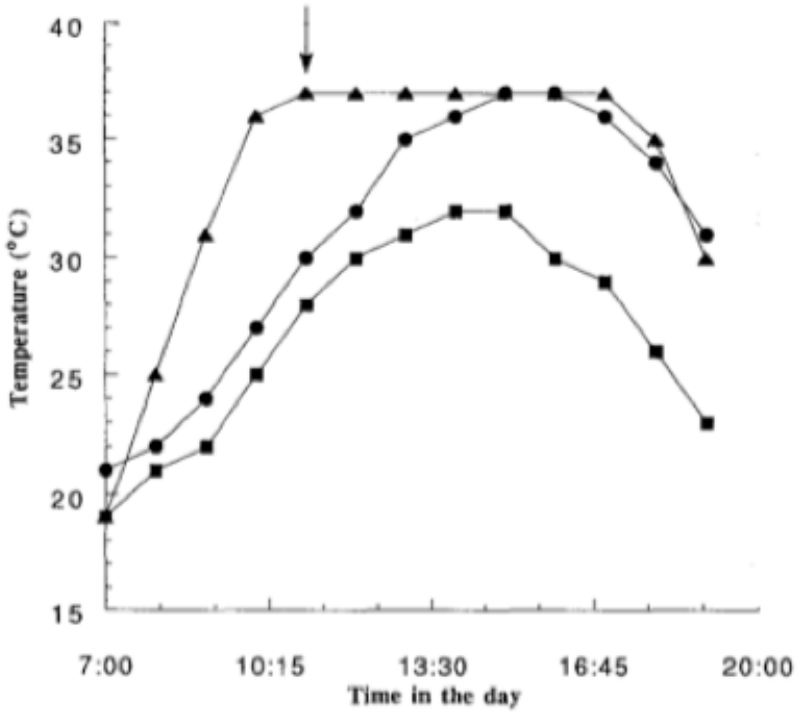


Figure 33: Daily changes in temperature of *Spirulina* cultures grown outdoors in ■: Open raceway; ●: Open raceway in greenhouse, and ▲: tubular PBR. (Richmond et al. [193]).

Table 3: Interrelationships between intensity of light source, the optimal cell density, the rate of mixing and the output rate of cell mass in *Spirulina platensis* (from Hu and Richmond [100] and Richmond and Zou [195])

Light µmol photon.$m^{-2}.s^{-1}$	Mixing rate L(air)$l^{-1}.min^{-1}$	Optimal cell density g dw.l^{-1}	Maximal output rate mg dw.$l^{-1}.hr^{-1}$
500	0.6	2.0	70
	2.0	5.0	100
	4.0	5.0	100
1800	0.6	6.0	200
	2.0	9.0	300
	4.0	17.0	400

Gas Exchange

CO_2 addition

As roughly 45-50% of microalgal biomass is made of carbon [12; 41; 71; 202], the low percentage of CO_2 in the air (0.033%) will quickly limit growth of the algae if supplementary carbon is not supplied.

Several methods have been used to distribute CO_2 into algae cultures, including bubbling through air stones, plastic dome exchangers with perforated PVC pipes, and injection into a pump used for aeration and mixing. Diffusion methods involve sparging the CO_2 through porous metal or plastic pipes to form very fine bubbles [208] in deep sumps. Care must be exercised when using spargers, however, as Sheehan et al. [208]obtained significant foaming when spargers were used in their raceway ponds. The foam caused an opaque cover, which interfered with light transmission and necessitated the use of an antifoaming compound to reduce the foam.

An advantage of closed PBRs over open ponds occurs in the reduction of CO_2 losses which will occur if CO_2 is simply bubbled into shallow open ponds. However, most open systems have devised methods to reduce such CO_2 losses which include increasing absorption by sparging into deep sumps or into suction sides of pumps [68; 131; 243] and trapping the CO_2 under floating gas exchanger [12; 28], thus prolonging its contact with the water. CO_2 utilization rates over 60% have also been obtained in open systems by maintaining high alkalinities and minimal CO_2 concentrations in the cultures [70; 134; 136; 242], without affecting the productivity of the algae. Obviously, this implies prior knowledge of the growth characteristics of the algae in terms of CO_2 utilization.

In PBRs where CO_2 is injected at a point (such as in tubular and helical PBRs), a CO_2 concentration gradient will be built up [37; 232] and the increase in pH will necessitate the addition of more CO_2 injection points, which add to the complexity and operating costs of PBRs.

In aerated cultures (both open ponds and PBRs), the CO_2 added can be used to control the pH level of the culture because the consumption of nitrates by the algae shifts the medium

to an alkaline pH. The addition of CO_2 acidifies the medium by the formation of carbonic acid and can be used to keep it within desirable limits. This is usually set up using a pH controller attached to a pH or CO_2 probe immersed in the culture. This controller activates a solenoid in response to higher pH levels in the cultures.

O_2 removal

If oxygen build up occurs in algae cultures to concentrations exceeding saturation, oxygen radicals may develop which cause photo-oxidative damage to the chlorophyll reaction centres and result in an inhibition of photosynthesis and a reduction in the productivity of the cultures. [153; 230]. [200]. The reduction of excess levels of dissolved oxygen in algae cultures is therefore, of paramount importance as many algal strains cannot survive in significantly O_2-oversaturated milieu longer than 2–3h [177].

The accumulation of O_2 can be a particular problem for some PBRs (especially the horizontal or near horizontal tubular PBRs) where supersaturated oxygen concentrations as high as 400% have been measured [84]. In horizontal PBRs, the dissolved oxygen cannot be removed within the bioreactor tube and degassers or gas exchange units must be used to alleviate this problem [52; 193]. These place a significant design constraint on the PBRs as the increase in oxygen concentrations in very long horizontal PBRs is such that the high density culture of algae will require so many degassing stations as to make the process impractical [226]. A failure to take this into consideration has been implicated as a causal factor in the failure of large-scale commercial ventures [191; 225].

Where there is an interface between the culture and the atmosphere, such as occurs in open systems, excessive oxygen is not usually a problem if the cultures are continually agitated or bubbled with air as the oxygen will leave the culture when it reaches the surface and the O_2 concentration will remain close to that of ambient air. This is an advantage that open ponds have over some PBRs other than aerated PBR cultures, which have an interface with the atmosphere and will not suffer from O_2 accumulation such as occurs in the tubular PBRs and the helical PBRs.

Scale-up

An advantage of open raceway ponds over many PBRs lies in the ease of scaling up. As long as the space for expansion exists, scale-up is very easy to accomplish.

In contrast, while many PBRs have been successfully operated at laboratory and pilot scales, very few have been scaled up to commercial scales. Notable exceptions are a large-scale tubular PBR producing *Chlorella* in Germany [177] and tubular reactors used to produce *Haematococcus* in Israel and Hawaii [162; 167]. Addavita Ltd., a UK-based company, also operates a commercial helical PBR targeted mainly towards the aquaculture industry.

Unlike in open systems, it is difficult to scale up PBRs and still maintain optimum light, temperature, mixing and mass transfer properties [231].

The easiest PBRs to scale up are the vertical bubble columns as additional units are simply added on to the available area. However, a disadvantage of the vertical bubble columns is the potential for shading by closely situated reactors, which may increase the land area required [48; 200] although the extent of the shadow thrown by the reactor will vary with the season. This was tested by Chinni Zittelli et al. [48] who conducted a full-scale simulation using plastic wrapped dummy columns to surround a few real columns of *T. suecica*. These authors reported little difference in the productivity of the simulated full-scale units as opposed to a single unit standing in full sunlight. The productivities obtained were 0.49 $g.L^{-}$

$^{1}.d^{-1}$ and 0.46 $g.L^{-1}.d^{-1}$ for the shaded and the free standing columns respectively. The authors gave an areal productivity measurement of 36.3 $g.m^{-2}.d^{-1}$ (This corresponded to 18.5 $g.m^{-3}.d^{-1}$ of exposed surface area (excluding the surface area of the internal cylinder).

Scale-up is relatively easy for some types of tubular systems such as the parallel flow tubular reactors as more parallel tubes can be connected to existing manifolds which share the same degassers and airlifts [193]. Inclined tubular PBRs can similarly be expanded by adding new tubes on to the upper and lower manifolds.

Helical PBRs can also be scaled-up relatively easily as only the number of parallel tubes needs to be increased. The advantage of these systems is that it allows relatively long tubes to be placed in a very small ground area and the coiled arrangement of the tubes in a vertical orientation, dilutes the incoming solar radiation enough to increase photosynthetic efficiency.

In contrast, serpentine type tubular reactors and horizontal PBRs are more difficult to scale up as they face the constraints of CO_2 and O_2 gradients and generating the required turbulence to force the culture through longer lengths of narrow tubes [153], if they are scaled up by lengthening the tubes. Scaling up by widening the tubes, however, decreases the surface area-to-volume ratio of the system (one of PBR's advantages over open systems) and decreases the volumetric productivity of the system. An additional disadvantage of horizontal PBRs is that scale-up requires as much land area for similar areal productivities as open raceways.

Scale-up in flat panel PBRs would require the construction of many different compartments and support materials. In addition, there would be some degree of shading if held vertically or inclined.

The scale-up of the inclined thin layer system (open pond) will also require construction of additional support material to support the inclined angles and is not easily achieved.

Nutrients

The nutrients added to algal cultures must provide the inorganic elements that make up the algal cell and include macronutrients, vitamins and trace elements. The macronutrients required are generally considered to be nitrogen, phosphorus and silicon [93]. Silicon is only required for diatoms, silicoflagellates and some chrysophyes. Three vitamins are usually used in culturing microalgae: vitamin B1 (thiamine HCL), vitamin B12 (cyanocobalamin) and vitamin H (biotin). Although all three vitamins can be added with no adverse effects, [176] many algae need only one or two of these vitamins [93]. For mass cultures, costs can therefore be reduced by only including the needed vitamins.

Typical trace metals used consist of chelated salts of iron, zinc, cobalt, manganese, selenium and nickel. The culture media used in the large-scale culture of microalgae are the same media used in the laboratory with a few modifications, which should depend on the growth requirements of the alga under cultivation.

There is very little published work that has been done to determine the optimal levels of nutrients to be added to mass algal cultures. Although macronutrients are usually required in a ratio of 16N: 1P [35], in practice, widely different ratios of the macronutrients are used, even when culturing the same microalga [186; 196]. To avoid nutrient limitation, nutrients are usually added to excess [3; 76; 200].

The costs of the nutrients have been estimated to range from 10-30% of the total production costs in open ponds [28; 30], and to reduce this, many producers recycle the medium that may still have nutrients. There is still uncertainty surrounding the potential for re-use of the media as some researchers found no deleterious effects on growth or productivity of the microalgae from re-using culture media compared to new media [242]. In contrast, Livansky et al [133], reported a reduction in algae productivity from re-using water and only a 16% reduction in new nutrient requirements. In any case, the reuse of spent media often results in the excessive accumulation of organic matter, which can increase the risks of contamination [149]. Another potential limitation on the reuse of spent media is imposed in high-density cultures by the occurrence of inhibitory secondary metabolites by certain microalgae when they are cultured at high densities [119; 195; 250]. However, as (with the exception of inclined thin layer systems) open ponds do not achieve high volumetric productivities, the accumulation of inhibitory metabolites is not expected to be an issue if the number of reuse cycles is limited.

Cleaning

Unlike open systems, which can be rapidly cleaned (except for large, unmixed systems), the enclosed nature and the high surface area-to-volume ratio of most enclosed PBRs make cleaning and disinfection difficult. This is exacerbated by the fact that wall growth and biofilms, which will occur on the inside surfaces of the PBRs, are not easily cleaned - especially in PBRs with very narrow light paths. This has the effect of increasing downtimes in the event of, for instance, a culture crash compared to open systems. Clean-in-place systems [50; 53] can help automate this process but they add significantly to the capital costs of PBR facilities.

Land

The availability and the costs of land are also important factors to be considered. When land is expensive, more intensive systems should be considered and this is an important disadvantage of open systems. However, this disadvantage is also shared by some PBR designs, such as the horizontal tubular PBR design. The vertically oriented PBRs, such as the helical, inclined tubular and inclined flat plate and vertical cylindrical PBRs, offer the advantage of allowing relatively large surface-to-volume areas and diluting the incoming solar radiation enough to increase photosynthetic efficiency.

The source and quality of water to be used are also significant factors. In open ponds, the larger the pond area, the more water is lost by evaporation However, this loss of water has been equated to the amount of water used to cool closed PBRs due to their tendency to store and accumulate heat. Tredici and Materassi [227] reported a water requirement of only 250 L per year to cool a tubular PBR but this figure will have to be multiplied by the number of PBRs available in a commercial scale setting.

Contamination

The open nature of pond culture systems restricts the number of species that can be successfully grown in monoculture. Contamination by zooplankton, other algae, protozoans and bacteria commonly occur in open pond systems [149; 208; 242]. These are minimized by culturing species which can survive and grow under highly selective conditions, such as high salt content for growing *Dunaliella* salina, high nutrient levels for growing *Chlorella* sp, *Nannochloropsis* sp. and *Phaeodactylum tricornutum* and high bicarbonate and high pH for growing *Spirulina* sp.

Despite these measures, contamination by other, unwanted species still occurs in these cultures [16], necessitating the operation of open cultures as batch cultures where the cultures are restarted at regular intervals using fresh, pure cultures. Indeed, several researchers have reported a disconnect between the lab and the field whereby the algae species that appeared most robust in the laboratory settings were easily overtaken by environmental strains in the field [208; 242]. From the Aquatic Species Programme (ASP) algae collection of over 3000 species, there was none that could dominate a raceway pond continually and have desirable biofuel properties [208]. Several researchers have therefore advocated that the best method to using an open system may be to select a native contaminant strain that takes over the ponds. Maintaining high culture densities minimizes or reduces the contamination by predators and competitors and this is more readily achieved in inclined systems than raceway ponds where the more dilute systems can be contaminated more easily.

Rainfall can cause significant problems in open ponds as it dilutes the cultures, reducing growth and more importantly, facilitates the emergence of predatory protozoans (ciliates and amoeba) [28; 149]. In fact, contamination by such predators and competing algae following rainfall has been given as one of the main reasons for failure in open algae cultures [189; 237; 239].

PBRs are less susceptible (but not immune) to contamination due to their closed or semi–closed designs and as such, may be able to be used to grow selected species for longer periods than open ponds.

Evaluation of productivities of open and closed systems

The productivity of algae, generally expressed in $g.m^{-2}$ of growth area, reflects the efficiency of the utilization of incident light and the land requirement to grow a given amount of algae. In contrast, the volumetric productivity of a system represents the efficiency with which a unit volume of the reactor is used. Although productivities are generally expressed in one or the other of these parameters, an efficient production system should entail high volumetric as well as high areal productivities, both of which should be optimised to reduce costs.

The productivity of the chosen system/s is the single most important parameter deciding the success or failure of an algae biomass venture and although PBRs were generally assumed to be more productive than open ponds, differences in geographical locations, climatic conditions, operating conditions and algal strains used make it difficult to compare productivities between different mass culture systems (see Table 4). In one exception, where Pedroni et al. [170] conducted a six-month side-by-side comparison of tubular PBRs and open raceway ponds, no differences were observed in the growth and daily productivity of the *Tetaselmis suecica* grown in both systems.

Both volumetric and areal productivities depend on the light path, cell density and the intensity of solar radiation. However, an inverse relationship generally occurs between the volumetric and areal productivities in relation to light penetration [3; 153; 212; 228; 233] as increasing the light path will often increase the areal yield of the culture systems (since more culture volume could be accommodated in a given area), but this often produces a dilute culture which will require more energy to mix, and higher processing costs downstream.

If an increase in the light path length is accompanied by an appropriate increase in light intensity, the biomass productivities in both short and long light path length reactors would be similar. However, the use of solar radiation precludes this option and a compromise must be made between both.

Richmond [196], proposed the adoption of a new parameter called the "reactor efficiency". This factor is obtained by dividing the volumetric productivity by the overall irradiated reactor surface required to produce that cell mass. This parameter should therefore give us the best reactor design which results in high volumetric output rates and yet would require the smallest volume-to-area ratio to produce that cell mass. However, when this parameter was applied to the productivity values obtained in this study, the reactor efficiency data was skewed towards smaller reactors. This skew is caused by the fact that a unit measurement (the volumetric output rate) was being divided by the overall areal measurement. Consider, for instance, two raceway ponds with surface areas of 10 and 100 m^2. If both ponds have the same areal and volumetric productivities (5 $g.L^{-1}.d^{-1}$ and 11 $g.m^{-2}.d^{-1}$), application of the reactor efficiency formula of Richmond would give efficiencies of 5 and 50 to the larger and smaller ponds, respectively, despite both having the same productivities. A better way to compare between systems still needs to be found.

The ability to support higher volumetric productivities is an important advantage of closed PBRs over open systems (except for inclined systems) as it results in a smaller footprint. Indeed, while increases in areal productivities may still achievable in both open and closed systems, the areal productivities generally obtained in both open and closed systems are comparable when the total irradiated surfaces of the PBRs are taken into account (see Table 4). Where higher areal productivities have been reported, these have not been related to the surface area of the PBRs, but rather to the occupied land area and were obtained by dilution of the light over a larger bioreactor surface area by taking advantage of the vertical height of the PBRs [123; 126; 127; 196; 227].

These higher areal yields that may be obtained in longer path length PBRs may not be of much significance, however, as the increased downstream processing costs and the capital costs of stacking expensive hardware to obtain dilute cultures will counter any benefits from the higher areal productivities. For instance, in the stacked design of Grobbelaar and Kurano [87] an improvement of only 37% in productivity was obtained by in effect, doubling the number of reactors used (stacking two flat plate PBRs on top of each other).

Growth inhibition

As described above, an increase in the light intensity (or a decrease in the light path) usually leads to increases in the productivity of the culture if all other conditions are optimal. However, at high concentrations, substances which can inhibit further growth of the cultures have been demonstrated [195; 250]. This inhibitory activity imposes an upper limit on the concentrations that may be achieved even when other factors are not limiting [119; 195]. The occurrence of these limiting factors eliminates any advantage that may be obtained in short light path length systems in favour of the more dilute systems. Thus, the relationships between the culture method, the optimal cell densities, light path, culture volumes and the level at which inhibitory activity occurs and its extent, need to be defined and optimized for the cultured species. This inhibition of growth is unlikely to be a major problem in open ponds due to their relatively dilute cultures. A situation in which it may occur may arise

when water and nutrients are continuously recycled without pretreatment between batches of algae.

Conclusion

As will be seen from the above discussions, there is no one ideal algae culture system. Each system will have to be evaluated based on its intended end use, the economics of the system and the technical expertise required and the geographical location where it will be used. As far as productivity goes, the increased volumetric productivities promised by PBRs have been shown to be matched and even exceeded by inclined, thin layer open systems (see Table 4), while the one valid side-by-side comparison of areal productivities conducted so far [170] has shown no differences in the areal productivities of both open systems and closed PBRs.

Photobioreactors do have the advantage of being less susceptible to contamination and this may be of particular importance in the culture of microalgae for biofuels as it may enable the culture of highly oleaginous species to the exclusion of others which is not possible in open systems.

In the literature, closed PBRs are said to offer the promise of more consistent production compared to the open systems. However, experience from the authors' facilities from growing algae in closed vertical cylindrical PBRs and vertical bags indicates that culture crashes and contamination are still issues. There was no corroboration of this from microalgae-to-biofuel companies themselves, but we consider it extremely likely that there will be culture crashes and contamination in PBRs. However, the frequency of these events is unknown.

Potential for further improvements in productivity exists in PBRs (for example, the stacked PBR system coupled with ordered L:D cycling in thin plates), but appears less so in open systems.

Table 4: Some microalgae production systems, species produced and productivities obtained.

Open systems						
Culture system	***Location***	***Species***	***Areal yield ($g.m^{-2}.d^{-1}$)***	***Volumetric yield ($g.L^{-1}.d^{-1}$)***	***Description***	***Reference***
Inclined	Czech Republic	*Chlorella sp.*	11.1	1.24	6 mm thick culture; volume = 2000L; exposed surface area=224m^2 (cultured in September)	Doucha and Livansky [70]
Inclined	Czech Republic	*Chlorella sp.*	23.5	2.63	6 mm thick culture, volume = 2,000L; exposed surface area=224m^2 (cultured in July)	Doucha and Livansky [70]
Inclined	Czech Republic	*Chlorella sp.*	18.1	1.8	8 mm thick culture, volume = 1,000L; exposed SA:100m^2 (cultured in October)	Doucha and Livansky [70]
Inclined	Czech Republic	*Chlorella sp.*	32.2	3.22	8 mm thick culture, volume = 1,000L; exposed SA:100m^2 (cultured in July)	Doucha and Livansky [70]
Raceway	Vietnam	*Spirulina sp.*	10	-		Kim [113]
Raceway	Hawaii	*Platymonas sp.*	26	0.22	Volume = 5,760L, 48m^2 raceway. With mixing foils	Sheehan et al. [208]
Raceway	Hawaii	*Platymonas sp.*	50	0.42	Volume = 5,760L, 48m^2 raceway. With mixing foils	Sheehan et al. [208]

Open systems						
Culture system	***Location***	***Species***	***Areal yield ($g.m^{-2}.d^{-1}$)***	***Volumetric yield ($g.L^{-1}.d^{-1}$)***	***Description***	***Reference***
Raceway	Hawaii	*Cyclotella cryptica*	30	0.25	Volume = 1,104L, 9.2m^2 raceway	Sheehan et al. [208]
Raceway	Hawaii	*T. suecica*	37.5	0.31	Volume = 1,104L, 9.2m^2 raceway	Sheehan et al. [208]
Raceway	Israel	*N. salina*	24.5	0.2	Volume = 300L, 2.5m^2 raceway	Richard et al., (cited by Sheehan et al. [208]
Raceway	Israel	*I. galbana*	28.1	0.23	Volume = 300L, 2.5m^2 raceway	Richard et al., (cited by Sheehan et al. [208]
Raceway	New Mexico	*Scenedesmus quadricauda*	14	0.06	Volume = 22,500L, 100m^2 raceway	Weismann and Goebel [242]
Raceway	New Mexico	*Chlorella sp.*	21	0.093	Volume = 22,500L, 100m^2 raceway	Weismann and Goebel [242]
Raceway	Israel	*Anabena siamensis*	12.9	0.086	Volume = 300L, 2.0m^2 raceway	Richmond et al. [193]
Raceway	Israel	*Anabena siamensis*[3]	31.5	0.21	Volume = 300L, 2.0m^2 raceway	Richmond et al. [193]

[3] Covered with polyethylene

*Yield per m^2 (occupied land area).d^{-1}

PBRS						
Culture system	***Location***	***Species***	***Areal yield ($g.m^{-2}.d^{-1}$)***	***Volumetric yield ($g.L^{-1}.d^{-1}$)***	***Description***	***Reference***
Helical		*Porphyridium cruentum*	20-25	0.29-0.36		Chaumont et al. [42]
Helical	Spain	*Phaeodactylum tricornutum*	21	1.4	Volume = 75L, 5.0m^2 illuminated surface area= total SA 10m^2/2. = 5.0m^2	Acien Fernandez et al. [2]
Helical	Italy	*A. platensis*	13.3; 113.7*	0.9	Ground area=0.95m^2 Volume = 120L; illuminated surface area=total SA 16.16m^2/2. = 8.08m^2	Tredici and Zitelli [228]
∝ reactor	Singapore	*Chlorella pyrenoidosa*	22.14; 72.5*	2.9	Ground area=0.95m^2 Volume = 300L; illuminated surface area=39.3m^2	Lee et al. [127]
Parallel flow PBR	Israel	*Anabena siamensis*	3.47	0.37	Volume = 140.5L; illuminated surface area=15m^2 3.2cm diameter tubes	Richmond et al. [193]
Parallel flow PBR	Israel	*Anabena siamensis*	4.95	0.55	Volume = 126L; illuminated surface area=14m^2 2.8cm diameter tubes	Richmond et al. [193]

**Occupied land area calculation includes shadow thrown by PBR.

PBRS						
Culture system	***Location***	***Species***	***Areal yield ($g.m^{-2}.d^{-1}$)***	***Volumetric yield ($g.L^{-1}.d^{-1}$)***	***Description***	***Reference***
Inclined with static mixers	Japan	*Chlorella sorokiniana*	20.89	0.67	58L	Ugwu et al. [233]
Serpentine, double layer	Spain	*P. tricornutum*	15.1; 20*	1.2	Volume=200L, Surface area = 15.1m^2, land area=12m^2	Acien-Fernandez et al. [3]
Serpentine, double layer	Spain	*P. tricornutum*	25.2; 31.7*	1.90	Volume=200L; surface area = 15.1m^2, land area=12m^2	Molina et al. [153]
MGM Horizontal Tubular	Hawaii	*H. pluvialis*	4 ; 1.64	0.016	Volume=25,000L, tube surface area=2,434m^2; land area=100m^2	Olaizola, [167]
MGM Horizontal Tubular	Hawaii	*H. pluvialis*	5.34; 13*	0.052	Volume=25,000L, tube surface area=2,434m^2; land area=100m^2	Olaizola, [167]
Vertical bubble column	Spain	*P. tricornutum*	29.1; 93**;	0,64	Volume=60L, tube surface area=1.32m^2; Land surface area=0.3m^2	Sanchez Miron et al. [200]
Horizontal tube	Spain	*P. tricornutum*	26.7; 34*	2.02	Vol=200L, Land surface area=12m^2; tube SA=15.1m^2	Sanchez Miron et al. [200]
L-shaped PBR	Korea	*Euglena gracilis*	9.5; 52.7*	0.11	Volume=1,000L; PBR surface area=11.96m^2; Land area=2.16m^2	Chae et al. [41]
Glass column PBR	Israel	*Isochrysis galbana*	1.04	1.6	Tube surface area=3.9m^2 Volume=25.5L	Hu and Richmond, [99]

PBRS						
Culture system	***Location***	***Species***	***Areal yield ($g.m^{-2}.d^{-1}$)***	***Volumetric yield ($g.L^{-1}.d^{-1}$)***	***Description***	***Reference***
Annular	Italy	*T. suecica*	17.6; 38.3*	0.46	Volume=120L; annular column placed in a field with dummy columns for a simulation. Irradiated surface area(outer cylinder only)=3.14m^2	Chini Zittelli et. al. [48]
Aerated pipe	Japan	*C. calcitrans*	37.3	0.266	Pipe dimensions not given Carried out in November and December	Sato et al. [203]

Artificially lighted systems						
Culture system	***Location***	***Species***	***Areal yield ($g.m^{-2}.d^{-1}$)***	***Volumetric yield ($g.L^{-1}.d^{-1}$)***	***Description***	***Reference***
Modular FPP	Italy	*Nannochloropsis sp.*	3.44	0.6	Volume=123L, Total surface area = 21.8m^2, light supplied at 115µmol photon.$m^{-2}.s^{-1}$ supplied on 1 side only	Chini-Zittelli et al. [47]
Modular FPP	Italy	*Nannochloropsis sp.*	4.8	0.85	Volume=123L, Total surface area = 21.8m^2, light supplied at 115µmol photon.$m^{-2}.s^{-1}$ supplied on both sides.	Chini Zitelli et. al. [47]

Artificially lighted systems						
Culture system	***Location***	***Species***	***Areal yield ($g.m^{-2}.d^{-1}$)***	***Volumetric yield ($g.L^{-1}.d^{-1}$)***	***Description***	***Reference***
Modular FPP	Italy	*Nannochloropsis sp.*	5.5	0.97	Volume=123L, Total surface area = 21.8m^2, light supplied at 230μmol photon.$m^{-2}.s^{-1}$ supplied on 1 side only	Chini Zitelli et. al. [47]
Modular FPP	Italy	*Nannochloropsis sp.*	8.2	1.45	Volume=123L, Total surface area = 21.8m^2, light supplied at 230μmol photon.$m^{-2}.s^{-1}$ supplied on both sides.	Chini Zitelli et. al. [47]
Turbidostat	Wales	*I. galbana*	14.8-21.4	0.13-0.19	Volume=80L, illuminated surface area=0.7m^2	Liang and Jones [130]
Turbidostat	Wales	*Tetraselmis suecica*	22.8-37.6	0.2-0.33	Volume=80L, illuminated surface area=0.7m^2	Liang and Jones [130]
Helical	Chile	*I. galbana*	10.5; 1.1	0.06	Ground area=0.41m^2 Volume = 70L; illuminated surface area=total SA 8/2. = 4$m^{2.}$	Uribe et al. [235]
Annular vertical PBR	Italy	*N. oculata*	4.64	0.21	10, 120L annular units, operated January to May, total surface area=5.3m^2. Used no temperature control. Combined artificial+natural light	Chinni-Zittelli et al. [49]

Artificially lighted systems						
Culture system	***Location***	***Species***	***Areal yield ($g.m^{-2}.d^{-1}$)***	***Volumetric yield ($g.L^{-1}.d^{-1}$)***	***Description***	***Reference***
Annular vertical PBR	Italy	*N. oculata*	5.11-6.4	0.23-0.28	10, 120L annular units, operated October to April, total surface area=5.3m^2, Used temperature control. Lower productivity in December, highest in April. Combined artificial+natural light	Chinni-Zittelli et al. [49]
Flat Panel airlift	Germany	*Chlorella vulgaris*	23.6	2.64	3L volume, total illuminated surface area=0.17m^2. culture carried out in a 15mm path length chamber	Degen et al. [64]
Flat Panel airlift	Germany	*Phaeodactylum tricornutum*	25.18	2.35	Total surface area=0.434m^2; volume= 4.7L (5L with 7% displacement for baffles)	Meiser et al. [144]

Mixed systems						
Culture system	***Location***	***Species***	***Areal yield ($g.m^{-2}.d^{-1}$)***	***Volumetric yield ($g.L^{-1}.d^{-1}$)***	***Description***	***Reference***
Mixed Flat panel PBR + Open ponds	Italy	*A. platensis*	0.18	0.18	Pond alone: volume=300L, Pond surface area=3.8m^2	Pushparaj et al. [179]
Mixed Flat panel PBR + Open ponds	Italy	*A. platensis*	2.4	2.10	Flat panel PBR alone: volume=18L, Illuminated surface area=12m^2	Pushparaj et al. [179]
Mixed Flat panel PBR + Open ponds	Italy	*A. platensis*	6.1	0.32	Mixed unit (pond and south facing flat panel PBR). Volume=282L; total surface area=15.8m^2	Pushparaj et al. [179]
Mixed Flat panel PBR + Open ponds	Italy	*A. platensis*	5.4	0.29	Mixed unit (pond and east facing flat panel PBR). Volume=282L; total surface area=15.8m^2	Pushparaj et al. [179]

*: calculated for this study

Appendix G

Appendix G. Heterotrophic and Mixotrophic Cultures

Heterotrophic culture

Although most microalgae grow phototrophically, some are capable of heterotrophic growth using organic substrates as the sole carbon and energy sources. Indeed, it appears that heterotrophy is more widespread among microalgae and cyanobacteria than previously thought as heterotrophs have been found from all the algal groups [14; 157].

The heterotrophic production of microalgae has many advantages over phototrophic growth. These include the large, pre-existing fermentation technology and knowledge base, the high degree of process control which ensures consistent, reproducible production, the elimination of the requirement for light, and lower harvesting costs. [8; 40; 46].

The heterotrophic cultivation of microalgae is a well established commercial reality. For instance, Martek Biosciences in Maryland commercially produces *Crypthecodinium cohnii* for the polyunsaturated fatty acid, DHA [8; 15] and Solarzyme, in California has produced biodiesel from algae biomass produced in fermenters. About half of the *Chlorella* biomass produced commercially in Japan is grown heterotrophically in fermenters [125]. Recently, Li et al. [129] have grown *Chlorella protothecoides* in a 11,000 L commercial fermenter for production of biodiesel.

Fermenters are available in a wide range of sizes ranging from 1 liter to over 500,000 liters, and the technology is well established and well described (for a review of fermenters, see [46]). A fermenter differs from a PBR in three main areas: 1) the carbon source is organic, 2), the need to supply oxygen for catabolism of the substrates and 3), the need to maintain sterility.

The most widely used source of organic carbon is glucose although other organic substrates such as acetate, fructose, citrate and ethanol have been used. The metabolism of the algae reduces the DO level in the fermenters, as adequate oxygen is required for the catabolism of the organic substrates. Hence, oxygen supply is usually the single most limiting factor preventing the achievement of a high cell concentration and a high growth rate in heterotrophically grown microalgae [57; 154].

Generally, heterotrophic cultivation has been found to increase the total lipid content in algae compared to phototrophically grown cells [129; 147; 216; 244]. In a comparison done by Miao and Wu [147], heterotrophically grown cells of *Chlorella* were found to accumulate lipids to 55.2% of the cellular dry weight as opposed to 14.57% in phototrophically grown cells (see Table 5 and Figure 35). The higher total lipids contained in heterotropically grown cells are thought to result from the accumulation of more storage lipids arising from the increased availability of carbon in the substrate [199], or the competition in phototrophic cultures of the lipid and photosynthetic enzymes for the available CO_2 [181].

Heterotrophic culture usually results in higher yields compared to autotrophic cultures (see Figure 34 [14]). For instance, Yu et al. [251] obtained a productivity of 0.731 $g.L^{-1}$ vs. 0.335 $g.L^{-1}$ for heterotrophic and autotrophic cultures of *Nostoc flagelliforme* respectively after 7 days growth. Similarly, Wen and Chen [244] obtained a productivity of 2.04 $g.L^{-1}$ and 0.5 $g.L^{-1}$ respectively, when *Nitzchia laevis* was grown heterotrophically and photoautotrophically for 8 days. In a few cases however, the heterotrophic growth has been lower than photoautotrophic growth: Fang et al. [74] reported a maximum productivity of 392 $mg.L^{-1}$ and 326 $mg.L^{-1}$ when *Nannochloropsis* sp. was cultured photoautotrophically and

heterotrophically, respectively, using glucose as the carbon source. The reason for this result is unknown but it highlights the need for optimization of the heterotrophic culture of individual strains prior to mass heterotrophic culture.

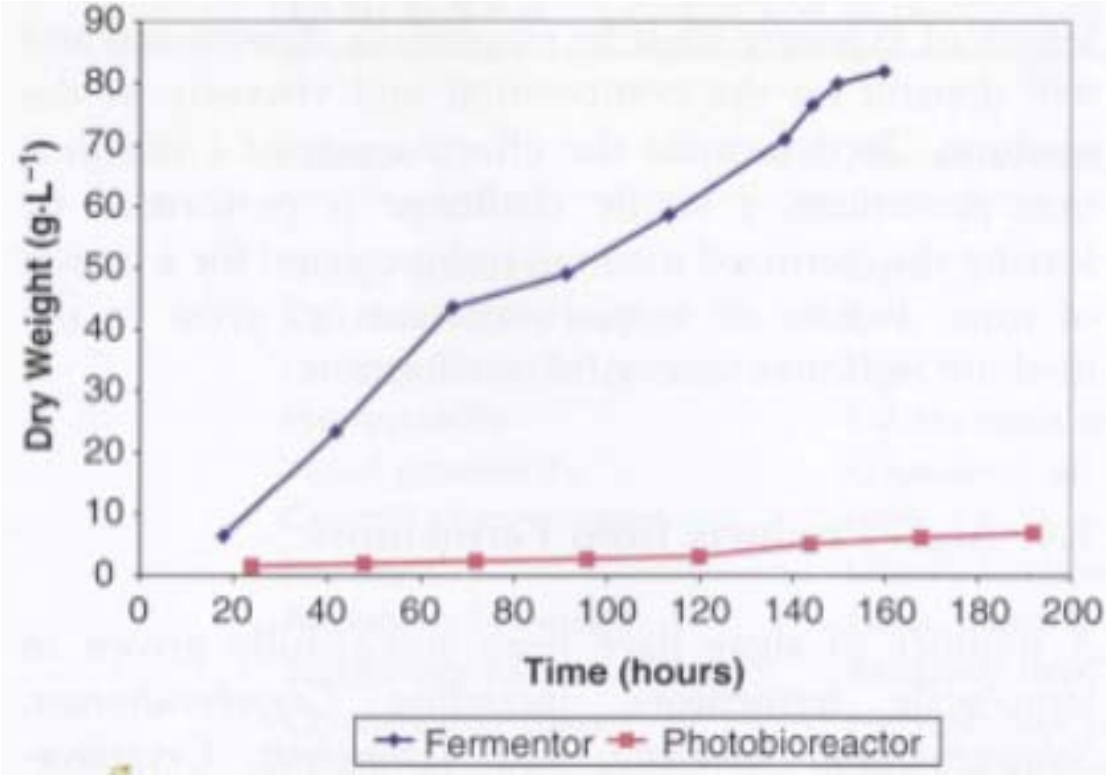


Figure 34: Comparison of growth of *Chlorella sp.* in a photobioreactor and a fermenter (Behrens [14]).

Heterotrophically grown algae can attain very high volumetric yields and they are susceptible to inhibition caused by the accumulation of inhibitory substances, as are phototrophs. Many researchers deal with this problem by using continuous perfusion to attain high yields. For instance, Chen and Johns [44] reported growing *Chlamydomonas reinhardtii* to high concentrations in the presence of inhibitors using continuous perfusion of the culture through hollow fibre cells. The highest cell concentration of *C. reinhardtii* obtained in perfusion culture was 9.0 $g.L^{-1}$, compared to 0.5 $g.L^{-1}$, 1.0 $g.L^{-1}$ and 1.5 $g.L^{-1}$ in batch, fed batch and chemostat cultures respectively.

A major disadvantage of heterotrophically grown cells is the reduction in levels of light-induced products (pigments, chlorophyll and carotenoids) which has been reported when algae are grown without light [43; 63; 166]. While not affecting the production of biofuels or oils for biofuel, this will limit or even exclude the production of some by-products or co-products that are light generated, thus limiting the usefulness of heterotrophic production in this aspect. On the other hand, the high level of process control assures continuous, year round, weather independent production – a major advantage not guaranteed in photoautotrophic production.

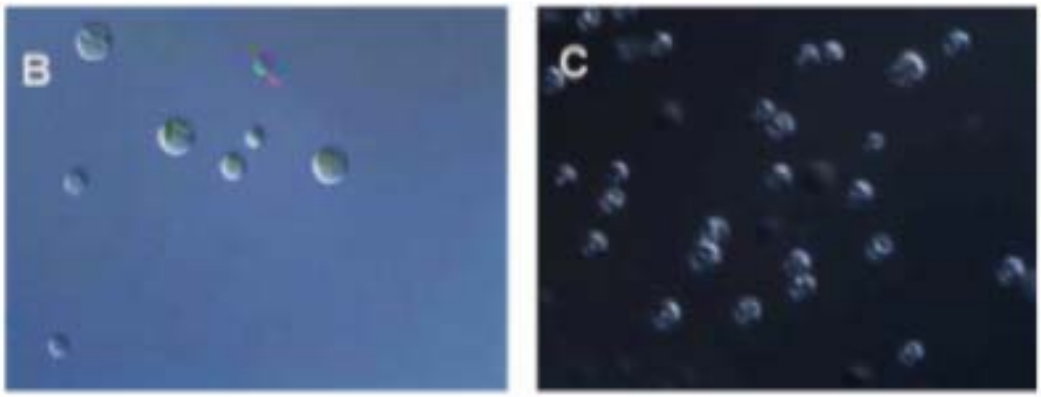


Figure 35: Cells of *C. protothecoides* grown under autotrophic (B), and heterotrophic (C) culture conditions under differential interference microscopy. Almost no lipid vesicles were observed in autotrophic *C. protothecoides* cells (B), while the cells of heterotrophic *C. protothecoides* were full of lipid vesicles (Miao and Wu [147]).

Table 5: Contents of the main chemical components of cells of autotrophic (AC) and heterotrophic (HC) *C. protothecoides* (Miao and Wu [147]).

Component	***AC***	***HC***
Protein	52.64 ± 0.26	10.28 ± 0.10
Lipid	14.57 ± 0.16	55.20 ± 0.28
Carbohydrate	10.62 ± 0.14	15.43 ± 0.17
Ash	6.36 ± 0.05	5.93 ± 0.04
Moisture	5.39 ± 0.04	1.96 ± 0.02
Others	10.42 ± 0.65	11.20 ± 0.61

Mixotrophic culture

Mixotrophic growth is a nutritional mode in which photoassimilation of CO_2 and the oxidative assimilation of organic carbon sources proceed simultaneously, thereby offering the potential of greatly increased productivities.

For species that can utilize both light energy and chemical substrates, the mixotrophic cultivation of microalgae may be suggested as a superior alternative to phototrophic and heterotrophic growth as both biomass and productivity increases have been reported when some microalgae were grown mixotrophically rather than phototrophically or heterotrophically [40; 55; 63; 74; 244; 251]. For instance, Lee et al. [122], obtained an optimal daytime productivity of 10.2 $g.L^{-1}.d^{-1}$ and 5.9 $g.L^{-1}.d^{-1}$ at night when *Chlorella sorokiniana* was cultured in a tubular PBR with added glucose. By comparison, the daily volumetric productivity of phototrophically cultured *Chlorella* in a similar PBR was about 3 times lower [124]. From an initial concentration of 2 $g.L^{-1}$, Lee et al. [122], reported yields of 11.1 $g.L^{-1}$ in 12h

in mixotrophic culture as opposed to phototrophic cultures which attained 2.2 $g.L^{-1}$ in the same time period. Similarly, Fang et al. [74] reported a maximum productivity of 550 $mg.L^{-1}$, 392 $mg.L^{-1}$ and 326 $mg.L^{-1}$ when *Nannochloropsis* sp. was cultured mixotrophically, photoautotrophically and heterotrophically, respectively, using glucose as the carbon source. Ceron-Garcia et al. [40] reported an increase in productivity of P. *tricornutum* of 9 and 8 times improvement in biomass concentration and biomass productivity compared to photoautotrophically grown cells and Chen and Zhang [45] reported a productivity 2.4 times higher than that obtained by photoautotrophically growing *Spirulina platensis*.

As they are grown in the presence of light, mixotrophic cultures are able to overcome a major disadvantage of purely heterotrophically grown microalgae, which is the inability of the cultures to produce light–induced products. However, in mixotrophic cultures, the concentrations of these light-induced products will depend on the relative heterotrophic and phototrophic growth rates: at high cell concentrations, the light becomes limiting and the autotrophic growth rate will be reduced relative to the heterotrophic growth rate. Thus, the accumulation of these light-induced compounds will be lower compared to phototrophic cultures [166; 249]. While this may have an effect on the yield of potentially useful by-products or co-products, it is expected that the increased biomass yield from heterotrophic or mixotrophic culture (as opposed to phototrophic growth) may be enough to offset this decrease to some extent, but by how much will depend on the cultured organism and the mode of culture.

Many workers have resolved this shortcoming by using a sequential culture process whereby the algae are first grown heterotrophically to achieve high densities and then, phototrophically to accumulate the desired light-induced pigments [94; 166]. Algae grown in such sequential systems display a slight decrease in biomass when they are switched to phototrophic growth but rapidly increase the pigment content to maximum or near maximum levels within 24 hours of switching to autotrophic growth [166].

It is important to ensure that the organic substrates are completely consumed in the heterotrophic phase. If they were carried over into the autotrophic phase, the cells would respire mainly by heterotrophic metabolism and self shading would still prevent the accumulation of light-induced products. In addition to this, the presence of organic material could serve as substrates for bacteria, leading to serious contamination problems [110]. This is especially problematic in the case of open or semi-open systems. Where organic nutrients are added in large-scale culture of microalgae (in Japan and Taiwan), they are slowly added at low levels during daytime in order to ensure that the algae cells can utilize it all and avoid having excess substrates which would encourage the growth of other microbial contaminants [126]. For the same reason, organic substrates are not added to these systems overnight [126].

High areal productivities have been reported for mixotrophic cultures. *Chlorella* cultures exhibit productivities as high as 127 $g.m^{-2}.d^{-1}$ daytime and 79 $g.m^{-2}.d^{-1}$, night time productivity, comparing favourably to the daily areal productivity of photosynthetic cultures of 35.8 – 41.4 $g.m^{-1}.d^{-1}$ [124]. This highlights another advantage of mixotrophic cultures (albeit one that is shared with heterotrophic cultures): the elimination of night time biomass losses.

Some studies have suggested that mixotrophic growth is the sum of heterotrophic as well as phototrophic growth [126]. However, this is not always the case as it would otherwise imply the optimization of both phototrophic and heterotrophic modes of growth at all phases of

the culture - a difficult task to achieve. For instance, when Wen and Chen [244] reported that the productivity in their mixotrophic culture was slightly lower than the sum of the phototrophic and heterotrophic cultures of *Nitzschia laevis* (Table 6), it was attributed to light limitation in the mixotrophic culture caused by rapid growth causing self shading, which was not obtained in the autotrophic cultures as they did not achieve such high densities. Yu et al. [251] reported that cell growth rates in mixotrophic cultures of *Nostoc flagelliforme* were always lower than the sum of the growth rates obtained in autotrophic and heterotrophic cultures for the first four days after inoculation and thereafter, it was higher.

Table 6: Kinetic parameters of growth and nutrient consumption of the diatom, *Nitzchia laevis* in different growth modes. Source: Wen and Chen [244]

Parameters[b]	***Growth modes***[c]			
	P	**M**	**H**	**P+H**
μ (d^{-1})	0.167 ± 0.019	0.466 ± 0.026	0.344 ± 0.034	0.511 ± 0.053
X_{max} ($g.L^{-1}$)	0.50 ± 0.028	2.27 ± 0.07	2.04 ± 0.08	2.52 ± 0.112
$Y_{X/Glu}$ ($g.g^{-1}$)	-	0.418 ± 0.002	0.372 ± 0.002	-
***q*Glu (d^{-1})**	-	1.11 ± 0.098	0.925 ± 0.058	-
$Y_{(X/N)}$ ($g\ mM^{-1}$)	0.199 ± 0.015	0.268 ± 0.008	0.403 ± 0.011	-

[a]Data are expressed as mean ± standard deviation of three replicates.\

[b]μ, specific growth rate; X_{max}, maximum cell density; $Y_{X/glu}$, cell yield based on glucose;

*q*Glu, specific glucose consumption rate; $Y_{(X/N)}$, cell yield based on nitrate.

[c]P, photoautotrophic culture; H, heterotrophic culture; M, mixotrophic culture; P+H, the sum of photoautotrophic and heterotrophic cultures.

Similar to what is obtained in heterotrophic growth, several authors have reported changes in the biochemical composition of the microalga depending upon the culture method. For instance, when *N. laevis* was grown phototrophically, heterotrophically and mixotrophically, the percentages of unsaturated fatty acids declined as the light levels declined. Thus, the values of the parameters followed the sequence: phototrophic > mixotrophic > heterotrophic. However, the total fatty acids were higher in the mixotrophic and heterotrophically grown cells than in the photoautotrophically grown cells.

The high degree of process control as well as the higher volumetric productivities and the ready availability of commercial fermentation equipment and knowledge all combine to offer great potentials for heterotrophic and mixotrophic techniques in the mass culture of microalgae. However, these techniques do not apply to all species of algae as some cannot utilize organic substrates and among those that can, some amount of process optimization is required as different strains respond differently to different organic substrates [157].

Appendix H

Appendix H. Harvesting

Harvesting is a crucial part of microalgae cultivation as its costs can constitute a significant proportion of the total production [30; 152; 162]. That harvesting is such a critical cost centre in the production of microalgae is due to the relatively dilute culture broths, which necessitate the processing of large volumes to obtain the biomass. This may be illustrated by a typical volumetric productivity of algae of 0.6 $g.L^{-1}.d^{-1}$ (from Table 4). With this level of productivity, 1,667 tons of water will have to be processed in order to recover 1 metric ton of dry algae.

It is essential to select a suitable method for the level of moisture that is acceptable in the harvested product. If the product is meant for anaerobic digestion, then a solids content of 3-4% is adequate [19]. However, for the recovery of oils or bioactive compounds, the solids content of the harvested biomass needs to be higher (15-25%) to reduce downstream processing costs. The value of the final product also plays a role in determining the efficiency required from the harvesting process. Most existing commercial algae production facilities produce high value products which may not be as sensitive to the constraint of high harvesting costs as producers of algae biomass destined for production of lower valued fuels. It is essential that efficient and low-cost harvesting methods are utilized for harvesting algal biomass.

Estimates for the cost of recovering biomass have ranged from 3.3% [162] to 30% of the total costs of production [90]. Regardless of the harvesting option that is selected, a few considerations apply which will increase the efficiency of the system. These include processing cultures with higher cell concentrations. For instance, in the earlier example, if the concentration used is 3.2 $g.L^{-1}.d^{-1}$ (from inclined open systems, shown in Table 4), then the amount of water that needs to be processed to obtain 1 ton of dried product decreases from 1,667 tons to only 313 tons of water.

Flocculation

Prior to separation, microalgae (especially small, planktonic algae) may be flocculated and harvested by sedimentation or floatation. In a second step, the flocculated microalgae may be harvested by various methods including sedimentation, floatation, centrifugation, filtration or micro straining. Microalgae cell surfaces are negatively charged [81; 209], the intensity of which, depends on the species, ionic strength of the medium, pH and other environmental conditions. In a stable culture, the electrical repulsion between the cells and the cell interactions with the surrounding water prevents the aggregation of the cells and contributes to the stability of the algal suspension. The neutralization of these surface charges destabilizes the algae culture, leading to the agglomeration of the algae into large clumps or "flocs", which can then be more readily separated from the culture medium. There are various methods of inducing flocculation in algal cultures and these are described below:

Chemical flocculation (inorganic chemicals)

Several authors have reported the use of chemical flocculation to obtain algae biomass [81; 95; 208]. The microalgae are induced to flocculate by the addition of inorganic chemicals, such as aluminum sulfate ($Al_2(SO_4)_3$) (alum), ferric sulfate $Fe_2(SO_4)_3$ or ferric chloride $FeCl_3$ or lime ($Ca(OH)_2$). The metal ions neutralize or reduce the negative surface charge of the cells, causing the formation of flocs and thus increasing the effective particle sizes and facilitating

separation. Flocculation can also be obtained with lime: this involves raising the pH with lime to the point at which $Mg(OH)_2$ is formed and acts as the flocculant.

High doses of flocculants are required for chemical flocculation [19, 209], and the associated high costs high costs of chemical flocculation led the ASP to conclude that chemical flocculation was too expensive for the production of biofuels [208]. Another disadvantage of chemical flocculation is that it incorporates the metal salts with the harvested biomass. Even if intended for use in biofuels, after the extraction of the active ingredients, the disposal of the residuals which contains the metal salts may present a disposal problem [19; 152].

Chemical flocculation (polyelectrolytes)

Polymeric organic flocculants (polyelectrolytes) are highly charged organic macromolecules or aggregates formed in aqueous solution by dissociation of charged units of these macromolecules. They operate in a similar fashion to inorganic flocculants by neutralizing the negative charges on algae cells. In addition to this, they also physically link the algae cells to each other through a process called bridging [222], thus producing more stable flocs. Only the cationic polyelectrolytes were found to be as efficient as flocculants [19; 208; 209; 222].

Lower levels of polyelectrolytes are required for flocculation in contrast to metallic flocculants [19; 152; 208] and this, together their reported lack of toxicity, has made them more attractive as flocculants as it eliminates the problems associated with the algae-flocculant mixture described above. The reported lack of toxicity may, however require further research as Heasmann et al. [95] reported significant mortality caused to Sidney rock oysters fed chitosan flocculated algae.

The flocculating capacity of polyelectrolytes is reduced in seawater (Figure 36) [19; 23; 152], as the cationic polymers fold tightly and fail to bridge microalgal cells at high ionic strengths. Despite this, flocculation has been reported in saline waters by several researchers although the optimal doses vary greatly and appear to be species dependent. The optimal flocculation of *Tetraselmis* chuii *Thalassiosira pseudonana* and *Isochrysis* sp. was obtained at a chitosan dosage of 40 $mg.L^{-1}$ [95; 96], 80 $mg.L^{-1}$ was required for *Chaetoceros calcitrans*, *Skeletonema costatum* and *Pavlova lutheri*, while *Chaetoceros muelleri* required 150 $mg.L^{-1}$ (Table 7). In addition to these, the optimal dose required for flocculation is also influenced by the chemical composition of the algal medium and the presence of dissolved organic material in the cultures [209].

During the course of the ASP, Sheehan et. al [208] reported that the higher costs of the polyelectrolytes meant that there was no observed difference in costs between inorganic and organic flocculants during large-scale trials. However, others have reported low costs for polyelectrolytes [9; 19; 96], attributed to improvements in commercial electrolyte processes and equipment [19]. Barclay et al. [9] estimated the cost of polymer needed to flocculate a ton of algae at $50.

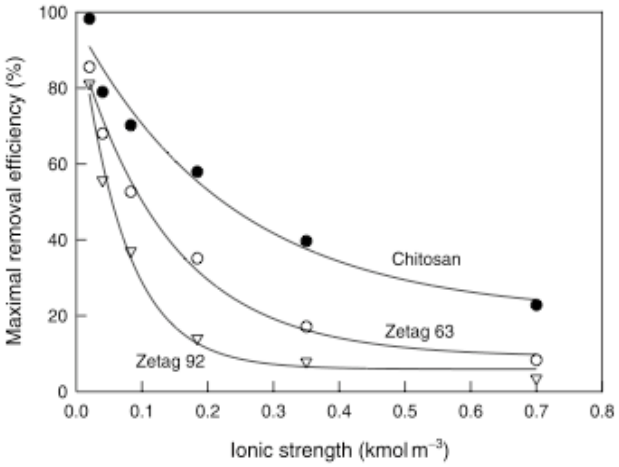


Figure 36: The effect of ionic strength on efficiency of cell removal by flocculation with commercial polymers. Polymers tested were the cationic chitosan, (400 kDA molar mass), Zetag 63 (a cationic polyacrylamide of 10,000 kDa molar mass), and Zetag 92 (a cationic polyacrylamide of 20,000 kDa (Bilanovic et al. [23]).

Table 7: Optimal chitosan dosage and associated flocculation data (Heasman et al. [95]).

Species	***Optimum chitosan dosage (mg.L^{-1})***	***Culture pH***	***pH after chitosan addition***	***Cell recovery (%)***
Chaetoceros muelleri	150	8.06	5.03	95
Chaetoceros calcitrans	80	7.29	5.27	80
Skeletoneman costatum	80	8.66	5.42	70
Thalassiosira pseudonana	40	8.29	6.31	90
Tetraselmis chuii	40	7.69	6.03	80
Pavlova lutheri	80	7.28	5.30	80
Tahitian Isochrysis	40	7.43	6.26	90

Bioflocculation

Some algae species naturally flocculate with no intervention [128; 162; 208; 242]. This normally occurs after transfer to settling ponds when the algae have been left quiescent for some

time. This self-flocculation (bioflocculation) has been used to successfully harvest microalgae biomass. When large filamentous *Scenedesmus quadricauda* was pumped into settling ponds, Weismann and Goebel [242] obtained harvesting efficiencies of over 90% after 6h. This was increased to 99% when the settlement time was increased to 9h. This bioflocculation is thought to be attributable to environmental stimuli some of which have been identified, including nitrogen limitation, pH and level of dissolved oxygen [19; 242]. Benemann and Oswald [19] reported on an effort to establish operating conditions under which *Microactinum sp.* cultures could be induced to bioflocculate in 0.1 ha ponds. However, relatively little was learned about the processes involved, and though they obtained high removal efficiencies, these were not reliable. The authors concluded that the processes were still unreliable and that the lack of understanding of the processes involved means that bioflocculation remains inconsistent and unreliable as a means for harvesting mass-produced cultures of microalgae. More recently, researchers have reported more consistent and rapid flocculation in pond reared microalgae when cultures in high energy culture were made quiescent. The formation of these aggregates has been attributed to aggregations between microalgae and bacteria [138], as well as formation of flocs around specific (undisclosed) strains of algae [182; 206].

pH changes

Sometimes, effective flocculation is achieved simply by changing the pH of the culture [26; 121]. Blanchemain and Grizeau [26] raised the pH of cultures of *S. costatum* to 10.2 and obtained flocculation and settling of the algae cultures. Similarly, Lee et al. [121], reported that a pH of 11, effectively gave better flocculating activity of *Botryococcus braunii* than the use of both metallic and organic flocculants (Table 8). In contrast, Levin et al. reported increased flocculation of *Chlorella sp.* at more acidic pH (4).

Table 8: Effect of pH values and flocculant concentrations on the flocculation of *Botryococcus braunii* (Lee et al. [121])

pH		**Aluminium sulphate**		**Pestan**	
Value	**Flocculating activity***	**Concentration ($mg.L^{-1}$)**	**Flocculating activity***	**Concentration ($mg.L^{-1}$)**	**Flocculating activity***
3	3.05	50	2.37	50	1.72
9	2.09	100	3.64	100	2.75
11	8.49	200	6.99	200	2.54
12	8.26	300	6.03	300	2.06

* Flocculating activity is a mean value of three measurements

The importance of pH regulation in increasing the efficiency of other flocculating agents is already well recognized [19; 23; 95; 152] and the use of pH manipulation by itself may overcome the limitation of toxic by-products being produced by the inorganic metal flocculants as it has no toxic residues. This simple approach is promising and may offer a low-cost method for initial harvesting of microalgae biomass. However, it must be understood that the effect of pH adjustment cannot be generally adopted for different algae species. While a medium

pH of 11 gave better flocculation of *B. braunii* compared to other organic and inorganic flocculants, at the same pH, when the trial alga was changed to *Chlorella sp.*, alum gave better flocculation than pH adjustment to 11 alone [121]. This variable flocculation potential obtained in different microalgae depends on the composition and the properties of the cell wall, extent and type of secretions, age, the presence of electrolytes in the media and other factors [7; 121; 209], which all need to be further investigated for a proper understanding and control of flocculation. In addition, the costs of adding alkali or CO_2 to large volumes of liquid in sufficient enough volumes to generate the required pH shift need to be properly evaluated. When this change in pH occurs naturally (caused by high algal productivities, leading to high pH values), it causes the precipitation of calcium and phosphate ions in the medium and flocculation of the algae (also called autoflocculation) [81; 209].

Magnetic Separation

Shelef et al. [209], reported on the use of high gradient magnetic filtration for the separation of algae. The method is based on the suspension of magnetic particles in the water (usually Fe_3O_4 magnetite). These magnetic particles were coagulated together with the algae and were then passed through a magnetic field focused on a porous screen which retained the magnetic flocs. Yadidia et al. cited by Shelef et al. [209], achieved algae removal over 90% with 5-13ppm FeCl as the flocculant and 500 -1200 ppm magnetite as magnetic seed for algae cultures. However, reliable cost estimates are not available for this technology.

Electroflocculation

Electroflocculation is a coagulation/flocculation process which provides an alternative technique for the removal of pollutants from wastewater [165; 198; 220]. It is based on the principle of the movement of electrically charged particles in an electric field in which active coagulant species are produced by oxidation of a metal anode.

The process involves the application of an electric current to sacrificial electrodes (usually aluminum or iron). The metal goes into solution at the anode, generating metal ions as coagulating agents, and hydrogen gas is released at the cathode. The reactions at the anode and cathode respectively are [165]:

Anode: $Fe(s) \ast Fe^{2+} + 2e^-$

$Fe^{2+} + 2OH^- \ast Fe(OH)_2$

Cathode: $2H_2O + 2e^- \ast 2OH + H_2$

In water treatment processes, the aluminum or iron coagulates with the pollutants and the production of hydrogen at the cathode produces bubbles which rise to the surface taking the flocs with them.

Electroflocculation has also been used in harvesting microalgae [96; 172; 220]. Since microalgae have a negative surface charge, they are attracted to the anode during electrolysis where they lose their negative charge and are then able to form aggregates (flocs) as described above. The flocs can be floated off as described above. Electroflocculation works irrespective of the size or morphology of the microalgae [172; 220], and a separation efficiency of over 96% has been reported for microalgae at a cost of 0.3 kWh/m^3 [172]. The harvesting efficiency results obtained have been mixed: while some report obtaining a thin layer of algal cells on the surface of their cultures, Heasmann et al. [96], working with seawater strains, reported a thick band of algal cells embedded within a matrix material that resulted

in a harvested product that was only 3-4 times more concentrated than the original product. It is likely that this can be remedied by the addition of polymers as can be seen from (**Figure 37**) where Tennant [220] obtained a thinner layer of algae cells following the addition of polymers.

Electroflocculation is a promising technology that offers a low-cost and efficient method of harvesting microalgae. This warrants further investigation to determine the optimal voltage required to obtain flocculation, duration of application, requirement for polymers and dose rates. A study was recently initiated to study these by a collaborative effort of the BC Ministry of Agriculture and Lands and the Alberta Research Council.

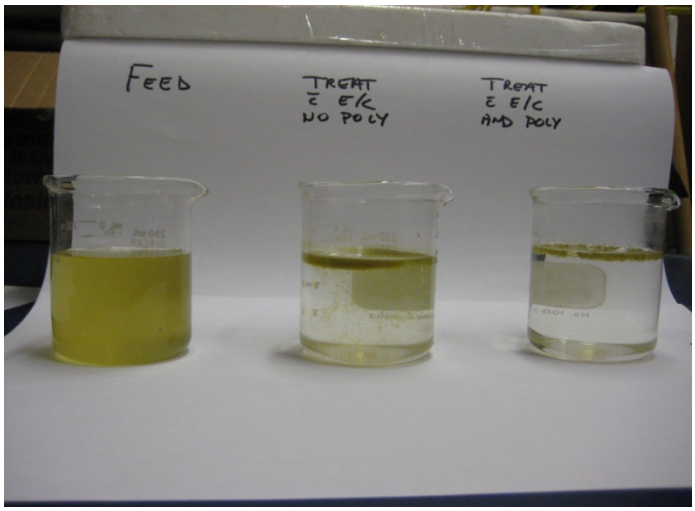


Figure 37: Flocculation of *Pavlova lutheri* by electro flocculation. Left: algae culture, middle: algae culture after electroflocculation, right: algae culture after flocculation with the addition of flocculation polymers. (Courtesy Bruce Tennant [220])

Ultrasound Separation

Bosma et al. [31] described a method of harvesting microalgae by the use of ultrasound induced flocculation, using high frequency, low sound amplitude waves which is then followed by sedimentation. The microalgae are pumped into a resonator chamber which consists of a transducer and a reflector. When the apparatus is turned on, it creates fields of maximum energy (bellies) and fields of minimum energy (nodes) (**Figure 38**). The microalgae are driven into the node planes, where they aggregate and settle when the field is nullified.

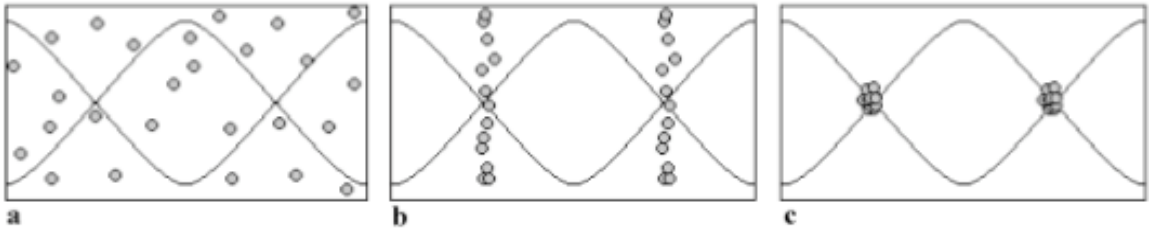


Figure 38: Principle of the ultrasound harvesting process. a) no ultrasonic field; b) Ultrasonic field has just been turned on and algae cells have migrated to the low pressure

node planes; c) the cells have aggregated into the knots of the ultrasonic field. (Bosma et al. [31])

Ultrasound separation has the advantage of maintaining the viability of the microalgae as it causes no shear, avoids mechanical failures as it has no moving parts and enables continuous operation. These authors reported harvesting efficiencies of up to 92% of *Monodus subterraneus*. as the maximum that could be obtained

However, scale up of the system is difficult as the resonator chamber needs to be cooled. The authors described a large system, which can handle only up to 1000L per day.

The energy costs for this method of harvesting are very high: neglecting the cost of cooling, Bosma et. al [31] estimated power costs of 345kW.day^{-1} for an 18L.d^{-1} unit.

Dissolved air floatation

After flocculation, the aggregated cells may be left to settle (sediment) (Figure 39), and are recovered by pumping off the surface liquid layer or the separation may be achieved by dissolved air floatation of the flocs [19; 27; 128] (Figure 40). This involves pressurizing some of the liquid in order to dissolve additional air. When mixed with the culture, at atmospheric pressure, the air comes out of solution as bubbles that attach to the flocs, making them float.

Dissolved air floatation was more effective in terms of solids concentration, yielding 5-8% solids vs. 2-5% in sedimentation [19; 128; 209], and required much less time for separation than sedimentation. The problem with initial designs was the complexity of the system required [19; 128]. However, Borodyanski and Konstantinov [27] obtained a patent for a simple froth separation device, which consisted of a telescoping chamber of adjustable height, into which the algae suspension is introduced at the base. Water with pressurized air is then introduced also at the base of the tank. The resulting bubbles give buoyancy to the flocs which are carried up in the froth and to an overflow at the top of the chamber. The movable telescopic design allows the height to be adjusted automatically to always maintain the position of the overflow to the height of the foam layer (Figure 41).

Figure 39: Harvesting of microalgae by sedimentation (Lundquist [139])

Figure 40: Harvesting of microalgae by flocculation – floatation (Lundquist [139])

Sedimentation

Sedimentation has also been described by some workers to harvest microalgae cells based on the tendency of microalgae to settle when the energy is withdrawn and they are made quiescent [19; 96; 206]. Sedimentation may or may not be coupled with flocculation. Heasman et al. [96] found no correlation between the sedimentation rates of seven different species of algae and their sizes, or dry cell weights (Table 9). These authors reported that even when enhanced by keeping the algae at lowered temperatures in the dark, this form of sedimentation was unsuitable for harvesting, requiring 3-4 days for stabilization of the sediments.

More often, sedimentation can also be used to harvest flocculated algae [81; 96; 209; 209], where the larger particle sizes of the flocs increases the settling velocities of the algae. Golueke and Oswald [81] had a harvesting efficiency of 85% following sedimentation of algae flocculated using alum.

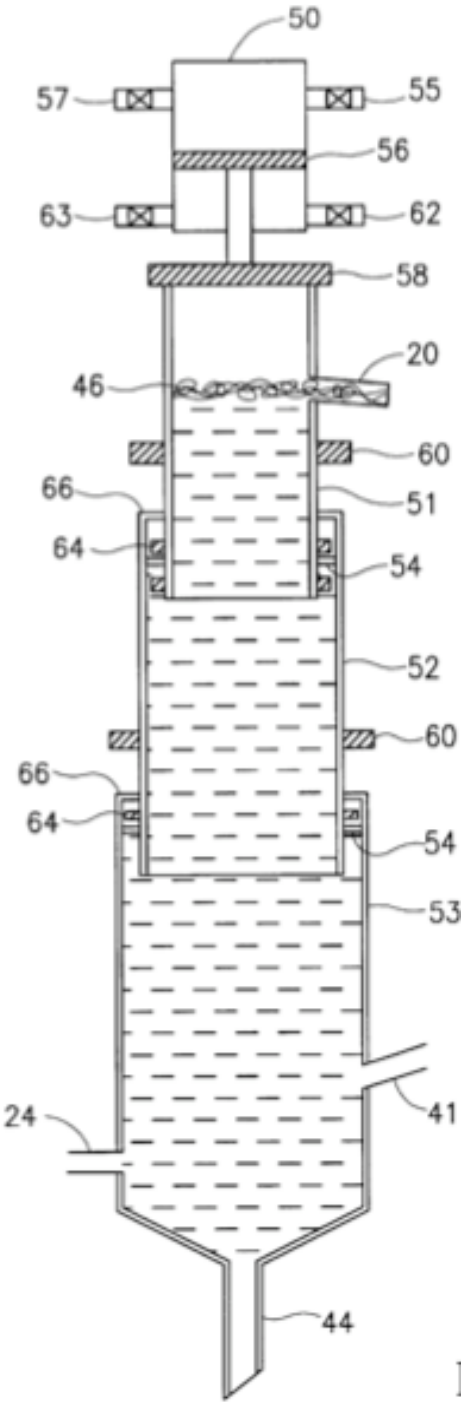


Figure 41: Microalgae separation apparatus by froth floatation (Borodyanski and Konstantinov [27]).

Key; 20=over flow outlet;

24=disperser for injection of pressurized water;

41=culture inlet;

44=outlet;

46=foam layer;

50=piston for adjusting column height;
51, 52, 53= stacked, concentric chamber rings;

54=rubber ring;

55=air valve for operating piston;
56=plunger;

57=air exit outlet;

60, 64=pressure projection rings; 62=air intake valve;

66=upper rim of second tube.

Table 9: Duration and efficiency of cell sedimentation for seven species of microalgae (Heasman et al. [96])

Species of microalgae	Cell dry weight* & (dimensions)	Initial density (cells.ml^{-1})	Time to harvest	Volume of settled slurry	Density of cells withing slurry (cells.ml^{-1})	Concentration factor	Estimated harvest efficiency
Tetraselmis chuii	96 (13.8 x 9.1)	0.20 x 10^6	95h	53ml	3.12 x 10^6	15.6x	83%
Skeletonema costatum small strain	11.5 ?	3.66 x 10^6	162h	55ml	55.75 x 10^6	15.2x	84%
Skeletonema costatum large strain	50 ?	0.29 x 10^6	18h	64ml	2.92 x 10^6	10.1x	645
Chaetoceros muellei	20 (7.4 x 5.4)	1.88 x 106	162h	119ml	3.76 x 10^6	2.0x	245
Chaetoceros calcitrans	15 (4.0 x 3.1)	8.25 x 10^6	162h	292ml	28.96 x 10^6	3.5x	1025
Pavlova lutheri	23 (6.9 x 4.9)	2.14 x 10^6	68h	61ml	25 x 10^6	11.7x	71%
Isochrysis sp. (Tahitian)	19 (8.5 x 5.40	4.44 x 10^6	92h	293ml	12.83 x 10^6	2.9x	85%

Lamellar settlers

Nakamura et al. [162] demonstrated the use of passive lamellar settlers in improving the natural settlement of microalgae. The lamellar settlers, widely used in wastewater applications, consist of a container with lamellae stacked at angles of 10° - 40° off the horizontal. It works by shortening the distance over which the particles need to travel before hitting a surface, thereby improving gravity settling, and inclining the lamellae causes the particles to slide to a collector. Lamellar settlers will eventually become fouled by particles that will not slide and may require frequent cleaning. While lamellar settlers improve the sedimentation of the algae, they are only useful if the cells have a specific gravity that is substantially heavier than the culture medium [162; 209].

Centrifugation

This is a well established industrial process with several companies producing commercial centrifugation units. Separation is obtained by the gravitational force attained by high centrifugal forces in rotating chambers. This can be described by Stokes' Law, which states that the settling velocity is proportional to the difference in density between the cell and the medium on the one hand, and the square of the radius of the cells.

The morphology and sizes of the cells being harvested also affect the costs and recovery as filamentous cells and large colonial cells will settle more readily than single smaller cells, thus requiring less residence time in the centrifuge. Nakamura et al. [162] investigated the influence of different cell sizes and morphologies on the flow rates that would permit recovery of 90% of the cells in a centrifuge. As can be seen from Figure 42, much higher volumes of liquid can be processed per unit time with the larger microalgae than the smaller ones. The effect that this would have on costs was determined by these authors who calculated that the harvesting costs in a large-scale facility would range from a low of 3.3% of the total costs for the larger strain AQ0015, to 17.6% of total costs for the smaller strain AQ0011.

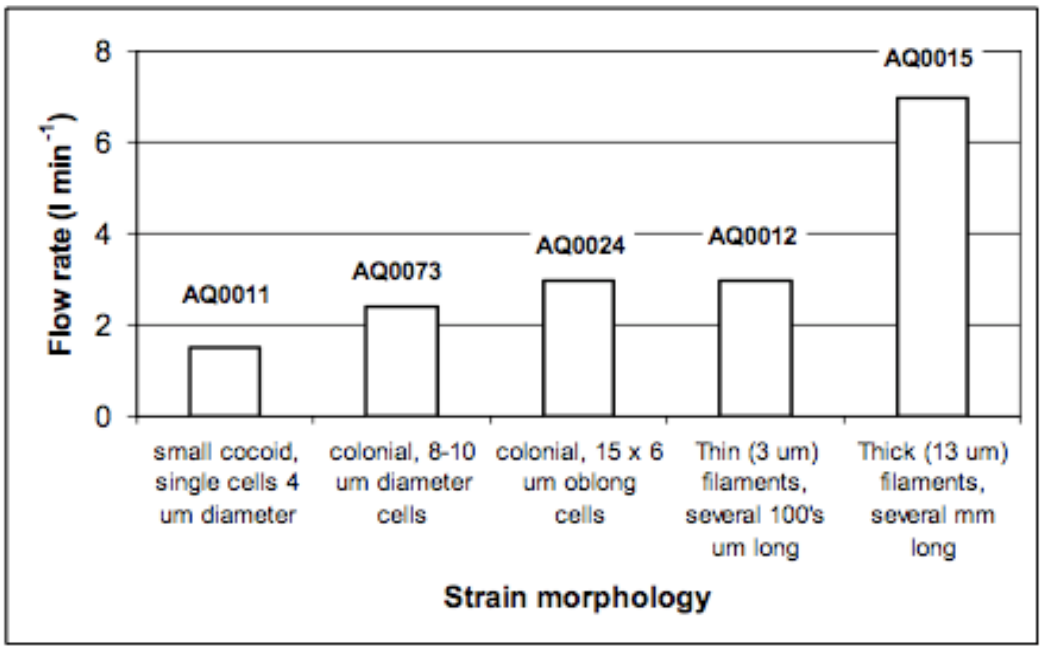


Figure 42: Maximum flow rates into the centrifuge that permit capture of 90% of the culture biomass (Nakamura et al. [162]).

Although centrifugation technology is well established, its use has to be optimized for the algae species being separated as design differences between different centrifuges affect performance in several critical aspects including harvest efficiency, operating costs and cell damage [96]. Heasmann et al. [95; 96], evaluated the performance of 9 species of microalgae in 3 different types of centrifuges and reported that cell viability differed significantly depending on species, method of centrifugation and interactions between the two. Damage to the cells ranged from 0% to 12% of the harvested biomass depending on type of centrifuge and species of algae. Similarly, harvesting efficiency also varied significantly depending on species, method of centrifugation and interactions between the two. (Detailed reviews of centrifuge types and classes have been undertaken by many researchers [95; 96; 152; 209]).

In fact, the failure of the first commercial attempt to culture *Dunaliella salina* in Israel in the 1970's was caused by using improperly sized centrifuges which were designed for yeast

cells. Despite the similarity in cell sizes, the lack of a cell wall meant that unacceptably high rates of lying occurred during harvesting, causing significant losses [19].

Recovery of cells by centrifugation can be rapid, but it is energy intensive. The energy required to harvest 1 ton of biomass at 333 mg.L^{-1} has been estimated at 3000 kWh [19; 81]. Molina Grima et al. [153] estimated the energy requirement for different types of centriofuge to range between 0.3 to 8kWh per m^3. The high costs of centrifugation led these authors to recommend the use of centrifugation as a secondary harvest method to concentrate an initial slurry (1-2% solids content) obtained by another method to a paste (15-20% solids content) as this could be done at more acceptable costs.

Filtration

Filtration involves introducing the particles onto a screen of given aperture sizes. The particles either pass through, or are retained on the screen according to their size. Harvesting by filtration often requires a secondary concentration step using filter presses or centrifuges. Three main technologies are highlighted here:

Pressure Filters

Filter presses operating under pressure and belt filters have been reported to operate satisfactorily for the recovery of large microalgae and have been reported in use in commercial *Spirulina* facilities [19; 205]. However, problems arise when small, unicellular cells are being filtered as the small sizes of these cells coupled with the presence of extracellular gelatinous material on cell surfaces cause clogging of the filters as well as breakthrough of the algae cells, resulting in poor performance [9]. This limits the usefulness of filtration for commercial-scale settling of biomass unless large or filamentous microalgae are grown [19; 152]. Low energy consumption requirements ranging from 0.2 to 0.88 kWh per m^3 have been reported for various types of pressure filters [153, 209].

Vacuum Filters

In vacuum filters, the driving force is provided by vacuum applied to the filtrate side of the medium. There are various types of vacuum filters (Reviewed in Shelef et al. [209]), and they come in large sizes for batch or truly continuous operation. Vacuum filters have high capital costs and similarly to the pressure filters, they are of limited effectiveness in the harvesting of small microalgae. Energy consumption ranging from 0.1 to 5.9 kWh per m^3 have been reported for vacuum filters [153, 209].

Microstrainers (microscreens)

Microstrainers consist of a rotating drum covered by a straining fabric. A backwash spray collects the particles into an axial trough. Large units are commercially available as they are commonly used in water purification units to remove very low concentrations of cyanobacteria in water supplies. These operate using a coarse (20-30 μm sized) screen to remove filamentous and mat forming algae.

Microstrainers have very low power requirements (0.02-0.2 kWh.m^3) [4] [209] and are relatively inexpensive to operate. Benemann et al. [20] estimated costs to be about 10% of

[4] Benemann, J., Koopman, B., Weissman, J. C., Eisenberg, D. M., & Oswald, W. J. (1977). Cultivation on sewage of microalgae harvestable by microstrainers. *Propared for Energy Research and Development Administration (Contract Nos. W-74-05-ENG-48 and E-(04-3)-34*

centrifugation costs. Wilde et al. [246] estimated costs for a large facility harvesting 10^6 $m^3.d^{-1}$ of liquid from a cooling reservoir containing very low algal concentrations (25 $mg.L^{-1}$) would be only $100-$150 per ton of dried algae. While promising for filamentous or mat forming microalgae, results from trials undertaken in the 1970's utilizing small unicellular microalgae were generally poor [19; 81; 209], limiting its use to strains of larger algae.

Conclusion

The expense associated with the large amounts of inorganic chemicals required for flocculation limits their usefulness in the harvesting of microalgae for biofuels. In contrast, the reduction in costs of organic polyelectrolytes have made them more cost competitive in the harvesting of microalgae and the reported lack of toxicity is also an added advantage over inorganic chemical flocculation.

Although no cost data have been generated, several authors have suggested that bioflocculation or autoflocculation are potentially the cheapest methods to induce flocculation in microalgae [19; 209]. However, the lack of consistency in the results limits their use on a large scale. More research is required to develop this technology into a reliable harvesting process.

Electroflocculation of the microalgae promises a rapid, efficient and cost-effective means of harvesting biomass. Despite this, metal from the sacrificial anode will be incorporated into the algae biomass and further research is required to quantify this effect. The apparent requirement for additional thickening of the floated flocs in seawater using polymers will also add to the costs of electroflocculation and should be the focus of further studies.

Although the costs associated with filtration are low, screen clogging and membrane fouling limits its suitability to larger species of microalgae. With the exception of large and filamentous algae, which can be recovered on filters, harvesting all other algae species will require centrifugation or filter presses as a secondary harvesting step after initial thickening of the algae suspension.

Centrifugation is one of the most reliable means of obtaining the biomass from the water. Although this method is reported to cause damage to the cells of some algae species, this is of little concern in the production of biofuels where the final products will be extracted anyway. Despite their usefulness as a universal harvesting technology, the high capital and running costs associated with centrifuges limit their use to second-stage filtration in the processing of biofuels.

Appendix I

Appendix I. Strain Selection

As different energy products can be obtained from microalgae, the desired end product should be the primary factor influencing the choice of species to be grown for biomass. For instance, a decision to produce bioethanol would require a species with a high amount of carbohydrate in the biomass and biodiesel would require high oil producing (oleaginous) microalgae. Also in the production of biogas, oleaginous algae are preferred as the conversion capacity of lipids into biogas is higher: 1390 L of biogas (composed of 72% CH_4, 28% CO_2) per kg dry organic matter, compared to proteins at 800 L biogas (composed of 60% CH_4, 40% CO_2) per kg dry organic matter and carbohydrates at 746 L biogas (composed of 50% CH_4, 50% CO_2) per kg dry organic matter [205]. Other factors that should be considered during strain selection include the growth rate and the optimal temperature range of the culture species in relation to the culture system to be used.

Conditions affecting lipid production

Under optimal growth conditions, the lipids in microalgae are mainly in the form of polyunsaturated membrane glycerolipids [72; 89]. However, under unfavourable conditions, cell division stops while the cells continue accumulating storage products at about the same rate [19; 208], resulting in the accumulation of neutral lipids (including hydrocarbons), mainly as triacylglycerols (TAGS). The interest in the triglycerides is due to their high specific calorific value of 9.3 kcal.g^{-1} as opposed to protein and carbohydrate with 5.1 kcal.g^{-1} and 4.5 kcal.g^{-1}, respectively. As described in the main report, the different microalgae accumulate different storage products in response to stress however, and this can be adapted to generate whatever storage product is of interest.

The fatty acid composition of microalgae is species specific (see Appendix C for fatty acid composition of several cultured microalgae), and this composition varies depending upon various factors, including nutrient starvation [10; 108; 111; 112; 146; 184; 210; 219], salinity changes, [5; 6; 58; 183; 185], pH changes [58], temperature [1; 6; 32; 58; 140; 187; 219], light intensity [34; 58; 73; 108; 163; 213; 215; 219] as well as the growth phase and age of the culture [22; 34; 59; 143].

The ASP programme of the US Department of Energy undertook a lot of research into the induction of lipids by nutrient manipulation and concluded that nutrient deficiency appears to be the most effective means for inducing changes in the lipid content and composition of the algae [208]. A major finding of their work was that while nutrient starvation does increase the lipid content of microalgae, it is also correlated with a decrease in the total cell and lipid productivity of the culture (Figure 43), both of which are important in the production of lipid based microalgae biofuels. Indeed, this has been recognized since the 1950's as Burlew [36] reported that the production of lipids would be most economical using nitrogen-sufficient rather than nitrogen-starved cells as the lower productivities obtained in the latter case, resulted in severely reduced total lipid productivities.

Given that there is no advantage to be gained by nutrient starvation of a culture, a two-phased approach has been suggested whereby the microalgae are grown under non-limiting conditions in a first phase, followed by culture under nitrogen-limiting (not starvation) conditions in the second phase [19]. This is because in a nutrient limited culture, the microalgae are growing with a constant but insufficient input of the limiting nutrient. This has the effect of imposing a growth rate limit on the microalgae as the intracellular levels of the limiting nutrient will determine the growth rate. In contrast, in a nutrient starved

culture (such as a batch culture), the microalgae are deficient in the limiting nutrient. Unless they receive a fresh supply, any cell growth that occurs, occurs at the expense of the limiting nutrients stored in the cell and declines as the stored levels of the limiting nutrients decline resulting in the reduced growth rates and productivities described above.

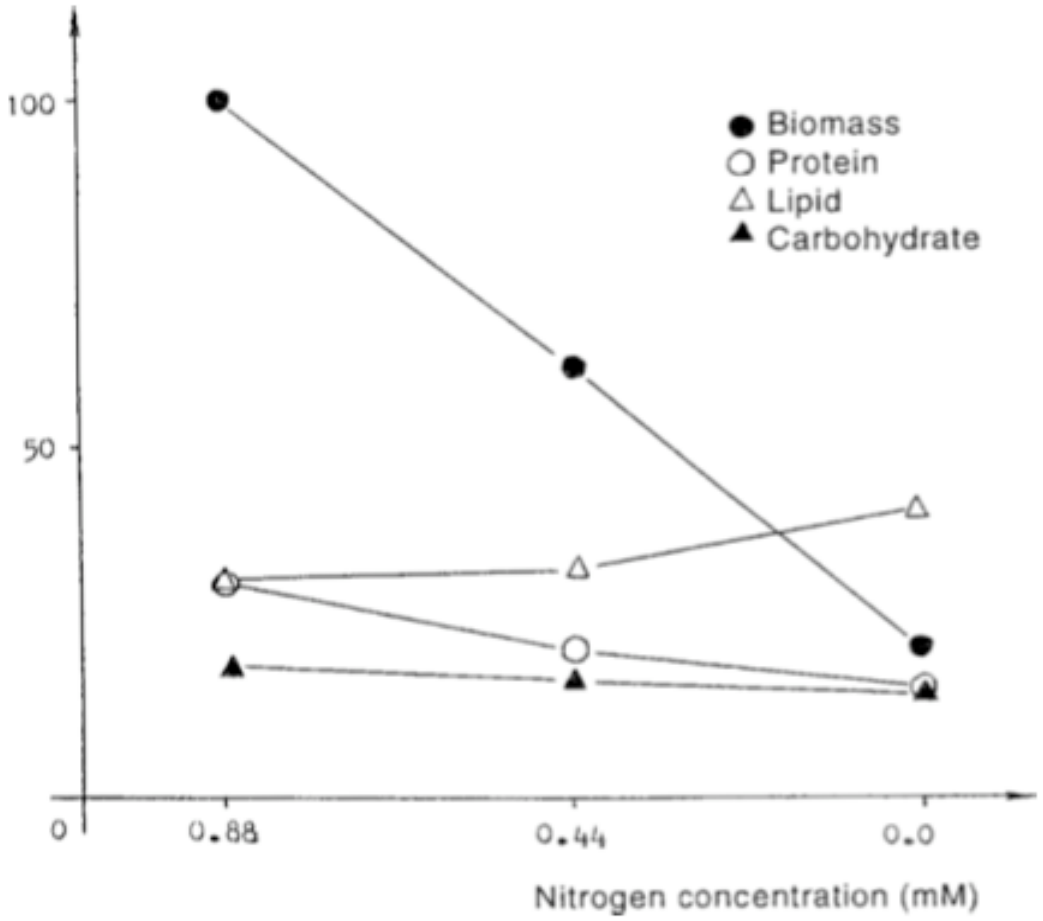


Figure 43: Changes in composition of lipid, protein and carbohydrate with the biomass of *Phaedactylum tricornutum* under different nitrogen concentrations after 10 d of growth. (Kaixian and Borowitzka [108])

Culture in nitrogen-limiting conditions will enable the microalgae to accumulate nutrients without the resultant decrease in biomass that nitrogen-deficient cultures will face (see Figure 43). To accomplish this, a good strategy may be to grow the microalgae first using nitrogen-sufficient nutrients followed by a later phase where the nitrogen content (or any other limiting factor) in the nutrients added is reduced. The level of nitrogen limitation and the nutrient addition rates required to achieve the desired increased lipid levels will need to be determined for each species and growth system.

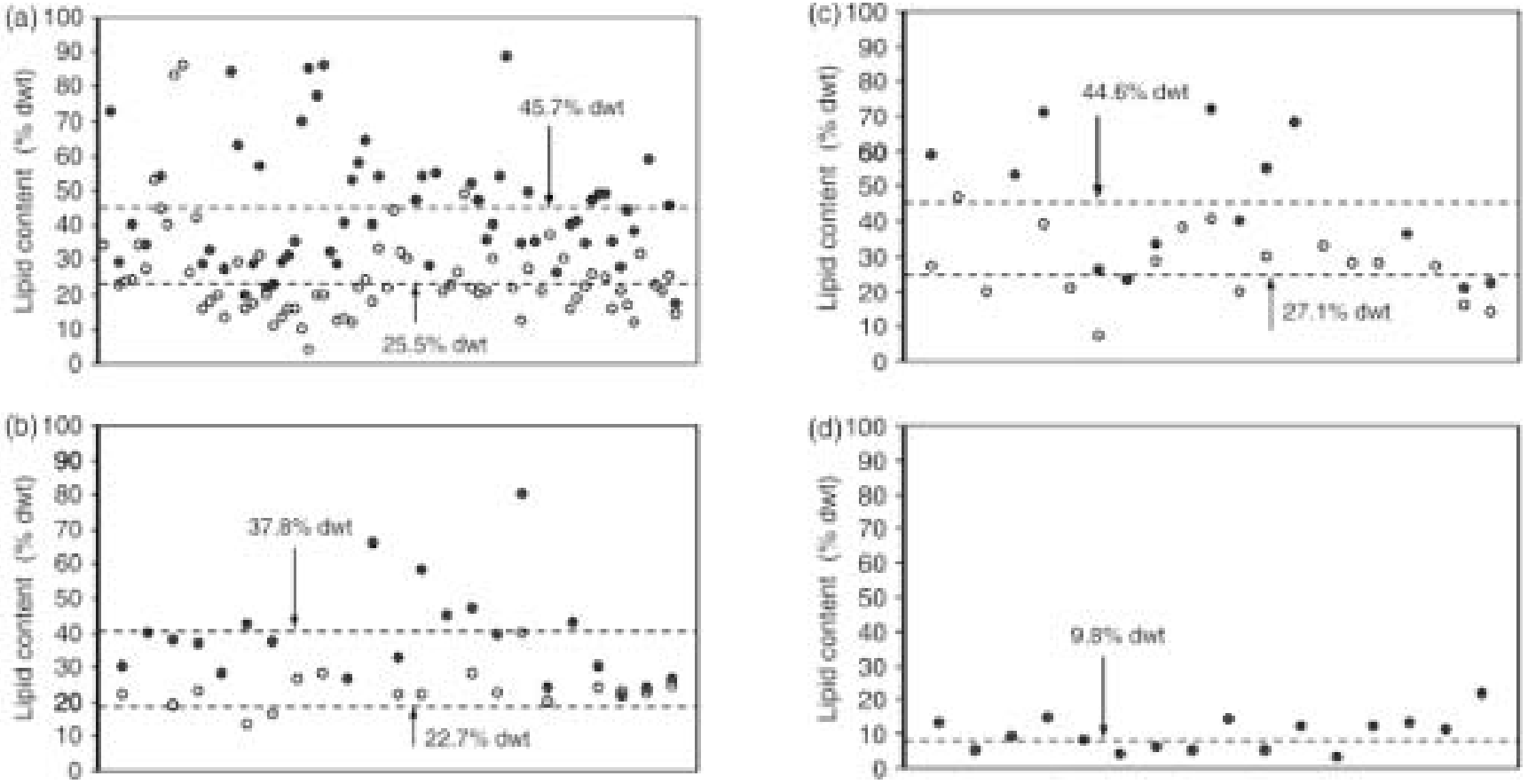


Figure 44: Cellular lipid content in various classes of microalgae and cyanobacteria under normal growth (○) and stress conditions (●). (a) Green microalgae; (b) diatoms; (c) oleaginous species/ strains (chrysophytes, haptophytes, eustigmatophytes, dinophytes, xanthophytes or rhodophytes). (d) cyanobacteria. The differences in cellular lipid content between cultures under normal growth and stress growth conditions were statistically significant for all three groups (a,b and c) of algae examined. (Schenk, et al. [205]).

Table 10: Proximate composition and generation times of some phototrophically cultured microalgae.

Strain	*Generation time (Hrs)*	*Optimal culture temperature (°C)*	*Protein (% dry weight)*	*Carbohydrate(% dry weight)*	*Lipids(% dry weight)*	*References*
Chlorella vulgaris	8.7[1]	22-26	51-58	12-17	14-22	Becker [(13)]
Chlorella pyrenoidosa	-	22-26	57	26	2	Becker [(13)]
Spirulina sp.	28.7[2]	18-22	61.4	3	8.5	Shimamatsu [(211)]
Arthrospira maxima	24.3[3]	18-22	60-71	13-16	6.0-7	Becker [(13)]
P. tricornutum	16.5[4]	18-22	35.2	32	13.3	Rivero-Rodriguez et al. [(197)]
I. galbana	8.7[5]	22-26	37	11.7	7.1	Ben-Amotz et al. [(18)]
Anabena cylindrica	-	18-22	43-56	25-30	4.0-7.0	Becker [(13)]
Tetraselmis suecica	11.3[6]	22-26	41-44	10.-13	30-32	Chinni Zitelli et al. [(48)]
C. calcitrans	9.3[7]	17-27	40.5	11.4	11.4	Rivero-Rodriguez et al. [(197)]
Euglena gracilis	11.6[8]	12-16	39-61	14-18	14-20	Becker [(13)]
Porphyridium cruentum	34.7[9]		28-39	40-57	9.- 14	Becker [(13)]
Scenedesmus sp.	13.9[10]	20-26	50-56	10.-17	12.-14	Becker [(13)]

Botryococcus braunii*	13.3[11]	18-22	22	14.1	44.5	Ben-Amotz et al. [(18)]
Ankistrodesmus sp.	8.3[11]	25	31.1	10.8	24.5	Ben-Amotz et al. [(18)]
Dunaliella salina	6.7[11]	22-26	29.3	16.3	25.3	Ben-Amotz et al. [(18)]
Nannochloris sp.	6.7[11]	25	33.1	13.2	20.8	Ben-Amotz et al. [(18)]
Chlamydomonas rheinhardii	-	18-22	48	17	21	Becker [(13)]
Aphnizomenon flos-aquae	-	12-16	43- 56	25-30	4.0-7.0	Becker [(13)]
Spirogyra sp.	-	25	6.0-20	33-64	11.0-21	Becker [(13)]
Synechococcus sp.	27.7[12]	22-26	63	15	11	Becker [(13)]
Thalassiosira pseudonana	8.8[13]	11-16	46	25	21	Brown et al. [(34)]

Note generation times calculated from: 1= Degen et al. [(64)]; 2=Oliviera et al. [(168)]; 3=Tredici and Zitelli [(228)]; 4=Caretto and Cattogio [(38)]; 5=Hu and Richmond [(99)]; 6=Chinni Zitelli et al. [(48)]; 7=Krichnavaruk et al. [(115)]; 8=Chae et al. [(41)]; 9=Csogor et al. [(61)]; 10=Nedbal et al. [(164)]; 11=Ben-Amotz et al. [(18)]; 12=Gilbert and Ray [(78)]; 13=Brown et al. [(34)]; *= green, active colonial stage. Note: Growth temperatures obtained from the Provasoli Guillard National Centre for Marine Phytoplankton. Strain temperatures may vary.

Appendix J

Appendix J. Maximum Productivity Model

For a realistic estimate of the maximum theoretical production in BC, an immaturity algae growth model (AlgaeG) was developed based on modifications of the basic theories described by Livansky [132]. To calculate maximal expected productivities in BC, the following assumptions were made:

- Ponds are very shallow so the water temperature approximates the air temperature. This model does not consider conditions of frozen water.
- CO_2, nutrients, mixing and all other factors (except for temperature and solar radiation) are at optimal levels for algae growth.
- There is no contamination or crashes of the algae cultures.
- Radiation and temperature are the only environmental conditions affecting or determining the growth of algae.
- Not all of the incoming photosynthetically active radiation (PAR) is intercepted by the algae, and the intercepted PAR corresponding to PAR_m is completely used for algae growth and metabolism.

This model is a daily algae growth model. It includes the following calculations:

The intercepted PAR_m is determined from daily solar radiation, the density and depth of algae in water, and the algae's extinction coefficient:

$$tPAR = 0.45(1-\alpha)R_a$$

$$PAR_m = tPAR(1-\exp(-\varepsilon X z))$$

where R_a is the daily solar radiation (W.m^{-2}), α is the reflection rate, *tPAR* is the total photosynthetically active radiation (W.m^{-2}), ε is the extinction coefficient (l.gm^{-1}), *X* is the algae concentration (g L^{-1}), z is the depth of algae in water (m) and PAR_m is the intercepted *PAR*.

The calculation of gross primary production, GPP, from intercepted PAR, activation energy of algae growth and temperature, is calculated using the formula

$$GPP = 0.03125hK\ln\left(\frac{PAR_m+(PAR_m^2+K^2)^{0.5}}{K}\right)$$

$$K = K_i \exp\left(\frac{E_l}{R}\left(0.003406+\frac{1}{T}\right)\right)$$

where *K* is the light saturation constant at actual temperature (W m^{-2}), *h* is the sunshine duration (h), *T* is the temperature (^{o}K), *R* is the gas constant (J mol^{-1} K^{-1}), E_l is algae photosynthesis activation energy (KJ mol^{-1}), and K_i is the value of K at 20 ^{o}C, and *GPP* the gross primary production (g m^{-2}).

The calculation of dark respiration based on algae's respiration activation energy and temperature,

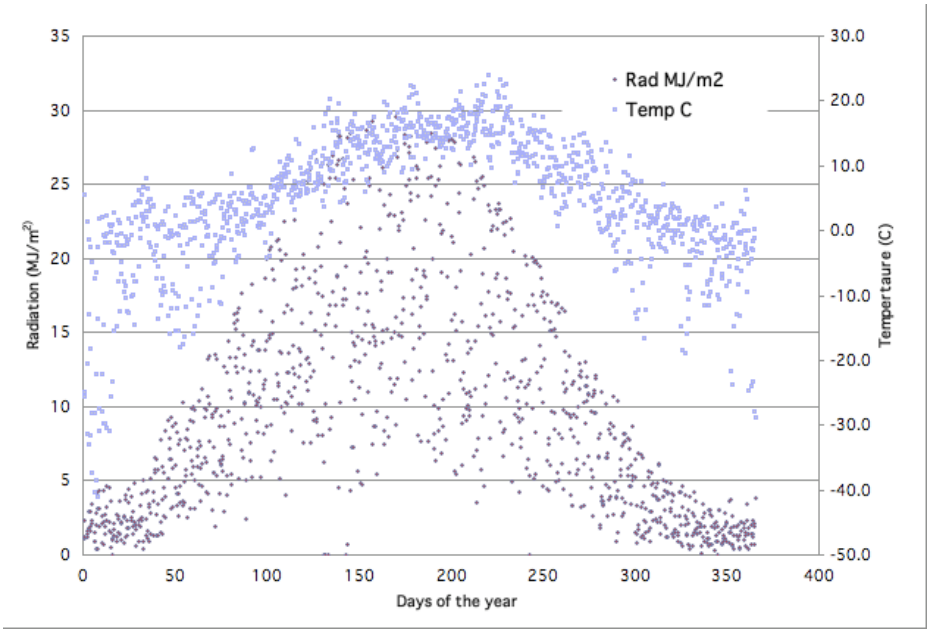
$$RESP = NPP_t U_i \exp(\frac{E_d}{R}(0.003406 + \frac{1}{T}))(24 - h)$$

where E_d is the algae activation energy in dark period (KJ mol^{-1}), u_i is the specific rate of respiration of algae in the dark period at 20 ^{o}C, and NPP_t is the accumulative net primary production until a given day (g m^{-2}), and *RESP* is the dry matter loss through dark respiration (g m^{-2}).

The model also included management sub-routines to determine harvest dates for maximum biomass productivity avoiding respiratory losses as well as pre-defined harvesting scenarios. The model was run using *Chlorella sp.* as a test organism at two different geographic locations in BC:

- Prince George: middle interior BC: Latitude: 53° 53.4'N; Longitude: 122° 40.8'W).
- Nanaimo: south BC: Latitude: 49° 3.000'N; Longitude: 123° 52.200'N).

Figure 45 shows the daily solar radiation and temperature input data for the model for Prince George between 1991 and 1993 for an unheated pond[5], and Figure 46 shows some model predictions calculated by the model based on the input data.



[5] Graph is a scatter plot with each point showing a daily value. For the years, 1991-1993, Each data point represented a different day in those three years and three points representing any one day. For instance a generic December 25th, has three separate points corresponding to the solar radiation values on December 25th, 1991, December 25th, 1992 and December 25th, 1993 respectively.

Figure 45 Daily solar radiation (MJ.m^{-2}) and average temperatures for Prince George from 1991 to 1993, used to model productivity of *Chlorella sp* in an open pond.

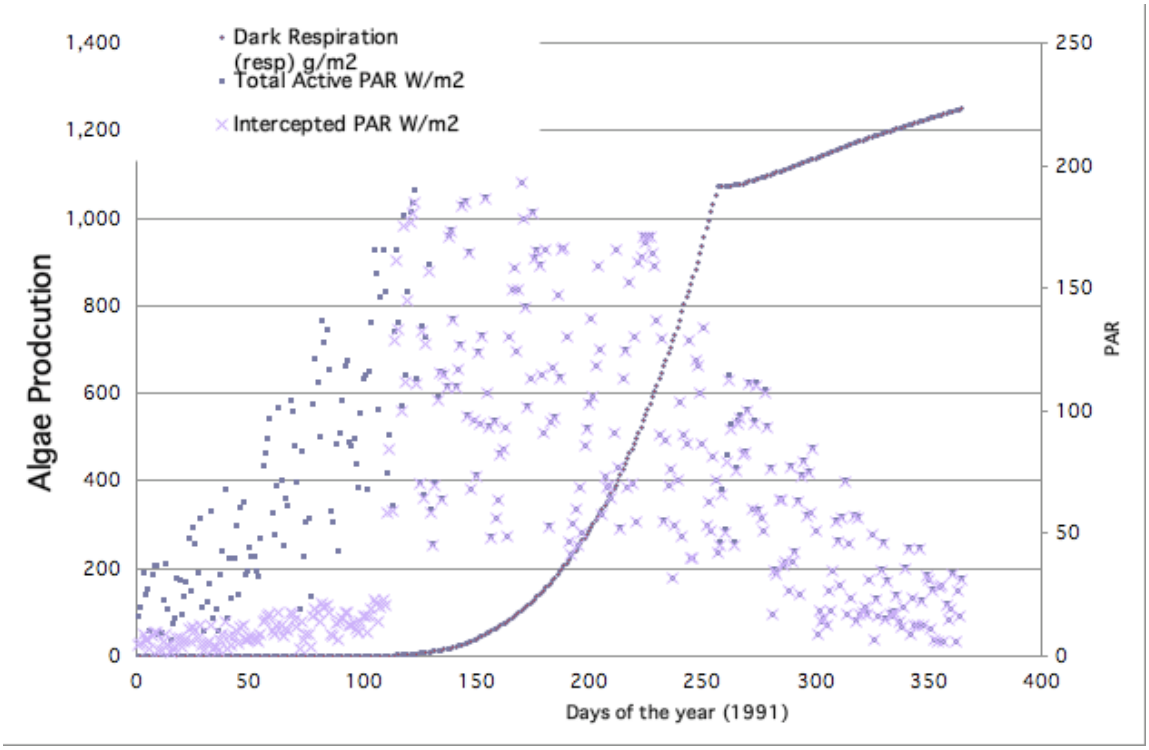


Figure 46: Model predictions for dark respiration, total active PAR and intercepted PAR for Prince George in 1991 (unheated)

Appendix K

Appendix K. Heat Transfer Model For Open Ponds

To understand the heating requirements for year round and/or seasonal operations with night heating, a heat transfer model was generated for open raceways. The heating would be provided by co-locating a large scale biomass plant with the algae plant as described in the next section.

Because the pond depth is much smaller than the width and length, the heat transfer can be assumed to be one-dimensional, occurring in the depth axis of the pond. The pond will gain heat via solar radiation during the day but as open ponds are subject to evaporative cooling, this component is ignored. Times when the air temperature is warmer than the water are also ignored as the analysis focuses on the maximum heat loss to understand if the reject heat of a typical biomass mass of 30 MWe that are prevalent in BC can heat up the ponds year round or at least at night, if run only during summers.

The maximum heat loss can be calculated by disregarding heat to the pond and adding the heat loss due to radiation to the sky, water evaporation, convective heat transfer caused by wind and via the bottom of the pond through the mud layer where:

$$Q_{\max} = Q_{rad,sky} + Q_{evap} + Q_{conv} + Q_{floor}$$

The heat loss by radiation to the sky is given by:

$$Q_{rad,sky} = A_w \varepsilon \sigma (T_w^4 - T_{sky}^4)$$

where A_w is the surface area of the pond, T_w is the pond water temperature, T_{sky} is the equivalent sky temperature, ε is the emissivity of the water taken as 0.8 and σ is the Stefan Boltzmann constant. Using the formulation of Livansky [132], the heat of evaporation can be calculated as:

$$Q_{evap} = A_w (0.2253 + 0.24644 V_{wind})(P_w - \phi P_a)^{0.82}$$

where V_{wind} is the wind velocity taken from historical data, P_w and P_a are the saturation vapor pressures of the water and air at their corresponding temperatures. The main heat loss is due to the convection at the air-water interface and is calculated as:

$$Q_{conv} = A_w h (T_a - T_w)$$

where h is the convective heat transfer coefficient given by [217]:

$$h = 0.075 \left(\frac{k_a}{L}\right)\left(\frac{V_{wind} L}{\nu_a}\right)^{0.8} \mathrm{Pr}_a^{\,0.33}$$

The heat loss via the bottom floor of the pond is quite small in comparison to the convection heat loss at the water-air interface [62] and this term is approximated by assuming a temperature gradient of 0.2°C and a force convection coefficient due to the mixing of 120 $W.m^{-2}K$, resulting in:

$$Q_{floor} = A_w h_{mud} (T_{floor} - T_w)$$

Results for the heat loss are shown in Table 11 and Figure 47, and were used as inputs in the next section. The table shows that convective heats transfer dominates the heat loss which can be significant during winter months, requiring over 1 kW.m^{-2}. During the summer months this value decreases below 0.1 kW.m^{-2}. The table also shows the results when the pond is designed to reduce the wind speed by 75%, which brings down the heat requirement from 556.0W.m^{-2} to 183.4W.m^{-2} during the span of a year.

Table 11: Heat loss for raceway pond located in Prince George. Covered wind is for the case where the pond is designed to slow down the wind by 75%.

Prince George									
Month	Air Temp Avg (C)	Rel Humid (%)	Daily Solar Rad Hor (kW/m^{2}d)	Wind covered (m/s)	Wind (m/s)	Earth Temp (C)	Heating Deg-Days	Twat (C)	Pwat (Pa)
Jan	-9.9	77.5	0.7	0.8	3.1	-10.4	865.0	18.0	9.51
Feb	-5.4	74.0	1.4	0.8	3.1	-8.8	655.0	18.0	9.51
Mar	-0.7	67.0	2.8	0.8	3.1	-4.8	580.0	18.0	9.51
Apr	4.7	59.0	4.3	0.8	3.1	0.5	399.0	18.0	9.51
May	9.4	59.5	5.1	0.7	2.8	7.1	267.0	18.0	9.51
Jun	13.1	62.5	5.9	0.6	2.5	11.8	147.0	18.0	9.51
Jul	15.3	65.5	5.6	0.6	2.2	14.0	84.0	18.0	9.51
Aug	14.6	68.5	4.8	0.6	2.2	13.2	105.0	18.0	9.51
Sep	9.8	72.5	3.1	0.6	2.5	7.2	246.0	18.0	9.51
Oct	4.8	73.0	1.7	0.8	3.3	1.2	409.0	18.0	9.51
Nov	-3.1	80.0	0.8	0.8	3.3	-5.5	633.0	18.0	9.51
Dec	-8.4	81.0	0.5	0.8	3.1	-10.0	818.0	18.0	9.51
Annual	3.7	70.0	3.1	0.7	2.9	1.4	5208.0	18.0	9.51
Month	Pair (Pa)	Qevap (W/m^2)	M Water (kg/month)	Q conv (W/m2) covered	Q conv (W/m2)	Q rad (W/m2)	Q soil (W/m2)	Q tot covered(W/m2)	Q tot (W/m2)
Jan	7.35	2.96	3.51	362.6	1099.2	129.5	18.0	513	1250
Feb	7.74	2.95	3.49	304.1	921.9	111.2	18.0	436	1054
Mar	8.12	3.13	3.71	243.0	736.7	91.1	18.0	355	849
Apr	8.54	3.38	4.00	172.9	524.0	66.6	18.0	261	612
May	8.90	2.98	3.53	103.0	312.3	44.1	18.0	168	377
Jun	9.17	2.51	2.97	53.6	162.5	25.6	18.0	100	209
Jul	9.32	2.10	2.48	26.7	80.9	14.3	18.0	61	115
Aug	9.27	1.97	2.34	33.6	101.8	17.9	18.0	71	140
Sep	8.93	2.09	2.48	89.7	272.0	42.2	18.0	152	334
Oct	8.55	2.74	3.25	180.4	546.7	66.1	18.0	267	634
Nov	7.93	2.67	3.17	288.3	873.9	101.5	18.0	410	996
Dec	7.48	2.73	3.24	343.1	1040.1	123.5	18.0	487	1184
Annual	8.44	2.68	3.18	183.42	556.0	69.47	18.00	273.57	646

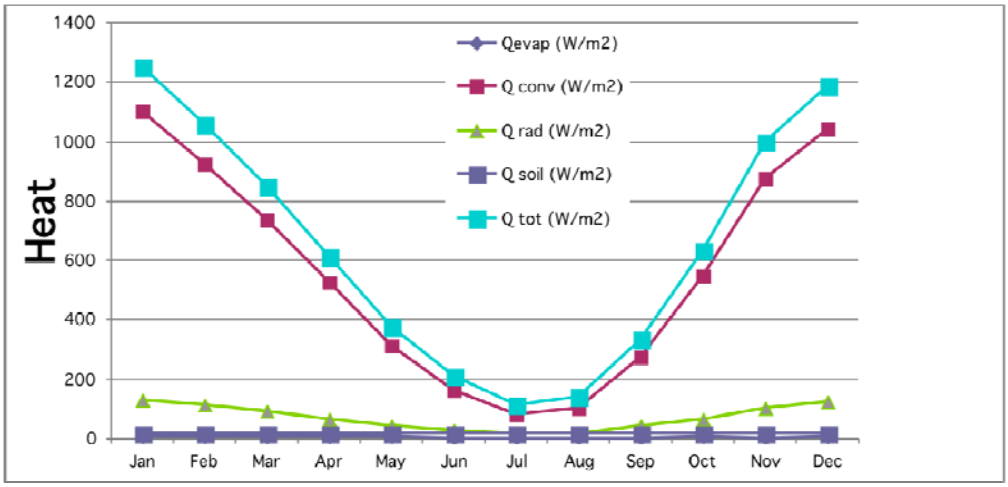


Figure 47: Heat loss for open pond for Prince George conditions

Biomass Power Plant

A biomass power plant model was developed to calculate the heat that could be theoretical extracted from (1) the flue gas containing sensible heat and latent heat from the fuel moisture and the fuel hydrogen that formed water during the thermal conversion process and from (2) the condenser used as part of the steam generation cycles that is used to condense the steam back to water. The analysis represents the theoretical limit based on a 4 km^2 algae plant. The heat transfer model was used to predict the heat loss for the Prince George area.

Biomass equations used in the model are shown in Figure 49. The biomass model takes into account the ultimate composition for the biomass fuel, the moisture in the air, the moisture in the biomass fuel, the higher heating value of the fuel, and the heat loss from the biomass plant to the environment. From the enthalpy of formation for the final combustion products and the flue gas temperature before the heat transfer tube is calculated using a thermodynamic equilibrium model.

Results are shown in Figure 48. It can be shown that assuming a maximum heat loss of 500 W/m^2, the biomass power plant cannot heat the pond even if we recuperate all the sensible heat and heat of formation heat as it exceeds the heat available by more than 3000%. This plant would produce over 4 million liters/year of biodiesel at 15% lipid content but it is clear that either a support structure needs to be added to cover the pond to reduce convection heat loss, or bioreactors are used instead to reduce heat loss or open ponds are used but they only operate for 6 months of the year and use heat energy to compensate for the heat loss during the night.

		Weight		Kmole/kg_{feed}	Volume
Feed Analysis	$Kg.mole^{-1}$	(dry)	(wet)	0.0705	100%
Carbon C ⇒	12	50.00%	25.00%	0.0208	29.54%
Hydrogen H_2 ⇒	2	6.00%	3.00%	0.0150	21.275
Oxygen O_2 ⇒	32	42.00%	21.00%	0.0066	9.30%
Nitrogen N_2 ⇒	28	2.00%	1.00%	0.0004	0.51%
Water H_2O ⇒	18	0.0%	50.00%	0.0278	39.38%
		14.178	**kg/kmole $_{feed}$**		

Biomass Combustor Analysis

Inputs		
Equipment Heat Losses	3%	%
Excess Air Supplied, a_{excess}	40%	%
T wood start, T_w	25	°C
Air temperature inlet, T_i	20	°C
T flue gas exit, T_e	250	°C
Relative humidity, φ_a	80%	%
Cycle eff	44%	
Overall eff	28%	

Outputs		
T flue gas exit, T_e	250 °C	
Flue gas max temperature, T_{max}	1196 °C	
Combust Energy, E_{HHV}	87998 KW	
Hydrogen Exhaust Loss, L_{LHV}	5232 KW	5.9%
Hog Water Exhaust Loss, L_{HW}	9689 KW	11.0%
General Equipment Losses, L_{eq}	2192 KW	2.5%
General Exhaust Losses, L_{exh}	14122 KW	16.0%
Total Losses, L_C	31235 KW	35.5%
Combustor Recovery, E_C	56763 KW	64.5%

Biomass Species	Moisture As Used	As used Tonne	Biomass (Dry tonne) (per year)	HHV kJ/kg Dry	Composition						
					C	H	O	S	Cl	N	P
Type	0.5	270,000	135,000	20,500	0.50	0.06	0.42	0.00	0.00	0.02	0.00

Algae Plant									
CO2 plant	7.87 kg/s	0.32	kg/s/MWe	Growth algae	16.00 g/m2/day	% CO2 used	17%		
MWe plant	25 MWe	1.00	MWe/MWe	Area algae	4.00 km2	CO2 algae	1.36 kg/s		
MWth condenser plant	32 MWth	1.27	MWth/MWe	Algae mass	0.74 kg/s	% heat used	3288%		
MW flue gas plant	29 MWth	1.16	MWth/MWe	Open race heat loss	500 W/m2	allowed heat	15.21 W/m2		
MW for heating	61 MWth	2.44	MWth/MWe	Max heat required	2000 MW	production BD	4.12 million l		

Figure 48: Input for biomass power production plant collated with algae plant

$$L_{Comb} = L_{LHV} + L_{HW} + L_{eq} + L_{eh}$$

$$\dot{E}_{LHV} = \dot{E}_{HHV} - \dot{E}_{LHV}$$

$$\%\dot{E}_{Comb} = \frac{\dot{E}_{HHV} - \dot{E}_{Conmb}}{\dot{E}_{HHV}}$$

$$\%L_X = \frac{\dot{E}_{HHV} - \dot{E}_X}{\dot{E}_{HHV}}$$

$$\eta_{Comb} = \frac{\dot{E}_{rec}}{\dot{E}_{HHV}}$$

$$\dot{m}_{f,yr} = \frac{\dot{m}_{f,yr}}{\left(\frac{weeks}{year}\right)\left(\frac{hours}{week}\right)\left(\frac{sec\ onds}{hour}\right)} \quad \left[\frac{kg}{s}\right]$$

$$P_g = P_{sat\ at\ T_i}$$

$$P_v = P_g \cdot \phi_a$$

$$\omega_a = \frac{0.622\ P_v}{P_{atm} - P_v}$$

$$\dot{E}_{HHV} = HHV_{dry} \cdot \dot{m}_{f,dry}$$

$$\dot{E}_{LHV} = LHV_{dry}\,\dot{m}_{f,dry}$$

$$\dot{E}_{A,HW} = \dot{E}_{LHV} - (mc)\dot{m}_{f,wet}\,h_{fg}$$

$$(mf)_{CO_2} = \frac{\dot{m}_{CO_2}}{\dot{m}_{total,\ flue\ gas}}$$

$$y_{CO_2} = \frac{N_{CO_2}}{N_{t,FG}}$$

$$\dot{E}_{A,eq} = \dot{E}_{A,HW}\left(1 - L_{eq}\right)$$

$$N_{CO_2} = \frac{\dot{m}_{CO_2}}{M_{CO_2}}$$

$$\dot{m}_{f,dry} = \dot{m}_{f,AR}\left(1 - \omega_{f,AR}\right)$$

$$P_{CO_2} = y_{CO_2} \cdot P_{atm}$$

$$\dot{m}_{f,wet} = \frac{\dot{m}_{f,dry}}{\left(1 - \omega_{f,wet}\right)}$$

$$LHV_{dry} = HHV_{dry} - M_H \cdot (mf)_H \cdot h_{fg}$$

$$c_{p,CO_2} = \frac{16.2 - 6530 \Big/ T_{m,\ flue\ gas} + 1410000 \Big/ \left(T_{m,\ flue\ gas}\right)^2}{M_{CO_2}} \cdot \left(\frac{Btu}{lbm \cdot °F}\right) \cdot \left(\frac{1.0551\ KJ}{Btu} \cdot \frac{2.2046\ lbm}{kg} \cdot \frac{1.8°F}{°C}\right) \quad \left[\frac{KJ}{kg \cdot °C}\right]$$

$$\left(\frac{\dot{m}_{a,dry}}{\dot{m}_{f,dry}}\right)_{dry,s} = \frac{(mf)_C\left(\frac{M_{O_2}}{M_C}\right) + (mf)_H\left(\frac{M_O}{M_{H_2}}\right) - (mf)_O}{0.232}$$

$$\dot{V}_t = \frac{\dot{m}_t}{\rho_{FG}}$$

$$\dot{m}_{a,dry,s} = \dot{m}_{f,dry} \cdot \left(\frac{\dot{m}_{a,dry}}{\dot{m}_{f,dry}}\right)_{dry,s}$$

$$(mf)_{C,wet} = (mf)_{C,dry}\left(1 - mc\right)$$

$$\dot{m}_{a,t} = \dot{m}_{a,dry}\left(1 + Excess\ \ Air\right)\left(1 + \omega_a\right)$$

$$T_{max} = \frac{\dot{E}_{A,EQ}}{c_{p,CO_2}\dot{m}_{CO_2} + c_{p,O_2}\dot{m}_{O_2} + c_{p,N_2}\dot{m}_{N_2} + c_{p,H_2O}\dot{m}_{H_2O}} + T_i$$

$$\dot{m}_{CO_2} = \dot{m}_{f,dry} \cdot (mf)_C\left(\frac{M_{CO_2}}{M_C}\right)$$

$$\dot{m}_{FG} = \dot{m}_{CO_2} + \dot{m}_{O_2} + \dot{m}_{N_2} + \dot{m}_{H_2O}$$

$$\rho_{FG} = \frac{y_{CO_2} \cdot 44 + y_{O_2} \cdot 32 + y_{N_2} \cdot 24 + y_{H_2O} \cdot 18}{0.8(24) + .2(32)}(0.0807096)\cdot\left(\frac{35.315\ ft^3}{m^3} \cdot \frac{kg}{2.2046\ lbm}\right) \quad \left[\frac{\text{kg}}{\text{m}^3}\right]$$

Figure 49: Biomass Boiler Model

Appendix L

Appendix L. Sample Questions/Problems to be Worked Out Before Starting an Algae Biofuels Venture

- How often will phototrophic cultures have to be restarted?
- Some algae strains produce ethers or oils that need to be cracked before they can be used as biodiesel feedstocks. Will this impede the ability to use algae for biofuels production?
- Are starter media, or possibly even several culture vessels of increasing size necessary to provide inoculum?
- Is enough land available close to a flue gas source?
- Will capital be available for an algae plant that plans to get flue gas off another operation (banks will not lend money if they perceive the possibility of the flue gas source ceasing operations a major risk)?
- Can raceways achieve significant labour cost decreases with larger installations?
- Considering the precipitation in BC, how much of a problem would grazing or contamination with undesirable species be in raceways in BC?
- If algae are grown with saltwater, how will that impact on the ability to use the algae cake, and its value?
- What species can be grown in BC, with what biomass and oil yields? Would species have to be changed between summer and winter?
- How often will culture crashes occur? How long will it take to recover each time?
- Will regulatory permission be granted to culture a genetically modified species in BC? How expensive will the approval processes and safety requirements be?
- Can oil production from fermenters be increased enough to reduce costs to the level of other plant oils?
- Can heterotrophic species of algae be found or genetically modified so they can use other, cost effective carbon sources?
- How does biodiesel from heterotrophic bacteria or yeast compare to other options such as using bacteria or yeast to make algae fermentatively?
- Where would the carbon source for fermentation come from, and would that mean dedicating large areas of land to e.g. sugar beet or corn production, and would that increase the footprint of biofuels from fermentation?

Appendix M

Appendix M. Personnel Tasks in a Phototrophic Microalgae Plant

Culture maintenance:

1. **Cleaning ponds :**
    a. Drain
    b. Rinse debris off
    c. Scrub tank (cannot be power washed as that would rip liner)
    d. Refill
    e. Disinfect water
    f. Neutralise disinfectant
    g. Inoculate
2. **Inoculating**
    a. Prepare inoculum
    b. Measure, mix, dissolve and apply nutrients. (May possibly be prepared in big batches, but may not be as efficient)
    c. Measure residual nutrients, prepare top ups
3. **Monitoring of cultures**
    a. Routine sampling of algae cultures
    b. Gross observations
    c. Microscopic observations
    d. Determination of biomass/ cell concentrations: (can be semi –automated)
4. Maintenance:
    a. Pond maintenance, repairs to ponds, tanks and liners,
    b. Repairs and maintenance of machinery, intake and circulating pumps, pipe, aerators, paddlewheels,
    c. Repairs to probes, meters, scientific and analytical equipment

Table showing tasks in a 400ha microalgae facility consisting of 988 ponds of 1 acre each. (For ease of management, the facility is divided into 40 units of 25 ponds each).

Task	*People required per unit*	*For whole facility*	*Description*
Cleaning	0.25	10	If we assume semi-continuous cultivation, with a 2 week harvesting cycle, and ponds completely drained and cleaned after 10 cycles. Each pond would then be cleaned once every 140 days (or 2.6 times a year). All the 988 ponds would then be cleaned 2,576 times a year (7 ponds per day). Pond cleaning is semi-automated, using tractors[6] with cleaning attachments and requires 1 FTE per pond.
Pond technicians	1.4	56	Facility divided into 40 groups of 25 ponds each. 1 technician is responsible for each group. Tasks include 1) daily taking samples from the ponds to analysts for determination of residual nutrients, 2): sampling for biologists for determination of biomass productivity, 3): sampling for algae biologists for microscopic analysis of microalgae cultures, 4): preparation of inocula, 5): mixing bulk nutrients and application of nutrients and pond repairs
GC analysts	0.08	5	Nutrient determination and analysis
Biologists	0.08	5	Determination of algae biomass and concentration[7]
Algae biologists	0.6	28	Microscopic evaluation of algae cultures. (Assume 1 biologist is responsible for 50 ponds/ day).
Maintenance	0.15	7	Assume 1 FTE is responsible for all the machinery and equipment for 200 ponds.
Total Biologists		**111**	

[6] Facility must be specially designed to leave room for the cleaning tractors to maneuver

[7] May be semi automated using automatic cell counters or spectrophotometers

Task	*People required per unit*	*For whole facility*	*Description*
Harvesting and oil extraction		83	A commercial scale algae plant uses ratios of between 1:1 and 1:2.4 of biologists to harvesting and processing FTEs. Depending on species being grown. Assuming economies of scale from our larger size, we assume a 1:0.75 ratio
Administration		**6**	
Total staff required (400ha facility)		**200**[8]	

[8] This includes only production staff and overhead (administration) staff. R&D personnel will be extra.

Appendix N

Appendix N. Detailed Rules and Regulations Applying to Microalgae Biomass Production Plants

Federal Rules and Regulations

Fisheries Act Fisheries Act (R.S., 1985, c. F-14)

This Act applies to all fish habitat and water bodies and is applicable to all lands regardless of ownership. The act broadly defines fish habitat to include spawning grounds and nursery, rearing, food supply and migration areas on which fish depend directly or indirectly in order to carry out their life processes. For more information visit http://laws.justice.gc.ca/en/F-14/index.html

Fisheries Act - Harmful Alteration of Fish Habitat (Section 35)

It is the responsibility of the proponent to consult with Fisheries and Oceans Canada to address potential impacts of the project (for instance construction of pipelines) on fish habitat. Fisheries and Oceans Canada generally strives to ensure "no net loss of habitat" and may require proponents to develop plans on how HADD can be reduced and/or how fish habitat can be created or enhanced to "replace" the negatively affected habitat.

Any activity with the potential to cause significant environmental harm can give rise to an environmental assessment under the Canadian Environmental Assessment Act. As a result, an environmental assessment will sometimes be required before Fisheries and Oceans Canada can issue an authorization under the HADD provisions of the Fisheries Act.

Fisheries Act – Deleterious Substances (Section 36 (3))

An interpretation of deleterious substances that make it pertinent to an algae facility is 'any water that contains a substance in such quantity or concentration […] that it would, if added to any other water […] likely [render it] deleterious to fish or fish habitat'. This definition would likely include any unused nutrients in effluent waters that cause eutrophication.

Fisheries Act – Petroleum Refinery Liquid Effluent Regulation

This Act applies to facilities for the processing of crude oil. It sets average authorized levels for the deposit of various deleterious substances, including oils and grease, phenols, sulphide, ammonia nitrate and other substances harmful to fish. The operator of the refinery is required to report to the Minister on how much of these substances are deposited on a regular basis. For more information visit http://laws.justice.gc.ca/en/ShowTdm/cr/C.R.C.-c.828///en

Navigable Waters Protection Act (R.S., 1985, c. N-22)

This act requires approval from Fisheries and Oceans Canada's Canadian Coast Guard before any work is 'built or placed in, on, over, under, through or across any navigable water'. Navigable waters are not fully defined by the Act, but the Coast Guard has adopted a very broad definition for the purposes of administering the Act. This includes bodies 'of

water capable, in its natural state, of being navigated by floating vessels of any description for the purpose of transportation, recreation or commerce. It also includes a canal and any other body of water created or altered for public use, as well as any waterway where the public right of navigation exists'. Works requiring approval are defined by the Act to include any 'bridge, boom, dam, wharf, dock, pier, tunnel or pipe and the approaches or other works necessary or appurtenant thereto [and] any structure, device or thing […] that may interfere with navigation'. These interpretations and definitions may potentially affect water intake and effluent discharge pipelines associated with a microalgae facility. For more information visit http://laws.justice.gc.ca/en/showtdm/cs/N-22.

Canadian Environmental Assessment Act (1992, c. 37)

The purpose of an EA is to identify the environmental effects of a proposed project and determine the need to mitigate or eliminate the effects before responsible authorities take actions in connection with them. An EA assessment can be triggered under various circumstances. These include:

- The proponent trigger (when a project is proposed),
- The funding trigger (when federal grants or financial assistance is secured for a project),
- The land trigger (when an interest is granted in land to enable a project to be carried out), and
- The law list trigger (when the government exercises a regulatory duty in relation to a project, such as issuing a permit or license). For more information visit http://laws.justice.gc.ca/en/showtdm/cs/C-15.2

Canadian Environmental Protection Act, 1999 (1999, c. 33)

This Act (CEPA) gives federal government the powers to request environment related information, notification and involvement from industry and establishes a National Pollutant Release Inventory where the public can find site specific information on the pollutants created by industrial facilities across Canada. Proponents who receive federal funding may be required to complete a CEPA review and are encouraged to consult a CEPA agent during the planning phases of the project to ensure compliance with all the relevant CEPA regulations. For more information visit http://laws.justice.gc.ca/en/showtdm/cs/C-15.31

Canadian Environmental Protection Act, 1999 (1999, c. 33) New Substances Notification regulations (Organisms)

This Act requires that a proponent notifies Environment Canada prior to importing or manufacturing a new substance (chemicals, polymers or animate products of biotechnology) that is not currently on the Domestic Substances List. The organisms that must be reported include all genetically modified or bio-adapted microbes as well as microbes intended for fermentation. For more information visit http://ec.gc.ca/substances/nsb/eng/home_e.shtml

Species at Risk Act (2002, c. 29)

Once a species is 'listed' as endangered, threatened or at risk it becomes an offence for any person to 'kill, harm, harass, capture or take an individual of a listed species or to damage or destroy the residence of an individual of a listed species'. Section 2(1) defines 'residence' as a dwelling-place, such as a den, nest or other similar area or place.

The act also protects habitats critical to the recovery and health of species at the population level. This provision applies to any critical habitat for aquatic species and migratory birds. For other species, it can apply to critical habitat on federal land and, in some circumstances, provincial land. For more information visit http://laws.justice.gc.ca/en/showtdm/cs/S-15.3

Provincial Rules and Regulations

Land Act (RSBC 1996) Chapter 245

Proponents should note sections of the Act reserving rights (and rights of access) to the subsurface resources under the land to the government (Section 50, 1a and b), as well as limitations on lease area (Section 22, 1) and terms (section 22, 2). For more information visit http://www.qp.gov.bc.ca/statreg/stat/L/96245_01.htm

Land Title Act (RSBC 1996) Chapter 250

This Act sets out rules for a range of issues about land ownership, registration and cancellation of titles, mortgages, subdivision of land, rules of disposition on death in cases of testacy and intestacy, tax sales, certificates of pending litigation, and statutory rights of way. For more information visit http://www.qp.gov.bc.ca/statreg/stat/L/96250_00.htm

Notice regarding pollution

Section 392 of the Act allows a waste management director to register a notice in the Land Title Office that serious pollution exists on a property. The waste management director has to be satisfied that the danger no longer exists before filing a notice to cancel the endorsement on the title.

Subdivision

If land is to be subdivided, a proponent must apply to an approval officer under the land title act. Even if all municipal requirements are met, the approving officer may reject a proposed subdivision under a range of circumstances, including the risks of flooding, erosion or avalanche, anticipated adverse effects of the development on the environment, or if the subdivision is determined to be against the public interest. The determination of 'public interest' includes aesthetic and environmental concerns and OCP policies.

Farm Practices Protection (Right to Farm) Act (RSBC 1996) Chapter 131

This Act includes definitions that specifically include microalgae cultivation for the production of biomass. The Act protects a farmer from a lawsuit resulting from farm operations, and Nuisance and Miscellaneous bylaws under certain sections of the Local Government Act, if the farmer is engaged in a 'normal farm practice' in the ALR, on land zoned for farming, or if he holds a valid aquaculture license. To be eligible for protection, a farmer must also comply with the Health Act, the Pesticide Control Act, the Waste Management Act, all regulations under these Acts, and any land use regulations.

A 'normal farm practice' is 'that conducted by a farm business in a manner consistent with proper and accepted customs and standards as established and followed by similar farm businesses under similar circumstances'. This includes practices that make 'use of innovative technology in a manner consistent with proper advanced farm management practices'.

The Farm Practices Board, established under the Natural Products Marketing (BC) Act, can order a farmer to stop a practice that is not normal, and if the farm practice is not normal, then the common law rules and local government bylaws dealing with nuisance will apply. While the production of biomass will likely be covered as a normal farm practice, it is unclear whether biomass processing will qualify.

Companion legislation in the Local Government Act requires a local government wishing to regulate or prohibit farm operations to first seek approval from the Minister. For more information visit http://www.qp.gov.bc.ca/statreg/stat/f/96131_01.htm

Waste Management Act (SBC 2003) Chapter 53

Proponents should note elements in the Act that include a broad prohibition against introducing waste into the environment without government approval (section 6 (2, 3, 4 and 5) and authorization for introducing waste into the environment where authorized by permits or regulations, or by other laws (Section 14 (1, 2, 3 and 4).

Section 118 (1) of the Act also allows for environmental certification of products by labels names, phrases or other symbols to indicate that the product or service meets prescribed environmental standards or was produced or provided using prescribed environmental practices. For more information visit
http://www.qp.gov.bc.ca/statreg/stat/e/03053_00.htm

Environmental Management Act [SBC 2003] Chapter 53

Section 6 (1, 2, 3 and 4) of the Waste Management Act prohibit introduction of waste into the environment. Land based microalgae biomass operation and oil and gas industries are prescribed under the Waste Discharge Regulations of the Environmental Management Act and as there are no established codes of practice for these activities yet, they will likely fall under the requirements of the Environmental Management Act. For more information visit http://www.qp.gov.bc.ca/statreg/stat/e/03053_00.htm and
http://www.qp.gov.bc.ca/statreg/reg/E/EnvMgmt/320_2004.htm

Environmental Management Act and Health Act, B.C. Reg. 131/92 Agricultural Waste Control Regulation

Section 30 states that 'agricultural products such as livestock, poultry, farmed game, fur bearing animals, animal and poultry feeds, forage silage, forage crops, vegetables and chemical fertilizers must be managed, used and stored in a manner that prevents the escape of agricultural waste that causes pollution'. This regulation will apply to fertilizers stored on site as nutrients for the production of biomass. This is important if nutrient recycling is to be practiced with or without the incorporation of an anaerobic digester. For more information visit http://www.qp.gov.bc.ca/statreg/reg/e/envmgmt/131_92.htm

Fisheries Act [RSBC 1996] Chapter 149

Section 3 further requires that precautions be taken against the release of farmed fish or plants into the environment. While it is unclear whether this will apply to microalgae, it is the responsibility of the proponent to verify this with the responsible Fisheries officials especially if non-native species are considered. For more information visit http://www.qp.gov.bc.ca/statreg/stat/F/96149_01.htm

Fish Protection Act [SBC 1997] Chapter 21

This Act includes provisions that prohibit dams, designates sensitive streams, limits Water Act approvals and licenses on sensitive streams, and allows the province to require local governments take actions to protect fish habitat.

Once a stream is designated under this Act, new licenses / approvals or amendments to old ones must be consistent with the Sensitive Streams Designation and Licensing Regulation. Water Act officials can only issue or amend licenses / approvals if they are satisfied that there are no significant adverse impacts on the protected fish population or that mitigation measures included in the approval or license will avoid any significant impacts. If mitigation can not fully address the problem, compensation measures elsewhere will fully compensate for adverse impacts. For more information visit http://www.qp.gov.bc.ca/statreg/stat/F/97021_01.htm

Water Act [RSBC 1996] Chapter 483

Under this Act the province owns and has the right to use or receive the flow of all water flowing in a natural watercourse anywhere in the province. Furthermore, no person can use, store or divert water without a license. One exception is that a person may make 'changes in and about a stream' under an Approval. This is defined as 'any modification to the nature of the stream including the land, vegetation, natural environment or flow of water within the stream, or any activity or construction within the stream channel that has or may have an impact on a stream'.

To have this right, a person must obtain a water license from the Comptroller of Water Rights in the Ministry of Sustainable Resource Management, or a Regional Water Manager in Land and Water BC. The Water Act limits who can hold a water license to a member of the public owning land (or a mine) near the water source. A water license allows the proponent to divert and use, for a specified purpose, a quantity of water specified in the license, store water, construct, maintain and operate the pipes, dams and other works set out in the license as necessary for the proper diversion and use of the water. For more information visit http://www.qp.gov.bc.ca/statreg/stat/W/96483_01.htm

Drainage, Ditch and Dyke Act [RSBC 1996] Chapter 102

Under this Act, Sections 105 and 106 give the commissioner unlimited powers to expropriate any sections or all of the land, with compensation being paid for taken or damaged lands (sections 108 and 109). For more information visit http://www.qp.gov.bc.ca/statreg/stat/D/96102_01.htm

Agricultural Land Commission Act [SBC 2002] Chapter 36

This Act establishes the Provincial Agricultural Land Commission. It also sets the Commission's mandate, which is to preserve agricultural land, encourage farming on that

land, and encourages local governments, first nations, the government and its agents to enable and accommodate farm use of agricultural land that is compatible with agriculture in their plans, bylaws and policies.

Land in the agricultural land reserve must be used for 'farm use' only. The Agricultural Land Reserve Use, Subdivision and Procedure Regulation lists land use activities that are considered 'farm use'. For more information visit
http://www.qp.gov.bc.ca/statreg/stat/A/02036_01.htm

Environmental Assessment Act [SBC 2002] Chapter 43

The BC legislation authorizes the assessment of environmental, economic, social, heritage and health effects of a 'reviewable project'. The goal is to identify and evaluate the effects of a project before it begins (or an existing project is modified) so that any negative or damaging effects may be avoided, or at the very least mitigated.

According to the Act, if a project is 'reviewable', the proponent may not proceed with the project unless an environmental assessment certificate has been issued for the project, or unless the executive director of the Environmental Assessment Office decides that an EA certificate is not required.

Projects may become reviewable through three routes:

- The Reviewable Project Regulation (passed under the Act) designates the project as reviewable,
- By special ministerial order (the Minister designates an otherwise not reviewable project as reviewable), or
- The project proponent applies for the project to be reviewed and the Minister orders it to become a reviewable project.

For most projects the Reviewable Projects Regulation will determine whether it is reviewable. For more information visit
http://www.qp.gov.bc.ca/statreg/stat/E/02043_01.htm

Wildlife Act [RSBC 1996] Chapter 488

Under section 3 of the Act, the Minister of Water, Land and Air Protection is authorized to acquire and administer land for the purpose of access to or the management of wildlife. The minister may also establish Wildlife Management Areas (WMA), Wildlife Sanctuaries and Critical Wildlife Areas where it is required for habitat for an endangered or threatened species. The land and resources within a WMA may not be used without first obtaining the written permission of the regional manager. It is an offence to alter, destroy or damage wildlife habitat or deposit on land or water any substance in a manner that is harmful to wildlife or wildlife habitat in a WMA, unless with written permission of a regional manager or as permitted by the regulations or a permit. For more information visit
http://www.qp.gov.bc.ca/statreg/stat/W/96488_01.htm

Local Bylaws, Rules and Regulations

As microalgae biomass production facilities are covered under the list of normal farm practices, the Agricultural Land Commission Act will supersede any local bylaws. However, presently, because renewable energy production is not considered a normal farm practice, it is unclear whether the further development of biomass to bioenergies will be considered normal farm practices.

Given that algae farms will include some industrial operations, such as backup generators, centrifuges, buildings, and oil storage, it may be that at least part of the facility will not be regarded a purely agricultural. If this is the case, proponents will have to comply with local government by laws and regulations surrounding the generation of odours, wastes, noise etc.

Local Government Act – Subdivision

Local government may pass various types of by-laws that will affect how and when lots can be subdivided. While the local government can pass these bylaws, the ultimate legal responsibility for approving rests with an approving officer under the authority of the provincial Land Title Act.

Local Government Act – Zoning Bylaws

'Zoning' refers to the ability of local governments to regulate the form and character of development. This is accomplished by passing bylaws dividing a municipality or regional district into land use 'zones'. In each zone the local government can limit how land can be used within that zone.

Official Community Plans

Official Community Plans contain policy statements on specific issues including commercial, industrial, institutional, agricultural, recreational and public utility land uses as well as restrictions on the use of land subject to hazardous conditions or environmentally sensitive to development. However, OCPs cannot regulate developments on land it does not control, such as land in the Agricultural Land Reserve.

Appendix O

Appendix O. Comparison of Algae and Polyvoltaics

This Appendix compares the phototrophic production of biodiesel from microalgae and photovoltaic (PV) power for Prince George. PV panels are a lot more efficient in using sunlight (about 14%, depending on the technology used, against a max. of 4% for microalgae). Solar radiation values were used to produce biodiesel from microalgae, assuming a raceway operated for 6 months per year. It was found that for Prince George 1.36 million l of oil per km^2 can be produced. It is calculated that a 1 kWp solar panel (kiloWatt Peak panel) would produce 995 kWh of power per year in Prince George, as shown in the table below. To compare biodiesel production and PV power production on an area basis to determine the revenue ratio (PV/BD revenue) and kilometers driven ratio (PV/BD km driven), it is required to make key assumptions:

- Biodiesel cost is $1 per l
- Electrical power costs in BC is 6c/kWhr
- To determine the area taken up for one kWp of PV panels, we take the 8 largest PV plants in the world and these average to 0.028 kWp/m^2. This value could be conservative with the lowering of the cost of thin-film panels on a kWp basis, which are less efficient on an area basis
- The efficiency ratio of an electrical delivery van using batteries versus a diesel engine is 2.1; for large trucks it is not possible to use batteries at this time.

Using these assumptions, we calculate that:

- PV to biodiesel km driven ratio is 4 times on an area basis, and
- The PV to biodiesel revenue ratio is 1.23 on an area basis, where 4 times more distance can be traveled using PV compared to biodiesel on an area basis, but the revenue stream is approximately the same.

These results show that although photovoltaics are more efficient to convert radiation to energy using panels compared to microalgae, PV power plants occupy more dead space required for maintenance and avoid shadows from tilted panels as compared to raceways. Still, the transportation system using electricity from PV is a lot more efficient than that using algae oil in a diesel engine, and less space would be necessary to run electric cars than to run diesel cars, keeping the distance driven the same.

Table AP.E.1: Comparison of biodiesel production to solar PV on area basis

Prince George Values					
Month	Solar Radiation ($kWh/m^2/day$)	AC Energy (kWh/kWp)	Solar Energy per area (kWh/m^2)	Energy Value/ kWp (Can$ at 6 c/kWh)	Assumptions
1	1.55	38	1.064	2.28	"Lat (deg N):", 53.88
2	2.74	62	1.736	3.72	"Long (deg W):", 122.68
3	3.78	93	2.604	5.58	"Elev (m): ", 676
4	5.16	117	3.276	7.02	"Weather Data:","CWEC"
5	4.94	113	3.164	6.78	"PV System Specifications"
6	5.35	116	3.248	6.96	"DC Rating:"," 1.0 kWp"
7	5.02	112	3.136	6.72	"DC to AC Derate Factor:"," 0.800"
8	5.11	113	3.164	6.78	"AC Rating:"," 0.8 kW"
9	4.23	92	2.576	5.52	"Array Type: Fixed Tilt"
10	2.97	70	1.96	4.20	"Array Tilt:"," 53.9"
11	1.69	39	1.092	2.34	"Array Azimuth:","180.0"
12	1.26	30	0.84	1.80	"Energy Specifications"
Year	3.65	995.0	27.9	59.70	"Cost of Electricity:"," 0.06 Can$/kWh"
PV area	0.03	kWp/m^2	HHV Biodiesel	45.6	MJ/kg
PV energy	100	MJ/m^2	BD density	850	kg/m^3
Electric car/IDE	2.1	eff ratio	BD cost	1.00	per l
PV revenue	1.6716	S/m^2	BD revenue	1.36	S/m^2
PV/BD km driven	4.00	$times/m^2$	Algae production	1.36	$l BD/m^2$ Prince George see report
PV/BD revenue	1.23	$times/m^2$	Algae production	52.7	MJ/m^2

APPENDIX P

Appendix P. References

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