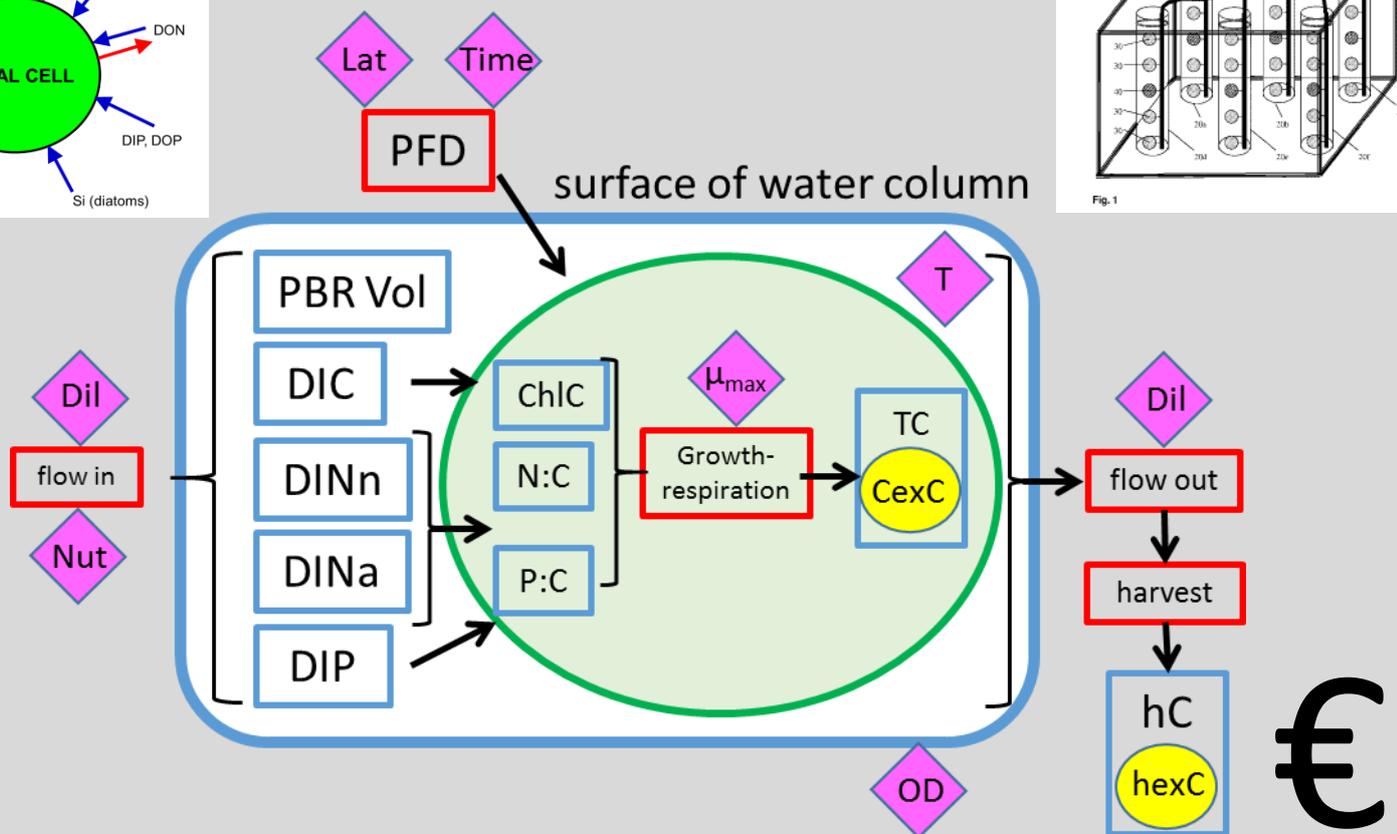
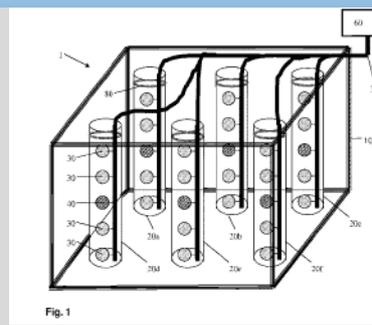
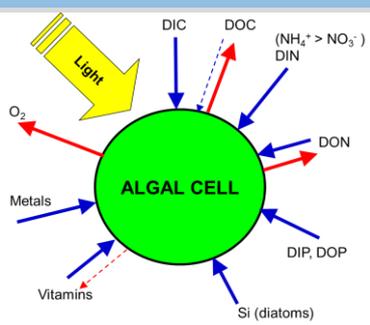


# Enhancing Microalgal Production

## Constructing Decision Support Tools Using System Dynamics Modelling

Kevin J Flynn



## Foreword to Draft DST e-Book

### PLEASE READ ME

This is a work under development as part of the deliverables for the EnhanceMicroAlgae project. “Beta” versions will be released during the course of the project, eventually leading by ca. early 2020 of a formal e-book with ISBN and various models to run as simulators of differently configured bioreactors for the commercial growth and exploitation of microalgae.

New chapters for this book will be delivered over the coming months. Be sure to check the project web site, <https://www.enhancemicroalgae.eu/>, for updates.

Inevitably, the document will mature over this period. There will also (alas inevitably) remain errors and omissions. The author welcomes any feedback; please contact him via [k.j.flynn@swansea.ac.uk](mailto:k.j.flynn@swansea.ac.uk).

Thank you

Kevin J Flynn

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The project website is: <https://www.enhancemicroalgae.eu/>



## Disclaimer

The contents of this work, and the allied simulation models, are directed towards the commercial production of microalgae. While the contents are offered free and in all good faith, neither the author nor the *EnhanceMicroAlgae* project accept any liability whatsoever for any commercial (or other) judgements made by any persons in consequence of the information contained herein or based upon the output of the models.

It is the responsibility of the end user to ensure that the models are run under conditions most closely aligned with their interests.

The simulation models for the DST were developed using Powersim software ([www.Powersim.com](http://www.Powersim.com)) Studio 10; they are presented for use under the Powersim Cockpit. The author, nor the *EnhanceMicroAlgae* project, nor the project funders, endorse Powersim products in any way.

## Glossary

*Items in italics are described elsewhere in this glossary*

$\alpha^C$ : the rate of photosynthesis per unit of C-biomass per photon.  $\alpha^C$  characterises the initial slope of a C-specific *PE curve* (e.g.,  $\text{gC gC}^{-1} \text{d}^{-1}$  vs *PFD*).

$\alpha^{\text{Chl}}$ : the rate of photosynthesis per unit of chlorophyll per photon.  $\alpha^{\text{Chl}}$  characterises the initial slope of a Chl-specific *PE curve* (e.g.,  $\text{gC gChl}^{-1} \text{d}^{-1}$  vs *PFD*).

**Acclimation:** changes in organism physiology in response to environmental factors. Often confused with *adaptation*, acclimation is an intra-generational response.

**Adaptation:** changes in organisms physiology that have come about through natural selection. Adaptation is an inter-generational response to changes in environmental factors. Cf. *acclimation*.

**Allelopath:** chemical involved in “signalling” between organisms. These signals may be negative between competitors, or positive between organisms of the same species. Allelopaths may be growth factors. Typically, they are of unknown chemical characteristics, which may be destroyed by heat.

**Anabolism:** biochemistry that is constructive, making new biomass, at the expense of energy consumption. Cf. *catabolism*. In reality there are simultaneous anabolic and catabolic processes occurring as cellular components are continuously built, maintained and turned over.

**Areal production:** production described in units of area (e.g.  $\text{gC m}^{-2} \text{d}^{-1}$ ). The area could be just that occupied by the bioreactor but, for financial calculations, it should include the total facility footprint. Exploiting a simple single layered *bioreactor*, the maximum rate of production is limited by the efficiency of the processes of photosynthesis to ca.  $3\text{-}5 \text{gC m}^{-2} \text{d}^{-1}$ . Cf. *volumetric production*.

**Axenic:** containing a single species. Usually implying bacteria-free. Cf. *unialgal*.

**Batch culture:** a culture scenario in which a single one-off culture is grown typically through different phases of the culture dynamics, to stationary phase. While the culture may be sampled continuously, the system never enters or approaches a *steady-state* condition except perhaps at stationary phase. Unlike *chemostat* or *turbidostat* cultures, in batch systems the growth rate may approach the maximum possible rate. Cf. *continuous culture*; *stretched batch culture*.

**Bioreactor:** a vessel in which microbes, such as microalgae are grown. Bioreactors come in different volumes (mL to 100’s of cubic metres) and different forms, from small glass flasks, to ponds dug in clay, to sophisticated arrangements of pipes and pumps made of exotic materials. See also *Photobioreactor*.

**Carbonic anhydrase (CA):** enzyme responsible for catalysing the conversion of  $\text{HCO}_3^-$  (usually the most abundant form of *DIC*) to  $\text{CO}_2$  (the form of *DIC* used by *RuBisCO*). *CA* activity may be internal or external.

**Catabolism:** biochemistry that consumes biomass, usually to generate energy. Cf. *anabolism*. In reality there are simultaneous anabolic and catabolic processes occurring as cellular components are continuously built, maintained and turned over.

**Chelating agent:** a chemical that holds on to other chemicals (usually for microalgal culturing an organic compound that binds onto iron, Fe, keeping it in suspension).

**Chemostat:** a *continuous culture* system of constant volume into which fresh medium is injected and at a similar rate expended medium complete with cells is withdrawn. At *steady-state* the organisms grow at the same rate as the specific dilution rate of the culture system. If the dilution rate is close to the maximum growth rate there is a risk of *washout*. See also *Turbidostat* and *Discontinuous culture*.

**Chl:** chlorophyll<sub>a</sub>, the core photopigment, usually augmented by various accessory pigments that collect energy across other parts of the *PAR* spectrum.

**Chl:C:** the ratio (usually as mass) of chlorophyll to C-biomass. This ratio varies between species (typically with a maximum of 0.06 g/g) and also increases during growth at low light and decreases with nutrient-stress. See also *photoacclimation*.

**Compensation point:** (*C<sub>p</sub>*) the *PFD* at which *gross photosynthesis* = concurrent respiration; i.e. *net photosynthesis* is zero.

**Continuous culture:** a culture system in which, logistics constraints aside, growth continues (usually at *steady-state*) for ever. Cf. *batch culture*.

**Dark reaction:** the plateau value of the *PE curve*, limited primarily by the activity of *RuBisCO*.

**DIC:** dissolved inorganic carbon, comprising  $\text{CO}_2$  (the substrate for *RuBisCO*, for photosynthesis), bicarbonate ( $\text{HCO}_3^-$ ) and carbonate ( $\text{CO}_3^{2-}$ ).

**DIN:** dissolved inorganic nitrogen, comprising ammonia ( $\text{NH}_3$ ), ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ).  $\text{NH}_4^+$  and  $\text{NO}_3^-$  are the usual main forms of DIN;  $\text{NH}_4^+$  is the “preferred” N-source in algal physiology but it is toxic at high residual concentrations (such as in undiluted anaerobic digestate liquors).

**DIP:** dissolve inorganic phosphorous,  $\text{PO}_4^{3-}$ .

**Discontinuous culture:** like a *chemostat* culture (where dilution is continuous) but with a recurring but temporally discrete replacement of a portion of the culture with fresh medium. Discontinuous removal has the advantage that a more useful volume is removed for processing. A period of maximum growth rate is not seen. If the gap between sampling is sufficient, and the proportion of culture replaced also significant, then the discontinuous culture approaches a *stretched-batch culture* system.

**DOC:** dissolved organic carbon (e.g. glucose).

**Down-shock:** response cellular physiology to application of stress (e.g., by nutrient exhaustion). Down-shock results in the de-repression of physiological processes that are repressed during *up-shock*.

**Exponential growth:** a rate of growth when organism-specific increase is constant. Best visualised as the linear (*steady-state*) portion of a plot of natural log (Ln) of cell numbers or biomass against time. The exponential growth rate does not necessarily equate to the maximum growth rate (though it is often confused with that).

**Gross photosynthesis:** photosynthesis disregarding concurrent respiration that consumes part of the products of C-fixation. Gross photosynthesis is zero when PFD=0 (i.e. in darkness). Cf. *net photosynthesis*.

**Heterotrophy:** nutrition and growth supported by organic sources of C. (Cf. *mixotrophy, osmotrophy, phagotrophy, phototrophy*).

**in vitro:** in test-tube, typically referring to quantification of materials extracted from organisms (which are invariably killed during the process). Cf. *in vivo*.

**in vivo:** in life, usually made in reference to measurements of processes or quantities within intact living organisms which are not usually killed in the process, though they may be damaged. Cf. *in vitro*.

**Inoculum:** cells introduced into a new culture system to initiate growth. Unless care is taken, typically cells in the inoculum are subjected to shock (light, temperature, pH) and often to nutrient *up-shock* as they encounter elevated nutrient concentrations.

**Light reaction:** the strictly light-dependant phase of photosynthesis. In a plot of photosynthesis against light (the *PE curve*), this is the initial linear slope before the curve levels off to be limited by the *dark reaction*. The light reaction rate is limited, in addition to the *PFD*, by the photopigment complement that captures photons, the value of  $\alpha^{Chl}$ , and *Chl:C*.

**Macronutrient:** nutrients that comprise the bulk of the biomass upon their assimilation and thus need to be added at high concentration to the growth medium. For microalgae these are C (usually as *DIC* supplied as bicarbonate and via aeration, but possibly also by *DOC*), N (as *DIN*), and P (as *DIP*). For diatoms, Si is also a macronutrient. Cf. *micronutrient*.

**Micronutrient:** nutrients that comprise a minor component of the biomass upon their assimilation, and are thus usually added to culture media at only low concentrations. These include Fe and other metal cofactors, and vitamins and other organic cofactors. Micronutrients are just as essential as are macronutrients. Cf. *macronutrients*.

**Mixotrophy:** combining *phototrophy* and *heterotrophy*. (Cf. *heterotrophy, osmotrophy, phagotrophy, phototrophy*.)

**Model:** a simplification of reality. Mathematical models range from simple statistics to complex *simulation* models running under differential calculus. Over-simplification renders models insufficiently realistic to enable them to provide a *simulation* of reality.

**N-quota:** the amount of N within the cell. The quota is usually described with reference to the cell (e.g., pgN cell<sup>-1</sup>), or the C content (e.g. gN gC<sup>-1</sup>). The value of N:C typically relates linearly to growth rate in N-limited cultures. The internal N is redistributed amongst daughter cells until the quota attains a minimum value, at which time C-specific growth halts.

**Net photosynthesis:** photosynthesis including concurrent respiration that consumes some part of the products of C-fixation. Net photosynthesis is zero when *PFD* is at the *compensation point*. Cf. *gross photosynthesis*.

**Nutrient deplete:** having less nutrient within the cell than is required to enable optimal (maximum) growth under current conditions, but growth can still continue. Cf. *nutrient limited*, *nutrient stress*, *nutrient sufficient*.

**Nutrient limited:** having so little of the nutrient in question that net growth halts.

**Nutrient replete:** having more nutrient within the cell than is required for optimal (maximum) growth under current conditions. Thus surplus P may be accumulated as polyphosphate, and cells grown on ammonium-N have a higher *nutrient-status* (higher N:C) than do cells grown on nitrate-N. Nutrient replete cells will have repressed biochemical routes to using alternative nutrients that are de-repressed during the development of *nutrient stress*.

**Nutrient-status:** a statement of physiological status, of *nutrient stress*, with reference to a particular nutrient. Maybe referenced as a quotient, so 0 indicates a very poor status (*nutrient limited*) and 1 is *nutrient sufficient*.

**Nutrient stress:** a condition between *nutrient sufficient* and *nutrient limited* during which various physiological processes are up- or down-regulated allowing the (de)repression of alternative biochemical pathways. Cf. *nutrient-status*. See also *down-shock* and *up-shock*.

**Nutrient sufficient:** having sufficient nutrient within the cell to support optimal (maximum) growth under current conditions. Cf. *nutrient replete*.

**Osmotrophy:** a form of *heterotrophy* in which nutrition and growth is supported by the use of dissolved organic sources of C. (Cf. *heterotrophy*, *mixotrophy*, *phagotrophy*, *phototrophy*.)

**P-quota:** the amount of P within the cell. The quota is usually described with reference to the cell (e.g., pgP cell<sup>-1</sup>), or the C content (e.g. gP gC<sup>-1</sup>). The value of P:C relates curvilinearly to growth rate in P-limited cultures (Cf. *N-quota*). The internal P is redistributed amongst daughter cells until the quota attains a minimum value.

**PAR:** photosynthetically active radiation; the portion of the light spectrum that is exploited by photosynthetic organisms. Coincidentally, this is the same as the visible spectrum for humans (light of wavelengths 400-700nm).

**PE curve:** the relationship between light (E) and net of gross photosynthesis (P), characterised by an initial upward slope (*light reaction*) and a plateau value (set by the maximum *dark reaction* rate).

**Phagotrophy:** a form of *heterotrophy* in which nutrition and growth is supported by the consumption (through engulfment) of particles of dissolved organic sources of C; usually those particles are other organisms and the phagotrophy is de facto predation. (Cf. *heterotrophy, mixotrophy, osmotrophy, phototrophy.*)

**Photoacclimation:** *acclimation* of microalgae to the supply and demand of photosynthesis balanced against light and nutrient (usually *DIN* or *DIP*) supply. Characterised by changes in *Chl:C* and often also changes in other photo-pigments.

**Photobioreactor:** a *bioreactor* specifically configured to be illuminated, usually to support the growth of photosynthetic organisms. Illumination may be by natural light and/or artificial light. Because light generates heat, photobioreactors often require cooling to prevent temperature increases that affect growth (see  $Q_{10}$ ).

**Phototrophy:** nutrition and growth supported by assimilation of inorganic sources of C (de facto,  $\text{CO}_2$ ) through photosynthesis. (Cf. *heterotrophy, mixotrophy, osmotrophy, phagotrophy.*)

**PFD:** photo flux density ( $\text{photons m}^{-2} \text{ s}^{-1}$ ); the number of photons hitting a stated area per time. A light meter for biological use may report this as *PAR PFD*, as just that part of the light energy spectrum of use for photosynthesis (wavelengths 400-700nm). Note that photons of different wavelengths contain different amounts of energy; a photon at 400nm contains approaching twice (i.e.,  $700/400$ ) of the energy of a photon at 700nm.

**Production:** implicitly as production rate, the rate of biomass generation expressed as *volumetric production* or *areal production*. Cf. *standing crop*.

**$Q_{10}$ :** the proportion by which biological process rates (e.g., growth rate) increases when temperature is increased by  $10^\circ\text{C}$ . Traditionally a value of  $Q_{10} = 2$  is used. The value is only useful within a narrow temperature window above which thermal death occurs rapidly.

**RuBisCO:** ribulose biphosphate carboxylase; the enzyme responsible for fixing CO<sub>2</sub> (a component of *DIC*, perhaps allied with *carbonic anhydrase*, *CA*). On account of it having a low efficiency (low  $k_{cat}$ ) and of the importance of primary production to life on Earth, RuBisCO is considered to be the most important single enzyme on the planet. At high O<sub>2</sub> concentrations (O<sub>2</sub> being a by-product of the *light reaction* of photosynthesis, q.v.), CO<sub>2</sub>-fixation by RuBisCO is inhibited.

**Si-quota:** the amount of Si within the diatom cell. The quota is usually described with reference to the cell (e.g., pgSi cell<sup>-1</sup>), or the C content (e.g. gSi gC<sup>-1</sup>). The value of the Si quota cannot be related usefully to growth rate because previously assimilated Si cannot be redistributed amongst daughter cells. Cf. *N-quota*, *P-quota*.

**Simulation:** operation of a *model* over a course of time with an output that aligns with reality.

**Simulator:** a *model* that is used to provide a *simulation*

**Specific growth rate:** growth rate made in reference to a specific component. A value of 0.693 d<sup>-1</sup> describes a doubling per day; 0.693 = Ln(2). It should be noted that depending on the reference component the value of the specific growth rate is not the same. Thus, cell-specific (cell cell<sup>-1</sup> d<sup>-1</sup>) differs from C-specific (C C<sup>-1</sup> d<sup>-1</sup>), and differs from N-specific (N N<sup>-1</sup> d<sup>-1</sup>), etc. Only in a culture growing at true steady-state in a heterogenous culture (organisms at all different stages of their cell cycle) will all specific growth rates be the same as averaged across the whole population. Unfortunately, because the units of the specific component cancel out, usually only the time unit is reported (e.g., d<sup>-1</sup>); full units should always be given.

**Standing crop:** the amount of biomass present at a given time, usually expressed per area or per volume. Cf. *production*.

**Steady-state:** a condition where all processes are progressing in unison, such that the *specific growth rate* as determined through reference to any/all components will be equal. The biochemistry of individual cells can be in steady-state while the population abundance is changing (not in steady-state). Steady-state is best achieved through growth in a *chemostat* or *turbidostat*. In steady-state, the growth rate is *exponential*. Growth in steady-state usually implies growth limited by a factor; non-decaying dead cells are also in steady-state. See also *specific growth rate*.

**Stretched batch culture:** a batch culture system into which fresh medium is added to balance the removal of volumes for sampling. This is a form of *discontinuous culture* in which the sample taken is so large and/or so infrequent, that the culture expresses a period of *batch culture* dynamics, including the potential for growth at the maximum possible rate.

**System Dynamics models:** a form of *model* in which specific attention is paid to the accounting of materials during the *simulation*.

**Tangential-flow filtration:** a filtration approach in which the suspension being filtered is passed over the face of the filter (at a tangent) to continuously removing particles from the face of the filter that would otherwise block the filter pores.

**Tuning:** a process as part of *validation* of a model during which model parameters are adjusted to achieve the best fit of the model output to real data.

**Turbidostat:** a continuous culture system that, in contrast to the operation of the *chemostat*, has a control of entry of fresh medium and simultaneous removal of spent medium and culture linked to the optical density of the culture suspension. Unlike the chemostat, dilution rates in a turbidostat can run close to the maximum growth rate without risk of *washout*.

**Validation:** a process through which the output from a *simulation model* is compared with the real world to convince the user that the *simulation* is fit for purpose.

**Volumetric production:** production described in units of volume (e.g.  $\text{gC m}^{-3} \text{d}^{-1}$ ). Because of self-shading within the microalgal suspension, optimising high *areal production* and high volumetric production can be challenging.

**Washout:** an event when the dilution rate of a culture system (*bioreactor*) exceeds the growth rate of the organism, so washing out the culture. Washout is common in a *chemostat* at high dilution rates but will not occur in a *tubidostat*.

**Unialgal:** single algal species. Often used to describe a culture that contains bacteria, but only one algal species. Cf *axenic*.

**Up-shock:** recovery of cellular physiology from stress (e.g., by supply of nutrients to a nutrient-starved culture). Up-shock results in the repression of physiological processes that were de-repressed during *down-shock*.

## Preface

The aim of this book is to provide the reader with a text that explains how to optimise the commercial production of microalgal biomass.

The target audience for this work includes, in no particular order:

- Undergraduate and postgraduate students of biology, process biotechnology and chemical engineering
- Engineers engaging in the design and optimisation of microalgal bioreactors
- Aquaculturists wishing to develop integrated platforms for the growth of shell or fin fish
- Pharmacologists and nutritionists exploring the commercial potential of whole microalgal biomass or of specific biochemicals
- Those engaged in wastewater treatment, or CO<sub>2</sub> removal, wishing to consider deploying microalgal bioreactors
- Venture capitalists who wish to understand more of the basics of microalgal biotechnology

Most texts and other works on the culture of microalgae emphasis only a few facets of the physical culture system and/or the biology. In reality, and because of the complex feedback processes that develop, an appreciation of all components is required. The system is highly dynamic, and things can happen, and go wrong, very quickly. Experiments, and especially large-scale experiments, are expensive in resources and time. An adequately constructed simulation platform, however, allows *in silico* experiments to be conducted quickly and safely.

Initial chapters (Part I) describe the critical components of the physical-chemical system used to grow the organisms, and also provide an introduction to the physiology of the organisms that are of importance to growth dynamics. Part II of the book is devoted to the construction of simulation platforms (model) with which the reader can explore the implications of changing different abiotic and biotic components of the system. Rather than just provide an “all-singing-all-dancing” model, the reader is led through a series of simpler models to provide a background level of understanding for this complex topic.

This text is produced in support of the Decision Support Tool development of the ERDF Atlantic Area project *EnhanceMicroAlgae* (2017-2021). There are also free-to-end-user models available at the project website (<https://www.enhancemicroalgae.eu/>).

If any errors or problems are encountered, please contact the author at [kjfp plankton@gmail.com](mailto:kjfp plankton@gmail.com).

## 1. General Introduction

This introduction gives a general overview of the topic; details are given in subsequent chapters.

### 1.1 A justification to the role of simulations in microalgal cultivation

Growing microalgae has attracted commercial interest for many decades. Few of those companies that started have managed to stay the course. Most that have survived have grown a crop for a very specialised yet robust market (*Spirulina* springs to mind). There has, however, long been a view that it must be possible to grow microalgae in some form of microbial-factory scenario, making use of waste nutrient streams (and thus helping to clean water) to support the growth of organisms under different ways to make best advantage of the flexible and rapid growth potential of these organisms. Such a view has emerged especially spurred on by interests in microalgal biodiesel, with the suggestion of microalgal based biorefineries (Greenwell et al. 2010).

The purpose of this work is not to provide a guide to making money from microalgae *per se*, but rather to provide a simulation platform that will enable those interested in entering this arena, and also those within it, with which they can explore different facets of the technology.

Simulations provide a way of quickly and relatively cheaply exploring (and usually rejecting) concepts. Most emphasis in the literature on modelling microalgae for biotechnology centres on the physics and chemistry rather than on the biology. This is, in the mind of the author, a mistake. The real challenge is in understanding and then exploiting the physiological flexibility of the organisms. Far too often the emphasis on non-biological aspects (such as the design of culture facilities) has been confused by using unrealistic biological input values, or biological models that so misrepresent the behaviour of real organisms that the conclusions may be brought into doubt. Scale-up is also a major challenge in microalgal biotechnology; exploitative processes that seem viable from calculations extrapolating from small laboratory flask systems fail to make the transition to the real world where Kg or tonnes of produce are required, rather than mg quantities.

For those who wish to explore modelling ecology in more general sense, and after all a bioreactor containing a growing algal suspension is an ecological system, please check the contents of the authors' companion volume, "*Dynamic Ecology*" (Flynn 2018). That book is available via <https://cronfa.swan.ac.uk/Record/cronfa40405>.

### 1.2 Target organisms

The target organisms of this work are phototrophic microalgae. While some facets of what follows also apply to the growth of purely heterotrophic microalgae, phototrophy presents various critical overriding features upon the commercial exploitation of these organisms. The mixotrophy (coupling of phototrophy and osmotrophy) of these organisms is also considered.

"Microalgae" is a collective generic terms for a very diverse group of mainly unicellular organisms that only share two features:

- i. They are microbial, requiring a microscope to observe them in any detail. Most cells are around  $10\mu\text{m}$  in diameter ( $1\text{mm} = 1000\mu\text{m}$ ).
- ii. They are algae, from which it is typically inferred that they contain pigments with which they can perform photosynthesis.

Microalgae are taxonomically extremely diverse, though the first split is between:

- prokaryote (bacteria-like) cyanobacteria; also called blue-green algae
- eukaryote protists

Some of these organisms have particular physiological characteristics that can be exploited, or on the converse may present challenges. For example:

- some cyanobacteria can (when starved of other N-sources) fix  $\text{N}_2$ -gas
- most diatoms (a group of protists) have cell walls of silicate
- many non-diatom protists are motile, and die if they lose their flagella in turbulent mixing. Many of those species in nature are also mixotrophic through combining phototrophy and phagotrophy (i.e., they eat their competitors and other organisms)
- fatty acid and/or starch content is highly variable between species and also (critically) varies with the nutritional state of the organism
- bacteria represent essential contaminants in many cultures (removing them can decrease growth rates as they produce critical biochemicals)

And so on.

While microalgal physiology has a long and rich history in academic research, much of it is confusing and liable for misinterpretation by the un-initiated. This is complicated further by the periodic renaming of organisms, and because strains and clones of the same species (especially when maintained in culture for many years, during which they mutate) rarely behave in the same way.

Microalgae and their physiology are explored in more detail in Chapters 2 and 3.

### 1.3 Biomass yield vs productivity

A common mistake in this subject arena is to confuse the algal biomass held within a culture vessel with productivity. In part this is perhaps a historic overlap with terminology used in a terrestrial agricultural context; yield of wheat or rice per hectare is viewed as a single crop gathered once, or perhaps twice, a year. This would give a productivity value of  $x$  tonnes per hectare per year. However, the time unit is often ignored, and the emphasis placed solely on the biomass at the time of harvest. This analogy is not helpful when considering microalgal cultivation.

The growth rate of microalgae is such that the biomass can, under optimal conditions, double every day or so. In a laboratory system, whole cultures (flasks or similar vessels) are often harvested, and emphasis is placed upon the amount of material collected at that time. The culture systems are then started over with an inoculum from a starter-culture of perhaps 2% of the volume of the main system. However, in operating a large bioreactor a partial harvest is more likely.

While achieving a high biomass is certainly important, what is at least of equal importance is the rate of productivity. Productivity of what is an allied and equally important issue. As an example, consider the topic of microalgal biofuels production:

The biochemical constituents of microalgae required for biodiesel production are the fatty acids accumulating in cells that are starving of N-nutrient. However, the growth of microalgal cells requires sufficient light and nutrient. A high biomass of microalgae self-limits growth by light; each cell shades light from its neighbours. And, as high biomass growth requires sufficient N-nutrient, then clearly there is a conflict between the growth and production of biomass, versus the synthesis of the fatty acids required for the support of biodiesel production. To optimise production thus requires an understanding of the physiology of the organisms as well as the physics of the systems (Kenny & Flynn 2017).

#### 1.4 Enhancing microalgal productivity

The overwhelming challenge in the commercial exploitation of microalgae centres upon maximising production rates. Specifically we need to maximise areal and volumetric production rates (AP and VP respectively). What does that mean?

Areal production is the rate of biomass produced per area (i.e., the foot-print of the facility) per day. Area is important in financial terms because it relates to ground-rental costs. Many workers measure biomass in terms of fresh or wet weight. Far better, and more meaningful from a simulation modelling perspective, is to define that growth in terms of carbon, so units for AP are as (for example)  $\text{gC m}^{-2} \text{d}^{-1}$ . C is the base for organism growth, C-metabolites control organism physiology,  $\text{CO}_2$  consumption is of importance from a “green economy” perspective, and so on. C-biomass can be estimated from dry weight or from biovolume (that is the product of {cell numeric abundance}  $\times$  {cell volume}).

Volumetric production refers to the rate of biomass production per volume of water per day (e.g.  $\text{gC m}^{-3} \text{d}^{-1}$ ). Volume is important as it relates to the consumption of water, nutrients, and the cost of harvesting etc.

In an ideal world it would be best to maximise both AP and VP, growing dense “pea-soup” suspensions. However, very quickly these ideals become self-defeating. A “pea-soup” suspension absorbs so much light that the growth of individual cells is light-limited. Not only is this bad in itself (decreasing productivity), but light limitation restricts or even prevents nutrient exhaustion, and that limits the flexibility of the production facility to provide different metabolites. Optimising AP and VP, while also providing metabolic flexibility is readily explored using simulations.

#### 1.5 Decision Support Tools

The inherent complexity and the roles of feedback processes in the physiology and culturing of microalgae make predicting what may happen very difficult. With knowledge, however, physiological responses to transient changes (such as changes in light or nutrient supply) may be exploited. It is for such reasons that mathematical models supporting simulations of microalgal growth may be of use.

What is a simulation vs a model? A model is a simplification of reality (often an extreme simplification, exemplified by a regression line through data), while a simulation has two important facets:

- i) a simulation requires that time as a variable – a simulation is not a simple steady-state representation; if you disturb the system something happens over the following period of study.
- ii) by definition a simulation must represent reality; and that capability can be exploited for “what-if?” analyses.

Simulation models are also excellent platforms for exploring financial consequences and viabilities. Further, depending on the software platform, you can explore the risks of operating the commercial facility in different ways. This is important, because all biological systems are temperamental, and certainly that is true of microalgal cultivation systems.

### 1.6 Concluding comments

This text provides you with information on the building and operation of *in silico* platform for exploring microalgal growth in the context of commercial or commercial-facing interests. The emphasis is on optimising production of biomass under nutrient sufficient or nutrient deplete conditions; irrespective of the details of the organism and the product that interests you, optimising production is ultimately the target.

In working through this book you will perhaps learn much about microalgal physiology. While this text is not specifically intended for that purpose, even those established researchers in the subject are often experts in only certain facets of the topic. A real benefit of building and operating simulation models is that the whole complexity and synergistic interactivity of the biological and non-biological systems come together. The approach is thus very powerful, though limited by the complexity of the models.

Chapters in Part II develop a theme and offers suggestions for experimentation. Unlike real systems, you cannot break anything, results come through very fast, and it will not bankrupt you either. The models described herein are available in a form that can be edited and modified using a commercial software platform. However, you can experiment and learn much from exploiting the free-to-use models. To use these models, you need to download the free Powersim Studio Cockpit from [https://www.powersim.com/main/download-support/technical\\_resources/service\\_releases/studio10cockpit/](https://www.powersim.com/main/download-support/technical_resources/service_releases/studio10cockpit/). Some of these models provide simple demonstrators for concepts; it would be best if you played with those models before moving on to the complex models.

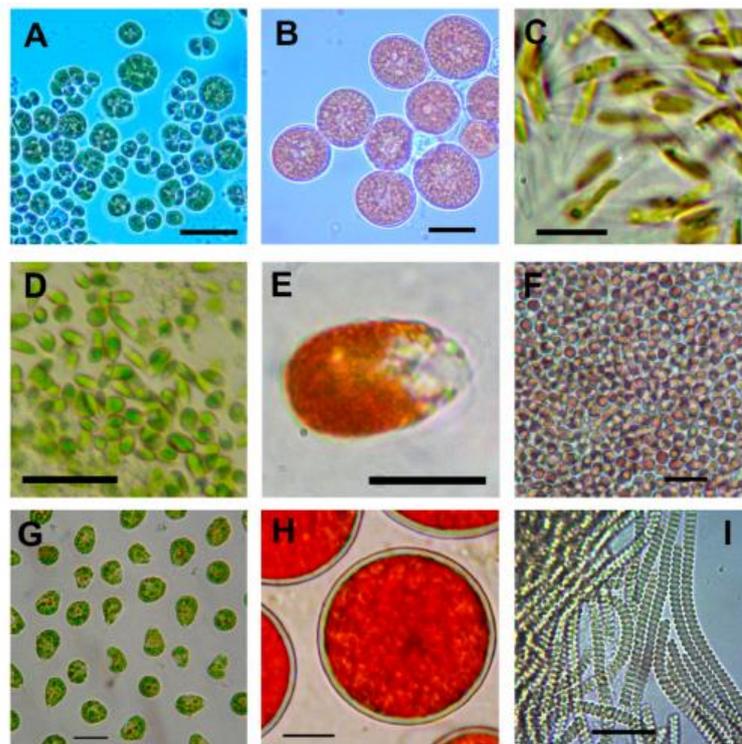
The models provided here are not described in great mathematical detail. What is provided are explanations for the conceptual basis of the mathematics. For those interested, the full code is available for each model, as is a version of the model that can be opened and modified/developed using the Powersim Studio platform. Anyone who is adept enough to explore the code will be able to work out how it functions; please also explore the companion e-book on Dynamic Ecology (Flynn 2018).

All the biological descriptions are based on peer-reviewed published research papers by the author and colleagues.

## 2. Microalgae – a (very) brief introduction

### 2.1 Introduction to microalgae

The term “microalgae” is used as a generic term to describe any microbial-scale “green” photosynthetic organism. Microalgae include prokaryote (bacteria-like) cyanobacteria, and also eukaryote protists. In reality, the genetic breadth of the organisms that are collectively termed “protist microalgae” approaches or exceeds that of all the other eukaryote (non-bacterial) life forms considered together. In short, the bucket term that describes “microalgae” is truly vast in its breadth. Of these, only the merest fraction (a few 10’s of species) have been considered from a commercial or biotechnological standpoint; Figure 1 gives some idea of the types commonly used.



**Fig. 2.1** Light micrographs of selected microalgae for commercial cultivation for various biotechnological applications. *Chlorococcum* sp. (A,B); source for mixed carotenoids including -carotene, astaxanthin, canthaxanthin, lutein), *Dunaliella salina* (D,E); the source for  $\beta$ -carotene) and *Haematococcus pluvialis* (G,H); the source for astaxanthin) are cultivated as two distinct growth phases: (1) green-phase ((A,D,G); for biomass generation) and (2) stress-phase ((B,E,H); for carotenoids and fatty acids accumulation). (C), *Phaeodactylum tricornutum* (the source for essential fatty acid EPA), (F), *Porphyridium cruentum* (source for natural pink colourant phycoerythrin and bioactive polysaccharides) and (I), *Arthrospira* (Spirulina) sp. (source for natural blue colourant phycocyanin and multiple health benefitting ingredients) are cultivated as single-phase actively growing biomass for the targeted biomolecules. All scale bars are 25  $\mu$ m, except for (C,E), which are 10  $\mu$ m. Figure and legend from Saha & Murray (2018)

Much of what follows has little impact on the construction or operation of simulation platforms for exploring the commercial growth of microalgae. Nonetheless, there are some basic features, and terminologies, that warrant introduction.

First a note of warning.

Some confusion may be caused by reference in the literature to non-photosynthetic “microalgae”. Protists may or may not need to perform photosynthesis, to generate at least certain key cellular components through photochemistry-linked biochemistry. “Microalgae” as a term specifically draws attention to an ability (if not an obligatory need) to engage in plant-like growth; it is perhaps disingenuous to refer to non-photosynthesising protist cultures as containing “microalgae”.

**In this book, “microalgae” specifically implies phototrophy. It should be noted, however, that both cyanobacteria and protist microalgae have potential to be mixotrophic by combining phototrophy and osmotrophy (the use of dissolved organic nutrients). Some of the protist microalgae may also have potential to engage in phagotrophy (feeding on particles, typically on bacteria, cyanobacteria or other protists).**

## 2.2 Microalgae vs Phytoplankton

Some 50% of the oxygen you are breathing right now was produced by the activity of microalgae growing as free-floating organisms in the ocean. These photosynthetic organisms are termed “phytoplankton”. Some phytoplankton just float, some can swim; none, by the definition of “plankton”, can move against the tides and currents. However, microalgae do not have to be planktonic; they can grow on, or even in, other substrates. Thus, microalgae may often grow in biofilms, on stones in rivers, on walls, on the sides of bottles, and (importantly for polar ecology) also within ice. For the bulk of commercial applications, however, microalgae are grown in suspensions, *de facto* as phytoplankton. However, growth of microalgae on surfaces (on bioreactor walls) is a nuisance that adversely affects commercial activity.

## 2.3 Prokaryote vs Eukaryote

### Prokaryote microalgae

Prokaryotes are bacteria-like organisms that lack internal compartmentalizations; no mitochondria, no chloroplasts, etc. Cyanobacteria, or blue-green algae (so called after the colour of the cyanophycin they contain) are prokaryote microalgae. They are bacteria that contain membranes arranged to hold light-absorbing pigments and the biochemical wherewithal to convert photons of light into chemical energy to support CO<sub>2</sub>-fixation (photosynthesis).

Most cyanobacteria, and all protist microalgae, use fixed (usually inorganic) forms of N-nutrient. Some cyanobacteria, however, can also fix N<sub>2</sub> gas into intracellular ammonium. These “diazotrophs” may either grow in filaments of cells where some cells fix CO<sub>2</sub> and others fix N<sub>2</sub>, or as single cells that separate the processes between light and dark phases of the day. The biochemical challenge that they face is that the processes of CO<sub>2</sub> and N<sub>2</sub> fixation cannot occur simultaneously within the same cell (and noting that prokaryotes lack internal structures with which they could separate conflicting chemical reactions) because a by-product of CO<sub>2</sub>-fixation (namely O<sub>2</sub>) poisons the

enzyme nitrogenase that fixes  $N_2$ . The process of  $N_2$ -fixation is also very expensive biochemically; diazotrophs only fix  $N_2$  if there is insufficient inorganic N (as nitrate or ammonium) in their growth medium.

No cyanobacterium is motile (while many bacteria are), but various species are buoyant either directly with gas vacuoles, or indirectly by their filamentous biomass trapping bubbles of  $O_2$  released during photosynthesis.

### Eukaryotic (protist) microalgae

Eukaryotes are organisms with cells that contain internal compartments, such as mitochondria and (of particular importance here) chloroplasts. Eukaryotes include all so-called higher life forms, from trees to humans. Eukaryotic microalgae are protists, and the original protists were heterotrophs through osmotrophy (using organic nutrients rather like current-day yeasts do), or phagotrophy (eating by engulfing food particles). The original protist microalgae evolved through acquiring the ability to photosynthesise from their prey. Thus, the first step was of eating a cyanobacteria, but rather than digesting it the prey were retained and continued to photosynthesise within the protist. It is no coincidence that the structure of chloroplasts resembles that of cyanobacteria. Later some of those photosynthetic prey were themselves protists, and following their assimilation into predatory protists additional layers of membranes and other biochemical features differentiated the developing evolutionary lines of what we see today as phototrophic protists. A taxonomic diagnostic feature of modern-day protist microalgae is the number of membranes around the chloroplast and the arrangement of the photosynthetic membranes (thylakoids).

Today we see protists that are still wholly phagotrophic (protozooplankton), and some which are wholly phototrophic (phytoplankton). Most, however, we now realise are actually photo-phago-mixotrophic being able to eat and photosynthesise (Mittra et al. 2016). Despite this mixotrophic potential (realised by combining phototrophy and osmotrophy and/or phagotrophy), most of these pigmented organisms, protist microalgae, are studied and grown as pure phototrophs. It is suspected that this culturing technique leads to the rapid loss of phagotrophy in organisms isolated from nature, while the emphasis on cultures as being axenic (unialgal, bacteria-free) has also restricted the number of species available for commercial exploitation to a small fraction of the real genetic diversity.

Protist microalgae are typically motile, though some important groups are not (notably the diatoms, which mostly have cell walls of silicate rather than of cellulose-like material). Some protist phytoplankton can swim using their two flagella (or in very small species, just 1 flagellum) up to 10m vertically every day to obtain light at the surface or nutrients/food at depth; not bad going for an organism of perhaps 0.01mm diameter. In culture, however, this motility is usually not seen due to the turbulent water conditions; indeed too much turbulence can kill some protist microalgae through removing their flagella.

## 2.4 Size

“Microalgae” are typically (by definition) microbial in size; that is, their surface (and internal) features cannot be observed by the naked eye. Indeed, little detail can be seen using a light microscope either. Most microalgae are also unicellular, growing as a single cell. A “typical” microalga is around 5-30  $\mu\text{m}$  in diameter; there are 1000  $\mu\text{m}$  in 1 mm, so 100 cells of a typical species would form a line just 1mm long. However, many tens or thousands of cells may clump together or form chains that are not only plainly visible to the naked eye, they may actually form a mass that can hinder pumping operations. Motile cells may also congregated at the surface of a flask of water, or (motile or non-motile cells) may appear as a mass on the bottom of a flask. Gentle swirling or other agitation (including aeration) will disperse any aggregations unless they are adhering to the vessel sides as a biofilm.

The shape of microalgae varies from spheres to pear-shape, long needles, double-bun shapes, and to weird asymmetric forms. Flagella (or in the very smallest motile species, just the one flagellum), if present may be apical or emerge from a more central location; the latter positioning (as in cryptophytes) make the cells swim in a wobbly fashion. Size may be reported as an “equivalent spherical diameter”, ESD; this considers the volume of the cell as a sphere, irrespective of whether it actually is, and converts that to a diameter (through manipulation of  $V=4/3\cdot\pi\cdot r^3$ , where  $r$  is the cell radius, and  $V$  is its volume).

The size and shape, together with the production of mucus, affects how the cells may be separated from their growth medium during harvesting or water purification. Long thin cells pass through meshes (filters) that would not allow passage of an equal-volume spherical cell, while conversely clumps of cells, especially with mucus, can block filters that may be expected to otherwise permit their passage.

Cell size, and to a lesser extent shape, is often affected by nutritional status. Thus, microalgae whose growth is limited by light or by availability of nitrogen nutrient tend to be smaller than normal, while those limited by availability of phosphorous are larger (and often also sticky, so they clump as well).

## 2.5 Colour

An obvious feature of microalgae is their colour. All phototrophic species contain the green pigment chlorophyll.a. This is a key photopigment in the biochemistry of photosynthesis, and a special form of this pigment, Chl.a<sub>P700</sub>, acts as a conduit for light energy collected by other chlorophyll molecules and from secondary pigments.

Chl.a absorbs light mainly in the red (ca. 650nm) and blue (ca. 450nm) sections of the visible spectrum. In doing so the pigment leaves green as the dominant visible spectra of light, hence microalgae containing mainly Chl.a appear green. To make additional use of photons of light in this “gap” in the visible spectrum, microalgae have secondary pigments. In eukaryotes, these include other chlorophylls and carotenoids; these typically confer a golden-orange colour to the organism. In cyanobacteria, major secondary pigments are phycocyanin (blue-green) and phycoerythrin (pink). These phyco-pigments are heavily proteinaceous and if the cyanobacteria are deprived of nitrogenous nutrition the organism degrades the pigment; such a change in colour can occur over a few hours and gives a ready indication of changes in cell nutrient status.

Microalgae also contain so-called sun-screen pigments, mycosporine-like amino acids (MAAs). These protect the organisms' DNA from UV damage. The concentration of both MAAs and of the photopigments reflect not only the radiant light levels (for protection) but also light-limiting conditions (where light-limited cells produce more pigment to capture more photons).

Different combinations of pigments can render a range of colours far beyond simply "green" or "golden-brown". Microalgae have been grown commercially to harvest pigments such as  $\beta$ -carotene and phycocyanin (as food colorants) and MAA (for making sun-lotions).

## 2.6 Photosynthesis

Photosynthesis, the fixation of CO<sub>2</sub> into organics (initially as sugars), requires light of the appropriate quantity (not too low, not too high) and quality (light in the visible spectrum), photosystems to capture photons and convert the energy into chemical energy (ATP and reductant), and also the enzymes of the Calvin cycle (most notably Ribulose biphosphate carboxylase; RuBisCO).

RuBisCO is arguably the most important single enzyme on Earth and, on account of it being a rather inefficient enzyme, it is also likely the most common enzyme as well. Importantly, the activity of this enzyme effectively limits the potential growth rate of phototrophs (Flynn & Raven 2017).

The whole photosynthetic machinery is subject (like all biochemical processes) to close regulation, but the main problem a phototrophic organism has is that it is not possible to modulate the biochemical machinery at the same pace as changes often occur in light. Too little capacity and the individual grows slightly slower (less competitively) than its neighbours; too much capacity and if light becomes too strong or nutrients become limiting then there is too much energy coming into the cell and damage occurs. With too much light, initially cells become photo-inhibited, but photodamage and death develops shortly after, as a function of accumulated photon dose. Too much photosynthesis can also result in super-saturation of O<sub>2</sub>, which is both directly dangerous for the cell and also inhibitory of CO<sub>2</sub>-fixing RuBisCO activity.

## 2.7 Nutrients and stoichiometry

Microalgae need other nutrients than just C. Nitrogen (N) and phosphorous (P) are quantitatively the next most important elements. Dissolved inorganic C (DIC; as carbonate, bicarbonate and dissolved CO<sub>2</sub>) is present in seawater at about 2mM concentration, and usually much less in freshwaters. Inorganic N (as ammonium or nitrate) is often supplied to cultures at around 1mM, though ammonium (the major N source present in anaerobic digestate liquor) is often toxic at levels above a few 100  $\mu$ M. The solubility of P (as phosphate) is limiting in seawater cultures, as phosphate precipitates out of solution above a concentration of ca. 35  $\mu$ M (depending on salinity and temperature). Silicon (Si), needed by diatoms, can also be problematic in culture medium, readily precipitating in certain marine media. On exhaustion of Si, diatom cultures can just crash, disappearing overnight as the cells collapse. The exception is the commonly grown diatom *Phaeodactylum*, which lacks any significant Si in its wall (that which it needs is often supplied from the dissolution of silica from the culture vessel glass into the slightly alkali sweater media). As we will see below, the ratios of these concentrations does not align well with that of algal biomass. This requires that dense cultures may need nutrients to be bled in so that residual concentrations are

not too high. CO<sub>2</sub> is usually bubbled in (often as CO<sub>2</sub>-enriched air); not only does this enable continuing photosynthesis but, as DIC also buffers the acidity-alkalinity of water, it is also vital to maintain the correct pH for growth.

Iron (Fe) is an important and potentially limiting nutrient unless a suitable chelating agent is used. When life evolved on Earth the planet atmosphere and waters were anaerobic and Fe-salts are soluble in such waters. However, microalgae were responsible for the greatest environmental disaster to ever impact Earth, by changing the environment to an oxidising one. This oxidation led to the formation of Fe-oxides, which are poorly soluble in water. Chelating agents (from the Greek for claw) help to keep Fe available in suspension for microalgae to acquire this element. In nature, chelating agents include organics leaked from degrading plant biomass (such as tannins); in cultures an artificial chelator such as EDTA is used. Without sufficient Fe, photosynthesis, respiration and synthesis of the enzymes of nitrate reduction are restricted.

Vitamins (especially B-group vitamins) and other cofactors (e.g., nickel is needed for the enzyme urease, to enable a microalga to exploit urine as a N-source) must also be supplied. Excess organic cofactors can promote the unwanted growth of bacteria, or fungus.

The ratio of C:N:P within organisms, referred to as the stoichiometric ratio, is highly variable in phototrophs and is especially so within microalgae (Geider & LaRoche 2002). The C:N:P ratio affects both growth rates and the chemical quality of the biomass; a high C content indicates an excess of carbohydrate and/or fatty acids, and a relative lack of proteins. The actual biochemical composition of the cells is largely reflected in commercial terms through taxonomic differences in carbohydrate and fatty acid content. These differences can be increased by careful exploitation of the impacts of nutrient stress.

Microalgae, like all phototrophs, also readily produce secondary metabolites. Primary metabolites are the protein amino acids, the nucleic acid bases and the suite of standard fatty acids and allied lipids. Secondary metabolites are other organic compounds that are not components of the major biochemical pathways. In most instances, the role (if indeed there is one) of these secondary metabolites is unknown; at least some appear as over-flow chemicals produced when normal biochemical processes are disturbed through imbalances in light and nutrient supply. They can, from a human perspective, be rather inert or useful (such as caffeine) but they can also be highly toxic (such as shell-fish toxins). The usefulness of secondary metabolites in medical science, in particular, is a subject of great interest. To optimise production of what are typically just a few fractions (<<1%) of total biomass, or possibly released (leaked) chemicals, requires close control over the growth of the organisms to exaggerate production of secondary metabolites.

An often neglected product of microalgal growth is released organics. These are compounds that are perhaps leaked rather than actively pumped out. They include sugars and amino acids, but all manner of (uncharacterised) other organics can accumulate in the water. Some 10-20% of C-fixation may be leaked allied with N and/or P depending on the stoichiometry of the chemicals.

## 2.8 Growth rates

Growth rates of microalgae do not even approach those of bacteria such as *E.coli*; microalgae may be “microbes”, but growth is rather slow. While *E.coli* has a generation time under optimal

conditions of ca. 20min, a typical microalga will double its biomass in ca. 24hs. Indeed, many synchronise their cell cycle to day-night (Nelson & Brand 1979), so they increase in biomass during the day with photosynthesis and go through the cell replication cycle during darkness.

Some microalgae can replicate much faster than this, but the activity of RuBisCO sets a limit to C-fixation of a few divisions per day (Flynn & Raven 2017). There is a problem then of claims in the scientific and grey literature of much higher growth rates. These most likely arise because of a misunderstanding of how to measure growth rate. This needs to be determined by an increase in C-biomass and not by any other approach. Growth may also be enhanced over short periods (ca. <6hrs) by raising the temperature, exploiting the potential doubling in enzymatic rates per 10°C (so-called  $Q_{10}=2$ ) before the enzymes denature and cell death ensues.

Another common misunderstanding is generated by use of the term “logarithmic” or “exponential” growth. Many reports do not actually determine this value correctly at all, or do so over an insufficient period (ideally several days) to produce a robust estimate of real growth rate. Only very thin cultures of microalgae can actually grow in true exponential phase at a maximum rate. Usually culture growth is linear because growth becomes self-limiting through self-shading as the increasingly dense culture cuts out light to the individual cell. This event is readily seen in simulations, and is a factor of importance that often surprises the uninitiated.

“Exponential production rates” and similar terms can also be confusing. Growth of the organism is of lesser importance during commercial production than is “growth of the product” (which, while it may be the whole biomass, is more often a mere fraction of it). A classic example of this confusion is the production of biodiesel by microalgae. Biodiesel is produced using fatty acids synthesised by microalgae primarily when they are entering N-deplete growth. This is a period when C-biomass-growth is slowing but N-specific growth may have completely halted; algal C:N thus increases. To maximise production of fatty acids requires a balancing act between nutrient limitation and continuing growth of microalgae in optically thin suspensions (maximising light for the individual cell) (Kenny & Flynn 2017).

## 2.9 Conclusions

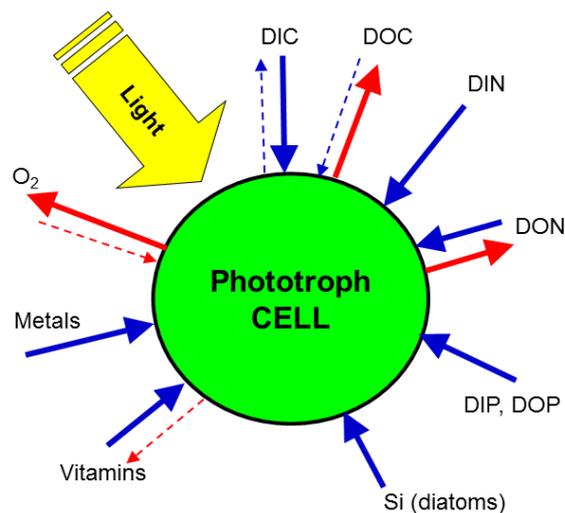
It will be apparent from the above that optimising the growth of microalgae is non-trivial. And this is before considering the vagaries of the weather for culture systems that rely on sunlight. There are additional issues of concern, or perhaps of interest, such as the growth of multi-species systems where competition and allelopathy (chemical signalling or interferences) develop, or for systems subjected to the entry of predators and disease. Developing simulation models provide approaches to explore options that would be costly in time and certainly financially through other routes. If the model does not describe what happens in reality then this indicates a gross failure in understanding of the commercial system being explored.

## 3. Algal Physiology

### 3.1 Introduction

In this chapter, we consider the key components of algal physiology that typically require representation in models. There are many facets of the physiology of any organism. As phototrophic protists and cyanobacteria, the physiology of “microalgae” is inevitably tightly bound to photosynthesis. However, that process (just the core enzyme, RuBisCO can account for 20% or so of cell-N) requires the acquisition of N, P, Fe and other factors as well as light and DIC.

Figure 3.1 gives an overview of the inputs and outputs of microalgal growth; in addition to those indicated, there is of course the major output which is biomass growth, and the consequential growth in cell numbers. Although indicated here as photosynthetic, with an input of light, growth is often not in continuous light. In darkness, unless organic substrates are being used to support heterotrophic growth, there is a loss of some portion of biomass previously accumulated during phototrophic growth in the light.



**Fig. 3.1.** Schematic of resource needs (arrows in), and releases (arrows out), from a phototrophic microalgal cell. Dissolved inorganic C (DIC) is consumed with light-enabled photosynthesis. Flows of O<sub>2</sub> are the converse to those of DIC; a net release of DIC occurs when respiration > C-fixation. A proportion of C-fixed is released as dissolved organics (DOC); many cells show an ability to acquire DOC during darkness and can grow either heterotrophically or mixotrophically. Nitrogen enters as dissolved inorganic (DIN) and dissolved organic (DON forms); many cells leak DON in the form of especially amino acids due to high internal concentrations, and can also use amino acids to provide heterotrophic or mixotrophic support of growth. Phosphorous is taken up as dissolved inorganic phosphate (DIP) but most if not all cells express an extracellular phosphatase activity when they are deprived of DIP, and can then use organic P (DOP). Diatoms use silicate (Si) for their cell walls. Many cells require vitamins; some may release them. Of the metals, iron (Fe) is quantitatively the most important.

Normally organism physiology operates to balance supply-and-demand, but for commercial applications there are times when the operator deliberately disturbs the balance of physiological processes to accentuate production of key metabolites. Through molecular biology approaches, the normal biochemical regulation can be further manipulated to enhance (over-express) or depress selected facets of growth or of specific metabolites.

The breadth of physiologies, culture system operations and commercial interests presents a myriad of combinations. It is not possible to explore even a meaningful fraction of those options empirically, but with models (provided you have trust in the model structure) you can easily, and very quickly, and inexpensively, work your way through them. The emphasis is thus on developing simulation platforms that can be readily modified to best fit the interests of the reader.

### 3.2 Temperature, salinity, pH, DIC and O<sub>2</sub>

Temperature affects all living entities. At the extremes, temperature may be so low that effective growth does not occur, or so high that proteins and lipid bilayers are denatured and death occurs. Between these extremes there is an increase in physiological rates (and thence of growth) with temperature that accords with the Arrhenius equation. In simple biological terms, and as required here, this relationship follows the form of:

$$\mu_T = \mu_{RT} \cdot Q_{10}^{[(T-RT)/10]}$$

Here,  $\mu_{RT}$  is the growth rate at the reference temperature RT,  $Q_{10}$  is a multiplier that defines how much faster is growth when temperature is elevated by 10°, and  $\mu_T$  is the resultant growth rate at temperature T.  $Q_{10}$  typically has a value around 2, so within a certain range, the growth rate doubles for a 10°C increase in temperature. In reality, the useful range of this relationship is ca. 10-25°C, and may be less. While the initial elevation of  $\mu$  with temperature is smooth, as per this equation, as it approaches a maximum value there is a sudden change in the relationship, and then a precipitous decline (with cell death) all of which may happen over a change in temperature of 5°C or so.

Different biochemical processes also exhibit different  $Q_{10}$  values; the light reactions of photosynthesis may have a quite different (lower)  $Q_{10}$  than those of the dark reactions and of other heterotrophic processes. Changes in temperature can thus be seen to have significant impacts on the growth of microalgae. In open shallow ponds, temperature can change significantly over the day. This can be to advantage, as higher day-light temperatures favour photosynthesis, while cooler night-time temperatures decrease respiration-linked loss of biomass in darkness. Evaporation of water from the pond during the day can mitigate temperature increases, but if the pond contains saline water, then salinity will also increase and non-saline water may need to be added to compensate.

Microalgae can grow at different salinities, and can do so often showing significant flexibility. Growth at elevated salinity promotes the production of extra osmoticums; these help the cell balance the osmotic pressure. The ability to grow at different salinities can be exploited to promote production of certain biochemicals (especially those used as osmoticums), to minimise growth of competitors or disease organisms. A classic biotechnological exploitation of this is the growth of *Dunaliella* sp. at high salinity which is used as a means to commercially produce glycerol, which is the osmoticum for this organism. Many marine species will grow at least as well, if not better, at ca. 50% seawater salinity; this may be because they waste less resources synthesising osmoticums.

Acidity has an important impact on microalgal growth. Acidity is typically described using the inverse logarithmic scale of pH. It is important to remember that a change in pH units of 1 means there is a 10-fold change in actual acidity, of the concentration of protons (i.e., of  $H^+$ ). And the change in  $H^+$  expressed as a change in pH of 0.2 units thus varies greatly depending on the starting pH. Proton gradients across cell membranes are of critical importance for biology, and the growth of microalgae itself changes the pH of the growth media. As they remove  $CO_2$  for photosynthesis so the pH increases (the water becomes more alkaline) and this can eventually halt growth and even kill some species. Furthermore, the dissolved inorganic C equilibrium (carbonate  $\leftrightarrow$  bicarbonate  $\leftrightarrow$   $CO_2$ ) buffers the pH, so as  $CO_2$  is removed so the buffering capacity decreases and subsequent changes in pH are even more likely. This has potential to change species succession (notably, in the context of ocean acidification, where the pH of seawater is decreased in consequence to atmospheric  $CO_2$  dissolving into the oceans; Flynn *et al.* 2015). There are additional (more modest) changes in pH through consumption of ammonium-N (pH decreases) and even lesser changes with consumption of other nutrients. Similar events, especially because of the high nutrient levels, can occur in mixed algal ponds. Preventing such changes, however, is relatively easy: the bioreactor simply needs a pH-linked  $CO_2$  injection or aeration system, that compensates for  $CO_2$  removal. Aeration also removes excess  $O_2$  during the day (which is inhibitory for  $CO_2$ -fixation), and adds  $O_2$  during darkness when a dense microalgal suspension could draw down  $O_2$  levels to dangerously low (anoxic) levels, especially in warm culture systems where gas solubility is decreased.

### 3.3 Algal growth dynamics

Microalgae typically, but not always, increase in abundance through a process of binary fission. A cell grows larger until it has attained sufficient size (and also sufficient time has elapsed) to enable the cell cycle to have been completed and cell division then occurs. That cell size is not fixed; depending on light, nutrient and temperature, the typical size at division varies. Further, in a culture of billions of individuals cell division may be essentially asynchronous, or on the contrary it may become entrained into part of the light-dark cycle and be more synchronous.

Cyanobacteria cells can undergo multiple forking, in which a series of rapid DNA replications occur with no significant increase in biomass. Thus, a single cell divides into 4, with a near constant total biomass. Likewise, a nutrient-starved eukaryote cell on re-supply with nutrients may either (from a small cell size) increase its biomass rapidly with no cell division, or (from a large cell size) divide with little significant biomass increase.

During the course of cellular growth various components are required. For a primary producer (phototroph), many of these components are inorganic, as DIC, DIN DIP etc. During growth, the elements associated with these nutrients are combined in different proportions and different ways to make the building blocks for cell growth (primary metabolites, such as amino acids and fatty acids). Some secondary metabolites are also produced (though these are usually of low N and P content), and may be of particular interest from a commercial perspective.

Given that the synthesis of cellular components and cell division do not occur in synchrony within a given cell, algal growth dynamics can never be in steady-state within an individual cell. However, given that there are typically millions of cells per mL ( $10^6$  cells mL<sup>-1</sup>), the system can be considered as operating as a heterogeneous, asynchronous collective. That is so unless steps are taken to deliberately generate a level of synchronicity; that may be readily achieved through manipulation of the light-dark cycle, but even so such synchronicity usually only lasts for a few cell divisions.

In crude terms, then, microalgal growth dynamics can usually be considered as following the traditional pattern of lag, log and stationary phases. In a light-dark illumination regime, that dynamic appears as a series of day-light increases in biomass and declines at night; considering cell numbers, the converse may be seen if cell division occurs (as is typical) in the dark phase.

In Figs. 3.2 and 3.3 are shown comparative operations of bioreactors of shallow and deep optical depths. These show the changes (growth) of algal C and N biomass during growth using ammonium as the N-source. Once the N-nutrient is exhausted, C-biomass growth continues (with excess C being deposited as starch and/or lipid) until the cell attains a critical minimum N:C elemental ratio. Note that from the plot of changes in biomass it is not possible to readily discern the period of exponential growth; this is, however, apparent as a straight phase in the plot of natural log ( $\log_e$ , Ln) against time. Also shown is the actual C and N specific growth rates. These systems were extremely N-stressed at time 0d (having a minimum N:C); the initial N-specific growth rate is thus very high as the cells rapidly accumulate N, and C-growth is in lag phase for the first day or so. During this time a nutrient-starved cell would be rebuilding its biochemical machinery, which would have been degraded during nutrient-starvation.

For 3 days or so (day 2 – 5) in the shallow system (Fig. 3.2), the system grows exponentially (straight sections in the Ln plot), and can be seen to be in cellular steady-state (C and N specific growth rates are the same). Then the ammonium is exhausted and N-specific growth drops to zero.

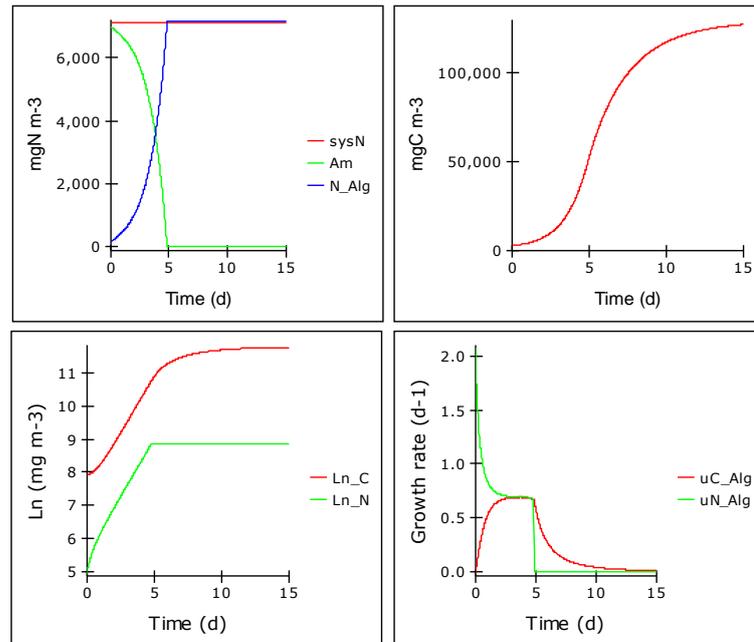
Contrast these dynamics in the shallow system with the growth dynamics in the deeper system (Fig. 3.3), where exponential growth never occurs (actually growth is linear, due to self-shading), and the cells are never in steady-state growth with balanced physiology ( $u_{C\_Alg}$  and  $u_{N\_Alg}$  are never matched and constant).

Except when under conditions of steady-state, different growth dynamics are reported depending on the parameter being used to reference the growth process. This is demonstrated in Figs. 3.2, 3.3, with reference to C, N. The same applies to Chl –specific growth and indeed reference to Chl-specific growth is particularly problematic as Chl synthesis and degradation can be very rapid in comparison with changes in C and N biomass. The situation is even worse if Chl is monitored using *in vivo* fluorescence as this signal (i.e., *in vivo* fluorescence per unit Chl) varies with nutrient status.

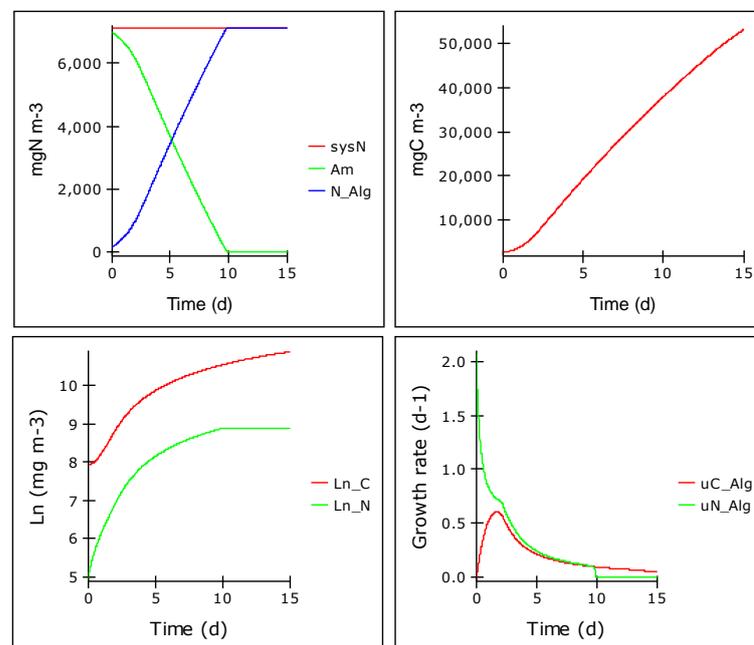
It is important to note that growth rate, technically termed the specific growth rate, has units of  $X \cdot X^{-1} \cdot \text{time}^{-1}$ . “X” could be as cell abundance, gC, Chl fluorescence, or whatever is measured.

**Providing the full units for specific growth (and not just  $\text{time}^{-1}$ ) is strongly encouraged to provide a reference for the reader.**

The lag phase of culture growth, occurring at inoculation, can be prolonged if care is not taken to balance abiotic conditions of temperature and pH in both volumes. The smoothest transition will involve large inoculum volumes of cells that are of at least reasonable physiological status (not stationary phase cells). However, such inoculation will also provide the shortest period of post lag growth before the culture approaches stationary phase again. The use of very small inoculums can result in very long lag phases, and sometimes in no growth at all. The latter may be a consequence of the disturbance of allelopathic chemical concentrations and of the balance of organic leakage and recovery from the cells (Flynn & Berry 1999).



**Fig. 3.2.** Simulated growth pattern in a system with a shallow (0.05m) optical depth, with ammonium as the limiting nutrient supplied at  $500\mu\text{M}$  ( $7\text{gN m}^{-3}$ ). Illumination is constant. The plots show, over the 15 day period, changes in external and cellular N (Am,  $N_{\text{Alg}}$ ) with a constant system N (sysN), growth of cellular C ( $C_{\text{Alg}}$ ), the natural log plot of cellular C and N ( $\text{Ln}_C$ ,  $\text{Ln}_N$ ), and the C- and N-specific growth rates ( $u_{C_{\text{Alg}}}$ ,  $u_{N_{\text{Alg}}}$ ). Compare with Fig. 3.3 for a deeper system.



**Fig. 3.3.** As Fig. 3.2, but for a deeper system, now of optical depth 0.5m. Note that growth is now linear because of the impact of self-shading within the developing culture system.

Only in batch cultures may the growth rate ( $\mu_T$ ) approach the maximum possible rate at that temperature ( $\mu_{maxT}$ ). Whether it actually does so depends on the nutrient status of the inoculum and the size of the inoculum. If the inoculum is large and of nutrient-stressed cells (e.g. from the end of a previous stationary-phase batch culture) rapid growth will not develop.

While the common perception is that growth of microalgae proceeds exponentially in a batch culture, that is actually not typically the case. Only in optically thin suspensions can exponential growth be attained; compare Figs. 3.2 and 3.3. Invariably at the types of nutrient concentrations, and thence cell densities, used in commercial platforms the rapidly increasing self-shading of cells results in a linear (and not exponential) growth style.

The balance of lag, log, stationary phases, with exponential and linear growth, also depends on the mode of system operation (batch, stretched-batch, discontinuous, continuous; see Glossary). It is also important to note that while most interest will be placed upon total biomass growth, that activity represents net growth, against the background of actual cell growth minus mortality. Cells that are stressed, and otherwise unable to grow under optimal conditions, are more likely to die. Cell death releases metabolites into the growth medium that promotes bacterial and fungal activity, and spoils the value of the algal crop.

### 3.4 Photosynthesis

Photosynthesis is the defining characteristic of phototrophic organisms. In crude terms the process is divided between the “light reactions” that convert energy in photons of light into usable chemical energy (as ATP and reductant), and the “dark reactions” that use the chemical energy to fix  $CO_2$  into sugars.

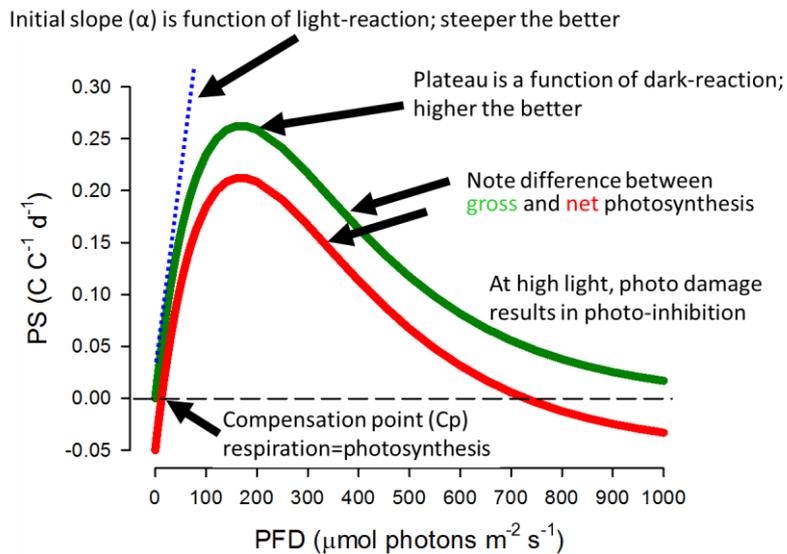
The light reaction rate is primarily a function of:

- the photon flux density (PFD) over the photosynthetically active radiation (PAR) part of the electromagnetic spectrum (which coincidentally aligns with what humans view as the visible spectrum, 400-700nm wavelength)
- the amount of pigment in the cell that captures the photons

The dark reaction rate is primarily a function of:

- the RuBisCO enzyme content of the cell (and of down-stream biochemistry)
- concentration of  $CO_2$  at the site of RuBisCO
- concentration of  $O_2$  at the site of RuBisCO ( $O_2$  is a by-product of the light reaction that competes with  $CO_2$  for RuBisCO activity)
- availability of ATP and the reductant NADPH (usually both photo-generated during the light reaction)

The relationship between light and photosynthesis is described by a photosynthesis-irradiance (PE) curve. This (Fig. 3.4) shows an initial linear section that relates to limitation at the light reaction, turning to a plateau value (relating to dark reaction limitation). After that, at higher PFD, there is often a downturn associated with photoinhibition and photodamage.



**Fig. 3.4** Photosynthesis-irradiance curve showing the relationship between gross vs net photosynthesis, limitations by “light” vs “dark” reactions. Here, respiration is shown as constant; in reality this is not so.

Note that gross photosynthesis is zero at 0 PFD, while for net photosynthesis the value is negative at 0 PFD (due to respiration), and is zero at a value of PFD termed the compensation point ( $C_p$ ). Although in this simple description respiration is shown as a constant (there is a constant difference between gross vs net photosynthesis), in reality, respiration increases with the rate of photosynthesis as the processes of anabolism increase.

In Fig. 3.4, photosynthesis is described as a C-specific term. Often it is described as Chl-specific, with units of  $C \cdot \text{Chl}^{-1} \cdot \text{time}^{-1}$ . For growth of the biomass, the C-specific term is the important value. The unit of time is also important; the daily (and not hourly) rate is important for production. This requires accounting for darkness, when respiration continues, consuming a proportion of the C-fixed during the light period. Thus, while the value of  $C_p$  is an instantaneous value, for the growth of the culture what is more important is the light dose over the whole day. The critical day-integrated light dose will thus be higher than its  $C_p$  equivalent as measured at (for example) midday.

During growth at different levels of irradiance, microalgae acclimate by altering their content of photopigments. This is photoacclimation. In crude terms, they become greener (to a limit, of course) when they grow with less light. Photoacclimation occurs to balance the supply and demand for photo-generated ATP and reductant. Too much photosynthesis leads to damage and cell death through production of superoxide radicals. Too much unused capacity also represents a waste of resources in synthesis and maintenance of the photosynthetic machinery. As a culture grows, the cell abundance increases and so each cell is shaded from the light source by an increasing number of cells in front of it. This shading prompts the individual cell to make more photopigments; most obviously Chl:C increases. Of course as each cell does this, and given that all the cells are being constantly mixed in the bioreactor, a positive feedback rapidly develops and the pigment level in each cell rises to the maximum (Chl:C tends towards its maximum value).

The nutrient status impacts the form of the PE curve in various ways. In comparison with a nutrient-replete cell, a nutrient deplete cell will likely have the following characteristics:

- less Chl:C as the cell down-regulates the need to capture light energy that it cannot safely use
- the value of C-specific  $\alpha$  ( $\alpha^C$ ) decreases – the initial slope of the PE curve decreases, though the Chl-specific equivalent ( $\alpha^{Chl}$ ) may alter rather less. Under Fe-limitation,  $\alpha^{Ch}$  is expected to change as Fe is core to the processes of the light reaction. Fe limitation is perhaps not likely in a commercial setting, but in laboratory cultures it is held in suspension with EDTA while in massive scale systems a natural chelating agent may be less efficient and super-saturating O<sub>2</sub> may also exacerbate precipitation of Fe oxides.
- less RuBisCO (which typically represents the largest single nitrogenous component in the cell); the PE curve plateau is thus lower.
- less ability to handle damaging PFDs so photoinhibition and photodamage occur at lower photon doses and occur more rapidly; the inhibition downturn is sharper.
- the respiration rate will decrease as metabolism shifts from anabolism (building new biomass) to catabolism (maintaining what is already present).

Photosynthesis needs to proceed with some degree of synchrony with the assimilation of macronutrients, such that over the day the biomass C,N,P accumulates. Macronutrients for microalgal growth include DIC (as the C-source for photosynthesis), DIN, DIP, and for the growth of diatoms (other than *Phaeodactylum*) also of Si. We assume that DIC is input into the system (typically as CO<sub>2</sub>-enriched air) at a rate to counter removal through photosynthesis; if that is not so then not only will growth be limited by DIC, but the pH of the growth medium will rapidly increase to lethal levels.

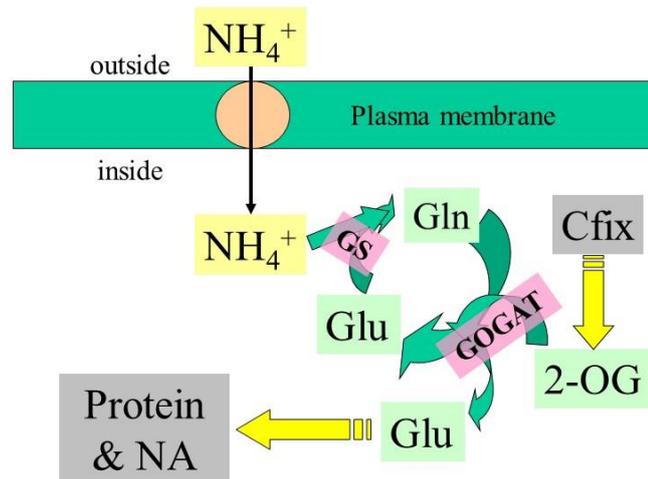
### 3.5 Nitrogenous nutrients

#### N-sources

The most common source of N used for experimentation on microalgal physiology is nitrate (NO<sub>3</sub><sup>-</sup>). However, the “preferred” source for physiology is ammonium (NH<sub>4</sub><sup>+</sup>); this is also the main component of regenerated N, such as that from anaerobic digestion. There are several important differences between these sources of DIN from physiological and operational perspectives.

- Growth using ammonium differs from that using nitrate, with various biochemical processes being repressed, cells contain higher levels of N-rich metabolites, and indeed cellular N:C is higher. These differences are associated with the fact that nitrate assimilation flows through ammonium during amino acid synthesis within the cell, and a high N-status represses the transport and assimilation of nitrate.
- The maximum growth rate need not be different between ammonium vs nitrate; this is despite the fact that the reduction of nitrate to ammonium is very expensive, accounting for ca. 20% of total photoreductant.
- Because evolution has led to microalgae being able to transport ammonium from very low concentrations in nature at high rates, at least some microalgae appear unable to control the accumulation of this substrate when exposed to the high concentrations of ammonium commonly added in cultures. In addition, ammonia (NH<sub>3</sub>), which forms an equilibrium product with ammonium (NH<sub>4</sub><sup>+</sup>), enters cells with no regulation when present at high concentration. High internal levels of ammonia/ammonium are toxic to the cells, so growth on ammonium nutrient may be poor or cell death may occur.

There are additional factors affecting diazotrophy, the fixation of  $N_2$ , into those species of cyanobacteria that possess the potential to synthesis the enzyme nitrogenase. This process is not only very expensive biochemically, but it is usually shut down by the presence (and thence assimilation) of sufficient nitrate or ammonium. There is thus a cascade of (de)repression regulatory processes; these are shown in Figs. 3.5 and 3.6.



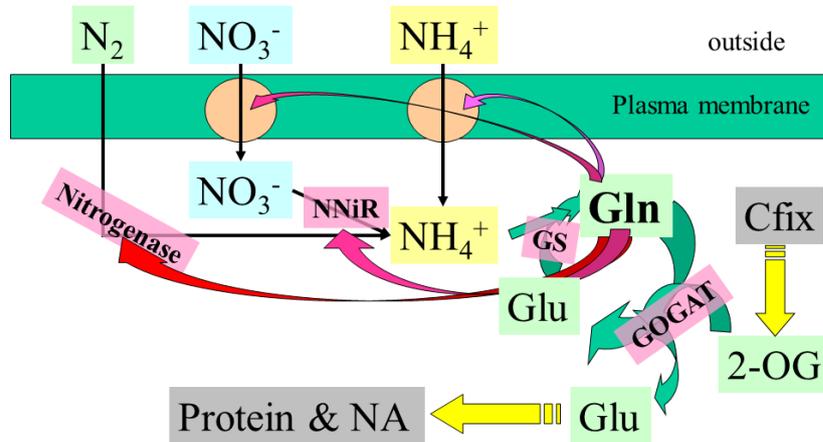
**Fig. 3.5.** Ammonium assimilation. Ammonium ( $NH_4^+$ ) is transported into the cell where it combines with the amino acid glutamate (Glu) to give the amino acid glutamine (Gln); this is enabled via the enzyme glutamine synthetase (GS). C is supplied, as shown here via C-fixation from photosynthesis, as 2-oxoglutaric acid (2-OG). Supported by the enzyme glutamine-oxoglutaric acid-amino transferase (GOGAT), 2-OG combines with Gln to produce 2 molecules of Glu; 1 Glu is syphoned off to support the synthesis of other amino acids, proteins and nucleic acids (NA), while the other Glu cycles around to assimilate the next molecule of  $NH_4^+$ .

The consequences of the emphasis on using nitrate are that operationally we know less about growing microalgae on what is more likely to be their most useful commercial N-source than we should. Entry into N-stress from ammonium vs nitrate nutrition is likely also different. Care also needs to be taken to carefully ramp up the availability of residual ammonium in the bioreactor as the biomass develops. Ammonium is not only toxic at high concentrations, but its removal leads to a decrease in pH conflicting with the increase in pH associated with DIC removal and is used to trigger  $CO_2$  injection.

Another source of N is urea. Urea is an organic N-source and is thus an excellent support medium for bacterial growth; care is thus required in its use in algal cultivation. As a N-source for microalgae, urea is not associated with the toxicity issues affecting ammonium, nor with the energetic issues affecting nitrate reduction. However, the urease enzyme requires nickel, so this metal needs to be provided as a micronutrient.

Some amino acids (e.g., arginine, glutamate, glutamine) can provide excellent sources of not only N but also of C. Some others are poor nutrients (notably histidine, which has an atypical structure

which is not easy to catabolise). Purines and pyrimidines can also be good N-sources. However, these organic nutrients will also support the growth of bacteria and fungi.



**Fig. 3.6.** (De)-repressive regulation of N-source acquisition. Through the scheme shown, the internal concentration of the first organic product of inorganic-N assimilation, glutamine (Gln), likely allied to the concentration of a C-substrate such as 2-oxoglutaric acid (2-OG) is implicated in the control of the transport of ammonium and nitrate, and the synthesis of enzymes for  $N_2$ -fixation (nitrogenase) and nitrate+nitrite reductases (indicated here as NNiR, though they are often separated within the cell, nitrite reductase being closely associated with chloroplasts in protist microalgae). Nitrogenase is only present in a few cyanobacteria; by this scheme it would only then be expressed if there was insufficient ammonium or nitrate available to repress its synthesis. By the same token, the ability to use nitrate is only de-repressed (enabled) if there is insufficient ammonium assimilation to raise levels of Gln. See also Fig. 3.5.

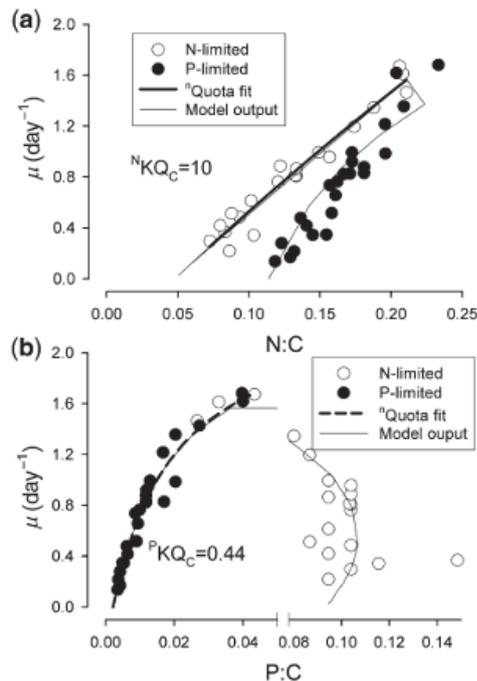
### N-quota growth relationship

The N-quota, describing the amount of N within the organisms as the value of N:C, is broadly linearly related to the potential growth rate between the values of the minimum quota ( $NC_{min}$ ) and the optimal value ( $NC_{opt}$ ). N:C can exceed  $NC_{opt}$  in cells growing using ammonium especially under low light conditions for the organisms (Fig. 3.7a).

## 3.6 Phosphorous nutrition

### P-sources

The usual source of P used for growing microalgae is inorganic phosphate ( $PO_4^{3-}$ ). However, microalgae (and microbes in general) rapidly express phosphatase enzyme activity (by which they can exploit organic P compounds) when they become P-stressed. Thus, marine microalgae (noting that seawater is alkaline) express an alkaline phosphatase, while freshwater microalgae (growing in acidic media) express an acid phosphatase. Microalgae may also express 5' nucleotidase activity (Flynn *et al.* 1986).



**Fig. 3.7.** Relationships between N:C and P:C with growth rate. Note how the relationships vary depending on whether N or P is limiting. The dashed line is a regression line fit through the nutrient limited points for N:C (panel (a)) or P:C (panel (b)). The thin lines (“Model output”) comes from a simulation, showing how well a mechanistic model of algal physiology can align with experimentally derived data. From Flynn (2008).

Phosphate is often used in experimental freshwater systems not only as the P-source but also to provide a pH buffer; such a usage is not practicable in large scale culture. Not only does this leave excess phosphate in the growth medium (which constitutes a potential eutrophication problem), phosphate fertilizer supplies are predicted to become increasingly limited over the coming decades as readily extracted phosphate mines are exhausted. It is thus all the more important to control the usage of this nutrient. (N-fertilizer, in contrast, can be synthesised using atmospheric  $N_2$  in the Bosch-Haber process.) In marine culture, phosphate precipitates out of solution at concentrations above ca.  $35\mu\text{M}$ . This is not to say that the culture cannot be loaded with more P than ca.  $1\text{mgP L}^{-1}$ , because, just as ammonium can be loaded carefully into the system to prevent high residual concentrations, so phosphate can be bled in to support algal growth while restricting residual concentrations.

#### P-quota growth relationship

The P-quota (P:C) relationship with growth is curvilinear, not linear as is that for N:C (Fig. 3.7). This has important implications. For species that can accumulate polyphosphate a large excess of P can be laid down within cells and the external P-nutrient concentration is rapidly depleted; the external concentration of P is thus not a good indicator of the P-status of cells.

Having exhausted the external P-nutrient, the curved form of the P-quota relationship means that cells can lower their P:C significantly with little obvious impact on growth rates. This affects the N:P ratio requirements of the growth medium (see Section 3.6).

### 3.7 N:P ratio

Ultimately, in a culture system that depletes the N and P nutrients, the cellular content of N:P will reflect that in the growth medium. However, unless that condition is met, then there will be an excess of one or other nutrient in the growth medium. This excess represents both a direct financial waste, but also a potential secondary problem as the excess nutrient represents a eutrophication risk. Logic is thus to adjust the nutrient addition to match the needs of the growth system. This could be monitored by analysis of residual nutrient concentrations to add new nutrients. Because, as noted in Section 3.6, some microalgae can accumulate polyphosphate within their cells, monitoring DIP may give an inaccurate impression of the P-status of the crop. It is thus preferable to add N and P nutrient in a set ratio in line with requirements within the microalgae.

By convention the added nutrient N:P is often given at a mole ratio of 16:1 (mass ratio of 7.22:1). Actually, however, because of the shape of the P-quota curve this likely represents a significant waste of P-nutrient. Cellular ratios of N:P may be lowered to less than 32:1, and perhaps even approaching 64:1, without undue problem (Mayers et al. 2014). This is particularly useful if anaerobic digestate is used as the nutrient source (Mayers et al. 2017) as the N:P (essentially as ammonium:phosphate) is usually very high, requiring either a removal of excess ammonium, or addition of phosphate.

### 3.8 Silicon nutrition

Diatoms are often very fast growing microalgae. Most diatoms have cell walls made of silicate, and thus require silicon as an essential nutrient. The exception to this is *Phaeodactylum tricorutum*, which has so little Si in its wall that usually sufficient dissolves off glass culture vessels into the alkali marine medium used for its culture; growth of this organism in plastic bioreactors without some silicon addition may be unsuccessful in consequence.

Si nutrition, and thence the growth of regular diatoms, is problematic for two reasons:

- i. Like phosphate, silicate precipitates out of solution at elevated concentration in marine medium.
- ii. In total contrast to the other macronutrients (and also to micronutrients), there is no relationship between Si-quota (i.e., cellular Si:C) and growth. That means that Si that has been previously accumulated into cells cannot be shared amongst daughter cells at cell division. New Si deposition occurs at each cell division; if there is no Si in the medium cell division stalls, and worse, the cells can collapse. The whole culture can thus die very rapidly if Si nutrient is exhausted.

### 3.9 Micronutrients

Micronutrients are just as important as macronutrients. These include especially Fe (a key metal in energetic systems, such as chloroplasts and mitochondria, but also for the reduction of nitrate to ammonium), and B-group vitamins. Because these nutrients are required at very low concentration, and the bioavailability can be highly problematic (for example, Fe salts precipitate out of solution), a vast excess of micronutrients are usually added.

To counter the bioavailability issue, especially for metals, chelating agents are often also added. In laboratory systems this is typically EDTA. In other systems (and in nature) this chelating action is provided by humics, tannins and other forms of dissolved organic matter which often come as partial degradation products of vegetative matter. Soil extract is another ingredient added in some systems to provide a soup of micronutrients. Both humics and soil extracts are of chemically undefined character and thus their source needs to be carefully controlled for repeatability (this is the reason why laboratory workers usually use EDTA). Similarly, anaerobic digestate quality is also variable.

### 3.10 Self-limiting growth

Microalgal cultures limit their own growth. This happens most obviously at high cell abundance (in dense cultures), though it can also occur in very thin cell suspensions (Flynn & Berry 1999) giving rise to the critical-inoculum problem. The latter represents a failure of a culture to start rapid growth unless a significant inoculum is added, or there is a very long lag phase until a critical cell abundance level is attained. To overcome this problem, usually growers of commercial microalgae gradually bulk up culture volumes; they do not pour a few 100 mL into a bioreactor of 1000L, for example. At the other extreme, limitation of growth at high cell abundance even in the presence of high nutrient levels is associated with abiotic events such as self-shading (and also elevated pH if CO<sub>2</sub> is not introduced to counter DIC removal), and biotically through chemical interferences.

#### Self-shading

Self-shading occurs in all suspensions of microalgae. It actually also occurs within cells, but the greater problem is between cells when growing in dense suspensions. And the critical issue is not volumetric abundance (i.e., cells m<sup>-3</sup>), but areal abundance (i.e., cells m<sup>-2</sup>). This is because light enters at a surface and is progressively absorbed as photons pass through the cell suspension. The optical depth of the bioreactor, and the nutrient loading (which ultimately controls the standing crop) interact with the surface irradiance (PFD PAR) to define the light available for the individual cell. If the growth medium contains coloured dissolved organics (such as from anaerobic digestates), then this also absorbs light. High rates of growth thus require grow in optically shallow systems (see Figs. 3.2 vs 3.3), though this conflicts with the need to optimise areal production (affecting ground rental for commercial growers).

#### Allelopathy

Allelopathy refers to chemical signalling or interactions between organisms. These may be positive or negative interactions (enhancing or decreasing growth, respectively) and they may be between cells of the same species, or in multi-species systems between organism types.

The whole subject of allelopathy is poorly understood, but is clearly a population-density dependant event. As an example of the complexity of such interactions, consider the interaction between *Dunaliella* and *Isochrysis* in the absence or presence of the predatory dinoflagellate *Oxyrrhis*. *Dunaliella* produces compounds that binds vitamin B<sub>12</sub> (Davies & Leftley, 1985) so in a suspension with *Isochrysis*, the latter does not grow if the *Dunaliella* cell abundance is high enough. Add the predator, which prefers *Dunaliella*, and this allelopathic control of *Dunaliella* over *Isochrysis* growth is released. However, while *Oxyrrhis* will graze *Isochrysis* when the latter is N-sufficient, *Isochrysis* produces a toxin when it is N-starved, so if the timing of the interactions is correct, the unlikely outcome is that ultimately *Isochrysis* becomes the dominant organism as the *Oxyrrhis* cannibalises itself (Flynn et al. 1996; Mitra & Flynn 2006). Mixed culture systems thus have the potential to be difficult to control.

### 3.11 Conclusions

As may be gleaned from the previous sections, from Fig. 3.1, and will also come from the contents of Chapter 4, the permutations of variables in microalgal cultivation are vast. To explore all of these empirically (through laboratory studies, let alone using pilot scale bioreactors) would be a near-impossible activity. Part II of this book provides a simulation-based platform for at least gaining a first order understanding of the production systems.

## 4. Culture Systems

### 4.1 Introduction

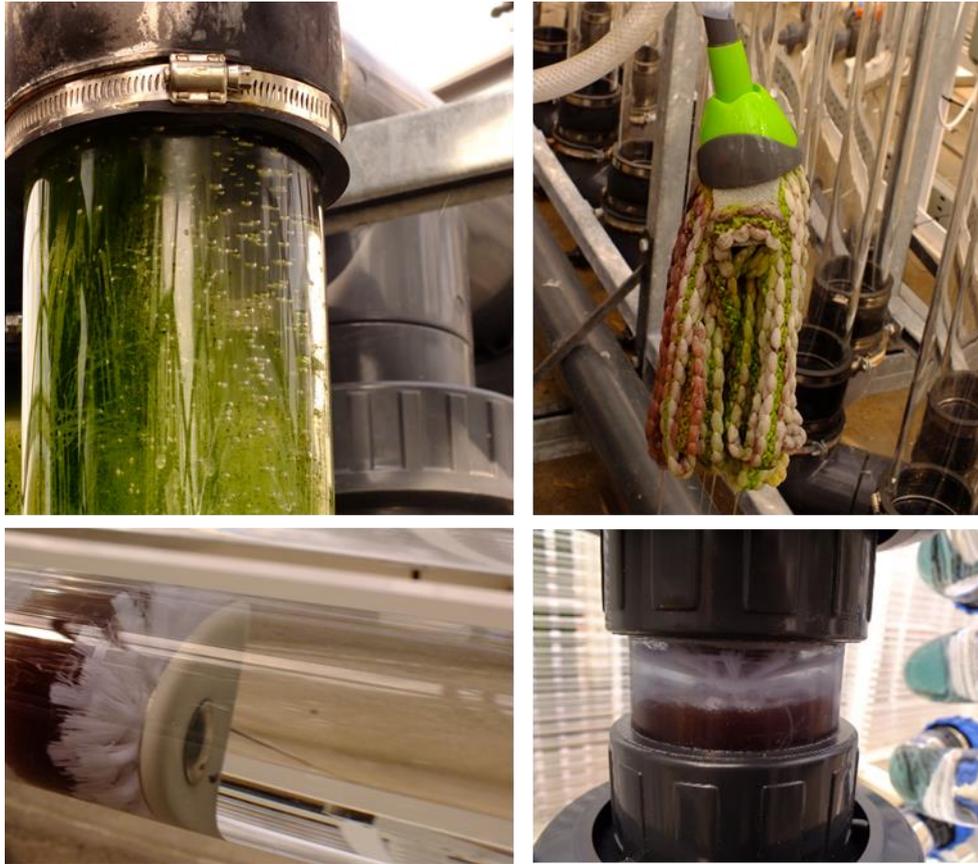
In this chapter we consider importance facets of the non-biological (abiotic) system affecting growth and harvesting of the microalgal crop. These are important factors that must be considered when configuring a simulator, if only to rule them out as being of little significance for the system being considered.

### 4.2 Reactor design – overview

Microalgae are generally grown in liquid suspension. Usually that means that the organisms are freely floating (planktonic). Although many protist microalgae can swim, their abilities to do so are feeble in comparison with the turbulence typically induced in bioreactors. Indeed, turbulence can kill many microalgae (and not just flagellates). Microalgae can, however, be grown on solid substrates as a biofilm, or on balls of an inert solid floating in the reactor. Indeed, a problem in many systems is to prevent the growth of microalgae on the walls of the bioreactor where they then prevent full light penetration, form a mass that is not readily harvestable, and can slough off causing blockages or otherwise fouling the system. Some species are buoyant, and some sink; both traits can cause problems during cultivation, though the trait can be of use as an aid to separating biomass from the growth medium during harvesting.

Growth on a solid substrate may be useful if the product of interest is exuded from the microalgae into the surrounding water, rather than the product being the algal biomass itself. There have been various trials of growing algae on alginate balls (e.g., Benasla & Hausler 2018). Growth in a biofilm or other solid substrate is inevitably slower due to diffusion gradients limiting nutrient acquisition by the cells, decreased diffusion of waste products away from cells, and decreased light penetration to those cells furthest away from the substrate surface which are shielded by overlying cells. That said, given the complexity and cost of harvesting to separate cells away from the exudate-containing medium, the use of solid substrates could well, on balance, be advantageous in some instances.

Typically, then, reactors are designed to maximise growth of microalgae in the main fluid stream, and minimise growth elsewhere. Thus, effort is expended on ensuring that there are no quiet corners in the fluid flow that may allow cells to accumulate. Micro-pellets, beads or a moving brush (Fig. 4.1) may be introduced to pass along tubes to slough off any biofilm growth.



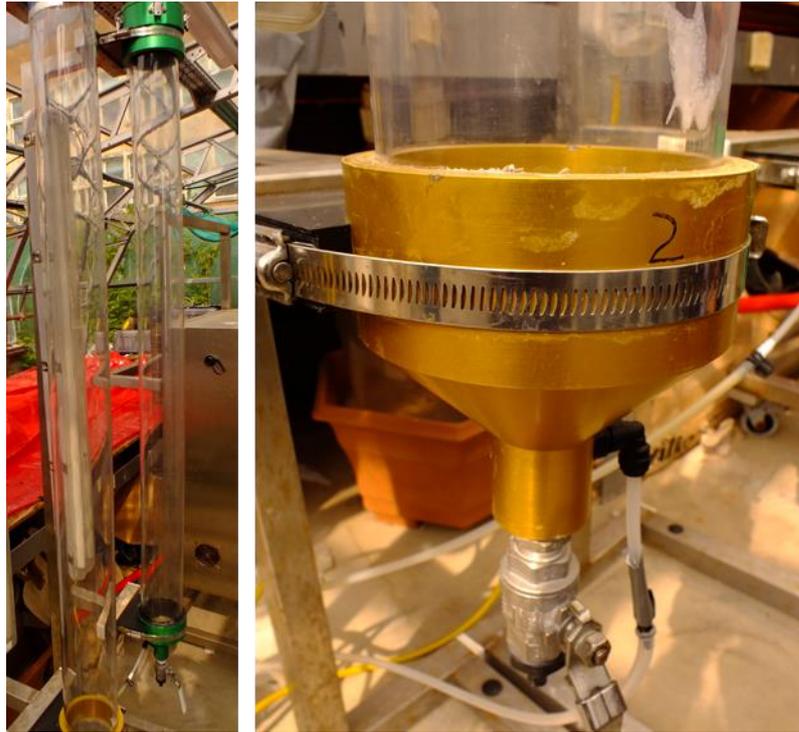
**Fig. 4.1.** Growth of microalgae on bioreactor walls can rapidly become highly significant (top-left), requiring the manual use of some form of brush or mop (top-right). A more high-tech option, that does not require the dismantling of the reactor, and indeed can be conducted during active culture growth, is to use a motile brush (lower panels). The brush moving through the glass tubes of a Varicon Phyco-Flow bioreactor (bottom-left) is otherwise housed in a section of the reactor when not being used (bottom-right). This particular reactor contained only water; in use the reactor takes on the dense colour of the culture (see also Fig. 4.6).

Reactors come in two basic forms:

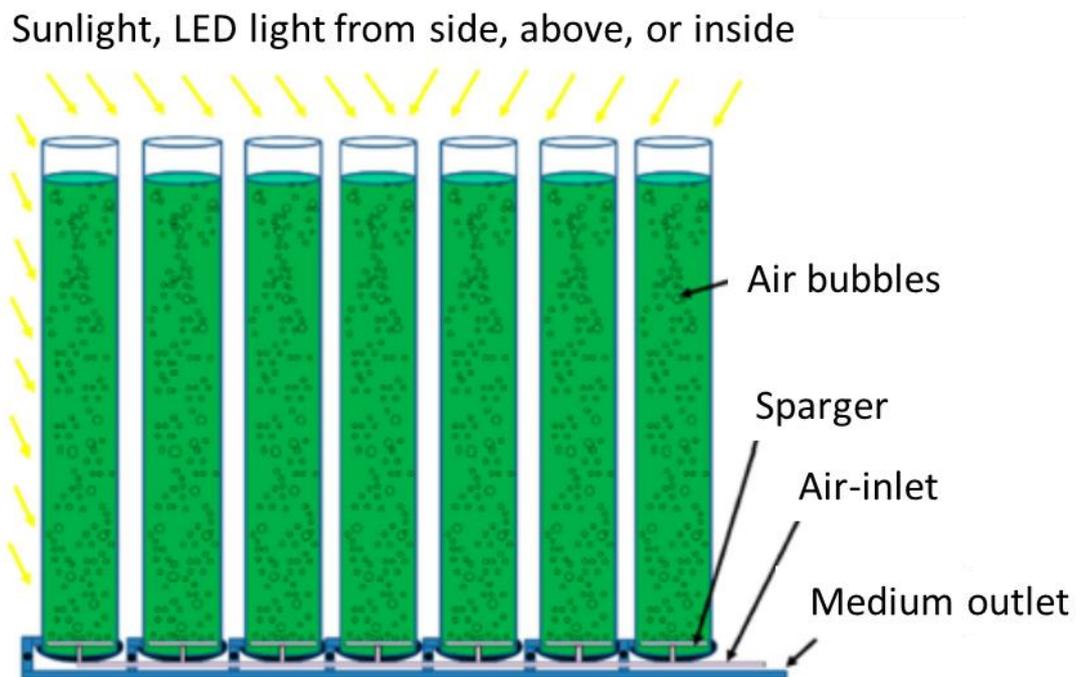
- Open volume reactors, typified by ponds, but also by flasks and similar vessels
- Closed volume reactors, typified by tubular or flat-plate reactors.

### Open Volume Reactors

Open volume reactors are relatively “cheap and cheerful” and are often used for batch cultures that are harvested in their entirety. At the most basic level, a simple flask could be considered as an open volume reactor. More commonly they are comprised of single tubes (glass or acrylic) of ca. 10cm diameter, with an aeration port at the bottom (Fig. 4.2). The type of reactor shown in Fig. 4.2 is scalable into a reactor format of multiple vertical-tubes (Fig. 4.3).

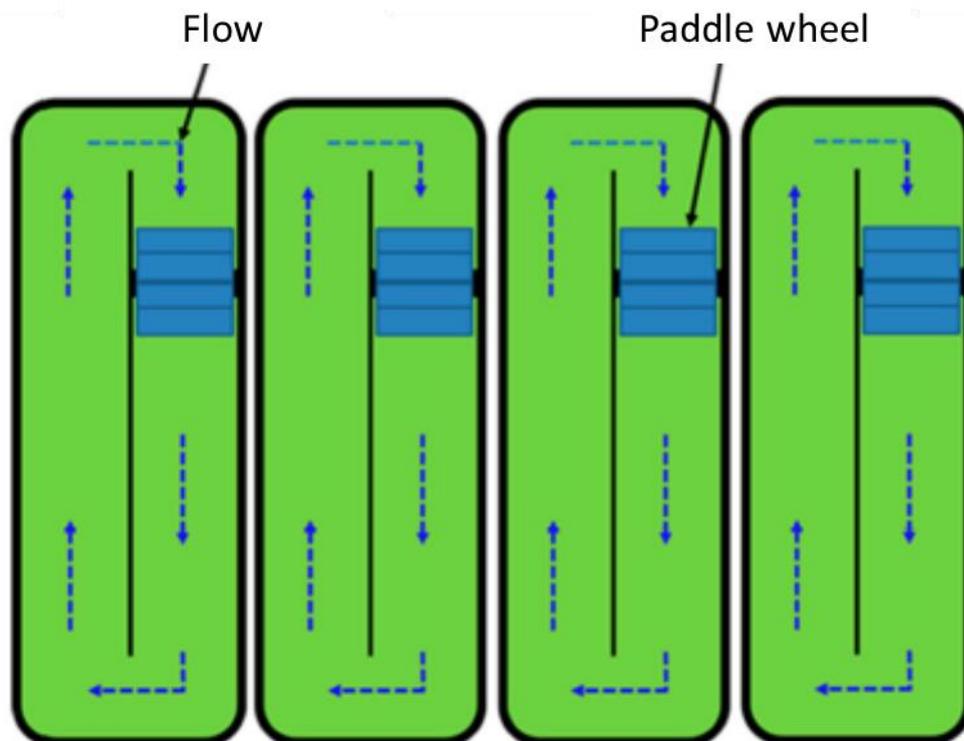


**Fig. 4.2.** Simple bioreactors for low volume (20-50L) batch cultures. These comprise lengths of acrylic tube with custom made end caps; detail of the lower cap (with aeration input) is shown on the right.



**Fig. 4.3.** Schematic of a modular PBR. Such a configuration can be scaled indefinitely but is not readily configured as a continuous-culture system but rather for batch growth. From Saha & Murray (2018).

At the other extreme, open reactors (as ponds; Fig. 4.4) can be truly massive and well suited to vast infrastructures with continuous harvesting. Such open reactors require some form of stirring (vigorous aeration, stirrer bars or paddles), and careful design to minimise dead zones where cells collect. Because paddles often do not work effectively in water shallower than ca. 30cm, ponds are most often optically deep; that is to say, the thickness of the algal suspension is such that light-limitation is common if not inevitable. This has important consequences for growth dynamics (Figs. 3.2 vs 3.3). Because pond reactors are typically totally open to the environment, contamination by other microalgae or by pests (infectious agents, or zooplanktonic grazers) can be common and highly damaging; the most successful crops in this regard are those species that grow in what may be termed extreme conditions of acidity (low pH) or high salinity which discourages growth of other species.



**Fig. 4.4.** Birds-eye schematic of a simple pond, “race-way”, culture system comprising outdoor rectangular ponds and paddle wheels to maintain a flow of mixed suspension. In addition to gas exchange at the surface, additional gas (CO<sub>2</sub>-augmented air) may be injected into the system. From Saha & Murray (2018).

### Closed Volume Reactors

Closed volume reactors are typically more expensive but are much more amenable to control. They are more likely used in continuous flow harvested systems, where a portion of the culture is harvested frequently. They require a pump to force the suspension around the system, and the consequential turbulence can damage or kill cells unless the pump is specifically designed and operated to minimise cavitation. Because the water is not directly open to the atmosphere, gas exchange ( $\text{CO}_2$  in,  $\text{O}_2$  out) needs to be more actively promoted; this is especially so with a horizontal tubular reactor, where bubbling in a (usually dark) chamber may be used to promote gas exchange. Closed volume reactors are optically shallow (a few cm), so they are better suited for production of high fatty acid products than are open volume reactors. Closed volume reactors are also much easier to keep as uni-algal, or perhaps even axenic with no bacteria, and are thus the reactor type that should be used when considering the growth of any genetically modified organism (GMO).



**Fig. 4.5.** Custom made multiple vertical-tube reactor with a total volume of 1000L. The diameter is 12cm. These acrylic tubes are connected at top and bottom so the culture is pumped through the whole system. Top-right shows the top caps, with degassing vents. Bottom-left shows the pH and  $\text{O}_2$  sensors in the return-from-pump branch of the system. Although of potentially infinite expansion, this reactor design is not amenable to easy-cleaning, requiring the removal of the upper unions and the use of a brush (see upper panels of Fig. 4.1).



**Fig. 4.6.** Commercial horizontal tube bioreactors; Varicon Biofence (left) and Phyco-Flow (right). The Biofence comprises narrow bore acrylic tubes that are grouped together for liquid flow, while the Phyco-Flow comprises larger bore but glass tubes which are linked end-to-end by curved connectors for an improved flow. The cleaning brush for the Phyco-Flow (see Fig. 4.1, lower panels) is housed between the orange taps in the dark vertical tube. To the left of that, and extending out of sight, is the gas-exchange tank which is particularly important in horizontal bioreactor configurations. Lower right shows the control panel and the dosing system for sterilizing the system prior to inoculation.

There are many different more exotic bioreactor designs, ranging from small bench-top systems to designs for reactors that float in the sea. Low volume reactors are best suited to studies of algal physiology; their low volume prevents the harvesting of significant biomass at a given time point, and they are not readily scalable either. Indeed, a fundamental challenge with most reactor designs is that of scalability and expandability. These are critical issues in commercial exploitation; the operator needs confidence that the system is reliable and if expanded then production is expanded pro rata with the system.

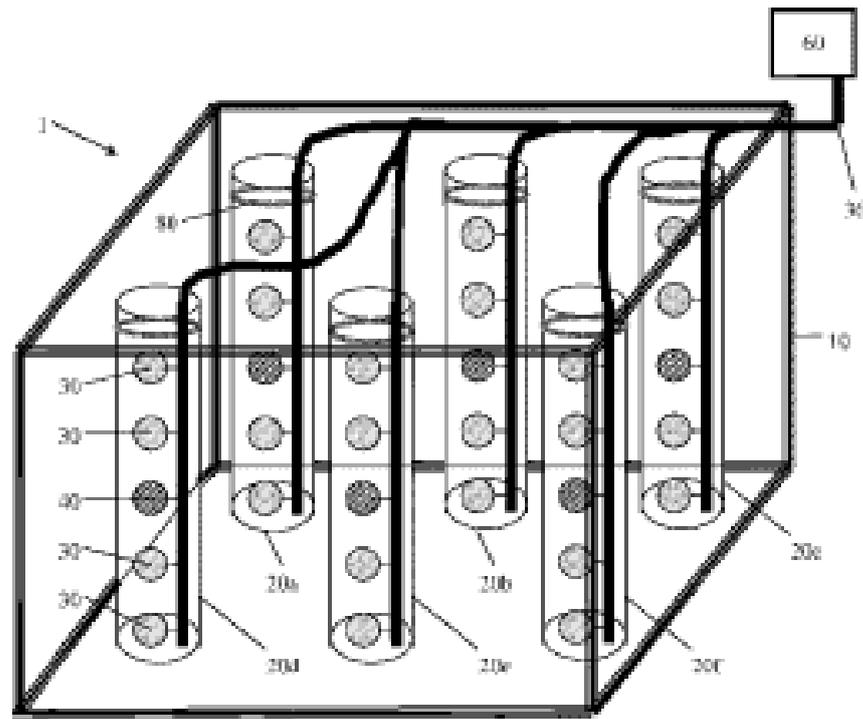


Fig. 1

**Fig. 4.7.** Schematic of an alternative closed reactor system, in which the volume is filled with the algal suspension and the lighting is provided via light arrays within rods. In the patent (GB2482083), the lighting was described with potential to be self-regulating to control energy consumption and limit the potential to over-illuminate thin cell suspensions or suspensions that were nutrient limited.

### 4.3 Reactor design – critical parameters

Irrespective of the reactor type and size, critical parameters in bioreactor design are:

- Cost per volume of culture (affecting infrastructure purchase price) and footprint (affecting ground rental).
- Optical depth (affecting microalgal growth rate and the potential for nutrient-limited growth).
- Lighting (natural and/or artificial), and proportion of the time cells spend in darkness.
- Temperature and pH control.
- Proportion of biomass lost from suspension (by adherence to the reactor walls, or into corners of the fluid system).
- Ease of maintenance (affecting down-time).

These are the types of parameters that need to be available in a simulator of microalgal growth for manipulation in “what-if?” scenarios and risk assessments.

## 4.4 Lighting

Light may be provided for free from the sun, but this is highly variable both with the seasons and on an almost minute-by-minute timeframe as clouds cross the sky. Because of the shape of the relationship between light and photosynthesis (the “PE-curve”; see Chapter 3) light is not limiting until the irradiance at the surface of the individual cell falls to below ca. 10% of maximum daylight levels. Indeed, full light can be distinctly deleterious, especially for nutrient-stressed cells which lack the physiological capacity to make good any photodamage. The problem, however, is that the cumulative microalgal biomass shades the individual cell as it is mixed within the bioreactor.

Natural (astronomical) light can be readily described in models with reference to the latitude of the growth facility, the date and locality-specific information on typical cloud cover. Artificial light can be used as the sole light source, but it can also be used to augment natural light, most obviously at night (though some species require a period of darkness; cell division in many species is synchronised to occur at night - Nelson & Brand 1979).

Both the quantity (photon flux) and the spectral quality (colour) of light is important. Human eyes are poor at detecting changes in quality and quantity; a light meter is required. For laboratory work, levels of irradiance are typically described using phrases such as “at the surface of the flask”; levels of light in the literature usually record values on the culture vessel face closest to the light source. Some researchers record light in the centre of a plain-water-filled vessel using a  $4\pi$  sensor (this looks like a small white ball on a stick) to better account for light coming from all angles, and also light being bounced around inside the vessel. In all instances there is the added issue of the spectral quality of light (a function of the light source, be it natural light, tungsten, fluorescent strip, LED etc.) and also of the calibration of the light sensor. LED lighting can be particularly problematic as light is often provided in a tight wavelength band (or several bands) rather than across a wide spectrum.

Coupled to the above is the subject of the action-spectrum of the phytoplankton photosystems, which acclimates to the light regime encountered by the organism itself. The action-spectrum describes the relationship between photosynthesis and light provided at each wavelength. As the organisms acclimate by synthesising different pigment types to capture photons across the wavelength range of 400-700nm, so the acclimative physiology of the phytoplankton themselves cause changes in PFD and the spectrum of residual light available to support the next period of photosynthesis.

Light is (or has been) recorded in various different units, such as foot-candles, lux, lumens, PFD, and  $\text{Wm}^{-2}$ , which do not easily relate to each other – see Thimijan & Heins (1983) for information on conversion factors. More worryingly, many experimentalists and most modellers pay scant regard to the light regime at all, even at the most basic descriptive level. Or they go to the other extreme and describe the light in great detail but fail to describe the microalgal physiology.

At the minimum we would expect the following information:

- Irradiance incident to the culture system, recorded as photosynthetically active radiation (PAR; 400-700nm wavelength) as it varies over the day.
- Optical depth of the culture suspension, and (if applicable) the proportion of the reactor volume in darkness.
- Absorbance coefficient of the growth medium (usually of minor concern but can be high if the medium contains anaerobic digestate or other sources of coloured dissolved organic matter, cDOM, such as tannins).

- Absorbance coefficient of the microalgal pigments (often related to the chlorophyll<sub>a</sub> content using taxonomic factors).

The unit of light is preferably as photon flux density (PFD; moles of photons m<sup>-2</sup> s<sup>-1</sup>), or as energy (W m<sup>-2</sup>).

#### 4.5 Temperature and humidity

Biochemistry, and thence whole-organism biological processes, are affected significantly by temperature. Any light source will heat the system, though heating from LEDs is minimal. As a rule of thumb, for a 10°C increase in temperature, biological rate processes double; this is often referred to as **Q<sub>10</sub>=2**. This is typically described mathematically using the **Arrhenius function**. The Arrhenius function takes various forms (depending on applications), but the cut-down version used for biology is:

$$U_T = U_{ref} \cdot Q_{10}^{\left[\frac{(T-T_{ref})}{10}\right]} \quad \text{Eq.4.1}$$

Here,  $U_{ref}$  is the process rate at the reference temperature,  $T_{ref}$ .  $Q_{10}$  is the multiplier for changes in the rate per 10°C, and  $U_T$  is the process rate at temperature  $T$ . Different components of physiology (e.g. photosynthesis vs respiration) may be affected differently, so while whole-organism growth may be assigned a  $Q_{10}$ , the balance of its biochemistry may be changed.

Temperature not only increases reaction rates, but it increases damage and thence turnover rates of proteins (enzymes). In consequence the relationship between temperature and organism physiology is highly complex (especially if temperature is changing over the day), and simple relationships are operable only over a narrow temperature span. However, in simple terms one may expect processes during the day to run faster than those at night, when it is cooler. In practice there is a lag in such response as it takes time for the water of the growth medium to heat and cool. All too easily in shallow reactors temperature can increase to lethal levels, though the high specific heat capacity of water slows the rate of change. In a reactor that is not operated in a temperature-controlled environment, if input weather conditions are conducive, significant diel changes in water temperature will thus develop.

Reactor water temperature is also altered by the temperature of the incoming water flows, and by heat exchange across the material that forms the reactor. Temperature is also affected by the incident irradiance, the air temperature, wind and humidity (which collectively affect evaporation), and also by cloud cover which affects dark radiation of heat back into space (most notably at night with no cloud cover). Evaporation from an open pond cools the water, but changes in pond water temperature (especially over the day-night, cycle) become increasingly apparent as the pond dries out because the residual water volume provides an ever-decreasing thermal buffer against temperature changes.

Extremes of evaporation also affect the salinity of pond water. This can be detrimental (as energy is wasted by the microalgae making osmoticums) but the process is used to advantage in the culturing of certain species (most notably the growth of *Dunaliella*, which synthesis both glycerol and carotenoid under such conditions).

## 4.6 pH and gas exchange

These factors are coupled because in many systems pH is buffered by carbonate, and carbonate concentrations are affected by the balance of CO<sub>2</sub> removal by photosynthesis and gas exchange of CO<sub>2</sub>. CO<sub>2</sub> entry into water directly from the atmosphere is very slow and is quite insufficient to balance even a slow rate of microalgal growth in dense cultures unless the water is strongly agitated.

There are 3 forms of dissolved inorganic C: CO<sub>2</sub> aqueous, HCO<sub>3</sub><sup>-</sup> (bicarbonate) and CO<sub>3</sub><sup>-2</sup> (carbonate). Collectively, the 3 forms of dissolved inorganic C are referred to as DIC. Depending on the pH, the balance between these shifts to mostly CO<sub>2</sub> at low pH (high acidity) versus high carbonate at high pH. These forms thus interchange as the system equilibrates, but it is a slow reaction. The substrate for photosynthesis (specifically for the enzyme RuBisCO) is CO<sub>2</sub>. Microalgae have to depend either directly upon CO<sub>2</sub> in the water, or use carbonic anhydrase to convert HCO<sub>3</sub><sup>-</sup> to CO<sub>2</sub> for use by RuBisCO

In small-scale laboratory cultures, pH is often held constant using an organic buffer (such as Tris). This is extremely expensive, and the organic buffer itself can act as a substrate promoting bacterial growth. Dense algal cultures require additional CO<sub>2</sub> input to counter the collective removal of DIC by the growth microalgae; this can be supplied in the form of additional bicarbonate or by entry of CO<sub>2</sub> gas. This addition not only maintains the DIC concentration but buffers the water as well. Typically, in large systems pH and CO<sub>2</sub> concentrations are simultaneously maintained using a pH-stat which governs the injection of CO<sub>2</sub> gas to balance the removal of CO<sub>2</sub> by algal growth. Usually CO<sub>2</sub> entry is coupled with air in a 5% v/v mixture, or similar.

Another, important, reason to aerate the system is to bring O<sub>2</sub> into the culture medium during the night (to prevent anaerobic conditions developing due to respiration) and conversely to remove excess O<sub>2</sub> during the day. It is important to remove O<sub>2</sub> during the light else it can become (super-) saturating and thence inhibitory to the action of RuBisCO in photosynthesis.

pH is also affected by consumption of ammonium as the N-source for growth. Most microalgal cultures grown in experiments are supplied with nitrate as the N-source. However, the more important source of inorganic N, which comes from anaerobic digestate and wastewater flows etc. is ammonium (NH<sub>4</sub><sup>+</sup>) and ammonia (NH<sub>3</sub>). The balance of NH<sub>4</sub><sup>+</sup> vs NH<sub>3</sub> depends on pH; at high pH NH<sub>3</sub> is favoured and can outgas. While ammonium is the preferred N-source by microalgae, at higher concentrations it is toxic. In part this is due to a direct external pH effect, in part due to an internal pH effect on entry of ammonium into the organism. Growth using ammonium must thus be controlled carefully.

Two other points on pH:

- pH is a logarithmic scale, so a small change in pH reflects a large change in acidity (1 pH unit reflects a 10-fold acidity range).
- Microalgal growth is typically adversely affected by high pH, and during growth pH increases markedly unless steps are taken to control it. Values above ca. pH9 can often lead to microalgal cell death.

Because of the aforementioned, most bioreactors involve some direct proactive pH control (which can involve direct acid/alkali injection) and/or aeration using CO<sub>2</sub>-enriched air. In simulations it is easiest to assume the pH is held constant and DIC availability is maintained.

#### 4.7 Harvesting: when and how much

Harvesting could at the extremes be of the entire reactor contents, or of a small volume continuously taken off as part of a chemostat-style operational regime. A chemostat is a culture system in which the volume is held constant as a continuous stream of fresh growth medium is pumped into the system, balanced by the exact same rate of removal of culture (i.e. medium containing microalgae and part-spent nutrients). Chemostats provide a continuous production (though at a low instantaneous biomass and volume) of organisms at a fixed physiological status; the growth rate is fixed by the dilution rate. To operate a chemostat properly assumes that the culture growth is asynchronous; for microalgae (whose cell cycle becomes synchronised by the day-night transition) this requires growth in continuous light. It is also not possible to achieve growth rate exceeding ca. 75% of maximum without an increasing risk of the culture being washed out.

More usually, a culture approach is deployed in which an intermediate proportion (not all as in a batch, and not some very small volume as in a chemostat) is removed periodically and the balance topped up with fresh medium. The balance of proportion and frequency is a major factor affecting biomass productivity and also the physiological quality of the cells (noting that a period of nutrient-stress may be desirable to stimulate production of certain metabolites, so “poor quality” in this context is not necessarily a bad thing). If the frequency of harvest is increased to continuous, a chemostat-like system is being run.

Handling the spent water can provide another logistic challenge, as can the preparation of the replacement medium. Cleaning the reactor, with its associated downtime and cost, are additional factors. All of these processes place additional requirements for space and thus affect the final areal productivity calculations for financial viability.

#### 4.8 Harvesting the particulate &/or the soluble crop

By far the easiest harvesting approach is when the microalgal biomass is fed directly into aquaculture facilities, as support for plankton-feeding animals (notably bivalves, the brine shrimp *Artemia*, or for rotifers). Challenges here are associated with having the appropriate balance of different microalgae species available at the correct rate of production (i.e., gC/m<sup>3</sup>/d). Invariably the animals will not require feed suspensions as dense as those attainable in bioreactors, so the feed can be dripped in or otherwise greatly diluted. The flow-through rate of water in the system must be optimised to minimise flushing out of uneaten prey.

More typically, the biomass is harvested from the culture. The vast bulk (99% or so) of even a dense microalgal suspension is essentially water, as the growth medium. Harvesting the biomass crop is thus a non-trivial undertaking. Furthermore, as the process proceeds so the quality of the resultant paste of algal biomass can deteriorate (biochemistry continues unless the temperature is decreased rapidly to near 0°C). Initial harvesting thus needs to be quick and at a low temperature.

Harvesting may be preceded by addition of chemicals to promote flocculation through which the microscopic cells stick together creating larger, more readily handled, aggregates. Addition of flocculants provides a source of expense and can also complicate recycling of the water. Flocculation also affects the physiological state of the cells (depending on the duration of the process and the approach taken) and thence the chemical quality of the product. More chemical flocculants (e.g., alum) need to be added to saline medium, though raising the pH can provide an alternative approach (Pérez et al. 2017).

Harvesting itself is usually undertaken by some combination of centrifugation and/or (tangential-flow) filtration. Excess salts may need to be washed out of the slurry as well, which can cause damage to the cells due to changes in osmotic pressure. The paste may then be taken to dryness (as a powder) during freeze drying; care must be taken not to expose the biomass to temperatures above ca. 60°C else fatty acids can deteriorate. Some 2/3<sup>ds</sup> of the actual algal cell itself can be water.

Harvesting of biochemicals released into the growth medium is more problematic than recovering cells, as the balance of water and salts must be removed. This procedure usually requires a series of filtration or flocculation to remove the biomass (which of course may be used to support a separate production line) and then removal of water using ultra-filtration. Growth of microalgae within alginate beads provides an alternative strategy, enabling ready removal of the algal biomass prior to ultra-filtration.

#### 4.9 Coupled bioreactor systems

Culture systems may be coupled in various ways. Most obviously, perhaps, are microalgal bioreactors connected to aquaculture facilities. The waste water from such systems (following suitable treatment) could be returned back into the bioreactor so that waste nutrients (ammonium and phosphate released as excreta from animals) can re-enter the microalgal culture system(s). Coupled systems may also include multi-species bioreactor combinations.

One may also envisage connected bioreactors such as:

- inoculation system (low volume, perhaps with lower light and greater optical depth),
- main culture system (higher volume, high light and low optical depth)
- final stage with elevated temperature (perhaps to near lethal levels), nutrient limitation (extreme light and very low optical depth), or changes in salinity, for a final 24hr period of incubation to induce particular physiological (biochemical) responses prior to harvesting.

The potential complexity of operating such systems is obvious but becomes more problematic when one considers the potential biotic interactions (Chapter 3).

#### 4.10 Conclusions

Most of the above topics impact upon the simulation process supporting a Decision Support Tool (DST) either directly or indirectly. The costs of accomplishing certain ends will be site- if not reactor- and organism- specific. For simulating commercial operations, viability could be explored by inputting a range of possible costs, or production implications of running sub-optimal configurations. Simulations could also be used to explore the implications of processes not running

reliably or not to full efficiency. While the worst that could happen with some systems is a missed delivery schedule, with a real-time linkage to aquaculture there could be a major loss of livestock if the microalgal system failed with no back-up.

Making sure the DST simulator closely matches reality is clearly important. It is to that topic that we now turn to in later chapters.

## 5. The Basics of Simulation

### 5.1 Introduction

This chapter introduces some of the critical aspects of model development and testing for simulations of microalgal production. For more complete details on the subject of building models, please see the e-book by Flynn (2018) which is specifically intended to guide a curious reader who has never used simulation approaches before. In Part II we will develop and explore different models.

It must be stressed at the outset that to use the DST models associated with this book does not require an in-depth understanding of simulation modelling. The models provided can be operated (as they are presented for free use) requiring only the selection of different parameters (selected from a range of options, considering the same types of factors you would consider when setting up a real bioreactor), running the model, and then checking the graphs of the output. This chapter is here to provide a background understanding; as with all models (statistical or simulation) there are caveats that the user needs to appreciate.

### 5.2 Systems dynamics models and the operational platform

The models described here are “systems dynamics” constructs. Such models describe materials flowing around a system over time. The models are not steady-state (though they can be run to steady-state), and hence operate with time as a variable, and the materials flowing in the simulated system are accounted for. So, for example, N in the nutrient nitrate is converted into N within the growing microalgal biomass over the simulation period.

The platform used in this work is Powersim Studio ( [www.powersim.com](http://www.powersim.com) ), a piece of software that operates on Microsoft Windows. Here, it is assumed that the typical reader is not so likely to also be a programmer, hence the use of a commercial software package. Models originally accompanying this book are available to operate free for the end-user upon downloading Powersim Cockpit from [www.powersim.com](http://www.powersim.com) . The models themselves could, however, be built on any platform that can support calculus running ordinary differential equations (ODEs).

The models are built from different types of components. In very simple terms, these components are:

- constants (values that remain unchanged in the simulation, defining things like nutrient inflow concentrations, maximum bioreactor volume, microalgal maximum growth rate),
- state variables (values that define measurable things that have a history, such as biomass, pigment and nutrient concentrations in the reactor), and
- auxiliaries (these are equations describing rates of change, transformations between units, and so on).

The values of auxiliaries vary depending on the values of constants and the current value of state variables. Importantly, some auxiliaries define the flows of materials into state variables. Thus, an auxiliary describing the biomass growth rate defines the transfer of nutrient-N into biomass-N.

### 5.3 The models

Models of different complexity are described in Part II of this book. Some are provided to give insight into how microalgae grow, and how their physiology acclimates to changes in conditions. Other models describe bioreactor systems of different complexity, or also provide scope for introducing financial aspects.

The models are provided in a form that the reader could, on acquiring the Powersim Studio software, develop models that better simulate their own specific culture systems. The reader would likely benefit from working their way through the examples in Flynn (2018) before making such modifications.

For the reader who does not have the time, or indeed the patience, to develop their own models, much can be explored and learnt by playing with the models provided free to the end user.

### 5.4 Parameterising the models

The models as presented describe growth of a generic organism in a generic culture system. To make these models better represent particular systems containing specific species, the values of constants defining critical components of the model need to be changed. This is a process called parameterisation.

The most obvious constants that need to be changed are those that define the maximum growth rate, maximum pigment content, initial nutrient concentration of the growth medium, and the optical depth of the reactor. Using the free models, such components can be changed by simply selecting from the provided options. To undertake such modifications in a more detailed and systematic fashion requires the modeller to undertake a process termed tuning.

Tuning involves changing the values of parameters that control the behaviour of the model so that the output better aligns with the performance of a real system for which data are available. This process can be undertaken manually, using data and knowledge already to hand. Alternatively, or in addition, a more complex tuning process can be undertaken; to do this requires access to extensive data series against which the model output is compared. How an automated procedure operates to achieve this tuning is described in Flynn (2018), but in essence the value of constants controlling the model are altered (increased, decreased) subtly at the start of each simulation and, several thousand simulations later, the programme identifies the values of the constants that give an output that most closely aligns with the real data series.

As a separate but important overlapping issue is one associated with units of biomass. For simulators that balance the cost of inputs and outputs it is necessary to consider biomass in terms of C,N,P mass, with units of g. Most often in the commercial world, microalgal biomass is described in terms of dry weight, which does not provide any indication of the gross chemical (e.g., C:N:P) quality and quantity. We can convert between data types by applying transforms; for example, gC is ca.  $1/3^{\text{rd}}$  of g dry weight.

It is important to balance units across the model. Thus, you cannot make algal dry weight from nutrient supplied as moles of ammonium-nitrate; you make algal-N from nutrient-N, both of these

being described using the same unit (e.g.,  $\text{gN m}^{-3}$ ), and the rate of production will thus have units of  $\text{gN m}^{-3} \text{d}^{-1}$ .

### 5.5 Minimal parameterisation

At a minimum the following information is required about a culture system and the crop organism in order to make the DST models perform in alignment with your interests. Not all models require all these data types.

- Total volume of the culture system: This is required as  $\text{m}^3$ ; there are 1000 L in  $1 \text{ m}^3$ . A volume of pure water of  $1 \text{ m}^3$  has a weight (mass) of 1 metric ton.
- Ground area of the bioreactor &/or of the facility: This is required as  $\text{m}^2$ .
- Dilution &/or harvesting rate: If the culture system is run in a fashion akin to a chemostat then the volume-specific dilution rate is needed (the unit is  $\text{d}^{-1}$ ). Organisms growing in a chemostat do so at the same rate as the dilution rate; so, a dilution rate of  $0.693 \text{ d}^{-1}$  will drive a growth rate of  $0.693 \text{ d}^{-1}$ , which equates to a doubling of biomass every day. If the system is harvested in a discontinuous fashion, with removal of a portion of the reactor volume being matched with an equal volume of fresh (algal-free) growth medium, then the volume being removed ( $\text{m}^3$ ) and the frequency (d) are required.
- Optical depth: This is required as m; there are 100 cm in 1 m. The optical depth is the distance from the surface of the culture vessel closest to the light source to the point furthest from that surface. In a pond, the optical depth is the pond depth. In a tubular reactor, depending on how light is delivered, the optical depth may be approximated to the radius of the tube.
- Irradiance: The units for this should ideally be as  $\text{W m}^{-2}$  or PFD (mole photons  $\text{m}^{-2} \text{time}^{-1}$ ). See Thimijan & Heins (1983) for transformations between light units. Also required is the L:D periodicity as a decimal proportion of the 24hr period as light (e.g., a 18:6 L:D cycle would have a value of  $18/24 = 0.75$ ).
- Nutrient concentrations in the blank medium: This needs to be given for the macronutrients, for example expressed as  $\text{mgN}$  and  $\text{mgP L}^{-1}$  ( $= \text{g m}^{-3}$ ).
- Volume of the microalgae inoculum: This is the volume of culture added as an inoculum to the bioreactor, required as  $\text{m}^3$ ; there are 1000 L in  $1 \text{ m}^3$ .
- Inoculation concentration: This is the biomass concentration in the inoculum; it is required as  $\text{gC m}^{-3}$ . This unit is the same numerically as  $\text{mgC L}^{-1}$ . In an ideal world this value would be measured by elemental analysis, but few have access to such equipment. However, C content can be estimated from a knowledge of the cell size and cell abundance; there are algorithms relating cell volume to C content for different types of microalgae of different cell size (Menden-Deuer & Lessard 2000). Alternatively, you can estimate the C content from the dry weight; transforms are in the range of 0.3–0.5 between cell C and dry weight (Heymans 2001; Geider and LaRoche 2002; Béchet et al. 2014), with the value expected to vary between species and also within species depending on the nutrient status.
- Maximum specific growth rate of the microalga: A maximum growth rate equating to a cell doubling per day is  $0.693 \text{ d}^{-1}$ . However, if growth is proceeding in a light-dark illumination cycle, then the growth rate over the light phase needs to be much higher than this because

growth de facto only occurs over part of the day. Thus, in a 12:12h L:D cycle, the maximum growth rate may need to be closer to  $2 \times 0.693 \text{ d}^{-1}$ . In reality it is not as simple as this because respiration continues in darkness.

It is assumed that the culture system is of constant temperature (unless indicated otherwise), constant pH, and that neither DIC ( $\text{CO}_2$ ), nor any micronutrients (vitamins, trace metals) are limiting.

### 5.6 Advanced parameterisation

A much-improved parameterisation will be achieved by having knowledge of the information described below. As presented, the models assume typical values for these parameters with scope for selecting different values.

- Minimum, optimal and maximum N-quota and P-quota: These describe the amount of N and P within the cells with reference to cellular-C, and are thus given as  $\text{gN gC}^{-1}$  and  $\text{gP gC}^{-1}$ . These values vary between organisms and have a significant impact upon the ability of organisms to grow under nutrient limiting conditions and to accumulate C-rich metabolites (lipids, starch, etc). A cell that exhausts its N-nutrient will have a N:C quota that gradually decreases as continuing photosynthesis brings in C (which it lays down as lipid and/or starch) until N:C reaches the minimum quota. Conversely, in that same culture scenario, the organism will accumulate P so that its P:C increases until it reaches a maximum quota value when uptake of phosphorus will be halted (see Chapter 3).
- Maximum Chl:C: This reflects the maximum extent of photoacclimation. The units are  $\text{gChl gC}^{-1}$ , where Chl is actually  $\text{Chl}_a$ . This value not only affects photosynthesis but a high value expressed by cells in the entire culture results in self-shading. In a dense algal culture self-shading results in the self-limitation of culture growth.
- $\alpha^{\text{Chl}}$ : This defines the initial slope of the PE curve expressed per unit of  $\text{Chl}_a$ . In organisms with a high content of secondary photopigment this value will be higher (assuming all else is equal).

The need for other information (including factors affecting financial aspects) depends on the model and applications. These will be considered in the appropriate chapters in Part II.

### 5.7 Collecting data for tuning and model validation

Validation is a process through which behaviour of the model is compared against a data series separate to that used for tuning. To rigorously compare the behaviour of your model with reality, you need data collected over a time course representative of the culture period, with the culture system operated under various conditions. You do not need an exhaustive number of data series; two would be the minimum (one for tuning, one for validation).

The total number of time points also needs to be sufficient to capture the spread of the dynamics (e.g., lag, log, stationary phases). A minimum of 6 time points are required; ideally there should be a sample taken every day at the start and/or end of the light phase of growth. The units need to be

transformed as required to be consistent with those used by the model; dimensions used here are g, d, and m.

The types of data that you could usefully collect routinely are:

- Residual nutrient concentrations in the bioreactor (gN, gP, gSi m<sup>-3</sup>)
- Irradiance at the bioreactor surface (as energy or PFD)
- Cell numbers &/or Chl<sub>a</sub>, (numbers or gChl m<sup>-3</sup>)
- Temperature, pH
- For open bioreactors (ponds) water depth if this is not maintained as constant (m)

Other types of data that you could usefully collect are:

- Biomass abundance (dry weight m<sup>-3</sup>, then transformed to gC m<sup>-3</sup>)
- Biomass elemental content (C,N,P; g m<sup>-3</sup>)
- Pigments (g m<sup>-3</sup>)
- Biomass protein &/or lipid &/or carbohydrate (g m<sup>-3</sup>, then transformed to gN &/or gC m<sup>-3</sup>)
- DIC if pH is not constant (gC m<sup>-3</sup>)
- Specific metabolites of interest (g m<sup>-3</sup>). These would include biochemicals released into the growth medium if those are of interest.

### 5.8 Financial aspects

To enable a cost-benefit analysis, costs are required of the major consumables (energy, nutrients, water, preparation and harvesting). There are also ground rental costs, and staffing costs. Potential values of the product(s) are also needed.

In addition, it is important to consider the % downtime of the bioreactor in between batches, periodic programmed maintenance, and risks that may decrease productivity (sometimes cultures simply do not take off, or crash for no obvious reason). These are all important factors.

### 5.9 Conclusions

The functioning of any simulator is only as good as the product of the mathematical description of the system, the data used for its parameterisation, and the data against which the model is tuned or otherwise validated. In the absence of detailed supporting data you can still use the DST models, but you need to be more cautious of interpreting the results. The models described in Part II are based upon algal physiology and are underpinned by several decades of published (peer reviewed) research.

## 6. Decisions Support Tool Use

### 6.1 Introduction

This aim of this book is to describe the functionality behind a Decision Support Tool (DST) for the commercial growth of microalgae. A DST is exactly that; it is a support tool and you need to use your own judgement in making the ultimate decision(s). All DSTs come with caveats, and you need to understand how the tool works to best make your own judgements. This chapter provides additional information that may help you.

### 6.2 What do you want vs what you can get

Ultimately a commercial-facing DST aims to ensure that you make, rather than lose, money. No DST can possibly guarantee that you will make money, and certainly it cannot guarantee that you will make a given amount of money! There are simply too many external factors, in addition to doubts within the DST itself. It is also possible that the behaviour of your system does not align with that of the DST. That is most likely if you are considering a GM strain.

For microalgae there are various potential products. These range from the whole biomass, to very specific compounds. You may be interested in using the technology to clean “grey” water, removing “waste” nutrients, while simultaneously making biomass and/or compounds of use elsewhere. Financial gain may thus come from various routes, some of which may be optimised physiologically by growing the microalgae under contrary conditions. Thus, for example, production of protein (which is N-containing) conflicts with the production of lipids that are synthesised when cells are N-stressed.

The models described in Part II of this book are not directed towards specific metabolites. Usually, however, you can associate specific metabolites with one of the following:

- General biomass production (invariably, the higher the biomass production rate the faster you will make your compound of interest; this requires growth, at least for most of the culture period, that is not limited by light nor nutrients).
- Protein production (production without exhausting N-nutrient)
- Lipid production (production typically exhausting the N-nutrient).
- Pigment production (production usually without exhausting nutrient, but may be enhanced using different light levels, or allied with other stresses)
- Changes in temperature, salinity, pH (often these are stress conditions, so you first need a high biomass production).

Remember that yield is not the same as production rate. A yield equates to a one-off harvest event; typically in this context the amount of material that would be recovered from a bioreactor if that reactor was totally drained. Often the word “production” is used in an ambiguous way in the literature (especially within the grey literature and at meetings etc.). For financial success you need a high production rate – that is you need a production of an amount of material within a given period

of time, and usually you will want to know how much space you will need to achieve that rate (e.g., Kg per hectare per week).

### 6.3 Modes of operating the DST

There are different ways of exploiting a DST. Most likely you will wish to work through all the options before making any decisions.

Education/Play/Experiment: There is no substitute with playing with the simulation platform, to explore the range of possible outcomes. With a simulator you can learn and explore the extremes of the system dynamics envelope quickly and at minimal cost (essentially, just your time). Even if you think you understand these systems well, more than likely you will learn something new, especially as some outcomes are contradictory or counter-intuitive.

What-if Tests: Often coupled implicitly with “Education/Play/Experiment”, you will likely conduct “what-if?” tests. These will typically identify the extremes of the operational envelope, but you will likely then hone your understanding as you conduct more explicit tests. There is a near-infinite range of conditions that you could explore; the most obvious of these will be useful for you to explore by manually altering input parameters into the model. Eventually however, likely you will begin to wish that there was a better way: there is ... it is “optimisation” (see next).

Optimise: Rather than work through permutations of parameters manually, some modelling software can do this automatically. So, you can instruct the software to maximise lipid production while minimising water and nutrient usage. Whether you can easily undertake such optimisations depends on the software you are using; Powersim Studio enables this functionality, but it is not available using the free models.

Risk Analysis: You have gained an understanding of how to maximise your profit, but no parameter is ever constant, so how does this affect decision making? Weather changes affect lighting and temperature regulation costs, input costs change, the value of your product change (usually coming down as production increases and the market reacts to availability); all these and other factors constitute risks. Again, the software may be able to help with this. For each of your input parameters you can assign a range of values (minimum, maximum, average). The simulation is then automatically run many times and the software will output your target interests with the average (typical) result together with confidence limits.

### 6.4 Conclusions

The next stage is to explore some of the simpler simulators and then work your way through to models that most clearly align with your own interests. There is every likelihood that you will develop interests beyond those offered here. To explore those options, you will need to modify, combine or otherwise develop your own models to simulate the scenarios that interest you.

## Disclaimer

While the contents of this work, and the allied models, are directed towards the commercial production of microalgae, and are offered free in all good faith, neither the author nor the *EnhanceMicroAlgae* project can accept any liability whatsoever for any commercial (or other) judgements made by any persons in consequence of the information contained herein, or the output of models.

It is up to the end user to ensure that the models are run under conditions most closely aligned with their interests.

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